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Handbook of Enzymes

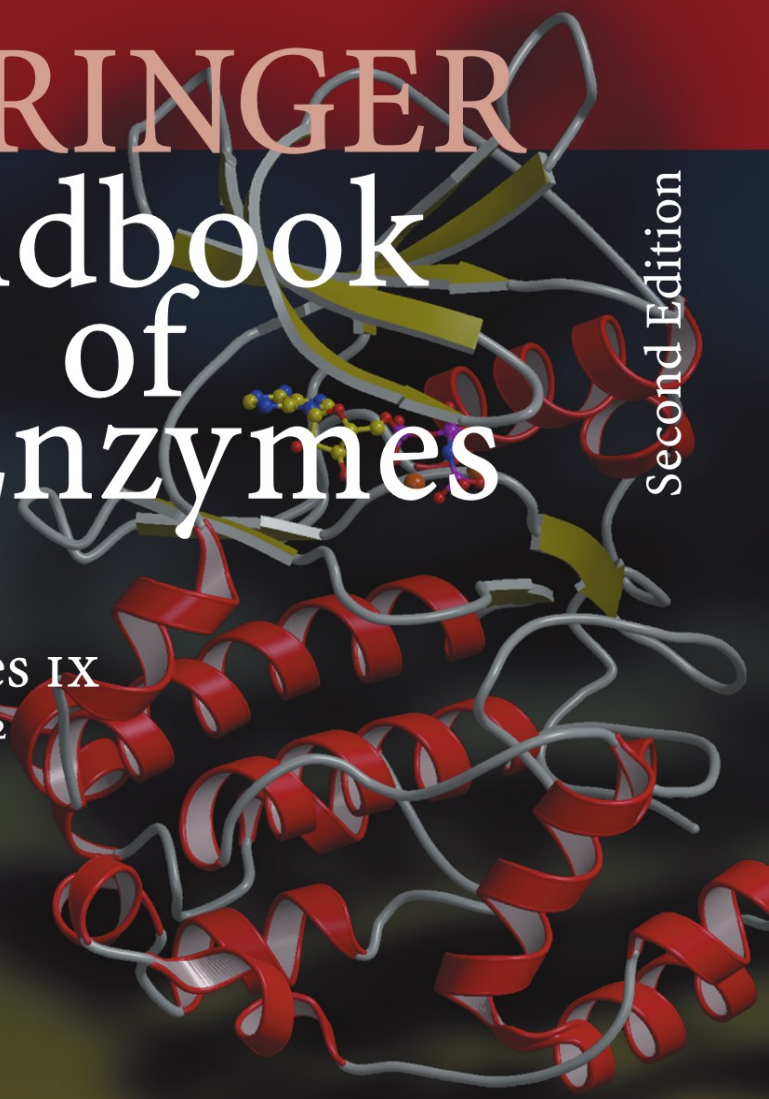
Second Edition

VOLUME 36

CLASS 2

Transferases IX

EC 2.7.1.38–2.7.1.112



Springer

Springer Handbook of Enzymes Volume 36

Dietmar Schomburg and
Ida Schomburg (Eds.)

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Volume 36

Class 2 · Transferases IX

EC 2.7.1.38–2.7.1.112

coedited by Antje Chang

Second Edition

Springer

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Attention all Users of the "Springer Handbook of Enzymes"

Information on this handbook can be found on the internet at
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A complete list of all enzyme entries either as an alphabetical Name Index or as the EC-Number Index is available at the above mentioned URL. You can download and print them free of charge.

A complete list of all synonyms (> 25,000 entries) used for the enzymes is available in print form (ISBN 3-540-41830-X).

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Preface

Today, as the full information about the genome is becoming available for a rapidly increasing number of organisms and transcriptome and proteome analyses are beginning to provide us with a much wider image of protein regulation and function, it is obvious that there are limitations to our ability to access functional data for the gene products – the proteins and, in particular, for enzymes. Those data are inherently very difficult to collect, interpret and standardize as they are widely distributed among journals from different fields and are often subject to experimental conditions. Nevertheless a systematic collection is essential for our interpretation of genome information and more so for applications of this knowledge in the fields of medicine, agriculture, etc. Progress on enzyme immobilisation, enzyme production, enzyme inhibition, coenzyme regeneration and enzyme engineering has opened up fascinating new fields for the potential application of enzymes in a wide range of different areas.

The development of the enzyme data information system BRENDA was started in 1987 at the German National Research Centre for Biotechnology in Braunschweig (GBF) and is now continuing at the Technical University Braunschweig, Institute of Bioinformatics & Systems Biology. The present book "Springer Handbook of Enzymes" represents the printed version of this data bank. The information system has been developed into a full metabolic database.

The enzymes in this Handbook are arranged according to the Enzyme Commission list of enzymes. Some 4,000 "different" enzymes are covered. Frequently enzymes with very different properties are included under the same EC-number. Although we intend to give a representative overview on the characteristics and variability of each enzyme, the Handbook is not a compendium. The reader will have to go to the primary literature for more detailed information. Naturally it is not possible to cover all the numerous literature references for each enzyme (for some enzymes up to 40,000) if the data representation is to be concise as is intended.

It should be mentioned here that the data have been extracted from the literature and critically evaluated by qualified scientists. On the other hand, the original authors' nomenclature for enzyme forms and subunits is retained. In order to keep the tables concise, redundant information is avoided as far as possible (e.g. if K_m values are measured in the presence of an obvious cosubstrate, only the name of the cosubstrate is given in parentheses as a commentary without reference to its specific role).

The authors are grateful to the following biologists and chemists for invaluable help in the compilation of data: Cornelia Munaretto and Dr. Antje Chang.

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Spring 2007

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List of Abbreviations

A	adenine
Ac	acetyl
ADP	adenosine 5'-diphosphate
Ala	alanine
All	allose
Alt	altrose
AMP	adenosine 5'-monophosphate
Ara	arabinose
Arg	arginine
Asn	asparagine
Asp	aspartic acid
ATP	adenosine 5'-triphosphate
Bicine	N,N'-bis(2-hydroxyethyl)glycine
C	cytosine
cal	calorie
CDP	cytidine 5'-diphosphate
CDTA	trans-1,2-diaminocyclohexane-N,N,N,N-tetraacetic acid
CMP	cytidine 5'-monophosphate
CoA	coenzyme A
CTP	cytidine 5'-triphosphate
Cys	cysteine
d	deoxy-
D-	(and L-) prefixes indicating configuration
DFP	diisopropyl fluorophosphate
DNA	deoxyribonucleic acid
DPN	diphosphopyridinium nucleotide (now NAD^+)
DTNB	5,5'-dithiobis(2-nitrobenzoate)
DTT	dithiothreitol (i.e. Cleland's reagent)
EC	number of enzyme in Enzyme Commission's system
E. coli	Escherichia coli
EDTA	ethylene diaminetetraacetate
EGTA	ethylene glycol bis(-aminoethyl ether) tetraacetate
ER	endoplasmic reticulum
Et	ethyl
EXAFS	extended X-ray absorption fine structure
FAD	flavin-adenine dinucleotide
FMN	flavin mononucleotide (riboflavin 5'-monophosphate)
Fru	fructose
Fuc	fucose
G	guanine
Gal	galactose

GDP	guanosine 5'-diphosphate
Glc	glucose
GlcN	glucosamine
GlcNAc	N-acetylglucosamine
Gln	glutamine
Glu	glutamic acid
Gly	glycine
GMP	guanosine 5'-monophosphate
GSH	glutathione
GSSG	oxidized glutathione
GTP	guanosine 5'-triphosphate
Gul	gulose
h	hour
H4	tetrahydro
HEPES	4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid
His	histidine
HPLC	high performance liquid chromatography
Hyl	hydroxylysine
Hyp	hydroxyproline
IAA	iodoacetamide
IC 50	50% inhibitory concentration
Ig	immunoglobulin
Ile	isoleucine
Ido	idose
IDP	inosine 5'-diphosphate
IMP	inosine 5'-monophosphate
ITP	inosine 5'-triphosphate
K_m	Michaelis constant
L-	(and D-) prefixes indicating configuration
Leu	leucine
Lys	lysine
Lyx	lyxose
M	mol/l
mM	millimol/l
m-	*meta-*
Man	mannose
MES	2-(N-morpholino)ethane sulfonate
Met	methionine
min	minute
MOPS	3-(N-morpholino)propane sulfonate
Mur	muramic acid
MW	molecular weight
NAD^+	nicotinamide-adenine dinucleotide
NADH	reduced NAD
$NADP^+$	NAD phosphate
NADPH	reduced NADP
NAD(P)H	indicates either NADH or NADPH

NBS	N-bromosuccinimide
NDP	nucleoside 5'-diphosphate
NEM	N-ethylmaleimide
Neu	neuraminic acid
NMN	nicotinamide mononucleotide
NMP	nucleoside 5'-monophosphate
NTP	nucleoside 5'-triphosphate
o-	*ortho-*
Orn	ornithine
p-	*para-*
PBS	phosphate-buffered saline
PCMB	*p*-chloromercuribenzoate
PEP	phosphoenolpyruvate
pH	$-\log10[H^+]$
Ph	phenyl
Phe	phenylalanine
PHMB	*p*-hydroxymercuribenzoate
PIXE	proton-induced X-ray emission
PMSF	phenylmethane-sulfonylfluoride
p-NPP	*p*-nitrophenyl phosphate
Pro	proline
Q_{10}	factor for the change in reaction rate for a 10°C temperature increase
Rha	rhamnose
Rib	ribose
RNA	ribonucleic acid
mRNA	messenger RNA
rRNA	ribosomal RNA
tRNA	transfer RNA
Sar	N-methylglycine (sarcosine)
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
Ser	serine
T	thymine
t_H	time for half-completion of reaction
Tal	talose
TDP	thymidine 5'-diphosphate
TEA	triethanolamine
Thr	threonine
TLCK	N^{α}-*p*-tosyl-L-lysine chloromethyl ketone
T_m	melting temperature
TMP	thymidine 5'-monophosphate
Tos-	tosyl- (*p*-toluenesulfonyl-)
TPN	triphosphopyridinium nucleotide (now $NADP^+$)
Tris	tris(hydroxymethyl)-aminomethane
Trp	tryptophan
TTP	thymidine 5'-triphosphate
Tyr	tyrosine
U	uridine

U/mg	μmol/(mg*min)
UDP	uridine 5'-diphosphate
UMP	uridine 5'-monophosphate
UTP	uridine 5'-triphosphate
Val	valine
Xaa	symbol for an amino acid of unknown constitution in peptide formula
XAS	X-ray absorption spectroscopy
Xyl	xylose

List of Deleted and Transferred Enzymes

Since its foundation in 1956 the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB) has continually revised and updated the list of enzymes. Entries for new enzymes have been added, others have been deleted completely, or transferred to another EC number in the original class or to different EC classes, catalyzing other types of chemical reactions. The old numbers have not been allotted to new enzymes; instead the place has been left vacant or cross-references given to the changes in nomenclature.

Deleted and Transferred Enzymes
For EC class 2.7.1.38–2.7.1.112 these changes are:

Recommended name	Old EC number	Alteration
mannitol kinase	2.7.1.57	deleted
protamine kinase	2.7.1.70	deleted, identical to EC 2.7.1.37
thymidine kinase	2.7.1.75	transferred to EC 2.7.1.21
NAD kinase	2.7.1.96	deleted, included in EC 2.7.1.86
opsin kinase	2.7.1.97	deleted, included in EC 2.7.1.125
phosphoenolpyruvate-fructose phosphotransferase	2.7.1.98	deleted

Index of Recommended Enzyme Names

Description of Data Fields

All information except the nomenclature of the enzymes (which is based on the recommendations of the Nomenclature Committee of IUBMB (International Union of Biochemistry and Molecular Biology) and IUPAC (International Union of Pure and Applied Chemistry) is extracted from original literature (or reviews for very well characterized enzymes). The quality and reliability of the data depends on the method of determination, and for older literature on the techniques available at that time. This is especially true for the fields *Molecular Weight* and *Subunits*.
The general structure of the fields is: **Information – Organism – Commentary – Literature**
The information can be found in the form of numerical values (temperature, pH, K_m etc.) or as text (cofactors, inhibitors etc.).
Sometimes data are classified as *Additional Information*. Here you may find data that cannot be recalculated to the units required for a field or also general information being valid for all values. For example, for *Inhibitors*, *Additional Information* may contain a list of compounds that are not inhibitory.
The detailed structure and contents of each field is described below. If one of these fields is missing for a particular enzyme, this means that for this field, no data are available.

1 Nomenclature

EC number
The number is as given by the IUBMB, classes of enzymes and subclasses defined according to the reaction catalyzed.

Systematic name
This is the name as given by the IUBMB/IUPAC Nomenclature Committee

Recommended name
This is the name as given by the IUBMB/IUPAC Nomenclature Committee

Synonyms
Synonyms which are found in other databases or in the literature, abbreviations, names of commercially available products. If identical names are frequently used for different enzymes, these will be mentioned here, cross references are given. If another EC number has been included in this entry, it is mentioned here.

CAS registry number

The majority of enzymes have a single chemical abstract (CAS) number. Some have no number at all, some have two or more numbers. Sometimes two enzymes share a common number. When this occurs, it is mentioned in the commentary.

2 Source Organism

For listing organisms their systematic name is preferred. If these are not mentioned in the literature, the names from the respective literature are used. For example if an enzyme from yeast is described without being specified further, yeast will be the entry. This field defines the code numbers for the organisms in which the enzyme with the respective EC number is found. These code numbers (form <_>) are displayed together with each entry in all fields of BRENDA where organism-specific information is given.

3 Reaction and Specificity

Catalyzed reaction

The reaction as defined by the IUBMB. The commentary gives information on the mechanism, the stereochemistry, or on thermodynamic data of the reaction.

Reaction type

According to the enzyme class a type can be attributed. These can be oxidation, reduction, elimination, addition, or a name (e.g. Knorr reaction)

Natural substrates and products

These are substrates and products which are metabolized in vivo. A natural substrate is only given if it is mentioned in the literature. The commentary gives information on the pathways for which this enzyme is important. If the enzyme is induced by a specific compound or growth conditions, this will be included in the commentary. In *Additional information* you will find comments on the metabolic role, sometimes only assumptions can be found in the references or the natural substrates are unknown.

In the listings, each natural substrate (indicated by a bold **S**) is followed by its respective product (indicated by a bold **P**). Products are given with organisms and references included only if the respective authors were able to demonstrate the formation of the specific product. If only the disappearance of the substrate was observed, the product is included without organisms of references. In cases with unclear product formation only a ? as a dummy is given.

Substrates and products

All natural or synthetic substrates are listed (not in stoichiometric quantities). The commentary gives information on the reversibility of the reaction,

on isomers accepted as substrates and it compares the efficiency of substrates. If a specific substrate is accepted by only one of several isozymes, this will be stated here.
The field *Additional Information* summarizes compounds that are not accepted as substrates or general comments which are valid for all substrates.
In the listings, each substrate (indicated by a bold **S**) is followed by its respective product (indicated by a bold **P**). Products are given with organisms and references included if the respective authors demonstrated the formation of the specific product. If only the disappearance of the substrate was observed, the product will be included without organisms or references. In cases with unclear product formation only a **?** as a dummy is given.

Inhibitors

Compounds found to be inhibitory are listed. The commentary may explain experimental conditions, the concentration yielding a specific degree of inhibition or the inhibition constant. If a substance is activating at a specific concentration but inhibiting at a higher or lower value, the commentary will explain this.

Cofactors, prosthetic groups

This field contains cofactors which participate in the reaction but are not bound to the enzyme, and prosthetic groups being tightly bound. The commentary explains the function or, if known, the stereochemistry, or whether the cofactor can be replaced by a similar compound with higher or lower efficiency.

Activating Compounds

This field lists compounds with a positive effect on the activity. The enzyme may be inactive in the absence of certain compounds or may require activating molecules like sulfhydryl compounds, chelating agents, or lipids. If a substance is activating at a specific concentration but inhibiting at a higher or lower value, the commentary will explain this.

Metals, ions

This field lists all metals or ions that have activating effects. The commentary explains the role each of the cited metal has, being either bound e.g. as Fe-S centers or being required in solution. If an ion plays a dual role, activating at a certain concentration but inhibiting at a higher or lower concentration, this will be given in the commentary.

Turnover number (min^{-1})

The k_{cat} is given in the unit min^{-1}. The commentary lists the names of the substrates, sometimes with information on the reaction conditions or the type of reaction if the enzyme is capable of catalyzing different reactions with a single substrate. For cases where it is impossible to give the turnover number in the defined unit (e.g., substrates without a defined molecular weight, or an undefined amount of protein) this is summarized in *Additional Information*.

Specific activity (U/mg)

The unit is micromol/minute/milligram of protein. The commentary may contain information on specific assay conditions or if another than the natural substrate was used in the assay. Entries in *Additional Information* are included if the units of the activity are missing in the literature or are not calculable to the obligatory unit. Information on literature with a detailed description of the assay method may also be found.

K_m-Value (mM)

The unit is mM. Each value is connected to a substrate name. The commentary gives, if available, information on specific reaction condition, isozymes or presence of activators. The references for values which cannot be expressed in mM (e.g. for macromolecular, not precisely defined substrates) are given in *Additional Information*. In this field we also cite literature with detailed kinetic analyses.

K_i-Value (mM)

The unit of the inhibition constant is mM. Each value is connected to an inhibitor name. The commentary gives, if available, the type of inhibition (e.g. competitive, non-competitive) and the reaction conditions (pH-value and the temperature). Values which cannot be expressed in the requested unit and references for detailed inhibition studies are summerized under *Additional information*.

pH-Optimum

The value is given to one decimal place. The commentary may contain information on specific assay conditions, such as temperature, presence of activators or if this optimum is valid for only one of several isozymes. If the enzyme has a second optimum, this will be mentioned here.

pH-Range

Mostly given as a range e.g. 4.0–7.0 with an added commentary explaining the activity in this range. Sometimes, not a range but a single value indicating the upper or lower limit of enzyme activity is given. In this case, the commentary is obligatory.

Temperature optimum (°C)

Sometimes, if no temperature optimum is found in the literature, the temperature of the assay is given instead. This is always mentioned in the commentary.

Temperature range (°C)

This is the range over which the enzyme is active. The commentary may give the percentage of activity at the outer limits. Also commentaries on specific assay conditions, additives etc.

4 Enzyme Structure

Molecular weight
This field gives the molecular weight of the holoenzyme. For monomeric enzymes it is identical to the value given for subunits. As the accuracy depends on the method of determination this is given in the commentary if provided in the literature. Some enzymes are only active as multienzyme complexes for which the names and/or EC numbers of all participating enzymes are given in the commentary.

Subunits
The tertiary structure of the active species is described. The enzyme can be active as a monomer a dimer, trimer and so on. The stoichiometry of subunit composition is given. Some enzymes can be active in more than one state of complexation with differing effectivities. The analytical method is included.

Posttranslational modifications
The main entries in this field may be proteolytic modification, or side-chain modification, or no modification. The commentary will give details of the modifications e.g.:
- proteolytic modification <1> (<1>, propeptide Name) [1];
- side-chain modification <2> (<2>, N-glycosylated, 12% mannose) [2];
- no modification [3]

5 Isolation / Preparation / Mutation / Application

Source / tissue
For multicellular organisms, the tissue used for isolation of the enzyme or the tissue in which the enzyme is present is given. Cell-lines may also be a source of enzymes.

Localization
The subcellular localization is described. Typical entries are: cytoplasm, nucleus, extracellular, membrane.

Purification
The field consists of an organism and a reference. Only references with a detailed description of the purification procedure are cited.

Renaturation
Commentary on denaturant or renaturation procedure.

Crystallization
The literature is cited which describes the procedure of crystallization, or the X-ray structure.

Cloning
Lists of organisms and references, sometimes a commentary about expression or gene structure.

Engineering
The properties of modified proteins are described.

Application
Actual or possible applications in the fields of pharmacology, medicine, synthesis, analysis, agriculture, nutrition are described.

6 Stability

pH-Stability
This field can either give a range in which the enzyme is stable or a single value. In the latter case the commentary is obligatory and explains the conditions and stability at this value.

Temperature stability
This field can either give a range in which the enzyme is stable or a single value. In the latter case the commentary is obligatory and explains the conditions and stability at this value.

Oxidation stability
Stability in the presence of oxidizing agents, e.g. O_2, H_2O_2, especially important for enzymes which are only active under anaerobic conditions.

Organic solvent stability
The stability in the presence of organic solvents is described.

General stability information
This field summarizes general information on stability, e.g., increased stability of immobilized enzymes, stabilization by SH-reagents, detergents, glycerol or albumins etc.

Storage stability
Storage conditions and reported stability or loss of activity during storage.

References
Authors, Title, Journal, Volume, Pages, Year.

Phosphorylase kinase 2.7.1.38

1 Nomenclature

EC number
2.7.1.38 (Protein kinases are in a state of review by the NC-IUBMB. This EC class will presumably be transferred to EC 2.7.11.19)

Systematic name
ATP:phosphorylase-b phosphotransferase

Recommended name
phosphorylase kinase

Synonyms
glycogen phosphorylase kinase
PSK-C3
phosphorylase b kinase
dephosphophosphorylase kinase
kinase, phosphorylase (phosphorylating)

CAS registry number
9001-88-1

2 Source Organism

<1> *Oryctolagus cuniculus* (New Zealand white (female) [3, 31]; New Zealand white (male) [13]; New Zealand white [45, 54, 56, 64]) [1, 3-15, 17-23, 25-27, 29, 31-34, 36, 37, 39, 40, 42, 44-51, 54-56, 58, 59, 61-64]
<2> *Canis familiaris* [2]
<3> *Mus musculus* [10, 15, 57, 60]
<4> *Rattus norvegicus* (male Wistar [14]) [10, 14, 19, 24, 38, 53]
<5> *Cavia porcellus* [10]
<6> *Bos taurus* [10, 16, 30, 32]
<7> *Gallus gallus* [10, 41, 43, 52]
<8> *Squalus acanthias* (i.e. Pacific dogfish [18]) [10, 15, 18]
<9> *Calliphoridae* (blowfly) [15]
<10> *Hyalophora cecropia* (silk moth) [35]
<11> *Saccharomyces cerevisiae* (commercial baker's yeast) [10, 28]

3 Reaction and Specificity

Catalyzed reaction

4 ATP + 2 phosphorylase b = 4 ADP + phosphorylase a (mechanism, <1> [22, 49, 55])

Reaction type

phospho group transfer

Natural substrates and products

S ATP + glycogen phosphorylase <1> (conversion to an AMP-independent form, key enzyme of neural and hormonal control of glycogen metabolism) [29]
P ?
S ATP + glycogen synthase <1, 4> (conversion to a glucose 6-phosphate dependent form, <1> [29, 31]; decreases activity of this substrate, <1, 4> [19]; key enzyme of neural and hormonal control of glycogen metabolism, <1> [29]) [19, 29, 31]
P ?
S ATP + phosphorylase b <1, 3-8> (i.e. EC 2.4.1.1 or glycogen phosphorylase, <1> [29, 55]; involved in glycogen metabolism regulation, <1> [1]; involved in glycogenolysis, <1, 3-8, 11> [10]; stimulates glycogenolysis in skeletal muscle, <1, 4> [19]; regulates conversion of inactive phosphorylase b into active phosphorylase a, <1,4> [19, 55]; vital process for short term energy supply to the cell, located at an interface between signalling and metabolic pathway, <1> [55]; key enzyme of neural and hormonal control of glycogen metabolism, <1> [29]) [1, 10, 19, 29, 55]
P ?

Substrates and products

S ATP + Ca^{2+}-dependent transport ATPase <1> (rabbit) [10]
P ?
S ATP + Lys-Arg-Glu-Gln-Ile-Ser-Val-Arg-Gly-Leu <1> [59]
P ADP + Lys-Arg-Glu-Gln-Ile-(phospho)Ser-Val-Arg-Gly-Leu <1> [59]
S ATP + Lys-Arg-Lys-Gln-Ile-Ser-Val-Arg-Gly-Leu <1> [59]
P ADP + Lys-Arg-Lys-Gln-Ile-(phospho)Ser-Val-Arg-Gly-Leu <1> [59]
S ATP + Lys-Arg-Lys-Gln-Ile-Ser-Val-Asp <3> [60]
P ?
S ATP + Lys-Arg-Lys-Gln-Ile-Ser-Val-Asp-Gly-Ile <3> [60]
P ?
S ATP + Lys-Arg-Lys-Glu-Ile-Ser-Val-Arg-Gly-Leu <1> [59]
P ADP + Lys-Arg-Lys-Glu-Ile-(phospho)Ser-Val-Arg-Gly-Leu <1> [59]
S ATP + Lys-Glu-Lys-Gln-Ile-Ser-Val-Arg-Gly-Leu <1> [59]
P ADP + Lys-Glu-Lys-Gln-Ile-(phospho)Ser-Val-Arg-Gly-Leu <1> [59]
S ATP + Lys-Pro-Val-Thr-Arg-Glu-Ile-Ser-Ile-Arg-NH_2 <3> (i.e. S-peptide) [60]
P ?
S ATP + Ser-Asp-Gln-Glu-Lys-Arg-Lys-Gln-Ile-Ser-Val-Asp <3> [60]

P ?
S ATP + Ser-Asp-Gln-Glu-Lys-Arg-Lys-Gln-Ile-Ser-Val-Asp-Gly-Ile <3> (i.e. phosphorylase b peptide (5-18)) [60]
P ?
S ATP + $\alpha\gamma\delta$ subunit complex <1> (autophosphorylation, by incorporation of phosphate into α subunit) [22]
P ADP + activated $\alpha\gamma\delta$ subunit complex <1> [22]
S ATP + casein <1, 4> (κ-casein, <1> [34, 44]; rabbit, <1> [10]; very poor substrate, <4> [24]; not, <1, 4, 7, 8> [14, 17, 18, 41]) [10, 24, 28, 34, 44]
P ?
S ATP + glycogen synthase <1, 3-4, 11> (glycogen synthase a, <1> [31]; at high concentration, from rabbit skeletal muscle, <1,4> [14, 24]; at high concentration, from yeast, <11> [28]; at the same rate as phosphorylase b, <1> [31]; inactivation of skeletal muscle glycogen synthase in the presence or absence of EGTA, <1, 4> [14]; phosphorylatable residue: Ser-7, <1> [10, 29, 31]; rabbit phosphorylase kinase, <1> [10]) [10, 14, 19, 24, 28, 29, 31, 34]
P ADP + phosphoglycogen synthase <1>
S ATP + histone H1 <1> [34]
P ?
S ATP + liver dephosphophosphorylase <1, 2> [2, 13]
P ?
S ATP + melittin <1> [56]
P ADP + phosphomelittin <1> [56]
S ATP + modified phosphorylase b <1> (modification at AMP-site) [8]
P ?
S ATP + myelin basic protein <1> [44]
P ?
S ATP + myosin light chain kinase <1> (rabbit) [15]
P ?
S ATP + nonactivated phosphorylase kinase <1, 3-8, 10> (i.e. autophosphorylation and autoactivation, <1, 3-8, 11> [10, 13, 17, 18, 20, 26, 37]; ATP can be replaced by dATP or adenosine 5'-(3-thiotriphosphate) with 50% and 10% efficiency, respectively, <8> [18]; presumably only in vitro, <1, 3-8, 11> [10]; in the presence of Mg^{2+} and Ca^{2+}, <10> [35]; phosphorylates α and β, not γ or δ subunits, <1> [37]; phosphorylation sites, <3> [60]; not, <7> [41]) [10, 13, 17, 18, 20, 26, 34, 35, 37, 43, 60]
P ADP + activated phosphorylase kinase <1, 3-8> [10, 13, 17, 18, 20, 26]
S ATP + peptides derived from glycogen synthase <1> (rabbit, overview) [10]
P ?
S ATP + phosphorylase b <1-11> (i.e. EC 2.4.1.1 or glycogen phosphorylase, <1> [13, 20, 29, 55]; r, <1> [9]; phosphorylase b from heart, <2> [2]; phosphorylation site: Ser-14, <1, 3-8, 11> [10, 29, 31, 60]; incorporation of terminal phosphate of ATP into phosphorylase b, <1> [13]; cosub-

strate: Mg-ATP complex, <1-11> [1-64]; ATP can be replaced by 8-azido-ATP and its 2',3'-dialdehyde derivative, not by any other natural nucleotide triphosphate, <1, 3-8, 11> [10]; main reaction, <1, 3-8, 11> [10]; binding studies with immobilized substrate, <1> [64]) [1-64]

P ADP + phosphorylase a <1, 3-11>

S ATP + sarcolemmal Na^+,K^+ ATPase <1> (rabbit) [10]

P ?

S ATP + sarcolemmal protein <1> [34]

P ?

S ATP + sarcoplasmic protein <1> [34]

P ?

S ATP + synthetic pentadecapeptide <1> (from amino-terminal of glycogen synthase, i.e. Pro-Leu-Ser-Arg-Thr-Leu-Ser-Val-Ser-Ser-Leu-Pro-Gly-Leu-Glu) [23]

P ?

S ATP + synthetic peptides derived from glycogen synthase <1> (overview, phosphorylation at the same site as glycogen synthase) [34]

P ?

S ATP + synthetic peptides derived from phosphorylase b <1, 3-9> (rabbit, <11> [10]; overview, <1,3-9,11> [10, 15, 34]) [10, 15, 34]

P ?

S ATP + synthetic tetradecapeptide <1, 8> (from amino-terminal of phosphorylase b, <1> [17]; i.e. Ser-Asp-Gln-Glu-Lys-Arg-Lys-Gln-Ile-Ser-Val-Arg-Gly-Leu, <1,8> [18, 23, 34, 49, 54]; phosphorylation site: Ser between Ile and Val, <8> [18]; substrate for holoenzyme and for catalytic γ subunit, <1> [49]) [17, 18, 23, 34, 49, 54]

P ?

S ATP + troponin I <1, 3> (rabbit phosphorylase kinase, <1> [10]; phosphorylation site (Thr-residue), <1> [10]; phosphorylation site, <1,3> [10, 60]; not rabbit or dogfish troponin I, <1,8> [18]) [10, 20, 26, 34, 60]

P ADP + phosphotroponin I <1, 3>

S ATP + troponin T <1> (not rabbit or dogfish troponin T, <1, 8> [18]) [34, 44]

P ADP + phosphotroponin T <1> [34, 44]

S GTP + phosphorylase b <11> (cosubstrate: Mg-UTP complex, <11> [28]; not (dogfish), <8> [18]; not, <1,3-6,8,11> [10, 16, 18, 32]) [28]

P GDP + phosphorylase a <11>

S UTP + phosphorylase b <11> (cosubstrate: Mg-UTP complex, <11> [28]; not (dogfish), <8> [18]; not, <1,3-6,8,11> [10, 16, 18, 32]) [28]

P UDP + phosphorylase a <11>

S Additional information <1, 3-8> (specificity, <1> [34]; $\gamma\delta$ complex catalyzes EGTA-insensitive phosphorylation of holoenzyme, <1, 3-8> [10]; no spontaneous or $MnSO_4$-induced dephosphorylation of activated enzyme, <1,4> [14]; no substrates are phosphorylase kinase γ subunit, <1> [17]; histone H2B, <1, 4> [14]; phosvitin, <1, 4, 7, 8> [18, 24, 41]; histone II^-A,

<1, 4, 8> [18, 24]; histone V-S, <4> [24]; troponin (whole complex), histone IIAS, <7> [41]; creatine phosphate, phosphoenolpyruvate, actin, parvalbumin, protamin, dogfish or rabbit myosin, adenosine 5'-(3-methyltriphosphate), 5'-adenylylimidodiphosphate (dogfish), <1, 8> [18]; polylysine, polyarginine, <1> [37]; Lys-Gln-Ile-Ser-Val-Arg, Ser-Asp-Gln-Glu-Lys-Arg-Lys-Gln-Gly-Ser-Gly-Arg-Gly-Leu, Lys-Gln-Ile-Thr-Val-Arg, Arg-Lys-Gln-Ile-Thr-Val-Arg, <1> [34]; ITP, CTP (dogfish), <1, 6, 8> [16, 18, 32]) [10, 14, 16-18, 24, 32, 34, 37, 41]

P ?

Inhibitors

(D)-Arg-(D)-Leu-(D)-Ser-(D)-Leu <1> (Ser-Asp-Gln-Glu-Lys-Arg-Lys-Gln-Ile-Ser-Val-Arg-Gly-Leu as substrate) [34]
(D)-Leu-(D)-Ser-(D)-Leu-(D)-Arg <1> [34]
(D)-Leu-(D)-Ser-(D)-Tyr-(D)-Arg-(D)-Arg-(D)-Tyr-(D)-Ser-(D)-Leu <1> [34]
$(NH_4)_2SO_4$ <1> (above 0.2 M, stimulates at 0.05-0.1 M) [13]
1,2-dimethoxyethane <1> (above 10% v/v, stimulates below) [22]
5'-adenylylimidodiphosphate <1> (substrate-directed dead end inhibitor) [49]
ADP <1> ($\gamma\delta$ subunit complex, <1> [22]; γ subunit, <1> [44]) [22, 44]
ATP <1, 4, 6> (total inhibition if ATP concentration exceeds that of divalent cation (i.e. Mg^{2+}), <1> [3]; total inhibition if ATP concentration exceeds that of divalent cation, <6> [16]; free ATP, reversible, <4> [38]; otherwise activating, <1,6> [3, 16]) [3, 16, 38]
actin <1> (inhibits activation of subunit γ-troponin C or subunit γ-calmodulin complexes) [54]
antibodies to δ subunit of phosphorylase kinase <4> [19]
antibodies to rabbit phosphorylase kinase <1> (rabbit) [10]
antibodies to rat testis calmodulin <4> (calmodulin or troponin (the latter at high concentrations) reverses) [19]
Arg-Lys-Gln-Ile-Thr-Val-Arg <1> (synthetic peptides as substrate) [34]
Ca^{2+} <1> (inhibition in millimolar, activation in micromolar range) [25]
calcineurin <1> (i.e. calmodulin-binding protein, blocks activation by calmodulin) [29]
calmodulin <1> (inhibits cAMP-dependent protein kinase mediated activation of phosphorylase kinase, kinetics) [46]
DTNB <1, 3, 8, 9> (only gradual loss of activity after more than 10 min, pH-dependent, <1> [6]) [6, 15]
EDTA <1, 8> (less effective than EGTA, <1> [4]; Ca^{2+} restores, <1, 8> [4, 18]; Ca^{2+} and Mg^{2+} partially protect, <1> [20]) [4, 18, 20]
EGTA <1, 3-8, 10> (strong, <1,6,8> [4, 16, 18]; partial, <4, 10> [24, 35]; non-activated enzyme, more effective than EDTA, <1> [4]; autophosphorylation, <1> [37]; together with trifluoperazime additive effect, <1> [23]; Ca^{2+} restores, <1, 3-8, 11> [10, 13, 16, 18, 22]; Ca^{2+} and Mg^{2+} partially protect, <1> [20]; irreversible upon prolonged incubation (liver enzyme), <1> [13]; kinetics, <1> [13]; effect on kinetic parameters, <1> [22]; influence on helical

structure, <1> [27]; $\alpha\gamma\delta$ and $\gamma\delta$ subunit complexes less sensitive than holoenzyme, <1> [22]; not, <1> [17]) [4, 10, 12-14, 16, 18, 20-24, 27, 35, 37, 41]
GTP <1, 6> ($\gamma\delta$ subunit complex, <1> [22]; weak, with ATP as substrate, <6> [16]) [16, 22]
D-glucose <1> (less effective than glucose 6-phosphate, pH 8.2) [3]
D-glucose 6-phosphate <1, 3, 8, 9> (pH 8.2, <1> [3]; Mg^{2+} protects, phosphorylase b as substrate, mechanism, kinetics, <1> [8]; no inhibition with modified phosphorylase b or a tetradecapeptide as substrate, <1> [8]; not ($\gamma\delta$ subunit complex), <1> [22]) [3, 8, 15]
heparin <1> (depending on pH it inhibits or activates nonactivated enzyme) [3]
hexametaphosphate <1> (pH 8.2) [3]
histone VIIS <1> ($\gamma\delta$ subunit complex) [22]
ITP <6> (weak, with ATP as substrate, <6> [16]; not ($\gamma\delta$ subunit complex), <1> [22]) [16]
Ile-Ser-Val-Arg-Gly <1> (Ser-Asp-Gln-Glu-Lys-Arg-Lys-Gln-Ile-Ser-Val-Arg-Gly-Leu as substrate) [34]
K252a <1> (microbial broth product, highly selective) [48]
KCl <3, 8, 9> (not, <1> [13]) [15]
Lys-Pro-Val-Thr-Arg-Glu-Ile-Val-Ile-Arg-NH_2 <3> (i.e. V-peptide) [60]
melittin <1> (model calmodulin-binding peptide, mechanism, kinetic, phosphorylase b as substrate) [56]
Mg^{2+} <1, 4> (in excess of ATP, <1,4> [14, 51]; free Mg^{2+}, only activated enzyme, reversible, <4> [38]; nonactivated and activated enzyme, <1, 4> [14]; not, <11> [28]) [14, 38, 51]
$MgADP^-$ <1> (product inhibition) [49]
Mn^{2+} <1, 4> (free Mn^{2+}) [22, 38, 44]
monospecific antibodies against α, β and γ subunits <1> (mechanism, kinetic, anti-β subunit reverses inhibition by anti-α at pH 6.8) [42]
NH_4Cl <1> (inhibits A1 and A2 activities by lowering of v_{max}, not A0, <1> [25]) [25, 26]
NaCl <1> (0.1 M) [13]
phenothiazin <1, 3-8> (blocks activation by extrinsic calmodulin) [10]
phosphorylase b <7> (high concentration) [41]
phosphotetradecapeptide <1> (product inhibition) [49]
poly-L-lysine <1> (strong, activated and nonactivated enzyme, stimulates autophosphorylation) [37]
polyaspartic acid <1, 3, 8, 9> (pH 8.2) [3, 15]
protamine <1, 3, 8, 9> (pH 8.2) [3, 15]
protein phosphatase <1> (type I, reverses autoactivation, <1> [50]; rabbit (not dogfish) kinase, <1> [18]) [18, 50]
quercetin <7> (ATP does not reverse) [41]
Ser-Asp-Gln-Glu-Lys-Arg-Lys-Gln-Ile-Asp-Val-Arg-Gly-Leu <1> (substrate-directed dead end inhibitor) [49]
staurosporine <1> [48]

synthetic peptide PhK13 <1> (derived from γ subunit region, residues 302-326, <1> [62]; kinetic, <1> [59, 62]; calmodulin reverses, <1> [59]; synergistic with PhK5, <1> [62]) [59, 62]
Synthetic peptide PhK5 <1> (derived from γ subunit region, residues 342-366, <1> [62]; kinetic, <1> [59, 62]; calmodulin reverses, <1> [59]; synergistic with PhK13, <1> [62]) [59, 62]
trifluoperazine <1> (nonspecific inactivation, at high concentrations, together with EGTA additive effect, <1> [23]; prevents activation by troponin C, <1> [29]) [23, 29]
UTP <1> (weak, $\gamma\delta$ subunit complex) [22]
Zn^{2+} <1> [3]
Additional information <1, 3-9, 11> (phosphorylase kinase α and β subunits suppress catalytic activity of γ subunit in holoenzyme, <1> [45]; γ subunit with autoinhibitory domains, <1> [59, 62]; inhibition study with modified γ subunit, <1> [59]; no inhibition by CTP, caffeine, cAMP, cGMP, IMP, D-glucose 6-phosphate ($\gamma\delta$ subunit complex), <1> [22]; no inhibition by D-glucose 1-phosphate ($\gamma\delta$ subunit complex), <1, 3, 8, 9> [3, 15, 22]; no inhibition by cAMP-binding protein, <1,11> [28, 50]; no inhibition by UDPglucose, <1, 3, 8, 9> [3, 15]; no inhibition by spermidine, spermine, F^-, <1> [3]; no inhibition by diethyldithiocarbamic acid, 2,2'-dipyridyl, <1> [4]; no inhibition by troponin (rabbit), <1> [10]) [3, 4, 10, 15, 22, 28, 45, 50, 59, 62]

Cofactors/prosthetic groups

2'-deoxy-ADP <1> (activation, can replace ADP) [58]
ADP <1, 3-8, 11> (activation, <1, 3-8, 11> [3, 10]; allosteric effector, <1,3-8,11> [10, 58]; kinetics, 8 binding sites per hexadecamer, <1> [58]; stimulates phosphorylase conversion and autophosphorylation, 8 mol ADP per mol $(\alpha\beta\gamma\delta)4$, <1, 3-8, 11> [10]; can partially replace ATP in the activation of nonactivated enzyme, <1> [3]) [3, 10, 58]
ATP <1, 3-8> (activation of nonactivated enzyme by phosphorylation of subunits A and B, not C <1> [3, 7]; by autophosphorylation or protein kinase phosphorylation, <1,3-8> [10]; not alone, only in the presence of Mg^{2+} or Mn^{2+}, <1,3-8> [10]; by phosphorylation of subunits α, β not γ, <1,3-8> [10]) [3, 7, 10]
calmodulin <1, 3-8> (requirement, <1, 3-8> [10, 19, 23, 29]; calmodulin containing enzyme i.e. tightly bound δ subunit, <1, 3-8> [10, 19, 23, 29]; calmodulin containing enzyme in the absence of Ca^{2+}, <1> [29]; activation of nonactivated enzyme and $\alpha\gamma\delta$ subunit complex, <1> [23]; activation of recombinant γ subunit at pH 6.8, slightly at pH 8.2, <3> [57]; additional activation <1, 3, 7> [31, 41, 57]; tightly bound molecule interacts with additional calmodulin in the presence of Ca^{2+}, <1> [29]; allosteric effector, <1, 3-8> [10]; interacts with α subunit, <1> [23]; in the presence of Ca^{2+} phosphorylase kinase binds a second molecule calmodulin (i.e. δ subunit) producing additional activation, <1, 4> [19]; in the presence of Ca^{2+}: 1 mol extrinsic calmodulin per mol $\alpha\beta\gamma\delta$ rabbit enzyme, <1> [10]; no activation of bovine red skeletal muscle or rabbit cardiac enzymes, <1> [10]; no activation of phos-

phorylated or proteolytically modified enzyme by δ'-subunit, <1> [31]; no additional activation <6> [30]) [10, 19, 23, 29-31, 41, 57]

Activating compounds

1,2-Dimethoxyethane <1> (activation, 10% v/v, stimulates phosphorylase kinase and $\alpha\gamma\delta$ (not $\gamma\delta$) subunit complex) [22]
Ca^{2+}-dependent protease <1, 3-8> (i.e. kinase-activating factor, <1> [3, 4]; or Ca^{2+}-activating factor, <1, 3-8, 11> [10]; proteolytic activation of nonactivated enzyme, <1, 3-8> [3, 10]; ir, <1, 3-8, 11> [10]) [3, 4, 10]
Casein protein kinase <1, 3-8> (activation of nonactivated enzyme) [10]
Catalytic subunit of cAMP-dependent protein kinase <1, 3-9> (activation of nonactivated enzyme, <1,3-9> [10, 15, 16, 19, 22, 24, 26, 30-32, 34, 43, 46]; or $\alpha\gamma\delta$ subunit complex, <1> [22]; by phosphorylation of α', <1, 6> [16, 32]; α, <1> [25, 31]; and β (not γ), <6> [16]; and β, <1> [25, 31, 32]; subunits, in the presence of ATP and Mg^{2+}, <1, 3-8> [10, 34]; Mn^{2+} stimulates, <1> [34]; kinetics, <1> [46]; at low Mg^{2+}-concentration, 2 phosphorylation sites, one Ser residue on α and β subunit each, <1, 4> [19]; activation by enhancing v_{max} selectively for A2 activity, <1> [25]; ATP cannot be replaced by 5'-AMP, 3'-AMP, 2',3'-AMP, CMP, CDP, CTP, UMP, UDP, UTP, GMP, GDP, GTP, IMP, IDP, ITP, <1, 3-8, 11> [10]; not, <1, 8> [18]) [10, 15, 16, 19, 22, 24, 25, 30-32, 34, 43, 46]
Catalytic subunit of cGMP-dependent protein kinase <1, 3-8> (activation of nonactivated enzyme, <1,3-8> [10]; not, <1,4> [19]) [10]
Chymotrypsin <1, 3-8> (proteolytic activation of nonactivated enzyme, <1,3-8> [10, 31, 43]; by limited proteolysis of α subunit, not γ or δ subunits, <1> [31]) [10, 31, 43]
Heparin <1, 3, 8, 9> (activation, <1, 3, 8, 9> [3, 15, 22, 28]; pH-dependent, <1, 3, 8, 9> [3, 15]; stimulates only holoenzyme, not subunit complexes, <1> [22]) [3, 15, 22, 28]
Papain <1, 3-8> (proteolytic activation of nonactivated enzyme) [10]
Poly-L-arginine <1> (strong, phosphorylase kinase as substrate, i.e. autophosphorylation) [37]
Poly-L-lysine <1> (strong, only with phosphorylase kinase as substrate, i.e. autophosphorylation, inhibits activity of activated and nonactivated enzyme with other substrates) [37]
Proteases <1, 3-9> (proteolytic activation of nonactivated enzyme, mechanism, <1,3-8,11> [10]) [10, 15]
Protein kinases <1, 3-8> (activation of nonactivated enzyme, phosphorylation sites, mechanism, <1, 3-8, 11> [10]; not (liver enzyme), <1> [13]; not, <8> [18]) [10]
Troponin <1> (i.e. complex of troponin C, I and T, activation, as effective as troponin C, forms complex with β subunit) [29]
Troponin C <1, 3-8> (activation, <1, 3-8> [10, 19, 29]; can replace extrinsic calmodulin, <1, 4> [19, 29]; presumably key event in vivo, coupling glycogenolysis and muscle contraction, <1, 4> [19]) [10, 19, 29]
Trypsin <1, 3-9> (proteolytic activation of nonactivated enzyme, <1,3-9> [3-5, 10, 15, 19, 23, 25, 30, 31, 41, 43]; strong, by limited proteolysis of α and β subunits (not γ), <1, 3, 8, 9> [15, 31]; strong, by limited proteolysis of α and

β subunits or δ subunits, <1> [31]; strong, by limited proteolysis of α and β subunits, <1, 3, 4, 8, 9> [15, 19, 31]; accompanied by loss of absolute requirement for Ca^{2+}, activates holoenzyme and $\alpha\gamma\delta$ subunit complex, not $\gamma\delta$ complex, <1,4> [19]; increase of pH 6.8 activity, not pH 8.2 activity, <6> [30]; at low concentration, <1> [3]; v_{max} enhancement of all three activities of the kinase: A0, A1 and A2, <1> [25]) [3-5, 10, 15, 19, 23, 25, 30, 31, 41, 43]
adenosine 3',5'-monophosphate <1, 3-8> (i.e. cAMP, activation of nonactivated enzyme, not alone, only in the presence of Mg^{2+} or Mn^{2+}, <1> [3]; cAMP mediated activation, <1, 4> [14]; cf. catalytic subunit of cAMP-dependent protein kinase, <1, 3-8> [10]; no enhancement or inhibition of this activation by various nucleotides and other compounds, overview, <1> [3]) [3, 10, 14, 16]
adenosine 3'-phosphate 5'-phosphosulfate <1> (activation, can replace ADP to some extent) [58]
adenosine 5'-phosphosulfate <1> (activation, can replace ADP to some extent) [58]
artificial thin filaments <1, 4> (activation, made by mixing actin, tropomyosin and troponin complex) [19]
glycogen <1, 3-9> (activation, <1, 3-9> [3, 10, 15, 22, 30, 64]; effect on nonactivated and activated enzyme, <6> [30]; increases apparent affinity for phosphorylase b, <1> [64]; allosteric effector, mechanism, <1, 3-8> [10]; pH-dependent, <1> [3]; stimulates phosphorylase kinase and $\alpha\gamma\delta$ (not $\gamma\delta$) subunit complex, <1> [22]; no significant effect on dogfish enzyme, <8> [18]) [3, 10, 15, 22, 30, 64]
Additional information <1, 3-9> (hexadecamer of $(\alpha\beta\gamma\delta)4$ with variable degree of activity depending on pH, metal ions, allosteric effectors, covalent modifications, etc., <1> [55]; three separate activities can be characterized by their response to Ca^{2+}, Mg^{2+}, NH_4Cl and pH: A0, A1 and A2, <1> [25, 26]; the nonactivated enzyme (i.e. dephospho-enzyme), <1, 3-8> [10]; the nonactivated enzyme is activated either by limited proteolysis, <1, 3-9> [3-5, 7, 10, 15, 19, 23, 25, 26, 30, 31, 41, 43]; phosphorylation by protein kinases, <1, 3-9> [3, 7, 10, 15, 25]; or autophosphorylation, <1, 3-9> [7, 10, 15, 26]; allosteric effectors, overview, <1, 3-8> [10]; no activation by substrates of phosphorylase b reaction, i.e. AMP or glucose 1-phosphate, <1, 8> [3, 18]; glucose 6-phosphate, UDPglucose, dogfish myosin, actin, tropomyosin, rabbit glycogen synthase, <1, 8> [18]; parvalbumin, <1, 4, 8> [18, 19]; poly-aspartic acid, hexametaphosphate, yeast nucleic acid, at pH 6.8, <1> [3]; adenine, adenosine, 5'-AMP, 2',5'-ADP, 3',5'-ADP, adenosine 2':3'cyclicphosphate 5'-monophosphate, α,β-methylene-ADP, adenosine 2'-phosphate 5'-phosphosulfate, adenosine 5'-diphosphoglucose, adenosine 5'-diphosphoribose, ADP-3'-diphosphate, adenylylimidodiphosphate, diadenosine diphosphate, <1> [58]; no autoactivation, <7> [41]; no activation of nonactivated enzyme by renin (with or without Ca^{2+}), thrombin (with or without Ca^{2+}), phospholipase D from Clostridium perfringens or phospholipase from Crotalus adamanteus, <1> [4]; no stimulation of autophosphorylation by poly-L-alanine, poly-L-asparagine, putrescine, spermidine or spermine, <1> [37]) [3-5, 7, 10, 15, 18, 19, 23, 25, 26, 30, 31, 37, 41, 43, 55, 58]

Metals, ions

$(NH_4)_2SO_4$ <1> (activation, 0.05-0.1 M, inhibits above 0.2 M) [13]
Ba^{2+} <1, 8> (activation, can replace Ca^{2+} with 60% efficiency, <1> [4]; activation, can replace Ca^{2+} with 26% efficiency, <8> [18]) [4, 18]
Ca^{2+} <1, 3-10> (requirement (trypsin activation leads to loss of absolute requirement), <1,4> [19]; requirement, <3,5-10> [9-11, 13-16, 18, 20-21, 25, 26, 29, 31-35, 38, 41, 63, 64]; required for activity and activation, <1, 3-8> [10, 26]; stimulates phosphorylase b binding to enzyme, but to a considerable lesser extent than Mg^{2+}, <1> [64]; stabilization, no absolute requirement for catalytic subunit γ_2, <1> [17]; activation, 0.0001-0.001 mM, inhibits above 0.001 mM, <4> [24]; stimulates autophosphorylation in micromolar range at pH 6.8, inhibits at millimolar range, <1> [26]; δ-subunit confers Ca^{2+}-sensitivity to the phosphorylase kinase reaction, <1,4> [19]; required for efficient substrate binding of active and nonactivated enzyme and for maximal catalysis of active enzyme, <1,4> [14]; allosteric mechanism, <1,3-8> [10]; 12 mol Ca^{2+} per mol $(\alpha\beta\gamma\delta)4$, <1> [11]; synergism with Mg^{2+}, <1,3-8> [10, 20, 21]; isolated δ-subunit from rabbit has 4 Ca^{2+}-binding sites of which 2 are lost at high ionic strength and 2 Mg^{2+}/Ca^{2+}-binding sites that can bind either ion, treatment of $\gamma\delta$-subunit complex with EGTA with following centrifugation leads to Ca^{2+}-independent catalytic activity, <1,3-8> [10]; binding studies, <1> [11]; irreversible activation of nonactivated kinase by preincubation together with a separate kinase-activating factor independent of cAMP, kinetics, <1> [4]; Ca^{2+}-independent activity: A0, <1> [25, 26]; not, <1> [1]) [4, 9-11, 13-21, 25, 26, 29, 31-35, 38, 41, 63, 64]
Co^{2+} <8> (activation, can replace Ca^{2+} with 10% efficiency, <8> [18]; not, <1> [4]) [18]
Fe^{3+} <8> (activation, can replace Ca^{2+} with 10% efficiency, <8> [18]; not, <1> [4]) [18]
Li^{+} <1, 3, 8, 9> (activation, <1, 3, 8, 9> [15, 18]; LiBr, <1, 3, 8, 9> [15]; can replace Ca^{2+} with 10% efficiency, <8> [18]) [15, 18]
Mg^{2+} <1-11> (requirement, <1-11> [1-64]; major role of Mg^{2+}: cosubstrate in Mg^{2+}-ATP complex, <1-11> [1-64]; required for activity and activation (by autophosphorylation), <4> [24]; required for activity phosphorylation by (cAMP-dependent protein kinase), <1,3-8> [10, 21, 24, 34, 38]; greatly enhances affinity for phosphorylase b, <1> [64]; free Mg^{2+} stimulates (nonactivated enzyme), <4> [38]; 10 mM, <1,4> [22, 38]; allosteric effector, rabbit δ-subunit has two Mg^{2+}/Ca^{2+}-binding sites that can bind either ion, <1,3-8> [10]; Mg^{2+} added in excess of ATP concentration stimulates, <1,3-8> [10]; synergism with Ca^{2+}, <1,3-8> [10, 20, 21]; effect of Mg^{2+} on Ca^{2+}-binding properties of nonactivated enzyme at pH 6.8, <1> [11]; enzyme catalyzes its own phosphorylation (i.e. α and β subunits, not $\gamma\delta$ subunit complex) in the presence of $MgATP^{2-}$ and Ca^{2+}, <1,3-8> [10]; can replace Mn^{2+}, <1> [1]; not, <1> [4]; free Mg^{2+} inhibits activated enzyme, <4> [38]) [1-64]
Mn^{2+} <1, 2, 6, 8> (requirement, <1, 2, 6, 8> [1-4, 16, 18, 22, 64]; activation, <1> [34]; optimal at ATP:Mg ratio of 1:1, <1> [1, 22]; can substitute for Mg^{2+} (less effective), <6> [16]; can substitute for Mg^{2+}, <1> [3, 22]; can substitute for Ca^{2+}, <1> [4]; at equimolar concentration of metal ion and ATP Mn^{2+}

more effective than Mg^{2+}, <1> [22]; enhances enzyme/phosphorylase b interaction more effectively, <1> [64]; can replace Ca^{2+} with 15% efficiency, <1, 8> [18]; stimulates activation by catalytic subunit of cAMP-dependent protein kinase, <1> [34]; free Mn^{2+} inhibits, <1> [22]) [1-4, 16, 18, 22, 34, 64]
Sr^{2+} <1, 8> (activation, can replace Ca^{2+} with 60% efficiency, <1> [4]; activation, can replace Ca^{2+} with 45% efficiency, <1,8> [18]) [4, 18]
phosphate <1-11> (requirement, phosphate containing enzyme, <1-11> [1-64]; nonactivated enzyme is activated by phosphorylation, <1, 3-8> [10]; α and β subunits are phosphorylated by protein kinases or autophosphorylation, <1,6> [31, 32]; $\gamma\delta$ subunit complex cannot phosphorylate itself but phosphorylates and activates native enzyme, even in the presence of EGTA or protein kinase inhibitor, <1> [22]; $\alpha\gamma\delta$ complex undergoes autophosphorylation: up to 4.2 mol phosphate/mol complex incorporated into α subunit, <1> [22]; contains 7.18-19 mol per mol $(\alpha\beta\gamma\delta)_4$ depending on phosphorylation status, <1,3-8> [10]; 20 mol/mol holoenzyme, phosphate content of subunits, <1> [39]) [1-64]
Additional information <1, 8> (three separate activities can be characterized by their response to Ca^{2+}, Mg^{2+}, NH_4Cl and pH: A0, A1 and A2, <1> [25, 26]; synopsis of activity by Ca^{2+}/Mg^{2+} and phosphorylation, <1> [55]; no activation by Cu^{2+}, Cd^{2+}, Sn^{2+}, Al^{3+}, <1, 8> [18]; Fe^{2+}, Zn^{2+} or Ni^{2+}, <1, 8> [4, 18]) [4, 18, 25, 26, 55]

Turnover number (min^{-1})

5.9 <1> (melittin) [56]
1415 <1> (tetradecapeptide) [56]
3423 <1> (phosphorylase b) [56]
5484 <1> (phosphorylase b, nonactivated enzyme) [22]
5958 <1> (phosphorylase b, $\alpha\gamma\delta$ subunit complex) [22]
6240 <1> (phosphorylase b, $\gamma\delta$ subunit complex) [22]

Specific activity (U/mg)

0.426 <4> [24]
0.53 <7> [41]
1.1-1.3 <1> [46]
2.8 <8> (dogfish) [18]
3.68 <1, 6> [16, 32]
4 <1> (pH 8.2) [11]
8.3 <6> [30]
10.2 <1> [31]
10.9 <3> (truncated form of phosphorylase kinase γ subunit) [60]
16.4 <7> (hind limb) [43]
19.6 <7> (brest) [43]
Additional information <1, 4, 11> [1, 3, 14, 28]

K_m-Value (mM)

0.002-0.0025 <7> (phosphorylase b, in the presence of glycogen) [43]
0.0098 <1> (melittin) [56]
0.01 <1> (phosphorylase b, pH 7.5, in the presence of glycogen) [3]

0.014 <3> (phosphorylase b, recombinant γ subunit, pH 6.8) [57, 60]
0.015-0.017 <1, 6> (phosphorylase b, <1,6> [3, 32]; bovine heart, <6> [32]; activated enzyme, pH 8.2, <1> [3]) [3, 32]
0.017-0.027 <1> ($MgATP^{2-}$, autophosphorylation) [10]
0.018 <1, 11> (ATP, <11> [28]; phosphorylase b, truncated form of phosphorylase kinase γ subunit, <1> [61]) [28, 61]
0.019-0.02 <1, 3-8> (phosphorylase b, <7> [10, 43]; pH 7, activated enzyme, <1,3-8,11> [10]) [10, 43]
0.025 <1> (ATP, proteolytic fragment of phosphorylase kinase) [13]
0.0276 <1> (phosphorylase b, holoenzyme) [56]
0.03-0.037 <1, 3-8> (ATP, <1> [13]; phosphorylase b, <1,3-8> [3, 4, 10, 57, 60]; pH 8.5, <1,3-8> [3, 10]; activated enzyme, pH 7.5, <1,3-8> [3, 4, 10]; recombinant γ subunit, pH 8.2, <3> [57, 60]) [3, 4, 10, 13, 57, 60]
0.04 <1> (phosphorylase b, before activation, pH 8.2) [3]
0.044-0.08 <1> (phosphorylase b, holoenzyme) [61]
0.05 <4> (ATP) [53]
0.07 <1, 4> ($MgATP^{2-}$, activated skeletal muscle enzyme, pH 8.2, <1,4> [14]; ATP (+ melittin), <1> [56]) [14, 56]
0.07-0.24 <1, 3-8> (phosphorylase b) [10]
0.08 <1, 4> ($MgATP^{2-}$, <1, 4> [14, 22, 44]; ATP, <1> [56]; activated rabbit skeletal muscle enzyme, pH 6, <1, 4> [1 4]; phosphorylase b, activated enzyme, <1> [22]; pH 8.2, γ subunit or holoenzyme, <1> [44]) [14, 22, 44, 56]
0.0824 <1> (phosphorylase b, γ subunit) [56]
0.084 <8> (phosphorylase b, pH 8.2, dogfish enzyme) [18]
0.094 <1> (phosphorylase b, pH 8.2, $\gamma\delta$ subunit complex) [22]
0.098 <1> ($MgATP^{2-}$, pH 8.2, γ subunit or holoenzyme) [44]
0.1 <4, 8> ($MgATP^{2-}$, nonactivated skeletal muscle enzyme, pH 6, <4> [14]; dogfish phosphorylase b, pH 8.2, <8> [18]) [14, 18]
0.11 <1> (phosphorylase b, pH 8.2, $\alpha\gamma\delta$ subunit complex) [22]
0.12 <1> (phosphorylase b, pH 7.5) [3]
0.125 <1> (phosphorylase b, before activation, pH 7.5) [3]
0.14 <1> (phosphorylase b, before activation, pH 7.5) [4]
0.14-0.22 <6> (ATP, at different phosphorylase b concentrations) [16]
0.19 <3> (phosphorylase b, nonactivated enzyme, pH 8.2) [60]
0.2 <3, 7> (ATP, <7> [43]; Ser-Asp-Gln-Glu-Lys-Arg-Lys-Gln-Ile-Ser-Val-Asp, Lys-Arg-Lys-Gln-Ile-Ser-Val-Asp, nonactivated enzyme, pH 8.2, <3> [60]) [43, 60]
0.2-2.3 <1> (peptides, from phosphorylase b, synthetic) [34]
0.21 <3> (S-peptide, recombinant γ subunit, pH 8.2) [60]
0.22 <1, 3, 6> (ATP, <1,6> [22, 32]; pH 8.2, nonactivated enzyme, <1> [22]; bovine heart, <6> [32]; S-peptide, activated enzyme, pH 6.8, <3> [60]) [22, 32, 60]
0.24 <1> (ATP, nonactivated enzyme, pH 7.5) [3]
0.25 <1> (phosphorylase b, <1> [3, 10, 22]; pH 7.6, <1> [3, 10]; pH 8.2, nonactivated enzyme, <1> [22]) [3, 10, 22]
0.27 <1> (phosphorylase b, nonactivated enzyme) [34]

0.28 <3> (S-peptide, recombinant γ subunit, pH 6.8) [60]
0.3085 <1> (tetradecapeptide) [56]
0.37 <1> (phosphorylase b, nonactivated enzyme, pH 7) [10]
0.38 <1> (ATP, activated enzyme, pH 7.5) [3]
0.4 <1> (ATP) [31]
0.45 <7> (ATP) [41]
0.47 <1> (tetradecapeptide) [49]
0.5 <1> (ATP, pH 8.2, $\alpha\gamma\delta$ subunit complex) [22]
0.6 <11> (GTP) [28]
0.7-3.5 <1> (peptides, from glycogen synthase, synthetic) [34]
0.9-1 <3> (Lys-Arg-Lys-Gln-Ile-Ser-Val-Asp-Gly-Ile, pH 8.2) [60]
0.95 <1> (ATP, pH 8.2, $\gamma\delta$ subunit complex) [22]
1.2 <3> (Ser-Asp-Gln-Glu-Lys-Arg-Lys-Gln-Ile-Ser-Val-Asp-Gly-Ile, nonactivated enzyme, pH 8.2) [60]
1.4 <11> (UTP) [28]
2 <3> (Ser-Asp-Gln-Glu-Lys-Arg-Lys-Gln-Ile-Ser-Val-Asp, activated enzyme, pH 8.2) [60]
3.5 <3> (Ser-Asp-Gln-Glu-Lys-Arg-Lys-Gln-Ile-Ser-Val-Asp-Gly-Ile, activated enzyme, pH 8.2) [60]
Additional information <1, 3-10> (kinetic properties, overview, <1,3-9> [10, 15]; pH-dependence of kinetic parameters, <1, 3, 8, 9> [3, 15]; kinetic parameters for recombinant wild-type γ subunit and its mutant form, <3> [60]; kinetic parameters for different enzyme forms, <1> [44]; kinetic studies, <1, 3-8, 11> [10]; effects of holoenzyme dissociation, <1, 3-8> [10]; kinetic data for peptides derived from glycogen synthase, <1, 3-8> [10, 34]; kinetic data for phosphorylase b, <1, 3-9> [10, 15, 34]; kinetic data of holoenzyme and catalytically active proteolytic fragment, <1> [13]; kinetic parameters of catalytically active γ subunit at pH 6.8 and 8.5, <1> [17]; effect of isolated δ subunit on kinetic parameters of nonactivated holoenzyme and $\alpha\gamma\delta$ complex, <1> [23]; kinetic properties of subunit complexes at pH 6.8 and 8.5, <1> [22]; kinetic properties of covalently modified and nonmodified phosphorylase kinase, <1> [25]; influence of Ca^{2+}, <4> [53]; influence of anti-subunit antibodies, <1> [42]; influence on kinetic parameters, glycogen decreases K_m-values for phosphorylase b, <6> [30]) [3, 10, 13-15, 17, 22, 23, 25, 30, 34, 35, 41, 42, 44, 53, 60]

pH-Optimum

6 <4> (nonactivated enzyme) [14]
7 <1, 4> (liver enzyme, <1> [13]; above, activated enzyme, <4> [14]; nonactivated enzyme, <4> [24]) [13, 14, 24]
7.6 <1> (above, nonactivated rabbit enzyme) [10]
8 <11> [28]
8.5 <7, 8> [18, 43]
8.8 <1, 6> [1, 16]
9.3 <7> [41]
9.5 <1> (muscle enzyme) [13]

Additional information <1, 3, 4, 6-9> (pI: 5.77 (nonactivated rabbit enzyme), <1> [6, 15]; activated enzyme has a higher pH-optimum than nonactivated enzyme, <4> [53]; nonactivated enzyme has only low activity at pH 6.8, <6> [30]; nonactivated kinase activity ratios at pH 6.8/8.2: between 0.01-0.05, activated kinase activity ratio: about 0.6, <1> [6]; activity ratios at pH 6.8/8.2: 0.01-0.02 (phosphorylase kinase a), 0.36 (phosphorylase kinase sa), 0.67 (phosphorylase kinase a'), <1> [9]; activity ratios pH 6.8/8.2 of nonactivated enzyme: 0.07 (in the presence of 0.05-0.07 M Ca^{2+}) and 0.23 (after calmodulin addition), <1> [31]; pH 6.8/8.2 activity ratios: 0.58, <7> [41]; 0.3-0.5, <7> [43]; 0.5-0.6 (γ subunit), <1> [44]; influence of activation by protein kinase on pH-activity profile, <4> [24]; pH-dependence of partial activities A1 and A2, <1> [25]; pH-activity profiles of $\alpha\gamma\delta$ and $\gamma\delta$ subunit complexes, <1> [27]) [3, 6, 9, 15, 24, 25, 27, 30, 31, 41, 43, 44, 53]

pH-Range

6-11.5 <1> (about half-maximal activity at pH 6 and 11.5, liver enzyme) [13]
6.2-7.6 <4> (about half-maximal activity at pH 6.2 and 7.6, nonactivated enzyme) [24]
6.2-8 <7> (progressive increase of activity, about half-maximal activity at pH 7.4) [43]
6.2-8.8 <6> (progressive increase of activity) [16]
6.2-9.3 <7> (progressive increase of activity from 10% to 100% of maximal activity, with half-maximal activity at pH 7) [41]
6.2-9.5 <1> (about half-maximal activity at pH 6.2 and about 70% of maximal activity at pH 9.5) [17]
6.5-8.5 <8> (progressive increase of activity, biphasic with dogfish phosphorylase b as substrate: then about half-maximal activity at pH 7.2-7.6 (dogfish activated enzyme)) [18]
6.6-9.1 <1> (about half-maximal activity at pH 6.6 and 9.1, activity increases up to pH 8, sharp drop above 9) [1]
6.8-8.5 <1> (about half-maximal activity at pH 6.8 and maximal activity at pH 8.5, activated rabbit enzyme) [18]
7.2-9 <11> (70% of maximal activity at pH 7.2 and and 9) [28]
8.1-8.5 <1> (about half-maximal activity at pH 8.1 and maximal activity at pH 8.5, nonactivated rabbit enzyme) [18]
9-10 <1> (about half-maximal activity at pH 9 and 10, muscle enzyme) [13]

Temperature optimum (°C)

30 <1, 3, 4, 6-8, 10, 11> (assay at) [1, 3, 4, 13, 14, 16-18, 24-28, 30-35, 37, 38, 40, 41, 44-46, 48, 54, 56, 59-63]
37 <2> (assay at) [2]

4 Enzyme Structure

Molecular weight

29000 <11> (gel filtration) [28]
86000 <1> (catalytically active $\gamma\gamma$ subunit complex, gel filtration) [17]

205000 <1> (trypsinized or chymotrypsinized enzyme form, gel filtration) [51]
243000 <1> ($\alpha\gamma\delta$ complex, gel filtration) [27]
Additional information <1, 3, 6, 8, 9> (amino acid composition [6, 15]; amino acid sequence in regulatory domain of γ subunit [60]; enzyme aggregates to high polymeric forms which arise as artifacts during isolation procedure due to sensitivity to high hydrostatic pressure, e.g. during sucrose density gradient centrifugation at very high angular velocities [6]; mechanism and structure [55]; MW of trypsinized enzyme [30]) [6, 15, 30, 55, 60]
1000000 <4> [53]
1220000 <1> (HPLC gel filtration) [39]
1260000 <1> (gel filtration) [51]
1300000 <1, 4, 6, 8> (gel filtration [13]; gel filtration [14, 24]; bovine, sucrose density gradient centrifugation [16]; gel filtration or sedimentation velocity analysis [18]) [13, 14, 16, 18, 24]
1320000 <7> (gel filtration) [43, 52]
1330000 <1> (nonactivated enzyme [6, 10]; analytical ultracentrifugation [6, 10, 15]) [6, 10, 15]

Subunits

dimer <1> (2 * 45000, catalytically active $\gamma\gamma$ subunit, SDS-PAGE) [17]
hexadecamer <1, 6> (4 * 118000-145000 + 4 * 108000-128000 + 4 * 44673 + 4 * 16680, ($\alpha\beta\gamma\delta$)4, SDS-PAGE, 2 isozymes that differ in size of the largest subunit (α: 118000-145000 and α': 133000-140000) [10]; 4 * 134000 + 4 * 125000 + 4 * 48000 + 4 * ?, $(\alpha\beta\gamma\delta)_4$, SDS-PAGE, the forth subunit is comigrating with calmodulin [16]; 4 * 140000 + 4 * 116000 + 4 * 45000 + 4 * 17500, $(\alpha\beta\gamma\delta)_4$, SDS-PAGE [24]; 4 * 140000 + 4 * 129000 + 4 * 44000 + 4 * 17000, $(\alpha'\beta\gamma'\delta)_4$, SDS-PAGE [43]; 4 * 140000 + 4 * 130000 + 4 * 46000 + 4 * 18000, $(\alpha\beta\gamma\delta)_4$, SDS-PAGE [30]; 4 * 145000 + 4 * 130000 + 4 * 45000 + 4 * 17000, $(\alpha\beta\gamma\delta)_4$, SDS-PAGE [29, 31]; 2 major isozymes in muscle: $(\alpha\beta\gamma\delta)_4$ and $(\alpha'\beta\gamma\delta)_4$ [32]) [10, 16, 24, 29-32, 43]
tetramer <1> (2 * 69000 + 2 * 44000, proteolytic form, SDS-PAGE) [51]
Additional information <1, 3-8> (partial amino acid composition of subunits [7]; amino acid composition of α [27, 39, 42]; β [39, 42]; γ [27, 39, 42]; δ [12, 27] subunits; amino acid sequence of α, β [47, 55]; α' [47]; γ subunit [36, 55]; γ' subunit is not identical with rabbit skeletal muscle γ subunit [43]; chicken γ and α' subunits compared to that of rabbit red muscle enzyme [52]; homology with catalytic subunit of cAMP dependent protein kinase [36]; spatial arrangement of subunits [10, 15]; molecular interaction and subunit structure [23]; structure/function relationships of subunits [55]; composed of 3 regulatory and 1 catalytic subunit [50]; α and β subunits are regulatory subunits controlled by phosphorylation and proteolysis, Ca^{2+}-sensitivity is conferred to δ subunit [61]; subunit α in isozymes that occur primarily in cells relying on glycolytic activity and α' in tissues with higher oxidative than glycolytic activity [10]; the catalytic γ subunit contains a kinase domain and a calmodulin binding domain [61]; the δ subunit is very similar to calmodulin but a tightly bound integral component of holoenzyme [10]; the δ subunit is

firmly bound to holoenzyme whereas δ subunit (i.e. calmodulin) is bound only in the presence of Ca^{2+} [19]) [7, 10, 12, 19, 23, 27, 36, 39, 42, 43, 47, 50, 52, 55, 61]

5 Isolation/Preparation/Mutation/Application

Source/tissue

cardiac muscle <1, 5, 6, 8> [2, 10, 15, 16, 30, 32]
fat body <10> (pupae) [35]
flight muscle <9> [15]
gizzard smooth muscle <7> [10, 41]
liver <1, 4, 8> [2, 10, 13-15, 24, 38, 50, 51, 53]
skeletal muscle <1, 3-8> [1, 3-15, 17-23, 27, 29, 31-34, 36, 37, 39, 42-49, 52, 54, 57, 58, 61-64]
Additional information <1, 3, 6, 8, 9> (tissue distribution in dogfish, <8> [18]; isozyme distribution in different tissues, <1, 3, 6, 8, 9> [15, 32]) [15, 18, 32]

Localization

cytosol <1, 4, 6, 8, 11> [1, 13, 14, 16, 18, 28]
glycogen particle <1, 3-8> (organelle-like particles, <1> [55]; together with other enzymes of glycogen metabolism linked together on glycogen particles, <1,4> [19]; due to protein-protein interactions in glycogen particles the proteins behave differently from those in cytosol, <1> [55]) [10, 19, 31, 55]
sarcoplasmic reticulum <1, 3-9> [10, 15]
soluble <1, 4, 6, 8, 11> [1, 13, 14, 16, 18, 28]

Purification

<1> (overview [10, 15]; liver [13]; partial [1, 4]; to near homogeneity (phosphorylase b is a persistent contaminant) [3]; to near homogeneity [13, 31]; as nonactivated enzyme [3]; as in vivo activated phosphorylase sa [9]; from protein-glycogen complex [31]; 2 isozymes separable by calmodulin affinity chromatography [10]; isolation of denatured subunits (from nonactivated enzyme) [45]; isolation of denatured subunits [10]; homogenous α, β and γ subunits [42]; α, α' and β subunits by preparative SDS-PAGE [47]; catalytic subunit (i.e. γ subunit, from holoenzyme by dissociation) [17]; catalytic subunit as expressed in Escherichia coli [61]; active γ subunit from inactive form by reverse-phase HPLC) [63]; δ subunit [12]; catalytically active proteolytic product of holoenzyme [13]; catalytically active $\alpha\gamma\delta$ complexes[23, 27]; catalytically active $\gamma\delta$ complexes [23]; native and proteolytically generated enzyme forms [51]; by affinity chromatography on calmodulin-Sepharose 4B [23]) [1, 3, 4, 6, 9, 10, 12, 13, 15, 17, 23, 27, 31, 36, 39, 42, 44, 45, 47, 51, 61, 63]
<2> (heart) [2]
<3> (recombinant γ-subunit, as expressed in Sf-9 cells) [57]
<4> (partial [14]; to near homogeneity from glycogen-rich pellet, from 1000 rats [24]) [14, 24]

<6> (to near homogeneity [16, 30]; nonactivated enzyme [30]; heart [32]) [16, 30, 32]
<7> (to near homogeneity) [41, 43]
<8> [18]
<11> (partial) [28]

Crystallization

<1> (muscle phosphorylase kinase catalytic domain of catalytic subunit, i.e. Phkγtrnc, in the presence of Mg-ATP, X-ray data) [61]

Cloning

<1, 3> (muscle phosphorylase kinase catalytic domain of catalytic subunit, expressed in Escherichia coli [61]; mouse catalytic γ subunit, Baculovirus-directed expression in Sf9 insect cells [57]) [57, 61]

6 Stability

pH-Stability

6 <1, 8> (below, rapid irreversible inactivation) [18]

Temperature stability

20 <1> (2 mg enzyme/ml, trypsin-activated enzyme, with or without ATP, 4 h stable) [5]
30 <1> (at least 30 min) [13]
37 <1> (50% loss of nonactivated enzyme activity within 15 min, 50% loss of $\alpha\gamma\delta$ subunit complex activity within 7 min, 90% loss of $\gamma\delta$ subunit complex activity within 5 min) [23]
40 <1> ($t_{1/2}$: 3 min, in 10% ethylene glycol, pH 8) [13]
45 <1> ($t_{1/2}$: 1 min, in 10% ethylene glycol, pH 8) [13]

General stability information

<1>, about 70% loss of activity during centrifugation for 5 h on a glycerol density gradient [13]
<1>, δ-subunit remains tightly bound to $\alpha\gamma\delta$ subunit complex even in the presence of 8 M urea [23]
<1>, effects of protein concentration, buffer and ATP on stability and dissociation behaviour of trypsin-activated enzyme [5]
<1>, in the presence of ATP nonactivated enzyme does not dissociate into catalytically active subunits as trypsin-activated enzyme does [5]
<1>, inactive γ subunit after reverse-phase HPLC can be reactivated by dilution into ice-cold, pH 8.2, Ca^{2+}/calmodulin containing buffer [63]
<1>, incubation of nonactivated enzyme with 100 mM ATP at 0°C dissociates the 23 S enzyme to active 7.5 S and 14 S subunits, with LiBr it produces 5 S subunits [17]
<1>, isoelectric focusing inactivates [6]
<1>, nonactivated or protein kinase-activated enzyme stable in the cold, not trypsin-activated enzyme [5]

<1>, rabbit muscle enzyme is subject to pressure denaturation leading to the formation of polydisperse aggregates [15]
<1>, unstable in the presence of Mg^{2+} [3]
<1>, with strong tendency to aggregate, unstable in high concentrations of ammonium sulfate for prolonged periods [31]
<8>, Ca^{2+}-free enzyme is unstable [18]
<8>, rapid irreversible inactivation during desalting by gel filtration or dialysis at ionic strength below 0.1, sucrose, glycerol, salts, SH-compounds or reagents does not stabilize, glycogen, glucose, glucose 1-phosphate, glucose 6-phosphate, mono-, di- and trinucleotides or divalent metal ions and protease inhibitors do not protect [18]
<8>, tends to aggregate upon standing [18]
<11>, 5%, w/v, glycerol stabilizes [28]
<1, 4>, unstable in dilute solutions [5, 14]

Storage stability

<1>, -20°C, in 50 mM sodium glycerophosphate, 0.1% v/v 2-mercaptoethanol, pH 7, 2 mM EDTA, 50% v/v glycerol, at least 1 year [31]
<1>, -20°C, partially purified, at least 2 months [1]
<1>, -25°C, in 50% ethylene glycol, at least 1 month [13]
<1>, 0-4°C, in 5-20% glycerol, 70% loss of activity within 5 h, more rapid inactivation in 5-20% sucrose [13]
<1>, 0°C, 2 mg enzyme/ml, trypsin-activated enzyme, with or without ATP, 30% loss of activity within 4 h [5]
<1>, 0°C, in 10% ethylene glycol, at least 1 week [13]
<1>, 20°C, 2 mg enzyme/ml, trypsin-activated enzyme, with or without ATP, 4 h [5]
<1>, frozen, at least 6 months [3]
<4>, -20°C, in 0.25 M sucrose, 0.1 M Tris-HCl, 0.5 mM DTT, pH 7.4, 50% glycerol, several weeks [14]
<4>, -70°C, in 20 mM triethanolamine-HCl, pH 7.5, 20% v/v glycerol, 1 mM DTT, 0.02% NaN_3, stable [24]
<6>, -70°C, several months [16]
<6>, frozen in liquid N_2, in 50 mM β-glycerophosphate, pH 7, 2 mM EDTA, 1 mM DTT, 10% sucrose, stable [30]
<8>, -15°C, 3 mg dogfish enzyme/ml, in 0.1 M glycerophosphate, 2 mM EDTA, pH 7, 0.3 M NaCl or in 0.1 M glycerophosphate, 2 mM EDTA, 10% sucrose, 1 mM ATP, pH 7, 1 mM DTT, about 50% loss of activity within 1 week [18]
<8>, -15°C, partially purified dogfish enzyme, lyophilized, almost indefinitely [18]

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Homoserine kinase 2.7.1.39

1 Nomenclature

EC number
2.7.1.39

Systematic name
ATP:L-homoserine O-phosphotransferase

Recommended name
homoserine kinase

Synonyms
HSK
homoserine kinase (phosphorylating)
kinase (phosphorylating), homoserine
kinase, homoserine (phosphorylating)

CAS registry number
9026-58-8

2 Source Organism

<1> *Hordeum vulgare* [1]
<2> *Pisum sativum* (L. cv. Pillert Fenomen [2]) [2]
<3> *Rhaphanus sativus* [3]
<4> *Escherichia coli* (K-12 [6, 11]; derepressed strain [11]) [4, 6, 11, 12, 14]
<5> *Rhodospirillum rubrum* (strain S1 [5]) [5]
<6> *Brevibacterium flavum* [7]
<7> *Triticum aestivum* [8]
<8> *Saccharomyces cerevisiae* [9, 10]
<9> *Arabidopsis thaliana* [13]
<10> *Methanococcus jannaschii* [15, 16]
<11> *Pseudomonas aeruginosa* [17]

3 Reaction and Specificity

Catalyzed reaction
ATP + L-homoserine = ADP + O-phospho-L-homoserine (<4>, ordered random mechanism with ATP preferentially binding before L-homoserine [4]; <6>, rapid equilibrium random bi bi mechanism [7]; <10>, the catalytic me-

chanism of the enzyme does not involve a catalytic base for activating the phosphoryl acceptor hydroxyl but instead is mediated via a transition state stabilization mechanism [16])

Reaction type

phospho group transfer

Natural substrates and products

- **S** ATP + L-homoserine <2, 3, 4, 5, 7, 8, 9, 10, 11> (<2, 3>, the enzyme catalyzes a reaction common to synthesis of Thr, Ile and Met in plants [2, 3]; <3>, the enzyme is a potential control point in the biosyntic pathway for Thr, Ile and Met [3]; <5>, enzyme of L-Thr synthesis [5]; <7>, the enzyme is not involved in the regulation of methionine, threonine and isoleucine biosynthesis [8]; <8>, the enzyme plays an important role in the regulation of the threonine biosynthesis [9]; <8>, enzyme of threonine biosynthesis [10]; <4>, enzyme in the aspartate pathway of amino acid biosynthesis [12]; <9>, formation of O-phospho-L-homoserine, a branch point intermediate in the pathways for Met and Thr in plants [13]; <4>, key enzyme in the aspartate pathway of amino acid biosynthesis in E. coli [14]; <10>, fourth enzyme in the aspartate pathway of amino acid biosynthesis [16]; <11>, enzyme catalyzes an obligatory step of threonine biosynthesis [17]) (Reversibility: ? <2, 3, 4, 5, 7, 8, 9, 10, 11> [2, 3, 5, 8, 9, 10, 12, 13, 14, 16, 17]) [2, 3, 5, 8, 9, 10, 12, 13, 14, 16, 17]
- **P** ADP + O-phospho-L-homoserine <2, 3, 4, 5, 7, 8, 9, 10, 11> [2, 3, 5, 8, 9, 10, 12, 13, 14, 16, 17]

Substrates and products

- **S** ATP + 4-hydroxynorvaline <4> (<4>, 0.3% of the activity with L-homoserine [4]) (Reversibility: ? <4> [4]) [4]
- **P** ADP + 4-phospho-L-norvaline
- **S** ATP + D-homoserine <4> (<4>, 32% of the turnover number with L-homoserine [12]) (Reversibility: ? <4> [12]) [12]
- **P** ADP + O-phospho-D-homoserine
- **S** ATP + L-2-amino-1,4-butanediol <4> (<4>, 7.9% of the turnover number with L-homoserine [12]) (Reversibility: ? <4> [12]) [12]
- **P** ADP + ?
- **S** ATP + L-2-amino-5-hydroxyvalerate <4> (<4>, 9.9% of the turnover number with L-homoserine [12]) (Reversibility: ? <4> [12]) [12]
- **P** ADP + L-2-amino-5-phosphovalerate
- **S** ATP + L-aspartate 4-semialdehyde <4> (<4>, 10% of the activity with L-homoserine [4]; <4>, 8.2% of the turnover number with L-homoserine [12]) (Reversibility: ? <4> [4,12]) [4, 12]
- **P** ?
- **S** ATP + L-homoserine <1-11> (Reversibility: ? <1-11> [1-17]) [1-17]
- **P** ADP + O-phospho-L-homoserine <1-11> [1-17]
- **S** ATP + L-homoserine ethyl ester <4> (<4>, 74% of the turnover number with L-homoserine [12]) (Reversibility: ? <4> [12]) [12]
- **P** ADP + O-phospho-L-homoserine ethyl ester

S ATP + L-homoserine isopropyl ester <4> (<4>, 74% of the turnover number with L-homoserine [12]) (Reversibility: ? <4> [12]) [12]
P ADP + O-phospho-L-homoserine isopropyl ester
S ATP + L-homoserine isubutyl ester <4> (<4>, 84% of the turnover number with L-homoserine [12]) (Reversibility: ? <4> [12]) [12]
P ADP + O-phospho-L-homoserine isobutyl ester
S ATP + L-homoserine methyl ester <4> (<4>, 80% of the turnover number with L-homoserine [12]) (Reversibility: ? <4> [12]) [12]
P ADP + O-phospho-L-homoserine methyl ester
S ATP + L-homoserine n-butyl ester <4> (<4>, 160% of the turnover number with L-homoserine [12]) (Reversibility: ? <4> [12]) [12]
P ADP + O-phospho-L-homoserine n-butyl ester
S ATP + L-homoserine n-propyl ester <4> (<4>, 76% of the turnover number with L-homoserine [12]) (Reversibility: ? <4> [12]) [12]
P ADP + O-phospho-L-homoserine n-propyl ester
S Additional information <4> (<4>, enzyme has inherent ATPase activity [12]) [12]
P ?

Inhibitors

(*p*-hydroxyphenyl)-glyoxal <4> [12]
1,2-amino-5-hydroxyvalerate <4> (<4>, substrate inhibition [12]) [12]
2-amino-3-(phosphonoethyl)thiopropionate <4> [12]
2-amino-5-phosphonovalerate <4> [12]
2-chloro-L-alanine <4> [11]
5'-adenylylimidodiphosphate <4> [4]
6-hydroxy-DL-Lys <2> (<2>, 5 mM, 92% inhibition [2]) [2]
L-2-aminobutyrate <4> [11]
L-2-aminobutyric acid <4> (<4>, 40 mM, 50% inhibition [6]) [6]
L-Arg <2> (<2>, 10 mM, 13% inhibition [2]) [2]
L-Cys <2, 4> (<2>, 10 mM, 25% inhibition [2]) [2, 11, 12]
L-Ile <2, 3, 4> (<2>, 7.5 mM, 90% inhibition [2]; <3>, no inhibition with the D-isomer [3]) [2, 3, 11]
L-Leu <2> (<2>, 10 mM, 20% inhibition [2]) [2]
L-Lys <2> (<2>, 10 mM, 35% inhibition [2]) [2]
L-Met <2, 4> (<2>, 10 mM, 17% inhibition [2]) [2, 11]
L-Orn <2> (<2>, 10 mM, 64% inhibition [2]) [2]
L-Ser <2, 4> (<2>, 10 mM, 10% inhibition [2]) [2, 11]
L-Thr <2, 3, 4, 6, 8> (<2>, 10 mM, 18% inhibition [2]; <6>, 8.1 mM, 50% inhibition [7]; <8>, half-maximal inhibition at 10 mM [9]; <4>, competitive to the substrate L-homoserine [11]; <4>, substrate inhibition [12]) [2, 3, 6, 7, 9, 11, 12]
L-Val <2, 4> (<2>, 16 mM, 86% inhibition [2]) [2, 11]
L-alanol <4> [12]
L-aspartate <5> (<5>, slight [5]) [5]
L-aspartate semialdehyde <4> (<4>, mixed inhibition versus L-homoserine and ATP [4]) [4]
L-glutamic acid <4> [12]

L-homocysteine <4> [11]
L-homoserine <4, 8> (<4>, above 0.75 mM [4]; <4>, substrate inhibition [14]) [4, 9, 12, 14]
L-homoserine α-methyl ester <4> (<4>, substrate inhibition [12]) [12]
L-homoserine ethyl ester <4> (<4> unlike the wild-type enzyme the mutant enzyme H202L is inhibited [12]) [12]
L-homoserine isopropyl ester <4> (<4> unlike the wild-type enzyme the mutant enzyme H202L is inhibited [12]) [12]
L-homoserine n-propyl ester <4> (<4> unlike the wild-type enzyme the mutant enzyme H202L is inhibited [12]) [12]
L-norvaline <4> [12]
O-phospho-DL-homoserine <2> (<2>, 10 mM, 91% inhibition [2]) [2]
O-phospho-L-serine <4> [12]
S-adenosyl-L-methionine <2, 3, 7> (<2>, 10 mM, 92% inhibition [2]; <7>, 10 mM, 26% inhibition [8]) [2, 3, 8]
α-amino-β-hydroxy valeric acid <4> [6]
diethyl dicarbonate <4> [14]
phosphohomoserine <6> (<6>, inhibits phosphorylation of L-homoserine [7]) [7]
pyridoxal 5'-phosphate <4> [14]
Additional information <1> (<1>, no feedback inhibition by Thr, Met or Ile [1]) [1]

Activating compounds

L-Cys <5> (<5>, slight activation [5]) [5]
NH_4^+ <1, 2, 4, 6> (<1>, monovalent cation required, K^+ or NH_4^+ [1]; <2>, 100 mM NH_4Cl results in 35% of the activation compared to 100 mM K^+ [2]; <4>, 30% of the activity obtained with 0.5 M K^+ [6]; <6>, not essential, but stimulates [7]) [1, 2, 6, 7]

Metals, ions

Co^{2+} <1> (<1>, divalent cation required, efficiency of activation in descending order: Mg^{2+}, Mn^{2+}, Zn^{2+}, Co^{2+} [1]; <2>, ineffective [2]) [1]
K^+ <1, 3, 4, 6, 9> (<1>, monovalent cation required, K^+ or NH_4^+ [1]; <2>, absolute requirement for monovalent cation. K_m: 21 mM [2]; <3>, KCl stimulates, maximum activation at 0.2 M [3]; <4>, activates [6]; <6>, 200 mM, enhances activity about twice [7]; <9>, requires 40 mM K^+ [13]) [1, 2, 3, 6, 7, 13]
Mg^{2+} <1, 2, 3, 4, 6> (<1>, divalent cation required, efficiency of activation in descending order: Mg^{2+}, Mn^{2+}, Zn^{2+}, Co^{2+} [1]; <2>, absolute requirement for divalent cation, Mg^{2+} or Mn^{2+} [2]; <3>, required [3]; <4>, absolute requirement, optimal activity between 10 mM and 20 mM [6]; <6>, stimulates [7]) [1, 2, 3, 6, 7]
Mn^{2+} <1, 6> (<1>, divalent cation required, efficiency of activation in descending order: Mg^{2+}, Mn^{2+}, Zn^{2+}, Co^{2+} [1]; <2>, absolute requirement for divalent cation, Mg^{2+} or Mn^{2+} [2]; <6>, can replace Mg^{2+} in stimulation [7]; <4>, high requirement [11]; <9>, requires 3 mM [13]) [1, 2, 7, 11, 13]
Zn^{2+} <1> (<1>, divalent cation required, efficiency of activation in descending order: Mg^{2+}, Mn^{2+}, Zn^{2+}, Co^{2+} [1]; <2>, ineffective [2]) [1]

Turnover number (min^{-1})

0.42 <4> (L-homoserine butyl ester, <4>, mutant R234L [12]) [12]
0.666 <4> (L-homoserine propyl ester, <4>, mutant R234L [12]) [12]
1.08 <4> (L-homoserine methyl ester, <4>, mutant R234L [12]) [12]
1.26 <4> (L-homoserine ethyl ester, <4>, mutant R234L [12]) [12]
12 <4> (L-homoserine, <4>, mutant R234L [12]) [12]
120 <4> (L-2-amino-1,4-butanediol, <4>, wild-type enzyme [12]) [12]
126 <4> (L-aspartate β-semialdehyde, <4>, wild-type enzyme [12]) [12]
150 <4> (L-2-amino-5-hydroxyvalerate, <4>, wild-type enzyme [12]) [12]
150 <4> (L-homoserine butyl ester, <4>, mutant H202L [12]) [12]
162 <4> (L-homoserine propyl ester, <4>, mutant H202L [12]) [12]
198 <4> (D-homoserine, <4>, wild-type enzyme [12]) [12]
246 <4> (L-homoserine ethyl ester, <4>, mutant H202L [12]) [12]
324 <4> (L-homoserine methyl ester, <4>, mutant H202L [12]) [12]
546 <4> (L-homoserine, <4>, mutant H202L [12]) [12]
816 <4> (L-homoserine ethyl ester, <4>, wild-type enzyme [12]) [12]
816 <4> (L-homoserine isopropyl ester, <4>, wild-type enzyme [12]) [12]
840 <4> (L-homoserine n-propyl ester, <4>, wild-type enzyme [12]) [12]
882 <4> (L-homoserine methyl ester, <4>, wild-type enzyme [12]) [12]
984 <4> (L-homoserine isobutyl ester, <4>, wild-type enzyme [12]) [12]
1098 <4> (L-homoserine, <4>, wild-type enzyme [12]) [12]
1746 <4> (L-homoserine n-butyl ester, <4>, wild-type enzyme [12]) [12]

Specific activity (U/mg)

0.024 <2> [2]
0.293 <6> [7]
3.09 <9> (<9>, L-homoserine-dependent ADP synthesis assay at pH 8.5 and 37°C [13]) [13]
3.1 <8> [10]
8.34 <7> [8]
Additional information <4> [11]

K_m-Value (mM)

0.11 <4> (ATP, <4>, mutant enzyme H202L [12]) [12]
0.11 <4> (L-homoserine, <4>, mutant enzyme H202L [12]) [12]
0.13 <4> (ATP, <4>, mutant enzyme R234H [12]) [12]
0.13 <4> (ATP, <4>, wild-type enzyme [12]) [12]
0.13 <4> (L-homoserine, <4>, mutant enzyme R234H [12]) [12]
0.14 <4> (L-homoserine, <4>, wild-type enzyme [12]) [12]
0.15 <4> (ATP, <4>, mutant enzyme H205Q [12]) [12]
0.15 <4> (L-homoserine, <4>, pH 7.8 [4]) [4]
0.21 <4> (ATP, <4>, mutant enzyme R234L [12]) [12]
0.24 <7> (L-homoserine) [8]
0.24 <4> (L-homoserine, <4>, pH 7.8, 37°C [11]) [11]
0.25 <8> (L-homoserine, <8>, pH 7.5, 30°C [9]) [9]
0.28 <4> (L-aspartate β-semialdehyde, <4>, wild-type enzyme [12]) [12]
0.3 <4> (ATP, <4>, pH 7.8, 27°C [6]) [6]
0.3 <4> (L-homoserine, <4>, pH 7.8, 27°C [6]) [6]

0.32 <9> (ATP) [13]
0.33 <7> (ATP) [8]
0.4 <1> (L-homoserine) [1]
0.4 <9> (L-homoserine) [13]
0.44 <5> (ATP, <5> pH 8, 28°C [5]) [5]
0.49 <4> (ATP, <4>, mutant enzyme H139L [12]) [12]
0.55 <4> (ATP, <4>, pH 7.8, 37°C [11]) [11]
0.58 <4> (L-aspartate semialdehyde, <4>, pH 7.8 [4]) [4]
0.6 <8> (ATP, <8>, pH 7.5, 30°C [9]) [9]
0.77 <6> (L-homoserine, <6>, pH 7.5, 30°C [7]) [7]
0.88 <4> (ATP, <4>, mutant enzyme R234C [12]) [12]
1 <1> (ATP) [1]
1.1 <4> (L-2-amino-5-hydroxyvalerate, <4>, wild-type enzyme [12]) [12]
1.2 <6> (ATP, <6>, pH 7.5, 30°C [7]) [7]
1.2 <4> (L-homoserine isopropyl ester, <4>, wild-type enzyme [12]) [12]
1.9 <4> (L-homoserine ethyl ester, <4>, wild-type enzyme [12]) [12]
2.5 <4> (L-homoserine, <4>, mutant enzyme H139L [12]) [12]
2.7 <2> (ATP, <2>, pH 8.5 [2]) [2]
3 <5> (L-homoserine, pH 8, 28°C [5]) [5]
3.5 <4> (L-homoserine n-propyl ester, <4>, wild-type enzyme [12]) [12]
3.7 <4> (L-homoserine, <4>, mutant enzyme H205Q [12]) [12]
3.8 <4> (4-hydroxynorvaline, <4>, pH 7.8 [4]) [4]
4.9 <4> (L-homoserine methyl ester, <4>, wild-type enzyme [12]) [12]
5.8 <4> (L-homoserine n-butyl ester, <4>, wild-type enzyme [12]) [12]
6.2 <4> (L-homoserine, <4>, mutant enzyme R234H [12]) [12]
6.7 <2> (L-homoserine, <2>, pH 8.5 [2]) [2]
6.9 <4> (L-homoserine, <4>, wild-type enzyme [12]) [12]
6.9 <4> (L-homoserine isobutyl ester, <4>, wild-type enzyme [12]) [12]
8.5 <4> (L-homoserine, <4>, mutant enzyme R234C [12]) [12]
11.6 <4> (L-2-amino-1,4-butanediol, <4>, wild-type enzyme [12]) [12]
31.8 <4> (D-homoserine, <4>, wild-type enzyme [12]) [12]
40.1 <4> (L-homoserine, <4>, mutant enzyme R234L [12]) [12]
58.2 <4> (L-homoserine, <4>, mutant enzyme H202L [12]) [12]

K_i-Value (mM)

0.1 <4> (L-homoserine, <4>, in 20% DMSO [14]) [14]
0.15 <4> (L-α-aminobutyric acid) [4]
0.2-0.5 <4> (L-alaniol, <4>, wild-type enzyme [12]) [12]
0.2-0.5 <4> (L-glutamic acid, <4>, wild-type enzyme [12]) [12]
0.2-0.5 <4> (L-norvaline, <4>, wild-type enzyme [12]) [12]
0.3 <4> (2-amino-3-(phosphonoethyl)thiopropionate, <4>, wild-type enzyme [12]) [12]
0.3 <4> (L-Thr, <4>, wild-type enzyme [12]) [12]
0.4 <4> (L-2-aminobutyrate, <4>, pH 7.8, 37°C [11]) [11]
0.46 <4> (L-Cys, <4>, wild-type enzyme [12]) [12]
0.6 <4> (L-Thr, <4>, pH 7.8, 37°C [11]) [11]
0.9 <4> (5'-adenylylimidodiphosphate, <4>, pH 7.4 [4]) [4]

0.9 <2> (L-Ile, <2>, pH 8.5 [2]) [2]
0.9 <2> (L-Val, <2>, pH 8.5 [2]) [2]
1 <4> (L-Cys, <4>, pH 7.8, 37°C [11]) [11]
1 <4> (L-Thr, <4>, pH 7.8, 27°C [6]) [6]
2 <4, 8> (L-homoserine, <4>, pH 7.6 [4]; <8>, pH 7.5, 30°C [9]) [4, 9]
2.7 <4> (O-phospho-L-serine) [12]
3 <4> (L-homoserine, <4>, aqueous sulution, pH 8 [14]) [14]
4 <4> (L-homocysteine, <4>, pH 7.8, 37°C [11]) [11]
4.3 <2> (L-Orn, <2>, pH 8.5 [2]) [2]
4.5 <4> (L-Ile, <4>, pH 7.8, 37°C [11]) [11]
10 <4> (L-Val, <4>, pH 7.8, 37°C [11]) [11]
10.4 <4> (2-amino-5-phosphovalerate) [12]
15 <4> (2-chloro-L-alanine, <4>, pH 7.8, 37°C [11]) [11]
27 <4> (L-Ser, <4>, pH 7.8, 37°C [11]) [11]
35 <4> (L-Met, <4>, pH 7.8, 37°C [11]) [11]
Additional information <4> [14]

pH-Optimum

6-8.5 <4> [14]
7.4 <6> (<6>, Tris buffer [7]) [7]
7.8 <4> [6]
8 <4, 9> (<4>, wild-type enzyme [12]) [12, 13]
8.3-9 <3> [3]
8.5 <2> [2]

pH-Range

8.1-9 <2> (<2>, the enzyme shows good activity in Tris-HCl buffer from pH 8.1 to pH 9.0 [2]) [2]

Temperature optimum (°C)

47 <9> [13]

4 Enzyme Structure

Molecular weight

55000 <6> (<6>, gel filtration [7]) [7]
60000 <4> (<4>, gel filtration [6,11]) [6, 11]
73000 <8> (<8>, gel filtration [10]) [10]
75000 <7> (<7>, gel filtration [8]) [8]
145000 <5> (<5>, gel filtration [5]) [5]
Additional information <2> (<2>, 2 peaks of MW 120000 Da and 240000 Da are detected by gel filtration [2]) [2]

Subunits

dimer <4, 7, 8> (<4>, 2 * 29000, SDS-PAGE [11]; <7>, 2 * 36000, SDS-PAGE [8]; <8>, 2 * 40000, SDS-PAGE [10]) [8, 10, 11]

5 Isolation/Preparation/Mutation/Application

Source/tissue

germ <7> [8]
leaf <3> [3]
seedling <1, 2> (<1> etiolated [1]) [1, 2]
shoot <2> (green [2]) [2]

Purification

<1> (partial [1]) [1]
<2> (partial [2]) [2]
<4> [4, 11]
<5> [5]
<6> (partial [7]) [7]
<7> [8]
<8> [10]

Crystallization

<10> (crystals are grown by the hanging drop vapor diffusion method [15,16]; crystal structure of the enzyme with ADP reveals a novel nucleotide binding fold. The N-terminal domain contains an unusual left-handed β,α,β unit, while the C-terminal domain has a central α-β plait fold with an insertion of four helices [15]; the enzyme ternary complexes with its amino acid substrate and ATP analogues determined by X-ray crystallography [16]) [15, 16]

Cloning

<4> (the combination of pTZ19u(ThrB) and the BL21(DE3) cell line is the best expression system with nearly 160 mg of enzyme produced in 4 liters of growth medium [14]) [14]
<8> [10]
<9> (expression of a His-tagged construct in Escherichia coli [13]) [13]
<11> (gene thrH is expressed in Escherichia coli [17]) [17]

Engineering

H139L <4> (<4>, mutant enzyme with diminished kinase activity and ATPase activity 150fold greater than that of the wild-type enzyme [12]) [12]
H202L <4> (<4>, K_m-value for L-homoserine and ATP remain unchanged, the K_i-value for substrate inhibition by L-homoserine increases about 8fold, the turnover-number decreases by 50%, unlike the wild-type enzyme the L-homoserine ethyl, isopropyl, and n-propyl esters show substrate inhibition [12]) [12]
H205Q <4> (<4>, K_m-value for ATP remains unchanged, ATPase activity is within a factor 2 of the wild-type enzyme, the kinase activity is less than 0.03% that of the wild-type enzyme [12]) [12]
R234C <4> (<4>, no observable homoserine kinase activity, the ATPase activity is nearly 20times that of the wild-type enzyme at pH 8.0. 7fold increase

in K_m-value for ATP. Mutant enzyme is sensitive to heat treatment and begins to precipitate at 55°C [12]) [12]
R234H <4> (<4>, mutant enzyme has a diminished kinase activity, 0.4% of that of the wild-type enzyme, and an enhanced ATPase activity, K_m-values for both substrates are unchanged [12]) [12]
R234L <4> (<4>, K_m-value for L-homoserine increases nearly 300fold, the turnover-number decreases by 90fold compared to the wild-type enzyme. Less than a 2fold change in K_m for ATP, the inherent ATPase activity increases by 3fold. The mutant enzyme has turnover-numbers for homoserine esters that are only 10% that of homoserine, but has higher affinity for the esters than for L-homoserine itself. L-Cys, a strong inhibitor of the wild-type enzyme, is 50fold less effective as inhibitor of the mutant enzyme. L-Thr no longer inhibits the mutant enzyme. Unlike the wild-type enzyme, addition of 10 mM L-homoserine to the mutant enzyme has no protective effect on the number of arginyl residues titrated with (*p*-hydroxyphenyl)glyoxal [12]) [12]

6 Stability

Temperature stability

50 <4> (<4>, stable up to in absence of ligands [4]) [4]
57 <4> (<4>, half-life is 10 min in absence of L-Thr ot L-homoserine [6]) [6]
Additional information <1, 2> (<1>, homoserine and $MgATP^{2-}$ protect against heat inactivation [1]; <2>, homoserine and $MgATP^{2-}$ do not protect against heat inactivation [2]) [1, 2]

General stability information

<9>, enzyme stored with 3 mM ATP loses 50% of its activity after two freezing cycles. In presence of 50% v/v glycerol the enzyme loses 50% of its activity after 10 cycles. In the basic storage buffer, the enzyme loses 80% of its activity after a single freeze-thaw cycle [13]

Storage stability

<2>, -25°C, purified enzyme is stable for at least 1 month [2]

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Pyruvate kinase 2.7.1.40

1 Nomenclature

EC number
2.7.1.40

Systematic name
ATP:pyruvate 2-O-phosphotransferase

Recommended name
pyruvate kinase

Synonyms
CTHBP
L-PK
R-type/L-type pyruvate kinase
THBP1
VEG17
cytosolic thyroid hormone binding protein
fluorokinase
kinase, fluoro- (phosphorylating)
kinase, pyruvate (phosphorylating)
phosphoenol transphosphorylase
phosphoenolpyruvate kinase
pyruvate kinase muscle isozyme
pyruvate phosphotransferase
pyruvic kinase
red cell/liver pyruvate kinase
vegetative protein 17

CAS registry number
9001-59-6

2 Source Organism

<1> *Bos taurus* [12, 42]
<2> *Felis catus* [46, 47]
<3> *Canis familiaris* [33]
<4> *Homo sapiens* [40, 41, 105]
<5> *Mus musculus* [48, 60]
<6> *Sus scrofa* (M1 isozyme [96]) [6, 17, 60, 96]
<7> *Oryctolagus cuniculus* [8, 49, 60]

<8> *Rattus norvegicus* (adult albino [51,52]; male Donryu [51]; female Wistar [52]) [43, 50-52, 60]
<9> *Columba sp.* [14]
<10> *Kinixys erosa* (African land tortoise [9]) [9]
<11> *Rana pipiens* (grass frog [38]) [38, 60]
<12> *Rana ridibunda* [27]
<13> *Anguilla rostrata* (American eel, kinase resembles mammalian M2-isozyme [25]) [25]
<14> *Antarctic fish* [60]
<15> *Chaenocephalus aceratus* (Loenberg, ice-fish [3]) [3]
<16> *Dicentrarchus labrax* (sea bass, marine teleost [2]) [2]
<17> *loach* [60]
<18> *Oncorhynchus kisutch* (salmon [35]; coho salmon [36]) [35, 36]
<19> *Platichthys flesus* (flounder [18]) [18, 26]
<20> *Oncorhynchus mykiss* [60]
<21> *Brochothrix thermosphacta* [7]
<22> *Busycotypus canaliculatum* (channelled whelk, gastropod mollusc [54]) [53, 54]
<23> *Cardium tuberculatum* (cockle [20]) [20]
<24> *Concholepas concholepas* [16, 31]
<25> *Mytilus galloprovincialis* (mussle [28]) [28]
<26> *Nautilus pompilius* [30]
<27> *Crassostrea sp.* [60]
<28> *Patella caerulea* (limpet [19]) [19]
<29> *Scapharca inaequivalvis* (mollusc [21]) [21]
<30> *Venus gallina* (Adriatic mollusc [21]) [21, 29]
<31> *Paralithodes camtschatica* (Alaskan king crab [60]) [60]
<32> *Cancer magister* (crustacean [34]) [34]
<33> *Schistocera gregaria* [60]
<34> *Periplaneta americana* (cockroach [22]) [22]
<35> *Tenebrio molitor* (coleoptera, larvae [23]) [23]
<36> *Entodinium sp.* (rumen ciliates [13]) [13]
<37> *Leishmania major* [15]
<38> *Trypanosoma brucei* [5, 32, 60, 66, 94]
<39> *Trypanoplasma borelli* (strain K-100 [67]) [67]
<40> *Cicer arietinum* (chickpea [11]) [11]
<41> *Gossypium hirsutum* [62]
<42> *Lycopersicon esculentum* [61]
<43> *Ricinus communis* (castor bean, var. Baker 296 [55, 57]; cv. Hale [56]; isoform Pka [106]) [55-57, 92, 104, 106]
<44> *Spinacia oleracea* (spinach [58]) [58, 92]
<45> *Chlorella reinhardtii* [4]
<46> *Chlorella kessleri* (chlorophyll-free mutant No. 20 [59]) [59]
<47> *Euglena gracilis* [60]
<48> *Selenastrum minutum* (green alga [63]) [63-65, 92]
<49> *Coprinus lagopus* [60]
<50> *Aspergillus niger* [1, 81]

<51> *Aspergillus nidulans* (wild-type strain WG 096 (yA2, paba A1) and transformant 9/402) [1, 68]
<52> *Dictyostelium discoideum* [10]
<53> *Mucor racemosus* (dimorphic phycomycete [69]) [69]
<54> *Mucor rouxii* [60, 83]
<55> *Neurospora crassa* [80, 82]
<56> *Phycomyces blakesleeanus* [24]
<57> *Rhodosporidium toruloides* (strain CBS 14 [75]) [75]
<58> *Saccharomyces cerevisiae* (Budweiser baker's yeast [39]; isoform Pyk2p [109]) [39, 58, 60, 108, 109]
<59> *Saccharomyces carlsbergensis* [39, 60]
<60> *Schizosaccharomyces pombe* (fission yeast [70]) [70]
<61> *Yarrowia lipolytica* (dimorphic yeast [71]) [71]
<62> *Acetobacter xylinum* [60]
<63> *Azotobacter vinelandii* [60]
<64> *Bacillus licheniformis* (A-5 [37]) [37]
<65> *Bacillus stearothermophilus* [72]
<66> *Bacillus subtilis* [60]
<67> *Brevibacterium flavum* [60]
<68> *Escherichia coli* (strain K12 [45, 60, 86] or B [45,60]) [45, 60, 86, 98, 115]
<69> *Halobacterium cutirubrum* (strain 9 [73]) [73]
<70> *Mycobacterium smegmatis* (strain CDC 46 [74]) [74]
<71> *Propionibacterium shermanii* [88]
<72> *Pseudomonas citronellolis* [84]
<73> *Salmonella typhimurium* (strain LT-2, highest activity in cells grown on glycolytic substrates, e.g. glucose, galactose, fructose or glycerol [76]) [76]
<74> *Streptococcus lactis* (strain C10 [44]) [44, 85]
<75> *Streptococcus mutans* (strain JC2 [77]) [77]
<76> *Thermoplasma acidophilum* (archaebacterium [78]) [78]
<77> *Thiobacillus neapolitanus* [60]
<78> *Veillonella parvula* [87]
<79> *Zymomonas mobilis* [79]
<80> *Thermoproteus tenax* [89]
<81> *Brassica napus* (PKc, cytosolic form [90]; PKp, plastidic form [111]) [90, 92, 111]
<82> *Musa cavendishii* (banana [91]) [91]
<83> *Cynodon dactylon* [92, 110, 113]
<84> *Brassica campestris* [92, 100]
<85> *Schizosaccharomyces pombe* [93]
<86> *Entosphenus japonicus* (lamprey [95]) [95]
<87> *Meganyctiphanes norvegica* (nordic krill, isoforms PK I and PK II [97]) [97]
<88> *Bacillus psychrophilus* [98]
<89> *Bacillus licheniformis* [98]
<90> *Vigna radiaita* (mung bean [99]) [99]
<91> *Chlamydia trachomatis* [101]

<92> *Toxoplasma gondii* [102]
<93> *Selenomonas ruminatum* (ruminal bacterium [103]) [103]
<94> *Synechococcus sp.* (cyanobacterium, strain PCC 6301 [107]) [107]
<95> *Setaria verticillata* [110]
<96> *Sorghum halepense* [110]
<97> *Digitaria sanguinalis* [110]
<98> *Amaranthus sp.* [110]
<99> *Microbispora thermodiastatica* [112]
<100> *Leishmania sp.* [114]

3 Reaction and Specificity

Catalyzed reaction

ATP + pyruvate = ADP + phosphoenolpyruvate (UTP, GTP, CTP, ITP and dATP can also act as donors, also phosphorylates hydroxylamine and fluoride in the presence of CO_2; <45> mechanism [4]; <2> catalyzes the addition of a proton and the loss of a phosphoryl group which is transferred to ADP [47]; <65, 69, 70> allosteric enzyme: homotropic [72-74]; <80> sigmoidal saturation curves with substrate and metal ions [89]; <82> model for allosteric regulation [91]; <43, 44, 48, 81, 83, 84> mechanism [92]; <85> sigmoidal kinetics with respect to phosphoenolpyruvate [93]; <6> hyperbolic kinetics [96]; <88, 89> allosteric enzyme [98]; <84> compulsory-ordered tri-bi mechanism [100])

Reaction type

phospho group transfer

Natural substrates and products

S ADP + phosphoenolpyruvate <1, 4, 12, 13, 23, 37, 40, 58, 64, 43, 44, 48, 81, 83, 84, 91> (<40> probably involved in supplying additional carbon-skeletons for ammonium assimilation [11]; <37> involved in regulation of metabolism of an aerobic organism capable of net glucose synthesis [15]; <12> rate-controlling enzyme of glycolytic flux [27]; <4> key enzyme in glycolysis [40]; <1, 4, 13, 23, 37, 58, 64> allosteric enzyme [15, 20, 25, 37, 39, 40, 42]; <43, 44, 48, 81, 83, 84> physiological role [92]; <91> final regulatory point in catabolic Embden-Meyerhoff-Parnas pathway [101]) (Reversibility: ? <1, 4, 12, 13, 23, 37, 40, 58, 64, 43, 44, 48, 81, 83, 84, 91> [11, 15, 20, 25, 27, 37, 39, 40, 42, 92, 100]) [11, 15, 20, 25, 27, 37, 39, 40, 42, 92, 101]

P ATP + pyruvate <37> [15]

Substrates and products

S ADP + phosphoenolpyruvate <1-98> (<2> catalyzes the addition of a proton and the loss of a phosphoryl group which is transferred to ADP [47]; <2, 3, 8, 38, 40, 43-45, 48, 65, 70, 74, 81, 84, 91> best nucleoside diphosphate substrate [4, 11, 33, 44, 46, 50, 55, 58, 64, 66, 72, 74, 90, 100, 101]; <19> other nucleoside diphosphates can replace ADP with a differ-

ent rank order of effectiveness for enzyme form I and II [18]; <1, 4, 13, 23, 37, 38, 64, 65, 68-70, 73> positive cooperativity for phosphoenolpyruvate [15, 20, 25, 37, 40, 42, 45, 66, 72-74, 76]; <65> no positive cooperativity for ADP [72]; <1> 4 phosphoenolpyruvate-binding sites/enzyme molecule [42]; <79> no allosteric activation [79]; <82, 94> ADP preferred substrate, UDP, IDP, GDP and CDP may also be used with lower effectivity [91, 107]; <84> poor substrates: UDP, GDP [100]) (Reversibility: ir <2, 48, 60, 76> [46, 47, 63, 70, 78]; ? <1, 3-47, 49-59, 61-75, 77-90> [1-45, 47-62, 64-69, 71-77, 79-89, 91-102, 107, 110]; r <93> [103]) [1-103, 107, 110]

P ATP + pyruvate <37> [15]

S CDP + phosphoenolpyruvate <2, 3, 8, 38, 43-45, 48, 65, 70, 75> (<2, 3, 8, 38, 43-45, 48, 65, 70, 75> less effective than ADP [4, 33, 46, 50, 55, 58, 64, 66, 72, 74, 77]; <45> reaction at 25% the rate of ADP [4]; <48> reaction with PKp-isozyme at 30%, with PKc-isozyme at 12% the rate of ADP [64]; <65, 75> poor substrate [72, 77]) (Reversibility: ? <2, 3, 8, 38, 43-45, 48, 65, 70, 75> [4, 33, 46, 50, 55, 58, 64, 66, 72, 74, 77]) [4, 33, 46, 50, 55, 58, 64, 66, 72, 74, 77]

P CTP + pyruvate

S GDP + phosphoenolpyruvate <2, 8, 23, 38, 40, 43-45, 48, 65, 74, 75> (<2, 8, 23, 38, 40, 43-45, 48, 65, 74, 75> less effective than ADP [4, 11, 20, 44, 46, 50, 55, 58, 64, 66, 72, 77]; <45> reaction at 55% the rate of ADP [4]; <48> reaction for PKc-isozyme at 71%, for PKp-isozyme at 39% the rate of ADP [64]) (Reversibility: ? <2, 8, 23, 38, 40, 43-45, 48, 65, 74, 75> [4, 11, 20, 44, 46, 50, 55, 58, 64, 66, 72, 77]) [4, 11, 20, 44, 46, 50, 55, 58, 64, 66, 72, 77]

P GTP + pyruvate

S IDP + phosphoenolpyruvate <3, 8, 23, 43, 45, 48, 65, 75> (<3, 8, 23, 43, 45, 48, 65, 75> less effective than ADP [4, 20, 33, 50, 55, 64, 72, 77]; <45> reaction at 53% the rate of ADP [4]; reaction with PKp-isozyme at 20%, with PKc-isozyme at 89% the rate of ADP <48> [64]) (Reversibility: ? <3, 8, 23, 43, 45, 48, 65, 75> [4, 20, 33, 50, 55, 64, 72, 77]) [4, 20, 33, 50, 55, 64, 72, 77]

P ITP + pyruvate

S TDP + phosphoenolpyruvate <75> (<75> poor substrate [77]) (Reversibility: ? <75> [77]) [77]

P TTP + pyruvate

S UDP + phosphoenolpyruvate <2, 3, 8, 38, 40, 43-45, 48, 65, 75> (<2, 3, 8, 38, 40, 43-45, 48, 65, 75> less effective than ADP [4, 11, 33, 46, 50, 55, 58, 64, 66, 72, 77]; <48> reaction at about 70% the rate of ADP, PKc-isozyme [64]; <48> reaction at about 31% the rate of ADP, PKp-isozyme [64]) (Reversibility: ? <2, 3, 8, 38, 40, 43-45, 48, 65, 75> [4, 11, 33, 46, 50, 55, 58, 64, 66, 72, 77]) [4, 11, 33, 46, 50, 55, 58, 64, 66, 72, 77]

P UTP + pyruvate

S ε-ADP + phosphoenolpyruvate <65> (<65> poor substrate [72]) (Reversibility: ? <65> [72]) [72]

P ?

S Additional information <38, 65, 69, 70> (<65> broad specificity for nucleoside diphosphates [72]; <38> preferred: purine nucleotides [66]; <65, 69, 70> allosteric enzyme: homotropic [72-74]; <5-8, 11, 14, 17, 20, 27, 31, 38, 47, 49, 54, 59, 58, 62, 63, 66-68, 77> specificity overview [60]) [60, 66, 72-74]

P ?

Inhibitors

2,3-diphosphoglycerate <9, 45, 48> [4, 14, 64]
2-oxoglutarate <32, 81, 94> (<81> 50% inhibition at 8.3 mM [111]; <94> at 5 mM, pH 7.0, 35% inhibition [107]; <22, 44> not [53, 58]) [34, 107, 111]
2-phosphoglycerate <48> (<48> only isozyme PKp, not PKc [64]) [64]
3-phosphoglycerate <48> (<22> not [53]) [64]
6-phosphogluconate <69> [73]
ADP <19, 22, 65> (<22, 65> at high concentrations [53, 72]; <65> substrate inhibition [72]; <19> above 1-2 mM [18]; <22> activators restore [54]; <22> fructose 1,6-diphosphate partially restores [53]) [18, 53, 54, 72]
AMP <22, 74, 91> (<22> MgAMP [53]; <74> in a cooperative manner with ATP [44]; <8, 57, 92> not [52, 75, 102]) [44, 53, 101]
ATP <1, 4, 8, 9, 12, 16, 18, 21, 22, 26, 32, 36, 37, 40, 45, 48, 57, 64, 70, 73, 74, 86, 88-91, 94> (<21, 36, 37, 86> strong [7, 13, 15, 95]; <86> 50% inhibition at 4 mM [95]; <1> above 6 mM [12]; <57> kinetics [75]; <36> enzyme form I [13]; <16> liver, not muscle enzyme [2]; <8> L- and R-type isozyme [43]; <8> phosphorylated enzyme is more sensitive than unphosphorylated enzyme [43]; <22, 32, 48> $MgATP^{2-}$ [34, 53, 54, 64]; <64> in the presence of Mg^{2+}, not Mn^{2+}, reversible by AMP [37]; <74> in a cooperative manner with AMP [44]; <57> ADP alleviates [75]; <70> glucose 6-phosphate reverses [74]; <26> fructose 1,6-diphosphate does not reverse [30]; <94> at 1 mM, pH 7.0, 50% inhibition [107]; <52> not [10]) [2, 4, 7, 11-15, 27, 30, 34, 35, 37, 40, 41, 43, 44, 53, 54, 64, 74-76, 95, 98, 99, 101, 107]
Ba^{2+} <70> (<70> in decreasing order of inhibitory efficiency: Ni^{2+}, Zn^{2+}, Cu^{2+}, Ca^{2+}, Ba^{2+} [74]) [74]
CTP <57> [75]
Ca^{2+} <8, 16, 30, 65, 70> (<8> strong at saturating phosphoenolpyruvate concentrations [52]; <70> in decreasing order of inhibitory efficiency: Ni^{2+}, Zn^{2+}, Cu^{2+}, Ca^{2+}, Ba^{2+} [74]) [2, 29, 50, 52, 72, 74]
Cd^{2+} <29, 30> (<29, 30> at physiological pH, activating below [21]) [21, 29]
Co^{2+} <29, 30> (<29, 30> at physiological pH, activating below [21]) [21, 29]
Cu^{2+} <16, 65, 70> (<70> in decreasing order of inhibitory efficiency: Ni^{2+}, Zn^{2+}, Cu^{2+}, Ca^{2+}, Ba^{2+} [74]) [2, 72, 74]
D-ribose 5-phosphate <48> (<48> only isozyme PKp, not PKc [64]) [64]
D-ribulose 1,5-bisphosphate <48> (<48> isozyme PKp, not PKc [64]) [64]
D-serine <8> [50]
GTP <37, 57, 91> [15, 75, 101]
ITP <37> [15]
K^+ <38> (<38> above 100 mM, activates below [66]) [66]

L-alanine <4, 8, 11, 13, 18, 22-26, 28, 34, 35> (<26> kinetics [30]; <22> allosteric inhibitor [53]; <23> phosphoenolpyruvate- and Mg^{2+}-dependent [20]; <24, 28> strong [19, 31]; <34> weak, flight muscle isozyme [22]; <24> weak, in the presence of Mn^{2+} [16]; <18, 24, 34> weak [16, 22, 35]; <28> D-fructose bisphosphate protects [19]; <8, 22, 26> fructose 1,6-diphosphate restores [30, 50, 53]; <8> L- and M2-type, not M1-type isozyme [43]; not <16, 52, 69> [2, 10, 73]) [16, 19, 20, 22, 23, 25, 28, 30, 31, 35, 38, 41, 43, 50, 53]
L-glutamate <40, 43, 44, 45, 81, 82, 84> (<44> strong, isozyme PKII, kinetics [58]; <45> dihydroxyacetone phosphate reverses [4]; <44> not isozyme PKI [58]; <81> 50% inhibition at 4 mM [90]) [4, 11, 58, 90, 91, 100, 104]
L-lactate <22, 23> [20, 53]
L-phenylalanine <1, 8, 13, 18, 19, 22, 24, 28> (<22> allosteric inhibitor [53]; <8, 24, 28> strong [19, 31, 52]; <18> weak [35]; <22> pH-dependent [53]; <28> not in the presence of Mn^{2+} [19]; <19> isozymes PK I and II differ in sensitivity to the inhibitor [26]; <8> L- and M2-type, not M1-type isozyme [43]; <1, 8, 28> D-fructose 1,6-diphosphate protects [12, 19, 50]; <22> alanine and D-fructose 1,6-diphosphate protect, kinetics [53]) [12, 19, 25, 26, 31, 35, 43, 50, 52, 53]
Mg^{2+} <65> (<65> free form [72]) [72]
$MgATP^{2-}$ <22, 32, 48, 82, 92> (<22> allosteric inhibitor [53]; <32> feed-back inhibition [34]; <22,32> fructose 1,6-diphosphate restores [34, 53]; <22> activators restore activity [54]) [34, 53, 54, 64, 91, 102]
N-ethylmaleimide <45, 48> (<48> isozyme PK1 is more sensitive than PK2 [63]) [4, 63]
NH_4^+ <38> (<38> above 100 mM, activates below [66]) [66]
Na^+ <12, 38> (<38> above 100 mM, activates below [66]) [27, 66]
Ni^{2+} <65, 70> (<70> in decreasing order of inhibitory efficiency: Ni^{2+}, Zn^{2+}, Cu^{2+}, Ca^{2+}, Ba^{2+} [74]) [72, 74]
Procion Blue MX-R <7> (<7> triazine dye, kinetics, ADP or ADP plus Mg^{2+} protect, not Mg^{2+} alone [49]) [49]
Sr^{2+} <65> [72]
UTP <40> [11]
Zn^{2+} <16, 29, 70> (<29> inhibition or activation, concentration-dependent behaviour [21]; <70> in decreasing order of inhibitory efficiency: Ni^{2+}, Zn^{2+}, Cu^{2+}, Ca^{2+}, Ba^{2+} [74]) [2, 21, 29, 74]
adenine <44> (<44> weak [58]) [58]
antibody <18> (<18> to bovine type L-kinase leading to partial inactivation of type K-kinase, not type M-kinase, to bovine, chicken and salmon type M-kinases leading partial inactivation of type K-kinase, to bovine type M-kinase leading to partial inactivation of type M-kinase [36]) [36]
arginine phosphate <22, 34> (<34> flight muscle isozyme: weak [22]; <22> allosteric inhibitor [53]; <22, 34> fructose 1,6-diphosphate restores [22, 53]) [22, 53]
aspartate <48> (<48> only isozyme PKp, not PKc [64]) [64]
carbamoyl phosphate <64> [37]
catecholamine <8> [43]

citrate <23, 26, 32, 34, 37, 40, 43-45, 48, 57, 81, 84, 94> (<34> weak, flight muscle isozyme [22]; <44> isozyme PKI, kinetics [58]; <44> not isozyme PKII [58]; <34> fat body isozyme [22]; <94> at 5 mM, pH 7.0, 40% inhibition [107]; <81> 50% inhibition at 18.4 mM [111]; <22> not [53]) [4, 11, 15, 20, 22, 30, 34, 55, 58, 64, 75, 100, 104, 107, 111]
L-cysteine <8> (<8> fructose 1,6-diphosphate protects [50]) [50]
D-fructose 1,6-diphosphate <40, 48, 69, 94> (<94> at 5 mM, pH 7.0, 60% inhibition [107]) [11, 64, 73, 107]
fumarate <84> [100]
glucagon <8> [43]
D-glucose 6-phosphate <21, 48> (<21> at high concentrations [7]; <48> only isozyme PKp, not PKc [64]; <22> not [53]) [7, 64]
L-glutamine <84> [100]
highly phosphorylated inositol derivatives <9> [14]
isocitrate <45, 48, 57, 84> (<48> only isozyme PKp, not PKc [64]; <22> not [53]) [4, 64, 75, 100]
malate <32, 45, 48> (<48> only isozyme PKp, not PKc [64]; <22> not [53]) [4, 34, 64]
oxalate <40, 43, 44, 45, 48, 81, 84, 87> (<44> kinetics [58]; <81> 50% inhibition at 4 mM [90]; <81> 50% inhibition at 0.23 mM [111]; <87> PK I, 80% inhibition at 0.3 mM, PK II, 50% inhibition at 0.3 mM [97]) [4, 11, 58, 64, 90, 97, 100, 104, 111]
oxaloacetate <40> (<22> not [53]) [11]
phosphate <22, 48, 64, 65, 69, 70, 74, 75, 93, 94> (<74, 75> strong [44,77]; <75> at high concentrations [77]; <22> activators restore [54]; <93> severe, restored by addition of D-fructose 1,6-diphosphate [103]; <94> at 5 mM, pH 7.0, 40% inhibition [107]; <22> not [53]) [37, 44, 54, 64, 72-74, 77, 103, 107]
phosphoglycolate <45, 48> (<48> only isozyme PKp, not PKc [64]) [4, 64]
L-proline <22> (<22> allosteric inhibitor, fructose 1,6-diphosphate restores [53]) [53]
pyruvate <43> (<43> product inhibition [55]) [55]
quercetin <81> (<81> 50% inhibition at 0.1 mM [90]) [90]
rutin <81> (<81> 50% inhibition at 0.07 mM [90]) [90]
sulfate <70, 74> [44, 74]
threonine <8> (<8> fructose 1,6-diphosphate protects [50]) [50]
tryptophan <32, 44> (<44> weak [58]) [34, 58]
tyrosine <8, 48> (<8> fructose 1,6-diphosphate partially protects [50]) [50, 64]
valine <8> (<8> fructose 1,6-diphosphate protects [50]) [50]
Additional information <6, 8, 22, 35, 37> (<8> phosphorylation by cAMP-dependent protein kinase, L- and R-type isozyme, [43]; <8> no phosphorylation [50]; <35> increasing buffer concentrations inhibit, least inhibitory: imidazole-HCl [23]; <22> interacting effects of various activators and inhibitors [54]; <6> no substrate inhibition at pH-optimum [17]; <37> no inhibition by several amino acids [15]; <22> no inhibition by acetyl-CoA, $NADP^+$, succinate, glycerol 1-phosphate, D-fructose 6-phosphate, D-octopine, *meso*-alanopine, NH_4Cl, Arg, Gly, taurine, creatine phosphate [53]) [15, 17, 23, 43, 53, 54]

Cofactors/prosthetic groups

AMP <36, 40, 44-46, 65, 69, 73, 76> (<36, 40, 44, 45, 73, 76> activation [4, 11, 13, 58, 76, 78]; <46, 65, 69> allosteric effector [59, 72, 73]; <36> enzyme form I [13]; <44> not at physiological concentrations [58]; <57, 75> not [75, 77]) [4, 11, 13, 58, 59, 72, 73, 76, 78]
ATP <1, 68> (<1, 68> activation [12, 45]; <1> below 4 mM, inhibits above 5 mM [12]; <68> isozyme PK II [45]) [12, 45]
CMP <40, 65> (<40, 65> activation [11, 72]; <65> allosteric [72]) [11, 72]
GDP <40, 65> (<40, 65> activation [11, 72]; <65> allosteric [72]) [11, 72]
GMP <40, 65> (<40, 65> activation [11, 72]; <65> allosteric [72]; <75> not [77]) [11, 72]
UDP <40> (<40> activation [11]) [11]

Activating compounds

3-phosphoglycerate <65> (<65> slight activation [72]) [72]
D-ribose 1-diphosphate,5-phosphate <38> (<38> activation, kinetics, much more effective than fructose 1,6-diphosphate or glucose 1,6-diphosphate [5]) [5]
6-phosphogluconate <81> (<81> at 1 mM, 200% of activity [111]) [111]
AMP <88, 89, 94, 99> (<94> at 1 mM or below [107]; <99> allosteric enzyme [112]) [98, 107, 112]
CO_2 <38> (<38> activation, kinetics [5]) [5]
D-fructose 1,6-diphosphate <1, 4-6, 8, 12, 13, 21-24, 32, 34, 37, 38, 56, 57, 58, 60, 68, 73, 74, 84, 85, 87, 93> (<1, 4-6, 8, 12, 13, 21-24, 32, 34, 37, 38, 56, 57, 60, 68, 73, 74> activation [5-7, 15, 17, 20, 22, 24, 25, 27, 31, 32, 34, 41, 42, 44, 45, 48, 50, 53, 54, 66, 70, 75, 76]; <58> isoform Pyk1p: is activated up to 8-fold with K_m lowered up to 30-fold, Pyk2p: activity and K_m only marginally affected [109]; <74> with Mn^{2+} [44]; <74> requirement with Mg^{2+} [44]; <56> heterotropic allosteric activator [24]; <4> major allosteric activator [41]; <38> allosteric activation together with phosphoenolpyruvate [32]; <22> stimulates aerobic isozyme more strongly than anoxic isozyme [54]; <22> synergism with Asp, PK-aerobic, not PK-anoxic [54]; <38> slight [5]; <60> more evident in the presence of glycerol [70]; <34> strong activation, fat body enzyme [22]; <21, 38, 73> kinetics [7, 66, 76]; <13> pH-dependent [25]; <87> only PK II, shifting sigmoidal kinetics to hyperbolic curves, decrease in K_m [97]; <1, 6, 8, 16, 34, 44, 52, 65, 75, 79, 92> not [2, 10, 22, 42, 52, 58, 72, 77, 79, 96, 102]) [5-7, 15, 17, 20, 22, 24, 25, 27, 31, 32, 34, 41, 42, 44, 45, 48, 50, 53, 54, 66, 70, 75, 76, 93, 97, 100, 103, 109]
D-fructose 2,6-diphosphate <22, 38, 39, 91> (<22, 38, 39> activation [5, 53, 67]; <38> best activator [5]; <24, 39, 91> allosteric effector [16, 67, 101]; <44> not [58]) [5, 53, 67, 101]
D-fructose 6-phosphate <74> (<74> requirement with Mg^{2+}, activation with Mn^{2+} [44]) [44]
D-fructose diphosphate <6, 9, 11, 21, 24, 28, 36, 38> (<6, 9, 11, 21, 24, 28, 36, 38> activation [5-7, 13, 14, 16, 19, 38]; <36> enzyme form I [13]; <11> cardiac and liver isozyme, together with phosphoenolpyruvate [38]; <38> allosteric effector [94]) [5-7, 13, 14, 16, 19, 38, 94]

D-glucose 1,6-diphosphate <38> (<38> slight activation [5]) [5]
D-glucose 1-phosphate <48> (<48> slight activation, not isozyme PKp [64]; <65> not [72]) [64]
D-glucose 6-phosphate <38, 48, 65, 68, 70, 74, 75, 84, 92> (<75> requirement [77]; <74> requirement with Mg^{2+}, activation with Mn^{2+} [44]; <48, 65, 70> activation [64, 72, 74]; <68> isozyme PK II [45]; <79> not [79]; <48> not: isozyme PKp [64]; <92> radically activates [102]) [44, 45, 64, 66, 72, 74, 77, 100, 102]
D-ribose 5-phosphate <65, 68, 69, 74, 75, 88, 89, 94> (<65, 68, 69, 75> activation [45, 72, 73, 77]; <74> requirement with Mg^{2+}, activation with Mn^{2+} [44]; <68> isozyme PK II [45]) [44, 45, 72, 73, 77, 98, 107]
D-ribulose 1,5-diphosphate <38, 45> (<38, 45> activation [4, 5]; <38> kinetics [5]; <38> much more effective than D-fructose 1,6-diphosphate or glucose 1,6-diphosphate [5]; <75> not [77]) [4, 5]
D-tagatose 1,6-diphosphate <74> (<74> requirement with Mg^{2+}, activation with Mn^{2+} [44]) [44]
D-tagatose 6-phosphate <74> (<74> requirement with Mg^{2+}, activation with Mn^{2+} [44]) [44]
GSH <38> (<38> activation, can replace dithiothreittol [66]) [66]
L-aspartate <81> (<81> Ka-value 0.31 mM, reverses inhibition by L-glutamate [90]) [90]
L-alanine <40> (<40> activation [11]; <69> not [73]) [11]
L-asparagine <22, 44, 48> (<22, 44, 48> activation [54, 58, 64]; <48> kinetics [64]; <44> synergistic activation with D-fructose 1,6-diphosphate of isozyme PK-aerobic, not PK-anoxic [58]) [54, 58, 64]
L-aspartate <93> [103]
L-cysteine <38> (<38> activation, can replace dithiothreitol [66]) [66]
dihydroxyacetone phosphate <45, 48, 74> (<45, 48, 74> activation [4, 44, 64]; <74> with Mn^{2+} [44]; <74> requirement with Mg^{2+} [44]; <48> kinetics [64]) [4, 44, 64]
dithiothreitol <38, 43> (<38> activation [66]; <43> required for optimal activity [56]) [56, 66]
erythrose 4-phosphate <74> (<74> requirement with Mg^{2+}, activation with Mn^{2+} [44]) [44]
glutamic acid <57> (<57> activation [75]) [75]
glutamine <40> (<40> activation [11]) [11]
glyceraldehyde 3-phosphate <45, 74> (<45, 74> activation [4, 44]; <74> with Mn^{2+} [44]; <74> requirement with Mg^{2+} [44]; <75> not [77]) [4, 44]
glycerol 3-phosphate <94> [107]
glycine <40> (<40> activation [11]) [11]
insulin <8> (<8> stimulation [43]) [43]
isoleucine <48> (<48> slight activation, kinetics [64]) [64]
methionine <40> (<40> activation [11]) [11]
monovalent anions <69> (<69> activation, in decreasing order of efficiency: Cl^-, Br^-, NO_3^- [73]) [73]
phosphate <1, 4, 9, 84> (<1, 4, 9> activation [12, 14, 40]) [12, 14, 40, 100]
phosphoenolpyruvate <93> [103]

phosphorylated hexoses <4, 9, 74> (<4, 9, 74> activation [14, 40, 44]; <74> with Mn^{2+} [44]; <74> requirement with Mg^{2+} [44]) [14, 40, 44]
D-ribulose 5-phosphate <45> (<45> activation [4]) [4]
Additional information <22, 64, 75, 79> (<64> the initial rate of catalysis is modulated by substrate activation by phosphoenolpyruvate and ADP, activation by AMP and inhibition by ATP, phosphate and carbamoyl phosphate [37]; <22> interacting effects of various activators and inhibitors [54]; <79> no activation by 6-phosphogluconate [79]; <75> no activation by glucose 1,6-diphosphate, 5'-IMP, 2',3'-AMP, 2',3'-GMP, 2',3'-UMP [77]) [37, 54, 77, 79]

Metals, ions

Ca^{2+} <12> (<12> activation [27];<65, 70, 75> not [72, 74, 77]) [27]
Cd^{2+} <29> (<29> multiphasical activation, at pH below physiological value, inhibits at physiological pH [21]) [21]
Co^{2+} <29, 65, 74> (<65> requirement [72]; <65> can replace Mg^{2+} [72]; <74> activation [44]; <29> multiphasical activation, at pH below physiological value, inhibits at physiological pH [21]) [21, 44, 72]
K^+ <1, 2, 4, 8, 12, 13, 22, 23, 26, 34, 37, 38, 43-45, 48, 57, 58, 60, 65, 69, 74, 75, 81, 82, 84, 85, 90, 91, 93> (<2, 23, 38, 43, 57-58, 65, 69, 74, 75, 82, 85, 91> requirement [20, 39, 44, 46, 56, 66, 72, 73, 75, 77, 91, 93, 101, 104]; <12, 26, 34, 37, 45> activation [4, 15, 22, 27, 30]; <69> best activator at optimal conditions, in decreasing order of efficiency: K^+, Rb^+, Cs^+, Na^+, NH_4^+, Li^+ [73]; <75, 81, 81, 90> only together with activating divalent cation [77, 90, 91, 99]; <45> strong [4]; <74> as good as NH_4^+ [44]; <58> K_m-value: 50 mM in the presence of fructose diphosphate [39]; <57> K_m-value 1.6 mM [75]; <81> apparent K_m-value: 0.48 mM [90]; <82> K_m-value 0.91 mM, hyperbolic saturation kinetics [91]; <2> 1 cation per active site [46]; <43, 84> fulfills requirement for monovalent cation [55, 100]; <38> inhibits above 100 mM [66]; <93> activates [103]; <79, 80, 94> not [79, 89, 107]) [4, 15, 20, 22, 25, 27, 30, 39-42, 44, 46, 50, 53-56, 58, 63, 64, 66, 70, 72, 73, 75, 77, 91-93, 99-101, 103, 104, 107]
Mg^{2+} <1, 2, 4, 5, 7-9, 11-13, 16, 18, 22, 24, 26, 30, 34, 36-38, 43-45, 48, 56, 58, 59, 64, 65, 70, 73-75, 80-82, 84, 85, 90, 91, 93, 94> (<2, 13, 16, 24, 36, 38, 43, 45, 58, 59, 64, 65, 70, 73, 75, 85, 90, 91, 94> requirement [2, 4, 5, 13, 16, 25, 37, 39, 46, 55, 56, 66, 72, 74, 76, 77, 93, 99, 101, 104, 107]; <90, 93, 94> either Mn^{2+} or Mg^{2+} are required [99, 103, 107]; <1, 9, 12, 18, 30, 37> activation [14, 15, 27, 29, 35, 42]; <43, 45, 81, 82, 84> fulfills absolute requirement for divalent cation [4, 55, 90, 91, 100]; <56> positive homotropic interaction with phosphoenolpyruvate and Mg^{2+} [24]; <73, 80> positive cooperativity [76, 89]; <16, 18, 38, 70> kinetics [2, 5, 35, 74]; <2> 2 cations per active site [46]; <34> K_m-value: 0.8 mM [22]; <58> K_m-value 2 mM in the presence of fructose diphosphate [39]; <43> K_m-value 0.45 mM for isozyme PKc, 1.6 mM for isozyme PKp [55]; <2,30> pH-dependent [29, 46]; <81> apparent K_m-value: 0,21 mM [90]; <82> K_m-value 0.27 mM, hyperbolic saturation kinetics [91]; <74> no activity in the absence of sugar phosphate activator [44]) [2, 4, 5, 13-16, 22, 24, 25, 27, 29, 30, 35, 37-44, 46, 48-50, 53-56, 58, 63, 64, 66, 72, 74, 76, 77, 89, 90, 91, 93, 99, 100, 101, 103, 104, 107]

Mn^{2+} <7, 16, 22-24, 29, 30, 34, 36, 38, 43, 45, 48, 64, 65, 70, 73-75, 80, 90, 93, 94> (<16, 24, 36, 38, 43, 45, 48, 64, 65, 70, 73, 75> requirement [2, 4, 5, 13, 16, 37, 55, 64, 66, 72, 74, 76, 77]; <90, 93, 94> either Mn^{2+} or Mg^{2+} are required [99, 103, 107]; <30, 74> activation [29, 44]; <23, 74> best activator [20, 44]; <75> only together with K^+ [77]; <43> fulfills absolute requirement for divalent cation [55]; <74> the activity of the sugar phosphate-activated enzyme is reduced to 50% if Mn^{2+} is omitted [44]; <29, 30> pH-dependent [21, 29]; <38, 70> kinetics [5, 74]; <34> K_m-value: 0.6 mM, flight muscle isozyme [22]; <43> K_m-value 0.05 mM for isozyme PKc, 0.5 mM for isozyme PKp [55]; <7> enzyme-bound [8]; <22, 23, 48, 65, 70> can partially replace Mg^{2+} [20, 53, 54, 64, 72, 74]; <80> positive cooperativity [89]) [2, 4, 5, 8, 13, 16, 20-22, 29, 37, 44, 53-55, 64, 66, 72, 74, 76, 77, 89, 99, 103, 107]

NH_4^+ <22, 23, 38, 43, 48, 57, 65, 69, 74, 75> (<22, 23, 38, 43, 48, 65, 74, 75> requirement [20, 44, 53-55, 64, 66, 72, 77]; <57> activation [75]; <74> as good as K^+ [44]; <22, 38, 48, 57, 65, 75> can replace K^+ [53, 54, 64, 66, 72, 75, 77]; <69> in decreasing order of efficiency: K^+, Rb^+, Cs^+, Na^+, NH_4^+, Li^+ [73]; <75> only together with activating divalent cation [77]; <57> kinetics [75]; <38> inhibits above 100 mM [66]; <94> not [107]) [20, 44, 53-55, 64, 66, 72, 73, 75, 77]

Na^+ <38, 65, 69> (<38> activation, can replace K^+ [32]; <65> can poorly replace K^+ [72]; <38> inhibits above 100 mM [66]; <69> in decreasing order of efficiency: K^+, Rb^+, Cs^+, Na^+, NH_4^+, Li^+ [73]; <57, 75, 93, 94> not [75, 77, 103, 107]) [32, 66, 72, 73]

Zn^{2+} <29> (<29> activation or inhibition, concentration-dependent behaviour [21]; <70> not [74]) [21]

divalent cation <1, 2, 40, 48, 74, 75> (<1, 2, 40, 48, 74, 75> requirement [11, 42, 44, 47, 64, 77]; <2, 40, 48, 75> requires both a divalent and a monovalent cation [11, 47, 64, 77]) [11, 42, 44, 47, 64, 77]

monovalent cation <1, 2, 23, 38, 40, 48, 69, 74, 75> (<1, 2, 23, 38, 40, 48, 74, 75> requirement [11, 20, 42, 44, 47, 64, 66, 77]; <2, 40, 48, 75> requires both a divalent and a monovalent cation [11, 47, 64, 77]; <69> in decreasing order of efficiency: K^+, Rb^+, Cs^+, Na^+, NH_4^+, Li^+ [73]; <70> not [74]) [11, 20, 42, 44, 47, 64, 66, 73, 77]

Additional information <22, 64, 65, 69, 70, 75, 43, 44, 48, 81, 83, 84> (<22> interacting effects of various activators and inhibitors [54]; <64> K-type allosteric properties only in the presence of Mg^{2+}, not Mn^{2+} [37]; <75> no activation by Li^+ [77]; <65, 70> no activation by Cu^{2+}, Ni^{2+} [72, 74]; <70> no activation by Ba^{2+} [74]; <65> no activation by Sr^{2+} [72]; <69> no activation by SCN^- [73]; <43, 75, 81, 82, 84> absolute requirement for a bivalent and a monovalent cation with Mg^{2+} and K^+ fulfilling this [77, 90, 91, 100, 104]; <43, 44, 48, 81, 83, 84> overview [92]) [37, 54, 72-74, 77, 90-92, 100, 104]

Specific activity (U/mg)

51 <43> (<43> pH 6.5, 30°C [104]) [104]
51 <81> (<81> pH 6.8, 24°C [90]) [90]
59 <82> (<82> pH 6.9, 25°C [91]) [91]

175 <89> (<89> 30°C [98]) [98]
191 <6> (<6> pH 7.4, 22°C [96]) [96]
201 <76> (<76> pH 7.5, 60°C [78]) [78]
202 <88> (<88> 30°C [98]) [98]
203 <43> (<43> pH 6.9, 30°C [56]) [56]
210 <64> (<64> pH 7.1, 30°C [37]) [37]
218 <48> (<48> isozyme PKp [65]) [65]
220 <70> [74]
233 <1> (<1> 25°C, pH 7.0, M-type isozyme from neck muscle [42]) [42]
250 <58> [60]
280 <79> [79]
307 <8> (<8> pH 7.4, 37°C, R-type isozyme [43]) [43]
330 <4> (<4> pH 8.0, 30°C, erythrocyte enzyme [41]) [41]
333 <65> (<65> pH 7.2, 30°C [72]) [72]
340 <58> (<58> pH 6.2, 30°C [39]) [39]
368 <22> (<22> isozyme PK-aerobic, pH 7.0, 20°C [53]) [53]
380 <5, 8> (<8> M1-type isozyme, pH 7.4, 37°C [43]; <5> M-type isozyme [48]) [43, 48]
397.5 <5> (<5> K-type isozyme [48]) [48]
420 <4> (<4> liver enzyme, pH 8.0, 30°C [41]) [41]
520 <8> (<8> M2-type isozyme, 25°C, pH 7.4 [43]) [43]
593 <8> (<8> pH 7.4, 37°C [52]) [52]
770 <8> (<8> M2-type isozyme, 37°C, pH 7.4 [43]) [43]
780 <8> (<8> M1-type isozyme, 37°C, pH 7.4 [43]) [43]
1341 <8> (<8> pH 7.1, 37°C [50]) [50]
1685 <53> (<53> isozyme PK5, pH 6.5, 30°C [69]) [69]
Additional information <1, 4, 6-9, 11, 13-15, 17, 20, 22-24, 27, 28, 31, 33, 38, 43-45, 47-49, 51, 53, 54, 57-59, 62, 63, 66-69, 73-75, 77> [3, 4, 12, 14, 19, 20, 25, 31, 38, 40, 42, 44, 45, 51, 53, 54, 57, 58, 60, 63, 66, 68, 69, 73, 75-77]

K_m-Value (mM)

0.024 <87> (phosphoenolpyruvate, <87> 15°, pH 7.0, isoform PK I, in winter [97]) [97]
0.026 <75> (GDP, <75> pH 7.5 [77]) [77]
0.03-0.05 <43, 48> (ADP, <48> PKc-isozyme [64]; <43> 25°C, pH 7.9 [55]) [55, 64]
0.033 <43> (phosphoenolpyruvate, <43> pH 7.5, 30°C [104]) [104]
0.033 <87> (phosphoenolpyruvate, <87> 15°, pH 7.0, isoform PK I, in summer [97]) [97]
0.037 <43> ($MgADP^-$, <43> pH 7.5, 30°C [104]) [104]
0.04-0.063 <1, 3, 6, 22, 26, 32, 34, 35, 43, 70, 75, 76> (phosphoenolpyruvate, <22> in the absence of fructose 1,6-diphosphate [54]; <22, 32, 35> in the presence of fructose 1,6-diphosphate [23, 34, 54]; <22> pH 7.0, 20°C [54]; <43> 2 isozymes with different kinetic mechanisms [55]; <43> 25°C, pH 7.9 [55]; <75> pH 7.5 [77]) [12, 17, 22, 23, 30, 33, 34, 54, 55, 74, 77, 78]

0.049-0.1 <12, 23, 32, 40, 43, 48, 75> (IDP, <8, 12, 23, 32, 40, 43, 48, 58> plus phosphoenolpyruvate [11, 20, 27, 34, 39, 43, 55, 64]; <43> 25°C, pH 7.9 [55]; <48> isozyme PKc [64]) [11, 20, 27, 34, 55, 64, 77]
0.05 <90> (phosphoenolpyruvate, <90> 30°C, pH 8.5 [99]) [99]
0.052 <81> (phosphoenolpyruvate, <81> 24°C, pH 8.0 [111]) [111]
0.064 <83> (phosphoenolpyruvate) [113]
0.075 <81> (ADP, <81> pH 6.8, 24°C [90]) [90]
0.082-0.4 <8, 38> (ADP, <8> L-type isozyme, pH 7.4, 37°C [43]; <8> pH 7.1, 37°C [50]; <38> pH 7.4, 25°C [66]) [43, 50, 66]
0.087 <43> ($MgADP^-$, <43> pH 6.5, 30°C [104]) [104]
0.089 <43> (phosphoenolpyruvate, <43> pH 6.5, 30°C [104]) [104]
0.09 <90> (phosphoenolpyruvate, <90> 30°C, pH 7.5 [99]) [99]
0.098 <82> (phosphoenolpyruvate, <82> hyperbolic saturation kinetics, pH 6.9, 25°C [91]) [91]
0.098-0.1 <38, 74> (GDP, <38> pH 7.5, 25°C [94]; <38> pH 7.4, 25°C [66]) [44, 66]
0.099 <58> (phosphoenolpyruvate, <58> pH 6.2, 30°C [39]) [39]
0.1 <84> (phosphoenolpyruvate, <84> pH 6.8, 30°C [100]) [100]
0.11 <84> (ADP, <84> pH 6.8, 30°C [100]) [100]
0.11 <93> (phosphoenolpyruvate, <93> allosteric enzyme, pH 7.0 [103]) [103]
0.12 <43, 57> (ADP, <43> plus UDP, isozyme PKc, 25°C, pH 7.9 [55]) [55, 75]
0.12 <82> (ADP, <82> hyperbolic saturation kinetics, pH 6.9, 25°C [91]) [91]
0.12 <81> (phosphoenolpyruvate, <81> pH 6.8, 24°C [90]) [90]
0.12 <90> (phosphoenolpyruvate, <90> 30°C, pH 6.5 [99]) [99]
0.14 <81> (ADP, <81> pH 8.0, 24°C [111]) [111]
0.15-0.16 <36, 45> (phosphoenolpyruvate, <36> enzyme form I [13]) [4, 13]
0.16 <58> (ADP, <58> pH 6.2, 30°C [39]) [39]
0.16 <90> (ADP, <90> 30°C, pH 8.5 [99]) [99]
0.16-0.17 <35, 40, 73, 79> (ADP, <73> isozyme II, 30°C, pH 6.8 [76]; <79> pH 6.5, 25°C [79]) [11, 23, 76, 79]
0.17 <90> (ADP, <90> 30°C, pH 7.5 [99]) [99]
0.18 <1, 8, 45, 48> (phosphoenolpyruvate, <1,45> plus ADP [4,42]; <1> L-type isozyme, 25°C, pH 7.0 [42]; <48> isozyme PKp [64]; <8> pH 7.4, 37°C [52]) [4, 42, 52, 64]
0.181 <87> (phosphoenolpyruvate, <87> 15°, pH 7.0, isoform PK II, in summer [97]) [97]
0.193 <87> (phosphoenolpyruvate, <87> 15°, pH 7.0, isoform PK II, in winter [97]) [97]
0.2 <64> (phosphoenolpyruvate, <64> pH 7.1, 30°C, in the presence of Mg^{2+}, 7fold lower in the presence of Mn^{2+} [37]) [37]
0.2-0.35 <1, 6, 22, 23, 26, 34, 36, 43, 48, 73> (ADP, <36,73> enzyme form I [13,76]; <73> 30°C, pH 6.8 [76]; <1> M-type isozyme, 25°C, pH 7.0 [42]; <43,48> PKp-isozyme [55,64]; <22> pH 7.0, 20°C [53]; <22> pH 7.0, 20°C [54]; <43> 25°C, pH 7.9 [55]) [12, 13, 17, 20, 22, 30, 42, 53-55, 64, 76]
0.22 <75> (phosphoenolpyruvate, <75> in the presence of glucose 6-phosphate, at pH 7.5 [77]) [77]

0.235 <83> (ADP) [113]
0.24 <90> (ADP, <90> 30°C, pH 6.5 [99]) [99]
0.24 <93> (ADP, <93> allosteric enzyme, pH 7.0 [103]) [103]
0.25-0.26 <43, 75> (GDP, <43> IDP, isozyme PKc, 25°C, pH 7.9 [55]; <75> UDP, at pH 7.5 [77]) [55, 77]
0.3-0.96 <8> (phosphoenolpyruvate, <8> L-type, pH 7.4, 37°C [43]) [43]
0.31 <86> (ADP, <86> pH 7.4, 30°C [95]) [95]
0.35 <93> (ATP, <93> allosteric enzyme, pH 7.0 [103]) [103]
0.35 <35> (phosphoenolpyruvate) [23]
0.39 <75> (ADP, <75> in the presence of glucose 6-phosphate, pH 7.5 [77]) [77]
0.4-0.57 <8, 9, 57> (phosphoenolpyruvate, <8> M2-type isozyme, pH 7.4, 37°C [43]; <8> pH 7.4, 37°C [52]) [14, 43, 52, 75]
0.4-0.6 <8, 9> (ADP, <8> R-type isozyme, pH 7.4, 37°C [43]; <8> pH 7.4, 37°C [52]) [14, 43, 52]
0.41 <38> (UDP, <38> pH 7.4, 25°C [66]) [66]
0.41 <86> (phosphoenolpyruvate, <86> pH 7.4, 30°C [95]) [95]
0.45 <94> (phosphoenolpyruvate, <94> allosteric enzyme, pH 7.5, 24°C [107]) [107]
0.48 <93> (pyruvate, <93> allosteric enzyme, pH 7.0 [103]) [103]
0.54 <94> (phosphoenolpyruvate, <94> allosteric enzyme, pH 7.0, 24°C [107]) [107]
0.67 <8> (GDP, <8> pH 7.1, 37°C [50]) [50]
0.7 <64> (ADP, <64> in the presence of Mg^{2+}, pH 7.1, 30°C [37]) [37]
0.7 <80> (ADP, <80> 50°C, pH 7.0 [89]) [89]
0.72-0.73 <44> (UDP, <44> pH 7.1, 25°C [58]) [58]
0.83 <8> (ADP, <8> in the presence of ATP, pH 7.4, 37°C [52]) [52]
0.88 <35> (phosphoenolpyruvate, <35> in the presence of alanine [23]) [23]
0.98-1 <8, 12> (ADP, <8> M1-type isozyme, pH 7.4, 37°C [43]) [27, 43]
1.1 <44> (GDP, <44> pH 7.1, 25°C [58]) [58]
1.25 <8> (UDP, <8> pH 7.1, 37°C [50]) [50]
1.4 <8> (phosphoenolpyruvate, <8> R-type isozyme, pH 7.4, 37°C [43]) [43]
1.4-1.5 <8> (ADP, <8> M2-type isozyme, pH 7.4, 37°C [43]) [43]
2.2 <43> (GDP, <43> isozyme PKp, 25°C, pH 7.9 [55]) [55]
2.4 <43> (UDP, <43> isozyme PKp, 25°C, pH 7.9 [55]) [55]
2.53 <38> (CDP, <38> pH 7.4, 25°C [66]) [66]
4 <43> (IDP, <43> isozyme PKp, 25°C, pH 7.9 [55]) [55]
6.8 <44> (CDP, <44> isozyme PKII, pH 7.1, 25°C [58]) [58]
9 <44> (CDP, <44> isozyme PKI, pH 7.1, 25°C [58]) [58]
Additional information <4-6, 8, 18, 19, 22, 25, 28, 35-39, 43, 44, 46, 58, 70, 75, 76, 81, 43, 44, 48, 81, 83-86, 95-98> (<4> allosteric enzyme [41]; <4> allosteric pattern dissappears at pH 5.9 [40]; <6, 19, 37, 38> kinetic study [5, 15, 17, 18]; <4> kinetic properties of phosphorylated and dephosphorylated kinases [41]; <46> kinetic properties of different MW-forms of Chlorella kinase [59]; <4, 18, 19, 22, 28, 36, 38, 46> kinetic properties [13, 18, 19, 32, 35, 41, 53, 59]; <35> kinetic properties compared to pyruvate kinase from other insects [23]; <25> alteration of kinetic properties by purification [28];

<5, 8, 22, 39, 43, 44, 58, 70, 75, 76> kinetic parameters [39, 48, 50, 54, 55, 58, 60, 67, 74, 77, 78]; <38> pH-dependence [32]; <5-8, 11, 14, 17, 20, 27, 31, 38, 47, 49, 54, 59, 58, 62, 63, 66-68, 77> kinetics overview [60]; <81> hyperbolic saturation kinetics for phosphoenolpyruvate, ADP, Mg^{2+}, K^+ [90]; <43, 44, 48, 81, 83, 84> assay and kinetics overview [92]; <83, 95-98> coupled assay for leaf crude extracts [110]) [5, 13, 15, 17-19, 23, 28, 32, 35, 39-41, 48, 50, 53-55, 58-60, 67, 74, 77, 78, 90, 93, 92, 95, 110]

K_i-Value (mM)

1.77 <92> ($MgATP^{2-}$, <92> 37°C, pH 7.0 [102]) [102]
3.7 <82> (L-glutamate, <82> cosubstrate $MgADP^-$, pH 7.5, 25°C [91]) [91]
3.9 <82> ($MgATP^{2-}$, <82> cosubstrate $MgADP^-$, pH 6.9, 25°C [91]) [91]
4 <82> ($MgATP^{2-}$, <82> cosubstrate $MgADP^-$, pH 7.5, 25°C [91]) [91]
4.4 <82> (L-glutamate, <82> cosubstrate phosphoenolpyruvate, pH 7.5, 25°C [91]) [91]
9.6 <82> ($MgATP^{2-}$, <82> cosubstrate phosphoenolpyruvate, pH 6.9, 25°C [91]) [91]
10 <82> ($MgATP^{2-}$, <82> cosubstrate phosphoenolpyruvate, pH 7.5, 25°C [91]) [91]
13.6 <82> (L-glutamate, <82> cosubstrate phosphoenolpyruvate, pH 6.9, 25°C [91]) [91]
19.2 <82> (L-glutamate, <82> cosubstrate $MgADP^-$, pH 6.9, 25°C [91]) [91]

pH-Optimum

4.5-7 <99> [112]
6 <38> [5]
6-6.6 <19> [26]
6.3-6.5 <13> [25]
6.5 <8, 15, 43, 48> (<8> at 0.125 mM phosphoenolpyruvate [52]; <48> isozyme PKp [64]; <43> broad [104]) [3, 50, 52, 64, 104]
6.5-7 <38> [66]
6.6 <88> (<88> 30°C [98]) [98]
6.6-6.9 <35> [23]
6.7 <3> [33]
6.8 <40, 45, 65, 73, 81, 84> (<65> 60°C [72]; <73> more active in HEPES than in other buffers [76]; <81> broad [90]) [4, 11, 72, 76, 90, 100]
6.8-7.2 <1> [12]
6.8-7.7 <75> [77]
6.9 <82> [91]
6.9-7.2 <43> (<43> isozyme PKc [55]) [55]
7 <8, 22, 37, 48, 70, 92, 94> (<37> in the absence of D-fructose 1,6-diphosphate [15]; <8> at saturating phosphoenolpyruvate concentration: 1.25 mM [52]; <22> broad [53,54]; <48> isozyme PKc [64]) [15, 52-54, 64, 74, 102, 107]
7-7.4 <64> [37]
7.2 <6, 65, 83> (<65> 30°C [72]) [6, 17, 72, 113]
7.3 <91> [101]
7.4 <6> [96]

7.5 <74, 76> [44, 78]
7.6 <37> (<37> in the presence of D-fructose 1,6-diphosphate [15]) [15]
7.8 <43> (<43> isozyme PKp [55]) [55]
8 <57, 81> [75, 111]
Additional information <22, 38> (<38> effect of pH-values on kinetic properties [5]; <22> pH-dependence of phosphoenolpyruvate affinity [53]) [5, 53]

pH-Range

4-6.8 <73> (<73> increase of activity in this range, decline at higher pH-values [76]) [76]
5.2-8 <48> (<48> isozyme PKp, about half-maximal activity at pH 5.2 and pH 8 [64]) [64]
5.5-7.4 <65> (<65> 60°C, about half-maximal activity at pH 5.5 and pH 7.4 [72]) [72]
5.5-7.5 <44> (<44> about 80% of maximal activity at pH 5.5 and pH 7.5 [58]) [58]
5.7-8.5 <8> (<8> 1.25 mM phosphoenolpyruvate, about half-maximal activity at pH 5.7 and 8.5, about 80% of maximal activity at pH 7.4 [52]) [52]
5.9-8.2 <48> (<48> isozyme PKc, about half-maximal activity at pH 5.9 and pH 8.2 [64]) [64]
6-7.3 <8> (<8> 0.125 mM phosphoenolpyruvate, about half-maximal activity at pH 6 and pH 7.3 [52]) [52]
6-8 <22> (<22> about 70% of maximal activity at pH 6 and about half-maximal activity at pH 8 [53]) [53]
6-8.3 <43> (<43> isozyme PKc, about half-maximal activity at pH 6 and pH 8.3 [55]) [55]
6.4-8.2 <65> (<65> 30°C, about half-maximal activity at pH 6.4 and pH 8.2 [72]) [72]
6.8-7.1 <70> [74]
7.2-8.3 <43> (<43> isozyme Pkp, about half-maximal activity at pH 7.2 and pH 8.3 [55]) [55]
7.6-8.2 <57> [75]

Temperature optimum (°C)

15 <11> (<11> assay temperature of cardiac and liver enzyme, value must not exceed 15°C [38]) [38]
20-21 <11> (<11> muscle enzyme, assay at [38]) [38]
25 <1, 4, 38, 43, 44, 48, 60, 68, 74, 79> (<1, 4, 38, 43, 44, 48, 60, 68, 74, 79> assay at [12, 40, 44, 45, 55, 57, 58, 63, 64, 66, 70, 79]) [12, 40, 44, 45, 55, 57, 58, 63, 64, 66, 70, 79]
28 <70> (<70> assay at [74]) [74]
30 <4, 43, 48, 53, 58, 59, 64, 65, 73> (<4, 43, 48, 53, 58, 59, 64, 65, 73> assay at [37, 39, 41, 56, 65, 69, 72, 76]) [37, 39, 41, 56, 65, 69, 72, 76]
37 <4, 8> (<4,8> assay at [41, 43, 50, 52]) [41, 43, 50, 52]
50 <88> [98]
60 <76> (<76> assay at [78]) [78]
Additional information <38> (<38> effect of temperature on kinetic properties [5]) [5]

4 Enzyme Structure

Molecular weight

100000 <60, 68> (<68> sucrose density gradient centrifugation [60]; <60> gel filtration [70]) [60, 70]
115000 <79> (<79> gel filtration [79]) [79]
120000-170000 <85> (<85> analytical ultracentrifugation [93]) [93]
152000 <9> (<9> gel filtration [14]) [14]
180000-190000 <75> (<75> gel filtration [77]) [77]
181000 <37> [15]
190000 <57, 59, 68, 73> (<68> isozyme PK II [45]; <57> gel filtration [75]; <73> isozyme PKII, gel filtration [76]) [39, 45, 75, 76]
195000-205000 <4> (<4> gel filtration [40]) [40]
200000 <40, 83, 93> (<40,83,93> gel filtration [11,103,113]) [11, 103, 113]
200000 <80> (<80> PAGE [89]) [89]
209400 <58> (<58> sedimentation equilibrium method [39]) [39]
210000 <19> (<19> PK II, sucrose density gradient centrifugation [26]) [26]
214000 <84> (<84> gel filtration [100]) [100]
215000 <1> (<1> L-type isozyme) [42]
216000 <53> (<53> isozyme PK1, PAGE) [69]
220000 <5, 73, 89> (<5> M4-type isozyme, glycerol density gradient sedimentation [48]; <73> isozyme PKII, gel filtration [76]; <89> gel filtration [98]) [48, 76, 98]
220000-240000 <92> (<92> gel filtration [102]) [102]
224000 <8, 45> (<45> native molecular mass [4]; <8> gel filtration [50]) [4, 50]
225000-240000 <68> (<68> isozyme PK I [45]) [45]
225500 <4, 5> (<5> M4-type isozyme, PAGE [48]; <4> sedimentation equilibrium method [40]) [48, 40]
227000 <53> (<53> average MW of all 5 isoforms, PAGE [69]) [69]
230000 <1, 5, 6, 24> (<1> M-type isozyme [42]; <5> K4-type isozyme, glycerol density gradient sedimentation, PAGE [48]; <6> sucrose density gradient centrifugation, M1 isozyme [96]) [16, 42, 48, 96]
230700 <70> (<70> gel filtration [74]) [74]
232000 <86> (<86> gel filtration [95]) [95]
232600 <22> (<22> PK-anoxic, gel filtration [53]) [53]
234000 <24> (<24> gel filtration [31]) [31]
235000 <34, 48, 74> (<34> [22]; <74> equilibrium sedimentation [44]; <48> isozyme PKp, FPLC gel filtration [65]) [22, 44, 65]
237000 <6> (<6> sucrose density gradient centrifugation [6]) [6]
237500 <22> (<22> PK-aerobic, gel filtration [53]) [53]
238000 <5, 28> (<28> native MW [19]; <5> K4-type isozyme, gel filtration [48]) [19, 48]
239000 <53> (<53> isozyme PK5, PAGE [69]) [69]

240000 <4, 8, 43, 48, 82> (<4> gel filtration [105]; <8> gel filtration [51]; <43> gel filtration [56]; <48> isozyme PK1 [63]; <82> gel filtration [91]) [51, 56, 63, 91, 105]
242000 <65> (<65> sedimentation analysis [72]) [72]
250000 <6, 8, 65, 76> (<6> gel filtration [17]; <7> muscle [60]; <65> gel filtration [72]; <76> PAGE [78]; <93> gel filtration [103]) [17, 60, 72, 78, 103]
265000 <6> (<6> liver, gel filtration [60]) [60]
270000 <38, 99> (<38> gel filtration [66]; <99> gel filtration [112]) [66, 112]
280000 <94> (<94> gel filtration [107]) [107]
282000 <23> (<23> gel filtration [20]) [20]
286000 <22> (<22> PK-aerobic, gel filtration [54]) [54]
299800 <22> (<22> PK-anoxic, gel filtration [54]) [54]
300000 <19, 25, 69> (<19> PK I, gel filtration [26]; <25> gel filtration [28]; <69> gel filtration [73]) [26, 28, 73]
305000 <43> (<43> gel filtration [57]) [57]
380000 <81> (<81> gel filtration [111]) [111]
400000 <46> (<46> growing and resting cells, FPLC gel filtration [59]) [59]
580000 <46> (<46> resting cells, FPLC gel filtration [59]) [59]
590000 <48> (<48> isozyme PK1 [63]) [63]
Additional information <2, 4, 5-8, 11, 14, 17, 20, 27, 31, 38, 39, 46, 47, 49, 51, 54, 59, 60, 62, 63, 65-68, 77> (<4> structure of liver and erythrocyte L-type kinases seems to be identical with an additional fragment present in erythrocytic enzyme [41]; <46> in vitro addition of substrates leads to higher MW enzyme species with MW of 730000, 1050000 and 1400000 [59]; <5, 51, 65> amino acid composition [48, 68, 72]; <39> comparison of amino acid sequences [67]; <2> 3-dimensional structure of cat M-type isozyme [47]; <5-8, 11, 14, 17, 20, 27, 31, 38, 47, 49, 54, 59, 58, 62, 63, 66-68, 77> overview [60]) [41, 47, 48, 59, 60, 67, 68, 70, 72]
2000000 <19> (<19> PK I [26]) [26]

Subunits

? <1, 8, 12, 37, 88, 89, 91, 99> (<1,8> x * 57000, SDS-PAGE [12,52]; <37> x * 59000 [15]; <12> x * 60000-65000 [27]; <88> x * 62570, deduced from gene sequence [98]; <89> x * 61940, deduced from gene sequence [98]; <91> x * 53500, SDS-PAGE [101]; <99> x * 55000, SDS-PAGE [112]) [12, 15, 27, 52, 98, 101, 112]
decamer <48> (<48> 10 * 57000, isozyme PK2, SDS-PAGE [63]) [63]
dimer <79, 85> (<79> 2 * 57000, SDS-PAGE [79]; <85> 2 * 55400, SDS-PAGE, predominantly a dimer, but also some tetramer [93]) [79, 93]
hexamer <81> (<81> 3 * 58000 + 3 64000, SDS-PAGE [111]) [111]
monomer <48> (<48> 1 * 210000, isozyme PKp, SDS-PAGE [65]) [65]
tetramer <1-7, 10, 21-24, 28, 43, 45, 51-53, 58, 65, 68, 69, 70, 73, 75, 76, 80, 83, 84, 85, 92, 94> (<80> 4 * 49000, SDS-PAGE [89]; <43> x * 56000 + x * 57000, SDS-PAGE [56]; <4> 2 * 60000 + 2 * 57000-58000, most important of the "aged" isozymes, PKR2, SDS-PAGE, derived from erythroblast homotetramer by partial proteolysis and transformation into various active heterotetrameric forms with two partially proteolyzed subunits [41]; <75> 4 * 44000, SDS-PAGE

[77]; <73> 4 * 47000, isozyme II, SDS-PAGE [76]; <10> 4 * 49680 [9]; <83> 4 * 50000, SDS-PAGE [113]; <58> 4 * 50000-52000, SDS-PAGE [39]; <93> 4 * 51300, SDS-PAGE [103]; <68> 4 * 51000, isozyme PK I, SDS-PAGE [45]; <1> 4 * 52000, L-type isozyme [42]; <73> 4 * 54000, isozyme I, SDS-PAGE [76]; <53> 4 * 54400, isozyme PK1, SDS-PAGE [69]; <85> 2 * 55400, SDS-PAGE, predominantly a dimer, but also some tetramer [93]; <84> x * 55000 + 4-x * 57000, SDS-PAGE [100]; <43> 2 * 56000 + 2 * 57000, SDS-PAGE [104]; <68> 4 * 56000, isozyme PK II, SDS-PAGE [45]; <81> 4 * 56000, SDS-PAGE [90]; <92> 4 * 57000, SDS-PAGE [102]; <45> 4 * 57000, SDS-PAGE [4]; <82> 4 * 57000, SDS-PAGE [91]; <70> 4* 57540, SDS-PAGE [74]; <21> 4 * 58000, SDS-PAGE [7]; <24> 4 * 58000, SDS-PAGE [31]; <53> 4 * 58000, SDS-PAGE, isozyme PK5 [69]; <5> 4 * 58600, M4-type isozyme, SDS-PAGE [48]; <6> 4 * 59000, SDS-PAGE [6]; <86> 4 * 59000, SDS-PAGE [95]; <5> 4 * 59500, K4-type isozyme, SDS-PAGE [48]; <6> 4 * 60000, SDS-PAGE, M1 isozyme [96]; <4> 4 * 60000, L-type isozyme, SDS-PAGE [41]; <8> S-type isozyme, SDS-PAGE [51]; <76> SDS-PAGE [78]; <4> 4 * 62000, SDS-PAGE [105]; <6> 4 * 62000, liver enzyme, SDS-PAGE [60]; <65> 4 * 62000-64000, SDS-PAGE [72]; <8> 4 * 62000-66000, SDS-PAGE [50]; <6> 4 * 63000, SDS-PAGE [17]; <22> 4 * 64400, PK-aerobic, SDS-PAGE [53]; <51> 4 * 65000,SDS-PAGE [68]; <52> 4 * 66000, tetrameric in low ionic strength buffer [10]; <94> 4 * 66000, SDS-PAGE [107]; <3> 4 * 69900 [33]; <23> 4 * 70000 [20]; <22> 4 * 71000, PK-aerobic, SDS-PAGE [54]; <22> 4 * 72600, PK-anoxic, SDS-PAGE [54]; <69> 4 * 73000, SDS-PAGE [73]) [3, 4, 6, 7, 9, 10, 17, 19, 20, 31, 33, 39-42, 45, 47, 48, 50-54, 56, 60, 68, 69, 72-74, 76-78, 89, 93, 96, 100, 102, 104, 105, 107, 113]

Additional information <1, 5-8, 11, 14, 17, 20, 27, 31, 38, 47, 49, 54, 59, 58, 62, 63, 66-68, 77, 43, 44, 48, 81, 83, 84> (<1> the isozymes are thought to be homotetramers, but hybrid isozymes result in vivo if a cell synthesizes 2 or more subunits simultaneously and in vitro after denaturation/renaturation of isozymic mixtures [42]; <5-8, 11, 14, 17, 20, 27, 31, 38, 47, 49, 54, 59, 58, 62, 63, 66-68, 77> structure overview [60]; <43, 44, 48, 81, 83, 84> overview [92]) [42, 60, 92]

Posttranslational modification

Additional information <65, 83> (<65> no glycoprotein [72]; <83> no S-S bonds [113]) [72, 113]

5 Isolation/Preparation/Mutation/Application

Source/tissue

Yoshida AH-130 cell <8> (<8> M2-type isozyme [43]) [43]
adipose tissue <8> [60]
ascites tumor cell <5> (Ehrlich and Yoshida [60]) [60]
brain <1, 8> (<8> M1-type isozyme [43]) [12, 43, 52]
cell culture <6-8, 11, 14, 17, 20, 27, 31, 33, 36, 38, 39, 45-49, 52-54, 57-79> (<64> vegetative [37]; <52> aggregating [10]; <53> hyphal and yeast [69]) [4, 10, 13, 37, 44, 45, 59, 60, 63-67, 69-79, 84-88]

cephalothorax <87> (<87> isoform PK II [97]) [97]
dental pulp <6> [17]
embryo <17> [60]
endosperm <43> (<43> germinating (from 5 days old seedlings) [56]; <43> developing [55,57]) [55-57]
erythrocyte <4, 8, 9, 12> (<8> R-type isozyme [43]) [14, 27, 40, 41, 43]
fat body <33, 34, 35> [22, 23, 60]
fetus <8> (M2-type isozyme [43]) [43]
foot <23, 24, 28-30> [16, 19-21]
fruit <82> [91]
heart <6, 11, 24, 8, 18> (<8,18> type M-isozyme [35,43]) [6, 16, 35, 43, 60]
hematopoietic system <8> (<8> R-type isozyme [43]) [43]
hepatopancreas <22, 25> [28, 54]
kidney <8, 68> (<68> M2-type: major, L-type: minor isozyme [45]) [45, 60]
larva <35> [23]
leaf <43, 44, 83, 95-98> [58, 104, 110]
liver <1, 4, 8, 11, 16, 19> (<8> L-type: major, M2-type: minor isozyme [43]) [2, 18, 26, 38, 41-43]
lung <8> (<8> M2-type isozyme [50]) [50]
mantle <27> [60]
muscle <5, 7, 8, 10, 11, 13, 14, 16, 18, 20, 22, 23, 26, 27, 29, 30, 33, 34, 68, 87> (<33, 34> flight muscle [22, 60]; <29, 30> posterior adductor [21, 29]; <27> adductor [60]; <23> foot muscle [20]; <26> retractor muscle [30]; <22> radular retractor muscle [53]; <18> cardiac [35]; <5, 7, 8, 10, 11, 18, 68> skeletal [9, 35, 38, 43, 45, 46, 48, 60]; <13, 16> white [2, 25]; <16, 22> red muscle [2, 53]; <15> striated muscle [3]; <5, 8, 18> type M-isozyme [35, 43, 48]; <87> abdominal muscle, PK I [97]) [2, 3, 8, 9, 16, 20-22, 25, 29-31, 33-35, 38, 42, 43, 45-49, 51, 53, 60, 97]
mycelium <50, 51, 56> [1, 24, 68]
pod <40> [11]
promastigote <37> [15]
sarcoma cell <8> (<8> S-type isozyme [51]) [51]
seed <41, 43, 84> [55, 57, 62, 100]
spadix <26> [30]
spleen <5> (<5> K-type isozyme [48]) [48]
Additional information <8, 29, 30> (<8, 29, 30> tissue distribution [21,43]) [21, 43]

Localization

chloroplast <48> (<48> 2 isozymes: a cytosolic and a chloroplastid one [55, 64, 65]) [64, 65]
cytosol <4, 5, 43, 48, 51, 81, 84> (<43, 48> 2 isozymes: a cytosolic and a plastid one [55, 63, 64]) [41, 48, 55, 56, 63, 64, 68, 90, 100, 104]
leucoplast <43> [57, 106]
plastid <43> (<43> 2 isozymes: a cytosolic and a plastid one [55]) [55]

Purification

<1> [12]
<1> (mammalian tissues contain at least 3 distinct and electrophoretically separable kinase isozymes: K-type predominant in fetal tissues, L-type in adult liver and kidney, M-type in brain, skeletal and cardiac muscle [42]) [42]
<3> [33]
<4> [40, 41]
<5> (K4- and M4-isozymes and a hybrid form [48]) [48]
<6> [6, 17, 60, 96]
<7> [60]
<8> (multiple isozymes [43]) [43, 50-52, 60]
<9> [14]
<10> [9]
<11> (major of 5 liver isozymes [38]) [38]
<12> [27]
<13> [25]
<15> [3]
<18> (2 isozymes: type M and type K [36]) [36]
<19> (2 forms, PK I and II) [18, 26]
<21> [7]
<22> (aerobic and anoxic variants, i.e. isozymes PK-aerobic and PK-anoxic) [53, 54]
<23> [20]
<24> (partial [16]) [16]
<25> [28]
<26> (partial [30]) [30]
<28> [19]
<29> (posterior adductor muscle [21]) [21]
<30> (partial [29]) [29]
<34> (2 isozymes, in flight muscle and fat body [22]) [22]
<36> (partial, 3 enzyme types: I, II and III [13]) [13]
<37> [15]
<38> (partial [66]) [66]
<40> (partial [11]) [11]
<43> (partial, several isozymes [55]) [55, 56, 104]
<44> (partial, 2 isoforms, separable by blue-agarose chromatography [58]) [58]
<45> (partial [4]) [4]
<46> (partial [59]) [59]
<48> (partial [63]; 2 immunologically unrelated isoforms, PK1 and 2, separable by anion exchange chromatography [63]; isozyme PKp [65]) [63, 65]
<51> (affinity chromatography on Mikacion brilliant yellow 6 GS-Sepharose CL-4B [68]) [68]
<52> [10]
<53> (two of 5 electrophoretic isoforms: PK1 and PK5 [69]) [69]
<56> (partial [24]) [24]
<58> [39, 60]

<59> [60]
<60> (partial, dye-ligand chromatography [70]) [70]
<64> (partial [37]) [37]
<65> (no isozymes [72]) [72]
<68> (2 non-interconvertible forms, isozyme PK I: at room temperature [45]) [45]
<69> (at room temperature, partial [73]) [73]
<70> [74]
<73> (2 isoforms, I and II [76]) [76]
<74> [44]
<75> [77]
<76> (at 45-50°C [78]) [78]
<79> (affinity chromatography [79]) [79]
<80> [89]
<81> [90, 111]
<82> [91]
<83> (partial [113]) [113]
<84> [100]
<86> [95]
<90> (partial [99]) [99]
<93> [103]
<94> [107]
<99> [112]
<43, 44, 48, 81, 83, 84> (overview [92]) [92]
<5-8, 11, 14, 17, 20, 27, 31, 38, 47, 49, 54, 59, 58, 62, 63, 66-68, 77> (partially purified enzyme preparations from various organisms: overview [60]) [60]

Renaturation

<10> (denaturation at 4 M guanidine chloride reversible by dilution at 20°C in a buffer containing 10 mM phosphoenolpyruvate and 1 mM L-Val, first order kinetics, renaturated catalytically active enzyme is a dimer [9]) [9]
<69> (the enzyme cannot be renatured [73]) [73]

Crystallization

<2> (skeletal muscle [46,47]; M1-isozyme, structure [46]; 3-dimensional structure of M-type isozyme [47]) [46, 47]
<7> (high-resolution X-ray analysis, EPR-measurements [8]) [8]
<8> (liver L-type isozyme, erythrocytic R-type isozyme, M1- and M2-type isozymes [43]) [43, 60]
<58> [60]
<68> [115]
<100> [114]

Cloning

<39> [67]
<50> [1]
<61> [71]
<80> [89]

<88> [98]
<89> [98]
<92> [102]

Engineering

F463V <38> (<38> reduced affinity for D-fructose 1,6-diphosphate and D-fructose 2,6-diphosphate [94]) [94]
R22G <38> (<38> strongly reduced affinity for D-fructose 1,6-diphosphate and fructose 2,6-diphosphate [94]) [94]
R510Q <4> (<4> similar kinetics as wild type, but dramatically decreased stability toward heat, more susceptible to ATP inhibition [105]) [105]
T298A <58> (<58> mutation of the proton donor, mutant is enzymatically active, with decrease in k_{cat}, K_m, altered dissociation constants of ligands [108]) [108]
T298S <58> (<58> mutation of the proton donor, mutant is enzymatically active, with decrease in k_{cat}, K_m, altered dissociation constants of ligands [108]) [108]
Additional information <43> (<43> various N-terminal deletions and chimeric fusions to examine translocation signlaing mechanism [106]) [106]

Application

medicine <4> (<4> mutation R510Q causes nonspherocytic hemolytic anemia [105]) [105]

6 Stability

pH-Stability

5 <65> (<65> below, inactivation within a few days, 4°C [72]) [72]
6.5 <69> (<69> stable at [73]) [73]
7-8 <73> (<73> at 25°C and 37°C, stable [76]) [76]

Temperature stability

20 <69> (<69> most stable at [73]) [73]
25 <43, 65, 73> (<43> in N-tris[hydroxymethyl]methyl-2-aminoethane-sulfonic acid buffer, pH 7.9, 1% glycerol, 0.04 mM DTT, isozyme PKp: $t_{1/2}$: 1.5 min, ADP and $MgCl_2$ preserve activity over 10 min, isozyme PKc: at least 30 min [55]; <65> in 25 mM Tris-HCl buffer, pH 7.5, 50 mM KCl, 5 mM $MgCl_2$, 50% ethylene glycol, inactivation within a few days [72]; <73> at pH 7-8, stable [76]) [55, 72, 76]
30 <70> (<70> up to, in 50 mM HEPES-potassium hydroxide buffer, pH 7.5, 0.1 M mercaptoethanol, 20% v/v glycerol, 30 min stable [74]) [74]
37 <73> (<73> at pH 7-8, stable [76]) [76]
40 <15, 76> (<15> 30 min, imidazole buffer stabilizes [3]; <76> negligible activity below, reversible at room temperature, irreversible at freezing temperatures [78]) [3, 78]
50 <48, 69> (<48> 3 min, isozyme PK1: 34% loss of activity, isozyme PK2: stable [63]; <69> 45 min, 15% loss of activity [73]) [63, 73]

55 <43, 68, 73, 99> (<68> in 5 mM phosphate buffer, pH 7.5, isozyme PKI: $t_{1/2}$: more than 1 h, isozyme PKII: $t_{1/2}$: 2 min [45]; <73> 15 min, 12% loss of activity, isozyme I, inactivation of isozyme II [76]; <43> 5 min, 10% loss of activity [104]; <99> 60 min, no loss of activity [112]) [45, 76, 104, 112]
60 <48, 69> (<69> $t_{1/2}$: 10 min [73]; <48> 3 min, 26% loss of activity, isozyme PK2 [63]) [63, 73]
65 <65> (<65> above, rapid inactivation, in the presence of 10 mM $MgCl_2$: $t_{1/2}$: more than 30 min, 50 mM KCl protects slightly, not phosphoenolpyruvate [72]) [72]
65 <81> (<81> 3 min, stable [90]) [90]
70 <65, 70, 76> (<65> 5 min, inactivation, $t_{1/2}$: 1 min [72]; <70> 30 min, in 50 mM HEPES-potassium hydroxide buffer, pH 7.5, 0.1 M 2-mercaptoethanol, 20% v/v glycerol, 85% loss of activity [74]; <76> 30 min stable [78]) [72, 74, 78]
90 <76> (<76> 10 min stable [78]) [78]
Additional information <44, 45, 58, 69, 92> (<45> heat-stable enzyme [4]; <44, 58, 69> cold-labile enzyme [39, 58, 73]; <58> D-fructose 1,6-diphosphate enhances rate of inactivation, 25% glycerol and/or KCl and $MgCl_2$ protect [39]; <92> no loss with one cycle of freezing and thawing [102]) [4, 39, 58, 73, 102]

General stability information

<8>, D-fructose 1,6-diphosphate and bovine serum albumin stabilize [43]
<8>, phosphate buffer, $MgSO_4$, 2-mercaptoethanol, D-fructose 1,6-diphosphate stabilize during purification at room temperature [43]
<11>, glycerol, 50%, stabilizes [38]
<22>, 0.5 M sucrose and 0.1 M KCl stabilize [54]
<38>, glycerol stabilizes during purification [66]
<38>, unstable in aqueous solution, thiol protecting agents and glycerol stabilize [66]
<43>, cytosolic isozyme is much more stable than plastid isozyme, dithiothreitol plus glycerol stabilize plastid isozyme PKp during purification, isozyme PKp requires dithiothreitol or 2-mercaptoethanol during purification, cytosolic isozyme PKc is stable to dilution into 50 mM N-tris[hydroxymethyl]methyl-2-aminoethane-sulfonic acid buffer, pH 7.9 [55]
<43>, even in the presence of glycerol or ethylene glycol, Mg^{2+} and dithioerythritol, at protein concentrations below 0.4 mg/ml, the enzyme is very unstable during extraction and purification [56]
<60>, dialysis against 10 mM sodium phosphate or Tris-HCl, pH 7.5, for 18 h at 4°C, inactivates [70]
<64>, $MgATP^{2-}$, NAD^+, NADH, 50% glycerol or sucrose partially stabilizes [37]
<64>, labile enzyme is stabilized in the active state throughout purification by avoiding extensive dilution to less than 40 mg protein/ml, and by inclusion of a stabilizing ligand mixture of phosphoenolpyruvate, phosphate and Mg^{2+} in supporting buffers if dilution is unavoidable [37]

<69>, highly unstable at less than 3 M sodium or potassium chloride, cannot be renatured [73]
<69>, optimal stability at pH 6.5 and room temperature [73]
<70>, 0.1 M KCl stabilizes enzyme in dilute solutions [74]
<75>, dialysis for 20 h against 40 mM Tris-HCl, Tris-maleate or morpholinepropanesulfonic acid-NaOH buffer, pH 7, inactivates, but stable to dialysis against phosphate buffer, pH 7 [77]
<75>, phosphate restores activity of dialyzed, partially purified, not fully purified, enzyme preparations [77]
<76>, freezing inactivates [78]

Storage stability

<4>, -70°C, enzyme precipitate in ammonium sulfate solution, several years [41]
<8>, -15°C, in 20 mM Tris-HCl, pH 6.8, 1 mM $MgCl_2$, 0.1 mM dithiothreitol, 2 months [52]
<22>, 4°C, in 25 mM imidazole-HCl buffer, pH 7.1, 10 mM 2-mercaptoethanol, 1 mM EDTA, 0.5 M sucrose and 0.04% NaN_3, concentrated by dialysis against solid polyethylene glycol, at least 4 weeks, isozyme PK-aerobic [53, 54]
<38>, 4°C, lyophilized lyzed trypanosomes, 3 h after resuspension, 20% loss of activity, storage in aqueous solution leads to a decrease in activity and cooperative nature of phosphoenolpyruvate-enzyme interaction [66]
<38>, glycerol, 25% w/v, or 0.1 mM dithiothreitol stabilizes, more effective under refrigerated conditions than at room temperature, KCl, $MgSO_4$, EDTA, phosphoenolpyruvate, ADP or $(NH_4)_2SO_4$ decreases stability at either 4°C or 17°C [66]
<43>, 5°C, isozyme PKp in 50 mM phosphate buffer, pH 7, 50%, v/v, glycerol, 2 mM dithiothreitol, several months and isozyme PKc in 50 mM phosphate buffer, pH 7, at least 1 week [55]
<44>, -20°C, in the absence of substrates, 70% loss of activity within 1 month, phosphoenolpyruvate reduces this loss somewhat, ADP increases it [58]
<44>, -80°C, in the absence of substrates, 95% loss of activity within 1 month, phosphoenolpyruvate reduces this loss somewhat, ADP increases it [58]
<44>, 4°C, in the absence of substrates, $t_{1/2}$: 1 month [58]
<44>, 8°C, isozyme PKI in the absence of substrates, less than 20% loss of activity within 1 month and isozyme PKII with $t_{1/2}$ of 5 days, 25% loss within 1 month in the presence of phosphoenolpyruvate or 60% loss of activity in the presence of ADP [58]
<58>, 2-4°C, in 90% saturated ammonium sulfate, at least 1 month [39]
<60>, -20°C, in 10 mM Tris-HCl or sodium phosphate buffer, pH 7.5, 50% v/v glycerol, 18 h [70]
<60>, 4°C, in 10 mM Tris-HCl or sodium phosphate buffer, pH 7.5, 50% v/v glycerol, 18 h [70]
<60>, glycerol stabilizes during storage [70]

<64>, -20°C, in potassium phosphate buffer, pH 7, $MgCl_2$, phosphoenolpyruvate and glycerol, 5 months [37]
<64>, 4°C, ammonium sulfate preparation, at least 5 months [37]
<65>, -20°C, in 25 mM Tris-HCl buffer, pH 7.5, 50 mM KCl, 5 mM $MgCl_2$, 50% ethylene glycol, at least 2 years [72]
<65>, 25°C, in 25 mM Tris-HCl buffer, pH 7.5, 50 mM KCl, 5 mM $MgCl_2$, 50% ethylene glycol, inactivation within a few days [72]
<65>, 4°C, in 25 mM Tris-HCl buffer, pH 7.5, 50 mM KCl, 5 mM $MgCl_2$, 50% ethylene glycol, at least 2 years, at pH-values below 5, inactivation within a few days [72]
<68>, 0-4°C, in 10 mM Tris, pH 7.5, 1 mM EDTA, 2 mM 2-mercaptoethanol, 1-10 mg isozyme PK I/ml, up to 9 months, in 10 mM Tris, 1 mM EDTA, 2 mM 2-mercaptoethanol, 0.15 M KCl, pH 8, 0.5-1 mg isozyme PK II/ml, up to 6 months [45]
<68>, K^+ stabilize during storage, NH_4^+ or Na^+ less efficiently [45]
<70>, -20°C, in 50 mM phosphate buffer, pH 7, 100 mM KCl, 2 mM phosphoenolpyruvate, 0.1% 2-mercaptoethanol, 50% v/v glycerol, at least 6 months [74]
<73>, 4°C, in 50 mM Tris-HCl buffer, isozyme I up to 10 days, isozyme II 4 days [76]
<73>, EDTA, 2-mercaptoethanol, dithiothreitol, glycerol and KCl stabilize, being more effective at refrigerated conditions than at room temperature [76]
<73>, frozen, in 50 mM Tris-HCl buffer, isozyme I at least 9 months, isozyme II 4 months [76]
<75>, 4°C, in 40 mM potassium phosphate buffer, pH 7, 10 mM 2-mercaptoethanol, 3 months [77]
<92>, 4°C, in 20 mM Tris-Cl, pH 7.0, 30% glycerol, 200 mM KCl, stable for 1 week [102]
<43, 48>, -80°C, frozen with liquid N_2, several months [56, 63]

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Glucose-1-phosphate phosphodismutase 2.7.1.41

1 Nomenclature

EC number
2.7.1.41

Systematic name
D-glucose-1-phosphate:D-glucose-1-phosphate 6-phosphotransferase

Recommended name
glucose-1-phosphate phosphodismutase

Synonyms
PgcM <4> (<4> bifunctional PgcM with β-phosphoglucomutase, EC 5.4.2.1, and glucose 1-phosphate phosphodismutase activities [3]) [3]
glucose 1-phosphate transphosphorylase
phosphodismutase
phosphodismutase, glucose 1-phosphate

CAS registry number
9026-25-9

2 Source Organism

<1> *Oryctolagus cuniculus* [1]
<2> *Sus scrofa* [2]
<3> *Escherichia coli* [1]
<4> *Bacillus subtilis* (wild-type strain 168 [3]) [3]

3 Reaction and Specificity

Catalyzed reaction
2 D-glucose 1-phosphate = D-glucose + D-glucose 1,6-bisphosphate

Reaction type
phospho group transfer

Natural substrates and products
S Additional information <4> (<4> carbohydrate metabolism [3]) [3]
P ?

Substrates and products

S D-glucose 1-phosphate + D-glucose 1-phosphate <1-4> (<4> PgcM uses the α- and β-forms of glucose 1-phosphate as substrates, higher affinity for the β-form, binding of substrates and cofactors trigger a conformational change of PgcM [3]) (Reversibility: ? <2-4> [1-3]; r <1> [1]) [1-3]

P D-glucose + D-glucose 1,6-bisphosphate <1-4> [1-3]

S Additional information <4> (<4> bifunctional PgcM with β-phosphoglucomutase, EC 5.4.2.1, and glucose 1-phosphate phosphodismutase activities [3]) [3]

P ?

Inhibitors

ATP <1> (<1> inhibits forward reaction above 2 mM [1]) [1]

Cu^{2+} <1> (<1> weak, 0.1 mM [1]) [1]

Mg^{2+} <1> (<1> inhibits reverse reaction at 0.5 mM, forward reaction: activation [1]) [1]

Mn^{2+} <1> (<1> inhibits forward reaction above 0.5 mM [1]) [1]

Additional information <1> (<1> not inhibited by 8 mM fluoride, cysteine, histidine, adenylic acid or phosphate [1]) [1]

Activating compounds

Additional information <1> (<1> forward reaction is not activated by ATP, cysteine, histidine, adenylic acid or phosphate [1]) [1]

Metals, ions

Mg^{2+} <1, 4> (<1> activation of forward reaction, optimal concentration: 1 mM, inhibition of reverse reaction, Mg^{2+} forms a complex with the product glucose-1,6-bisphosphate [1]; <4> $MgCl_2$ as cofactor, optimal concentration: 10-15 mM [3]) [1, 3]

K_m-Value (mM)

0.006 <4> (β-D-glucose 1-phosphate) [3]

0.021 <4> (α-D-glucose 1-phosphate) [3]

pH-Optimum

7.6 <1> (<1> forward reaction, in the presence of Mg^{2+} [1]) [1]

Temperature optimum (°C)

30 <1> [1]

4 Enzyme Structure

Subunits

trimer <4> (<4> 3 * 28000, PgcM with β-phosphoglucomutase and glucose 1-phosphate phosphodismutase activities, estimated from the amino acid sequence data [3]; <4> 3 * 26500, PgcM with β-phosphoglucomutase and glucose 1-phosphate phosphodismutase activities, SDS-PAGE [3]) [3]

5 Isolation/Preparation/Mutation/Application

Source/tissue

muscle <2> (<2> 4 different enzymic activities of D-glucose 1,6-bisphosphate synthesis: in muscle glucose 1-phosphate transphosphorylase activity is the major activity, but not in brain [2]) [2]
skeletal muscle <1> (<1> muscles of back and hind legs [1]) [1]

Localization

soluble <4> (<4> recombinant PgcM with β-phosphoglucomutase and glucose 1-phosphate phosphodismutase activities [3]) [3]

Purification

<1> (partial, not separated from phosphoglucomutase [1]) [1]
<4> (recombinant PgcM expressed in Bacillus megaterium DSM 319 [3]) [3]

Cloning

<4> (pgcM gene, encoding PgcM, a 226 amino acids protein with β-phosphoglucomutase and glucose 1-phosphate phosphodismutase activities, is cloned, sequenced and used to construct a plasmid-based overexpression system for PcgM in Bacillus megaterium DSM 319 [3]) [3]

6 Stability

Temperature stability

95 <4> (<4> 30 min, stable, PgcM with β-phosphoglucomutase and glucose 1-phosphate phosphodismutase activities [3]) [3]

General stability information

<1>, stable to 3-4 h dialysis against distilled water, prolonged or repeated dialysis inactivates [1]

Storage stability

<1>, 6°C, within 1 week, inactivation [1]
<1>, frozen, partially purified preparation, at least 4 weeks, stable [1]

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Riboflavin phosphotransferase 2.7.1.42

1 Nomenclature

EC number
2.7.1.42

Systematic name
α-D-glucose-1-phosphate:riboflavin 5'-phosphotransferase

Recommended name
riboflavin phosphotransferase

Synonyms
G-1-P phosphotransferase
glucose-1-phosphate phosphotransferase
phosphotransferase, riboflavin
riboflavine phosphotransferase

CAS registry number
9026-26-0

2 Source Organism

<1> *Escherichia coli* (neapolitanus, strain B-25 [1]) [1]

3 Reaction and Specificity

Catalyzed reaction
D-glucose 1-phosphate + riboflavin = D-glucose + FMN

Reaction type
phospho group transfer

Natural substrates and products
S α-D-glucose 1-phosphate + riboflavin <1> [1]
P α–glucose + FMN
S Additional information <1> (<1> may be involved in biosynthesis of phosphate compounds that are essential for bacterial growth [1]) [1]
P ?

Substrates and products

- **S** D-glucose 1-phosphate + 6-methyl-9-(D-1'-ribityl)-isoalloxazine <1> (<1> 93% of activity compared to riboflavin [1]) (Reversibility: ? <1> [1]) [1]
- **P** D-glucose + 6-methyl-9-(D-1'-ribityl)-isoalloxazine 5'-phosphate
- **S** D-glucose 1-phosphate + araboflavin <1> (<1> 93% of activity compared to riboflavin [1]) (Reversibility: ? <1> [1]) [1]
- **P** D-glucose + araboflavin 5'-phosphate
- **S** D-glucose 1-phosphate + isoriboflavin <1> (<1> 73% of activity compared to riboflavin [1]) (Reversibility: ? <1> [1]) [1]
- **P** D-glucose + isoriboflavin 5'-phosphate
- **S** α-D-glucose 1-phosphate + riboflavin <1> (Reversibility: ? <1> [1]) [1]
- **P** α-D-glucose + FMN
- **S** β-D-glucose 1-phosphate + riboflavin <1> (<1> about 50% of activity compared to α-glucose 1-phosphate [1]) (Reversibility: ? <1> [1]) [1]
- **P** β-D-glucose + FMN
- **S** Additional information <1> (<1> ATP, diphosphate, adenylate, glucose 6-phosphate, fructose 1,6-diphosphate, riboflavin ketoic acid and hydroxyethylisoalloxazine are no substrates [1]) [1]
- **P** ?

Inhibitors

$(NH_4)_2MoO_4$ <1> (<1> 0.5%: 100% inhibition [1]) [1]
EDTA <1> (<1> 2 mM: 3% inhibition [1]) [1]
KH_2AsO_4 <1> (<1> 5 mM: 25% inhibition [1]) [1]
$KHSO_4$ <1> (<1> 5 mM: 13% inhibition, 50 mM: 100% inhibition [1]) [1]
$MgSO_4$ <1> (<1> 0.1 mM: 7% inhibition [1]) [1]
NaF <1> (<1> 25 mM, 100% inhibition [1]) [1]
D-fructose <1> (<1> 0.2 M: 24% inhibition [1]) [1]
D-glucose <1> (<1> 0.2 M: 84% inhibition [1]) [1]
maltose <1> (<1> 0.2 M: 20% inhibition [1]) [1]

Specific activity (U/mg)

0.12 <1> (<1> pH 5.4, 30°C [1]) [1]

pH-Optimum

5.3 <1> (<1> broad [1]) [1]

pH-Range

4-8 <1> (<1> 75% of maximal activity at pH 4, 20% of maximal activity at pH 8 [1]) [1]

Temperature optimum (°C)

30 <1> (<1> assay at [1]) [1]

5 Isolation/Preparation/Mutation/Application

Purification
<1> (partial [1]) [1]

6 Stability

Storage stability
<1>, 0°C, 1 week, stable [1]

References

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Glucuronokinase 2.7.1.43

1 Nomenclature

EC number
2.7.1.43

Systematic name
ATP:D-glucuronate 1-phosphotransferase

Recommended name
glucuronokinase

Synonyms
glucuronokinase, glucurono-
kinase, glucurono- (phosphorylating)

CAS registry number
9026-62-4

2 Source Organism

<1> *Phaseolus aureus* [1, 3]
<2> *Nicotiana tabacum* [3]
<3> *Lilium longiflorum* [2-4]
<4> *Zea mays* [3]
<5> *Glycine max* [3]

3 Reaction and Specificity

Catalyzed reaction
ATP + D-glucuronate = ADP + 1-phospho-α-D-glucuronate

Reaction type
phospho group transfer

Natural substrates and products
S ATP + D-glucuronate <1-5> [1-4]
P ADP + 1-phospho-D-glucuronate

Substrates and products
S ATP + D-glucuronate <1-5> (Reversibility: r <1> [1]; ? <2-5> [2-4]) [1-4]
P ADP + 1-phospho-D-glucuronate <1> [1]

S ITP + D-glucuronate <3> (<3> 3% of the activity with ATP [2]) (Reversibility: ? <3> [2]) [2]
P IDP + 1-phospho-D-glucuronate
S dATP + D-glucuronate <3> (<3> 3% of the activity with ATP [2]) (Reversibility: ? <3> [2]) [2]
P dADP + 1-phospho-D-glucuronate
S Additional information <1, 3> (<1> no substrate: UTP, GTP, CTP [1]; <3> less than 1% of the activity with ATP: GTP, CTP, UTP, TTP [2]) [1, 2]
P ?

Inhibitors

ADP <3> (<3> competitive to ATP [2]) [2]
AMP <3> (<3> with ATP as substrate [2]) [2]
CDP <3> (<3> with ATP as substrate [2]) [2]
GDP <3> (<3> with ATP as substrate [2]) [2]
GTP <3> (<3> with ATP as substrate [2]) [2]
ITP <3> (<3> with ATP as substrate [2]) [2]
TTP <3> (<3> with ATP as substrate [2]) [2]
UDP <3> (<3> with ATP as substrate [2]) [2]
UDP-D-glucuronate <3> [4]
β-D-glucuronic acid 1-phosphate <3> (<3> 50% inhibition at 1-3 mM [2]) [2, 4]
dATP <3> (<3> with ATP as substrate [2]) [2]

Metals, ions

Co^{2+} <1> (<1> can partially replace Mg^{2+} in activation [1]) [1]
Mg^{2+} <1> (<1> divalent cation required, Mg^{2+} most effective [1]) [1]
Mn^{2+} <1> (<1> can partially replace Mg^{2+} in activation [1]) [1]

Specific activity (U/mg)

0.46 <3> (<3> pH 7.2, 30°C [4]) [4]

K_m-Value (mM)

0.5-0.6 <3> (D-glucuronate, <3> pH 7.2, 30°C [4]) [4]
1.9 <3> (ATP, <3> pH 7.2, 30°C [4]) [4]

K_i-Value (mM)

0.18 <3> (β-D-glucuronic acid 1-phosphate, <3> pH 7.2, 30°C [4]) [4]
0.55 <3> (UDP-D-glucuronate, <3> pH 7.2, 30°C [4]) [4]

Temperature optimum (°C)

30 <3> (<3> assay at [2,4]) [2, 4]
37 <1> (<1> assay at [1]) [1]

5 Isolation/Preparation/Mutation/Application

Source/tissue

callus <1-5> [3]
pollen <1-5> [2-4]
seedling <1-5> [1, 3]

Localization
particle-bound <1> [1]
soluble <1> [1]

Purification
<3> (partial [2,4]) [2, 4]

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[4] Leibowitz, M.D.; Dickinson, D.B.; Loewus, F.A.; Loewus, M.: Partial purification and study of pollen glucuronokinase. Arch. Biochem. Biophys., **179**, 559-564 (1977)

Galacturonokinase 2.7.1.44

1 Nomenclature

EC number
2.7.1.44

Systematic name
ATP:D-galacturonate 1-phosphotransferase

Recommended name
galacturonokinase

Synonyms
D-galacturonic acid kinase
kinase, galacturono- (phosphorylating)

CAS registry number
9026-63-5

2 Source Organism

<1> *Phaseolus aureus* (mung bean [1]) [1]
<2> *Pisum sativum* (pea [1]) [1]
<3> *Fagopyrum esculentum* (buckwheat [1]) [1]
<4> *Raphanus sativus* (radish [1]) [1]
<5> *Petroselinum hortense* (parsley [1]) [1]

3 Reaction and Specificity

Catalyzed reaction
ATP + D-galacturonate = ADP + 1-phospho-α-D-galacturonate

Reaction type
phospho group transfer

Natural substrates and products
S ATP + D-galacturonate <1-5> (<1-5> involved in formation of pectin [1]) [1]
P ADP + 1-phospho-α-D-galacturonate

Substrates and products
S ATP + D-galacturonate <1-5> (Reversibility: ? <1-5> [1]) [1]
P ADP + 1-phospho-α-D-galacturonate <1-5> [1]

S Additional information <1-5> (<1-5> D-glucuronic acid, D-galactose and L-arabinose are no substrates, ATP cannot be replaced by other nucleotides [1]) [1]
P ?

Metals, ions
Co^{2+} <1-5> (<1-5> divalent metal ion is required, lower activity than Mg^{2+} or Mn^{2+} [1]) [1]
Mg^{2+} <1-5> (<1-5> divalent metal ion is required, Zn^{2+} or Ni^{2+} are ineffective [1]) [1]
Mn^{2+} <1-5> (<1-5> divalent metal ion is required, higher activity than Mg^{2+}. Zn^{2+} or Ni^{2+} are ineffective [1]) [1]

Temperature optimum (°C)
37 <1-5> (<1-5> assay at [1]) [1]

5 Isolation/Preparation/Mutation/Application

Source/tissue
leaf <5> [1]
root <4> [1]
seed <1-3> (germinating [1]) [1]

Purification
<1> (partial [1]) [1]
<2> (partial [1]) [1]
<3> (partial [1]) [1]
<4> (partial [1]) [1]
<5> (partial [1]) [1]

6 Stability

General stability information
<1-5>, mercaptoethanol, 0.05 M, is necessary for preservation of activity in dialysis buffer, lowering its concentration to 0.01 M reduces enzyme activity by 30 to 50%, omission of mercaptoethanol results in complete loss of activity [1]

Storage stability
<1-5>, 4°C, 0.05 M mercaptoethanol, stable up to 10 days [1]

References

[1] Neufeld, E.F.; Feingold, D.S.; Ilves, S.M.; Kessler, G.; Hassid, W.Z.: Phosphorylation of D-galacturonic acid by extracts from germinating seeds of Phaseolus aureus. J. Biol. Chem., **236**, 3102-3105 (1961)

2-Dehydro-3-deoxygluconokinase 2.7.1.45

1 Nomenclature

EC number
2.7.1.45

Systematic name
ATP:2-dehydro-3-deoxy-D-gluconate 6-phosphotransferase

Recommended name
2-dehydro-3-deoxygluconokinase

Synonyms
2-keto-3-deoxy-D-gluconic acid kinase
2-keto-3-deoxygluconate kinase
2-keto-3-deoxygluconokinase
ketodeoxygluconokinase
kinase, 2-keto-3-deoxyglucono- (phosphorylating)

CAS registry number
9026-54-4

2 Source Organism

<-3> no activity in *Azotobacter agilis* (grown on glucose) [1]
<-2> no activity in *Neurospora crassa* [1]
<-1> no activity in *Salmonella typhimurium* [1]
<1> *Escherichia coli* (K-12 [2]) [1, 2]
<2> *Aerobacter aerogenes* [1]

3 Reaction and Specificity

Catalyzed reaction
ATP + 2-dehydro-3-deoxy-D-gluconate = ADP + 6-phospho-2-dehydro-3-deoxy-D-gluconate

Reaction type
phospho group transfer

Natural substrates and products

S ATP + 2-dehydro-3-deoxy-D-gluconate <1> (<1> enzyme is induced by galacturonate or glucuronate and is controlled by a single structural gene [2]) [2]
P ADP + 6-phospho-2-dehydro-3-deoxy-D-gluconate

Substrates and products

S ATP + 2-dehydro-3-deoxy-D-gluconate <1, 2> (Reversibility: ? <1,2> [1,2]) [1, 2]
P ADP + 6-phospho-2-dehydro-3-deoxy-D-gluconate <1, 2> [1]
S CTP + 2-dehydro-3-deoxy-D-gluconate <1, 2> (<1,2> 8% of the activity with ATP [1]) (Reversibility: ? <1,2> [1]) [1]
P CDP + 6-phospho-2-dehydro-3-deoxy-D-gluconate
S GTP + 2-dehydro-3-deoxy-D-gluconate <1, 2> (<1,2> 34% of the activity with ATP [1]) (Reversibility: ? <1,2> [1]) [1]
P GDP + 6-phospho-2-dehydro-3-deoxy-D-gluconate
S ITP + 2-dehydro-3-deoxy-D-gluconate <1, 2> (<1,2> 75% of the activity with ATP [1]) (Reversibility: ? <1,2> [1]) [1]
P IDP + 6-phospho-2-dehydro-3-deoxy-D-gluconate
S UTP + 2-dehydro-3-deoxy-D-gluconate <1, 2> (<1,2> 7% of the activity with ATP [1]) (Reversibility: ? <1,2> [1]) [1]
P UDP + 6-phospho-2-dehydro-3-deoxy-D-gluconate
S Additional information <1> (<1> no substrate: 2-deoxy-D-ribose, 2-deoxy-D-glucose, D-gluconate, 2-keto-D-gluconate, 2-oxoglutarate, 2-oxo-3-deoxy-6-phosphogluconate [2]) [2]
P ?

Inhibitors

Mn^{2+} <1> (<1> divalent cation required, most effective at a ratio of Mn^{2+} and ATP of 1:3, deviation from this ratio is inhibitory at several concentration levels [1]) [1]

Metals, ions

Co^{2+} <1> (<1> divalent cation required [1]) [1]
Mg^{2+} <1> (<1> divalent cation required [1]) [1]
Mn^{2+} <1> (<1> divalent cation required, most effective at a ratio of Mn^{2+} and ATP of 1:3, deviation from this ratio is inhibitory at several concentration levels [1]) [1]

Specific activity (U/mg)

Additional information <1> (<1> assay method [1]) [1]

K_m-Value (mM)

1 <1> (2-dehydro-3-deoxy-D-gluconate) [1, 2]

pH-Optimum

5.6-6 <1> (<1> acetate buffer [1]) [1]
6 <1> [2]

pH-Range

4.7-8 <1> (<1> pH 4.7: about 50% of maximum activity, pH 8: about 85% of maximum activity, acetate buffer [1]) [1]

5 Isolation/Preparation/Mutation/Application

Purification

<1> [1, 2]

Cloning

<1> [2]

6 Stability

Temperature stability

52 <1> (<1> half-life: 6-7 min [2]) [2]

Storage stability

<1>, -15°C, retains more than 50% of activity after 2 weeks [1]

References

[1] Cynkin, M.A.; Ashwell, G.: Uronic acid metabolism in bacteria: IV. Purification and properties of 2-keto-3-deoxy-D-gluconokinase in Escherichia coli. J. Biol. Chem., **235**, 1576-1579 (1969)

[2] Pouyssegur, J.; Stoeber, F.: Study of the common degradative pathway of hexuronates in Escherichia coli K 12. Purification, properties and individuality of 2-keto-3-deoxy-D-gluconokinase. Biochimie, **53**, 771-781 (1971)

L-Arabinokinase 2.7.1.46

1 Nomenclature

EC number
2.7.1.46

Systematic name
ATP:L-arabinose 1-phosphotransferase

Recommended name
L-arabinokinase

Synonyms
kinase (phosphorylating), L-arabino-
kinase, L-arabino- (phosphorylating)

CAS registry number
37277-99-9

2 Source Organism

<1> *Phaseolus aureus* [1, 2]
<2> *Arabidopsis thaliana* (wild type and arabinose-deficient mutant [3]) [3, 4]

3 Reaction and Specificity

Catalyzed reaction
ATP + L-arabinose = ADP + β-L-arabinose 1-phosphate

Reaction type
phospho group transfer

Natural substrates and products
S ATP + L-arabinose <1, 2> (<2> arabinose salvage pathway is not essential for normal growth [3]) (Reversibility: ? <1, 2> [1, 3]) [1, 3]
P ADP + β-L-arabinose 1-phosphate <1> [1]

Substrates and products
S ATP + L-arabinose <1, 2> (Reversibility: ? <1,2> [1-4]) [1-4]
P ADP + β-L-arabinose 1-phosphate <1> [1]

Specific activity (U/mg)
1.44 <1> [2]

5 Isolation/Preparation/Mutation/Application

Source/tissue
seed <2> [3]
seedling <1> [1, 2]

Localization
membrane <1> [2]

Purification
<1> [2]

Cloning
<2> (expression in Escherichia coli [4]) [4]

6 Stability

Temperature stability
4 <1> (<1> 24 h, complete loss of activity [2]) [2]

General stability information
<1>, no stabilization by substrates, higher ionic strength, glycerol, EDTA, different pH values or other stabilizing methods [2]

Storage stability
<1>, 4°C, 24 h, complete loss of activity, no stabilization with substrates, higher ionic strength, glycerol, EDTA, differnt pH values or other stabilizing methods [2]

References

[1] Neufeld, E.F.; Feingold, D.S.; Hassid, W.Z.: Phosphorylation of D-galactose and L-arabinose by extracts from Phaseolus aureus seedlings. J. Biol. Chem., **235**, 906-909 (1960)
[2] Hoo Chan, P.; Hassid, W.Z.: One step purification of D-galactose and L-arabinose kinases from Phaseolus aureus seedlings by ATP-sepharose affinity chromatography. Anal. Biochem., **64**, 372-379 (1975)
[3] Dolezal, O.; Cobbett, C.S.: Arabinose kinase-deficient mutant of Arabidopsis thaliana. Plant Physiol., **96**, 1255-1260 (1991)
[4] Sherson, S.; Gy, I.; Medd, J.; Schmidt, R.; Dean, C.; Kreis, M.; Lecharny, A.; Cobbett, C.: The arabinose kinase, ARA1, gene of Arabidopsis is a novel member of the galactose kinase gene family. Plant Mol. Biol., **39**, 1003-1012 (1999)

D-Ribulokinase 2.7.1.47

1 Nomenclature

EC number
2.7.1.47

Systematic name
ATP:D-ribulose 5-phosphotransferase

Recommended name
D-ribulokinase

Synonyms
kinase (phosphorylating), D-ribulo-
kinase, D-ribulo- (phosphorylating)
Additional information (cf. EC 2.7.1.16)

CAS registry number
9026-40-8

2 Source Organism

<1> *Aerobacter aerogenes* (mutant, constitutive for D-ribulokinase [2]) [1, 2]
<2> *Klebsiella aerogenes* (expressed as constitutive enzyme in Escherichia coli K12 construct strain NC629) [3]

3 Reaction and Specificity

Catalyzed reaction
ATP + D-ribulose = ADP + D-ribulose 5-phosphate (<1> random bi bi reaction mechanism [2])

Reaction type
phospho group transfer

Natural substrates and products
S ATP + D-ribulose <1> (<1> inducible enzyme of ribitol catabolism [1,2]) (Reversibility: ? <1> [1, 2]) [1, 2]
P ?

Substrates and products

S ATP + D-arabitol <2> (<2> poor substrate, not L-isomer [3]) (Reversibility: ? <2> [3]) [3]
P ?
S ATP + D-ribulose <1, 2> (<1> specific for D-isomer [1]; <1> ATP cannot be replaced by ITP, GTP or ADP [1]) (Reversibility: ? <1,2> [1-3]) [1-3]
P ADP + D-ribulose 5-phosphate <1, 2> [1-3]
S ATP + ribitol <2> (<2> poor substrate [3]) [3]
P ?

Inhibitors

ADP <1> (<1> product inhibition [2]) [2]
Mg^{2+} <1> (<1> inhibition at a Mg/ATP ratio above 2, 11% inhibition at a ratio of 3 [1]) [1]
Additional information <1> (<1> no inhibition by D-ribulose 5-phosphate [2]) [2]

Activating compounds

2-mercaptoethanol <1> (<1> activation [1,2]) [1, 2]

Metals, ions

Ca^{2+} <1> (<1> activation, can replace Mg^{2+} with 23% efficiency [1]) [1]
Mg^{2+} <1> (<1> requirement [2]; <1> optimum Mg/ATP-ratio: 1-2, inhibits at higher values [1]) [1, 2]
Mn^{2+} <1> (<1> activation, can replace Mg^{2+} with 54% efficiency [1]) [1]

Specific activity (U/mg)

84 <2> [3]
152 <1> [2]
Additional information <1> [1]

K_m-Value (mM)

0.4 <2> (D-ribulose, <2>, pH 7.8, 28°C [3]) [3]
0.83 <1> (ATP, <1> pH 7.7, 27°C [1]) [1]
0.92 <1> (D-ribulose, <1> pH 7.7, 37°C [1]) [1]
140 <2> (D-arabitol, <2>, pH 7.8, 28°C [3]) [3]
220 <2> (ribitol, <2>, pH 7.8, 28°C [3]) [3]
Additional information <1> (<1> kinetic study at 28°C [2]) [2]

pH-Optimum

7-9.5 <1> (<1> broad [1]) [1]

pH-Range

6-10.5 <1> (<1> pH 6.0: about 70% of maximal activity, pH 10.5: about half-maximal activity at pH 10.5 [1]) [1]

4 Enzyme Structure

Molecular weight

112000 <2> (<2> recombinant enzyme expressed in E. coli strain NC629, gel filtration [3]) [3]
116000 <1> (<1> gel filtration [2]) [2]

Subunits

dimer <1, 2> (<1> 2 * 59000, SDS-PAGE [2]; <2> 2 * 60000, recombinant enzyme expressed in E. coli strain NC629, SDS-PAGE [3]) [2, 3]

5 Isolation/Preparation/Mutation/Application

Purification

<1> (partial [1]) [1, 2]
<2> [3]

Cloning

<2> (expression in Escherichia coli K12 strain NC629 harbouring multi-copy λprbt-101 prophage which carries the rbt-operon [3]) [3]

6 Stability

General stability information

<1>, D-ribulose stabilizes [2]
<1>, EDTA or 2-mercaptoethanol stabilizes during purification [2]

Storage stability

<1>, 4°C, about 10% loss of activity within 6 months [2]
<2>, -20°C, in 50% v/v glycerol, in the dark, 1 month [3]

References

[1] Fromm, H.J.: D-Ribulokinase from Aerobacter aerogenes. J. Biol. Chem., **234**, 3097-3101 (1959)
[2] Stayton, M.M.; Fromm, H.J.: Purification, properties, and kinetics of D-ribulokinase from Aerobacter aerogenes. J. Biol. Chem., **254**, 3765-3771 (1979)
[3] Neuberger, M.S.; Hartley, B.S.; Walker, J.E.: Purification and properties of D-ribulokinase and D-xylulokinase from Klebsiella aerogenes. Biochem. J., **193**, 513-524 (1981)

Uridine kinase 2.7.1.48

1 Nomenclature

EC number
2.7.1.48

Systematic name
ATP:uridine 5'-phosphotransferase

Recommended name
uridine kinase

Synonyms
UCK1 <8> [18]
UCK2 <9> [18]
kinase, uridine (phosphorylating)
pyrimidine ribonucleoside kinase
uridine phosphokinase
uridine-cytidine kinase <5> [9]

CAS registry number
9026-39-5

2 Source Organism

<1> *Escherichia coli* [7, 17]
<2> *Bos taurus* (calf [14,15]) [14, 15]
<3> *Mus musculus* (several isoenzymes [3]) [1, 3, 5, 8, 10, 12, 13, 17]
<4> *Bacillus stearothermophilus* [2, 6]
<5> *Rattus norvegicus* (2 forms of liver uridine kinase in foetus [11]) [2, 6, 9, 11, 16]
<6> *Homo sapiens* [4, 16, 17]
<7> *Saccharomyces cerevisiae* [17]
<8> *Homo sapiens* (uridine-cytidine kinase 1 [18]) [18]
<9> *Homo sapiens* (uridine-cytidine kinase 2 [18]) [18, 19]

3 Reaction and Specificity

Catalyzed reaction

ATP + uridine = ADP + UMP (<3> sequential mechanism [10]; <4,5> ping-pong mechanism [6,9]; <3> sequential addition of both substrates to the enzyme to form a ternary complex [13])

Reaction type

phospho group transfer

Natural substrates and products

S ATP + uridine <3-5> (<3> rate-limiting enzyme in anabolism of uridine and cytidine [10]; <3> rate limiting enzyme of pyrimidine salvage pathway [10]; <4,5> first enzyme of pyrimidine salvage pathway [6]; <3> enzyme is part of the anabolic pathway by which the preformed pyrimidine nucleosides are salvaged for nucleic acid biosynthesis [8]) (Reversibility: ? <3-6> [6, 8, 10]) [6, 8, 10]

P ADP + UMP <3-5> [6, 8, 10]

Substrates and products

S ATP + 2-thiocytidine <8, 9> (<8> recombinant uridine kinase 1, 20% of activity with uridine [18]; <9> recombinant uridine kinase 2, 86% of activity with uridine [18]) (Reversibility: ? <8,9> [18]) [18]

P ADP + 2-thiocytidine 5'-monophosphate <8, 9> [18]

S ATP + 3'-C-ethynylcytidine <6> (<6> recombinant uridine kinase, 20% of activity with uridine [17]) (Reversibility: ? <6> [17]) [17]

P ADP + 3'-C-ethynylcytidine 5'-monophosphate <6> [17]

S ATP + 3'-C-ethynyluridine <6> (<6> recombinant uridine kinase, 20% of activity with uridine [17]) (Reversibility: ? <6> [17]) [17]

P ADP + 3'-C-ethynyluridine 5'-monophosphate <6> [17]

S ATP + 3,4,5,6-tetrahydrouridine <8, 9> (<8> recombinant uridine kinase1, 13% of activity with uridine [18]; <9> recombinant uridine kinase 2, 81% of activity with uridine [18]) (Reversibility: ? <8,9> [18]) [18]

P ADP + 3,4,5,6-tetrahydrouridine 5'-monophosphate <8, 9> [18]

S ATP + 3-deazauridine <3, 9> (<9> recombinant uridine kinase 2, 49% of activity with uridine [18]) (Reversibility: ? <3,9> [10,18]) [10, 18]

P ADP + 3-deazauridine 5'-monophosphate <3, 9> [10, 18]

S ATP + 3-methyluridine <9> (<9> recombinant uridine kinase 2, 18% of activity with uridine [18]) (Reversibility: ? <9> [18]) [18]

P ADP + 3-methyluridine 5'-monophosphate <9> [18]

S ATP + 4-thiouridine <8, 9> (<8> recombinant uridine kinase 1, 122% of activity with uridine [18]; <9> recombinant uridine kinase 2, 125% of activity with uridine [18]) (Reversibility: ? <8,9> [18]) [18]

P ADP + 4-thiouridine 5'-monophosphate <8, 9> [18]

S ATP + 5-azacytidine <3, 6> (<3> 39.1% of the activity with uridine [12]) (Reversibility: ? <3,6> [4,8,12]) [4, 8, 12]

P ADP + 5-azacytidine 5'-monophosphate <3, 6> [4, 8, 12]

S ATP + 5-bromouridine <8, 9> (<8> recombinant uridine kinase 1, 50% of activity with uridine [18]; <9> recombinant uridine kinase 2,6% of activity with uridine [18]) (Reversibility: ? <8,9> [18]) [18]
P ADP + 5-bromouridine 5'-monophosphate <8, 9> [18]
S ATP + 5-fluorocytidine <3> (Reversibility: ? <3> [1]) [1]
P ADP + 5-fluorocytidine 5'-monophosphate <3> [1]
S ATP + 5-fluorocytidine <8, 9> (<8> recombinant uridine kinase 1, 37% of activity with uridine [18]; <9> recombinant uridine kinase 2, 184% of activity with uridine [18]) (Reversibility: ? <8,9> [18]) [18]
P ADP + 5-fluorocytidine 5'-monophosphate <8, 9> [18]
S ATP + 5-fluorouridine <3, 6, 9> (<3> 66.9% of the activity with uridine [12]; <6> recombinant uridine kinase [17]; <9> recombinant uridine kinase 2, 89% of activity with uridine [18]) (Reversibility: ? <3, 6, 9> [1, 8, 10, 12, 17, 18]) [1, 8, 10, 12, 17, 18]
P ADP + 5-fluorouridine 5'-monophosphate <3, 6, 9> [1, 8, 10, 12, 17, 18]
S ATP + 5-hydroxyuridine <8, 9> (<8> recombinant uridine kinase 1, 14% of activity with uridine [18]; <9> recombinant uridine kinase 2, 12% of activity with uridine [18]) (Reversibility: ? <8,9> [18]) [18]
P ADP + 5-hydroxyuridine 5'-monophosphate <8, 9> [18]
S ATP + 5-methoxyuridine <8, 9> (<8> recombinant uridine kinase 1, 122% of activity with uridine [18]; <9> recombinant uridine kinase 2, 92% of activity with uridine [18]) (Reversibility: ? <8,9> [18]) [18]
P ADP + 5-methoxyuridine 5'-monophosphate <8, 9> [18]
S ATP + 5-methylcytidine <8, 9> (<8> recombinant uridine kinase 1, 9% of activity with uridine [18]; <9> recombinant uridine kinase 2, 30% of activity with uridine [18]) (Reversibility: ? <8,9> [18]) [18]
P ADP + 5-methylcytidine 5'-monophosphate <8, 9> [18]
S ATP + 6-azacytidine <3, 9> (<3> 60.4% of the activity with uridine [12]; <9> recombinant uridine kinase 2, 75% of activity with uridine [18]) (Reversibility: ? <3,9> [8,12,18]) [8, 12, 18]
P ADP + 6-azacytidine 5'-monophosphate <3, 9> [8, 12, 18]
S ATP + 6-azauridine <2, 3, 8, 9> (<3> 65.5% of the activity with uridine [12]; <8,9> recombinant uridine kinase 1, 38% of activity with uridine, recombinant uridine kinase 2, 148% of activity with uridine [18]) (Reversibility: ? <2,3,8,9> [1,8,10,12,14,18]) [1, 8, 10, 12, 14, 18]
P ADP + 6-azauridine 5'-monophosphate <2, 3, 8, 9> [1, 8, 10, 12, 14, 18]
S ATP + N^4-acetylcytidine <8, 9> (<8> recombinant uridine kinase 1, 142% of activity with uridine [18]; <9> recombinant uridine kinase 2, 84% of activity with uridine [18]) (Reversibility: ? <8,9> [18]) [18]
P ADP + N^4-acetylcytidine 5'-monophosphate <8, 9> [18]
S ATP + N^4-aminocytidine <8> (<8> recombinant uridine kinase 1, 20% of activity with uridine [18]) (Reversibility: ? <8> [18]) [18]
P ADP + N^4-acetylcytidine 5'-monophosphate <8> [18]
S ATP + N^4-anisoylcytidine <8, 9> (<8> recombinant uridine kinase 1, 20% of activity with uridine [18]; <9> recombinant uridine kinase 2, 12% of activity with uridine [18]) (Reversibility: ? <8,9> [18]) [18]
P ADP + N^4-anisoylcytidine 5'-monophosphate <8, 9> [18]

S ATP + N^4-benzoylcytidine <8, 9> (<8> recombinant uridine kinase 1, 68% of activity with uridine [18]; <9> recombinant uridine kinase 2, 51% of activity with uridine [18]) (Reversibility: ? <8,9> [18]) [18]
P ADP + N^4-benzoylcytidine 5'-monophosphate <8, 9> [18]
S ATP + cyclopentenylcytosine <6> (<6> recombinant uridine kinase, 12% of activity with uridine [17]) (Reversibility: ? <6> [17]) [17]
P ADP + cyclopentenylcytosine 5'-monophosphate <6> [17]
S ATP + cytidine <1, 3, 6, 8, 9> (<3> 35.6% of the activity with uridine [12]; <3> 50% of activity with uridine [8,12]) (Reversibility: ? <1, 3, 6, 8, 9> [1, 3, 4, 7, 8, 12, 17, 18]) [1, 3, 4, 7, 8, 12, 17, 18]
P ADP + CMP <1, 3, 6, 8, 9> [1, 3, 4, 7, 8, 12, 17, 18]
S ATP + uridine <1-6, 8, 9> (<3> ATP most effective as phosphate donor [12]; <4,5> very low activity with CTP and UTP [6]; <3> no activity with CTP and UTP [1]; <3> no activity with thymine riboside, deoxyribosides, cytosine arabinoside, adenosine, purine deoxyribosides, UMP, CMP and deoxyribosides [8,12]; <1> dGTP and GTP are the most efficient phosphate donors, activity with ATP and dATP is 10% and with dUTP, dCTP and dTTP 5% of that with GTP or dGTP [7]; <8,9> no activity with adenosine, guanosine and deoxyribonucleotides, no activity with CTP and UTP [18]) (Reversibility: ? <1-6,8,9> [1-15,18]) [1-15, 18]
P ADP + UMP <1-6, 8, 9> [1-15, 18]
S GTP + 5-fluorocytidine <1> (<1> 40% of activity with uridine [7]) (Reversibility: ? <1> [7]) [7]
P GDP + 5-fluorocytidine 5'-monophosphate <1> [7]
S GTP + 5-fluorouridine <1> (<1> 60% of activity with uridine [7]) (Reversibility: ? <1> [7]) [7]
P GDP + 5-fluorouridine 5'-monophosphate <1> [7]
S GTP + 6-azauridine <1> (<1> 31% of activity with uridine [7]) (Reversibility: ? <1> [7]) [7]
P GDP 6-azauridine 5'-monophosphate <1> [7]
S GTP + N-acetylcytidine <1> (<1> 57% of activity with uridine [7]) (Reversibility: ? <1> [7]) [7]
P GDP + N-acetylcytidine 5'-monophosphate <1> [7]
S GTP + cytidine <1> (Reversibility: ? <1> [7]) [7]
P CMP + GDP <1> [7]
S GTP + uridine <1, 3-5, 8, 9> (<4, 5> weak activity [6]; <3> 23% of the activity with ATP [8]) (Reversibility: ? <1, 3-5, 8, 9> [1, 6-8, 12, 18]) [1, 6-8, 12, 18]
P UMP + GDP <1, 3-5, 8, 9> [1, 6-8, 12, 18]
S ITP + uridine <3-5> (<4,5> weak activity [6]) (Reversibility: ? <3-5> [6]) [1, 6]
P IDP + UMP <3-5> [1, 6]
S dATP + cytidine <6> (<6> recombinant uridine kinase [17]) (Reversibility: ? <6> [17]) [17]
P dADP + CMP <6> [17]

S dATP + uridine <3-5, 6> (<3> 20.5% of activity with ATP [12]; <3> 88% of activity with ATP [8]; <6> recombinant uridine kinase [17]) (Reversibility: ? <3-5,6> [6,8,9,12,17]) [6, 8, 9, 12, 17]
P dADP + UMP <3-5, 6> [6, 8, 9, 12, 17]
S dCTP + cytidine <6> (<6> recombinant uridine kinase [17]) (Reversibility: ? <6> [17]) [17]
P dCDP + CMP <6> [17]
S dCTP + uridine <3-5> (<4,5> weak activity [6]; <3> 49.7% of activity with ATP [12]; <3> 50% of activity with ATP [8]) (Reversibility: ? <3-5> [6,8,12]) [6, 8, 12]
P dCDP + UMP <3-5> [6, 8, 12]
S dGTP + uridine <1, 3-5> (<3> 23.5% of activity with ATP [12]; <3> 21% of activity with ATP [8]) (Reversibility: ? <1,3-5> [6-9,12]) [6-9, 12]
P dGDP + UMP <1, 3-5> [6-9, 12]
S dTTP + uridine <4, 5> (<4,5> weak activity [6]) (Reversibility: ? <4,5> [6]) [6]
P dTDP + UMP <4, 5> [6]
S dUTP + cytidine <6> (<6> recombinant uridine kinase [17]) (Reversibility: ? <6> [17]) [17]
P dUDP + CMP <6> [17]
S dUTP + uridine <3-5, 6> (<3> 67% of activity with ATP [8]; <3> 66.7% of activity with ATP [12]; <6> recombinant uridine kinase [17]) (Reversibility: ? <3-5,6> [6,8,9,12,17]) [6, 8, 9, 12, 17]
P dUDP + UMP <3-5, 6> [6, 8, 9, 12, 17]

Inhibitors

5'-O-nitro-5-fluorouridine <3> [3]
5'-amino-5-deoxyuridine <3> [8]
5'-azido-5'-deoxycytidine <3> [3]
5'-azido-5'-deoxyuridine <3> [3]
5'-azido-5-deoxyuridine <3> [8]
5'-deoxyuridine <3> [8]
5'-iodo-5'-deoxyuridine <3> [8]
Ag^+ <2> [15]
CTP <1-5> (<4> enzyme shows a temperature dependence of CTP inhibition [6]; <5> inhibition is partially lost upon ageing of the enzyme and CTP becomes effective as a phosphate donor [9]; <4> 80-85% inhibition at approx. 2 mM [2]; <5> complete inhibition at approx. 2 mM [2]; <5> 0.56 mM, 50% inhibition [6]; <2> 0.6 mM, approx. 80% inhibition of soluble enzyme, complete inhibition of Pb^{2+}-precipitated enzyme [14]; <2> 0.1 mM, complete inhibition [15]) [2, 6, 7, 9, 10, 14, 15]
UTP <1-5> [6, 7, 9, 10, 14]
p-chloromercuribenzoate <3> (<3> 0.17 mM, 67% inhibition, 0.69 mM, 82% inhibition, glutathione protects [1]) [1]
Additional information <3> (<3> no substrate inhibition of cytidine and 5-fluorouridine at substrate concentrations higher than 0.5 mM [1]) [1]

Activating compounds

2-mercaptoethanol <2> (<2> 1-2.5 mM, 40-50% activation of Pb^{2+}-precipitated uridine kinase [15]) [15]
dithiothreitol <2> (<2> 1-2.5 mM, 40-50% activation of Pb^{2+}-precipitated uridine kinase [15]) [15]

Metals, ions

Co^{2+} <3> (<3> 13 mM, 66% of activity with Mg^{2+} [8,12]) [8, 12]
Fe^{2+} <3> (<3> can partially replace Mg^{2+} [1,8,12]; <3> 13 mM, 65% of activity with Mg^{2+} [8,12]; <3> 59% of activity with Mg^{2+} [1]) [1, 8, 12]
Mg^{2+} <3-5> (<3-5> required for full activity [1,6,8,9,11,12]; <5> optimal Mg^{2+} to ATP ratio at 12 mM ATP: 1.25 [11]; <3> between 0.5/1 and 1/1 [1]; <3> maximal activity at 13 mM [12]) [1, 6, 8, 9, 11, 12]
Mn^{2+} <3> (<3> can partially replace Mg^{2+} [1, 8, 12]; <3> 44 mM, 68% of activity with Mg^{2+} [1]; <3> 13 mM, 80% of activity with Mg^{2+} [8, 12]) [1, 8, 12]
Additional information <2, 3> (<3> not activated by Ca^{2+} and Cu^{2+} [1, 8, 12]; <2> no metalloenzyme [14]; <2> freeze dried preparations of uridine kinase precipitated by Pb^{2+} or Zn^{2+} are highly active [15]) [1, 8, 12, 14]

Specific activity (U/mg)

0.06 <5> (<5> uridine kinase from adult animal [11]) [11]
0.88 <5> [6, 9]
3.03 <3> [8, 12]
4.33 <4> [6]
57.49 <5> (<5> uridine kinase from foetal animal [11]) [11]
80.5 <3> [1]
200 <1> [7]
283 <3> [10]
Additional information <5, 6> (<5> in hepatomas of slow and intermediate growth rates, uridine kinase activity increases 1.5-2.6fold, in hepatomas of rapid growth rates activity increases 5-6fold over that in normal livers [16]; <6> 5-13fold increase of uridine kinase activity in ovarian carcinomas over the activity in normal ovaries [16]) [16]

K_m-Value (mM)

0.023 <3> (cytidine, <3> pH 7.4, 37°C [1]) [1]
0.03 <3> (5-fluorouridine, <3> pH 7.4, 37°C [1]) [1]
0.04 <3> (uridine, <3> pH 8.0, 22°C [10]) [10]
0.045 <3> (cytidine, <3> pH 7.5, 25°C [8, 13]) [8, 13]
0.048 <3> (uridine, <3> pH 7.4, 37°C [1]) [1]
0.05 <8> (uridine, <8> pH 7.6, 37°C, recombinant uridine kinase 2 [18]) [18]
0.052 <5> (uridine, <5> 37°C, adult liver uridine kinase [11]) [11]
0.057 <3> (cytidine, <3> pH 8.0, 22°C [10]) [10]
0.069 <3> (5-fluorouridine, <3> pH 8.0, 22°C [10]) [10]
0.075 <6> (cytidine) [4]

0.086 <8> (cytidine, <8> pH 7.6, 37°C, recombinant uridine kinase 2 [18]) [18]
0.13 <1> (cytidine, <1> pH 7.8, 37°C [7]) [7]
0.14 <6> (uridine) [4]
0.15 <3> (cytidine) [3]
0.15 <3> (uridine, <3> pH 7.5, 25°C [13]) [13]
0.15 <3> (uridine, <3> pH 7.5, 25°C [8]) [8]
0.2 <3> (3-deazauridine, <3> pH 8.0, 22°C [10]) [10]
0.21 <4> (uridine, <4> pH 7.5, 10°C [6]) [2, 6]
0.23 <3> (uridine) [3]
0.27 <5> (uridine, <5> 37°C [2]) [2]
0.3 <8> (cytidine, <8> pH 7.6, 37°C, recombinant uridine kinase 1 [18]) [18]
0.3 <8> (uridine, <8> pH 7.6, 37°C, recombinant uridine kinase 1 [18]) [18]
0.34 <3> (6-azauridine, <3> pH 8.0, 22°C [10]) [10]
0.349 <3> (ATP) [3]
0.35 <1> (uridine, <1> pH 7.8, 37°C [7]) [7]
0.37 <5> ($MgATP^{2-}$, <5> 37°C, foetal liver uridine kinase [11]) [11]
0.4 <5> (uridine, <5> 37°C, foetal liver uridine kinase [11]) [11]
0.45 <3> (ATP, <3> pH 8.0, 22°C [10]) [10]
0.5 <3> (ATP, <3> pH 7.4, 37°C [1]) [1]
0.5 <6> (uridine, <6> pH 7.6, 37°C, ovarian carcinoma [16]) [16]
0.64 <4> (uridine, <4> pH 7.5, 37°C [6]) [2, 6]
0.8 <5> (ATP, <5> pH 7.6, 37°C, normal liver [16]) [16]
0.8 <5> (uridine, <5> pH 7.6, 37°C, normal liver [16]) [16]
1.6 <4> (uridine, <4> pH 7.5, 60°C [6]) [2, 6]
2.1 <3> (ATP, <3> pH 7.5, 25°C, cosubstrate cytidine [8]) [8]
2.1 <3> (uridine, <3> pH 7.5, 25°C, cosubstrate cytidine [13]) [13]
3.6 <3> (ATP, <3> pH 7.5, 25°C, cosubstrate uridine [8, 13]) [8, 13]
5 <5> (ATP, <5> pH 7.6, 37°C, hepatoma cells [16]) [16]
5 <5> (uridine, <5> pH 7.6, 37°C, hepatoma cells [16]) [16]
11 <6> (5-azacytidine) [4]
11.5 <6> (uridine, <6> pH 7.6, 37°C, normal ovary [16]) [16]

K_i-Value (mM)

0.037 <3> (5'-azido-5'-deoxycytidine) [3]

pH-Optimum

5.5-8 <3> [1]
6.5-7 <3> [8, 12]
7.2-7.6 <5> (<5> normal liver and hepatoma cells [16]) [16]
7.8 <1> (<1> broad optimum [7]) [7]

pH-Range

5-9 <3> (<3> approx. 50% of maximal activity at pH 5.0, approx. 70% of maximal activity at pH 9.0 [1]) [1]

Temperature optimum (°C)

22 <3> (<3> assay at [10]) [10]
37 <1, 3-5> (<1,3-5> assay at [1,6,7,10,12]) [1, 6, 7, 10, 12]
60 <4> (<4> higher activity than at 10°C and 37°C [2,6]) [2, 6]

4 Enzyme Structure

Molecular weight

90000 <1> (<1> gel filtration [7]) [7]
109000 <4> (<4> sucrose density gradient centrifugation [2]) [2]
160000 <3> (<3> gel filtration [8,12]) [8, 12]
Additional information <3> (<3> 120000 Da polymeric and 30000 Da monomeric form, gel filtration, sucrose density gradient centrifugation [5]) [5]

Subunits

? <3, 8, 9> (<3> x * 31000, enzyme exists in multiple interconvertible oligomeric forms, SDS-PAGE [10]; <8,9> x * 30000, recombinant uridine kinase 1 and 2, SDS-PAGE [18]) [10, 18]
Additional information <3> (<3> 120000 Da polymeric and 30000 Da monomeric form, gel filtration, sucrose density gradient centrifugation [5]) [5]

5 Isolation/Preparation/Mutation/Application

Source/tissue

Ehrlich ascites carcinoma cell <3> [1, 5, 10]
Hela-S3 cell <6> (<6> uridine kinases mRNA expression is higher than in normal tissue [17]) [17]
Novikoff ascites tumor cell <5> [1, 6, 9]
adenocarcinoma cell <6> (<6> SW480 colorectal cells, uridine kinases mRNA expression is higher than in normal tissue [17]) [17]
adipose tissue <5> (<5> 36% of liver activity [16]) [16]
bone marrow <5> (<5> 184% of liver activity [16]) [16]
bone marrow <6> (<6> uridine kinases mRNA expression [17]) [17]
brain <2, 5, 8> (<5> 248% of liver activity [16]; <8> low levels of uridine kinase 1 mRNA [18]) [14, 15, 16, 18]
fibrosarcoma cell <6> [17]
heart <5, 8> (<5> 85% of liver activity [16]; <8> uridine kinase 1 mRNA [18]) [16, 18]
hepatoma <5> (<5> hepatoma 3924A cells, 2fold increase in activity during early log phase of prolifration [16]) [16]
kidney <6, 8> (<6> uridine kinases mRNA expression [17]; <8> uridine kinase 1 mRNA [18]) [17, 18]
large intestine <6> (<6> uridine kinases mRNA expression [17]) [17]

leukemia cell line <3, 6> (<3> L-1210 cell line [3]; <6> lymphoblastic CEM cell line [4]; <6> leukemia cells Hl-60, K562 and Molt-4, uridine kinases mRNA expression is higher than in normal tissue [17]) [3, 4]
leukocyte <6> (<6> peripheral blood leukocyte, uridine kinases mRNA expression [17]) [17]
liver <5, 6, 8> (<5> foetal and adult [11]; <6> uridine kinases mRNA expression [17]; <8> uridine kinase 1 mRNA [18]) [11, 16, 17, 18]
lung <5> (<5> 142% of liver activity [16]) [16]
lung <6> (<6> uridine kinases mRNA expression [17]) [17]
lung cancer cell <6> (<6> A549 lung carcinoma cells, uridine kinases mRNA expression is higher than in normal tissue [17]) [17]
lymph node <6> (<6> uridine kinases mRNA expression [17]) [17]
lymphoma cell <6> (<6> Raji and Daud Burkitt's lymphoma cells, uridine kinases mRNA expression is higher than in normal tissue [17]) [17]
mastocytoma cell <3> (<3> P815 cell line [8,12,13]) [8, 12, 13]
ovarian cancer cell <6> [16]
ovary <6> (<6> uridine kinases mRNA expression [17]) [16, 17]
placenta <8, 9> (<9> uridine kinase 2 mRNA [18]; <8> low levels of uridine kinase 1 mRNA [18]) [18]
renal cortex <5> (<5> 160% of liver activity [16]) [16]
skeletal muscle <5, 8> (<5> 95% of liver activity [16]; <8> uridine kinase 1 mRNA [18]) [16, 18]
small intestine <6, 8> (<6> uridine kinases mRNA expression [17]; <8> low levels of uridine kinase 1 mRNA [18]) [17, 18]
spleen <5> (<5> 212% of liver activity [16]) [16]
spleen <6, 8> (<6> uridine kinases mRNA expression [17]; <8> low levels of uridine kinase 1 mRNA [18]) [17, 18]
testis <5> (<5> 140% of liver activity [16]) [16]
thymus <5> (<5> 468% of liver activity [16]) [16]

Localization

soluble <6> [4]

Purification

<1> (ultracentrifugation, DEAE-cellulose, affinity chromatography on 5'amino-5'-deoxyuridine [7]) [7]
<2> (ammonium sulfate, Pb^{2+}-precipitation [14]) [14]
<3> (partial purification [5]; protamine sulfate, CM-cellulose, ammonium sulfate, DEAE-cellulose, zone electrophoresis [1]; DEAE-cellulose, ammonium sulfate, hydroxyapatite [8,12]; DE-52, ammonium sulfate, ATP-agarose column [10]) [1, 3, 5, 8, 10, 12]
<4> (streptomycin, ammonium sulfate, calcium phosphate, DEAE-cellulose [2]) [2, 6]
<5> (streptomycin, ammonium sulfate, DEAE-cellulose [11]; streptomycin, ammonium sulfate, calcium phosphate, Sephadex G-200 [6,9]) [6, 9, 11]
<6> (recombinant His-tagged uridine kinase, Ni-nitrilotriacetic acid-Sepharose [17]) [17]

Crystallization

<9> (crystals of uridine kinase 2 and in complex with CTP, UTP, cytidine, ATPγS and with both cytidine and ATP, the latter complex diffracted to 1.8 A resolution [19]) [19]

Cloning

<1> [17]
<3> [17]
<6> (expression in Escherichia coli [17]) [17]
<7> [17]
<8, 9> (expression of UCK1 and 2 in Escherichia coli [18]) [18]

6 Stability

pH-Stability

4-7.5 <2> (<2> complete loss of activity below pH 4.0 [15]) [15]

Temperature stability

45 <5> (<5> foetal liver uridine kinase, 50% loss of activity after 10 min, adult liver uridine kinase, no loss of activity after 10 min [11]) [11]
50 <2> (<2> 3% loss of activity after 10 min [15]) [15]
59.5 <4, 5> (<5> 79% loss of activity after 4 min [2]; <4> no loss of activity after 30 min [2]) [2]
100 <2> (<2> dry uridine kinase precipitated with Pb^{2+}, 30% loss of activity after 1 h, dry uridine kinase precipitated with Zn^{2+}, 25% loss of activity after 1 h [15]) [15]
Additional information <3, 5> (<5> ATP protects foetal liver uridine kinase completely for 30 min at 37°C in crude extract [11]; <3> stabilized against thermal inactivation by uridine and ATP [5]) [5, 11]

General stability information

<1>, enzyme is unstable in crude extracts, especially in the presence of Mg^{2+} [7]
<2>, stability of insoluble metal ion precipitated form of the enzyme [15]
<3>, complete loss of activity after storage at -18°C for 24 h in the presence of 0.1 mM dithiothreitol [12]
<3>, pure enzyme is very sensitive to freezing [10]
<3>, some loss of activity after repeated freezing and thawing [8]
<3>, to achieve stability phosphate buffers are used throughout purification and for storage of enzyme fractions [12]
<5>, ATP protects the enzyme completely for 30 min at 37°C in crude extract [11]

Storage stability

<1>, 4°C, 100 mM Tris-HCl, pH 7.8, 6 months, no loss of activity [7]
<2>, 5°C, Pb^{2+} or Zn^{2+}-precipitated uridine kinase, 6 months, 10-15% loss of activity [15]
<3>, -15°C, 2 to 3 months, occasional thawing, no loss of activity [1]

<3>, -18°C, phosphate buffer, pH 7.4-8.2, several months, no loss of activity [12]
<3>, -20°C, 50% glycerol, several months, no loss of activity [10]
<3>, 0°C, 8 weeks, 20% loss of activity [10]
<4>, 4°C, sterile conditions, at least 2 years, no loss of activity enzyme [6]
<5>, -20°C, 50% glycerol, at least 6 months, no loss of activity [6, 9]
<5>, -20°C, several weeks, no loss of activity [11]
<5>, 4°C, at least 1 month, no loss of activity [6, 9]

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Hydroxymethylpyrimidine kinase 2.7.1.49

1 Nomenclature

EC number
2.7.1.49

Systematic name
ATP:4-amino-5-hydroxymethyl-2-methylpyrimidine 5-phosphotransferase

Recommended name
hydroxymethylpyrimidine kinase

Synonyms
EC 2.7.1.14 <4> (<4> hydroxymethylpyrimidine kinase [2]) [2]
EC 2.7.1.35 <4> (<4> pyridoxal kinase [2]) [2]
hydroxymethylpyrimidine kinase (phosphorylating)
pyridoxal kinase <4> [2]

CAS registry number
9026-55-5

2 Source Organism

<1> *Arabidopsis thaliana* [3]
<2> *Brassica napus* (rape [3]) [3]
<3> *Escherichia coli* (nucleotide sequence of thiD [5]) [5]
<4> *Escherichia coli* (K-12 [2]; BL21(DE3) [4]; BL21(DE3) (pLysS) [5]) [2, 4, 5]
<5> *Saccharomyces cerevisiae* (bakers' yeast [1]) [1]

3 Reaction and Specificity

Catalyzed reaction
ATP + 4-amino-5-hydroxymethyl-2-methylpyrimidine = ADP + 4-amino-5-phosphomethyl-2-methylpyrimidine (CTP, UTP and GTP can act as donors)

Reaction type
phospho group transfer

Natural substrates and products

- **S** ATP + 2-methyl-4-amino-5-hydroxymethylpyrimidine <1, 2, 4, 5> (<1, 2, 4, 5> involved in thiamine biosynthetic pathway, first steps [1-5]) (Reversibility: ? <1, 2, 4, 5> [1-5]) [1-5]
- **P** ADP + 2-methyl-4-amino-5-hydroxymethylpyrimidine monophosphate <1, 2, 4, 5> [1-5]

Substrates and products

- **S** ATP + 4-amino-5-hydroxy-2-methylpyrimidine <1, 2, 4, 5> (Reversibility: ? <1, 2, 4, 5> [1-5]) [1-5]
- **P** ADP + 4-amino-5-phospho-2-methylpyrimidine <1, 2, 4, 5> [1-5]
- **S** ATP + pyridoxal <4> (Reversibility: ? <4> [2]) [2]
- **P** ADP + pyridoxal 5'-phosphate
- **S** ATP + pyridoxamine <4> (Reversibility: ? <4> [2]) [2]
- **P** ADP + pyridoxamine 5'-phosphate
- **S** ATP + pyridoxine <4> (Reversibility: ? <4> [2,4]) [2, 4]
- **P** ADP + pyridoxine 5'-phosphate <4> [2, 4]
- **S** CTP + 5-hydroxy-2-methylpyrimidine <5> (Reversibility: ? <5> [1]) [1]
- **P** CDP + 5-phospho-2-methylpyrimidine <5> [1]
- **S** GTP + 5-hydroxy-2-methylpyrimidine <5> (Reversibility: ? <5> [1]) [1]
- **P** GDP + 5-phospho-2-methylpyrimidine <5> [1]
- **S** UTP + 5-hydroxy-2-methylpyrimidine <5> (Reversibility: ? <5> [1]) [1]
- **P** UDP + 5-phospho-2-methylpyrimidine <5> [1]
- **S** Additional information <2, 4> (<4> enzyme is probably different from previously isolated HMP kinase, because the subunit molecular masses are significantly different [4]; <4> bifunctional hydroxymethylpyrimidine kinase/phosphomethylpyrimidine kinase [5]; <2> bifunctional hydroxypyrimidine kinase/thiamin-phosphate pyrophosphorylase [3]) [3-5]
- **P** ?

Inhibitors

2-methyl-4-amino-5-hydroxymethylpyrimidine <4> (<4> competitive inhibition [2]) [2]
pyridoxine <4> (<4> inhibits competitively the phosphorylation of 2-methyl-4-amino-5-hydroxymethylpyrimidine [2]) [2]
Additional information <5> (<5> stable to treatment with *p*-hydroxymercuribenzoate [1]) [1]

Metals, ions

Additional information <5> (<5> no metal requirement [1]) [1]

Turnover number (min^{-1})

0.006 <4> (pyridoxine, <4> pH 8.0, 25°C [4]) [4]
0.007 <4> (2-methyl-4-amino-5-hydroxymethylpyrimidine, <4> pH 8.0, 25°C [4]) [4]
0.007 <4> (ATP, <4> pH 8.0, 25°C [4]) [4]

Specific activity (U/mg)
0.329 <4> [5]
4.344 <4> [2]

K_m-Value (mM)
0.0285 <4> (pyridoxine, <4> pH 8.0, 25°C [4]) [4]
0.0335 <4> (2-methyl-4-amino-5-hydroxymethylpyrimidine, <4> pH 8.0, 25°C [4]) [4]
0.066 <4> (pyridoxine, <4> pH 6.2, 37°C [2]) [2]
0.11 <4> (2-methyl-4-amino-5-hydroxymethylpyrimidine, <4> pH 6.2, 37°C [2]) [2]
0.1431 <4> (ATP, <4> pH 8.0, 25°C [4]) [4]
0.24 <4> (pyridoxal, <4> pH 6.2, 37°C [2]) [2]
2.4 <4> (pyridoxamine, <4> pH 6.2, 37°C [2]) [2]

K_i-Value (mM)
0.0027 <4> (pyridoxine, <4> pH 6.2, 37°C [2]) [2]
0.09 <4> (2-methyl-4-amino-5-hydroxymethylpyrimidine, <4> pH 6.2, 37°C [2]) [2]

pH-Optimum
5.6 <4> (<4> substrate pyridoxine [2]) [2]
6 <4> (<4> substrate 2-methyl-4-amino-5-hydroxymethylpyrimidine [2]) [2]

pH-Range
5-6 <4> (<4> about half-maximal activity at pH 5.0 and pH 6.0, pyridoxine as substrate [2]) [2]
5.5-6.5 <4> (<4> about half-maximal activity at pH 5.5 and pH 6.5, 2-methyl-4-amino-5-hydroxymethylpyrimidine as substrate [2]) [2]

4 Enzyme Structure

Molecular weight
27500 <4> (<4> SDS-PAGE [5]) [5]
31000 <4> (<4> gel filtration [5]) [5]
43000-44000 <4> (<4> gel filtration [2]) [2]
Additional information <4> (<4> gel filtration indicates the enzyme is tetrameric in the native state [4]) [4]

Subunits
monomer <4> (<4> 1 * 43000-44000, SDS-PAGE [2]; <4> 1 * 27500, SDS-PAGE [5]) [2, 5]
tetramer <4> (<4> 4 * 33000, SDS-PAGE [4]; <4> 4 * 33534, predicted from amino acid sequence [4]) [4]

5 Isolation/Preparation/Mutation/Application

Source/tissue
leaf <2> [3]
root <2> [3]
stem <2> [3]

Localization
cytosol <4> [2]
plastid <1, 2> [3]

Purification
<4> (recombinant enzyme [5]) [2, 4, 5]
<5> (partially [1]) [1]

Cloning
<1> (single-copy gene in BAC clone T23L3 [3]) [3]
<2> (cDNA clone pBTH1 encoding bifunctional hydroxymethylpyrimidine kinase/thiamin-phosphate diphosphorylase established in Escherichia coli DH5α, expressed in Escherichia coli NI500 and Salmonella typhimurium DM456 by functional complementation [3]) [3]
<3> [5]
<4> (pET overexpression system [4]; Escherichia coli BL21(DE3) (pLysS) transformed with pTRD63 carrying ORF2, single gene thiD/J encodes 2 distinct enzyme activities, HMP kinase and HMP-P kinase, functional complemetation of Escherichia coli NI500, overexpression of ThiD [5]) [4, 5]

Application
synthesis <4> (<4> useful reagent for the preparation of intermediates on the thiamin biosynthetic pathway [4]) [4]

6 Stability

Temperature stability
55 <5> (<5> stable to heating for 10 min [1]) [1]

General stability information
<5>, protected from inactivation by cysteine [1]

Storage stability
<4>, -70°C, stored in 10% glycerol, loses half of its activity after 1 month [4]

References

[1] Lewin, I.M.; Brown, G.M.: The biosynthesis of thiamine. III. Mechanism of enzymatic formation of the pyrophosphate ester of 2-methyl-4-amino-5-hydroxymethylpyrimidine. J. Biol. Chem., **236**, 2768-2771 (1961)

[2] Mizote, T.; Nakayama, H.: Purification and properties of hydroxymethylpyrimidine kinase from Escherichia coli. Biochim. Biophys. Acta, **991**, 109-113 (1989)
[3] Kim, Y.S.; Nosaka, K.; Downs, D.M.; Kwak, J.M.; Park, D.; Chung, I.K.; Nam, H.G.: A Brassica cDNA clone encoding a bifunctional hydroxymethylpyrimidine kinase/thiamin-phosphate pyrophosphorylase involved in thiamin biosynthesis. Plant Mol. Biol., **37**, 955-966 (1998)
[4] Reddick, J.J.; Kinsland, C.; Nicewonger, R.; Christian, T.; Downs, D.M.; Winkler, M.E.; Begley, T.P.: Overexpression, purification and characterization of two pyrimidine kinases involved in the biosynthesis of thiamin: 4-amino-5-hydroxymethyl-2-methylpyrimidine kinase and 4-amino-5-hydroxymethyl-2-methylpyrimidine phosphate kinase. Tetrahedron, **54**, 15983-15991 (1998)
[5] Mizote, T.; Tsuda, M.; Smith, D.D.; Nakayama, H.; Nakazawa, T.: Cloning and characterization of the thiD/J gene of Escherichia coli encoding a thiamin-synthesizing bifunctional enzyme, hydroxymethylpyrimidine kinase/phosphomethylpyrimidine kinase. Microbiology, **145**, 495-501 (1999)

Hydroxyethylthiazole kinase 2.7.1.50

1 Nomenclature

EC number
2.7.1.50

Systematic name
ATP:4-methyl-5-(2-hydroxyethyl)-thiazole 2-phosphotransferase

Recommended name
hydroxyethylthiazole kinase

Synonyms
4-methyl-5-(β-hydroxyethyl)thiazole kinase
4-methyl-5-β-hydroxyethylthiazole kinase
TH kinase
THZ kinase
ThiK
hydroxyethylthiazole kinase/thiamine-phosphate pyrophosphorylase <1> (<1> bifunctional enzyme with hydroxyethylthiazole kinase and thiamine-phosphate pyrophosphorylase activity [2]) [2]
kinase, hydroxyethylthiazole (phosphorylating)

CAS registry number
9026-56-6

2 Source Organism

<1> *Saccharomyces cerevisiae* (mutant resistant to 2-amino-4-methyl-5-β-hydroxyethylthiazole, an antimetabolite of 4-methyl-5-β-hydroxyethylthiazole, deficient in activity of both EC 2.5.1.3 and EC 2.7.1.50 [2]; bifunctional enzyme with hydroxyethylthiazole kinase and thiamine-phosphate pyrophosphorylase activity [2]) [1, 2]
<2> *Bacillus subtilis* [3]

3 Reaction and Specificity

Catalyzed reaction
ATP + 4-methyl-5-(2-hydroxyethyl)-thiazole = ADP + 4-methyl-5-(2-phosphoethyl)-thiazole

Reaction type

phospho group transfer

Natural substrates and products

- **S** ATP + 4-methyl-5-(2-hydroxyethyl)thiazole <1, 2> (<1> enzyme involved in biosynthesis of thiamine [1]; <1> the bifunctioinal enzyme hydroxyethylthiazole kinase/thiamine-phosphate pyrophosphorylase catalyzes two sequential steps in the synthesis of thiamin monophosphate from hydroxyethylthiazole [2]; <2> the enzyme is a salvage enzyme in the thiamin biosynthetic pathway and enables the cell to use recycled 4-methyl-5-β-hydroxyethylthiazole as an alternative to its synthesis from 1-deoxy-D-xylulose-5-phosphate, cysteine, and tyrosine [3]) (Reversibility: ? <1, 2> [1, 2, 3]) [1, 2, 3]
- **P** ADP + thiazole monophosphate

Substrates and products

- **S** ADP + 4-methyl-5-(2-hydroxyethyl)thiazole <1> (<1> 3.4% of the activity with ATP [2]) (Reversibility: ? <1> [2]) [2]
- **P** AMP + thiazole monophosphate
- **S** ATP + 4-methyl-5-(2-hydroxyethyl)thiazole <1, 2> (<2> phosphate transfer occurs by an inline mechanism [3]) (Reversibility: ? <1,2> [1,2,3]) [1, 2, 3]
- **P** ADP + thiazole monophosphate <1> [1]
- **S** CTP + 4-methyl-5-(2-hydroxyethyl)thiazole <1> (<1> 13.7% of the activity with ATP [2]) (Reversibility: ? <1> [2]) [2]
- **P** CDP + thiazole monophosphate
- **S** GTP + 4-methyl-5-(2-hydroxyethyl)thiazole <1> (<1> 3.2% of the activity with ATP [2]) (Reversibility: ? <1> [2]) [2]
- **P** GDP + thiazole monophosphate
- **S** UTP + 4-methyl-5-(2-hydroxyethyl)thiazole <1> (<1> 75.1% of the activity with ATP [2]) (Reversibility: ? <1> [2]) [2]
- **P** UDP + thiazole monophosphate
- **S** dATP + 4-methyl-5-(2-hydroxyethyl)thiazole <1> (<1> 24.8% of the activity with ATP [2]) (Reversibility: ? <1> [2]) [2]
- **P** dADP + thiazole monophosphate

Inhibitors

2-aminohydroxyethylthiazole <1> [2]

PCMB <1> (<1> 0.01 mM, 98.5% inhibition, inhibition prevented by addition of 2-mercaptoethanol [2]) [2]

Metals, ions

Co^{2+} <1> (<1> 10 mM, stimulates as effectively as Mg^{2+} [2]) [2]

Mg^{2+} <1> (<1> 10 mM, stimulates as effectively as Co^{2+} [2]; <1> required [1]) [1, 2]

Mn^{2+} <1> (<1> stimulates, 42.7% as effectively as Mg^{2+} [2]) [2]

Specific activity (U/mg)

0.53 <1> [2]

K_m-Value (mM)

0.0057 <1> (hydroxyethylthiazole, <1> pH 7.5, 37°C [2]) [2]
0.79 <1> (ATP, <1> pH 7.5, 37°C [2]) [2]

K_i-Value (mM)

0.0021 <1> (2-aminohydroxyethylthiazole, <1> pH 7.5, 37°C [2]) [2]

pH-Optimum

7.7 <1> [2]

pH-Range

7.4-9.2 <1> (<1> pH 7.4: about 80% of activity maximum, pH 9.2: about 25% of activity maximum [2]) [2]

4 Enzyme Structure

Molecular weight

470000 <1> (<1> bifunctional enzyme with hydroxyethylthiazole kinase and thiamine-phosphate pyrophosphorylase activity, gel filtration [2]) [2]

Subunits

octamer <1> (<1> 8 * 60000, bifunctional enzyme with hydroxyethylthiazole kinase and thiamine-phosphate pyrophosphorylase activity, SDS-PAGE [2]) [2]
Additional information <2> (<2> as determined from crystallization data the enzyme is a trimer of identical subunits, the active site is formed at the interface between two subunits within the trimer [3]) [3]

5 Isolation/Preparation/Mutation/Application

Purification

<1> (bifunctional enzyme with hydroxyethylthiazole kinase and thiamine-phosphate pyrophosphorylase activity [2]) [2]

Crystallization

<2> (crystallization by vapor diffusion equilibration, crystal structure at 1.5 A resolution [3]) [3]

6 Stability

Storage stability

<1>, -80°C, about 50% loss of activity after 1 month [2]

References

[1] Camiener, G.W.; Brown, G.M.: The biosynthesis of thiamine. 2. Fractionation of enzyme system and identification of thiazole monophosphate and thiamine monophosphate as intermediates. J. Biol. Chem., **235**, 2411-2417 (1960)
[2] Kawasaki, Y.: Copurification of hydroxyethylthiazole kinase and thiaminephosphate pyrophosphorylase of Saccharomyces cerevisiae: characterization of hydroxyethylthiazole kinase as a bifunctional enzyme in the thiamine biosynthetic pathway. J. Bacteriol., **175**, 5153-5158 (1993)
[3] Campobasso, N.; Mathews, II; Begley, T.P.; Ealick, S.E.: Crystal structure of 4-methyl-5-β-hydroxyethylthiazole kinase from Bacillus subtilis at 1.5 A resolution. Biochemistry, **39**, 7868-7877 (2000)

L-Fuculokinase 2.7.1.51

1 Nomenclature

EC number
2.7.1.51

Systematic name
ATP:L-fuculose 1-phosphotransferase

Recommended name
L-fuculokinase

Synonyms
L-fuculose kinase
kinase, L-fuculo- (phosphorylating)

CAS registry number
9026-64-6

2 Source Organism

<1> *Escherichia coli* (strain O-111 B4 [1]; K-12 mutant strain 1102 derived from parent strain 1000 [3]) [1, 3]
<2> *Escherichia freundii* [1]
<3> *Salmonella typhimurium* (wild-type and mutant strains [2]) [2]
<4> *Aerobacter aerogenes* (PRL-R3, strain 176 [3]) [3]

3 Reaction and Specificity

Catalyzed reaction
ATP + L-fuculose = ADP + L-fuculose 1-phosphate

Reaction type
phospho group transfer

Natural substrates and products
S ATP + L-fuculose <1, 3, 4> (<3> involved in L-fucose metabolism, inducible [2]) [2, 3]
P ADP + L-fuculose 1-phosphate

Substrates and products

- **S** ATP + D-fructose <1, 2> (<1,2> phosphorylation at about 45% the rate of L-fuculose [1]) (Reversibility: ? <1,2> [1]) [1]
- **P** ADP + D-fructose 1-phosphate
- **S** ATP + D-ribulose <1, 2> (<1,2> phosphorylation at about 40% the rate of L-fuculose, not L-ribulose [1]) (Reversibility: ? <1,2> [1]) [1]
- **P** ADP + D-ribulose 1-phosphate
- **S** ATP + D-xylulose <1, 2> (<1,2> phosphorylation at about 40% the rate of L-fuculose [1]) (Reversibility: ? <1,2> [1]) [1]
- **P** ADP + D-xylulose 1-phosphate
- **S** ATP + L-fuculose <1, 2> (<1,2> specific for ATP, cannot be replaced by GTP, ITP, CTP or UTP, no substrates are L-fucose, D-psicose, D-tagatose, L-sorbose, 6-deoxy-L-sorbose or L-rhamnulose [1]) (Reversibility: ? <1,2> [1]) [1]
- **P** ADP + L-fuculose 1-phosphate <1, 2> [1]

Metals, ions

Ca^{2+} <1, 2> (<1,2> activation, about 50% as efficient as Mn^{2+} or Mg^{2+} [1]) [1]
Co^{2+} <1, 2> (<1,2> activation, about 50% as efficient as Mn^{2+} or Mg^{2+} [1]) [1]
Mg^{2+} <1, 2> (<1,2> requirement, as efficient as Mn^{2+} [1]) [1]
Mn^{2+} <1, 2> (<1,2> requirement, as efficient as Mg^{2+} [1]) [1]

Specific activity (U/mg)

0.106-1.24 <3> (<3> several wild-type and mutant strains [2]) [2]
0.966 <1, 2> (<1> pH 7.8, 37°C [1]) [1]

K_m-Value (mM)

1.4 <1, 2> (L-fuculose, <1> pH 7.8, 37°C [1]) [1]

pH-Optimum

7.8 <1, 2> [1]

pH-Range

6-8.6 <1, 2> (<1,2> about half-maximal activity at pH 6 and 8.6 [1]) [1]

Temperature optimum (°C)

37 <1, 2> (<1,2> assay at [1]) [1]

5 Isolation/Preparation/Mutation/Application

Purification

<1, 2> [1]

References

[1] Heath, E.C.; Ghalambor, M.A.: The metabolism of L-fucose: I. The purification and properties of L-fucose kinase. J. Biol. Chem., **237**, 2423-2426 (1962)
[2] Old, D.C.; Mortlock, R.P.: The metabolism of D-arabinose by Salmonella typhimurium. J. Gen. Microbiol., **101**, 341-344 (1977)
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Fucokinase 2.7.1.52

1 Nomenclature

EC number
2.7.1.52

Systematic name
ATP:6-deoxy-β-L-galactose 1-phosphotransferase

Recommended name
fucokinase

Synonyms
L-fucose kinase
fucokinase
fucose kinase
kinase, fuco- (phosphorylating)

CAS registry number
37278-00-5

2 Source Organism

<1> *Sus scrofa* [1, 3, 4, 5]
<2> *Canis familiaris* [2]
<3> *Rattus norvegicus* [6]
<4> *Homo sapiens* [7]

3 Reaction and Specificity

Catalyzed reaction
ATP + 6-deoxy-β-L-galactose = ADP + 6-deoxy-β-L-galactose 1-phosphate (<1>, the mechanism involves anomeric oxygen retention [4])

Reaction type
phospho group transfer

Natural substrates and products
S ATP + 6-deoxy-L-galactose <1, 2> (<1,2>, i.e. L-fucose, serves to utilize free L-fucose for glycoprotein synthesis [2,3]; <1>, enzyme is part of a salvage pathway for reutilization of L-fucose [5]) (Reversibility: ? <1, 2> [2, 3, 5]) [2, 3, 5]
P ADP + 6-deoxy-L-galactose 1-phosphate

Substrates and products

S ATP + 6-deoxy-L-galactose <1-4> (<1>, β-L-fucose isomer required [4]; <1,2> highly specific for ATP [2,3]; <1,2>, higly specific for L-fucose [2,3]) (Reversibility: ? <1-4> [1-7]) [1-7]
P ADP + 6-deoxy-L-galactose 1-phosphate <1, 2> (<1,2>, β-L-fucose 1-phosphate [1,2,5]) [1, 2, 5]
S ATP + D-arabinose <1> (<1>, 28% of the activity with L-fucose [1]; <1>, 9.3% of the activity with L-fucose [5]) (Reversibility: ? <1> [1,5]) [1, 5]
P ?
S ATP + D-glucose <1> (<1>, 107% of the activity with L-fucose [1]; <1>, 2.5% of the activity with L-fucose [5]) (Reversibility: ? <1> [1,5]) [1, 5]
P ?
S ATP + D-ribose <1> (<1>, 87% of the activity with L-fucose [1]) (Reversibility: ? <1> [1]) [1]
P ?
S ATP + L-arabinose <1> (<1>, 31% of the activity with L-fucose [1]) (Reversibility: ? <1> [1]) [1]
P ?
S ATP + L-rhamnose <1> (<1>, 41% of the activity with L-fucose [1]) (Reversibility: ? <1> [1]) [1]
P ?
S CTP + 6-deoxy-L-galactose <1> (<1>, 60% of the activity with ATP [1]; <2>, no activity [2]) (Reversibility: ? <1> [1]) [1]
P CDP + 6-deoxy-L-galactose 1-phosphate
S GTP + 6-deoxy-L-galactose <1> (<1>, 56% of the activity with ATP [1]; <2>, no activity [2]) (Reversibility: ? <1> [1]) [1]
P GDP + 6-deoxy-L-galactose 1-phosphate
S ITP + 6-deoxy-L-galactose <1> (<1>, 2.5% of the activity with L-fucose [5]) (Reversibility: ? <1> [5]) [5]
P IDP + 6-deoxy-L-galactose 1-phosphate
S UTP + 6-deoxy-L-galactose <1> (<1>, 59% of the activity with ATP [1]; <2>, no activity [2]) (Reversibility: ? <1> [1]) [1]
P UDP + 6-deoxy-L-galactose 1-phosphate
S Additional information <1> (<1>, less than 5% of the activity with L-fucose is observed with: D-mannose, D-galactose, D-xylose, L-xylose, D-fucose and L-fucolose [1]) [1]
P ?

Inhibitors

1,1,1-trichlorethyl 6-deoxy-α-L-galactopyranoside <3> (<3>, 10 mM, 87% inhibition [6]) [6]
4,6-dideoxy-4-azido-α-L-glucopyranose <3> (<3>, 10 mM, 9% inhibition [6]) [6]
4,6-dideoxy-4-fluoro-L-gluco-pyranose <3> (<3>, 10 mM, 30% inhibition [6]) [6]
4,6-dideoxy-4-iodo-L-glucopyranose <3> (<3>, 10 mM, 75% inhibition [6]) [6]
4,6-dideoxy-L-xylo-hexopyranose <3> (<3>, 10 mM, 74% inhibition [6]) [6]

ADP <1> (<1>, 4.9 mM, 96% inhibition, competitive with ATP [3]) [3]
AMP <1> (<1>, 5.0 mM, 14% inhibition in presence of ATP [3]) [3]
CDP <1> (<1>, 4.9 mM, 16% inhibition in presence of ATP [3]) [3]
CTP <1> (<1>, 4.9 mM, 30% inhibition in presence of ATP [3]) [3]
D-arabinose <1> (<1>, 0.5 mM, 20% inhibition. 10 mM, 84% inhibition [5]) [5]
GDP-L-fucose <1> (<1> competitive with L-fucose [3]) [3, 5]
GTP <1> (<1>, 0.1 mM, 59% inhibition in presence of ATP [3]) [3]
L-fucose <1, 2> (<2> substrate inhibition at high concentrations [2]; <1> 0.5 mM [5]) [2, 5]
L-galactose <2> (<2> weak [2]) [2]
TTP <1> (<1>, 4.8 mM, 45% inhibition in presence of ATP [3]) [3]
methyl 3,6-dideoxy-α-L-xylo-hexopyranoside <3> (<3>, 10 mM, 17% inhibition [6]) [6]
methyl 4,6-dideoxy-4-iodo-α-L-glucopyranoside <3> (<3>, 10 mM, 54% inhibition [6]) [6]
methyl 4,6-dideoxy-α-L-xylo-hexopyranoside <3> (<3>, 10 mM, 9% inhibition [6]) [6]
methyl 6-deoxy-2-O-dodecanoyl-α-L-galactopyranoside <3> (<3>, 10 mM, 37% inhibition [6]) [6]
methyl 6-deoxy-α-L-galactopyranoside <3> (<3>, 10 mM, 89% inhibition [6]) [6]
octyl 6-deoxy-α-L-galactopyranoside <3> (<3>, 10 mM, 40% inhibition [6]) [6]

Activating compounds

GDP-α-D-mannose <1> (<1> stimulation [3]) [3]

Metals, ions

Ca^{2+} <1> (<1>, absolute requirement for a divalent cation, 85% of the activation with Mg^{2+} [1]) [1]
Co^{2+} <1, 2> (<1>, absolute requirement for a divalent cation, 15% of the activation with Mg^{2+} [1]; <2>, divalent cation required, $CoSO_4$ exhibits 12% of the activity with $MgSO_4$ [2]; <1>, divalent cation required, Co^{2+} stimulates with maximal activity at 3-5 mM, maximal activity is about 35-30% compared to activation by Mg^{2+} [5]) [1, 2, 5]
Fe^{2+} <1> (<1>, absolute requirement for a divalent cation, 29% of the activation with Mg^{2+} [1]; <1>, divalent cation required, Fe^{2+} stimulates nearly to the same degree as Mg^{2+}, optimum activity at 10 mM [5]) [1, 5]
Mg^{2+} <1, 2> (<1>, absolute requirement for a divalent cation, Mg^{2+} is most efficient [1]; <2>, divalent cation required Mg^{2+} is slightly more effective than Mn^{2+} [2]; <1>, absolute requirement for a divalent cation, best stimulation at 3 mM [5]) [1, 2, 5]
Mn^{2+} <1, 2> (<1>, absolute requirement for a divalent cation, 87% of the activation with Mg^{2+} [1]; <2>, divalent cation required, $MnSO_4$ exhibits 90% of the activity with $MgSO_4$ [2]; <1>, divalent cation required, Mn^{2+} stimulates with maximal activity at 3-5 mM, maximal activity is about 35-30% compared to activation by Mg^{2+} [5]) [1, 2, 5]

Specific activity (U/mg)

0.003275 <1> [1]
0.0091 <2> (<2> radiometric assay [2]) [2]
0.6 <1> [3]
1.273 <1> [5]

K_m-Value (mM)

0.017 <2> (L-fucose, <2> pH 6.5, 37°C [2]) [2]
0.026 <2> (L-fucose, <2> pH 8.3, 37°C [2]) [2]
0.027 <1> (L-fucose, <1>, pH 8.0, 37°C [5]) [5]
0.03 <1> (L-fucose, <1>, pH 7.4, 37°C [3]) [3]
0.12 <1> (L-fucose, <1> pH 8, 37°C [1]) [1]
0.29 <2> (ATP, <2> pH 6.5, pH 37°C [2]) [2]
0.6 <1> (ATP, <1>, pH 8.0, 37°C [5]) [5]
0.63 <2> (ATP, <2> pH 8.3, pH 37°C [2]) [2]
1 <1> ($MgATP^{2-}$, <1>, pH 7.4, 37°C [3]) [3]

K_i-Value (mM)

0.01 <1> (GDP-L-fucose) [5]
0.5 <3> (4,6-dideoxy-L-xylo-hexopyranose, <3>, pH 7.4, 37°C [6]) [6]
1.1 <3> (methyl 6-deoxy-α-L-galactopyranoside, <3>, pH 7.4, 37°C [6]) [6]
5 <3> (4,6-dideoxy-4-iodo-L-glucopyranose, <3>, pH 7.4, 37°C [6]) [6]
5.5 <3> (1,1,1-trichlorethyl 6-deoxy-α-L-galactopyranoside, <3>, pH 7.4, 37°C [6]) [6]

pH-Optimum

6.5 <1, 2> (<2> two optima: pH 6.5 and pH 8.3 [2]) [2, 3]
6.6 <1> [3]
8 <1> (<1> in Tris buffer about 80% of the activity in HEPES buffer [5]) [1, 5]
8.3 <2> (<2> two optima: pH 6.5 and pH 8.3 [2]) [2]

pH-Range

7-9.5 <1> (<1> pH 7: about 50% of maximal activity, pH 9.5: about 60% of maximal activity [1]) [1]

4 Enzyme Structure

Molecular weight

309200 <1> (<1> gel filtration [3]) [3]
440000 <1> (<1>, gel filtration [5]) [5]
494000 <2> (<2> gel filtration [2]) [2]

Subunits

? <4> (<4>, x * 110000, SDS-PAGE [7]) [7]
tetramer <1> (<1> 4 * 78000, SDS-PAGE [3]; <1>, 4 * 110000, SDS-PAGE [5]) [3, 5]

5 Isolation/Preparation/Mutation/Application

Source/tissue
aorta <1> [5]
brain <1> [5]
heart <1> [5]
kidney <1> [5]
liver <1, 3> [1, 5, 6]
lung <1> [5]
pancreas <1> [5]
spleen <1> [5]
thyroid gland <1, 2> [2-4]

Localization
cytosol <1> [5]

Purification
<1> (partial [1]) [1, 3, 5]
<2> [2]

Cloning
<4> (expression of full-length protein and a deletion mutant lacking the first 363 amino acids of the N-terminus are expressed in Escherichia coli BL21 cells. Both proteins display fucokinase activity. Identification of amino acid sequence [7]) [7]

Engineering
Additional information <4> (<4>, deletion mutant lacking the first 363 amino acids of the N-terminus expressed in E. coli BL21 cells display fucokinase activity [7]) [7]

Application
synthesis <1> (<1>, the enzyme is a valuable biochemical tool to prepare activated L-fucose derivatives for fucosylation reactions, enzyme is valuable for the formation of radiolabeled fucose 1-phosphate [5]) [5]

6 Stability

General stability information
<1>, ovalbumin stabilizes [1]
<1>, substrates do not stabilize [1]
<1>, unstable in the presence of KCl [3]
<2>, EDTA stabilizes during purification [2]
<2>, glycerol, sucrose, DTT or 2-mercaptoethanol stabilizes [2]
<2>, ovalbumin does not stabilize purified enzyme [2]

Storage stability

<1>, -16°C, about 30% loss of activity within 24 h and about 60% loss of activity within 72 h [1]
<1>, -16°C, in the presence of stabilizing ovalbumin, about 30% loss of activity within 48 h [1]
<1>, -20°C, in the presence of 2-mercaptoethanol and glycerol, quite stable [3]
<1>, 4°C, unstable in saline solution [3]
<1>, frozen, unstable in saline solution [3]
<2>, 2-4°C, most stable in the presence of glycerol and a sulfhydryl reagent [2]
<2>, frozen, in the presence of glycerol or sucrose and a sulfhydryl reagent, up to 1 week [2]
<2>, frozen, rapid loss of activity, glycerol or sucrose and DTT or 2-mercaptoethanol stabilize, no stabilization by Mg^{2+}, EDTA, KCl or ovalbumin [2]

References

[1] Ishihara, H.; Massaro, D.J.; Heath, E.C.: The metabolism of L-fucose. 3. The enzymatic synthesis of β-L-fucose 1-phosphate. J. Biol. Chem., **243**, 1103-1109 (1968)
[2] Richards, W.L.; Serif, G.S.: Canine thyroid fucokinase. Biochim. Biophys. Acta, **484**, 353-367 (1977)
[3] Kilker, R.D.; Shuey, D.K.; Serif, G.S.: Isolation and properties of porcine thyroid fucokinase. Biochim. Biophys. Acta, **570**, 271-283 (1979)
[4] Butler, W.; Serif, G.S.: Fucokinase, its anomeric specificity and mechanism of phosphate group transfer. Biochim. Biophys. Acta, **829**, 238-243 (1985)
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[6] Zeitler, R.; Danneschewski, S.; Lindhorst, T.; Thiem, J.; Reutter, W.: Inhibition of L-fucokinase from rat liver by L-fucose analogues in vitro. J. Enzyme Inhib., **11**, 265-273 (1997)
[7] Hinderlich, S.; Berger, M.; Blume, A.; Chen, H.; Ghaderi, D.; Bauer, C.: Identification of human L-fucose kinase amino acid sequence. Biochem. Biophys. Res. Commun., **294**, 650-654 (2002)

L-Xylulokinase 2.7.1.53

1 Nomenclature

EC number
2.7.1.53

Systematic name
ATP:L-xylulose 5-phosphotransferase

Recommended name
L-xylulokinase

Synonyms
kinase, L-xylulo- (phosphorylating)

CAS registry number
37278-01-6

2 Source Organism

<1> *Aerobacter aerogenes* [1]
<2> *Erwinia uredovora* (mutant strains DM101 and DM121 [2]) [2]

3 Reaction and Specificity

Catalyzed reaction
ATP + L-xylulose = ADP + L-xylulose 5-phosphate

Reaction type
phospho group transfer

Natural substrates and products
S ATP + L-xylulose <1> (<1> involved in pentose metabolism [1]) [1]
P ADP + L-xylulose 5-phosphate

Substrates and products
S ATP + L-xylulose <1, 2> (<1> specific for L-isomer, no phosphorylation of D-xylulose, D-/L-ribulose, D-/L-xylose, D-/L-arabinose, D-lyxose, D-ribose, D-/L-glucose, D-fructose, L-sorbose, D-mannose, D-galactose, D-altrose, D-/L-fucose, L-rhamnose, L-erythrulose, D-/L-arabitol, xylitol, ribitol or erythritol [1]) (Reversibility: ? <1,2> [1,2]) [1, 2]
P ADP + L-xylulose 5-phosphate <1, 2> [1, 2]

Activating compounds
glutathione <1> (activation) [1]

Metals, ions
Mg^{2+} <1> (<1> requirement [1]) [1]
Additional information <1> (<1> no activation by Mn^{2+} [1]) [1]

Specific activity (U/mg)
340.6 <1> (<1> pH 7.5 [1]) [1]

K_m-Value (mM)
0.4 <1> (D-xylulose, <1> pH 7.5 [1]) [1]

pH-Optimum
7.5 <1> [1]

5 Isolation/Preparation/Mutation/Application

Purification
<1> [1]

References

[1] Anderson, R.L.; Wood, W.A.: Purification and properties of L-xylulokinase. J. Biol. Chem., **237**, 1029-1033 (1962)
[2] Doten, R.C.; Mortlock, R.P.: Characterization of xylitol-utilizing mutants of Erwinia uredovora. J. Bacteriol., **161**, 529-533 (1985)

D-Arabinokinase 2.7.1.54

1 Nomenclature

EC number
2.7.1.54

Systematic name
ATP:D-arabinose 5-phosphotransferase

Recommended name
D-arabinokinase

Synonyms
kinase, D-arabino- (phosphorylating)

CAS registry number
37278-02-7

2 Source Organism

<1> *Propionibacterium pentosaceum* (strain E14 [1]) [1]

3 Reaction and Specificity

Catalyzed reaction
ATP + D-arabinose = ADP + D-arabinose 5-phosphate

Reaction type
phospho group transfer

Natural substrates and products
S ATP + D-arabinose <1> [1]
P ADP + D-arabinose 5-phosphate

Substrates and products
S ATP + D-arabinose <1> (Reversibility: ? <1> [1]) [1]
P ADP + D-arabinose 5-phosphate <1> [1]
S CTP + D-arabinose <1> (<1> 51% of activity with ATP [1]) (Reversibility: ? <1> [1]) [1]
P CDP + D-arabinose 5-phosphate
S GTP + D-arabinose <1> (<1> GTP is as effective as ATP [1]) (Reversibility: ? <1> [1]) [1]
P GDP + D-arabinose 5-phosphate

S ITP + D-arabinose <1> (<1> 88% of activity with ATP [1]) (Reversibility: ? <1> [1]) [1]
P IDP + D-arabinose 5-phosphate
S UTP + D-arabinose <1> (<1> 64% of activity with ATP [1]) (Reversibility: ? <1> [1]) [1]
P UDP + D-arabinose 5-phosphate
S Additional information <1> (<1> D-ribose may be a substrate, D-glucose, D-galactose, D-mannose, D-fructose, D-xylose and L-arabinose are no substrates [1]) [1]
P ?

Metals, ions

Mg^{2+} <1> (<1> requirement [1]) [1]
$MnSO_4$ <1> (<1> Mn^{2+} can replace Mg^{2+} [1]) [1]
Additional information <1> (<1> Ca^{2+} is inactive [1]) [1]

Specific activity (U/mg)

37 <1> (<1> pH 8.0, 37°C [1]) [1]

K_m-Value (mM)

0.83 <1> (ATP, <1> pH 8.0, 37°C [1]) [1]
1.2 <1> (D-arabinose, <1> pH 8.0, 37°C [1]) [1]

pH-Optimum

9.5 <1> [1]

pH-Range

5.5-10.5 <1> (<1> 40% of maximal activity at pH 5.5, 60% of maximal activity at pH 10.5 [1]) [1]

Temperature optimum (°C)

37 <1> (<1> assay at [1]) [1]

5 Isolation/Preparation/Mutation/Application

Purification

<1> (strain E14, partial [1]) [1]

6 Stability

General stability information

<1>, 0.1 M $(NH_4)_2SO_4$ stabilizes during purification [1]

Storage stability

<1>, 0°C, crude extract, stable for several weeks [1]
<1>, 0°C, partially purified enzyme: 46% loss of activity after 1 week, complete loss of activity within 2 weeks [1]

References

[1] Volk, W.A.: Purification and properties of D-arabinokinase from Proprionibacterium pentosaceum. J. Biol. Chem., **237**, 19-23 (1962)

Allose kinase 2.7.1.55

1 Nomenclature

EC number
2.7.1.55

Systematic name
ATP:D-allose 6-phosphotransferase

Recommended name
allose kinase

Synonyms
D-allokinase
D-allose-6-kinase
allokinase
kinase, allo- (phosphorylating)

CAS registry number
9031-78-1

2 Source Organism

<1> *Aerobacter aerogenes* [1]

3 Reaction and Specificity

Catalyzed reaction
ATP + D-allose = ADP + D-allose 6-phosphate

Reaction type
phospho group transfer

Natural substrates and products
S ATP + D-allose <1> (<1> first step of Embden-Meyerhof-Parnas pathway [1]) [1]
P ADP + D-allose 6-phosphate

Substrates and products
S ATP + D-allose <1> (Reversibility: ? <1> [1]) [1]
P ADP + D-allose 6-phosphate <1> [1]
S ATP + D-galactose <1> (<1> 3.5% of activity with D-allose [1]) (Reversibility: ? <1> [1]) [1]

P ADP + D-galactose 6-phosphate
S ATP + D-glucose <1> (<1> 20% of activity with D-allose [1]) (Reversibility: ? <1> [1]) [1]
P ADP + D-glucose 6-phosphate
S ATP + D-ribose <1> (<1> 6.3% of activity with D-allose [1]) (Reversibility: ? <1> [1]) [1]
P ADP + D-ribose 6-phosphate
S ATP + allitol <1> (<1> 2.8% of activity with D-allose [1]) (Reversibility: ? <1> [1]) [1]
P ADP + allitol 6-phosphate
S Additional information <1> (<1> CTP: 50% of activity with ATP, ITP: 43% of activity with ATP [1]) [1]
P ?

Inhibitors

EDTA <1> (<1> 10 mM: 50% inhibition [1]) [1]
Hg^{2+} <1> (<1> strong [1]) [1]
p-chloromercuribenzoate <1> (<1> strong, addition of GSH reduces inhibition to 13% [1]) [1]

Metals, ions

Ca^{2+} <1> (<1> 37% as effective as Mg^{2+} [1]) [1]
Mg^{2+} <1> (<1> requirement [1]) [1]

Specific activity (U/mg)

1.93 <1> (<1> 25°C, pH 6.5 [1]) [1]

K_m-Value (mM)

0.98 <1> (D-allose, <1> 25°C, pH 6.5 [1]) [1]

pH-Optimum

6.5 <1> [1]

pH-Range

5-7.5 <1> (<1> 40% of maximal activity at pH 5, 50% of maximal activity at pH 7.5 [1]) [1]

Temperature optimum (°C)

25 <1> (<1> assay at [1]) [1]

5 Isolation/Preparation/Mutation/Application

Purification

<1> (partial [1]) [1]

6 Stability

pH-Stability

5-8 <1> (<1> 0°C, 10 min, 10% remaining activity at pH 5, 80% remaining activity at pH 8 [1]) [1]

General stability information

<1>, GSH stabilizes [1]

Storage stability

<1>, 0°C, 0.01 M glycerol, 0.002 M KH_2PO_4, 0.001 M EDTA, 16 h, 70-80% remaining activity [1]

References

[1] Gibbins, L.N.; Simpson, F.J.: The purification and properties of D-allose-6-kinase from Aerobacter aerogenes. Can. J. Biochem., **9**, 769-779 (1963)

1-Phosphofructokinase 2.7.1.56

1 Nomenclature

EC number
2.7.1.56

Systematic name
ATP:D-fructose-phosphate 6-phosphotransferase

Recommended name
1-phosphofructokinase

Synonyms
D-fructose-1-phosphate kinase
fructose 1-phosphate kinase
kinase, 1-phosphofructo- (phosphorylating)
phosphofructokinase 1

CAS registry number
37278-03-8

2 Source Organism

<1> *Aerobacter aerogenes* (wild-type strain PRL-R3 [2]; fructose 6-phosphate kinase-deficient mutant of a uracil auxotroph of strain PRL-R3 [4]) [1, 2, 4]
<2> *Bacteroides symbiosus* [3]
<3> *Clostridium pasteurianum* [5, 6]
<4> *Escherichia coli* (K12 strain jOD5 [7]) [7, 10]
<5> *Pseudomonas doudoroffii* (wild-type strain 75 [8]) [8]
<6> *Pseudomonas putida* [9]

3 Reaction and Specificity

Catalyzed reaction
ATP + D-fructose 1-phosphate = ADP + D-fructose 1,6-bisphosphate

Reaction type
phospho group transfer

Natural substrates and products

- **S** ATP + D-fructose 1-phosphate <1, 2, 6> (<1,2> inducible enzyme [1,3]; <1> involved in D-fructose metabolism [1]; <6> sole pathway of D-fructose metabolism in several Pseudomonas species [9]) (Reversibility: ? <1, 2, 6> [1, 3, 9]) [1, 3, 9]
- **P** ADP + D-fructose 1,6-bisphosphate <1, 2, 6> [1, 3, 9]

Substrates and products

- **S** ATP + D-fructose 1-phosphate <1-6> (<1> specific for D-fructose 1-phosphate, no activity with L-fructose 1-phosphate, D-fructose, D-mannose 6-phosphate [2, 4]; <1, 2, 4, 5> no activity with D-fructose 6-phosphate, [1-4, 7, 8]; <1,4> no activity with D-glucose 6-phosphate, D-glucose 1-phosphate, [2,4,7]; <1,2> no activity with sorbose 1-phosphate [3,4]) (Reversibility: ? <1-6> [1-9]) [1-9]
- **P** ADP + D-fructose 1,6-bisphosphate <1-6> [1-9]
- **S** CTP + D-fructose 1-phosphate <1, 4> (<4> 10% of activity with ATP [7]; <1> poor substrate [2]) (Reversibility: ? <1,4> [2,7]) [2, 7]
- **P** CDP + D-fructose 1,6-bisphosphate <1, 4> [2, 7]
- **S** GTP + D-fructose 1-phosphate <1, 2, 4-6> (<5> 69% of activity with ATP [8]; <4> 60% of activity with ATP [7]; <1> 35% of activity with ATP [2]) (Reversibility: ? <1, 2, 4-6> [2, 3, 7-9]) [2, 3, 7-9]
- **P** GDP + D-fructose 1,6-bisphosphate <1, 2, 4-6> [2, 3, 7-9]
- **S** ITP + D-fructose 1-phosphate <1, 2, 5, 6> (<5> 45% of activity with ATP [8]; <1> 43% of activity with ATP [2]) (Reversibility: ? <1, 2, 5, 6> [2, 3, 8, 9]) [2, 3, 8, 9]
- **P** IDP + D-fructose 1,6-bisphosphate <1, 2, 5, 6> [2, 3, 8, 9]
- **S** TTP + D-fructose 1-phosphate <1> (<1> poor substrate [2]) (Reversibility: ? <1> [2]) [2]
- **P** TDP + D-fructose 1,6-bisphosphate <1> [2]
- **S** UTP + D-fructose 1-phosphate <1, 2, 4> (<4> 20% of activity with ATP [7]; <1> poor substrate [2]) (Reversibility: ? <1,2,4> [2,3,7]) [2, 3, 7]
- **P** UDP + D-fructose 1,6-bisphosphate <1, 2, 4> [2, 3, 7]

Inhibitors

ADP <3-6> (<4> kinetics [7]; <5> ATP reverses [8]; <3> competitive vs. ATP [5]; <6> 2 mM, 45% inhibition, 5 mM ATP completely relieves inhibition [9]) [5, 7-9]

ATP <1, 3, 5, 6> (<5,6> free form [8,9]; <1,5,6> at a Mg^{2+}/ATP ratio below 2 [2,8,9]; <1> additional Mg^{2+} reverses [2]) [2, 5, 8, 9]

CTP <3> (<3> if concentration exceeds Mg^{2+} concentration [5]) [5]

Cu^{2+} <3> (<3> 0.5 mM, 50% inhibition in the presence of 3 mM Mg^{2+} [5]) [5]

D-fructose 1,6-bisphosphate <1, 3, 4> (<4> kinetics [7]; <3> noncompetitive vs. fructose 1-phosphate [5]) [2, 5, 7]

D-fructose 6-phosphate <1> [2]

GTP <1, 3> (<1> at a Mg^{2+}/GTP ratio below 2 [2]) [2, 5]

Hg^{2+} <3> (<3> 0.002 mM, 50% inhibition in the presence of 3 mM Mg^{2+} [5]) [5]

ITP <1, 3> (<1> at a Mg^{2+}/ITP ratio below 2 [2]) [2, 5]

K^+ <5> (<5> 300 mM, activates at 30 mM [8]) [8]
NaCl <5> [8]
Ni^{2+} <3> (<3> 3 mM, 50% inhibition in the presence of 3 mM Mg^{2+} [5]) [5]
SO_4^{2-} <3, 5, 6> (<5> above 25 mM [8]) [5, 8, 9]
UTP <3> (<3> if concentration exceeds Mg^{2+} concentration [5]) [5]
citrate <1> [2]
phenylmethanesulfonyl fluoride <4> (<4> 1 mM, 65% irreversible inhibition, 1.5 M ATP protect [7]) [7]
Additional information <1-6> (<1> not inhibited by nucleoside monophosphates or diphosphates, D-mannose 6-phosphate, D-fructose, L-fructose 1-phosphate [2]; <2> not inhibited by sorbose 1-phosphate [3]; <1, 3, 4, 6> not inhibited by D-glucose 6-phosphate, [2, 5, 7, 9]; <1, 3, 4> not inhibited by D-glucose 1-phosphate [2, 5, 7]; <1, 3, 5, 6> not inhibited by phosphate, AMP [2, 5, 8, 9]; <3> not inhibited by monovalent cations, glucose, ADPglucose, UDPglucose, NAD(H), NADP(H), dihydroxyacetone phosphate, glyceraldehyde 3-phosphate, 3-phosphoglycerate, CoA, acetyl-CoA, acetoacetyl-CoA [5]; <3, 5, 6> not inhibited by cAMP, pyruvate, phosphoenolpyruvate [5, 8, 9]; <5> not inhibited by L-glutamate [8]; <6> not inhibited by 6-phosphogluconate, 2-oxo-3-deoxy-6-phosphogluconate [9]; <1> not inhibited by ATP [2]; <2-6> not inhibited by D-fructose 6-phosphate [3, 5, 7-9]; <5, 6> not inhibited by CTP and UTP [8, 9]; <3, 5, 6> not inhibited by citrate [5, 8, 9]) [2, 3, 5, 7-9]

Activating compounds

Additional information <6> (<6> no activation by pyruvate, phosphoenolpyruvate, AMP, cAMP, citrate, fructose 6-phosphate, glucose 6-phosphate, 6-phosphogluconate or 2-keto-3-deoxy-6-phosphogluconate [9]) [9]

Metals, ions

Co^{2+} <3, 4> (<4> 5 mM, 25% of activity with Mg^{2+} [7]; <3> 3 mM, 80% of activity with Mn^{2+} [5]) [5, 7]
Fe^{2+} <3> (<3> 3 mM, 50% of activity with Mn^{2+} [5]) [5]
K^+ <1, 4, 5, 6> (<5> activation at 30 mM, inhibiton at 300 mM [8]; <1> 40 mM, 3fold increase of activity [4]; <6> 30-50 mM, 2.3fold activation [9]; <4> 50 mM, 3fold increase of maximal activity [10]) [4, 8, 9, 10]
Mg^{2+} <1-6> (<1-6> required for activity [1-9]; <5> can replace Mn^{2+} [8]; <1> maximal activity at a Mg^{2+}/ATP ratio of at least 2 [4]; <5,6> actual substrate: $MgATP^{2-}$ [8,9]; <3> 3 mM, 84% of activity with Mn^{2+} [5]; <6> sigmoidal response, maximal activity at 5 mM [9]) [1-9]
Mn^{2+} <2-5> (<2-5> required for activity [3,5-8]; <2,3> can replace Mg^{2+} [3,5]; <4> 5 mM, 50% of activity with Mg^{2+} [7]) [3, 5-8]
NH_4^+ <5, 6> (<5> stimulation [8]; <6> 30-50 mM, 1.5fold activation [9]) [8, 9]
Na^+ <6> (<6> 30-50 mM, 1.5fold activation [9]) [9]
Zn^{2+} <3> (<3> 3 mM, 34% of activity with Mn^{2+} [5]) [5]
Additional information <2, 4, 5> (<6> not activated by phosphate [9]; <4> not activated by Ca^{2+}, Cu^{2+} and Fe^{2+} [7]; <2> not activated by Ca^{2+} [3]; <5> not activated by Na^+ [8]) [3, 7, 8]

Specific activity (U/mg)
0.27 <1> (<1> activity in crude extracts [1]) [1]
4.02 <6> [9]
4.2 <5> [8]
6.9-81.6 <2> [3]
47 <1> [2, 4]
Additional information <1, 3> [1, 5]

K_m-Value (mM)
0.12 <4> (ATP, <4> pH 7.6, 37°C [7]) [7]
0.13 <4> (D-fructose 1-phosphate, <4> pH 7.1, 30°C, in the presence of 50 mM KCl [10]) [10]
0.14 <3> (ATP, <3> pH 8.0, 25°C [5]) [5]
0.16 <6> (D-fructose 1-phosphate, <6> pH 8.0, 25°C [9]) [9]
0.19 <6> (D-fructose 1-phosphate, <6> pH 9.0, 25°C [9]) [9]
0.25 <4> (D-fructose 1-phosphate, <4> pH 7.6, 37°C [7]) [7]
0.3 <1, 5> (D-fructose 1-phosphate, <1> pH 7.5, 25°C [4]) [1, 4, 8]
0.34 <5> (ATP) [8]
0.35 <4> (ATP, <4> pH 7.1, 30°C, in the presence of 5 mM KCl [10]) [10]
0.36 <4> (D-fructose 1-phosphate, <4> pH 7.1, 30°C, in the absence of KCl [10]) [10]
0.41 <6> (ATP, <6> pH 8.0, 25°C [9]) [9]
0.6 <4> (ATP, <4> pH 7.1, 30°C, in the presence of 50 mM KCl [10]) [10]
0.62 <3> (D-fructose 1-phosphate, <3> pH 8.0, 25°C [5]) [5]
0.67 <3> (ITP, <3> pH 8.0, 25°C [5]) [5]
0.7 <1> (ATP) [2]
0.75 <1> (D-fructose 1-phosphate) [2]
0.8 <1> (ATP) [1]

K_i-Value (mM)
0.28 <4> (ADP, <4> pH 7.6, 37°C [7]) [7]
6.1 <4> (D-fructose-1,6-bisphosphate, <4> pH 7.6, 37°C [7]) [7]

pH-Optimum
7.5 <1> [2, 4]
7.8 <4> (<4> 50 mM Tris-HCl [7]) [7]
7.8-9.2 <3> [5]
8-8.8 <2> (<2> 50% activity at pH 7.1 [3]) [3]
8.5 <4> (<4> 50 mM NH_4HCO_3 [7]) [7]
9 <5, 6> [8, 9]

pH-Range
5.5-10.2 <6> (<6> very low activity at pH 5.5, approx. half-maximal activity at pH 6.3 and pH 10.2 [9]) [9]
7-8 <5> (<5> 44% of maximal activity at pH 7 and 81% of maximal activity at pH 8 [8]) [8]
7-8.7 <1> (<1> approx. 80% of maximal activity at pH 7. approx. half-maximal activity at pH 8.7 [2]) [2]
7-9.8 <3> (<3> approx. half-maximal activity at pH 7 and 9.8 [5]) [5]

Temperature optimum (°C)
25 <1, 2, 6> (<1,2,6> assay at [1,3,9]) [1, 3, 9]
28 <5> (<5> assay at [8]) [8]
37 <4> (<4> assay at [7]) [7]

4 Enzyme Structure

Molecular weight
57000 <4> (<4> gel filtration [7]) [7]
62500 <3> (<3> low-speed sedimentation equilibrium [5]) [5]
63100 <3> (<3> gel filtration [5]) [5]
63200 <3> (<3> sucrose density gradient centrifugation [5]) [5]
75000 <1> (<1> gel filtration [2,4]) [2, 4]

Subunits
dimer <3, 4> (<3> 1 * 21400 + 1 * 35500, SDS-PAGE, after treatment with 8 M urea and 2-mercaptoethanol [5]; <3> 1 * 28500 + 1 * 40000, SDS-PAGE, after treatment with 2-mercaptoethanol [5]; <4> 2 * 30000, SDS-PAGE [7]; <4> 2 * 34000, recombinant enzyme, SDS-PAGE [10]) [5, 7, 10]

5 Isolation/Preparation/Mutation/Application

Localization
soluble <2> [3]

Purification
<1> (protamine sulfate, ammonium sulfate, heat, Sephadex G-100, DEAE-cellulose, partially purified [1]) [1, 2, 4]
<2> (partial [3]) [3]
<3> (CM-Sephadex, calcium phosphate gel, Sephadex G-100, DEAE-Sephadex, hydroxyapatite [5]) [5, 6]
<4> (DEAE-cellulose, ammonium sulfate, hydroxylapatite, Sephadex G-75, DEAE-cellulose [7]; ammonium sulfate, Sephadex G25, DEAE-Sepharose [10]) [7, 10]
<5> (partial [8]) [8]
<6> (ammonium sulfate, DEAE-Sephadex, partially purified [9]) [9]

Cloning
<4> (overexpression in Escherichia coli [10]) [10]

6 Stability

General stability information

<2>, dialysis against water, 0.9% NaCl or buffer, stable to [3]
<2>, partially purified enzyme withstands freeze-thawing, purified enzyme loses 50% of activity by freezing and thawing [3]
<3>, Tris-HCl buffer in purification and electrophoresis leads to partial denaturation and loss of activity, stable in phosphate buffer [5]
<3>, freeze-thawing, stable to [5]

Storage stability

<1>, -20°C, 100 mM $(NH_4)_2SO_4$, pH 7.5, at least 8 months [2]
<1>, -20°C, 100 mM $(NH_4)_2SO_4$, pH 7.5, several months, no loss of activity [4]
<1>, -20°C, crude enzyme extract prepared in water, at least 3 weeks [2]
<2>, 4°C, a few days [3]
<3>, -20°C, 50 mM phosphate buffer, pH 7.5, 1 year, no loss of activity [5]
<4>, -80°C, 20% glycerol, 2 years, no loss of activity [10]
<5>, -15°C, 3 days, 70% loss of activity [8]
<5>, 4°C, 1 week, about 30% loss of activity [8]
<6>, 4°C, 0.8-1.3 mg protein/ml in 50 mM phosphate buffer, pH 7, 0.5 mM dithiothreitol, 5-10% loss of activity [9]

References

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Mannitol kinase 2.7.1.57

1 Nomenclature

EC number
2.7.1.57 (deleted)

Recommended name
mannitol kinase

2-Dehydro-3-deoxygalactonokinase 2.7.1.58

1 Nomenclature

EC number
2.7.1.58

Systematic name
ATP:2-dehydro-3-deoxy-D-galactonate 6-phosphotransferase

Recommended name
2-dehydro-3-deoxygalactonokinase

Synonyms
2-keto-3-deoxygalactonate kinase (phosphorylating)
2-keto-3-deoxygalactonokinase
2-oxo-3-deoxygalactonate kinase
KDGal kinase <3> [3]
KDGalA kinase <5> [4]

CAS registry number
37278-050

2 Source Organism

<1> *Escherichia coli* (strains NCTC 9001(neotype), B, Crookes, K10, PA309, X289, DF 1070 gnd edd and A1201 kga [2]) [2]
<2> *Gluconobacter liquefaciens* [1]
<3> *Mycobacterium butyricum* [3]
<4> *Salmonella typhimurium* (LT-2 [2]) [2]
<5> *Stenotrophomonas maltophilia* (Ac, (DSM 14322), previously named Pseudomonas sp. strain Ac [4]) [4]

3 Reaction and Specificity

Catalyzed reaction
ATP + 2-dehydro-3-deoxy-D-galactonate = ADP + 2-dehydro-3-deoxy-D-galactonate 6-phosphate

Reaction type
phospho group transfer

Natural substrates and products

S ATP + 2-dehydro-3-deoxy-D-galactonate <1, 2, 4, 5> (<1> exogenous galactonate catabolism [2]; <5> L-glucitol catabolism [4]) (Reversibility: ? <1, 2, 4, 5> [1, 2, 4]) [1, 2, 4]

P ADP + 2-dehydro-3-deoxy-6-phosphogalactonate <1, 2, 4, 5> [1, 2, 4]

Substrates and products

S ATP + 2-dehydro-3-deoxy-D-galactonate <1, 2, 4, 5> (Reversibility: ? <1, 2, 4, 5> [1,2,4]) [1, 2, 4]

P ADP + 2-dehydro-3-deoxy-6-phosphogalactonate <1, 2, 4, 5> [1, 2, 4]

S Additional information <1, 2, 4> (<2> induced by growth on galactose medium [1]; <1,4> enzyme only present in galactonate grown cells, can not be detected in glycerol-, gluconate-, or galactose-grown cells [2]) [1, 2]

P ?

Specific activity (U/mg)

0.04 <5> (<5> in presence of KDGalA 6-P aldolase [4]) [4]
0.06 <4> [2]
0.086 <3> [3]
0.18 <1> (<1> strain Crookes [2]) [2]
0.24 <1> (<1> strain K10 [2]) [2]

5 Isolation/Preparation/Mutation/Application

Localization

membrane <2> (<2> protoplasmic [1]) [1]

Purification

<1> (partial [2]) [2]
<2> (partial [1]) [1]
<3> (partial [3]) [3]

6 Stability

Storage stability

<1>, 4°C, 50 mM imidazole-HCl, 0.5 mM DTT, 0.6 mM $FeSO_4$ buffer, pH 7.0, remains stable after 4 days [2]

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N-Acetylglucosamine kinase 2.7.1.59

1 Nomenclature

EC number
2.7.1.59

Systematic name
ATP:N-acetyl-D-glucosamine 6-phosphotransferase

Recommended name
N-acetylglucosamine kinase

Synonyms
2-acetylamino-2-deoxy-D-glucose kinase
ATP:2-acetylamino-2-deoxy-D-glucose 6-phosphotransferase
CaNag5p <6> (<6> part of cluster with 6 genes, product from gene CaNag5 [12]) [12]
acetylaminodeoxyglucokinase
acetylglucosamine kinase(phosphorylating)

CAS registry number
9027-48-9

2 Source Organism

<1> *Streptococcus pyogenes* (strain 523 serotype 14) [1]
<2> *Escherichia coli* [2]
<3> *Sus scrofa* (hog [3,5]) [3, 5]
<4> *Capra hircus* [3]
<5> *Homo sapiens* [4, 9, 12]
<6> *Candida albicans* [6, 12]
<7> *Rattus norvegicus* [7, 8, 10]
<8> *Plasmodium falciparum* (malaria parasite [9]) [9]
<9> *Mus musculus* (sequence with additional 92 bp sequence found in lung, SwissProt-ID: Q9QZ08 [11]) [11]
<10> *Homo sapiens* (SwissProt-ID: Q9UJ70) [11]
<11> *Mus musculus* [13]

3 Reaction and Specificity

Catalyzed reaction

ATP + N-acetyl-D-glucosamine = ADP + N-acetyl-D-glucosamine 6-phosphate

Reaction type

phospho group transfer

Natural substrates and products

- **S** ATP + N-acetyl-D-glucosamine <1-11> (<5> part of glycoprotein synthesis pathway [4]; <3> involved in UDP-N-acetylglucosamine formation [5]; <6> first reaction of inducible N-acetylglucosamine catabolic pathway, enables organism to grow on N-acetylglucosamine as sole carbon source [6, 12]; <11> first enzyme of N-acetyl-D-glucosamine salvage pathway [13]) (Reversibility: ? <1-11> [1-13]) [1-13]
- **P** ADP + N-acetyl-D-glucosamine 6-phosphate <1-11> [1-13]

Substrates and products

- **S** ATP + D-glucose <1, 2, 6, 7> (<1> at equal rate than N-acetyl-D-glucosamine [1]; <2> 15% of the rate with N-acetyl-D-glucosamine [2]; <7> very poor substrate, 4.5% of the rate with N-acetyl-D-glucosamine [8]) (Reversibility: ? <1, 2, 6, 7> [1, 2, 7, 8, 12]) [1, 1, 2, 7, 8, 12]
- **P** ADP + D-glucose 6-phosphate <1, 2, 7> [1, 2, 7, 8]
- **S** ATP + D-mannose <6> (Reversibility: ? <6> [12]) [12]
- **P** ?
- **S** ATP + N-acetyl-D-glucosamine <1-11> (<3,6> highly specific [3,6]) (Reversibility: ? <1-11> [1-13]) [1-13]
- **P** ADP + N-acetyl-D-glucosamine 6-phosphate <1-11> [1-13]
- **S** ATP + N-acetyl-D-mannosamine <5, 7, 9> (<5, 7> roughly 50% of activity [4, 7]; <7> 78% of the rate with N-acetyl-D-glucosamine [8]; <9> recombinant enzyme, same rate than with N-acetyl-D-glucosamine [11]) (Reversibility: ? <5, 7, 9> [4, 7, 8, 11]) [4, 7, 8, 11]
- **P** ADP + N-acetyl-D-mannosamine 6-phosphate
- **S** CTP + N-acetyl-D-glucosamine <6> (Reversibility: ? <6> [6]) [6]
- **P** CDP + N-acetyl-D-glucosamine 6-phosphate
- **S** GTP + N-acetyl-D-glucosamine <3> (<3> 30% of the activity with ATP [3,5]) (Reversibility: ? <3> [3, 5]) [3, 5]
- **P** GDP + N-acetyl-D-glucosamine 6-phosphate
- **S** ITP + N-acetyl-D-glucosamine <2> (<2> 75% of the activity with ATP [2]) (Reversibility: ? <2> [2]) [2]
- **P** IDP + N-acetyl-D-glucosamine 6-phosphate
- **S** dATP + N-acetyl-D-glucosamine <6> (Reversibility: ? <6> [6]) [6]
- **P** dADP + N-acetyl-D-glucosamine 6-phosphate
- **S** Additional information <1-3, 5, 6, 7, 9> (<1, 3> D-fructose, D-mannose, D-maltose, D-galactose, N-acetyl-D-galactosamine, N-acetyl-D-mannosamine do not serve as substrates [1, 3]; <2> N-methyl-D-glucosamine, D-glucosamine, N-acetyl-D-galactosamine, N-acetyl-D-mannosamine do not

serve as substrates, very low activities with UTP, GTP and CTP [2]; <3> no activity with UTP, CTP, ADP or phosphoenol pyruvate [3]; <7> no activity with D-galactose and D-fructose, very low activities with N-acetyl-D-galactosamine: 7.6%, D-glucosamine: 11.2%, D-mannosamine: 2.7%, D-galactosamine: 2.1%, D-mannose: 4.5% and D-xylose: 2.1% [8]; <9> D-fructose, D-mannose, D-maltose, D-galactose, N-acetyl-D-galactosamine, ribose, fucose do not serve as substrates [11]; <5,6> D-fructose, D-galactose, galactosamine, N-acetyl-D-galactosamine, mannosamine, N-acetyl-D-mannosamine do not serve as substrates [12]) [1-3, 8, 11, 12]

P ?

Inhibitors

5,5'-dithiobis(2-nitrobenzoic acid) <7> (<7> substrates partially protect against inactivation [10]) [10]
ADP <2, 3, 7> (<2,3> strong competitive inhibitor [2,3]) [2, 3, 7]
CTP <3> [3, 5]
D-glucosamine <2> [2]
D-glucose <1> (<1> competitive with N-acetyl-D-glucosamine [1]) [1]
N-acetyl-D-glucosamine <1, 9> (<1> competitive with glucose [1]; <9> inhibition of activity with N-acetyl-D-mannosamine [11]) [1, 11]
N-acetyl-D-glucosamine <7> (<7> inhibits phosphorylation of D-glucose [7]) [7]
N-acetyl-D-glucosamine-6-phosphate <3, 5, 8> (<3> non-competitive [3,5]) [3, 5, 9]
N-acetyl-D-mannosamine <7, 9> (<7> inhibits phosphorylation of D-glucose [7]; <9> no inhibition of activity with N-acetyl-D-glucosamine [11]) [7, 11]
N-ethylmaleimide <6, 7> (<7> substrates partially protect against inactivation [10]) [6, 10]
N-methyl-D-glucosamine <2> [2]
UDP-N-acetylglucosamine <3> [3, 5]
UTP <3> [3, 5]
arsenite/2,3-dimercaptopropanol <7> (<7> substrates partially protect against inactivation [10]) [10]
diamide <7> (<7> substrates partially protect against inactivation [10]) [10]
diphosphate <3> [3, 5]
iodoactamide <7> (<7> substrates partially protect against inactivation [10]) [10]
p-chloromercuribenzoate <3, 5, 6> (<3> complete inhibition at 0.4 mM [3]; <5> irreversible [4]; <3> reversible with cysteine or 2-mercaptoethanol [3,5]) [3-6]
p-hydroxymercuribenzoate <1> (<1> no protection by substrate, protection but not reversal of inhibition by thioethanol, complete inhibition above 0.00125 mM, cysteine reverses inhibition [1]) [1]
periodate <7> (<7> substrates partially protect against inactivation [10]) [10]
phosphate <3> [3, 5]

Activating compounds

2- mercaptoethanol <3> (<3> reverses inhibition with *p*-chloromercuribenzoate and loss of activity during storage [3]) [3, 5]
cysteine <1, 3> (<1> reverses inhibition with *p*-hydroxymercuribenzoate [1]; <3> reverses inhibition with *p*-chloroxymercuribenzoate [3,5]) [1, 3, 5]

Metals, ions

Ba^{2+} <6> (<6> can partially replace Mg^{2+} in activation [6]) [6]
Ca^{2+} <6> (<6> can partially replace Mg^{2+} in activation [6]) [6]
Co^{2+} <3, 5, 6> (<3,5,6> can partially replace Mg^{2+} in activation [3-6]) [3-6]
Mg^{2+} <3, 5-8> (<3,5-8> required [3-7,9]) [3-7, 9]
Mn^{2+} <3, 5, 6> (<3,5,6> can partially replace Mg^{2+} in activation [3-6]) [3-6]
Additional information <3, 5> (<3> no activity with Ca^{2+}, Ba^{2+} [3,5]; <5> slight activities with Ni^{2+}, Zn^{2+}, Cu^{2+} [4]) [3, 4, 5]

Turnover number (min^{-1})

1.8 <6> (N-acetyl-D-glucosamine, <6> 37°C, pH 7.5 [12]) [12]
1.95 <5> (N-acetyl-D-glucosamine, <5> 37°C, pH 7.5 [12]) [12]

Specific activity (U/mg)

0.00009 <5> (<5> 37°C, pH 7.5 [9]) [9]
0.00012 <8> (<8> 37°C, pH 7.5 [9]) [9]
0.009 <4> (<4> spleen, 37°C, pH 9.0 [3]) [3]
0.025 <3> (<3> spleen, 37°C, pH 9.0 [3]) [3]
0.137 <9> (<9> enzyme expressed in E. coli, 37°C, pH 7.5 [11]) [11]
1.28 <5> (<5> purified enzyme, 37°C, pH 8.0 [4]) [4]
4.6 <2> (<2> purified enzyme, 37°C, pH 7.8 [2]) [2]
9.5 <6> (<6> purified enzyme, 30°C, pH 7.6 [6]) [6]
25 <9> (<9> purified enzyme from E. coli, 37°C, pH 7.5 [11]) [11]
39 <7> (<7> purified enzyme from liver, 32°C, pH 7.5 [8]) [8]
42 <7> (<7> purified enzyme from kidney, 32°C, pH 7.5 [8]) [8]
52.4 <7> (<7> purified enzyme, 37°C, pH 7.5 [10]) [10]
90 <3> (<3> purified enzyme, 37°C, pH 9.5 [3,5]) [3, 5]
250 <1> (<1> purified enzyme, 30°C, pH 7.4 [1]) [1]

K_m-Value (mM)

0.00005 <2> (N-acetyl-D-glucosamine, <2> 37°C, pH 7.8 [2]) [2]
0.00014 <2> (ATP, <2> 37°C, pH 7.8 [2]) [2]
0.00025 <2> (ITP, <2> 37°C, pH 7.8 [2]) [2]
0.00077 <1> (N-acetyl-D-glucosamine, <1> 30°C, pH 7.4 [1]) [1]
0.0011 <1> (D-glucose, <1> 30°C, pH 7.4 [1]) [1]
0.005 <2> (D-glucose, <2> 37°C, pH 7.8 [2]) [2]
0.04 <7> (N-acetyl-D-glucosamine, <7> enzyme from kidney, 32°C, pH 7.5 [7]) [7]
0.05 <7> (N-acetyl-D-glucosamine, <7> 37°C, pH 7.5 [10]) [10]
0.06 <7> (N-acetyl-D-glucosamine, <7> enzyme from liver, 32°C, pH 7.5 [7]) [7]
0.089 <5> (N-acetyl-D-glucosamine, <5> 37°C, pH 7.5 [9]) [9]

0.1 <7> ($MgATP^{2-}$, <7> enzyme from kidney with N-acetyl-D-glucosamine as substrate, 32°C, pH 7.5 [7]) [7]
0.11 <5> (N-acetyl-D-glucosamine, <5> 37°C, pH 8.0 [4]) [4]
0.23 <7> ($MgATP^{2-}$, <7> enzyme from liver with N-acetyl-D-glucosamine as substrate, 32°C, pH 7.5 [7]) [7]
0.376 <6> (N-acetyl-D-glucosamine, <6> 37°C, pH 7.5 [12]) [12]
0.426 <6> (D-mannose, <6> 37°C, pH 7.5 [12]) [12]
0.445 <5> (N-acetyl-D-glucosamine, <5> 37°C, pH 7.5 [12]) [12]
0.483 <6> (D-glucose, <6> 37°C, pH 7.5 [12]) [12]
0.95 <7> (N-acetyl-D-mannosamine, <7> enzyme from liver, 32°C, pH 7.5 [7]) [7]
1 <7> (N-acetyl-D-mannosamine, <7> 37°C, pH 7.5 [10]) [10]
1 <7> (N-acetyl-D-mannosamine, <7> enzyme from kidney, 32°C, pH 7.5 [7]) [7]
1.1 <3> (N-acetyl-D-glucosamine, <3> 37°C, pH 9.0 [3,5]) [3, 5]
1.33 <6> (N-acetyl-D-glucosamine, <6> 30°C, pH 7.6 [6]) [6]
1.8 <3> (ATP, <3> 37°C, pH 9.0 [3,5]) [3, 5]
1.8 <5> (ATP, <5> 37°C, pH 8.0 [4]) [4]
1.82 <6> (ATP, <6> 30°C, pH 7.6 [6]) [6]
410 <7> (D-glucose, <7> enzyme from kidney, 32°C, pH 7.5 [7]) [7]
600 <7> (D-glucose, <7> enzyme from liver, 32°C, pH 7.5 [7]) [7]
Additional information <11> (<11> K_m for both substrates tested for wild type and all mutant enzymes [13]) [13]

K_i-Value (mM)

0.0009 <1> (N-acetyl-D-glucosamine, <1> for reaction with glucose, 30°C, pH 7.4) [1]
0.001 <2> (N-methyl-D-glucosamine, <2> 37°C, pH 7.8 [2]) [2]
0.0018 <1> (D-glucose, <1> for reaction with N-acetyl-D-glucosamine, 30°C, pH 7.4) [1]
0.01 <2> (D-glucosamine, <2> 37°C, pH 7.8 [2]) [2]
0.9 <3> (ADP, <3> 37°C, pH 9.0 [3]) [3]

pH-Optimum

7-8 <1, 6> (<1> strongly decreasing activity outside this range [1]) [1, 6]
7.4 <7> [7]
7.5-9.5 <2> [2]
7.8-8 <5> [4]
8.6-9.4 <3> [3, 5]

4 Enzyme Structure

Molecular weight

39000 <7, 11> (<7> SDS-PAGE, second band at 90000 Da shows no activity [8,10]; <7> identification of 90000 Da band, found to be another protein [10]; <11> SDS-PAGE, wild type protein [13]) [8, 10, 13]
41000 <9> (<9> SDS-PAGE, from E. coli [11]) [11]

42000 <11> (<11> SDS-PAGE, from E. coli [13]) [13]
75000 <6> (<6> gel filtration [6]) [6]
77000 <5> (<5> gel filtration [4]) [4]
80000 <7> (<7> gel filtration [8,10]) [8, 10]
373000 <9> (<9> calculated from DNA sequence [11]) [11]
374000 <10> (<10> calculated from DNA sequence [11]) [11]

Subunits

dimer <7> (<7> postulated due to kinetic data [7]; <7> 2 * 39000, SDS-PAGE, gel filtration [8,10]) [7, 8, 10]

5 Isolation/Preparation/Mutation/Application

Source/tissue

brain <3, 4, 9, 10> [3, 11]
erythrocyte <5> [9]
gastric mucosa <5> [4]
heart <9, 10> [11]
kidney <3, 4, 7, 9, 10> [3, 7, 8, 11]
liver <3, 4, 7, 9, 10> [3, 7, 8, 10, 11]
lung <9, 10> [11]
muscle <4, 9, 10> [3, 11]
spleen <3, 4, 9, 10> [3, 5, 11]
testis <9, 10> (<9,10> very high activity [11]) [11]
Additional information <10> (<10> present in cancer cell lines [11]) [11]

Localization

cytoplasm <7> [10]
soluble <7> [7]

Purification

<1> [1]
<2> [2]
<3> [3, 5]
<5> (fusion protein from Escherichia coli [12]) [4, 12]
<6> (fusion protein from Escherichia coli [12]) [6, 12]
<7> (from liver and kidney [7]) [7, 10]
<9> (from E. coli [11]) [11]
<11> (from E. coli [13]) [13]

Cloning

<5> (functionally expressed in Escherichia coli as glutathione-S-transferase fusion protein, active enzyme [12]) [12]
<6> (functionally expressed in Escherichia coli as glutathione-S-transferase fusion protein, active enzyme [12]) [12]

<9> (functionally expressed in Escherichia coli BL21, active enzyme, high rate of protein expression in transfected cells, inclusion bodies [11]) [11]
<10> [11]
<11> (6 point mutations in cysteine residues, expression in Escherichia coli BL21 [13]) [13]

Engineering

C131S <11> (<11> strongly decreased activity, very low affinity for N-acetyl-D-glucosamine [13]) [13]
C143S <11> (<11> strongly decreased activity, very low affinity for ATP [13]) [13]
C211S <11> (<11> slightly decreased activity [13]) [13]
C217S <11> (<11> decreased activity, low affinity for N-acetyl-D-glucosamine [13]) [13]
C268S <11> (<11> decreased activity [13]) [13]
C45S <11> (<11> very slightly decreased activity, no function in substrate binding [13]) [13]

6 Stability

pH-Stability

5 <5> (<5> 50% loss of activity within 2 min, no protection with added substrate [4]) [4]
6.2-8.5 <7> (<7> relatively stable at pH 6.2, rapid loss of activity above pH 8.5 [7]) [7]
9.5 <2> (<2> no loss of activity within 30 min at 30°C [2]) [2]

Temperature stability

60 <1> (<1> 25% loss of activity within 1 min in presence of 13% ammonium sulfate, stable for 1 h in presence of ammonium sulfate and substrate [1]) [1]
60 <5> (<5> 84% loss of activity within 2 min, no protection with added substrate [4]) [4]
65 <2> (<2> heat treatment for 5 min included in purification procedure [2]) [2]
65 <6> (<6> complete inactivation with 2 min [6]) [6]
70 <1> (<1> complete inactivation with 1 min [1]) [1]
70 <3> (<3> complete inactivation with 2 min [3]; <3> 50% loss of activity within 2 min [5]) [3, 5]

General stability information

<1>, ammonium sulfate partially stabilizes against temperature inactivation [1]
<1>, dialysis at 4°C for 17 h against 0.005 M Tris buffer, pH 7.6, 92% loss of activity, 33% loss of activity in presence of 0.1 M N-acetyl-D-glucosamine or glucose, stable in presence of 0.2 M or 2.5 [1]
<1>, substrate at 0.2 M stabilizes, unstable in absence of substrate at 4°C [1]

<5>, unstable to dialysis and gel filtration, 0.013 mM substrate protects against inactivation [4]
<3, 6>, stable to dialysis [3, 6]
<3, 6>, unstable to freezing and thawing, heat and low pH [3, 5, 6]

Storage stability

<1>, -25°C, stable for several months [1]
<2>, -20°C, stable for several months [2]
<3>, 4°C, 0.02 M potassium phosphate buffer, pH 7.6, 0.001 M EDTA, 0.01 M 2-mercaptoethanol, stable for at least a week [3, 5]
<5>, -20°C, stable for at least 6 months [4]
<5>, 4°C, stable for at least 3 days [4]
<6>, 0-2°C, 20 mM potassium phosphate, pH 7.6, 1 mM EDTA, 10 mM 2-mercaptoethanol, stable for at least 1 week [6]
<7>, -10°C, several months, no loss of activity [8]

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N-Acylmannosamine kinase 2.7.1.60

1 Nomenclature

EC number
2.7.1.60

Systematic name
ATP:N-acyl-D-mannosamine 6-phosphotransferase

Recommended name
N-acylmannosamine kinase

Synonyms
ATP:N-acetylmannosamine 6-phosphotransferase
EC 5.1.3.14/EC 2.7.1.60 <2, 4> [9-11, 13]
EC 2.7.1.59/EC 2.7.1.60 <3> (<3> bifunctional, N-acetylglucosamine kinase and N-acetylmannosamine kinase activity [14]) [14]
ManAc kinase <4> [8, 10]
N-acetylmannosamine kinase
N-acyl-D-mannosamine kinase
UDP-GlcNAc-epimerase/ManNAc kinase <2-4> [8-13]
acetylamidodeoxymannokinase
acetylmannosamine kinase
acylaminodeoxymannokinase
acylmannosamine kinase
acylmannosamine kinase (phosphorylating)

CAS registry number
9027-53-6

2 Source Organism

<1> *Escherichia coli* (strain B [3]) [3]
<2> *Homo sapiens* (hepatoma cell line HepG2 [7]; human cell lines promyeolytic leukemia HL-60, HeLa S3, chronic myelogenous leukaemia K-562, lymphoblastic leukaemia MOLT-4, Burkitt's lymphoma Raji, colorectal adenocarcinoma SW480, lung carcinoma A549 and melanoma G361 [13]) [7, 13, 14]
<3> *Mus musculus* (mouse [12,14]; NMRI [12]) [12, 14]
<4> *Rattus norvegicus* (rat, albino [2]; Wistar [6-8,12]; hepatoma 7777 [7]; Sprague-Dawley [5]) [1, 2, 5-12]
<5> *Salmonella typhimurium* (LT-2 [4]) [3, 4]
<6> *Staphylococcus aureus* [3]

3 Reaction and Specificity

Catalyzed reaction

ATP + N-acyl-D-mannosamine = ADP + N-acyl-D-mannosamine 6-phosphate (acts on the acetyl and glycolyl derivatives)

Reaction type

phospho group transfer

Natural substrates and products

S ATP + N-acetyl-D-mannosamine <1-6> (<5> highly specific for ATP and N-acetyl-D-mannosamine [3]; <4> metabolic pathway between hexoses and sialic acids [2]; <4> enzyme is involved in sialic acid metabolism [5]; <4> involved in N-acetylneuraminic acid metabolism, key enzyme in N-acetylneuraminic acid biosynthesis [6,9-11]) (Reversibility: ? <1-6> [1-12]) [1-12]

P ADP + N-acetyl-D-mannosamine 6-phosphate <1-6> [1-12]

Substrates and products

S ATP + N-acetyl-D-mannosamine <1-6> (<5> highly specific for ATP and N-acetyl-D-mannosamine [3]) (Reversibility: ? <1-6> [1-12]) [1-12]

P ADP + N-acetyl-D-mannosamine-6-phosphate <1-6> [1-12]

S ATP + N-glycolylmannosamine <4> (Reversibility: ? <4> [2]) [2]

P N-glycolylmannosamine-6-phosphate

S Additional information <3-5> (<5> glucose, mannose, fructose, glucosamine, N-acetylglucosamine and N-acetylgalactosamine are inactive as substrates [3]; <4> N-acetylglucosamine and N-glycolylglucosamine, N-acetylgalactosamine, mannosamine, glucosamine, galactosamine, mannose, galactose, fructose, glucose, glucose-1-phosphate and glucose-6-phosphate are inactive as substrates, the phosphoryl donor ATP cannot be replaced by GTP, UTP, CTP, TTP, dATP or phosphoenolpyruvate [2]; <4> D-glucose, D-mannose, N-acetyl-D-glucosamine and N-acetyl-D-galactosamine are not phosphorylated [1]; <5> no enzyme found when bacteria grow in presence of glucose [4]; <2-4> UDP-GlcNAc 2-epimerase and ManNAc kinase are parts of one bifunctional enzyme, catalyzes the first 2 steps in N-acetylneuraminic acid biosynthesis [8, 10, 12, 13]; <3> displays N-acetylglucosamine kinase activity as well as N-acetylmannosamine kinase activity [14]) [1-4, 8, 10, 12-14]

P ?

Inhibitors

3-O-methyl-N-acetyl-D-glucosamine <4> (<4> non-competitive inhibition [7]) [7]
5,5'-dithiobis(2-nitrobenzoic acid) <4> [9]
N-ethylmaleimide <4> [9]
arsenite/2,3-dimercaptopropanol <4> [9]
diamide <4> [9]
iodoacetamide <4> [9]

p-chloromercuribenzoate <5> (<5> 2-mercaptoethanol protects from inactivation [3]) [3]
periodate <4> [9]

Activating compounds

NaCl <4> (<4> ManNAc kinase activity is increased by presence of 200 mM [10]) [10]

Metals, ions

Cd^{2+} <4> (<4> less effective [10]) [10]
Co^{2+} <5> (<5> can replace Mg^{2+}, but is less active [3]) [3, 4, 10]
Mg^{2+} <4, 5> (<4,5> required for activity [2,3]) [2-4, 10]
Mn^{2+} <5> (<5> can replace Mg^{2+}, but is less active [3]) [3, 4, 10]
Ni^{2+} <4> (<4> less effective [10]) [10]
Additional information <5> (<5> Ca^{2+}, Na^{+} and K^{+} have no effect [3,4]; <4> Ca^{2+}, Ba^{2+}, Cu^{2+}, Fe^{2+}, Sn^{2+} and Zn^{2+} shows no effect on enzyme activity, monovalent cations Li^{+}, Na^{+}, K^{+}, Rb^{+} or Cs^{+} cannot replace Mg^{2+} [10]) [3, 4, 10]

Specific activity (U/mg)

0.84 <4> (<4> recombinant enzyme [11]) [11]
2.5 <4> (<4> activity of fraction with N-glycolylmannosamine [2]) [2]
2.76 <4> (<4> activity of fraction with N-glycolylmannosamine [2]) [2]
4 <4> (<4> activity of fraction with N-acetylmannosamine [1,2]) [1, 2]
4.114 <4> [8]
19.3 <5> [4]
58 <5> [3]

K_m-Value (mM)

0.093 <4> (N-acetyl-D-mannosamine, <4> pH 7.5, 37°C, hexamer [8]) [8]
0.121 <4> (N-acetyl-D-mannosamine, <4> pH 7.5, 37°C, dimer [8]) [8]
0.2 <4> (N-acetyl-D-mannosamine, <4> pH 8.1, 37°C [2]) [2]
0.75 <5> (N-acetyl-D-mannosamine, <5> pH 7.6, 37°C [3]) [3, 4]
1.15 <5> (ATP, <5> pH 7.6, 37°C [3]) [3, 4]
1.18 <4> (ATP, <4> pH 7.5, 37°C, hexamer [8]) [8]
1.2 <4> (N-glycolylmannosamine, <4> pH 8.1, 37°C [2]) [2]
1.67 <4> (ATP, <4> pH 7.5, 37°C, dimer [8]) [8]
2 <4> (ATP, <4> pH 8.1, 37°C [2]) [2]

K_i-Value (mM)

0.08 <4> (3-O-methyl-N-acetyl-D-glucosamine, <4> pH 7.4, 37°C [7]) [7]

pH-Optimum

6.2-8 <5> [4]
6.2-8.1 <5> [3]
6.5-8 <5> [3]

4 Enzyme Structure

Molecular weight

37300 <3> (<3> amino acid sequence, predicted from an open reading frame [14]) [14]
37400 <2> (<2> amino acid sequence, predicted from an open reading frame [14]) [14]
70000 <3> (<3> Western blot analysis [12]) [12]
75000 <4> (<4> gel filtration, SDS-PAGE [8,11]) [8, 11]
79000 <2> (<2> assembles to dimer or hexamer [13]) [13]
79200 <3> [12]
150000 <4> (<4> gel filtration, ion of the monomeric 75000 Da protein self associate as dimer [8]) [8]
450000 <4> (<4> gel filtration, ion of the monomeric 75000 Da protein self associate as hexamer [8]; <4> gel filtration [11]) [8, 11]

Subunits

dimer <2, 4> (<4> 2 * 75000, self associates as a dimer, gel filtration, SDS-PAGE, catalyzes only phosphorylation of N-acetylmannosamine, incubation with UDP-N-acetylglucosamine leads to reassembly of the fully active hexamer [8,11]) [8, 11, 13]
hexamer <2-4> (<4> 6 * 75000, self associates as a hexamer, gel filtration, SDS-PAGE, catalyzes both enzyme activities, UDP-GlcNAc-epimerase/ManNAc kinase [8]) [8, 11-13]

5 Isolation/Preparation/Mutation/Application

Source/tissue

HL-60 cell <2> [13]
HeLa s3 cell <2> [13]
HepG2 cell <2> [7]
K-562 cell <2> [13]
Morris hepatoma 7777 cell <4> [7]
blood <4> [6]
brain <3> [12]
colonic mucosa <4> (<4> normal mucosa, non-malignant mucosa, tumour [5]) [5]
embryo <3> [12]
erythrocyte <4> [6]
intestinal mucosa <4> [6, 8]
kidney <3, 4> [2, 12]
liver <2-4> [1, 2, 8-13]
lung <3> [12]
placenta <2> [13]
salivary gland <4> [6, 8]
spleen <4> [2, 6]

testis <3> [14]
Additional information <4> (<4> no enzymatic activity in brain, heart and muscle [2]; <4> only in sialoglycoprotein-secreting tissues [6]) [2, 6]

Localization
cytoplasm <4> (<4> soluble cell fraction [6]) [6]
cytosol <4> [6, 8, 11]

Purification
<4> [1, 2, 8-10]
<5> [3, 4]

Cloning
<2> (cDNA cloned and sequenced, expressed in Escherichia coli HB101 or INVαF' [13]) [13, 14]
<3> (cDNA cloned and sequenced, expressed in Escherichia coli HB101 or INVαF' [12]; cloned and functionally expressed in Escherichia coli BL21 [14]) [12, 14]
<4> (overexpression of active enzyme by using the baculovirus/Sf9 system [11]) [11]

Engineering
D413K <4> (<4> loss of kinase activity [11]) [11]
D413L <4> (<4> loss of kinase activity [11]) [11]
D413N <4> (<4> loss of kinase activity [11]) [11]
H110A <4> (<4> loss of epimerase activity [11]) [11]
H132A <4> (<4> loss of epimerase activity [11]) [11]
H155A <4> (<4> loss of epimerase activity [11]) [11]
H157A <4> (<4> loss of epimerase activity [11]) [11]
H45A <4> (<4> loss of epimerase activity [11]) [11]
R420M <4> (<4> loss of kinase activity [11]) [11]

Application
medicine <2> (<2> clinical relevance of the enzyme in the basic defect in sialuria [13]) [13]

6 Stability

Temperature stability
70 <5> (<5> stable to heating for a short period in a water-bath [3]) [3]

General stability information
<4>, hexameric enzyme is completely stable with 0.1 mM UDP [8]
<4>, purified enzyme is unstable to ammonium sulfate precipitation or freezing and thawing [2]
<5>, fairly stable, also stable to dialysis [3, 4]

Storage stability

<4>, -70°C, no loss of activity observed for several months [8]
<4>, 0°C, loses about 50% of its activity in 24 hours, stable for at least 1 week in presence of 0.01 M N-acylmannosamine [2]
<5>, 4°C, loses about 30% of its activity during 7 days of storage [4]
<5>, 4°C, loses about 33% of its activity during 7 days of storage [3]

References

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[3] Banerjee, S.; Ghosh, S.: Purification and properties of N-acetylmannosamine kinase from Salmonella typhimurium. Eur. J. Biochem., **8**, 200-206 (1969)
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Acyl-phosphate-hexose phosphotransferase 2.7.1.61

1 Nomenclature

EC number
2.7.1.61

Systematic name
acyl-phosphate:D-hexose phosphotransferase

Recommended name
acyl-phosphate-hexose phosphotransferase

Synonyms
acyl phosphate-hexose phosphotransferase
acyl phosphate:hexose phosphotransferase
hexose phosphate:hexose phosphotransferase
phosphotransferase, acyl phosphate-hexose

CAS registry number
37278-06-1

2 Source Organism

<1> *Aerobacter aerogenes* (PRL-R3) [1-3]

3 Reaction and Specificity

Catalyzed reaction
acyl phosphate + D-hexose = an acid + D-hexose phosphate (phosphorylates D-glucose and D-mannose on O-6, and D-fructose on O-1 or O-6; <1> rapid equilibrium random bi bi mechanism [3])

Reaction type
phospho group transfer

Natural substrates and products
S D-glucose + D-mannose 6-phosphate <1> [1, 2]
P D-glucose 6-phosphate + D-mannose
S D-glucose + acetyl phosphate <1> [1-3]
P D-glucose 6-phosphate + acetate
S acyl phosphate + D-hexose <1> [2]
P an acid + D-hexose phosphate

Substrates and products

S D-fructose + acetyl phosphate <1> (Reversibility: ? <1> [1,2]) [1, 2]
P D-fructose 1-phosphate + D-fructose 6-phosphate + acetate <1> (<1> D-fructose 1-phosphate is the predominant product [1]) [1]
S D-glucose + D-fructose 1-phosphate <1> (<1> 15% of the activity with acetyl phosphate or carbamoyl phosphate [1,2]) (Reversibility: ? <1> [1,2]) [1, 2]
P D-glucose 6-phosphate + D-fructose
S D-glucose + D-fructose 6-phosphate <1> (<1> 25% of the activity with acetyl phosphate or carbamoyl phosphate [1,2]) (Reversibility: ? <1> [1,2]) [1, 2]
P D-glucose 6-phosphate + D-fructose
S D-glucose + D-glucose 1-phosphate <1> (<1> 5% of the activity with acetyl phosphate or carbamoyl phosphate [1,2]) (Reversibility: ? <1> [1,2]) [1, 2]
P D-glucose 6-phosphate + D-glucose
S D-glucose + D-mannose 6-phosphate <1> (<1> 71% of the activity with acetyl phosphate or carbamoyl phosphate [1,2]) (Reversibility: ? <1> [2]; r <1> [1]) [1, 2]
P D-glucose 6-phosphate + D-mannose
S D-glucose + D-ribose 5-phosphate <1> (<1> 18% of the activity with acetyl phosphate or carbamoyl phosphate [1,2]) (Reversibility: ? <1> [1,2]) [1, 2]
P D-glucose 6-phosphate + D-ribose
S D-glucose + D-sorbitol 6-phosphate <1> (<1> 2.5% of the activity with acetyl phosphate or carbamoyl phosphate [1,2]) (Reversibility: ? <1> [1,2]) [1, 2]
P D-glucose 6-phosphate + D-sorbitol
S D-glucose + acetyl phosphate <1> (Reversibility: ? <1> [1-3]) [1-3]
P D-glucose 6-phosphate + acetate <1> [1-3]
S D-glucose + carbamoyl phosphate <1> (Reversibility: ? <1> [1,2]) [1, 2]
P D-glucose 6-phosphate + carbamate
S D-mannitol + acetyl phosphate <1> (Reversibility: ? <1> [1,2]) [1, 2]
P ?
S D-mannose + acetyl phosphate <1> (Reversibility: ? <1> [1,2]) [1, 2]
P D-mannose 6-phosphate + acetate <1> [1]
S N-acetylglucosamine + acetyl phosphate <1> (<1> 10% of the activity with glucose [3]) (Reversibility: ? <1> [3]) [3]
P ?

Inhibitors

D-mannose <1> (<1> inhibits phosphorylation of D-glucose, competitive to acetyl phosphate or D-mannose 6-phosphate [1]) [1]
N-acetylglucosamine <1> [3]
phosphate <1> [3]
Additional information <1> (<1> not inhibitory: EDTA [2]) [2]

Metals, ions

Additional information <1> (<1> no stimulation by addition of various cations [2]) [2]

Specific activity (U/mg)

1.3 <1> (<1> pH 7.5, 25°C [1,2]) [1, 2]

K_m-Value (mM)

0.16 <1> (D-glucose, <1> pH 7.5, 25°C [1,2]) [1, 2]
0.4 <1> (acetyl phosphate, <1> carbamoyl phosphate, D-mannose 6-phosphate, pH 7.5, 25°C [1,2]) [1, 2]
2 <1> (D-ribose 5-phosphate, <1> pH 7.5, 25°C [1,2]) [1]
12 <1> (D-mannose, <1> pH 7.5, 25°C [1,2]) [1, 2]
67 <1> (mannitol, <1> pH 7.5, 25°C [1,2]) [1]

pH-Optimum

9 <1> [1, 2]

pH-Range

7.5-9 <1> (<1> pH 7.5: 50% of maximum activity, pH 9: maximum activity) [1, 2]

Temperature optimum (°C)

25 <1> (<1> assay at [1]) [1]
28 <1> (<1> assay at [3]) [3]

4 Enzyme Structure

Molecular weight

150000 <1> (<1> gel filtration [3]) [3]

5 Isolation/Preparation/Mutation/Application

Purification

<1> [1-3]

6 Stability

General stability information

<1>, stable to repeated freezing and thawing [1, 2]

Storage stability

<1>, -20°C, stable [1]

References

[1] Anderson, R.L.; Kamel, M.Y.: Acyl phosphate:hexose phosphotransferase (hexose phosphate:hexose phosphotransferase). Methods Enzymol., **9**, 392-396 (1966)

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Phosphoramidate-hexose phosphotransferase 2.7.1.62

1 Nomenclature

EC number
2.7.1.62

Systematic name
phosphoramidate:hexose 1-phosphotransferase

Recommended name
phosphoramidate-hexose phosphotransferase

Synonyms
phosphoramidate-hexose transphosphorylase
phosphoramidic-hexose transphosphorylase
phosphotransferase, phosphoramidate-hexose
Additional information (may be identical with EC 3.1.3.9)

CAS registry number
9031-45-2

2 Source Organism

<1> *Escherichia coli* (Crookes strain, ATCC 8739 [1]) [1]

3 Reaction and Specificity

Catalyzed reaction
phosphoramidate + hexose = NH_3 + hexose 1-phosphate (may be identical with EC 3.1.3.9 glucose-6-phosphatase)

Reaction type
phospho group transfer

Natural substrates and products
S PNH_2 + glucose <1> (<1> phosphoramidate, i.e. PNH_2, is delivered by KO_3PNH_3 [1]) [1]
P glucose 1-phosphate + NH_3

Substrates and products
S PNH_2 + 2-deoxy-D-glucose <1> (Reversibility: ? <1> [1]) [1]
P 2-deoxy-D-glucose 1-phosphate + NH_3
S PNH_2 + 3-O-methylglucose <1> (Reversibility: ? <1> [1]) [1]

P 3-O-methylglucose 1-phosphate + NH_3
S PNH_2 + fructose <1> (Reversibility: ? <1> [1]) [1]
P fructose 1-phosphate + NH_3 <1> [1]
S PNH_2 + glucosamine <1> (Reversibility: ? <1> [1]) [1]
P glucosamine 1-phosphate + NH_3
S PNH_2 + glucose <1> (<1> glucose is best substrate, phosphoramidate, i.e. PNH_2, is delivered by KO_3PNH_3 [1]) (Reversibility: ? <1> [1]) [1]
P glucose 1-phosphate + NH_3 <1> [1]
S PNH_2 + mannose <1> (Reversibility: ? <1> [1]) [1]
P mannose 1-phosphate + NH_3 <1> [1]

Inhibitors

F^- <1> (<1> 1.5 mM, at pH 8.7 8% inhibition, at pH 6.5 90% inhibition [1]) [1]

Specific activity (U/mg)

22.7 <1> (<1> 37°C, pH 8.7 [1]) [1]

K_m-Value (mM)

12.5 <1> (D-glucose, <1> 37°C, pH 8.7 [1]) [1]

pH-Optimum

8.7 <1> (<1> assay at [1]) [1]

Temperature optimum (°C)

37 <1> (<1> assay at [1]) [1]

5 Isolation/Preparation/Mutation/Application

Purification

<1> [1]

6 Stability

Temperature stability

55 <1> (<1> pH 7.5, more than 30 min, no loss of activity [1]) [1]
100 <1> (<1> 10 min, about 60% loss of activity [1]) [1]

Storage stability

<1>, 4°C or -15°C, 6 years stable [1]

References

[1] Smith, R.A.; Theisen, M.C.: Phosphoramidate-hexose transphosphorylase. Methods Enzymol., **9**, 403-407 (1966)

Polyphosphate-glucose phosphotransferase 2.7.1.63

1 Nomenclature

EC number
2.7.1.63

Systematic name
polyphosphate:D-glucose 6-phosphotransferase

Recommended name
polyphosphate-glucose phosphotransferase

Synonyms
PGPTase
inorganic polyphosphate:D-glucose 6-phosphotransferase
phosphotransferase, polyphosphate-glucose
poly(P) glucokinase
poly(P)/ATP-glucomannokinase
polyP-GK
polyphosphate glucokinase
polyphosphate-D-(+)-glucose-6-phosphotransferase
polyphosphate-glucose 6-phosphotransferase
polyphosphate/ATP-glucomannokinase

CAS registry number
9033-50-5

2 Source Organism

<1> *Mycobacterium phlei* [1, 2, 13]
<2> *Mycobacterium aurum* [2]
<3> *Mycobacterium gordonae* [2]
<4> *Mycobacterium jucho* [6]
<5> *Mycobacterium pellegrino* [6]
<6> *Mycobacterium smegmatis* [2]
<7> *Mycobacterium thermoresistible* [2]
<8> *Mycobacterium tuberculosis* (strain H37Ra [7]) [7, 9, 10, 13]
<9> *Mycobacterium sp.* [6]
<10> *Nocardia minima* [5, 13]
<11> *Propionibacterium shermanii* [3, 4, 8, 13]
<12> *Microlunatus phosphovorus* (strain NM-1 [11]) [11]

<13> *Arthrobacter sp.* (strain KM [12]) [12]
<14> *Propionibacterium arabinosum* [13]
<15> *Corynebacterium xerosis* [13]

3 Reaction and Specificity

Catalyzed reaction

$(phosphate)_n$ + D-glucose = $(phosphate)_{n-1}$ + D-glucose 6-phosphate (<11> with long chain polyphosphates, the reaction proceeds by a processive type mechanism, with short polyphosphates the mechanism is nonprocessive [3]; <8> in the polyphosphate-dependent reaction the enzyme follows an ordered bi bi sequential mechanism with polyphosphate binding to the enzyme first and glucose 6-phosphate dissociating last. The ATP-dependent glucokinase reaction is consistent with an ordered bi bi sequential mechanism, with ATP binding to the enzyme first and glucose 6-phosphate leaving last [9])

Reaction type

phospho group transfer

Natural substrates and products

S $(phosphate)_n$ + D-glucose <11> (<11>, constitutive enzyme, enzyme is involved in metabolism of glucose [13]) (Reversibility: ? <11> [13]) [13]
P $(phosphate)_{n-1}$ + D-glucose 6-phosphate

Substrates and products

S $(phosphate)_n$ + D-glucose <1-13> (<1> i.e. poly$(P)_n$, no substrates are monophosphate, diphosphate or triphosphate [2]; <1>, no utilization of triphosphate or tetrapolyphosphate [1]; <11> the enzyme utilizes polyphosphate much more efficiently than it does ATP, with a turnover number/Kpolyphosphate to turnover number/KATP ratio of 2800 [8]; <8> polyphosphate is utilized nonprocessively with a preference for longer chains [9]) (Reversibility: ir <12,13> [11,12]; ? <1-11> [1-10]) [1-12]
P $(phosphate)_{n-1}$ + D-glucose 6-phosphate <1, 8, 13> (<8>, tetrapolyphosphate appears to precede the formation of tripolyphosphate which accumulates as the ultimate product [7]) [1, 2, 7, 12]
S $(phosphate)_n$ + D-mannose <12, 13> (<12> slowly [11]; <13> about 40% of the activity with glucose [12]) (Reversibility: ? <12,13> [11,12]) [11, 12]
P $(phosphate)_{n-1}$ + D-mannose 6-phosphate
S $(phosphate)_n$ + glucosamine <12> (<12> rate similar to that of glucose [11]) (Reversibility: ? <12> [11]) [11]
P $(phosphate)_{n-1}$ + D-glucosamine 6-phosphate
S ATP + D-glucose <8, 10, 11, 13> (<11> the enzyme utilizes polyphosphate much more efficiently than it does ATP, with a turnover number/Kpolyphosphate to turnover number/KATP ratio of 2800 [8]; <12>, no activity with ATP [11]) (Reversibility: ? <8, 10, 11, 13> [3, 8, 9, 10, 12, 13]) [3, 8, 9, 10, 12, 13]
P ADP + D-glucose 6-phosphate

S ATP + D-mannose <13> (<13>, about 30% of the activity with glucose [12]) (Reversibility: ? <13> [12]) [12]
P ADP + D-mannose 6-phosphate
S CTP + D-glucose <8, 11> (Reversibility: ? <8,11> [8,13]) [8, 13]
P CDP + D-glucose 6-phosphate
S GTP + D-glucose <8, 11> (Reversibility: ? <8,11> [8,13]) [8, 13]
P GDP + D-glucose 6-phosphate
S TTP + D-glucose <8> (Reversibility: ? <8> [13]) [13]
P TDP + D-glucose 6-phosphate
S UTP + D-glucose <8, 11> (Reversibility: ? <8,11> [8,13]) [8, 13]
P UDP + D-glucose 6-phosphate
S XTP + D-glucose <8> (Reversibility: ? <8> [13]) [13]
P XDP + D-glucose 6-phosphate
S dATP + D-glucose <11> (Reversibility: ? <11> [8]) [8]
P dADP + D-glucose 6-phosphate
S Additional information <12, 13> (<12>, no activity with ATP [11]; <13>, the enzyme also shows weak NAD kinase activity and fructokinase activity with polyphosphate or ATP [12]) [11, 12]
P ?

Inhibitors

ADP <8, 11> (<8,11>, inhibits reaction with ATP [8,9]) [8, 9]
AMP <8> (<8> inhibits reaction with polyphosphate and glucose or ATP and glucose [9]) [9]
ATP <8> (<8>, at high concentrations competitive substrate inhibition with respect to glucose, ATP-dependent reaction [9]) [9]
D-fructose 6-phosphate <8> (<8> inhibits reaction with polyphosphate and glucose or ATP and glucose [9]) [9]
D-glucose <1> (<1> above 150 mM, substrate inhibition [2]) [2]
D-glucose 6-phosphate <8, 11> (<11>, inhibits reaction with ATP [8]; <8>, inhibits reaction with polyphosphate and glucose [9]) [8, 9]
D-xylose <8, 11> (<8,11>, inhibits reaction with ATP [8,9]; <8> inhibits reaction with polyphosphate and glucose or ATP and glucose [9]) [8, 9]
Mg^{2+} <1> (<1> molar ratio polyphosphate/Mg below 0.5, activation above, EDTA partially reverses [2]) [2]
NaCl <10> (<10> above 100 mM [5]) [5]
poly(P) <1> (<1> above 0.05 mM, substrate inhibition [2]) [2]
tetrapolyphosphate <11> (<11>, inhibits reaction with ATP [8]) [8]
tripolyphosphate <1> (<1>, when present in excess together with Graham's salt at pH 7.2 [1]) [1]

Metals, ions

Ca^{2+} <1> (<1> activation, about 30% as efficient as Mg^{2+} [2]) [2]
Co^{2+} <12, 13> (<12>, divalent cations activate in the order of decreasing effectiveness: Mg^{2+}, Mn^{2+}, Co^{2+}, Zn^{2+} [11]; <13>, divalent cation required, 86% of the activity with Mg^{2+} for the polyphosphate-dependent activity and 30% of the activity with Mg^{2+} for ATP-dependent activity, 5 mM [12]) [11, 12]

Cu^{2+} <13> (<13>, divalent cation required, 11% of the activity with Mg^{2+} for the polyphosphate-dependent activity and no activation of ATP-dependent activity, 5 mM [12]) [12]
Fe^{2+} <13> (<13>, divalent cation required, no activation of the polyphosphate-dependent activity and 15% of the activity with Mg^{2+} for ATP-dependent acticity, 5 mM [12]) [12]
Mg^{2+} <1, 8, 12, 13> (<1,8> activation [1,2,7]; <1> molar ratio poly(P)/Mg^{2+} above 0.5 inhibition below [2]; <12> requires 1-10 mM Mg^{2+} [11]; <13>, divalent cation required, maximal activition of polyphosphate-dependent glucokinase and ATP-dependent glucokinase activity, 5 mM [12]) [1, 2, 7, 11, 12]
Mn^{2+} <1, 13> (<1> activation, as effective as Mg^{2+} [2]; <12>, divalent cations activate in the order of decreasing effectiveness: Mg^{2+}, Mn^{2+}, Co^{2+}, Zn^{2+} [11]; <13>, divalent cation required, 82% of the activity with Mg^{2+} for the polyphosphate-dependent activity and 88% of the activity with Mg^{2+} for ATP-dependent activity, 5 mM [12]) [2, 11, 12]
Zn^{2+} <1, 12> (<1> activation, about 30% as efficient as Mg^{2+} [2]; <12>, divalent cations activate in the order of decreasing effectiveness: Mg^{2+}, Mn^{2+}, Co^{2+}, Zn^{2+} [11]) [2, 11]

Turnover number (min^{-1})

420 <11> (UTP, <11> pH 7.5, 30°C [8]) [8]
444 <11> (CTP, <11> pH 7.5, 30°C [8]) [8]
660 <11> (GTP, <11> pH 7.5, 30°C [8]) [8]
840 <11> (dATP, <11> pH 7.5, 30°C [8]) [8]
1500 <11> (ATP, <11> pH 7.5, 30°C [8]) [8, 13]
2460 <14> (poly(P_{400})) [13]
3300 <14> (poly(P_{35})) [13]
3360 <11> (poly(P_{31}), <11> pH 7.5, 30°C [8]) [8]
3420 <11> (poly(P_{35})) [13]
3780 <14> (ATP) [13]
5220 <12> (poly(P8)) [11]
6480 <8> (ATP, <8> pH 7.5 [9]) [9]
6480 <8> (D-glucose, <8> pH 7.5 [9]) [9]
6960 <8> (ATP, <8> pH 8.6 [9]) [9]
6960 <8> (D-glucose, <8> pH 8.6 [9]) [9, 13]
9780 <8> (poly(P_{400})) [13]
10980 <12> (poly(P_{700})) [11]
11760 <8> (poly(P_{35})) [13]
16200 <12> (poly(P_{30})) [11]
22260 <11> (poly(P_{400})) [13]

Specific activity (U/mg)

15.3 <11> [3]
110 <13> (<13> reaction with ATP and glucose [12]) [12]
130 <11> [4]
140 <1> [2]
203 <8> (<8>, polyphosphate-dependent activity [10]) [10]
220 <13> (<13>, reaction with polyphosphate and glucose [12]) [12]

K_m-Value (mM)

0.000005 <11> (poly(P_{400})) [13]
0.00008 <8> (poly(P_{400})) [13]
0.0012 <11> (poly(P_{31}), <11> pH 7.5, 30°C [8]) [8]
0.0012 <11> (poly(P_{35})) [13]
0.002 <11> (poly(P_{724}), <11> pH 7.5, 30°C [3]) [3]
0.004 <11> (poly(P_{575}), <11> pH 7.5, 30°C [3]) [3]
0.0046 <8> (poly(P_{35})) [13]
0.0067 <11> (poly(P_{430}), <11> pH 7.5, 30°C [3]) [3]
0.0072 <1> (poly(P_{15}), <1>, pH 8.5, 30°C, soluble enzyme [2]) [2]
0.0086 <11> (poly(P_{347}), <11> pH 7.5, 30°C [3]) [3]
0.0088 <14> (poly(P_{35})) [13]
0.012 <12> (poly(P_8)) [11]
0.013 <11> (poly(P_{75}), <11> pH 7.5, 30°C [3]) [3]
0.02 <13> (hexametaphosphate, <13> pH 7.0, 30°C [12]) [12]
0.025 <11> (poly(P_{209}), <11> pH 7.5, 30°C [3]) [3]
0.033 <8> (poly(P_{10}), <8> pH 7.5 [7]) [7]
0.037 <11> (poly(P_{138}), <11> pH 7.5, 30°C [3]) [3]
0.037 <14> (poly(P_{400})) [13]
0.06 <8> (D-glucose, <8> pH 7.5, reaction with ATP [9]) [9]
0.06 <12> (poly(P_{700})) [11]
0.082 <8> (poly(P_{10}), <8> pH 8.5 [7]) [7]
0.13 <1> (poly(P_{15}), <1>, pH 8.5, 30°C, immobilized enzyme [2]) [2]
0.15 <11> (ATP) [13]
0.15 <13> (mannose, <13> pH 7.0, 30°C [12]) [12]
0.175 <1> (poly(P), <1> pH 8.5, 37°C, in terms of acid-labile phosphate [1]) [1]
0.18 <11> (poly(P_{68}), <11> pH 7.5, 30°C [3]) [3]
0.2 <13> (ATP, <13> pH 7.0, 30°C [12]) [12]
0.22 <8> (D-glucose, <8> pH 8.6, reaction with ATP [9]) [9]
0.28 <1> (D-glucose, <1>, pH 8.5, 37°C [1]) [1]
0.4 <14> (ATP) [13]
0.48 <1> (D-glucose, <1>, pH 8.5, 30°C, soluble enzyme [2]) [2]
0.5 <13> (glucose, <13> pH 7.0, 30°C [12]) [12]
0.6 <1> (D-glucose, <1>, pH 8.5, 30°C, immobilized enzyme [2]) [2]
0.8 <11> (GTP, <11> pH 7.5, 30°C [8]) [8]
0.88 <8> (ATP, <8> pH 7.5 [9]) [9, 13]
0.94 <11> (poly(P_{40}), <11> pH 7.5, 30°C [3]) [3]
1.1 <11> (D-glucose, <11> pH 7.4 [4]) [4]
1.2 <11> (dATP, <11> pH 7.5, 30°C [8]) [8]
1.4 <8> (ATP, <8> pH 8.6 [9]) [9]
1.5 <11> (ATP, <11> pH 7.5, 30°C [8]) [8]
2.6 <11> (UTP, <11> pH 7.5, 30°C [8]) [8]
3.8 <12> (poly(P_{30})) [11]
5.9 <11> (CTP, <11> pH 7.5, 30°C [8]) [8]

K_i-Value (mM)
6.6 <8> (ATP, <8> pH 7.5 [9]) [9]
7.4 <8> (ATP, <8> pH 8.6 [9]) [9]
Additional information <8, 11> [8, 9]

pH-Optimum
5.5 <12> [11]
6.4-8.3 <1> (<1> broad plateau [2]) [2]
7.5 <13> (<13> polyphosphate- and ATP-dependent mannokinase activity, polyphosphate and ATP-dependent glucokinase activity [12]) [12]
8.5 <1> [1]

pH-Range
6-8.4 <1> (<1> very little activity below pH 6.0 and above pH 8.4 [2]) [2]
6-9.5 <13> (<13> pH 6.0: about 25% of maximal activity, pH 9.5: about 35% of maximal activity, polyphosphate-dependent and ATP-dependent mannokinase activity, polyphosphate and ATP-dependent glucokinase activity [12]) [12]

Temperature optimum (°C)
30 <12> [11]
45 <13> (<13> polyphosphate-dependent and ATP-dependent mannokinase activity, polyphosphate and ATP-dependent glucokinase activity [12]) [12]

4 Enzyme Structure

Molecular weight
30000 <11> (<11> about, gel filtration [4]) [4]
59000 <10> (<10> gel filtration [5]) [5]
63000 <11> (<11>, gel filtration [13]) [13]
64000 <12> (<12> gel filtration [11]) [11]
66000 <8> (<8> gel filtration [13]) [13]
113000 <1> (<1> sucrose density gradient centrifugation [1]) [1]
275000-280000 <1> (<1> non-denaturing PAGE, gel filtration [2]) [2]

Subunits
? <1, 8> (<8> x * 27400, calculation from nucleotide sequence [10]; <8>, x * 33000, SDS-PAGE [10]; <1> x * 34000, SDS-PAGE [2]) [2, 10]
dimer <8, 11, 12, 14> (<8,11,14>, 2 * 30000, about, SDS-PAGE [13]; <12> 2 * 32000, SDS-PAGE [11]) [11, 13]
monomer <11> (<11> 1 * 31000 [4]) [4]

5 Isolation/Preparation/Mutation/Application

Localization
cytosol <1> [2]

Purification

<1> (partial [1]) [1, 2]
<8> (partial [7]; recombinant enzyme [10]) [7, 10]
<10> (partial [5]) [5]
<11> (partial [3]; not separable from ATP-glucokinase [3,8]) [3, 4, 8]
<12> [11]
<13> [12]

Cloning

<8> (expression in Escherichia coli [10,13]) [10, 13]
<13> [12]

6 Stability

pH-Stability

3.5 <1> (<1> $t_{1/2}$: 24 h at 3°C or at 37°C plus 0.3 M KCl [1]) [1]
5-7 <1> (<1> 24 h stable at 3°C [1]) [1]
8.1-8.4 <1> (<1>, immobilized enzyme is stable [2]) [2]

Temperature stability

37 <1> (<1> 24 h, pH 4-5: precipitation and 80% of activity retained, above pH 5.5: inactivation within 24 h, 0.3 M KCl stabilizes [1]) [1]
40 <13> (<13> 5 min, 50% loss of activity [12]) [12]

General stability information

<1>, KCl, 0.3 M, stabilizes [1]
<1>, dialysed enzyme preparations are largely inactive, Mg^{2+} restores activity [2]
<1>, dialysis against distilled water for 10 h leads to decreased activity [1]
<1>, enzyme immobilized on corn stover is stable for one months at pH 8.5, 30°C [2]

Storage stability

<1>, frozen, partially purified preparation, several weeks [1]

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Inositol 3-kinase 2.7.1.64

1 Nomenclature

EC number
2.7.1.64

Systematic name
ATP:myo-inositol 1-phosphotransferase

Recommended name
inositol 3-kinase

Synonyms
inositol 1-kinase
kinase, inositol 1- (phosphorylating)
myo-inositol 3-kinase
myoinositol kinase

CAS registry number
37278-07-2

2 Source Organism

<1> *Lemna gibba* [2]
<2> *Spinacia oleracea* (spinach [1]) [1]
<3> *Triticum aestivum* (wheat [1,3]) [1, 3]
<4> *Acer pseudoplatanus* [4]
<5> *Phaseolus aureus* [1, 4]
<6> *Escherichia coli* [4]
<7> *Bos taurus* [4]

3 Reaction and Specificity

Catalyzed reaction
ATP + myo-inositol = ADP + 1D-myo-inositol 3-phosphate

Reaction type
phospho group transfer

Natural substrates and products
S ATP + myo-inositol <1> (<1> biosynthesis of phytic acid [2]) [2]
P ADP + myo-inositol 1-phosphate

Substrates and products

S ATP + myo-inositol <1-7> (Reversibility: ? <1-7> [1-4]) [1-4]
P ADP + myo-inositol 1-phosphate <1-7> [1-4]
S ATP + myo-inositol <3> (Reversibility: ? <3> [3]) [3]
P ADP + 1L-myo-inositol 1-phosphate <3> [3]
S CTP + myo-inositol <4-7> (<4-7> weak activity [4]) (Reversibility: ? <4-7> [4]) [4]
P CDP + myo-inositol 1-phosphate <4-7> [4]
S GTP + myo-inositol <4-7> (<4-7> no actiity with TTP [4]) (Reversibility: ? <4-7> [4]) [4]
P GDP + myo-inositol 1-phosphate <4-7> [4]
S UTP + myo-inositol <4-7> (Reversibility: ? <4-7> [4]) [4]
P UDP + myo-inositol 1-phosphate <4-7> [4]

Metals, ions

Mg^{2+} <3> (<3> required for activity [3]) [3]

pH-Optimum

7.8 <4-7> [4]

pH-Range

6.7-8.7 <5> (<5> approx. 25% of maximal activity at pH 6.7, approx. 75% of maximal activity at pH 8.7 [4]) [4]

5 Isolation/Preparation/Mutation/Application

Source/tissue

cell culture <1, 4> [2, 4]
germ <3> [1, 3]
leaf <2> [1]
liver <7> [4]
seed <5> [4]

Purification

<2> (ammonium sulfate, partial purification [1]) [1]
<3> (ammonium sulfate, partial purification [1]) [1]
<5> (ammonium sulfate, density-gradient centrifugation, partial purification [1]) [1]

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Scyllo-inosamine 4-kinase 2.7.1.65

1 Nomenclature

EC number
2.7.1.65

Systematic name
ATP:1-amino-1-deoxy-*scyllo*-inositol 4-phosphotransferase

Recommended name
scyllo-inosamine 4-kinase

Synonyms
ATP:inosamine phosphotransferase
kinase, *scyllo*-inosamine (phosphorylating)
scyllo-inosamine kinase

CAS registry number
37278-08-3

2 Source Organism

<1> *Streptomyces bikiniensis* (ATCC 11062 [1,2]) [1, 2]
<2> *Streptomyces griseus* (ATCC 12475 [1]) [1]

3 Reaction and Specificity

Catalyzed reaction
ATP + 1-amino-1-deoxy-*scyllo*-inositol = ADP + 1-amino-1-deoxy-*scyllo*-inositol 4-phosphate

Reaction type
phospho group transfer

Natural substrates and products
S ATP + *scyllo*-inosamine <1> (<1> reaction involved in biosynthesis of streptidine from myo-inositol [1]) [1]
P ADP + 1-amino-1-deoxy-*scyllo*-inositol 4-phosphate

Substrates and products
S 2'-deoxy-ATP + *scyllo*-inosamine <1> (Reversibility: ? <1> [1]) [1]
P 2'-deoxy-ADP + *scyllo*-inosamine phosphate <1> [1]

S ATP + 1-amino-1-deoxy-*scyllo*-inositol <1> (<1> i.e. scyllo-inosamine [1,2]; <1> no activity with guanidinodeoxy-*scyllo*-inositol [2]; <1> no activity with streptidine, myoinosamine-2, DL-myoinosamine-4 and neoinosamine-2 [1]; <1> no activity with CTP, UTP, dTTP or GTP [2]) (Reversibility: ? <1> [1,2]) [1, 2]
P ADP + 1-amino-1-deoxy-*scyllo*-inositol 4-phosphate <1> (<1> *scyllo*-inosamine-phosphate [1]) [1, 2]
S ATP + 1D-1-guanidino-3-amino-1,3-dideoxy-*scyllo*-inositol <1> (Reversibility: ? <1> [2]) [2]
P 1D-1-guanidino-3-amino-1,3-dideoxy-*scyllo*-inositol 6-phosphate + ADP <1> [2]
S ATP + 2-deoxystreptamine <1> (Reversibility: ? <1> [1]) [1]
P ?
S ATP + streptamine <1> (Reversibility: ? <1> [1,2]) [1, 2]
P ?

Inhibitors

formamidine disulfite <1> [1]
p-chloromercuribenzoate <1> [1]

Metals, ions

Mg^{2+} <1> (<1> or Mn^{2+} required [2]; <1> required for activity [1]) [1, 2]
Mn^{2+} <1> (<1> or Mg^{2+} required [2]) [2]

pH-Optimum

7.4 <1> (<1> assay at [2]) [2]

5 Isolation/Preparation/Mutation/Application

Source/tissue

mycelium <1, 2> [1, 2]

Purification

<1> (ammonium sulfate, Sephadex G-100 or DEAE-cellulose [2]) [2]

6 Stability

General stability information

<1>, mercaptan required during purification [2]
<1>, purified enzyme preparations are stabilized by addition of serum albumin and mercaptans [2]

References

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Undecaprenol kinase 2.7.1.66

1 Nomenclature

EC number
2.7.1.66

Systematic name
ATP:undecaprenol phosphotransferase

Recommended name
undecaprenol kinase

Synonyms
C_{55}-isoprenoid alcohol kinase
C_{55}-isoprenoid alcohol phosphokinase
C_{55}-isoprenyl alcohol phosphokinase
isoprenoid alcohol kinase
isoprenoid alcohol phosphokinase
isoprenoid-alcohol kinase
kinase (phosphorylating), isoprenoid alcohol
kinase, isoprenoid alcohol (phosphorylating)
polyisoprenol kinase

CAS registry number
9068-22-8

2 Source Organism

<1> *Staphylococcus aureus* [1, 2, 3, 7, 8, 9, 10, 11]
<2> *Lactobacillus plantarum* (ATCC 8014 [4]) [4, 5]
<3> *Klebsiella aerogenes* [6]
<4> *Streptococcus mutans* (mutant strain Tn-1 [12]) [12]

3 Reaction and Specificity

Catalyzed reaction
ATP + undecaprenol = ADP + undecaprenyl phosphate

Reaction type
phospho group transfer

Natural substrates and products

S Additional information <1, 4> (<1> product of the reaction is a substrate for the synthesis of lipid intermediates in peptidoglycan synthesis [1]; <1> the product of the reaction, C_{55}-isoprenoid alcohol monophosphate functions in the biosynthesis of bacterial peptidoglycan and lipopolysaccharides [3]; <4>, the enzyme predominantly affects growth of the cells under stress conditions [12]) [1, 3, 12]
P ?

Substrates and products

S ATP + (E,E,E)-geranylgeraniol <2> (<2> 5%, of the activity with ATP [4]; <2> 28% of the activity with ATP [5]) [4, 5]
P ADP + (E,E,E)-geranylgeraniyl phosphate
S ATP + (Z,E,E)-geranylgeraniol <2> (<2> 5% of the activity with undecaprenol [4]) [4]
P ADP + (Z,E,E)-geranylgeraniyl phosphate
S ATP + C_{55}-isoprenoid alcohol <1> (Reversibility: ? <1> [3]) [3]
P ADP + C_{55}-isoprenoid alcohol monophosphate
S ATP + dolichol <2> (<2> 13% of the activity with undecaprenol [4]; <2> 26% of the activity with undecaprenol [5]) (Reversibility: ? <2> [4,5]) [4, 5]
P ADP + dolichyl phosphate
S ATP + ficaprenol <1, 3> (Reversibility: ? <1,3> [1,3,6]) [1, 3, 6]
P ADP + ficaprenyl phosphate <3> [6]
S ATP + solanesol <1, 2> (<2> about 85% of the activity with undecaprenol [4,5]; <1>, poor substrate [3]; <1> no activity [1]) [3, 4, 5]
P ADP + solanesyl phosphate
S ATP + undecaprenol <2> (<2> best substrate [4,5]) (Reversibility: ? <2> [4,5]) [4, 5]
P ADP + undecaprenyl monophosphate <2> [4]

Inhibitors

1-butanol <1> [11]
K_2HPO_4 <2> (<2> above 10 mM [4]) [4]
KCl <2> (<2> above 10 mM [4]) [4]
Mg^{2+} <2> (<2> above 10 mM [4]) [4]
Mn^{2+} <1> (<1> complete inhibition at 0.01 M [1]) [1]
NH_4Cl <2> (above 10 mM [4]) [4]
Na_2HPO_4 <2> (<2> above 10 mM [4]) [4]
NaCl <1, 2> (<1> above 0.5 M [3,11]; <2> above 10 mM [4]) [3, 4, 11]
NaF <2> (<2> above 5 mM [4]) [4]
guanidine hydrochloride <1> (<1>, strong inhibition above 100 mM [11]) [3, 11]
urea <1> (<1>, 8 M, 20% inhibition [11]) [11]

Activating compounds

DMSO <2> (<2> required [4]) [4]
Triton X-100 <2> (<2> required [4]) [4]

detergents <1> (<1> overview: activation by detergents, only neutral detergents with short chain and unsaturated chain hydrophobes are successful activators at 25°C [10]) [10]
ditetradecanoylphosphatidylcholine <2> (<2>, good activator [5]) [5]
lysophosphatidylglycerol <2> (<2>, good activator [5]) [5]
phospholipid cofactor <1, 2> (<1> absolute requirement for a phospholipid cofactor, mixture of phosphatidylglycerol and cardiolipin, either of which is effective alone in restoring the activity of the enzyme [2]; <2> requirements for endogenous and exogenous phospholipids. Enzyme requires a fluid lipid surface for activity, lysophosphatidylglycerol and ditetradecanoylphosphatidylcholine are the best activators [5]; <1> reactivation of apoprotein by synthetic lecithins [7]; <1> lipid requirement of the enzyme is nonelectrostatic in nature [8]; <1> absolute requirement for phospholipid and for detergent [8]; <1> overview: activation by commercial phospholipids and glycolipids, lipids providing a hydrated, loosely packed, highly fluid environment are often effective activators [9]; <1> phospholipid cofactor required [3]) [2, 3, 5, 7-9, 11]

Metals, ions

Co^{2+} <2> (<2> can partially replace Mg^{2+} in activation, 25% of the activation with Mg^{2+}, optimal concentration is 10 mM [4]; <1> no effect [1]) [4]
Mg^{2+} <1, 2> (<2> required, optimal activation at 10 mM [4]; <1> divalent cation required, Mg^{2+} most effective, optimal concentration: 0.01 M [1]; <1,2> optimum concentration: 0.01 M [1,4]) [1, 4]
Mn^{2+} <1, 2> (<1,2> can partially replace Mg^{2+} in activation [1,4]; <2> 50% of the activation with Mg^{2+}, optimal concentration is 2.5 mM [4]; <1> one-fourth of the activity of Mg^{2+} at 0.001 M [1]) [1, 4]
NaCl <1> (<1> 0.5 M, stimulates about 2-fold [3,11]) [3, 11]
NaF <2> (<2> 1.25 mM stimulates [4]) [4]

Specific activity (U/mg)

0.54 <1> [11]
Additional information <1, 2> [3, 5]

K_m-Value (mM)

0.0055 <2> (dolichol) [5]
0.008 <2> (undecaprenol) [5]
0.014 <2> (dolichol, <2>, pH 8.0, 33°C [4]) [4]
0.014 <2> (undecaprenol, <2>, pH 8.0, 33°C [4]) [4]
0.057 <1> (ATP, <1> pH 8.5, 25°C, reaction with C_{55}-isoprenoid alcohols from Streptococcus faecalis or ficaprenol [11]) [3, 11]
0.4 <1> (ATP, <1> pH 8.2, 37°C, reaction with ficaprenol [1]) [1]
0.5 <1> (ficaprenol, <1> pH 8.2, 37°C [1]) [1]
2 <2> (ATP, <2> pH 8.0, 33°C, reaction with undecaprenol [4]) [4]

pH-Optimum

7.5-9 <2> [4]
8.2 <1> [1]
8.5 <1> [3, 11]

pH-Range

7.2-8.8 <1> (<1> pH 7.2: about 80% of maximal activity, pH 8.8: about 70% of maximal activity [1]) [1]

Temperature optimum (°C)

30-35 <2> [4]

4 Enzyme Structure

Molecular weight

17000 <1> (<1> apoprotein, gel filtration [3,11]) [3, 11]

Subunits

monomer <1> (<1> 1 * 17000, SDS-PAGE [3,8]) [3, 8]

Posttranslational modification

lipoprotein <1> (<1> absolute requirement for a phospholipid cofactor, mixture of phosphatidylglycerol and cardiolipin, either of which is effective alone in restoring the activity of the enzyme [2]; <2> requirements for endogenous and exogenous phospholipids. Enzyme requires a fluid lipid surface for activity, lysophosphatidylglycerol and ditetradecanoylphosphatidylcholine are the best activators [5]; <1> reactivation of apoprotein by synthetic lecithins [7]; <1> lipid requirement of the enzyme is nonelectrostatic in nature [8]; <1> absolute requirement for phospholipid and for detergent [8]; <1> overview: activation by commercial phospholipids and glycolipids, lipids providing a hydrated, loosely packed, highly fluid environment are often effective activators [9]; <1> phospholipid cofactor required [3]) [2, 3, 5, 7, 8, 9, 11]

5 Isolation/Preparation/Mutation/Application

Localization

membrane <1-3> (<2> bound to [4]; <1> exclusively found in the cell membrane fraction [1]) [1, 3, 4, 5, 6, 7, 8, 11]

Purification

<1> [3, 8, 11]
<2> (partial [5]) [5]
<3> (partial [6]) [6]

6 Stability

Temperature stability

100 <1> (<1> pH 8.2, aqueous suspension in Tris-HCl, presence of substrate, $t_{1/2}$: 10 min [1]; <1> solution in 1-butanol, activation of nearly 50% after 20 min [1]; <1> 30 min, about 30% loss of activity [3,11]) [1, 3, 11]

Organic solvent stability

1-butanol <1> (<1> highly stable [1]) [1]

General stability information

<1>, enzyme is quite stable to SDS, recovered from SDS gel slices after solubilization in 1% SDS, 1% 2-mercaptoethanol [11]
<1>, relatively unstable in suspension in water [1]
<1>, remarkably stable towards SDS, 8 M urea and SH-reagents [3, 11]

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1-Phosphatidylinositol 4-kinase 2.7.1.67

1 Nomenclature

EC number
2.7.1.67

Systematic name
ATP:1-phosphatidyl-1D-myo-inositol 4-phosphotransferase

Recommended name
1-phosphatidylinositol 4-kinase

Synonyms
PI 4-kinase
PI kinase
PI4K
PtdIns 4-kinase
kinase, phosphatidylinositol (phosphorylating)
phosphatidylinositol 4-kinase
phosphatidylinositol kinase
type II phosphatidylinositol kinase

CAS registry number
37205-54-2

2 Source Organism

<1> *Homo sapiens* [1, 9, 11, 12, 13, 26, 27, 30, 34, 39, 41, 42, 46, 47, 52]
<2> *Rattus norvegicus* [2, 3, 6, 22, 23, 29, 35, 39, 40, 45, 50, 53]
<3> *Bos taurus* [4, 10, 15, 19, 20, 25, 33, 39, 43, 45]
<4> *Triticum aestivum* [5]
<5> *Oryctolagus cuniculus* [7]
<6> *Saccharomyces cerevisiae* (strain ade5 MATa [8]; S288C [16]; phosphatidylinositol 4-kinases, Stt4p and Pik1p [51]) [8, 16, 17, 18, 28, 36, 51]
<7> *Arabidopsis sp.* [49]
<8> *Mus musculus* [21, 39, 54]
<9> *Sus scrofa* [24]
<10> *Ovis aries* [31]
<11> *Legionella micdadei* [32]
<12> *Dunaliella parva* [37]
<13> *Daucus carota* [14, 38, 44]
<14> *Spinacia oleracea* [48]

3 Reaction and Specificity

Catalyzed reaction

ATP + 1-phosphatidyl-1D-myo-inositol = ADP + 1-phosphatidyl-1D-myo-inositol 4-phosphate (<6> sequential bi-bi reaction mechanism. Phosphatidylinositol binds prior to ATP, and phosphatidylinositol 4-phosphate is the first product released in the reaction [8]; <6> sequential reaction mechanism [18,36])

Reaction type

phospho group transfer

Natural substrates and products

S ATP + 1-phosphatidyl-1D-myo-inositol <1, 2, 3, 6, 7, 8> (<1>, regulation by Ca^{2+} may act as a negative feedback system [13]; <2> phosphatidylinositol 4-kinase is a component of glucose transporter-containing vesicles. It may play a role in defining the fusogenic properties necessary to mediate membrane movement between the glucose transporter vesicles, plasma membranes and micosomes [29]; <1, 2, 3, 8> PI4K92 is a key enzyme regulating Golgi disintegration/reorganization during mitosis probably via phosphorylation by cyclin-dependent kinases on well-defined sites. PI4K55 is involved in the production of second messengers, diacylglycerol and inositol 1,4,5-triphosphate at the plasma membrane, moreover, in the endocytotic pathway in the cytoplasm [39]; <2> the enzyme is an integral component of the early signal transduction machinery during T-cell activation by concanavalin A and is actively regulated by protein tyrosine phosphorylation-dephosphorylation [40]; <3> phosphorylation of phosphatidylinositol at the D-4 position is an essential step in the biosynthesis of phosphatidylinositolpolyphosphates [45]; <1> enzyme is involved in the regulation of endosomal membrane traffic in mammalian cells [47]; <7> type III enzymes are responsible for distinct phosphoinositide pools [49]; <2> generation of 1-phosphatidyl-1D-myo-inositol 4-phosphate by the enzyme on synaptic vesicles may be the first step in the resynthesis of phosphatidylinositol 4,5-bisphosphate [50]; <6> Stt4p activity is required for maintenance of vacuole morphology, cell wall integrity, and actin cytoskeleton organization. In contrast Pik1p is essential for normal secretion, Golgi and vacuole membrane dynamics and endocytosis [51]; <8> exocytosis of insulin-containing secretory granules depends on phosphatidylinositol 4-kinase activity. Phosphatidylinositol 4-kinase serves as a metabolic sensor and regulates priming of secretory granules in pancreatic β cells [54]) (Reversibility: ? <1, 2, 3, 6, 7, 8> [13, 29, 39, 40, 45, 47, 49, 50, 51, 54]) [13, 29, 39, 40, 45, 47, 49, 50, 51, 54]

P ADP + 1-phosphatidyl-1D-myo-inositol 4-phosphate

Substrates and products

S 2'-dATP + 1-phosphatidyl-1D-myo-inositol <3> (Reversibility: ? <3> [4]) [4]

P 2'-dADP + 1-phosphatidyl-1D-myo-inositol 4-phosphate [1]

S ADP + 1-phosphatidyl-1D-myo-inositol <3> (<3> 12% of the activity with ATP [20]) (Reversibility: ? <3> [20]) [20]
P AMP + 1-phosphatidyl-1D-myo-inositol 4-phosphate
S ATP + 1-phosphatidyl-1D-myo-inositol <1-14> (<6> reverse reaction is facored in vitro [36]) (Reversibility: r <6> [36]; ? <1-14> [1-35,37-54]) [1-54]
P ADP + 1-phosphatidyl-1D-myo-inositol 4-phosphate
S CTP + 1-phosphatidyl-1D-myo-inositol <3> (<3> 10% of the activity with ATP [20]) (Reversibility: ? <3> [20]) [20]
P CDP + 1-phosphatidyl-1D-myo-inositol 4-phosphate
S GTP + 1-phosphatidyl-1D-myo-inositol <3, 8> (<3> 5% of the activity with ATP [20]; <2> no activity [35]) (Reversibility: ? <3,8> [4,20,21]) [4, 20, 21]
P GDP + 1-phosphatidyl-1D-myo-inositol 4-phosphate [1]
S ITP + 1-phosphatidyl-1D-myo-inositol <3> (<3> 11% of the activity with ATP [20]) (Reversibility: ? <3> [20]) [20]
P IDP + 1-phosphatidyl-1D-myo-inositol 4-phosphate
S TTP + 1-phosphatidyl-1D-myo-inositol <3> (<3> 3% of the activity with ATP [20]) (Reversibility: ? <3> [20]) [20]
P TDP + 1-phosphatidyl-1D-myo-inositol 4-phosphate
S UTP + 1-phosphatidyl-1D-myo-inositol <3> (<3> 18% of the activity with ATP [20]) (Reversibility: ? <3> [20]) [20]
P UDP + 1-phosphatidyl-1D-myo-inositol 4-phosphate
S dATP + 1-phosphatidyl-1D-myo-inositol <3> (<3> 104% of the activity with ATP [20]) (Reversibility: ? <3> [20]) [20]
P dADP + 1-phosphatidyl-1D-myo-inositol 4-phosphate

Inhibitors

2'(3')-O-(2,4,6-trinitrophenyl)ATP <10> [31]
2'-dATP <1> [9]
2,3-dihydroxybenzaldehyde <1> [12]
3'-dATP <1> [9]
5'-AMP <1> (<1> 0.5 mM, 10% inhibition [26]) [26]
5,5'-dithiobis(2-nitrobenzoate) <10> (<10> 0.03 mM, 10 min, 95% inhibition [31]) [31]
8-bromo-ATP <6> (<6> no effect of enzyme activity in absence of spermidine, rather potent inhibitor in presence of stimulating amounts of spermidine [8]) [8]
ADP <1, 2, 3, 6, 9, 10, 12> (<6> 5 mM, 90% inhibition [17]; <9> IC50: 0.12 mM. 1 mM, 92% inhibition [24]; <1> 0.5 mM, 58% inhibition [26]; <1> inhibition of mPIK-I and mPIK-III [34]; <2> 0.5 mM, 77.4% inhibition [35]; <13> up to 0.1 mM, no effect [44]) [1, 9, 17, 19, 24, 26, 31, 34, 35, 37]
AMP <1, 2, 3, 9, 12> (<9>, 1 mM, 48% inhibition [24]; <2> 0.5 mM, 70.8% inhibition [35]) [1, 9, 19, 24, 35, 37]
ATP <2> (<2> above 4 mM [6]) [6]
ATPγS <6, 10> (<6> 5 mM 90% inhibition [17]) [17, 31]

CDP <2, 12> (<2> 0.5 mM, 72.8% inhibition [35]) [35, 37]
CTP <2, 12> (<2> 0.5 mM, 38% inhibition [35]) [35, 37]
Ca^{2+} <1, 2, 3, 4, 6, 8, 9, 13> (<1,4>, inhibits in the presence of Mg^{2+} [1,5]; <3> reversibly inhibited by free Ca^{2+} in the nanomolar and low micromolar range, dependening on the concentration of Mg^{2+} [15]; <6>, IC50: 5.6 mM [18]; <3> 0.3 mM, 50% inhibition. Increasing Mg^{2+} concentrations antagonize this inhibition [20]; <8> competitive inhibitor of Mg^{2+} [21]; <2> 1 mM [22]; <9> inhibits Mg^{2+}-stimulated activity, I50: 0.4 mM [24]; <1> inhibition depends in Ca^{2+} concentration [30]; <6> 10 mM, 50% inhibition [36]; <13> 0.01 mM, 90% inhibition [38]; <13> 0.01 mM to 1 mM: no effect [44]) [1, 2, 3, 5, 8, 15, 18, 20, 21, 22, 24, 30, 33, 36, 38, 53]
Co^{2+} <1> [30]
Cu^{2+} <2> (<2> 0.25 mM $CuCl_2$, complete inhibition [3]) [3]
Cutsum <2> (<2> above 2% v/v [6]) [6]
F^- <2, 6> [3, 16]
Fe^{2+} <1> [30]
GDP <1, 9> (<9> 1 mM, 27% inhibition [24]; <1> 0.5 mM, 4% inhibition [26]) [24, 26]
GMP <9> (<9> 1 mM, 20% inhibition [24]) [24]
GTP <1, 2, 9, 12> (<9> 1 mM, 24% inhibition [24]) [9, 24, 35, 37]
Hg^{2+} <6> [36]
$HgCl_2$ <6> (<6> 5 mM, complete inhibition [18]) [18]
K^+ <2, 6> [3, 16]
KCl <3> (<3> slight inhibition [15]) [15]
La^{3+} <2> (<2> 0.2 mM, 78% inhibition [22]) [22]
Li^+ <6> [16]
Lubrol PX <9> (<9> 1%, 72% inhibition [24]) [24]
MES <3> (<3> IC50: 4 mM [33]) [33]
Mg^{2+} <5> (<5> above 20 mM PI kinase I and II are inhibited. PI kinase III is only slightly inhibited at 50 mM [7]) [7]
$MgADP^-$ <6> (<6> IC50: 0.5 mM [18]) [18]
Mn^{2+} <3, 10> (<3> above 0.5 mM [10]; <10> above 1 mM [31]) [10, 31]
N^6-dimethylamine-adenosine 5'-triphosphate <6> (<6> no effect of enzyme activity in absence of spermidine, rather potent inhibitor in presence of stimulating amounts of spermidine [8]) [8]
NEM <2, 6, 9> (<2> 0.1 mM, complete loss of activity [2]; <6> 5 mM, complete inhibition [18]; <9> 0.1 mM, 42% inhibition [24]) [2, 18, 24, 36]
Na^+ <2, 6> [3, 16]
NaCl <3, 5> (<5> above 0.1 mM [7]; <3> slight inhibition [15]) [7, 15]
NaF <2> (<2> 60 mM, 73% inhibition [2]; <2>, 70% inhibition at 1 mM, complete inhibition at 10 mM [3]) [2, 3]
Ni^{2+} <1> [30]
PCMB <1, 2, 6, 9> (<2> 0.1 mM, complete loss of activity [2]; <6> 5 mM, complete inhibition [18]; <9> 0.1 mM, 98% inhibition [24]; <1> 0.01 mM, 74% inhibition [27]) [2, 18, 24, 27]
SDS <2> (<2> 0.05%, complete [35]) [35]

Triton X-100 <1, 2, 3, 9, 13> (<3> enzyme type I is inhibited at concentrations above 0.2% [10]; <2> above 0.4% w/v [22]; <9> 0.2%, 11% inhibition [24]; <1> inhibits activity of mPIK-I but rather weakly enhances mPIK-III activity [34]; <13> 0.3% v/v, 30% decrease of activity [38]) [6, 10, 22, 23, 24, 34, 38]
UTP <2, 12> (<2> 0.5 mM, 69% inhibition [35]) [35, 37]
Wortmannin <1, 3, 14> (<1> IC50: about 300 nM [41]; <3> type II enzyme is inhibited by low concentrations, type III enzyme requires mM concentrations for inhibition [45]; <14> IC50 for QI is approximately 0.007 mM, enzyme form QII is inhibited 30% at 0.01 mM [48]) [41, 45, 48]
adenosine <1, 2, 3, 6, 9, 10, 12, 13, 14> (<1>, IC50: 0.09 mM [1]; <3> enzyme type I is resistant, enzyme type II is inhibited [10]; <6> no inhibition [17]; <6>, 10 mM, 30% inhibition [17]; <9> I50: 0.07 mM. 1 mM, 93% inhibition [24]; <1> 0.5 mM, 67% inhibition [26]; <3> I50: 0.07 mM [33]; <13> 0.1 mM, no effect [44]; <14> 35% inhibition at 1 mM, 20% inhibition at 4 mM and above, enzyme form QI and QII [48]) [1, 9, 10, 11, 12, 17, 19, 24, 26, 29, 30, 31, 33, 37, 41, 44, 48, 53]
arachidonic acid <1> (<1> 0.5 mg/ml, 91.3% inhibition of mPIK-III [34]) [34]
cAMP <1, 2, 12> (<1> 0.5 mM, 32% inhibition [26]; <2> 0.5 mM, 10.8% inhibition [35]) [9, 26, 35, 37]
caffeine <1> (<1> 6.5 mM, 18% inhibition [26]) [26]
cetyltrimethylammonium bromide <2> [6]
deoxycholate <9, 10> (<9> 10 mM, 35% inhibition [24]) [24, 31]
formycin A <1> [12]
guanosine <9> (<9> 1 mM, 50% inhibition [24]) [24]
heparin <6> [8]
inosithin <2> (<2>, at high concentration [2]) [2]
iodoacetate <2> (<2> 0.1 mM, complete loss of activity [2]) [2]
orobol <1> [12]
phosphate <6> (<6> 20 mM, 50% inhibition [17]) [17]
phosphatidic acid <2> (<2> slight [22]) [22]
phosphatidylcholine <3, 11> (<3> inhibits type 3 kinase more strongly than type 2 kinase [19]; <11> 0.6 mM, 45% inhibition [32]) [19, 32]
phosphatidylethanolamine <9, 11, 12> (<9> 0.1 mM, 24% inhibition [24]; <11> 0.6 mM, 67% inhibition [32]) [24, 32, 37]
phosphatidylglycerol <9, 11> (<9> 0.1 mM, 63% inhibition [24]; <11> 0.6 mM, 68% inhibition [32]) [24, 32]
phosphatidylinositol 4,5-bisphosphate <1, 3, 8, 11> (<1> 50% inhibition when added in equimolar amounts to phosphatidylinositol [11]; <3> 0.5 mg/ml, 59% inhibition [20]; <11> 0.6 mM, 50% inhibition [32]) [11, 20, 21, 32]
phosphatidylinositol 4-phosphate <3, 8, 11> (<3> 0.5 mg/ml, 42% inhibition [20]; <11> 0.6 mM, 80% inhibition [32]) [19, 20, 21, 32]
phosphatidylserine <1, 9, 11> (<9> 0.1 mM, 33% inhibition [24]; <11> 0.6 mM, 28% inhibition [32]; <1> 0.5 mg/ml, 64.3% inhibition of mPIK-III [34]) [24, 32, 34]

quercetin <1, 2, 9> (<1> IC50: 0.004 mM [52]; <2> 1 mM, 36% inhibition [35]; <9> I50: 0.1 mM [24]) [24, 35, 52]
sodium cholate <1> (<1> inhibits activity of both mPIK-I and mPIK-III [34]) [34]
sodium deoxycholate <2> [6]
sodium mersalyl <9> (<9> 0.1 mM, 78% inhibition [24]) [24]
theophylline <1> (<1> 2 mM, 21% inhibition [26]) [26]
toyocamycin <1> (<1> IC50 is 0.0033 mg/ml [12]) [12]

Activating compounds

2-mercaptoethanol <2> (<2> enhances activity [3]) [3]
3-mercaptopropionic acid <2> (<2> enhances activity [3]) [3]
Cutsum <2> (<2>, stimulates [3]) [3]
EDTA <2> (<2>, 2 mM, marked stimulation, even in presence of 30 mM Mg^{2+} [3]) [3]
GSH <2> (<2> enhances activity [3]) [3]
PIK-A49 <13> (<13> purification of the phosphatidylinositol 4-kinase activator from carrot cells [14]) [14]
Triton X-100 <2, 3, 6, 10, 11, 12, 13> (<2, 6, 10>, stimulates [3, 17, 31]; <3> enzyme type I is stimulated at low concentrations, enzyme type II is stimulated by concentrations of Triton up to 1% [10]; <3> type 2 and type 3 enzyme are maximally active in 0.1-0.5% Triton X-100 [19]; <2> 0.4% stimulates 2fold [22]; <2> maximal activity at 0.2% w/v [29]; <11> 0.25-0.5%, stimulates [32]; <3> optimal activity at about 0.1% w/v [33]; <3> maximal stimulation at 0.1% w/v [33]; <1> inhibits activity of mPIK-I but rather weakly enhances mPIK-III activity [34]; <2> 0.1-1.0% w/v, stimulates [35]; <12> activity depends on presence of a surfactant. Optimal concentration is 3.7 mM [37]; <13> activity is enhances 5fold in presence of low concentrations of Triton X-100, 0.05-0.3% v/v [38]; <1> optimal concentration is 0.1% [41]; <13> maximal activity occurs when the substrate is added as Triton X-100/phosphatidylinositol mixed micelles [44]; <2> stimulates [53]) [3, 10, 17, 19, 22, 29, 31, 32, 33, 34, 35, 37, 38, 41, 44, 53]
Tween-20 <2> (<2>, weak stimulation [3]) [3]
cetyltrimethyl ammonium bromide <2> (<2>, weak stimulation [3]) [3]
dimethylsulfoxide <1> (<1> 1.0%, activation to 160% of the original activity [27]) [27]
heparin <1> (<1> 0.01 mM, activation to 116% of the orginal activity [27]) [27]
histone <1> (<1> stimulates [9]) [9]
inosithin <2> (<2>, at low concentrations [2]) [2]
octyl β-D-glucoside <11> (<11>, 10-20 mM, stimulates [32]) [32]
phosvitin <1> (<1> stimulates [9]) [9]
poly(L-lysine) <1, 3> (<1,3> stimulates [9,33]) [9, 33]
polyarginine <1> (<1> stimulates [9]) [9]
sodium cholate <6> (<6> stimulates 3.8fold at 14 mM [16]) [16]
sodium deoxycholate <2> (<2>, stimulates [3]) [3]
sodium taurocholate <2> (<2>, weak stimulation [3]) [3]

spermidine <1> (<1> half-maximal stimulation at 1.5 mM [9]) [9]
spermine <1> (<1> 2.0 mM, activation to 121% of the original activity [27]) [27]
spermine <2> (<2> stimulates [53]) [53]
trifluoperazine <3> (<3> stimulates activity of the purified enzyme less efficiently than that of the membrane-bound enzyme [33]) [33]
Additional information <2> (<2>, activity of the enzyme is dependent on SH groups [3]) [3]

Metals, ions

Ca^{2+} <11, 12, 14> (<11>, divalent cation requirement is best satisfied by Mg^{2+} and Ca^{2+} [32]; <12> enzyme requires divalent cations, Mg^{2+} with an optimal concentration of 30 mM, Ca^{2+} with an optimal concentration of 10 mM or Mn^{2+} with an optimal concentration of 10 mM. Mn^{2+} is the best activator. Mn^{2+}, Mg^{2+} or Ca^{2+} activate synergistically [37]; <13> 0.01-1 mM, no effect [44]; <14> inhibits enzyme form QI and QII at millimolar concentrations [48]) [32, 37, 48]
Co^{2+} <9> (<9> divalent metal ions stimulate in decreasing order of efficiency: Mg^{2+}, Fe^{2+}, Mn^{2+}, Fe^{3+}, Co^{2+} [24]) [24]
Fe^{2+} <9> (<9> divalent metal ions stimulate in decreasing order of efficiency: Mg^{2+}, Fe^{2+}, Mn^{2+}, Fe^{3+}, Co^{2+} [24]) [24]
Fe^{3+} <9> (<9> divalent metal ions stimulate in decreasing order of efficiency: Mg^{2+}, Fe^{2+}, Mn^{2+}, Fe^{3+}, Co^{2+} [24]) [24]
Mg^{2+} <1, 2, 3, 4, 5, 6, 8, 9, 10, 11, 12, 13, 14> (<1> less than 1 mM is required [1]; <2> Mg^{2+} or Mn^{2+} required. Mg^{2+} at 15 mM gives maximal activity with 5 mM ATP [2]; <2>, enzyme is activated by Mn^{2+} or Mg^{2+} [3]; <4,6>, required [5,8]; <5>, the three enzyme forms are completely dependent on Mg^{2+}, optimal activity at 4-10 mM [7]; <3> divalent cation required, maximal activity at 5-10 mM [10]; <1> absolute requirement [13]; <3> highly dependent on Mg^{2+}, maximal stimulation at 60 mM [15]; <6> dependent on, maximal activity at 10 mM [16]; <6> dependent on 10 mM Mg^{2+} [18]; <3>, K_m for type 2 kinase is 0.6 mM, K_m for type 3 kinase is 2.6 mM [19]; <3> activates, maximal activity at 2-10 mM [20]; <8> dependent on Mg^{2+} or Mn^{2+}. K_m for Mg^{2+} is about 5 mM [21]; <2> optimal activity in presence of at least 20 mM $MgCl_2$ [22]; <2> preferred divalent cation, maximal activity at 15 mM [23]; <9> divalent metal ions stimulate in decreasing order of efficiency: Mg^{2+}, Fe^{2+}, Mn^{2+}, Fe^{3+}, Co^{2+} [24]; <1> optimal activation at 10 mM [30]; <10> most effective divalent cation activator, maximal activation at 10 mM [31]; <11>, divalent cation requirement is best satisfied by Mg^{2+} and Ca^{2+} [32]; <3> stimulates [33]; <1> required, optimal concentrations for mPIK-I and mPIK-III at 5-10 mM, optimal concentration for cPIK-I and cPIK-II is 20-40 mM [34]; <2> the enzyme requires 5-20 mM Mg^{2+} or 1-2 mM Mn^{2+} [35]; <6> dependent on [36]; <12> enzyme requires divalent cations, Mg^{2+} with an optimal concentration of 30 mM, Ca^{2+} with an optimal concentration of 10 mM or Mn^{2+} with an optimal concentration of 10 mM. Mn^{2+} is the best activator. Mn^{2+}, Mg^{2+} or Ca^{2+} activate synergistically [37]; <13> divalent cation required. At 1-5 mM, Mn^{2+} is more effective than Mg^{2+} in increasing enzyme activity. Maximal activity occurs at 25-

40 mM [44]; <14> Mg^{2+} is the preferred divalent cation [48]; <2> 10-12 mM, effective divalent cation activator [53]) [1, 2, 3, 5, 7, 8, 10, 13, 15, 16, 18, 19, 20, 21, 22, 23, 24, 30, 31, 32, 33, 34, 35, 36, 37, 44, 48, 53]
Mn^{2+} <1, 2, 3, 4, 6, 10, 12, 14> (<2>, Mg^{2+} or Mn^{2+} required [2]; <2>, enzyme is activated by Mg^{2+} or Mn^{2+} [3]; <4>, can partially substitute for Mg^{2+} [5]; <2>, the enzyme is dependent on Mg^{2+} [6]; <3> optimal concentration for enzyme type I is 1-20 mM, optimal activity for enzyme type II is 0.5 mM [10]; <6> 0.5 mM can substitute for Mg^{2+} [18]; <8> dependent on Mg^{2+} or Mn^{2+} [21]; <2> can only partially replace Mg^{2+}. Activity at 2 mM or 5 mM is approximately 40% of the activity at 15 mM mM $MgCl_2$ [23]; <1> optimal activation at 0.5 mM [30]; <10> can partially replace Mg^{2+} at concentrations below 1 mM [31]; <2> the enzyme requires 5-20 mM Mg^{2+} or 1-2 mM Mn^{2+} [35]; <6>, can not substitute for Mg^{2+} [36]; <12> enzyme requires divalent cations, Mg^{2+} with an optimal concentration of 30 mM, Ca^{2+} with an optimal concentration of 10 mM or Mn^{2+} with an optimal concentration of 10 mM. Mn^{2+} is the best activator. Mn^{2+}, Mg^{2+} or Ca^{2+} activate synergistically [37]; <13> divalent cation required. At 1-5 mM, Mn^{2+} is more effective than Mg^{2+} in increasing enzyme activity [44]; <14> Mn^{2+} can partially substitute for Mg^{2+} in the reaction catalyzed by the QII enzyme but not in that catalyzed by QI. Mn^{2+} acts synergistically, with suboptimal Mg^{2+} concentrations to activate not only the QII enzyme, but also to some extent QI [48]; <2> can not substitute for Mg^{2+} in activation [53]) [2, 3, 5, 6, 10, 18, 21, 23, 30, 31, 35, 37, 44, 48]
Mn^{2+} <9> (<9> divalent metal ions stimulate in decreasing order of efficiency: Mg^{2+}, Fe^{2+}, Mn^{2+}, Fe^{3+}, Co^{2+} [24]) [24]
NaCl <12> (<12> enhances activity up to about 1.3fold at 1 M [37]) [37]

Turnover number (min^{-1})

166 <6> (1-phosphatidyl-1D-myo-inositol, <6> pH 8.0, 30°C [36]) [8, 36]
166 <6> (ATP, <6> pH 8.0, 30°C [36]) [8, 36]

Specific activity (U/mg)

0.0051 <3> [33]
0.0075 <13> [44]
0.027 <3> [25]
0.044 <1> [27]
0.05994 <2> [53]
0.077 <12> [37]
0.0933 <6> [16]
0.143 <2> [35]
0.581 <1> [41]
1.05 <1> [1]
1.149 <10> [31]
1.18 <1> [30]
2.7 <3> [20]
4.3 <6> [17]
4.75 <6> [8]
5.08 <6> [18]
8.824 <9> [24]

K_m-Value (mM)

0.016 <1> (1-phosphatidyl-1D-myo-inositol, <1> pH 7.2, 30°C [1]) [1]
0.017 <3> (phosphatidylinositol, <3> room temperature, enzyme type 2 [19]; <3> pH 7.0, 25°C [33]) [19]
0.018 <3> (ATP, <3> pH 8.3, enzyme type II [10]; <3> pH 7.0, 30°C [20]) [10, 20]
0.02 <1> (ATP, <1> pH 7.4, 25°C [9]) [9, 11]
0.022 <2, 10> (phosphatidylinositol, <10> pH 7.4, 30°C [31]) [31, 53]
0.027 <12> (phosphatidylinositol, <12>, pH 7.8, 28°C, at 0.75 mM Triton X-100 [37]) [37]
0.035 <1> (ATP, <1> pH 7.5, 30°C [27]) [27]
0.036-0.04 <13> (ATP) [38]
0.04 <1, 3> (phosphatidylinositol, <1> pH 7.5, 30°C [27]) [27, 33]
0.044 <3> ($MgATP^{2-}$, <3> in presence of 1 mM $MgCl_2$ [15]) [15]
0.05 <2> (ATP) [53]
0.05 <6> (phosphatidylinositol, <6> pH 7.5, 34°C [17]) [17]
0.054 <3> (ATP, <3> room temperature, enzyme type 2 [19]) [19]
0.055 <3> (ATP, <3> pH 7.0, 30°C [33]) [33]
0.055 <12> (phosphatidylinositol, <12>, pH 7.8, 28°C, at 1.5 mM Triton X-100 [37]) [37]
0.06 <9> (ATP, <9> pH 7.5, 30°C [24]) [24]
0.062 <3> ($MgATP^{2-}$, <3> in presence of 20 mM $MgCl_2$ [15]) [15]
0.067 <10> (ATP, <10> pH 7.4, 30°C [31]) [31]
0.07 <6> (phosphatidylinositol, <6> pH 8.0, 30°C [36]) [36]
0.071 <6> (phosphatidylinositol, <6> pH 8.5, 30°C [16]) [16]
0.074 <1> (ATP) [1]
0.075 <1> (ATP, <1> pH 7.4, 30°C, enzyme form mPIK-III [34]) [34]
0.1 <1> (ATP, <1> pH 7.2, 37°C [30]) [30]
0.1 <5, 6> (ATP, <5>, PI kinase I [7]; <6> pH 7.5, 34°C [17]) [7, 17]
0.1-0.15 <5> (ATP, <5>, PI kinase III [7]) [7]
0.115 <2> (phosphatidylinositol, <2> pH 7.4, 30°C [35]) [35]
0.12 <9> (phosphatidylinositol, <9> pH 7.5, 30°C [24]) [24]
0.127 <3> (phosphatidylinositol, <3> room temperature, enzyme type 3 [19]) [19]
0.13 <2> (ATP, <2> pH 7.5, 30°C, in presence of at least 20 mM $MgCl_2$ [22]) [22]
0.14 <8> (ATP, <8> pH 7.1, 25°C [21]) [21]
0.15 <2> (ATP, <2> pH 7.4, 30°C [35]) [35]
0.15 <3> (ATP, <3> pH 7.4, 30°C [25]) [25]
0.152 <1> (ATP, <1> pH 7.4, 30°C, enzyme form cPIK-I and cPIK-II [34]) [34]
0.17 <14> (phosphatidylinositol, <14> pH 7.5, enzyme form QII [48]) [48]
0.2 <5> (ATP, <5>, PI kinase II [7]) [7]
0.2 <1> (phosphatidylinositol, <1> pH 7.2, 37°C [30]) [30]
0.21 <6> (ATP, <6> pH 8.5, 30°C [16]) [16]
0.217 <1> (ATP, <1> pH 7.4, 30°C, enzyme form mPIK-I [34]) [34]
0.23 <14> (phosphatidylinositol, <14> pH 7.5, enzyme form QI [48]) [48]

0.25 <3> (ATP, <3> pH 8.3, enzyme type I [10]) [10]
0.3 <6> ($MgATP^{2-}$, <6> pH 8.0, 30°C [36]) [36]
0.3-0.5 <6> (ATP) [8]
0.36 <6> ($MgATP^{2-}$, <6> pH 7.0, 30°C [18]) [18]
0.39 <12> (ATP, <12> pH 7.8, 28°C, at 2 mM $MgCl_2$ and 10 mM $MnCl_2$ [37]) [37]
0.4 <13> (ATP, <13> pH 8.0, 25°C [44]) [44]
0.44 <14> (ATP, <14>, pH 7.5, enzyme form QII [48]) [48]
0.49 <14> (ATP, <14>, pH 7.5, enzyme form QI [48]) [48]
0.62 <8> (GTP, <8> pH 7.1, 25°C [21]) [21]
0.742 <3> (ATP, <3> room temperature, enzyme type 3 [19]) [19]
1 <1> (ATP, <1> 37°C [41]) [41]
1 <1> (phosphatidylinositol, <1> 37°C [41]) [41]
1.5 <11> (ATP, <11> pH 7.2, 37°C [32]) [32]
1.7 <12> (ATP, <12> pH 7.8, 28°C, at 30 mM $MgCl_2$ [37]) [37]
Additional information <5, 6> (<6> kinetic analysis using Triton X-100/ phosphatidylinostitol-mixed micelles [28]) [7, 28]

K_i-Value (mM)

0.0019 <1> (Ca^{2+}, <1> pH 7.2, 37°C, 45000 Da enzyme [30]) [30]
0.0025 <1> (Ca^{2+}, <1> pH 7.2, 37°C, 50000 Da enzyme [30]) [30]
0.01 <1> (ADP, <1> pH 7.4, 25°C, in absence of spermidine [9]) [9]
0.012 <1> (3'-dATP, <1> pH 7.4, 25°C, in absence of spermidine [9]) [9]
0.013 <1> (GTP, <1> pH 7.4, 25°C, in absence of spermidine [9]) [9]
0.014 <1> (2'-dATP, <1> pH 7.4, 25°C, in presence of 3 mM spermidine [9]) [9]
0.018 <2, 3> (adenosine, <3> room temperature, enzyme type 2 [19]) [19, 53]
0.02-0.03 <2> (adenosine) [29]
0.025 <12> (ADP, <12> pH 7.8, 28°C [37]) [37]
0.027 <3> (AMP, <3> room temperature, enzyme type 2 [19]) [19]
0.032 <10> (2'(3')-O-(2,4,6-trinitrophenyl)ATP, <10> pH 7.4, 30°C [31]) [31]
0.035 <10> (adenosine, <10> pH 7.4, 30°C [31]) [31]
0.04 <1> (N^6-dimethylamino-adenosine 5'-triphosphate, <1> pH 7.4, 25°C, in presence of 3 mM spermidine [9]) [9]
0.045 <3> (adenosine, <3> type II enzyme [45]) [45]
0.05 <10> (ADP, <10> pH 7.4, 30°C [31]) [31]
0.07 <1> (adenosine, <1> pH 7.4, 25°C, in absence of spermidine [9]) [9]
0.08 <1> (adenosine, <1> pH 7.2, 37°C, 45000 Da enzyme [30]) [30]
0.088 <3> (ATP, <3> type II enzyme [45]) [45]
0.1 <1> (Ca^{2+}, <1> pH 7.2, 30°C [1]) [1]
0.1 <1> (adenosine, <1> pH 7.2, 37°C, 50000 Da enzyme [30]) [30]
0.11 <1> (8-bromo-ATP, <1> pH 7.4, 25°C, in presence of 3 mM spermidine [9]) [9]
0.18 <10> (ATPγS, <10> pH 7.4, 30°C [31]) [31]
0.235 <3> (phosphatidylinositol 4-phosphate, <3> room temperature [19]) [19]
0.24 <3> (AMP, <3> room temperature, enzyme type 2 [19]) [19]
0.365 <1> (adenosine, <1> 37°C [41]) [41]

0.5 <1> (cAMP, <1> pH 7.4, 25°C, in absence of spermidine [9]) [9]
0.53 <1> (AMP, <1> pH 7.4, 25°C, in absence of spermidine [9]) [9]
0.57 <3> (ADP, <3> room temperature, enzyme type 3 [19]) [19]
0.774 <3> (phosphatidylinositol 4-phosphate, <3> room temperature [19]) [19]
0.775 <12> (adenosine, <12> pH 7.8, 28°C [37]) [37]
1.2 <12> (AMP, <12> pH 7.8, 28°C [37]) [37]
1.52 <3> (adenosine, <3> room temperature, enzyme type 3 [19]) [19]
1.68 <12> (CTP, <12> pH 7.8, 28°C [37]) [37]
1.68 <12> (cAMP, <12> pH 7.8, 28°C [37]) [37]
1.75 <12> (GTP, <12> pH 7.8, 28°C [37]) [37]
1.9 <1> (N^6-dimethylamino-adenosine 5'-triphosphate, <1> pH 7.4, 25°C, in absence of spermidine [9]) [9]
2.82 <3> (AMP, <3> room temperature, enzyme type 3 [19]) [19]
2.9 <12> (CDP, <12> pH 7.8, 28°C [37]) [37]
3.6 <12> (UTP, <12> pH 7.8, 28°C [37]) [37]
12 <8> (Ca^{2+}, <8> pH 7.1, 25°C [21]) [21]

pH-Optimum

6-7 <3> [20]
6-8 <10> [31]
6.5 <13> (<13> when the substrate is added in form of phosphatidylinositol/phosphatidylserine mixed micelles, 1 mM phosphatidylinositol in 0.025% v/v final concentration of Triton X-100 [38]) [38]
6.5-7 <4> (<4> phosphorylation of endogenous 1-phosphatidyl-1D-myo-inositol without Triton X-100 [5]) [5]
6.5-7.5 <5, 13> (<5> PI kinase III [7]; <13> in presence of endogenous substrate [38]) [7, 38]
6.5-8 <2> (<2> activity in presence of 0.25% Triton X-100 [23]) [23]
6.5-8.6 <4> (<4> phosphorylation of endogenous 1-phosphatidyl-1D-myo-inositol without 0.01% Triton X-100 [5]) [5]
7 <3, 6, 11, 13> (<3> broad, around [33]; <13> when phosphatidylinositol/Triton X-100 mixed micelles, 1 mM phosphatidylinositol in 0.025% v/v final concentration of Triton, are used [38]) [18, 32, 33, 38]
7-8 <8> [21]
7-9 <2> [22]
7.4 <2> (<2>, broad [35]) [35]
7.5 <1> (<1> cPIK-I and cPIK-II [34]) [34]
7.5-9 <5> (<5> PI kinase I [7]) [7]
7.7 <2> [6]
7.8 <12> [37]
8 <6> [8]
8-8.5 <13> [44]
8.3 <2> [2]
8.5 <6> [16]
8.5-9 <1> (<1> mPIK-I and mPIK-III [34]) [34]
9 <5> (<5> PI kinase II [7]) [7]

pH-Range

5-8 <3> (<3> pH 5.0: about 40% of maximal activity, pH 8.0: about 50% of maximal activity [20]) [20]
5.3-9 <2> (<2> pH 5.3: about 80% of maximal activity, pH 8.6: about 60% of maximal activity [23]) [23]
5.5-8 <10> (<10> rapid decrease of activity above pH 8.0 and below pH 5.5 [31]) [31]
5.5-9 <1> (<1> pH 5.5: 60% of maximal activity for mPIK-I and 45% of maximal activity of mPIK-III, pH 8.5-9.0: pH-optimum for enzyme form mPIK-I and mPIK-III [34]) [34]
6-8.5 <5> (<5> pH 7.5: about 75% of maximal activity, pH 10.5: about 40% of maximal activity, PI kinase III [7]) [7]
6-9 <1, 6> (<6> active from pH 6.0 to pH 9.0 with Tris maleate buffer [16]; <1>, activity of cPIK-I and cPIK-III is almost undetectable at pH 6.0, pH 9.0: about 80% of maximal activity [34]) [16, 34]
6-9.5 <6> (<6> pH 6.0: about 75% of maximal activity, pH 9.5: about 60% of maximal activity [18]) [18]
6.3-8.7 <2> (<2> pH 6.3: about 50% of maximal activity, pH 8.7: about 80% of maximal activity [2]) [2]
6.5-8.5 <13> (<13> pH 6.5: about 40% of maximal activity, pH 8.0-8.5: optimium [44]) [44]
7-8 <14> (<14> pH 7.0: about 70% of maximal activity, pH 8.0: about 45% of maximal activity [48]) [48]
7-8.5 <14> (<14> pH 7.0: about 50% of maximal activity, pH 8.5: about 40% of maximal activity, enzyme form QII [48]) [48]
7.3-9 <2> (<2> pH 7.3: about 75% of maximal activity, pH 9.0: about 95% of maximal activity [6]) [6]
7.5-10.5 <5> (<5> pH 7.5: about 50% of maximal activity, pH 10.5: about 65% of maximal activity, PI kinase I [7]) [7]

Temperature optimum (°C)

28 <12> (<12> at 30 mM $MgCl_2$ [37]) [37]
30 <6> [18]
40 <6> [36]
42 <12> (<12> at 2 mM $MgCl_2$ and 10 mM $MnCl_2$ [37]) [37]

Temperature range (°C)

20-40 <6> (<6> 20°C: about 65% of maximal activity, 40°C: about 65% of maximal activity [18]) [18]
20-50 <6> (<6> 20°C: about 20% of maximal activity, 50°C: about 15% of maximal activity [36]) [36]

4 Enzyme Structure

Molecular weight

55000 <3> (<3>, enzyme type 2, sucrose density gradient centrifugation [19]) [19]
60000 <8> (<8> gel filtration [21]) [21]
76000 <1> (<1> mPIK-I, gel filtration [34]) [34]
80000 <1, 2, 3> (<3> enzyme type II, gel filtration [10]; <1> mPIK-III, gel filtration [34]; <2>, gel filtration [35]) [10, 34, 35]
83000 <13> (<13> gel filtration [44]) [44]
150000 <10> (<10> gel filtration [31]) [31]
200000 <3> (<3> enzyme type I, gel filtration [10]) [10]
230000 <3> (<3> enzyme type 3, sucrose density gradient centrifugation [19]) [19]
550000 <1> (<1> cPIK-I and cPIK-II, gel filtration [34]) [34]
Additional information <1> (<1> the enzyme binds substantial amounts of Triton X-100 and is actually present in detergent-containing solutions as a complex with a molecular weight of 120000 Da. It seems likely that the active species of the enzyme is a single 55000 Da polypeptide chain bound to essentially one Triton X-100 micelle [1]) [1]

Subunits

? <1, 2, 3, 6, 9, 10, 14> (<6> x * 35000, SDS-PAGE [36]; <1,3> x * 45000, SDS-PAGE [25,30]; <6>, x * 45000, on storage the enzyme degrades to MW 30000 Da and 35000 Da but retains full activity, SDS-PAGE [8]; <1> x * 50000, SDS-PAGE [30]; <3> x * 54305, calculation from nucleotide sequence [45]; <2,6,9,10> x * 55000, SDS-PAGE [18,24,31,53]; <1> x* 56000, SDS-PAGE [11]; <1> x * 59000, SDS-PAGE [27]; <14> x * 65000, SDS-PAGE [48]; <6> x * 125000, SDS-PAGE [17]) [8, 11, 17, 18, 24, 25, 27, 30, 31, 36, 45, 48, 53]
monomer <1, 2, 3, 13> (<2> 1 * 76000, SDS-PAGE [35]; <1,3> 1 * 55000, SDS-PAGE [1,20]; <13> 1 * 80000, SDS-PAGE [44]; <14> x * 120000, SDS-PAGE [48]) [1, 20, 35, 44, 48]
Additional information <1> (<1> two enzyme species a 45000 Da enzyme and a 50000 Da enzyme [30]) [30]

Posttranslational modification

phosphoprotein <1, 2, 3> (<2> the enzyme is an integral component of the early signal transduction machinery during T-cell activation by concanavalin A and is actively regulated by protein tyrosine phosphorylation-dephosphorylation [40]; <1> phosphoprotein, additionally phosphate is incorporated by incubation with ATP/Mg or ATP/Mn. Phosphorylation sites are mapped by MALDI-MS and LC-MS/MS at the following positions: S258, T263, S266, S277, S294, T423, S496, T504 [41]; <3> enzyme exhibits autophosphorylation that is enhanced by Mn^{2+} ions. Isolated C-terminal catalytic domain still displays autophosphorylation. Phosphorylation of endogenous or overexpressed PI4Kβ is also observed in COS-7 cells [43]) [40, 41, 43]

5 Isolation/Preparation/Mutation/Application

Source/tissue

A-431 cell <1> [1, 9, 42]
OVCAR-5 cell <1> [52]
T-lymphocyte <8> [21]
adipocyte <2> [29]
adrenal <3> (<3> PI4KII and PI4KIII [45]) [15, 45]
adrenal medulla <3> [33]
brain <2, 3, 10> (<3> myelin [25]; <2> the type IIα enzyme is concentrated both at the synapse and in the region of the Golgi complex in neuronal perikarya [50]) [2, 3, 4, 19, 25, 31, 35, 39, 43, 45, 50]
erythrocyte <1> [11, 26, 27, 30]
heart <2> [2]
kidney <2> (<2>, cortex [6]) [2, 6]
leaf <14> [48]
liver <2, 9> [2, 22, 23, 24]
lung <2> [2]
lymphocyte <2> (spleen; <2> type II enzyme [40]) [40, 53]
myelin <3> [25]
neuronal cell <1, 2, 3, 8> [39]
ovarian cancer cell <1> [52]
pancreas <8> [54]
pancreatic β cell <8> [54]
platelet <1> [13, 34]
renal cortex <2> [6]
reticulocyte <5> [7]
root <4> (<4> dark grown [5]) [5]
shoot <4> (<4> dark grown [5]) [5]
spleen <2> (<2> lymphocyte [40,53]) [2, 40, 53]
suspension culture <13> [38, 44]
uterus <3> [10, 20]

Localization

Golgi apparatus <1> (<1> PI4KIIα is primarily Golgi-associated. Platelet-derived growth factor promotes PI4KIIβ recruitment to membrane ruffles [46]) [46]
chromaffin granule <3> [15, 33, 45]
cytoplasm <1, 2, 3, 8> (<1,2,3,8> neuronal cells [39]) [39]
cytosol <1, 5> (<1> enzyme form cPIK-I and cPIK-II [34]; <1> PI4KIIβ is primarily cytosolic and it associates peripherally with plasma membranes, endoplasmic reticulum and the Golgi apparatus [46]) [7, 34, 46]
endoplasmic reticulum <1> (<1> highly active pool of type II phosphatidylinositol 4-kinase is localized in a p97/valosin-containing-protein-rich fraction of the endoplasmic reticulum [42]) [42]
endosome <1> (<1> endosome [47]) [47]

lysosomal membrane <2> [22]
lysosome <2> (<2>, membrane [22]) [22]
membrane <1, 2, 3, 6, 8, 10, 14> (<6> associated [16, 18]; <3,8> integral membrane protein [21, 33]; <1, 2, 3, 10> bound to [27, 31, 33, 35]; <1> enzyme form mPIK-I and mPIK-II [34]; <1, 2, 3, 8> PI4K230 is distributed evenly on membranes that are ultra structurally cisterns of the rough endoplasmic reticulum, outer membranes of mitochondria, multivesicular bodies and are in close vicinity of synaptic contacts [39]; <3> type II enzyme is almost exclusively found in membrane fraction [45]; <14>, firmly associated with plasma membrane [48]) [1, 4, 9, 11, 16, 18, 21, 25, 27, 30, 31, 33, 35, 39, 45, 48]
microsome <1, 2, 6, 9> [2, 16, 24, 47]
nucleolus <1, 2, 3, 8> (<1, 2, 3, 8> enzyme PI4K230 [39]) [39]
nucleus <1, 2, 3, 13> (<1, 2, 3, 8> enzyme form PI4K92 [39]) [38, 39]
plasma membrane <2, 4, 13, 14> (<14> firmly associated with plasma membrane [48]) [5, 14, 23, 29, 48]
soluble <6, 12> [17, 37]
transporter vesicle <2> (<2> glucose-transporter [29]) [29]
Additional information <2> (<2> no or minimal activity can be ascribed to mitochondria, lysosomes, Golgi membranes or endoplasmic reticulum [23]) [23]

Purification

<1> (enzyme type II [11]; mPIK-I and mPIK-III from membrane fraction and cPIK-I and cPIK-II from cytoslic fraction [34]; type III kinase, isoform PI4K92 is expressed as His6 tagged protein in Sf9 cells [41]; COS-7 cells transfected with the HA-tagged PI 4-kinase construct [47]) [1, 11, 27, 30, 34, 47]
<2> (enzyme type IIα [50]; type II enzyme [53]) [35, 50, 53]
<3> (phosphatidylinositol 4-kinase type I and type II [10]; glutathione S-transferase PI4Kβ fusion protein [43]; partial [45]) [4, 10, 20, 25, 33, 4, 45]
<5> (PI kinase I, II and III [7]) [7]
<6> (partial [16]) [8, 16, 17, 18, 28, 36]
<9> [24]
<10> [31]
<12> (partial [37]) [37]
<13> [44]
<14> (partial, enzyme form QI and QII [48]) [48]

Cloning

<1> (isoform PI4K92 is expressed as His6 tagged protein in Sf9 cells reaching a level of approximately 5% of cellular proteins [41]) [41, 46]
<2> [45]
<3> (glutathione S-transferase PI4Kβ fusion protein, expression in Escherichia coli [43]) [43]
<7> (type III enzyme expressed in Spodoptera frugiperda [49]) [49]

Engineering

PI4K230 Δ1-1189 <1> (<1> deletion mutant with 97000 Da compared to wild-type enzyme of 230000 Da, the enzyme is stable but activity is not detectable [39]) [39]
PI4K230 Δ1-1427 <1> (<1> deletion mutant with 68000 Da compared to wild-type enzyme of 230000 Da, the enzyme is stable but activity is not detectable [39]) [39]
PI4K230 Δ1-1531 <1> (<1> deletion mutant with 56000 Da compared to wild-type enzyme of 230000 Da, the enzyme is stable but activity is not detectable [39]) [39]
PI4K230 Δ1-872 <1> (<1> deletion mutant with 130000 Da compared to wild-type enzyme of 230000 Da, the enzyme is stable, 0.048 mM/mg*min compared to 0.058 mM/mg*min for the wild-type enzyme [39]) [39]
Additional information <3> (<3> creation of a glutathione S-transferase PI4Kβ fusion protein that is biologically active and phosphorylates phosphatidylinositol in ist 4-position with wortmannin sensitivity and kinetic parameters that are identical to thoses of purified wild-type enzyme [43]) [43]

6 Stability

pH-Stability

7-8 <3> (<3> optimal stability of enzyme type I and type II [10]) [10]

Temperature stability

25 <8> (<8> enzyme is stable [21]) [21]
30 <6> (<6> labile above [8]; <6> unstable above [18,36]) [8, 18, 36]
33 <8> (<8> 3 min, 50% inactivation [21]) [21]
40 <6> (<6> $t_{1/2}$: 2.1 min [18]) [18]
50 <6> (<6> 20 min, complete inactivation [36]) [36]
56 <8> (<8> cell lysate, 5 min, virtually all activity is lost [21]) [21]
60 <6> (<6>, 10 min, complete inactivation [18]) [18]

Storage stability

<1>, -20°C, 50% glycerol, stable for 4 weeks [27]
<1>, -20°C, buffer containing 30% glycerol and 2 mM DTT, minimal loss of activity after 2 weeks [1]
<2>, -20°C, 6 months, 50% loss of activity [2]
<2>, -20°C, brain homogenate in 0.32 M sucrose, stable for up to 6 d [3]
<6>, -80°C, about 50% loss of activity after 2 weeks [16]
<6>, -80°C, purified enzyme is completely stable for at least 2 months [36]
<6>, 8°C, 30% loss of activity after 4 h [16]

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1-Phosphatidylinositol-4-phosphate 5-kinase 2.7.1.68

1 Nomenclature

EC number
2.7.1.68

Systematic name
ATP:1-phosphatidyl-1D-myo-inositol-4-phosphate 5-phosphotransferase

Recommended name
1-phosphatidylinositol-4-phosphate 5-kinase

Synonyms
MSS4p
PI4P 5-kinase
PI4P5K
PIP kinase
PIP-5kin
PIP5K
PIP5Ks
PtdIns(4)P 5-kinase
diphosphoinositide kinase
phosphatidylinositol 4-phosphate kinase
phosphatidylinositol-4-phosphate 5-kinase

CAS registry number
9032-61-5

2 Source Organism

<1> *Rattus norvegicus* [1, 3, 7, 8, 9, 10, 12, 15, 29, 31]
<2> *Bos taurus* [2, 5, 11, 14, 17, 21]
<3> *Homo sapiens* [3, 4, 13, 19, 33]
<4> *Saccharomyces cerevisiae* [6, 20, 22]
<5> *Triticum aestivum* [16]
<6> *Mus musculus* [18, 25, 26, 27, 32, 34, 35]
<7> *Schizosaccharomyces pombe* [23]
<8> *Arabidopsis thaliana* [24]
<9> *Ovis aries* [28]
<10> *Candida albicans* [30]

3 Reaction and Specificity

Catalyzed reaction

ATP + 1-phosphatidyl-1D-myo-inositol 4-phosphate = ADP + 1-phosphatidyl-1D-myo-inositol 4,5-bisphosphate

Reaction type

phospho group transfer

Natural substrates and products

S ATP + 1-phosphatidyl-1D-myo-inositol 4-phosphate <1, 2, 3, 4, 6, 7> (<4> enzyme activity is regulated by cAMP levels. This regulation may be critical in the initiation of cell growth for which 1-phosphatidyl-1D-myo-inositol 4,5-bisphosphate seems to be necessary [6]; <1> the enzyme from liver membrane is possible regulated by a G-protein [10]; <2> the enzyme may be under control of phosphatidic acid level in membranes [14]; <6> in addition to regulating early steps in endocytosis, the enzyme acts through 1-phosphatidyl-1D-myo-inositol 4,5-bisphosphate to regulate endosomal trafficking and/or fusion [18]; <2> the product 1-phosphatidyl-1D-myo-inositol 4,5-bisphosphate is a key precursor in phosphoinositide signaling that also regulates some proteins and cellular processes directly [21]; <4> the MSS4 gene product functions in regulation of actin-binding proteins through generation of 1-phosphatidyl-1D-myo-inositol 4,5-bisphosphate from 1-phosphatidyl-1D-myo-inositol 4-phosphate in or near the plasma membrane [22]; <7> the enzyme catalyzes the last step in the synthesis of phosphatidylinositol 4,5-bisphosphate, which is a precursor of diacylglycerol and inositol 1,4,5-triphosphate and is also involved in regulation of actin cytoskeleton remodeling and membrane traffic. The enzyme is regulated by casein kinase Cki1 [23]; <8> enzyme is induced by water stress and abscisic acid. The enzyme is involved in water-stress signal transduction [24]; <6> enzyme type 1β is essential for epidermal growth factor receptor-mediated endocytosis [25]; <6> PIPKIα activity is involved in the actin remodeling that is a prerequisite for efficient phagocytosis. PIPKIα appears to contribute to the transient changes in 1-phosphatidyl-1D-myo-inositol 4,5-bisphosphate levels that are associated with, and likely required for, the recruitment and regulation of actin-modulating proteins [26]; <6> PIP-5kina-α functions as a downstream effector for RhoA/ROCK to couple lysophosphatidic acid signaling to neurite retraction presumably through its product phosphatidylinositol 4,5-bisphosphate [27]; <1> type I phosphatidylinositol 4-phosphate 5-kinase directly interacts with ADP-ribosylation factor 1 and is responsible for phosphatidylinositol 4,5-bisphosphate synthesis in the Golgi compartment [29]; <10> the enzyme is stimulated during temperature-induced morphogenesis, i.e. switch to the hyphal growth form [30]; <1> the enzyme form PIPKIγ may cooperate with synaptojanin in the regulation of actin and synaptic vesicle traffic [31]; <6> PIP 5-kinase α is a critical mediator of thrombin- and Rac-dependent actin assembly

[32]; <3> Rho-kinase is involved in the Rho-controlled synthesis of 1-phosphatidyl-1D-myo-inositol 4,5-bisphosphate by phosphatidylinositol-4-phosphate 5-kinase [33]; <6> the enzyme plays an essential role during neurite retraction in response to a number of diverse stimuli [35]; <6> the activity of the enzyme is regulated by the reversible balance between cAMP-dependent protein kinase-dependent phosphorylation and protein phosphatase 1-dependent dephosphorylation [34]) (Reversibility: ? <1, 2, 3, 4, 6, 7> [6, 10, 18, 22, 23, 27, 29, 31, 32, 33, 34, 35]) [6, 10, 14, 18, 21, 22, 23, 24, 25, 26, 27, 29, 30, 31, 32, 33, 34, 35]

P ADP + 1-phosphatidyl-1D-myo-inositol 4,5-bisphosphate

Substrates and products

S ATP + 1-phosphatidyl-1D-myo-inositol 4-phosphate <1-10> (Reversibility: ? <1-10> [1-32]) [1-32]

P ADP + 1-phosphatidyl-1D-myo-inositol 4,5-bisphosphate

S ATP + phosphatidylinositol 3-phosphate <3> (Reversibility: ? <3> [19]) [19]

P ADP + phosphatidylinositol 3,4-bisphosphate <3> [19]

S ATP + phosphatidylinsositol 3,4-bisphosphate <3> (Reversibility: ? <3> [19]) [19]

P ADP + phosphatidylinositol 3,4,5-trisphosphate

S GTP + 1-phosphatidyl-1D-myo-inositol 4-phosphate <1, 3> (<3> 0.05 mM GTP is 2fold more active than ATP with type II enzyme. 0.05 mM GTP is 5fold more active than ATP with the type I enzyme [4]; <1> 50% of the activity with ATP [8]; <9> no activity [28]) (Reversibility: ? <1,3> [4,8]) [4, 8]

P GDP + 1-phosphatidyl-1D-myo-inositol 4,5-bisphosphate

Inhibitors

1-phosphatidyl-1D-myo-inositol 4,5-bisphosphate <1, 2, 3, 7> (<1> product inhibition [1]; <3> 20 nM, maximal inhibition [3]; <2> 50% inhibition at equimolar concentrations of product and substrate [5]; <3> half-maximal inhibition at a ratio of 1-phosphatidyl-1D-myo-inositol 4,5-bisphosphate to 1-phosphatidyl-1D-myo-inositol 4-phosphate of 1.5:1 [13]; <7> 0.1 mM, 55% inhibition [23]) [1, 3, 5, 9, 13, 23]

2'(3')-O-(2,4,6-trinitrophenyl)ATP <9> (<9> competitive [28]) [28]

ADP <1, 2> [10, 11]

ATP <1> (<1> above 4 mM [15]) [15]

Ca^{2+} <1, 2> (<1>, inhibits in presence of optimal Mg^{2+} concentrations [1]; <2>, free Ca^{2+}, IC50: about 0.0001 mM [11]) [1, 11]

Cutsum <1> [15]

EDTA <1> (<1> slight inhibition between 0.5-5 mM [15]) [15]

GSSG <1> (<1> 0.1 mM, 17% inhibition [1]) [1]

NEM <1> (<1> 1 mM, 26% inhibition [1]) [1]

PCMB <1> (<1> 0.1 mM, complete inhibition [1]) [1]

Triton X-100 <1, 5> (<5> above 0.015%, phosphorylation of endogenous substrate is inhibited [16]; <1>, 0.5-3.0% w/v, 40% inhibition [1]) [1, 15, 16]

cetyltrimethylammonium bromide <1> [15]

cysteine <1> (<1> 1 mM, 49% inhibition [1]) [1]
heparin <1, 3> (<3> type II kinase [4]; <1> IC50: 0.002 mg/ml, competitive towards 1-phosphatidyl-1D-myo-inositol 4-phosphate [8]) [4, 8]
phosphatidic acid <7> (<7> 0.1 mM, 125% stimulation [23]) [23]
phosphatidylinositol <7> (<7> 0.1 mM, 125% stimulation [23]) [23]
phosphatidylserine <7> (<7> 0.1 mM, 125% stimulation [23]) [23]
quercetin <1> (<1> ID50: 0.0002 mM, competitive towards ATP or GTP [8]) [8, 10]
sodium deoxycholate <1> [15]
spermine <7> (<7> 0.03 mg/ml, 50% inhibition [23]) [23]
trifluoperazine <2> (<2> IC50: about 0.015 mM [11]) [11]
wortmannin <4> (<4> phosphatidylinositol 4-kinase activity of STT4, but not of PIK1 is potently inhibited [20]) [20]

Activating compounds

Triton X-100 <3, 5> (<3>, the optimal activity obtained in Triton X-100 is 6fold higher than that obtained in the absence of Triton X-100 [13]; <5> 0.3% stimulates phosphorylation of exogenous substrate 2-3fold [16]) [13, 16]
acetylcholine <1> (<1> 0.01 mM or 0.1 mM, each in presence of 0.1 mM eserine, stimulates the enzyme in homogenates by 30-40% [1]) [1]
heparin <3> (<3> type I enzyme is stimulated at low concentrations [4]) [4]
myelin basic protein <1> (<1> enhances activity when phosphatidylinositol 4-phosphate/phosphatidylethanolamine vesicles are used as substrate [9]) [9]
phosphatidic acid <2, 10> (<2> stimulates in a concentration-dependent manner up to 20fold when an equal molar ratio of phosphatidic acid to phosphatidylinositol 4-phosphate is attained [14]; <10> stimulates [30]) [14, 30]
spermidine <1> (<1> enhances activity [9]) [9]
spermine <1, 3, 7> (<3> 2 mM, 4fold increase in activity of type I enzyme, no effect on type II enzyme [4]; <1> 1 mM, 3fold stimulation. Stimulation decreases to half at physiological ionic strength and is not affected appreciably by variations in the concentration of ATP and $MgCl_2$ [7]; <1> strong activation in presence of micromolar concentrations, at 2 mM Mg^{2+} optimal spermine concentration is 0.1-0.2 mM spermine [8]; <1> enhances activity severalfold. Causes a shift in the $MgCl_2$ saturation curve from sigmoidal to hyperbolic, lowering the Mg^{2+} concentration required for optimum kinase activity to the physiological range. 0.6 mM, 4fold increase of phosphorylation activity in phosphatidylinositol/phosphatidylethanolamine vesicles [9]; <7> 0.5 mM, 300% increase in activity [23]) [4, 7, 8, 9, 23]

Metals, ions

Ca^{2+} <1> (<1>, activates to a smaller extent than Mg^{2+} [1,10]) [1, 10]
Co^{2+} <1> (<1>, activates to a smaller extent than Mg^{2+} [1]; <2> stimulates [11]; <1> 2 mM, activates [15]) [1, 11, 15]
Fe^{2+} <1> (<1> 2 mM, slight inhibition [15]) [15]
Mg^{2+} <1, 3, 5> (<1>, activates [1,15]; <1> required, optimal concentration is 20-30 mM [8]; <1> maximal activity at 10 mM $MgCl_2$ [9]; <1>, required, K_m: 10 mM [10]; <2> stimulates [11]; <1>, required, optimal stimulation between 10-30 mM [12]; <3> K_m: 2 mM, Mg^{2+} is three times more effective than Mn^{2+}

[13]; <1> 2 mM, dependent on [15]; <5> with exogenous substrate maximal activity is obtained at 5 mM Mg^{2+}. Maximal phosphorylation of endogenous substrate is in shoot plasma membranes at around 12.5 mM Mg^{2+}. In root membranes 5-40 mM Mg^{2+} stimulate the phosphorylation of the endogenous substrate [16]) [1, 8, 9, 10, 11, 12, 13, 15, 16]
Mn^{2+} <1, 3, 5> (<1>, activates to a smaller extent than Mg^{2+} [1,10]; <3> K_m: 0.2 mM, Mg^{2+} is three times more effective than Mn^{2+} [13]; <1> 2 mM, activates [15]; <5>, stimulatory effect of Mg^{2+} can only be partly substituted by Mn^{2+} [16]) [1, 10, 13, 15, 16]
Zn^{2+} <1> (<1> 2 mM, slight inhibition [15]) [15]

Specific activity (U/mg)

0.0057 <1> [12]
0.015 <3> (<3> membrane bound type II enzyme [4]) [4]
0.023 <3> (<3> cytosolic enzyme [4]) [4]
0.085 <2> [2]
0.117 <3> [13]
0.171 <2> [17]
1 <1> [8]
1.021 <1> [10]
1.704 <7> [23]
1.75 <9> [28]

K_m-Value (mM)

0.0012 <3> (1-phosphatidyl-1D-myo-inositol 4-phosphate, <3> in 1-phosphatidyl-1D-myo-inositol 4-phosphate in membranes, type I enzyme [4]) [4]
0.0014 <3> (1-phosphatidyl-1D-myo-inositol 4-phosphate, <3> in 1-phosphatidyl-1D-myo-inositol 4-phosphate liposomes, type I enzyme [4]) [4]
0.002 <3> (ATP, <3> pH 7.4, 23-25°C [13]) [13]
0.005 <3> (1-phosphatidyl-1D-myo-inositol 3-phosphate, <3> pH 7.5, enzyme type Iβ [19]) [19]
0.005 <2> (1-phosphatidyl-1D-myo-inositol 4-phosphate) [17]
0.005 <3> (ATP, <3> type II enzyme [4]) [4]
0.006 <3> (1-phosphatidyl-1D-myo-inositol 4-phosphate, <3> in 1-phosphatidyl-1D-myo-inositol 4-phosphate micelles, type I enzyme [4]) [4]
0.006 <3> (phosphatidylinositol 3,4-bisphosphate, <3> pH 7.5, enzyme type Iβ [19]) [19]
0.008 <7> (ATP, <7> pH 7.5 [23]) [23]
0.01 <1> (1-phosphatidyl-1D-myo-inositol 4-phosphate, <1> pH 7.5, 25°C [10]) [10]
0.019 <2> (ATP) [17]
0.025 <1, 3> (ATP, <3> type I enzyme [4]; <1> pH 7.5, 22°C [8]) [4, 8]
0.04 <3> (1-phosphatidyl-1D-myo-inositol 4-phosphate, <3> in 1-phosphatidyl-1D-myo-inositol 4-phosphate liposomes, type II enzyme [4]) [4]
0.043 <9> (ATP, <9> pH 7.4, 30°C [28]) [28]
0.047 <3> (1-phosphatidyl-1D-myo-inositol 4-phosphate, <3> pH 7.5, enzyme type Iα [19]) [19]

0.05 <3> (1-phosphatidyl-1D-myo-inositol 4-phosphate, <3> pH 7.5, enzyme type IIα [19]) [19]
0.06 <3> (1-phosphatidyl-1D-myo-inositol 4-phosphate, <3> in 1-phosphatidyl-1D-myo-inositol 4-phosphate micelles, type II enzyme [4]) [4]
0.065 <3> (1-phosphatidyl-1D-myo-inositol 3-phosphate, <3> pH 7.5, enzyme type Iα [19]) [19]
0.065 <1> (ATP, <1> pH 7.5, 25°C [10]) [10]
0.08 <3> (phosphatidylinositol 3,4-bisphosphate, <3> pH 7.5, enzyme type IIα [19]) [19]
0.12 <3> (phosphatidylinositol 3-phosphate, <3> pH 7.5, enzyme type IIα [19]) [19]
0.129 <2> ($MgATP^{2-}$, <2> pH 7.0, 25°C [11]) [11]
0.13 <2> ($MgATP^{2-}$, <2> pH 7, 25°C, at 5 mM Mg^{2+} [11]) [11]
0.133 <1> (GTP, <1> pH 7.5, 22°C [8]) [8]
0.2 <5> (ATP) [16]
0.25 <1> (1-phosphatidyl-1D-myo-inositol 4-phosphate, <1> pH 7.4, 37°C [1]) [1]
0.262 <3> (1-phosphatidyl-1D-inositol 4-phosphate, <3> pH 7.5, enzyme type Iβ [19]) [19]
Additional information <1, 7> (<1> K_m-value for 1-phosphatidyl-1D-myo-inositol 4-phosphate is 0.0033 mg/ml [8]; <7> K_m for 1-phosphatidyl-1D-myo-inositol 4-phosphate is 1.0 mol% [23]) [8, 23]

K_i-Value (mM)

0.035 <1> (quercetin, <1> pH 7.5, 25°C [10]) [10]
0.055 <9> (2'(3')-O-(2,4,6-trinitrophenyl)ATP, <9> pH 7.4, 30°C [28]) [28]

pH-Optimum

6.5 <1> [15]
6.5-7 <5> (<5> shoot and root plasma membrane, endogenous substrate [16]) [16]
7.2 <2> [11]
7.3 <1> [1, 9]
7.5 <7> [23]
7.5-8.2 <5> (<5> shoot plasma membrane, exogenous substrate [16]) [16]
7.8-8.2 <5> (<5> root plasma membrane, exogenous substrate [16]) [16]

pH-Range

5-8 <1> (<1> pH 5.0: about 65% of maximal activity, pH 8.0: about 75% of maximal activity [15]) [15]
5.5-8.5 <7> (<7> pH 5.5: about 35% of maximal activity, pH 8.5: about 60% of maximal activity [23]) [23]
6.1-7.9 <1> (<1> pH 6.1: about 85% of maximal activity, pH 7.9: about 85% of maximal activity [1]) [1]
6.5-8.5 <2> (<2> pH 6.5: about 35% of maximal activity, pH 8.5: about 60% of maximal activity [11]) [11]

4 Enzyme Structure

Molecular weight

130000 <9> (<9> gel filtration [28]) [28]
150000 <3> (<3>, gel filtration [13]) [13]

Subunits

? <1, 2, 3, 7> (<1> x * 45000, SDS-PAGE [12]; <3> x * 53000, SDS-PAGE [4,13]; <7> x * 63000, SDS-PAGE [23]; <2> x * 110000, SDS-PAGE [2]) [2, 4, 12, 13, 23]
dimer <9> (<9> x * 60000, SDS-PAGE [28]) [28]

Posttranslational modification

phosphoprotein <7> (<7> phosphorylation by Cki1 in vitro decreases the activity of the enzyme [23]) [23]

5 Isolation/Preparation/Mutation/Application

Source/tissue

3T3 cell <6> [34]
HEK-293 cell <3, 6> [18, 33]
N1E-115 cell <6> (<6> neuroblastoma [27]) [27, 35]
adrenal medulla <2> [11]
brain <1, 2, 9> (<2> moderate expression of PIP5KIβ, low expression of PIP5KIα [21]) [1, 2, 3, 8, 9, 12, 14, 17, 21, 28, 31]
erythrocyte <2, 3> (<3> two form of PIP kinase: type I kinase is membrane bound and type II kinase is both membrane bound and cytosolic [4]) [4, 13, 21]
heart <2> (<2> high expression of PIP5KIβ and PIP5KIα [21]) [21]
hematopoietic cell line <6> [26]
kidney <1, 2> (<1> cortex [15]; <2> high expression of PIP5KIβ [21]) [15, 21]
liver <1, 2> (<2> low expression of PIP5KIα [21]) [7, 10, 21, 29]
lung <2> (<2> low expression of PIP5KIα [21]) [21]
pancreas <2> (<2> moderate expression of PIP5KIβ, high expression of PIP5KIα [21]) [21]
placenta <2> (<2> high expression of PIP5KIβ [21]) [21]
platelet <3> [3]
renal cortex <1> [15]
retina <2> [5]
root <5> (<5> dark-grown [16]) [16]
shoot <5> (<5> dark-grown [16]) [16]

Localization

Golgi apparatus <1> [15, 29]
cytoplasm <6> [26]

cytosol <1, 3> (<3> type I kinase is membrane bound and type II kinase is both membrane bound and cytosolic [4]) [4, 9, 12, 31]
membrane <1, 2, 3, 9> (<3> type I kinase is membrane bound and type II kinase is both membrane bound and cytosolic [4]; <3> associated with, peripheral membrane protein [13]; <10> associated [30]) [2, 4, 12, 13, 14, 17, 21, 28, 30]
plasma membrane <1, 2, 4, 5, 7> (<2>, synaptosomal plasma membrane [14]; <4> MSS4P [22]) [7, 10, 14, 16, 22, 23]
soluble <1, 2, 3> [3, 11]

Purification

<1> (partial [8]) [1, 8, 10, 12]
<2> [2, 11, 17, 21, 23]
<3> (cytosolic enzyme type II enzyme and membrane-bound type I and type II enzyme [4]) [4, 13, 19]
<7> [23]
<9> [28]

Cloning

<2> (enzyme form PIP5KIα [21]) [21]
<3> (expression in COS cells [19]) [19]
<6> (expression of mutant enzymes K181A, D309N, D309N/R429Q and R427Q in Escherichia coli, expression of mutant enzymes in human MG-63 osteosarcoma cells [26]) [26]
<8> (expression in Escherichia coli [24]) [24]

Engineering

D309N <6> (<6> greatly reduced kinase activity towards 1-phosphatidyl-1D-myo-inositol 4-phosphate [26]) [26]
D309N/R427Q <6> (<6> greatly reduced kinase activity towards 1-phosphatidyl-1D-myo-inositol 4-phosphate [26]) [26]
K181A <6> (<6> greatly reduced kinase activity towards 1-phosphatidyl-1D-myo-inositol 4-phosphate [26]) [26]
R427Q <6> (<6> greatly reduced kinase activity towards 1-phosphatidyl-1D-myo-inositol 4-phosphate [26]) [26]

6 Stability

General stability information

<2>, sucrose and PEG 20000 in the assay medium are required to stabilize the enzyme during phosphorylation reaction [17]

Storage stability

<1>, -80°C, stable for at least 3 months [8]
<2>, -20°C, 50 MM Tris-HCl, pH 8.0, 1 mM EDTA, 0.25 M sucrose, 0.3% v/v 2-mercaptoethanol, 0.1% Triton X-100, 0.1% PEG 20000, 0.05 mM ATP, 0.1 mM PMSF, stable for several months [17]

<3>, -70°C, stable in concentrated form [13]
<3>, 4°C, storage causes the 53000 Da enzyme to be slowly degraded to a 45000 Da peptide [13]
<9>, 4°C, about 10% loss of activity after overnight storage [28]

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Protein-N^{π}-phosphohistidine-sugar phosphotransferase 2.7.1.69

1 Nomenclature

EC number
2.7.1.69

Systematic name
protein-N^{π}-phosphohistidine:sugar N-pros-phosphotransferase

Recommended name
protein-N^{π}-phosphohistidine-sugar phosphotransferase

Synonyms
BglF protein
PEP-dependent phosphotransferase enzyme II
PEP-sugar phosphotransferase enzyme II
PTS permease
enzyme II of the phosphotransferase system (cf. EC 2.7.3.9)
enzyme IIl4ac
gene bglC RNA formation factors
gene glC proteins
glucose permease
mannitol transport protein enzyme II mtl
phosphoenolpyruvate-sugar phosphotransferase enzyme II
phosphohistidinoprotein-hexose phosphoribosyltransferase
phosphohistidinoprotein-hexose phosphotransferase
phosphoprotein factor-hexose phosophotransferase
phosphotransferase, phosphohistidinoprotein-hexose
protein, specific or class, gene bglC
ribonucleic acid formation factor, gene glC
sucrose phosphotransferase system II

CAS registry number
37278-09-4

2 Source Organism

<1> *Bacillus subtilis* [1, 3, 17, 24]
<2> *Escherichia coli* [2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 18, 19, 21, 23, 25, 27, 29, 30, 31, 32]
<3> *Salmonella typhimurium* [4, 13, 14]
<4> *Staphylococcus aureus* (strain 305 A [15]) [4, 15, 20, 33]
<5> *Streptococcus faecalis* (26487 [16]) [16]

<6> *Klebsiella sp.* [4]
<7> *Streptococcus sp.* [4]
<8> *Lactobacillus sp.* [4]
<9> *Bacillus sp.* [4]
<10> *Clostridium sp.* [4]
<11> *Mycoplasma sp.* [4]
<12> *Klebsiella pneumoniae* [22]
<13> *Mycoplasma capricolum* [26]
<14> *Streptomyces lividans* [28]
<15> *Streptomyces coelicolor* [28]
<16> *Streptomyces griseofuscus* [28]

3 Reaction and Specificity

Catalyzed reaction
protein N$^{\pi}$-phosphohistidine + sugar = protein histidine + sugar phosphate

Reaction type
phospho group transfer

Natural substrates and products
S sugar + phosphohistidine-containing protein <2, 14, 15, 16> (<2> part of the bacterial phosphotransferase system, EC 2.7.1.69 comprises a group of sugar-specific enzymes, which, together with the soluble enzymes of the PTS (phosphoenolpyruvate, enzyme I EC 2.7.3.9, and histidine-containing protein (HPr)), are responsible for the translocation of sugars across membranes [4,18]; <2>, the mannitol-specific enzyme is responsible for the transport and phosphorylation of D-mannitol [10]; <2> physiological functions of the mannitol enzyme II: 1. chemoreception, 2. unidirectional and exchange group translocation, 3. phosphoenolpyruvate-dependent and mannitol-1-phosphate-dependent sugar phosphorylation, 4. autoinduction of phosphotransferase system protein synthesis, 5. control of non-phosphotransferase-system permease activity, 6. control of adenylate cyclase activity [18]; <2> inducible enzyme [19]; <2> in the absence of β-glucosides, the enzyme phosphorylates BglG, a positive regulator of bgl operon transcription, thus inactivating BglG. In the presence of β-glucosides, it activates BglG by dephosphorylating it and, at the same time, transports β-glucosides into the cell and phosphorylates them [27]; <14,15> fructose-specific enzyme IIfruc is fructose-inducible [28]; <16> fructose-specific enzyme IIfruc is constitutively expressed [28]) (Reversibility: ? <2, 14, 15, 16> [4, 10, 18, 19, 29]) [4, 10, 18, 19, 28]
P ?

Substrates and products
S sugar + phosphohistidine-containing protein <1-15> (<2> a group of sugar-specific enzymes, which, together with the soluble enzymes of the PTS (phosphoenolpyruvate, enzyme I (EC 2.7.3.9), and histidine-contain-

ing protein (HPr)), are responsible for the translocation of sugars across membranes [4,18]; <4> isoenzyme IIlac is specific for lactose and thiomethyl-β-galactopyranoside [4]; <7> isoenzyme IIlac is specific for lactose, thiomethyl-β-galactopyranoside and galactose. Isoenzyme IIglc is specific for glucose, 2-deoxyglucose and glucosamine. Isoenzyme IIgal is specific for galactose. Isoenzyme IIscr is specific for sucrose. Isoenzyme IIfru is specific for fructose [4]; <8> isoenzyme IIglc is specific for glucose and α-methylglucopyranoside. Isoenzyjme IIlac is specific for lactose, lactulose and lactobionic acid. Isoenzyme IIxtl is specific for xylitol and D-arabitol. Isoenzyme IIgal is specific for galactose. Isoenzyme IIrtl is specific for ribitol [4]; <9> Isoenzyme IImtl is specific for mannitol. Isoenzyme IIman is specific for mannose. Isoenzyme IIglc is specific for glucose and α-methylglucopyranoside. Isonzyme IItre is specific for trehalose [4]; <10> isoenzyme IIglc is specific for glucose. Isoenzyme IIfru is specific for fructose. Isoenzyme IIscr is specific for sucrose. Isoenzyme IImtl is specific for mannitol and sorbitol [4]; <11> isoenzyme IIglc is specific for glucose and α-methylglucopyranose [4]; <2> N-acetylglucosamine [5]; <2> isoenzyme IIGlc enzyme, reaction with α-methylglucopyranoside [6]; <2> mannitol-specific enzyme, reaction with mannitol and mannitol 1-phosphate [7]; <2> enzyme IIMan, reaction with methyl α-glucoside [8]; <2> mannitol-specific enzyme catalyzes both the phosphoenolpyruvate-dependent and the mannitol 1-phosphate-dependent phosphorylation of D-mannitol with high specificity for the accepting sugar and the phosphoryl donor [10]; <2> mannitol-specific enzyme [11,12]; <3> glucose-specific enzyme catalyzes the phosphoenolpyruvate-dependent phosphorylation of methyl-α-D-glucopyranoside [13]; <4> galactoside-specific enzyme, reaction with *o*-nitrophenyl-β-D-galactoside [15]; <1,5> phosphorylation of glucose [16,17]; <2> phosphoenolpyruvate-dependent and mannitol 1-phosphate-dependent phosphorylation of mannitol [18,19]; <4> phosphorylation of β-galactosidase by the lactose phosphotransferase system [20]; <12> sorbose permease [22]; <2> mannitol-specific enzyme [23]; <1> fructose permease [24]; <2> glucose permease, mannitol permease and mannose permease [25]; <2> in the absence of β-glucosides, the enzyme phosphorylates BglG, a positive regulator of bgl operon transcription, thus inactivating BglG. In the presence of β-glucosides, it activates BglG by dephosphorylating it and, at the same time, transports β-glucosides into the cell and phosphorylates them [27]; <14,15,16> fructose-specific enzyme II activity [28]; <2> isoenzyme EIIGlc phosphorylates:glucose, 1-deoxy-D-glucose with 119% of the activity with glucose,α-1-fluoro-D-glucose at 98% of the activity with D-glucose, β-1-fluoro-D-glucose at 112% of the activity with D-glucose, mannose at 5% of the activity with D-glucose, 2-deoxy-D-glucose at 17% of the activity with D-glucose, 2-fluoro-D-glucose at 18% of the activity with D-glucose, allose at 3% of the activity with D-glucose, 3-deoxy-D-glucose at 8% of the activity with D-glucose, 3-fluoro-D-glucose at 53% of the activity with D-glucose, 4-deoxy-D-glucose at 2% of the activity with D-glucose, 4-fluoro-D-glucose at 7% of the activity with D-glucose.

EIIMan phosphorylates: glucose, 1-deoxy-D-glucose with 42% of the activity with glucose, α-1-fluoro-D-glucose at 79% of the activity with D-glucose, β-1-fluoro-D-glucose at 102% of the activity with D-glucose, mannose at 169% of the activity with D-glucose, 2-deoxy-D-glucose at 120% of the activity with D-glucose, 2-fluoro-D-glucose at 120% of the activity with D-glucose, allose at 9% of the activity with D-glucose, 3-deoxy-D-glucose at 34% of the activity with D-glucose, 3-fluoro-D-glucose at 91% of the activity with D-glucose, 4-deoxy-D-glucose at 0.3% of the activity with D-glucose, 4-fluoro-D-glucose at 5% of the activity with D-glucose [29]; <2> methyl-α-glucoside. G320V mutation allows the isoenzyme EIIGlc to transport mannitol [32]; <4> *o*-nitrophenyl-β-D-galactopyranoside [33]) (Reversibility: ? <1-15> [1-33]) [1-33]

P sugar phosphate + histidine-containing protein <2, 3, 5> (<2, 3, 5> glucose is phosphorylated at position 6 [4, 3, 16]; <2> fructose is phosphorylated at position 1 [4]; <2> mannitol is phosphorylated at position 1 [11]) [4, 11, 13, 16]

S Additional information <2> (<2> the enzyme catalyzes an exchange reaction in which a phosphoryl moiety is transferred from one molecule of the heat stable phosphocarrier protein Hpr to another [7]) [7]

P ?

Inhibitors

2-(N-dansyl)aminoethyl-β-D-thiogalactoside <4> [33]

5-thioglucose <5> [16]

D-gluco-hexadialdo-1,5-pyranose <2> (<2> which exist as gem-diols in aqueous solution [29]) [29]

NEM <2> (<2> inhibits phosphoenol-dependent phosphorylation of mannitol and mannitol 1-phosphate, no effect on activity of exchange reaction [7]; <2> enzyme reconstituted in phospholipid vesicles [11]) [7, 11]

PCMB <2> (<2> partial inactivation of exchange reaction [7]) [7]

SDS <4> (<4> complete inhibition complete inhibition of phosphorylation of β-galactoside at 0.015% w/v, reversible, only slight inhibition of phosphorylation of methyl-α-glucoside [20]) [20]

diethyldicarbonate <2> (<2> inhibits exchange reaction [7]) [7]

ferricyanide <2> (<2> inhibits glucose-specific enzyme, mannose-specific enzyme is less sensitive [8]; <2> phosphorylated enzyme is less susceptible than non-phosphorylated enzyme [9]) [8, 9]

D-glucose <2> [29]

isopropyl-β-D-thiogalactoside <4> [33]

D-mannitol <2> (<2>, strong substrate inhibition at neutral pH in the transphosphorylation reaction. No substrate inhibition for phosphoenolpyruvate-dependent reaction [10]) [10, 32]

D-mannitol 1-phosphate <2> (<2>, strong substrate inhibition at neutral pH in the transphosphorylation reaction [10]) [10]

D-mannose <5> [16]

methyl α-D-gluco-hexodialdo-1,5-pyranoside <2> (<2> which exist as gem-diols in aqueous solution [29]) [29]
methyl α-D-glucopyranoside <2> [29]
phenazine methosulfate <2> (<2> inhibits glucose-specific enzyme [8]) [8, 9]
plumbagin <2> (<2> inhibits glucose-specific enzyme [8]) [8, 9]
sodium deoxycholate <4> (<4> complete inhibition of phosphorylation of β-galactoside at 0.33% w/v, reversible, only slight inhibition of phosphorylation og methyl-α-glucoside [20]) [20]
Additional information <2> (<2> antibodies directed against the enzyme inhibit phosphoenolpyruvate-dependent activity to a greater extent than the transphosphorylation [10]) [10]

Activating compounds

Lubrol PX <2> (<2> purified enzyme is dependent on Lubrol PX and phospholipids for maximal activity [10]) [10]
phosphatidylglycerol <3> (<3> required for enzyme in sonicated suspension [13]) [13]
phospholipids <2> (<2> purified enzyme is dependent on Lubrol PX and phospholipids for maximal activity [10]) [10]

Specific activity (U/mg)

1.77 <3> [13]
4.52 <4> (<4> IICBlac [33]) [33]
5.3 <2> [19]
10.1 <2> [6]
52 <4> (<4> the value corrected for enzyme II decay is 110 [15]) [15]
Additional information <2, 3> (<2, 3> 2 assay systems: assay I measuring the phosphoenolpyruvate-dependent phosphorylation of sugar in presence of the enzymes of the phosphotransferase system [9,14]; <2> assay II measuring the transphosphorylation from sugar to sugar in presence of EC 2.7.1.69 alone [9]) [9, 14, 15]

K_m-Value (mM)

0.009 <2> (methyl-α-D-glucoside, <2> wild-type enzyme [32]) [32]
0.011 <2> (D-mannitol) [10]
0.023 <2> (methyl-α-D-glucopyranoside, <2> pH 7.5, 37°C, glucose-specific enzyme in presence of 3 mM ferrocyanide [8]) [8]
0.036 <2> (methyl-α-D-glucopyranoside, <2> pH 7.5, 37°C, glucose-specific enzyme in presence of 3 mM ferricyanide [8]) [8]
0.04 <5> (2-deoxy-D-glucose, <5> 25°C [16]) [16]
0.04 <5> (D-glucose, <5> 25°C [16]) [16]
0.19 <2> (β-1-fluoro-D-glucose, <2> pH 7.5, enzyme EIIGlc [29]) [29]
0.2 <2> (1-deoxy-D-glucose, <2> pH 7.5, enzyme EIIGlc [29]) [29]
0.21 <2> (α-1-fluoro-D-glucose, <2> pH 7.5, enzyme EIIGlc [29]) [29]
0.22 <2> (D-glucose, <2> pH 7.5, enzyme EIIGlc [29]) [29]
0.32 <2> (D-mannose, <2> pH 7.5, enzyme EIIMan [29]) [29]
0.45 <2> (2-fluoro-D-glucose, <2> pH 7.5, enzyme EIIMan [29]) [29]

0.47 <2> (β-1-fluoro-D-glucose, <2> pH 7.5, enzyme EIIMan [29]) [29]
0.48 <2> (α-1-fluoro-D-glucose, <2> pH 7.5, enzyme EIIMan [29]) [29]
0.49 <2> (2-deoxy-D-glucose, <2> pH 7.5, enzyme EIIMan [29]) [29]
0.49 <2> (3-fluoro-D-glucose, <2> pH 7.5, enzyme EIIGlc [29]) [29]
0.49 <2> (D-glucose, <2> pH 7.5, enzyme EIIMan [29]) [29]
0.5 <4> (o-nitrophenyl-β-D-galactopyranoside, <4>, pH 7.5, IICBLac [33]) [33]
0.61 <2> (3-fluoro-D-glucose, <2> pH 7.5, enzyme EIIMan [29]) [29]
0.7 <2> (methyl-α-D-glucopyranoside, <2> pH 7.5, 37°C, mannose-specific enzyme in presence of 3 mM ferrocyanide [8]) [8]
1.1 <2> (1-deoxy-D-glucose, <2> pH 7.5, enzyme EIIMan [29]) [29]
1.1 <2> (methyl-α-D-glucopyranoside, <2> pH 7.5, 37°C, mannose-specific enzyme in presence of 3 mM ferricyanide [8]) [8]
1.2 <2> (2-fluoro-D-glucose, <2> pH 7.5, enzyme EIIGlc [29]) [29]
1.5 <2> (2-deoxy-D-glucose, <2> pH 7.5, enzyme EIIGlc [29]) [29]
1.7 <2> (4-fluoro-D-glucose, <2> pH 7.5, enzyme EIIGlc [29]) [29]
2 <2> (3-deoxy-D-glucose, <2> pH 7.5, enzyme EIIMan [29]) [29]
4 <2> (4-deoxy-D-glucose, <2> pH 7.5, enzyme EIIGlc [29]) [29]
4.1 <2> (3-deoxy-D-glucose, <2> pH 7.5, enzyme EIIGlc [29]) [29]
4.8 <2> (D-mannose, <2> pH 7.5, enzyme EIIGlc [29]) [29]
5 <2> (4-fluoro-D-glucose, <2> pH 7.5, enzyme EIIMan [29]) [29]
5 <2> (D-mannitol, <2> wild-type enzyme [32]) [32]

K_i-Value (mM)

0.003 <2> (D-gluco-hexodialdo-1,5-pyranose, <2> pH 7.5 [29]) [29]
0.009 <2> (α-D-gluco-hexodialdo-1,5-pyranoside, <2> pH 7.5 [29]) [29]
0.011 <4> (isopropyl-β-D-thiogalactoside, <4>, pH 7.5, isoenzyme IICBLac [33]) [33]
0.1 <5> (mannose, <5> 25°C [16]) [16]
0.15 <4> (2-(N-dansyl)aminoethyl-β-D-thiogalactoside, <4>, pH 7.5, isoenzyme IICBLac [33]) [33]
0.62 <5> (5-thioglucose, <5> 25°C [16]) [16]

pH-Optimum

6 <2> (<2> mannitol-dependent transphosphorylation [11,18]; <2> enzyme reconstituted in phospholipid vesicles [11]) [11, 18]
6.5 <2> (<2> mannitol-dependent transphosphorylation, phosphoenolpyruvate-dependent transphosphorylation, 2 optima: pH 6.5 and pH 8.5 [10]) [10]
7 <2> (<2> mannitol-dependent transphosphorylation, phosphoenolpyruvate-dependent transphosphorylation, two optima: pH7.0 and pH 9.0 [19]) [19]
7.5 <2> (<2> exchange reaction in which a phosphoryl moiety is transferred from one molecule of the heat stable phosphocarrier protein Hpr to another [7]) [7]
8.5 <2> (<2> phosphoenolpyruvate-dependent transphosphorylation, 2 optima: pH 6.5 and pH 8.5 [10]) [10]
9 <2> (<2> phosphoenolpyruvate-dependent transphosphorylation, two optima: pH 7.0 and pH 9.0 [19]) [19]
9.5 <2> (<2> phosphoenolpyruvate-dependent transphosphorylation [18]) [18]

pH-Range

5.2-9.2 <2> (<2> less than 50% of maximal activity above pH 9.2 and below pH 5.2, phosphoenolpyruvate-dependent transphosphorylation [10]) [10]
5.5-9 <2> (<2> pH 5.5: about 50% of maximal activity, pH 9.0: about 65% of maximal activity, mannitol-dependent reaction [11]) [11]
6-7.5 <2> (<2> less than 50% of maximal activity above pH 7.5 and below pH 6, mannitol-dependent transphosphorylation [10]) [10]
6-9 <2> (<2> pH 6.0: about 85% of maximal activity, pH 9.0: about 65% of maximal activity, exchange reaction in which a phosphoryl moiety is transferred from one molecule of the heat stable phosphocarrier protein Hpr to another [7]) [7]

4 Enzyme Structure

Molecular weight

16700 <13> (<13> analytical ultracentrifugation [26]) [26]
18130 <2> (<2> mass spectrometry [31]) [31]
105000 <3> (<3> analytical ultracentrifugation [13]) [13]
400000 <4> (<4>, gel filtration [15]) [15]

Subunits

? <2, 3, 4> (<3> x * 40000, SDS-PAGE [13]; <4> x * 55000, SDS-PAGE, gel filtration of SDS-treated enzyme [15]; <2> x * 60000, SDS-PAGE [10,18,19]; <2> x * 67893, mannitol-specific enzyme, calculation from nucleotide sequence of cDNA [12]; <2> x * 68356, N-acetylglucosamine-specific enzyme, calculation from nucleotide sequence of cDNA [5]) [5, 10, 12, 13, 15, 18]
Additional information <2, 3, 4, 6, 7, 8, 9, 10, 11> (<2, 3, 4, 6, 7, 8, 9, 10, 11>, except Escherichia coli IIman all EII species consist of a single polypeptide chain but higher molecular weight complexes have been observed in Salmonella typhimurium IIglc, E. coli IImtl and IIman [4]; <3> enzyme IIglc appears as a dimer during gel filtration and analytical ultracentrifugation [4]; <2> enzyme IImtl is cross-linked to a dimer through an activity-linked thiol using bifunctional maleimides and oxidizing agents. Enzyme IIman which consists of two polypeptide chains totaling 59000 Da has an apparent molecular mass of 240000 Da upon gel filtration, suggesting a higher molecular weight complex [4]; <2> Enzyme IIGlc is active as a monomer. A dimeric form is detected by chemical cross-linking and by zonal sedimentation at 4°C. Upon mild oxidation a disulfide bond is formed between the subunits of the dimer. Oxidized IIGlc is more stable than the reduced form, but is inactive because it cannot be phosphorylated by the cytoplasmic subunit IIGlc of the glucose permease [6]; <2> at high concentrations the enzyme exists in an aggregated or oligomeric state, this form is more active than the monomeric or dissociated form of the enzyme in catalyzing the vectorial mannitol transphosphorylation [12]; <3> the solubilized enzyme exists as a complex of MW 105000 Da [13]; <2> the membrane-integrated enzymes II are largely dimeric, whereas the soluble enzymes II are largely monomeric [25]) [4, 6, 12, 13, 25]

5 Isolation/Preparation/Mutation/Application

Localization

membrane <2, 3, 4, 5> (<2> integral membrane protein [10,18]; <2> a considerable portion of its polypeptide chain must also extend into a hydrophilic environment, presumably to cytoplasm [10]; <2> the NH_2-terminal half of the enzyme resides within the membrane [12]; <2> enzymes II of the phosphotransferase system exist in two physically distinct forms, one tightly integrated into the membrane and one either soluble or loosely associated with the membrane [25]) [5, 6, 8, 10, 12, 13, 14, 15, 16, 18, 19, 25]

soluble <2> (<2> enzymes II of the phosphotransferase system exist in two physically distinct forms, one tightly integrated into the membrane and one either soluble or loosely associated with the membrane [25]) [25]

Purification

<2> (membrane subunit IIGlc purified from overproducing Escherichia coli [6]; IImtl [9]; enzymatically active C-terminal domain of enzyme IImtl [21]) [6, 9, 10, 19, 21]

<3> [13]

<4> (isoenzyme IIlac [15]; histidine-tagged transmembrane component IICBLac [32]) [15, 32]

<5> [16]

<13> (recombinant enzyme IIAglc [26]) [26]

Renaturation

<2> (reconstitution of mannitol-dependent mannitol transphosphorylation [11]) [11]

Crystallization

<1> (hanging-drop vapor diffusion method, IIA domain [1]; crystals are obtained in 57-62% saturated ammonium sulfate solution, 0.5% w/v [3]; IIB subunit of fructoase permease [24]) [1, 3, 24]

<2> (hanging-drop vapor diffusion method, IIA domain [2]; mannitol-specific IIA domain [23]) [2, 23]

<12> (crystal structure of the IIBSor domain of the sorbose permease solved to 1.75 A resolution [22]) [22]

Cloning

<2> (enzyme IIN-acetylglucosamine [5]; enzymatically active C-terminal domain of enzyme IImtl [21]; subcloning and expression of the three individual domains IIAbgl, IIBbgl and IICbgl, as well as truncated BglF proteins which lack one domain [27]; BglF derivative in which the order of the domains is CBA [30]) [5, 12, 21, 27, 30]

<4> (construction of a recombinant IICBLac with a C-terminal with a C-terminal affinity tag of six His residues and of a singly IIBlac-His protein, expression in Escherichia coli [33]) [33]

<13> (cloned into a regulated expression vector, expression in Escherichia coli [26]) [26]

Engineering

C461D <1> (<1> Cys461 and His620 are required for the function of the enzyme. A replacement of these residues results in loss of glucose repression of bglPH operon expression and β-xylosidase synthesis [17]) [17]
G320V <2> (<2> G320V-EIIGlc, mutation results in a G320 to V substitution that allows EIIGlc to transport mannitol [32]) [32]
H620D <1> (<1> Cys461 and His620 are required for the function of the enzyme. A replacement of these residues results in loss of glucose repression of bglPH operon expression and β-xylosidase synthesis [17]) [17]
H90D <2> (H90DE-EIIAGlc does not undergo phosphorylation to create an unstable phosphoacyl but possibly forms an abortive complex with the other PTS proteins limiting their phosphorylation [31]) [31]
Additional information <2> (<2> BglF derivative IICBAbgl, in which the order of the domains is different from the wild-type enzyme, behaves like wild-type enzyme in its ability to be phosphorylated and to phosphorylate BglG in vitro and in vivo. However, it can not catalyze phosphorylation of β-glucosides in vitro nor their phosphorylation in vivo and can not catalyze BglG dephosphorylation in vitro and in vivo [30]) [30]

6 Stability

Temperature stability

Additional information <13> (<13> the enzyme undergoes reversible, two-state thermal unfolding with T_m: 70°C [26]) [26]

General stability information

<2>, repeated freezing and thawing does not affect the phosphorylation activity but freezing can alter the physical properties [6]
<4>, stabilization of IIBLac-His for several weeks is achieved in 50% glycerol and by storage at -20°C [33]

Storage stability

<2>, -70°C [9, 10]
<2>, -70°C, long-term storage in 25 mM sodium phosphate buffer, pH 7.0, 1% polydisperse octyl-oligooxyethylene, 1 mM EDTA, 10% glycerol, short-term storage is possible without glycerol [6]
<2>, -70°C, stable for at least 2 months [19]
<2>, 4°C, stable for several days [19]
<3>, -70°C, several weeks [13]
<3>, 4°C, 1 week [13]

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Protamine kinase 2.7.1.70

1 Nomenclature

EC number
2.7.1.70 (transferred to EC 2.7.1.37)

Recommended name
protamine kinase

Shikimate kinase 2.7.1.71

1 Nomenclature

EC number
2.7.1.71

Systematic name
ATP:shikimate 3-phosphotransferase

Recommended name
shikimate kinase

Synonyms
AroK
AroL
SKI
SKII
kinase (phosphorylating), shikimate
kinase, shikimate (phosphorylating)
shikimate kinase II

CAS registry number
9031-51-0

2 Source Organism

<1> *Salmonella typhimurium* [1]
<2> *Phaseolus mungo* [2]
<3> *Escherichia coli* (strain K12 [3,9]; two isoenzymes: SK1 and SK2 [3]; shikimic acid kinase I [12]; shikimate kinase II [9]) [3, 9, 12, 14, 20]
<4> *Hansenula henricii* [4]
<5> *Hansenula fabianii* [4]
<6> *Hansenula anomala* [4]
<7> *Candida utilis* [4]
<8> *Pichia guilliermondii* [4]
<9> *Lodderomyces elongisporus* [4]
<10> *Rhodosporidium sphaerocarpum* (enzyme aggregate contains 5 activities: EC 1.1.1.25, EC 2.7.1.71, EC 4.2.1.10, EC 4.2.3.4 and EC 2.5.1.19 [4]) [4]
<11> *Rhodosporidium toruloides* (enzyme aggregate contains 5 activities: EC 1.1.1.25, EC 2.7.1.71, EC 4.2.1.10, EC 4.2.3.4 and EC 2.5.1.19 [4]) [4]
<12> *Rhodotorula rubra* (enzyme aggregate contains 5 activities: EC 1.1.1.25, EC 2.7.1.71, EC 4.2.1.10, EC 4.2.3.4 and EC 2.5.1.19 [4]) [4]

<13> *Saccharomyces lipolytica* (enzyme aggregate contains 5 activities: EC 1.1.1.25, EC 2.7.1.71, EC 4.2.1.10, EC 4.2.3.4 and EC 2.5.1.19 [4]) [4]
<14> *Saccharomyces cerevisiae* (ARO 1 [11]; enzyme aggregate contains 5 activities: EC 1.1.1.25, EC 2.7.1.71, EC 4.2.1.10, EC 4.2.3.4 and 2.5.1.19 [4,11]) [4, 11]
<15> *Neurospora crassa* (enzyme aggregate contains 5 activities: EC 1.1.1.25, EC 2.7.1.71, EC 4.2.1.10, EC 4.2.3.4 and EC 2.5.1.19 [4,7,13]) [4, 7, 13]
<16> *Schizosaccharomyces pombe* (enzyme aggregate contains 5 activities: EC 1.1.1.25, EC 2.7.1.71, EC 4.2.1.10, EC 4.2.3.4 and EC 2.5.1.19 [5]) [5]
<17> *Methanococcus jannaschii* [18]
<18> *Sorghum bicolor* [8]
<19> *Spinacia oleracea* [10]
<20> *Mycobacterium tuberculosis* [15, 22]
<21> *Erwinia chrysanthemi* [6, 16, 17, 19, 21, 23]

3 Reaction and Specificity

Catalyzed reaction

ATP + shikimate = ADP + shikimate 3-phosphate (<21> the enzyme binds substrates randomly and in a synergistic fashion [21])

Reaction type

phospho group transfer

Natural substrates and products

S ATP + shikimate <3, 17, 19> (<3> SK2 is the isoenzyme that normally functions in aromatic biosynthesis in the cell, SK1 functions only when high intracellular levels of shikimate occurs [3]; <19> energy charge plays a role in regulating shikimate kinase, thereby controlling the shikimate pathway [10]; <17> the enzyme catalyzes the committed step in the seven-step biosynthesis of chorismate [18]) (Reversibility: ? <3, 17, 19> [3, 10, 18]) [3, 10, 18]
P ADP + shikimate 3-phosphate <3> [3]

Substrates and products

S 2'-deoxyATP + shikimate <19> (Reversibility: ? <19> [10]) [10]
P 2'-deoxyADP + shikimate 3-phosphate
S ATP + shikimate <1-21> (Reversibility: ? <1-21> [1-23]) [1-23]
P ADP + shikimate 3-phosphate <3, 18> [3, 8, 14]
S Additional information <19> (<19> no activity with XTP, ITP, GTP, TTP, CTP and UTP [10]) [10]
P ?

Inhibitors

$(NH_4)_2SO_4$ <18> (<18> 5 mM, 29% inhibition [8]) [8]
ADP <2> [2]
ADP <18, 19> (<18> 4 mM, 44% inhibition [8]; <19> 0.5 mM; 12% inhibition [10]) [8, 10]

AMP <18> (<18> 4 mM, 27% inhibition [8]) [8]
EDTA <2> [2]
Na_2SO_4 <18> (<18> 5 mM, 27% inhibition [8]) [8]
NaCl <18> (<18> 250 mM, 50% inhibition [8]) [8]
caffeic acid <18> (<18> 1 mM, 44% inhibition [8]) [8]
p-coumaric acid <18> (<18> 1 mM, 14% inhibition [8]) [8]
shikimate 3-phosphate <2> [2]
Additional information <1, 17> (<1> no inhibition by 1 mM Phe, Tyr, Trp, chorismate or prephenate [1]; <17> no inhibition by shikimate up to 10 mM [18]) [1, 18]

Metals, ions

Ca^{2+} <2, 3, 18> (<2> requirement for Mg^{2+} can partially be replaced by Mn^{2+}, Ca^{2+}, Co^{2+} and Cd^{2+} [2]; <3> divalent cations required, Mg^{2+} is most effective, but significant activity is obtained with Fe^{2+}, Ca^{2+}, Mn^{2+} or Co^{2+}, isoenzyme SK2 [3,9]; <18> divalent cation required, Mg^{2+} and Mn^{2+} are most effective, Ca^{2+} activates to a lesser extent [8]) [2, 3, 8, 9]
Cd^{2+} <2> (<2> requirement for Mg^{2+} can partially be replaced by Mn^{2+}, Ca^{2+}, Co^{2+} and Cd^{2+} [2]) [2]
Co^{2+} <2, 3, 18> (<2> requirement for Mg^{2+} can partially be replaced by Mn^{2+}, Ca^{2+}, Co^{2+} and Cd^{2+} [2]; <3> divalent cations required, Mg^{2+} is most effective, but significant activity is obtained with Fe^{2+}, Ca^{2+}, Mn^{2+} or Co^{2+}, isoenzyme SK2 [3,9]; <18> divalent cation required, Mg^{2+} and Mn^{2+} are most effective, Co^{2+} activates to a lesser extent [8]) [2, 3, 8, 9]
Fe^{2+} <3, 18> (<3> divalent cations required, Mg^{2+} is most effective, but significant activity is obtained with Fe^{2+}, Ca^{2+}, Mn^{2+} or Co^{2+}, isoenzyme SK2 [3,9]; <18> divalent cation required, Mg^{2+} and Mn^{2+} are most effective, Fe^{2+} activates to a lesser extent [8]) [3, 8, 9]
Mg^{2+} <2, 3, 18, 19, 21> (<2> requirement for Mg^{2+} can partially be replaced by Mn^{2+}, Ca^{2+}, Co^{2+} and Cd^{2+} [2]; <3> divalent cations required, Mg^{2+} is most effective, but significant activity is obtained with Fe^{2+}, Ca^{2+}, Mn^{2+} or Co^{2+}, isoenzyme SK2 [3,9]; <18> divalent cation required, Mg^{2+} and Mn^{2+} are most effective. Optimal Mg^{2+} concentration is 10 mM [8]; <19> divalent cation required, Mg^{2+} is most effective followed by Mn^{2+}. Optimal Mg^{2+} concentration is 2.4 mM [10]; <21> in the enzyme that contains bound $MgADP^-$ the binding of Mg^{2+} in the active site involves direct interaction with two protein side-chains [17]) [2, 3, 8, 9, 10, 17]
Mn^{2+} <2, 3, 18, 19> (<2> requirement for Mg^{2+} can partially be replaced by Mn^{2+}, Ca^{2+}, Co^{2+} and Cd^{2+} [2]; <3> divalent cations required, Mg^{2+} is most effective, but significant activity is obtained with Fe^{2+}, Ca^{2+}, Mn^{2+} or Co^{2+}, isoenzyme SK2 [3,9]; <18> divalent cation required, Mg^{2+} and Mn^{2+} are most effective [8]; <19> divalent cation required, Mg^{2+} is most effective followed by Mn^{2+} [10]) [2, 3, 8, 9, 10]
Ni^{2+} <18> (<18> divalent cation required, Mg^{2+} and Mn^{2+} are most effective, Ca^{2+} activates to a lesser extent [8]) [8]

Turnover number (min^{-1})

1380 <21> (ATP, <21> pH 7.0, 25°C, mutant enzyme C13S [21]) [21]
1380 <21> (shikimate, <21> pH 7.0, 25°C, mutant enzyme C13S [21]) [21]
2100 <21> (ATP, <21> pH 7.0, 25°C, wild-type enzyme [21]) [21]
2100 <21> (shikimate, <21> pH 7.0, 25°C, wild-type enzyme [21]) [21]
2400 <21> (ATP, <21> pH 7.0, 25°C, mutant enzyme C162S [21]) [21]
2400 <21> (shikimate, <21> pH 7.0, 25°C, mutant enzyme C162S [21]) [21]

Specific activity (U/mg)

0.05718 <18> [8]
8.39 <19> [10]
100 <3> (<3> isoenzyme SK2 [3]; <3> shikimate kinase II [9]) [3, 9]
410 <3> (<3> isoenzyme SK1 [3]) [3]

K_m-Value (mM)

0.048 <17> (ATP) [18]
0.075 <21> (shikimate, <21> pH 7.0, 25°C, mutant enzyme C13S [21]) [21]
0.11 <18> (ATP, <18> pH 9.0 [8]) [8]
0.16 <3> (ATP, <3> isoenzyme SK2, at 1 mM shikimate [3]) [3, 9]
0.2 <3, 18> (shikimate, <18> pH 9.0 [8]; <3> isoenzyme SK2, at 5 mM ATP [3]) [3, 8, 9]
0.25 <2> (shikimate, <2> pH 8.6 [2]) [2]
0.28 <21> (shikimate, <21> pH 7.0, 25°C, mutant enzyme C162S [21]) [21]
0.38 <2> (ATP, <2> pH 8.6 [2]) [2]
0.414 <17> (shikimate) [18]
0.67 <21> (ATP, <21> pH 7.0, 25°C, mutant enzyme C162S [21]) [21]
5 <3> (shikimate, <3> above, isoenzyme SK1, at 5 mM [3]) [3]
Additional information <19> [10]

pH-Optimum

8.6-9 <2> [2]
9 <18> (<18> or higher, in Tris-HCl buffer or bis-Tris propane buffer [8]) [8]
9.5 <19> [10]

pH-Range

6-9.5 <19> (<19> pH 6.0: about 50% of maximal activity, pH 9.5: optimum [10]) [10]
6.2-9 <18> (<18>, pH 6.2: about 20% of maximal activity in Tris propane buffer, pH 7.8: about 30% of maximal activity, pH 9.0: optimum [8]) [8]

4 Enzyme Structure

Molecular weight

13000 <4, 8, 9> (<4,8,9> gel filtration [4]) [4]
14000 <5, 6, 7> (<5,6,7> gel filtration [4]) [4]
19000 <3> (<3> isoenzyme SK1, gel filtration [3]) [3]
21400 <3> (<3> isoenzyme SK2, gel filtration [2,9]) [2, 9]
28000 <19> (<19> gel filtration [10]) [10]

30000 <1> (<1> about, gel filtration [1]) [1]
Additional information <10, 11, 12, 13, 14, 15, 16> (<10> molecular weight of enzyme aggregate containing 5 activities: EC 1.1.1.25, EC 2.7.1.71, EC 4.2.1.10, EC 4.2.3.4 and EC 2.5.1.19 is 270000 Da determined by gel filtration [4]; <11,12,14> molecular weight of enzyme aggregate containing 5 activities: EC 1.1.1.25, EC 2.7.1.71, EC 4.2.1.10, EC 4.2.3.4 and EC 2.5.1.19 is 280000 Da determined by gel filtration [4]; <13,15> molecular weight of enzyme aggregate containing 5 activities: EC 1.1.1.25, EC 2.7.1.71, EC 4.2.1.10, EC 4.2.3.4 and EC 2.5.1.19 is 290000 Da determined by gel filtration [4]; <16> molecular weight of enzyme aggregate containing 5 activities: EC 1.1.1.25, EC 2.7.1.71, EC 4.2.1.10, EC 4.2.3.4 and EC 2.5.1.19 is 140000-145000 Da determined by gel filtration or glycerol density gradient centrifugation [5]; <14> MW calculated from nucleotide sequence of the pentafunctional arom polypeptide is 174555 Da [11]; <3> identification of the gene [12]; <15> aggregate containing 5 activities: EC 1.1.1.25, EC 2.7.1.71, EC 4.2.1.10, ED 4.2.3.4 and EC 2.5.1.19 has a MW of 270000 Da determined by glycerol-density-gradient centrifugation [13]) [4, 5, 11, 12, 13]

Subunits

monomer <3, 19> (<3> 1 * 17000, isoenzyme SK2, SDS-PAGE [2,9]; <19> 1 * 31000, SDS-PAGE [10]) [2, 9, 10]
Additional information <15> (<15> 95% of the functional enzyme system in crude extracts exists in a dimeric form and both polypeptide chains of the homodimer are required for full activity of each of the five enzymes [7]; <15> aggregate containing 5 activities: EC 1.1.1.25, EC 2.7.1.71, EC 4.2.1.10, EC 4.2.3.4 and EC 2.5.1.19 is composed of two subunits of 165000 Da [13]) [7, 13]

5 Isolation/Preparation/Mutation/Application

Source/tissue

cell extract <3, 4> [3-15]
leaf <19> [10]
seedling <2> [2]
shoot <18> [8]
stem <18> [8]

Localization

chloroplast <19> [10]

Purification

<1> [1]
<2> (partial [2]) [2]
<3> (isoenzyme SK1 and SK2 [3]; shikimate kinase II [9]) [3, 9]
<16> (enzyme aggregate contains 5 activities: EC 1.1.1.25, EC 2.7.1.71, EC 4.2.1.10, EC 4.2.3.4 and EC 2.5.1.19 [5]) [5]
<17> [18]

<18> [8]
<19> [10]
<21> [19]

Renaturation

<21> (the enzyme is fully unfolded in 4 M urea. Approximately 95% of the enzyme activity can be recovered on dilution of the urea from 4 to 0.36 M. Refolding occurs in at least four kinetic phases, the slowest of which corresponds with the regain of shikimate binding and enzyme activity [19]; when the enzyme is unfolded by incubation in 4 M urea, addition of NaCl or Na_2SO_4 leads to relatively slow refolding of the enzyme. The refolded enzyme can bind shikimate, though more weakly than the native enzyme. The refolded enzyme does not appear to be capable of binding nucleotides, nor does it possess detectable catalytic activity. The refolding process brought about by addition of salt in the presence of 4 M urea is not associated with any change in the fluorescence of the probe 8-anilino-1-naphthalenesulfonic acid [6]) [6, 19]

Crystallization

<3> [14]
<20> (crystal structure of the enzyme in complex with $MgADP^-$ determined at 1.8 A resolution [15]) [15]
<21> (vapor-diffusion method using NaCl as precipitant [16]; mutant enzyme K15M, sitting-drop vapor diffusion [21]) [16, 21]

Cloning

<3> (cloning of aroK encoding shikimate kinase I. AroK protein, i.e. shikimate kinase I and AroL protein, i.e. shikimate kinase II are of comparable length and the homology between them extends the entire length of the two enzymes [20]) [20]
<17> (protein is expressed in Escherichia coli as N-terminal fusion with His6 tag and a TEV-protease cleavage site [18]) [18]
<20> (aroK-endoded shikimate kinase, cloned and overexpressed in soluble form in Escherichia coli [22]) [22]
<21> (wild-type and mutant enzyme expressed in Escherichia coli [21]) [21]

Engineering

C13S <21> (<21> enzymatically active mutant, turnover-number is 65% of that of the wild-type enzyme [21]) [21]
C162S <21> (<21> turnover-number is 1.14fold higher than that of the wild-type enzyme [21]) [21]
D34N <21> (<21> inactive mutant enzyme [21]) [21]
K15M <21> (<21> inactive mutant enzyme, increased thermostability and affinity for ATP when compared to the wild-type enzyme, the organization of the P-loop and flanking regions is heavily disturbed [21]) [21]

6 Stability

Temperature stability

40 <21> (<21> midpoint of protein unfolding transition is 39.7°C for the wild-type enzyme, 39°C for the mutant enzyme C13S and 43.0°C for the wild-type enzyme K15M in absence of ligands [21]) [21]

General stability information

<21>, the enzyme is fully unfolded in 4 M urea [19]

<21>, unfolding of the enzyme by guanidinium chloride, in the absence of ligands there is a loss of structure over the range of 1-3 M guanidinium chloride [23]

Storage stability

<3>, -20°C, protein concentration 1 mg/ml in 0.05% M Tris-HCl buffer, pH 7.5, 10 mM $MgCl_2$, 0.1 M NaCl, 1 mM DTT, 10% loss of activity after 1 week, 90% loss of activity after 2 months [9]

<15>, -20°C, 50% glycerol, aggregate containing 5 activities: EC 1.1.1.25, EC 2.7.1.71, EC 4.2.1.10, 5-dehydroquinate synthase and 3-enoyl-pyruvylshikimate 5-phosphate synthase is stable for at least 1 month [13]

<15>, 4°C, 50% glycerol, aggregate containing 5 activities: EC 1.1.1.25, EC 2.7.1.71, EC 4.2.1.10, 5-dehydroquinate synthase and 3-enoyl-pyruvylshikimate 5-phosphate synthase is stable for at least 19 days [13]

<18>, -18°C, 30% v/v glycerol, stable for several months with gradual loss of activity [8]

<19>, -20°C, in presence of 15% glycerol, stable for up to 4 weeks without loss of activity [10]

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Streptomycin 6-kinase 2.7.1.72

1 Nomenclature

EC number
2.7.1.72

Systematic name
ATP:streptomycin 6-phosphotransferase

Recommended name
streptomycin 6-kinase

Synonyms
SM 6-kinase
kinase, streptidine (phosphorylating)
kinase, streptomycin 6- (phosphorylating)
streptidine kinase
streptidine kinase (phosphorylating)
streptomycin 6-O-phosphotransferase
streptomycin 6-kinase (phosphorylating)
streptomycin 6-phosphotransferase

CAS registry number
37278-11-8

2 Source Organism

<1> *Streptomyces bikiniensis* (strain ATCC 11062 [1,2]) [1-3]
<2> *Streptomyces griseus* (strain HUT 6037 [4]) [4]
<3> *Streptomyces glebosus* (strain ATCC 14607 [3]) [3]

3 Reaction and Specificity

Catalyzed reaction
ATP + streptomycin = ADP + streptomycin 6-phosphate (dATP can replace ATP, dihydrostreptomycin, streptidine and 2-deoxystreptidine can act as acceptors)

Reaction type
phospho group transfer

Natural substrates and products

S ATP + streptomycin <1-3> (<1> streptomycin is secreted [1,2]; <1> detoxification of a potent inhibitor of protein biosynthesis [3]; <2> self-protection of streptomycin producer, because the product streptomycin 6-phosphate does not inhibit protein synthesis [4]) (Reversibility: ? <1-3> [1-4]) [1-4]
P ADP + streptomycin 6-phosphate <1-3> [1-4]

Substrates and products

S 3'-deoxydihydrostreptomycin + ATP <1> (Reversibility: ? <1> [2,3]) [2, 3]
P ADP + 3'-deoxydihydrostreptomycin 6-phosphate
S ATP + dihydrostreptomycin <1-3> (Reversibility: ? <1-3> [1-4]) [1-4]
P ADP + dihydrostreptomycin 6-phosphate <1-3> [1-4]
S ATP + streptomycin <1-3> (Reversibility: ? <1-3> [1-4]) [1-4]
P ADP + streptomycin 6-phosphate <1-3> [1-4]
S dATP + streptomycin <1> (Reversibility: ? <1> [3]) [3]
P dADP + streptomycin 6-phosphate
S dihydrostreptomycin 3'-α-phosphate + ATP <1> (Reversibility: ? <1> [2,3]) [2, 3]
P ADP + 3'-α-phosphate 6-phosphate
S dihydrostreptomycin 3'-α-phosphate + ATP <1> (Reversibility: ? <1> [2,3]) [2, 3]
P ADP + 3'-α-phosphate 6-phosphate
S streptidine + ATP <1, 2> (Reversibility: ? <1,2> [1,3,4]) [1, 3, 4]
P streptidine 6-phosphate + ADP <1> (<1> O-phosphorylstreptidine + ADP [3]) [1]
S Additional information <1, 2> (<1> no activity with streptomycin derivatives that contain 3'-α-aldehyde groups [2]; <1> no activity with N-amidinostreptamine, N'-amidinostreptamine, N-amidinoinosamine, dTTP [1]; <1,2> no activity with GTP, CTP, UTP [1,4]) [1, 4]
P ?

Inhibitors

ADP <2> (<2> noncompetitive [4]) [4]
AMP <2> (<2> noncompetitive [4]) [4]
$AgNO_3$ <2> (<2> strong inhibition [4]) [4]
EDTA <2> (<2> strong inhibition [4]) [4]
adenosine <2> (<2> competitive [4]) [4]
dihydrostreptomycin <1> [1]
diphosphate <2> (<2> noncompetitive [4]) [4]
streptomycin <1> [1]
Additional information <1> (<1> no inhibition by sulfhydryl reagents, formamidine disulfide, *p*-chloromercuribenzoate [1]) [1]

Cofactors/prosthetic groups

ATP <1-3> [1-4]

Metals, ions
Co^{2+} <2> (<2> 10 mM, rather less stimulatory than Mg^{2+} [4]) [4]
Cu^{2+} <2> (<2> 10 mM, rather less stimulatory than Mg^{2+} [4]) [4]
Fe^{2+} <2> (<2> 10 mM, slight stimulation [4]) [4]
Mg^{2+} <1-3> (<1-3> required [1,3,4]; <2> maximal activity with 10 mM [4]) [1, 3, 4]
Mn^{2+} <1-3> (<2> 10 mM, slight stimulation [4]; <1,3> can replace Mg^{2+} in activation [1,3]) [1, 3, 4]
Zn^{2+} <2> (<2> 10 mM, slight stimulation [4]) [4]
Additional information <2> (<2> not affected by Ca^{2+}, 0.2-100 mM [4]) [4]

Specific activity (U/mg)
11.35 <2> (<2> purified enzyme [4]) [4]
Additional information <1> (<1> assay methods [3]) [3]

K_m-Value (mM)
0.4 <2> (ATP) [4]
3.5 <2> (streptomycin) [4]
11 <2> (dihydrostreptomycin) [4]
28 <2> (streptidine) [4]

K_i-Value (mM)
0.8 <2> (adenosine) [4]
3 <2> (diphosphate) [4]
5 <2> (ADP) [4]
6 <2> (ATP) [4]

pH-Optimum
7.4 <1, 3> (<1,3> assay at [1,3]) [1, 3]
9 <1> (<1> assay at [3]) [3]
Additional information <2> (<2> pI: 6.6 [4]) [4]

Temperature optimum (°C)
35 <1> (<1> assay at [3]) [3]

4 Enzyme Structure

Molecular weight
38000 <2> (<2> gel filtration [4]) [4]

Subunits
monomer <2> (<2> 1 * 36000, SDS-PAGE [4]) [4]

5 Isolation/Preparation/Mutation/Application

Source/tissue
mycelium <1, 3> [1, 3]

Purification

<1> (partial [1]) [1, 4]
<2> [4]

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[2] Walker, J.B.; Skorvaga, M.: Phosphorylation of streptomycin and dihydrostreptomycin by Streptomyces. Enzymatic synthesis of different diphosphorylated derivatives. J. Biol. Chem., **248**, 2435-2440 (1973)
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[4] Sugiyama, M.; Sakamoto, M.; Mochizuki, H.; Nimi, O.; Nomi, R.: Purification and characterization of streptomycin 6-kinase, an enzyme implicated in self-protection of a streptomycin-producing micro-organism. J. Gen. Microbiol., **129**, 1683-1687 (1983)

Inosine kinase 2.7.1.73

1 Nomenclature

EC number
2.7.1.73

Systematic name
ATP:inosine 5'-phosphotransferase

Recommended name
inosine kinase

Synonyms
guanosine-inosine kinase
inosine-guanosine kinase
kinase, inosine (phosphorylating)

CAS registry number
37237-46-0

2 Source Organism

<1> *Mus musculus* [1]
<2> *Helianthus tuberosus* [2]
<3> *Spirulina platensis* [3]
<4> *Escherichia coli* [4, 7]
<5> *Curtobacterium flaccumfaciens* [5]
<6> *Brevibacterium acetylicum* (ATCC 953 [5]) [5]
<7> *Exiguobacterium acetylicum* [6]

3 Reaction and Specificity

Catalyzed reaction
ATP + inosine = ADP + IMP (<4> ordered bi bi mechanism in which guanosine is the first substrate to bind and GMP is the last product to be released [7])

Reaction type
phospho group transfer

Substrates and products

S ATP + deoxyguanosine <4> (Reversibility: ? <4> [7]) [7]
P ADP + dGMP
S ATP + guanosine <2, 4> (Reversibility: ? <2,4> [2,4]) [2, 4]
P ADP + GMP
S ATP + inosine <1, 2, 3, 4> (<4> about 60% of the activity with guanosine [4]) (Reversibility: ? <1, 2, 3, 4> [1, 2, 3, 4, 5, 6, 7]) [1, 2, 3, 4, 5, 6, 7]
P ADP + 5'-IMP <1, 4> [1, 4]
S ATP + xanthosine <4> (Reversibility: ? <4> [7]) [7]
P ADP + XMP
S CTP + inosine <4> (Reversibility: ? <4> [7]) [7]
P CDP + IMP
S UTP + guanosine <4> (<4> UTP shows 20% of the activity with ATP [4]) (Reversibility: ? <4> [4]) [4]
P UDP + GMP
S UTP + inosine <4> (<4> UTP shows 20% of the activity with ATP [4]) (Reversibility: ? <4> [4,7]) [4, 7]
P UDP + IMP
S dATP + guanosine <4> (Reversibility: ? <4> [4]) [4]
P dADP + GMP
S dATP + inosine <4> (Reversibility: ? <4> [4,7]) [4, 7]
P dADP + IMP

Inhibitors

Cu^{2+} <4> [4]
GDP <4> [7]
GTP <4> [7]
Zn^{2+} <4> [4]
guanosine <2> (<2> inhibits phosphorylation of inosine [2]) [2]
inosine <2> (<2> inhibits phosphorylation of guanosine [2]) [2]

Activating compounds

K^+ <2, 4> (<2> increases activity, optimum concentration: 40 mM [2]; <4> the enzyme requires both Mg^{2+} and K^+ [4]) [2, 4]
Mg^{2+} <1, 2, 4> (<2> reaction is dependent on presence of divalent cation [2]; <1> required, optimal concentration: 5 mM [1]; <4> enzyme requires both Mg^{2+} and K^+ [4]) [1, 2, 4]
Mn^{2+} <1> (<1> can partially replace Mg^{2+} in activation [1]) [1]

Specific activity (U/mg)

3.7 <4> [4]

K_m-Value (mM)

0.0061 <4> (guanosine, <4> pH 7.2, 30°C [4]) [4]
0.014 <2> (guanosine) [2]
0.07 <2> (inosine) [2]
0.078 <1> (inosine) [1]
0.166 <3> (inosine, <3> pH 7.4 [3]) [3]
0.51 <4> (ATP, <4> pH 7.2, 30°C, guanosine kinase reaction [4]) [4]

0.66 <4> (dATP, <4> pH 7.2, 30°C, inosine kinase reaction [4]) [4]
0.71 <4> (ATP, <4> pH 7.2, 30°C, inosine kinase reaction [4]) [4]
1.45 <2> ($MgATP^{2-}$) [2]
2.1 <4> (inosine, <4> pH 7.2, 30°C [4]) [4]
2.4 <4> (dATP, <4> pH 7.2, 30°C, guanosine kinase reaction [4]) [4]

pH-Optimum
6.8-7.6 <2> [2]
6.9 <4> (<4> inosine kinase [4]) [4]
7.4 <1> [1]
8.2 <4> (<4> guanosine kinase [4]) [4]
8.5-9 <3> [3]

pH-Range
6-10.5 <3> (<3> pH 6.0: about 40% of maximal activity, pH 10.5: about 85% of maximal activity [3]) [3]
6.5-8.2 <1> (<1> pH 6.5: about 60% of maximal activity, pH 8.2: about 70% of maximal activity, Tris-maleate buffer [1]) [1]

Temperature optimum (°C)
26-39 <4> (<4> inosine kinase [4]) [4]
38 <4> (<4> guanosine kinase [4]) [4]

4 Enzyme Structure

Molecular weight
85000 <4> [7]

Subunits
? <4> (<4> x * 48400, calculation from nucleotide sequence [4]) [4]
dimer <4> (<4> 2 * 45000 [7]) [7]

5 Isolation/Preparation/Mutation/Application

Source/tissue
Ehrlich ascites carcinoma cell <1> [1]

Localization
mitochondrion <2> (<2> intermembrane space [2]) [2]

Purification
<4> (recombinant enzyme [4]) [4, 7]

Cloning
<4> [4]

Application

nutrition <7> (<7> practical possibility of producing 5'-GMP by phosphorylation of guanosine using a guanosine-inosine kinase coupled with ATP regeneration [6]) [6]

6 Stability

Temperature stability

37 <1> (<1> rapid loss of activity [1]) [1]

General stability information

<2>, very low stability [2]

References

[1] Pierre, K.J.; LePage, G.A.: Formation of inosine-5-monophosphate by a kinase in cell-free extracts of Ehrlich ascites cells in vitro. Proc. Soc. Exp. Biol. Med., **127**, 432-440 (1968)

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Deoxycytidine kinase 2.7.1.74

1 Nomenclature

EC number
2.7.1.74

Systematic name
NTP:deoxycytidine 5'-phosphotransferase

Recommended name
deoxycytidine kinase

Synonyms
2'-deoxycytidine kinase
ara-C kinase
arabinofuranosylcytosine kinase
dCK
deoxycytidine-cytidine kinase
kinase, deoxycytidine (phosphorylating)
Additional information (possibly identical with EC 2.7.1.76)

CAS registry number
9039-45-6

2 Source Organism

<1> *Mus musculus* [1, 5, 19, 21, 25, 27]
<2> *Bos taurus* [2-4, 6-8, 17]
<3> *Homo sapiens* [9-20, 24-26, 28-45]
<4> *Sus scrofa* [17]
<5> *Aotus trivirgatus* (owl monkey [21]) [19, 21]
<6> *Gallus gallus* [21]
<7> *Lactobacillus acidophilus* [22, 23]

3 Reaction and Specificity

Catalyzed reaction
NTP + deoxycytidine = NDP + dCMP (cytosine arabinoside can act as acceptor, all natural nucleoside triphosphates except dCTP can act as donors; <3, 7> mechanism [12, 22, 30, 43]; <3> ordered A-B random B-Q reaction sequence [30]; <3> random bi-bi mechanism [38])

Reaction type

phospho group transfer

Natural substrates and products

- **S** ATP + deoxycytidine <1-3, 5> (<3> key anabolic enzyme for activation of purine or pyrimidine deoxynucleosides as well as cytidine arabinoside and other anti-tumor drugs [9]; <1,3,5> key enzyme in anabolic phosphorylation of deoxyribonucleosides and their analogues [19]) (Reversibility: ? <1-3, 5> [8, 9, 19]) [8, 9, 19]
- **P** ADP + ?
- **S** UTP + deoxycytidine <3> (<3> UTP is physiological phosphate donor [42]) (Reversibility: ? <3> [42]) [42]
- **P** UDP + dCMP

Substrates and products

- **S** 2',2'-difluoro-2'-deoxyguanosine + ATP <3> (Reversibility: ? <§> [§/]) [37]
- **P** ADP + 2',2'-difluoro-2'-deoxyguanosine 5'-phosphate
- **S** 2'-O-methyl-cytidine + ATP <3> (Reversibility: ? <3> [37]) [37]
- **P** ADP + 2'-O-methyl-cytidine 5'-phosphate
- **S** 2'-fluoro-2'-deoxyarabinosylcytidine + ATP <3> (Reversibility: ? <3> [37]) [37]
- **P** ADP + 2'-fluoro-2'-deoxyarabinosylcytidine 5'-phosphate
- **S** 2'-fluoro-2'-deoxycytidine + ATP <3> (Reversibility: ? <3> [37]) [37]
- **P** ADP + 2'-fluoro-2'-deoxycytidine 5'-phosphate
- **S** 2-chloro-2'-deoxyadenosine + NTP <1, 3> (Reversibility: ? <1,3> [25,37]) [25, 37]
- **P** NDP + 2-chloro-2'-deoxy-AMP
- **S** 3'-O-methyl-2'-deoxycytidine + ATP <3> (Reversibility: ? <3> [37]) [37]
- **P** ADP + 3'-O-methyl-2'-deoxycytidine 5'-phosphate
- **S** 4'-thio-2'-deoxycytidine + UTP <3> (Reversibility: ? <3> [42]) [42]
- **P** UDP + 4'-thio-2'-deoxycytidine 5'-phosphate
- **S** 4'-thio-β-D-arabinofuranosylcytosine + UTP <3> (Reversibility: ? <3> [42]) [42]
- **P** UDP + 4'-thio-β-D-arabinofuranosylcytosine 5'-phosphate
- **S** 9-β-D-arabinofuranosylguanine + NTP <1, 3> (Reversibility: ? <1, 3> [25]) [25]
- **P** NDP + 9-β-D-arabinofuranosylguanine 5'-phosphate
- **S** ATP + 2'-deoxycytidine <1-3, 7> (<1> best phosphate donor [1,16]; <3> best phosphate donor cytosolic isozyme II and mitochondrial isozyme [10]; <2> in the presence of DTT less effective than dUTP, UTP, dTTP or GTP, without DTT only less effective than dUTP [3]) (Reversibility: ? <1-3, 7> [1-10, 12-16, 20, 22, 24]) [1-10, 12-16, 20, 22, 24]
- **P** ADP + 5'-dCMP <1-3> [1-10]
- **S** ATP + 5-(3-pyridyl)-2'-deoxycytidine <3> (<3> cytosolic enzyme, poor substrate [35]) (Reversibility: ? <3> [35]) [35]
- **P** ADP + 5-(3-pyridyl)-2'-deoxycytidine 5'-phosphate

S ATP + 5-(4-pyridyl)-2'-deoxycytidine <3> (<3> cytosolic enzyme, poor substrate [35]) (Reversibility: ? <3> [35]) [35]
P ADP + 5-(4-pyridyl)-2'-deoxycytidine 5'-phosphate
S ATP + cytidine <2, 3> (<2> poor: β-isomer [7]; <2> not: α-isomer [7]; <3> not [10]) (Reversibility: ? <2,3> [6-8,13,14]) [6-8, 13, 14]
P ADP + ?
S ATP + thymidine <3> (<3> cytosolic enzyme, poor substrate [35]) (Reversibility: ? <3> [35]) [35]
P ADP + TMP
S CTP + 2'-deoxycytidine <2, 3> (<3> as good as GTP, UTP, dATP, mitochondrial isozyme [10]; <3> poor [14]) (Reversibility: ? <2, 3> [2, 6, 10, 14, 16]) [2, 6, 10, 14, 16]
P CDP + 2'-deoxy-CMP
S GTP + 2'-deoxycytidine <2, 3> (<2> best phosphate donor [2]; <3> as good as CTP, dATP or UTP, mitochondrial isozyme [10]; <3> as good as ATP [14]; <3> as good as dGTP, dTTP or dUTP, cytosolic isozyme I [10]) (Reversibility: ? <2,3> [2,3,6,10,14,16,20]) [2, 3, 6, 10, 14, 16, 20]
P GDP + 2'-deoxy-CMP
S NTP + 2',3'-dideoxycytidine <1, 3> (Reversibility: ? <1,3> [25]) [25]
P NDP + 2',3'-dideoxycytidine 5'-phosphate
S NTP + 2',2'-difluoro-2'-deoxycytidine <3> (<3> best acceptor substrate, best phosphate donors: UTP or NTP-mixture, better than ATP [18]; <3> cytosolic isozyme I, not mitochondrial isozyme [10]) (Reversibility: ? <3> [10,18]) [10, 18]
P NDP + 2',2'-difluoro-2'-deoxycytidine 5'-phosphate
S NTP + 2'-3'-dideoxycytidine <1> (<1> poor [19]) (Reversibility: ? <1> [19]) [19]
P NDP + 2'-3'-dideoxycytidine 5'-phosphate
S NTP + 5'-deoxy-5'-fluoroadenine <2> (Reversibility: ? <2> [7]) [7]
P NDP + 5'-deoxy-5'-fluoroadenine 5'-phosphate
S NTP + 9-β-D-arabinofuranosyl-2-fluoroadenine <3> (Reversibility: ? <3> [18]) [18]
P NDP + 9-β-D-arabinofuranosyl-2-fluoroadenine 5'-phosphate
S NTP + β-D-2'-deoxythioguanosine <2> (<2> not α-isomer [7]) (Reversibility: ? <2> [7]) [7]
P NDP + ?
S NTP + cytosine arabinoside <1-3> (<2> i.e. 1-β-arabinofuranosyl cytosine, L-isomer better substrate than D-isomer [7]; <3> D-isomer [10,18]; <3> best phosphate donors: UTP or NTP-mixture, even better than ATP [18]; <2> ATP can be replaced by GTP, dATP, dGTP or dTTP [2]; <3> cytosolic isozyme I [10]; <3> no acceptor substrate for cytosolic isozyme II or mitochondrial isozyme [10]) (Reversibility: ? <1-3> [1-3, 7, 10, 13-15, 18, 20, 37, 42]) [1-3, 7, 10, 13-15, 18, 20, 37, 42]
P NDP + cytosine arabinoside 5'-monophosphate <2> [2]
S NTP + deoxyadenosine <1-3, 5, 7> (<3> cytosolic isozyme I [10]; <1> poor [19]; <1> not [5]; <3> not mitochondrial isozyme [10]) (Reversibil-

ity: ? <1-3, 5, 7> [3, 8-10, 13-16, 19, 22-25, 37, 39]) [3, 8-10, 13-16, 19, 22-25, 37, 39]

P NDP + dAMP

S NTP + deoxycytidine <1-7> (<2> best acceptor substrate at low substrate concentrations [8]; <2> β-D-2'-deoxyribonucleosides are more efficient than corresponding β-D-arabinonucleosides or β-ribonucleosides, rather nonspecific for base moiety of nucleoside substrate [7]; <2> specificity [7]; <2> poor substrate: β-5-azacytidine [7]; <1-3> enzyme shows little specificity towards phosphate donor [1, 2, 13, 14, 18]; <2> in order of decreasing activity: dGTP, CTP, ATP, dATP, dTTP, UTP, GTP, [6]; <3> poor substrate: arabinosyl-CTP [10]; <2,3> no substrate: dCTP [6,16]; <3> no substrate: nucleoside diphosphates and their deoxy derivatives [14]; <3> no substrate: uridine arabinoside, cytosine [10]; <2> no substrate: thymidine [6]; <2,3> no substrate: guanosine [6,10]; <2,3> no substrate: adenosine, uridine [6,7,10]; <1> no substrate: phosphate [1]; <1> no substrate: deoxyuridine [5]; <2> no substrate: ribavirin [7]; <2> no substrate: β-γ-methylene diphosphonate analogue of dTTP [8]) (Reversibility: ? <1-7> [1-25,35,37,39,42]) [1-25, 35, 37, 39, 42]

P NDP + dCMP <1-3> [1-10]

S NTP + deoxyguanosine <2, 3> (<3> cytosolic isozyme I [10]; <1> not [5]; <3> not mitochondrial isozyme [10]) (Reversibility: ? <2, 3> [3, 4, 6-8, 10, 13-16, 24, 37, 39]) [3, 4, 6-8, 10, 13-16, 24, 37, 39]

P NDP + dGMP

S NTP + deoxythymidine <3> (<3> mitochondrial isozyme, better than deoxycytidine, not cytosolic isozyme I [10]) (Reversibility: ? <3> [10]) [10]

P NDP + dTMP

S NTP + deoxyuridine <3> (<3> mitochondrial isozyme, not cytosolic isozyme I [10]; <3> cytosolic enzyme, poor substrate [35]) (Reversibility: ? <3> [10,35]) [10, 35]

P NDP + dUMP

S UTP + 2'-deoxycytidine <1-3> (<1> best phosphate donor at low substrate levels [1]; <1,3> as good as CTP, GTP or dATP [1, 10, 18]; <3> poor [14]) (Reversibility: ? <1-3> [1,3,6,10,14,16,18]) [1, 3, 6, 10, 14, 16, 18]

P UDP + 2'-deoxy-CMP

S arabinosyladenosine + ATP <3> (Reversibility: ? <3> [37]) [37]

P arabinosyladenosine 5'-phosphate + ADP

S β-D-3'-hydroxymethyl-2',3'dideoxycytidine + ATP <3> (Reversibility: ? <3> [37]) [37]

P ADP + β-D-3'-hydroxymethyl-2',3'dideoxycytidine 5'-phosphate

S dCTP + 2'-deoxycytidine <7> (<7> only in the absence of ATP [22]) (Reversibility: ? <7> [22]) [22]

P dCDP + 2'-deoxy-CMP

S dGTP + 2'-deoxycytidine <2, 3> (<2,3> best phosphate donor [6,14]; <3> cytosolic isozyme I [10]; <3> poor substrate of mitochondrial isozyme [10]) (Reversibility: ? <2,3> [2,6,10,14,16]) [2, 6, 10, 14, 16]

P dGDP + 2'-deoxy-CMP

S dTTP + 2'-deoxycytidine <2, 3> (Reversibility: ? <2, 3> [2-4, 6, 15, 16]) [2-4, 6, 15, 16]
P dTDP + 2'-deoxy-CMP
S dUTP + 2'-deoxycytidine <2, 3> (<2> best phosphate donor [3]; <3> best phosphate donor, cytosolic enzyme I, poor, mitochondrial isozyme [10]) (Reversibility: ? <2,3> [3,10]) [3, 10]
P dUDP + 2'-deoxy-CMP
S Additional information <3, 7> (<3> enzyme exists in different conformational states with different substrate kinetic properties [9]; <3> presumably one common nucleoside acceptor site [15]; <3> purine deoxynucleoside activity inseparably associated with deoxycytidine kinase protein [16]; <3> several isozymes: cytosolic deoxycytidine kinase I and II, plus mitochondrial isozyme [10]; <3> multisubstrate enzyme, that also phosphorylates purine deoxyribonucleotides [9]; <7> enzyme has two separate active sites for deoxycytidine and deoxyadenosine activity [22]; <3> reacts with both enantiomers of β-deoxycytidine, β-deoxyguanidine, β-deoxyadenosine, and α-D-deoxycytidine is also substrate [31]; <3> reacts with both enantiomers of β-deoxyadenosine, β-arabinofuranosyl-adenine and β-deoxyguanine [34]; <3> remarkably relaxed enantioselectivity with respect to cytidine derivatives in β configuration [36]; <3> lack of enantioselectivity for D- and L-analogues of cytidine and adenosine [43]) [9, 10, 15, 16, 22, 31, 34, 43]
P ?

Inhibitors

2'-O-methylcytidine <3> [37]
2-chloroadenosine <3> [14]
3'-O-methyl-2'deoxycytidine <3> [37]
4'-thio-β-D-arabinofuranosylcytosine <3> (<3> IC50 0.000024 mM [42]) [42]
5'-O-ethyl-2'-deoxycytidine <3> [37]
5'-O-methyl-2'-deoxycytidine <3> [37]
5-chloro-4'-thio-β-D-arabinofuranosylcytosine <3> (<3> IC50 0.051 mM [42]) [42]
5-fluoro-2'-deoxycytidine <7> [22]
5-fluoro-4'-thio-β-D-arabinofuranosylcytosine <3> (<3> IC50 0.0000020 mM [42]) [42]
ADP <2, 3> (<3> kinetics [12,13]; <2> not [3]) [6, 12, 13]
Ba^{2+} <3> [14]
CDP <2> (<2> strong [6]) [6]
CMP <3> (<2> not [3]) [13]
CTP <2, 3> (<3> strong, ATP as substrate [24]) [6, 13, 24]
Cu^{2+} <3> [14]
GDP <2, 3> (<2> not [3]) [6, 13]
GSSG <2> (<2> thiol reagents protect or reverse [3]) [3]
GTP <2, 3> (<3> weak, ATP and deoxycytidine as substrate [24]; <3> not, deoxyadenosine as substrate [10,24]) [6, 24]
$HgCl_2$ <1-3> (<2> strong, thiol reagents protect [3]) [1-3, 14]

3-indoleacetic acid <1> (<1> weak [1]) [1]
IMP <3> [13]
N-ethylmaleimide <1, 2> (<1> weak [1]; <2> thiol reagents protect or reverse [3]) [1, 3]
N^6-phenyladenosine <2> [7]
NaCl <3> (<3> at 0.2-0.4 M, stimulation of 2'-deoxycytidine and 2-chlorodeoxyadenosine phosphorylation, but inhibition of 2'-deoxyguanosine phosphorylation [33]) [33]
P^1,P^4-diadenosine 5'-tetraphosphate <3> (<3> not di-, tri- or pentaphosphate derivative [13]) [13]
SDS <1> [1]
Tris-maleate buffer <2> [6]
UDP <2, 3> (<2, 3> strong [6, 10, 13]; <3> only cytosolic isozyme I [10]) [3, 4, 6, 8, 10, 13, 30]
UMP <3> (<3> strong [13]; <2> not [3]) [13]
UTP <2, 3> (<3> ATP as substrate [24]; <3> not mitochondrial isozyme [10]; <3> not with deoxyadenosine as substrate [24]) [6, 10, 13, 24]
adenosine arabinoside <3> (<3> weak, not with deoxyadenosine as substrate [24]) [24]
α-D-2'-deoxythioguanosine <2> [7]
cytidine <2, 3, 7> (<2> α-isomer [7]; <3> deoxycytidine as substrate [14]; <2> cytosine arabinoside as substrate [3]; <3> weak [24]; <1,2> not [1,6]) [3, 7, 14, 22, 24]
cytosine arabinoside <1-3> (<3> ATP as substrate [24]; <1-3> deoxycytidine as substrate [1, 2, 14, 24]; <3> strong, deoxyadenosine as substrate [24]; <2> deoxyguanosine as substrate [3]; <3> IC50 0.0000055 mM [42]) [1-3, 14, 24]
cytosine arabinoside monophosphate <3> (<3> strong [13]) [13]
cytosine arabinoside triphosphate <3> (<3> strong [10]; <3> weak [20]) [10, 13, 20]
dADP <2> (<2> strong [6]) [6]
dAMP <3> (<3> strong [13]; <1,2> not [1,3]) [13]
dATP <2, 3> (<2,3> strong [6,13,24]; <7> not [22]) [6, 13, 24]
dCDP <1-3> (<2> strong, ATP as substrate [6]) [1-3, 6, 13]
dCMP <1-3> (product inhibition, strong <2,3> [6, 8, 10, 13]; <2> ATP as substrate [6]; <3> only cytosolic isozyme I [10]; <2> dTTP reverses [8]) [1-3, 6, 8, 10, 13]
dCTP <1-3, 7> (<1> strong, pH-dependent [1]; <2,7> ATP as substrate [6, 22]; <3> cytosine arabinoside as substrate [10, 20]; <2> reversible by UTP, dUTP [3]; <2> reversible by UDP [4]; reversible by dTTP <2> [3, 4]; <2> no reversion by β-γ-methylene diphosphonate analogue of dTTP [8]; <2, 3> kinetics [4, 13, 15]; <3> isozyme II [10]; <7> moderate phosphate donor in the absence of ATP [22]; <3> no inhibition at physiological concentrations [18]) [1-4, 6, 8, 10, 12, 13, 15, 18, 20, 22, 24, 30]
dCTP <1, 3> (<3> at 0.013 mM, substrates ATP, deoxycytosine, 30% inhibition [25]; <1> at 0.013 mM, substrates ATP, deoxycytosine, 50% inhibition [25]) [25]
dGDP <2> (<2> strong [6]; <3> not [10]) [6]

dGMP <3> (<3> strong [13]; <1> not [1]) [13]
dGTP <2, 3, 7> (<3,7> ATP as substrate [22,24]) [6, 22, 24]
dTDP <2> (<2> strong [6]) [3, 6, 8]
dTTP <2, 3, 7> (<3> strong [10]; <3,7> weak [22,24]; <3,7> ATP as substrate [22,24]; <2> at increased deoxycytidine concentrations [4]; <3> only mitochondrial isozyme [10]) [4, 6, 10, 22, 24]
dUDP <2, 3> (<2,3> strong [6,10]; <3> only cytosolic isozyme I [10]) [6, 10]
dUMP <3> (<3> strong [13]; <1,2> not [1,3]) [13]
dUTP <3> [13]
deoxyadenosine <2, 3, 7> (<7> kinetics [22]; <3,7> deoxycytidine [14,15,22]; <2> cytosine arabinoside or deoxyguanosine as substrate [3]; <3,7> cytosolic isozyme I, weak [10,22]; <2> not [2]) [3, 10, 14, 15, 22, 24]
deoxycytidine <2, 3> (<2,3> cytosine arabinoside as substrate [2,3,10,20]; <2,3> deoxyadenosine as substrate [3,15,24]; <2> cytidine as substrate [6]; <2,3> deoxyguanosine as substrate [3,6,15]; <3> at concentrations greater than 0.003 mM, UTP as substrate, noncompetitive [30]) [2, 3, 6, 10, 15, 20, 24, 30]
deoxyguanosine <2, 3, 7> (<3> deoxycytidine as substrate [14,15]; <2> cytosine arabinoside as substrate [3]; <3> deoxyguanosine as substrate, cytosolic isozyme I, weak, [10,24]; <2> not [2,6]) [3, 10, 14, 15, 22, 24]
deoxythymidine <3> (<3> weak [10]; <2> cytosolic isozyme I, not [3]) [10]
dideoxyadenosine <3> [13]
dideoxycytidine <3> [13, 14]
dinitrophenol <1> (<1> weak [1]) [1]
dioxane <1> [1]
non-substrate inhibitors <2> [7]
nucleoside analogs <2> (<2> related to deoxycytidine, deoxyadenosine or deoxyguanosine [8]) [8]
nucleosides <3> (<3> overview, ATP and deoxyadenosine or deoxycytidine as substrate [24]) [14, 24]
p-chloromercuribenzoate <2> (<2> thiol reagents protect or reverse [3]) [3]
sodium diphosphate <3> (<3> not monophosphate [13]) [13]
substrate derivatives <2> (<2> overview [7]) [7]
trypsin <7> (<7> substrates or dNTP protect corresponding active site [22]) [22]
urea <1> [1]
uridine arabinoside <3> (<3> weak, cytosolic isozyme I [10]) [10]
Additional information <1-3> (<3> deoxycytidine phosphorylating ability shows different inhibition pattern than deoxyadenosine activity [9]; <1> relatively insensitive to SH-inhibitors and detergents [1]; <2,3> little or no inhibition by AMP, dTMP, adenosine, guanosine [3,14,24]; <1-3> little or no inhibition by deoxyuridine [1,3,14]; <3> little or no inhibition by ribavirin [14]; <2> no inhibition by ribavirin [7]; <3> overview [14]; <3> activity of

enzyme is reduced by hyperosmotic treatment of cells [29]; <1,3> no inhibition by uridine, thymidine [1, 14]; <3> no inhibition by inosine, xanthosine, S-adenosylhomocysteine, S-adenosylmethionine [14]; <1> no inhibition by TTP [1]) [1, 3, 7, 9, 14, 24, 29]

Activating compounds

1-β-D-arabinosylcytosine <3> (<3> i.e. cytarabine, 0.001 mM, stimulation [28]) [28]
2'-deoxythymidine-5'-thiosulphate <3> (<3> in intact cells, activation, reversed by 2'-deoxycytidine [45]) [45]
2-chloro-2'-arabino-fluoro-2'-deoxyadenosine <3> (<3> 0.001 mM, stimulation [28]) [28]
2-chloro-2'-deoxy-2'-fluoro-arabinosyladenine <3> (<3> stimulation [40]) [40]
2-chloro-2'-deoxyadenosine <3> (<3> i.e. cladribine, 0.001 mM, stimulation [28]; <3> up to 0.01 mM, up to 4-fold increase in activity [40]) [28, 40]
2-fluoro-1-β-D-arabinosyladenine <3> (<3> i.e. fludarabine, 0.001 mM, stimulation [28,40]) [28, 40]
ATP <3> (<3> substrate activation, at high concentrations of deoxycytidine [30]) [30]
CHAPS <3> (<3> slight stimulation, deoxycytidine as substrate [9]) [9]
NaCl <3> (<3> at 0.2-0.4 M, stimulation of 2'-deoxycytidine and 2-chlorodeoxyadenosine phosphorylation, but inhibition of 2'-deoxyguanosine phosphorylation [33]) [33]
NaF <3> (<3> 15 mM, 1 h, activity about twice as high as initial level [43]) [43]
TTP <3> (<3> stimulation, cytosolic isozyme I [10]) [10]
UDP <3> (<3> stimulation, mitochondrial isozyme [10]) [10]
Zwittergent 3-14 <3> (<3> slight stimulation, deoxycytidine as substrate [9]) [9]
aphidicolin <3> (<3> at 0.006-0.012 mM, 2-3 fold activation [26]) [26]
arabinosylcytidine <3> (<3> stimulation [40]) [40]
bovine serum albumin <2> (<2> activation [3]) [3]
calyculin A <3> (<3> inhibitor of protein phosphatases, activation [29]) [29]
deoxyadenosine <3> (<3> activation, deoxycytidine as substrate, mitochondrial isozyme [10]) [10]
deoxyguanosine <3> (<3> activation, deoxycytidine as substrate, mitochondrial isozyme [10]) [10]
dithiothreitol <1-3> (<1-3> activation [3,5,15]; <2> not [2]) [3, 5, 15]
etoposide <3> (<3> 0.001 mM, stimulation [28]) [28]
γ-radiation <3> (<3> 0.5-2 GY dosage, activation [29]) [29]
sodium cholate <3> (<3> slight stimulation, deoxycytidine as substrate [9]) [9]
uridine arabinoside <3> (<3> activation, deoxycytidine as substrate, mitochondrial isozyme [10]) [10]
Additional information <3> (<3> not activating: 2-chloro-riboadenosine [28]) [28]

Metals, ions

Ca^{2+} <1-3> (<1-3> activation [1-3,6,8,14]; <1,2> can replace Mn^{2+} or Mg^{2+} to some extent [1,2]; <2> about 55% as efficient as Mg^{2+} [3]) [1-3, 6, 8, 14]
$CdCl_2$ <2> (<2> activation, in the absence of dithiothreitol [3]) [3]
Co^{2+} <2, 3> (<2,3> activation [3,8,14]; <2> about 40% as efficient as Mg^{2+} [3]; <3> about 30% as efficient as Mg^{2+} [14]) [3, 8, 14]
Fe^{2+} <1-3> (<1-3> activation [1,3,8,14]; <1> can replace Mn^{2+} or Mg^{2+} to some extent [1]; <3> about 60% as efficient as Mg^{2+} [14]; <2> about 45% as efficient as Mg^{2+} [3]) [1, 3, 8, 14]
Mg^{2+} <1-3, 7> (<1> requirement, 2 mM [1]; <2> more efficient than Mn^{2+} or Ca^{2+} [2]; <2,3> Mg^{2+}/ATP complex is the reactive species [4,6,12,13,15]; <2,3> maximal activity at a ATP:Mg^{2+}-ratio of 1:1 [6,13]; <2> below: decrease of activity [6]) [1-9, 12-15, 22, 23]
Mn^{2+} <1-3> (<1-3> requirement [1-3,6,8,14]; <1> 0.5 mM [1]; <3> about 90% as efficient as Mg^{2+} [14]; <2> about 80% as efficient as Mg^{2+} [3]) [1-3, 6, 8, 14]
Ni^{2+} <2> (<2> activation [3,6,8]; <2> about 20% as efficient as Mg^{2+} [3]; <3> not [14]) [3, 6, 8]
Sr^{2+} <2> (<2> activation [3, 8]; <2> about 15% as efficient as Mg^{2+} [3]) [3, 8]
Zn^{2+} <2, 3> (<2,3> activation [3,6,8,14]; <2> about 40% as efficient as Mg^{2+} [3]; <3> about 30% as efficient as Mg^{2+} [14]; <1> not [1]) [3, 6, 8, 14]
Additional information <1-3> (<1-3> no activation by Ba^{2+}, Cu^{2+} [1,3,14]; <3> no activation by monovalent cations [14]) [1, 3, 14]

Turnover number (min^{-1})

80000 <1> (deoxycytidine, <1> cytosine arabinoside-induced cells [5]) [5]
4700000 <1> (deoxycytidine) [5]

Specific activity (U/mg)

0.00088 <1> (<1> cytosine arabinoside-induced cells [5]) [5]
0.0048 <1> (<1> 37°C, pH 7.0 [1]) [1]
0.00523 <2> (<2> 37°C, pH 8.0 [6]) [6]
0.00632 <2> (<2> 37°C, pH 8.0 [2]) [2]
0.012 <2> (<2> 37°C, pH 8.0 [3]) [3]
0.0206 <2> (<2> 37°C, pH 8.0 [8]) [8]
0.048 <3> (<3> mitochondrial isozyme, pH 7.5, 37°C [10]) [10]
0.072 <3> (<3> cytoplasmic isozyme I, pH 7.5, 37°C [10]) [10]
0.0846 <1> (<1> 37°C, pH 8.0 [5]) [5]
0.11-0.15 <3> (<3> pH 8.0, 37°C [15]) [15]
0.2 <3> [11]
4 <3> (<3> cytidine as substrate, 37°C, pH 7.0 [14]) [14]
7.2 <3> (<3> deoxyguanosine as substrate, 37°C, pH 7.0 [14]) [14]
8 <3> (<3> 37°C, pH 7.0 [13,14]) [13, 14]
13.5 <3> (<3> deoxyadenosine as substrate, 37°C, pH 7.0 [14]) [14]

K_m-Value (mM)

0.00005 <3> (2'-deoxycytidine, <3> cosubstrate UTP, 37°C, pH 7.6 [25]) [25]
0.0001 <1> (2'-deoxycytidine, <1> cosubstrate UTP, 37°C, pH 7.6 [25]) [25]
0.00013 <3> (deoxycytidine, <3> cosubstrate UTP 37°C, pH 8.0 [42]) [42]
0.00016 <3> (2'-deoxycytidine, <3> cosubstrate ATP, 37°C, pH 7.6 [25]) [25]
0.00018 <1> (2'-deoxycytidine, <1> cosubstrate ATP, 37°C, pH 7.6 [25]) [25]
0.00032 <3> (4'-thio-2'-deoxycytidine, <3> cosubstrate ATP 37°C, pH 8.0 [42]) [42]
0.00041 <3> (β-D-arabinofuranosylcytosine, <3> cosubstrate UTP 37°C, pH 8.0 [42]) [42]
0.0005 <3> (UTP, <3> cosubstrate 2'-deoxycytidine, 37°C, pH 7.4 [30]) [30]
0.00057 <3> (deoxycytidine, <3> cosubstrate ATP 37°C, pH 8.0 [42]) [42]
0.0008-0.0015 <3> (deoxycytidine, <3> 37°C, pH 7.0 [13,14]; <3> pH 8.0, 37°C [15]) [13-15]
0.0009 <3> (GTP, <3> cosubstrate 2'-deoxycytidine, 37°C, pH 7.4 [30]) [30]
0.00099 <3> (2-chloro-2'-deoxyadenosine, <3> cosubstrate UTP, 37°C, pH 7.6 [25]) [25]
0.001 <3> (ATP, <3> cosubstrate 2'-deoxycytidine, 37°C, pH 7.4 [30]) [30]
0.001 <3> (dTTP, <3> cosubstrate 2'-deoxycytidine, 37°C, pH 7.4 [30]) [30]
0.0013 <3> (CTP, <3> cosubstrate 2'-deoxycytidine, 37°C, pH 7.4 [30]) [30]
0.002 <2, 3> (cytosine arabinoside, <2> L-isomer, 25°C, pH 7.6 [7]; <3> deoxycytidine, cytosolic isozyme I, pH 7.5, 37°C [10]) [7, 10]
0.0033-0.0093 <1, 3> (deoxycytidine, <1> 37°C, pH 8.0 [5]) [5, 16, 20, 24]
0.0063 <1> (2-chloro-2'-deoxyadenosine, <1> cosubstrate UTP, 37°C, pH 7.6 [25]) [25]
0.007 <3> (cytosine arabinoside, <3> cytosolic isozyme I, pH 7.5, 37°C [10]) [10]
0.014 <2> (deoxycytidine, <2> 37°C, pH 8.0 [2]) [2]
0.014-0.0167 <1-3> (deoxycytidine, <1> cytosine arabinoside-induced cells, 37°C, pH 8.0 [5]; <3> mitochondrial isozyme, pH 7.5, 37°C [10]; <1,2> 37°C, pH 8.0 [2,5]; <3> pH 7.5, 37°C [10]) [2, 5, 10]
0.015 <3> (β-D-arabinofuranosylcytosine, <3> cosubstrate ATP 37°C, pH 8.0 [42]) [42]
0.017 <1> (2-chloro-2'-deoxyadenosine, <1> cosubstrate ATP, 37°C, pH 7.6 [25]) [25]
0.019 <3> (2'-deoxyadenosine, <3> cosubstrate UTP, 37°C, pH 7.6 [25]) [25]
0.02 <3> (4'-thio-β-D-arabinofuranosylcytosine, <3> cosubstrate UTP 37°C, pH 8.0 [42]) [42]
0.02-0.04 <2, 3> (cytosine arabinoside, <2,3> D-isomer [7,20]; <2> 37°C, pH 8.0 [2]; <2> pH 7.6, 25°C [7]; <3> pH 7.0, 37°C [13,14]; <3> pH 8.0, 37°C [15]) [2, 7, 13-15, 20]
0.024 <3> (2-chloro-2'-deoxyadenosine, <3> cosubstrate ATP, 37°C, pH 7.6 [25]) [25]
0.088 <3> (4'-thio-β-D-arabinofuranosylcytosine, <3> cosubstrate ATP 37°C, pH 8.0 [42]) [42]
0.11 <2> (ATP, <2> cosubstrate deoxycytidine, 37°C, pH 8.0 [2]) [2]
0.12 <3> (deoxyadenosine, <3> pH 8.0, 37°C [15]) [15]

0.15 <3> (2',3'-dideoxycytidine, <3> cosubstrate UTP, 37°C, pH 7.6 [25]) [25]
0.15 <1> (2'-deoxyadenosine, <1> cosubstrate UTP, 37°C, pH 7.6 [25]) [25]
0.15 <3> (deoxyadenosine, <3> pH 8.0 [24]) [24]
0.15 <3> (deoxyguanosine, <3> pH 8.0, 37°C [15]) [15]
0.2 <2> (ATP, <2> cosubstrate cytosine arabinoside, 37°C, pH 8.0 [2]) [2]
0.23 <3> (2',3'-dideoxycytidine, <3> cosubstrate ATP, 37°C, pH 7.6 [25]) [25]
0.33-0.43 <3> (deoxyguanosine, <3> 37°C, pH 7.0 [13,14]) [13, 14, 24]
0.45 <3> (cytidine, <3> 37°C, pH 7.0 [13,14]) [13, 14]
0.48 <1> (2',3'-dideoxycytidine, <1> cosubstrate UTP, 37°C, pH 7.6 [25]) [25]
0.48 <3> (2'-deoxyadenosine, <3> cosubstrate ATP, 37°C, pH 7.6 [25]) [25]
0.5 <3> (deoxyadenosine, <3> 37°C, pH 7.0 [13,14]) [13, 14]
0.55 <1> (2'-deoxyadenosine, <1> cosubstrate ATP, 37°C, pH 7.6 [25]) [25]
0.64 <3> (deoxyguanosine, <3> 37°C, pH 7.5 [16]) [16]
0.67 <1> (2',3'-dideoxycytidine, <1> cosubstrate ATP, 37°C, pH 7.6 [25]) [25]
0.89 <3> (deoxyadenosine, <3> 37°C, pH 7.5 [16]) [16]
1.4 <2> (cytidine, <2> 37°C, pH 8.0 [6]) [6]
2 <3> (9-β-D-arabinofuranosylguanine, <3> cosubstrate UTP, 37°C, pH 7.6 [25]) [25]
3 <1> (9-β-D-arabinofuranosylguanine, <1> cosubstrate UTP, 37°C, pH 7.6 [25]) [25]
3 <3> (9-β-D-arabinofuranosylguanine, <3> cosubstrate ATP, 37°C, pH 7.6 [25]) [25]
3 <2> (deoxyguanosine, <2> 37°C, pH 8.0 [6]) [6]
5 <1> (9-β-D-arabinofuranosylguanine, <1> cosubstrate ATP, 37°C, pH 7.6 [25]) [25]
Additional information <1-3, 7> (<1, 2, 3, 7> kinetic study [1, 6, 12, 22]; <2> linear kinetics with deoxyguanosine or cytidine, bimodal kinetics with deoxycytidine as substrate [6,8]; <2> K_m-values of a variety of substrate derivatives [7]; <3> K_m-values of D-and L-cytidine analogues [36]; <3> K_m-values of D- and L-analogues of cytidine and adenosine [43]) [1, 6-9, 12, 13, 15, 22, 36, 43]

K_i-Value (mM)

0.0004 <3> (UDP, <3> cosubstrate UTP, 37°C, pH 7.4 [30]) [30]
0.0015 <3> (dCMP, <3> cosubstrate UTP, 37°C, pH 7.4 [30]) [30]
0.0069 <3> (UDP, <3> cosubstrate deoxycytidine, 37°C, pH 7.4 [30]) [30]
0.02 <3> (3'-O-methyl-2'deoxycytidine, <3> 37°C, pH 7.6 [37]) [37]
0.022 <3> (dCMP, <3> cosubstrate deoxycytidine, 37°C, pH 7.4 [30]) [30]
0.032 <3> (5'-O-ethyl-2'-deoxycytidine, <3> 37°C, pH 7.6 [37]) [37]
0.23 <3> (5'-O-methyl-2'-deoxycytidine, <3> 37°C, pH 7.6 [37]) [37]
0.3 <3> (2'-O-methylcytidine, <3> 37°C, pH 7.6 [37]) [37]

pH-Optimum

5-7 <1> (<1> broad [1]) [1]
5.5-8.5 <3> (<3> plateau, higher activity in Tris than in phosphate buffer [15]) [15]
6-10 <2> (<2> broad plateau, with very slight optima at pH 6 and 10 [3]; <2> nearly flat response to pH-variations [8]) [3, 8]
7 <3> (<3> broad [14]) [14]
8 <2> [6]

pH-Range

5-10 <1> (<1> maximal activity at pH 5 and about half-maximal activity at pH 10 [1]) [1]
5.8-10 <3> (<3> about half-maximal activity at pH 5.8 and pH 10, about 90% of maximal activity at pH 6.5 and pH 9 [14]) [14]
7-10 <2> (<2> about 80% of maximal activity at pH 7 and about 65% of maximal activity at pH 10 [6]) [6]

Temperature optimum (°C)

25 <2> (<2> assay at [7]) [7]
37 <1-4> (<1-4> assay at [1-6,8,10,12-17]) [1-6, 8, 10, 12-17]
50 <3> [9]

Temperature range (°C)

27-37 <1> (<1> moderately sensitive to variations of incubation temperature [1]) [1]
37-55 <3> (<3> about half-maximal activity at 37°C and 55°C [9]) [9]

4 Enzyme Structure

Molecular weight

56000 <2> (<2> gel filtration, equilibrium density and sucrose density gradient centrifugation [3,8]) [3, 8]
59300 <3> (<3> sedimentation equilibrium determination, PAGE, both in the presence of protease inhibitors [16]) [16]
60000 <1, 3> (<1,3> gel filtration [1,14,33]; <3> sedimentation equilibrium [38]) [1, 14, 33, 38]
61000 <3> (<3> gel filtration, glycerol gradient centrifugation [15]) [15]
68000 <3> [20]
70000 <3> (human cytoplasmic isozymes [10]) [10]

Subunits

? <3> (<3> x * 52000, SDS-PAGE, T-ALL, B-CLL, AML, CML cells, x * 30000, SDS-PAGE, AML, CML, B-ALL, MOLT-4 cells [39]) [39]
dimer <1, 3, 7> (<3> 2 * 30000, SDS-PAGE [15,33,38]; <1> 2 * 30000, SDS-PAGE [19]; <3> 2 * 30500, SDS-PAGE [14]; <7> 1 * 27200 + 1 * 29000, SDS-PAGE, heterodimers in quarternary structure [23]) [14, 15, 19, 23, 33, 38]
monomer <3> (<3> 1 * 52000, SDS-PAGE, mitochondrial isozyme [10]) [10, 16]

Posttranslational modification

phosphoprotein <3> (<3> inactivation of purified enzyme by protein phosphatases [26]; <3> activation of enzyme is mediated by phosphorylation [29]; <3> no changes in kinetic parameters of recombinant enzyme after treatment with protein kinases A or C [41]) [26, 29, 41]

5 Isolation/Preparation/Mutation/Application

Source/tissue

BALL-1 cell <3> [24]
BG-1 cell <3> [10]
CCRF-CEM cell <3> [24]
ML-1 cell <3> [10]
MOLT-4 cell <3> [13, 14, 18, 39]
MOLT-4F cell <3> [24]
P-815 cell <1> [5]
RPMI-8402 cell <3> [24]
carcinoma <3> [10]
carcinoma cell line <1> (<1> P815-neoplasm cell line [5]) [5]
kidney <1, 5, 6> [21]
leukemia cell <1-4> (<1> L1210 [1]; <3> ML-1 cells [10]; <3> from peripheral blood [10]; <3> from spleen [9,11,15,35,37]; <3> from acute myeloid leukemia (AML) patients [10,20]; <2-4> lymphocytes [17]; <3> leukemic T-lymphoblasts [12-16, 18]; <3> cell line MOLT-4 [13,14,18,39]; <3> various spontaneous leukemic cells [38]) [1, 9-18, 20, 35, 37, 39]
liver <1, 3-5> (<4> lymphomic [17]) [17, 19]
lymph node <4> (<4> normal and leukemic [17]) [17]
lymphoblast <3> (<3> T-lymphoblasts, cell lines MOLT 4F, CCRF, CEM and RPMI 8402, B-lymphoblasts cell lines BALL 1 and EBV (Epstein-Barr-virus) transformed B-lymphoblasts [24]) [24]
lymphocyte <3> (<3> from human leukemia patients [17]; <3> normal, acute myeloid leukemic and HL60 promyelocytic cells [26]; <3> tonsillar lymphocyte [28,43]; <3> normal cells and various leukemic cell lines [40]) [17, 26, 28, 29, 40, 41, 43, 45]
spleen <1, 3, 5> [19, 9, 11, 15]
thymus <2> [2-4, 6-8, 17]
Additional information <3> (<3> very low activity in normal spleens and placentas [15]) [15]

Localization

cytosol <1-3, 5, 6> (<3> 2 isozymes: I and II [10]; <3> native enzyme, perinuclear and cellular membrane area [32]) [1, 2, 5, 6, 8, 10, 19, 21, 32, 35]
mitochondrion <1-6> (<3> outer membrane [10]; <3> not [15]) [10, 17, 21]
nucleus <3> (<3> only when overexpressed [32]) [32]
viral nucleocapsid <3> (<3> Epstein-Barr-Virus [24]) [24]
Additional information <1, 5, 6> (<1,5,6> subcellular distribution [21]) [21]

Purification

<1> (partial [1]; spleen [19]) [1, 5, 19]
<2> (partial [2, 6]; preparative PAGE [8]; affinity chromatography [17]) [2, 3, 6, 8, 17]
<3> (mitochondrial isozyme (extracted with digitonin) [10]; partial [10, 20]; spleen [15]; affinity chromatography [10, 14-17]) [10, 13-17, 20]
<4> (affinity chromatography [17]) [17]
<7> (affinity chromatography [23]) [23]

Renaturation

<3> (after elution from Sephadex column, reactivation by incubation with 50 mM dithiothreitol and 1 mg/ml bovine serum albumin [43]) [43]

Cloning

<1, 3> [25]

Application

medicine <1, 3> (<3> direct inhibition of DNA polymerases by aphidicolin stimulated enzyme in normal, acute myeloid leukaemic and HL60 promyelocytic cells [26]; <1> recombinant enzyme in human A-549 lung carcinoma cells or murine NIH3T3 fibroblast cells, increase in cytotoxicity of cytosine arabinoside, 5'-aza-2'-deoxycytidine, decrease in toxicity of 2',2'-difluorodeoxycytidine [27]) [26, 27]

6 Stability

pH-Stability

6 <2> (<2> $t_{1/2}$: 2 min at 60°C [3]) [3]
8 <2> (<2> moderately stable, $t_{1/2}$: 7.9 min at 60°C [3]) [3]
10 <2> (<2> unstable at 60°C [3]) [3]

Temperature stability

22 <2> (<2> at least 24 h, in the presence of dithiothreitol [3]) [3]
37 <3> (<3> at least 6 h stable [9]) [9]
50 <1> (<1> 10 min, 10% loss of activity [1]) [1]
54 <3> (<3> inactivation [9]) [9]
60 <1, 2> (<1> 10 min, about 65% loss of activity [1]; <2> $t_{1/2}$: 7.9 min at pH 8, $t_{1/2}$: 2 min at pH 6, unstable at pH 10 [3,8]; <2> ATP, dCTP or glycerol stabilizes against heat inactivation [8]; <2> dCTP enhances heat stability at pH 6, dithiothreitol slightly, not deoxycytidine or other nucleosides [3]) [1, 3, 8]
Additional information <3> (<3> temperature stability of deoxycytidine kinase activity differs appreciably from deoxyadenosine kinase activity [9]) [9]

General stability information

<1>, bovine serum albumin does not stabilize [1]
<1>, protamine treatment, $MnCl_2$-treatment or ammonium sulfate fractionation inactivates during purification [1]

<2>, 2-mercaptoethanol stabilizes [3]
<2>, SH-reducing agents stabilize [8]
<3>, dTTP, ATP or deoxycytidine stabilizes [9]
<3>, dithiothreitol, $MgCl_2$, ATP, KCl and glycerol stabilize [14]
<3>, very unstable at low protein concentrations [14]
<1, 2>, dithiothreitol stabilizes [5, 8]
<2, 3>, bovine serum albumin stabilizes [8, 9]

Storage stability

<1>, -20°C, 3 months [1]
<1>, 4°C, at least 1 week [1]
<1>, glycerol, bovine serum albumin, 2-mercaptoethanol or EDTA does not enhance storage stability [1]
<2>, -20°C, in 5 mM DTT and 50% glycerol, stable [8]
<2>, -30°C, several months in the presence of 2-mercaptoethanol [3]
<2>, 4°C, if purified in the absence of 2-mercaptoethanol it loses most of its activity within 2 days, DTT restores [3]
<2>, dithiothreitol is more effective than 2-mercaptoethanol in stabilizing during storage [8]
<3>, -70°C, concentrated enzyme solution in Tris-buffer with $MgCl_2$ and mercaptoethanol, at least 12 months [15]
<3>, -85°C, in 1 mg/ml bovine serum albumin, 20% v/v glycerol, 0.2 M potassium phosphate, 2 mM ATP, 2.4 mM $MgCl_2$ and 0.025 M DTT, more than 12 months [14]
<3>, 4°C, concentrated enzyme solution in Tris-buffer with $MgCl_2$ and mercaptoethanol, at least 2 weeks [15]
<3>, 4°C, $t_{1/2}$: 7 days with and $t_{1/2}$: 72 h without bovine serum albumin [9]

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Thymidine kinase 2.7.1.75

1 Nomenclature

EC number
2.7.1.75 (transferred to EC 2.7.1.21)

Recommended name
thymidine kinase

Deoxyadenosine kinase 2.7.1.76

1 Nomenclature

EC number
2.7.1.76

Systematic name
ATP:deoxyadenosine 5'-phosphotransferase

Recommended name
deoxyadenosine kinase

Synonyms
dCyd kinase/dAdo kinase I <4> [6]
dGuo kinase/dAdo kinase II <4> [6]
deoxycytidine kinase/deoxyadenosine kinase, dCK/dAK <6> [12]
deoxyguanosine kinase/deoxyadenosine kinase, dGK/dAK <6> [12]
kinase, deoxyadenosine (phosphorylating)
purine-deoxyribonucleoside kinase
Additional information <1> (<1> may be identical with EC 2.7.1.113, i.e. deoxyguanosine kinase [1]; <2> enzyme may be identical with EC 2.7.1.20, i.e. adenosine kinase [2]; <3> enzyme may be identical with EC 2.7.1.20, i.e. adenosine kinase [14]) [1, 2, 14]

CAS registry number
37278-12-9

2 Source Organism

<1> *Bos taurus* (calf [1]) [1, 4, 8]
<2> *Rattus norvegicus* [2, 3]
<3> *Homo sapiens* (newborn [10]) [9, 10, 14]
<4> *Lactobacillus acidophilus* (strain R-26 [6]) [5-7]
<5> *Mus musculus* [11]
<6> *Lactobacillus acidophilus* (SwissProt-ID: Q59483, strain R-26 [12]) [12, 13, 16]
<7> *Mycoplasma pneumoniae* [15]
<8> *Mycoplasma mycoides* (subspecies mycoides SC [15]) [15]
<9> *Acholeplasma laidlawii* [15]
<10> *Mycoplasma arginini* [15]

3 Reaction and Specificity

Catalyzed reaction

ATP + deoxyadenosine = ADP + dAMP (<4> random sequential substrate addition [5])

Reaction type

phospho group transfer

Natural substrates and products

- **S** ATP + deoxyadenosine <1, 3, 5, 6> (<1> involved in biosynthesis of nucleoside monophosphates from preformed deoxyribonucleosides [4]; <3> key anabolic enzyme for activation of purine and pyrimidine deoxyribonucleosides as well as cytosine arabinoside and other anti-tumour drugs [9]; <5> involved in nucleoside metabolism [11]; <6> dGK/dAK plays an essential role in generating the dexyribonucleotide precursors, dGTP and dATP, for DNA metabolism [12]) (Reversibility: ? <1, 3, 5, 6> [4, 9, 11, 12]) [4, 9, 11, 12]
- **P** ADP + 5'-dAMP <1, 3, 5, 6> [4, 9, 11, 12]

Substrates and products

- **S** ATP + 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide <2> (<2> trivial name ribavirin [3]) (Reversibility: ? <2> [3]) [3]
- **P** ADP + 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide 5'-monophosphate <2> [3]
- **S** ATP + adenosine <2, 3> (<2> kinetic data suggest that different catalytic sites of deoxyadenosine kinase or a different enzyme are responsible for deoxyadenosine and adenosine kinase activities [3]) (Reversibility: ? <2, 3> [3, 14]) [3, 14]
- **P** ADP + AMP <2, 3> [3, 14]
- **S** ATP + cytidine <1> (<1> approx. 18% of activity with deoxyadenosine [8]) (Reversibility: ? <1> [8]) [8]
- **P** ADP + CMP <1> [8]
- **S** ATP + deoxyadenosine <1-10> (<1> transfers a phospho group from specific nucleoside 5'-triphosphate donors to 5'-position of deoxyadenosine [1]; <1> no activity with adenosine and guanosine [1]; <2> no activity with cytidine, uridine, guanosine, deoxyguanosine and thymidine [3]; <4> enzyme bears two separate but interacting active sites for deoxyadenosine and deoxycytidine kinase activity [5]; <4> enzyme exists in two heterodimeric complexes, complex I: deoxycytidine/deoxyadenosine kinase and complex II: deoxyguanosine/deoxyadenosine kinase [6]; <3> enzyme has both adenosine kinase and deoxyadenosine kinase activity [14]) (Reversibility: ? <1-10> [1-9,11,12,15]) [1-9, 11, 12, 14, 15]
- **P** ADP + dAMP <1-10> [1-9, 11, 12, 14, 15]
- **S** ATP + deoxycytidine <1, 3, 4, 8> (<3> deoxycytidine kinase is a multisubstrate enzyme that also phosphorylates deoxyadenosine, it exists in different conformational states with different substrate kinetic properties

[9]; <1> approx. 12% of activity with deoxyadenosine [8]) (Reversibility: ? <1, 3, 4, 8> [5, 8, 9, 15]) [5, 8, 9, 15]
P ADP + dCMP <1, 3, 4, 8> [5, 8, 9, 15]
S ATP + deoxyguanosine <1, 8> (<8> approx. 40% of activity with deoxyadenosine [15]) (Reversibility: ? <1, 8> [1, 8, 15]) [1, 8, 15]
P ADP + dGMP <1, 8> [1, 8, 15]
S CTP + deoxyadenosine <8> (Reversibility: ? <8> [15]) [15]
P CDP + dAMP <8> [15]
S GTP + deoxyadenosine <1, 8> (<8> 10% of activity with ATP [15]) (Reversibility: ? <1,8> [8,15]) [8, 15]
P GDP + dAMP <1, 8> [8, 15]
S TTP + deoxyadenosine <8> (Reversibility: ? <8> [15]) [15]
P TDP + dAMP <8> [15]
S UTP + deoxyadenosine <1, 8> (<1> 90% of activity with ATP [8]) (Reversibility: ? <1,8> [8,15]) [8, 15]
P UDP + dAMP <1, 8> [8, 15]
S dTTP + deoxyadenosine <1> (<1> 75% of activity with ATP [8]) (Reversibility: ? <1> [8]) [8]
P dTDP + dAMP <1> [8]

Inhibitors

CHAPS <3> (<3> at critical micelle concentration [9]) [9]
Hg^{2+} <1> (<1> 0.01 mM, 25% inhibition, 0.1 mM, almost complete inhibition [8]) [8]
adenine arabinoside <4> [5]
adenosine <2, 4> [3, 5]
dADP <1> (<1> 0.05 mM, 70% inhibition [1]) [1]
dAMP <1> (<1> 0.2 mM, 36% inhibition [1]) [1]
dATP <1, 4, 6> (<4> kinetic, mechanism, protects corresponding active site against trypsin inactivation [6]; <1> 0.05 mM, 68% inhibition [1]; <6> 0.5 mM, 90% inhibition of recombinant dAK, end product inhibition [12]) [1, 6, 12]
dCDP <1> (<1> 0.001 mM, 60% inhibition [1]) [1]
dCMP <1> (<1> 0.01 mM, 88% inhibition [1]) [1]
dCTP <1, 4> (<4> kinetic, mechanism, protects corresponding active site against trypsin inactivation, enhances inhibition by dATP [6]; <1> 0.0005 mM, 59% inhibition [1]) [1, 6]
dGDP <1> (<1> 0.2 mM, 46% inhibition [1]) [1]
dGMP <1> (<1> 0.5 mM, 32% inhibition [1]) [1]
dGTP <1, 4> (<4> kinetic, mechanism, protects corresponding active site against trypsin inactivation, enhances inhibition by dATP [6]; <1> 0.2 mM, 64% inhibition [1]) [1, 6]
deoxyadenosine <2> (<2> substrate ribavirin [3]) [3]
deoxyguanosine <1> (<1> substrate deoxyadenosine [1]) [1]
p-chloromercuribenzoate <2> (<2> 0.0007 mM, 50% inhibition of ribavirin and deoxadenosine kinase activity [3]) [3]
ribavirin <2> (<2> deoxyadenosine as substrate [3]) [3]

trypsin <4> (<4> substrates protect, end-products, i.e. dATP, dGTP or dCTP, protect corresponding active sites [6]) [6]
Additional information <1> (<1> not inhibited by adenosine [1]) [1]

Activating compounds

2'-O-cyclocytidine <4> (<4> activation [5]) [5]
5-fluoro-2'-deoxycytidine <4> (<4> 0.4 mM, 275% activation of deoxyadenosine phosphorylation, half-maximal activation at 0.028 mM [5]) [5]
cytosine arabinoside <4> (<4> activation [5]) [5]
deoxycytidine <4> (<4> 30 mM, 584% activation of deoxyadenosine phosphorylation, half-maximal activation at 0.1 mM [5]) [5]
deoxycytidine monophosphate <4> (<4> 10 mM, 409% activation of deoxyadenosine phosphorylation, half-maximal activation at 1.6 mM [5]) [5]
deoxyguanosine <4, 6> (<4> 5 mM, 330% activation of deoxyadenosine phosphorylation, half-maximal activation at 2.2 mM [5]; <6> 0.1 mM, 5fold activation of recombinant dAK [12]) [5, 12]

Metals, ions

Ca^{2+} <1> (<1> activation, can replace Mg^{2+} [8]) [8]
Mg^{2+} <1, 2, 4> (<1,2,4> required for activity [1,3,5,8]) [1, 3, 5, 8]
Mn^{2+} <1> (<1> activation, can replace Mg^{2+} [8]) [8]

Turnover number (min^{-1})

21.6 <8> (UTP, <8> pH 7.6, 37°C, recombinant enzyme [15]) [15]
36.6 <8> (deoxyguanosine, <8> pH 7.6, 37°C, recombinant enzyme [15]) [15]
37.2 <8> (CTP, <8> pH 7.6, 37°C, recombinant enzyme [15]) [15]
50.4 <8> (ATP, <8> pH 7.6, 37°C, recombinant enzyme [15]) [15]
73.8 <8> (deoxycytidine, <8> pH 7.6, 37°C, recombinant enzyme [15]) [15]
88.8 <8> (deoxyadenosine, <8> pH 7.6, 37°C, recombinant enzyme [15]) [15]
98.4 <8> (TTP, <8> pH 7.6, 37°C, recombinant enzyme [15]) [15]

Specific activity (U/mg)

0.06 <2> (<2> substrate ribavarin [3]) [3]
0.073 <1> [8]
Additional information <1, 5> (<1> 0.00006 mmol/ml min [1]; <5> activity increases 5.4fold between days 7 and 9 of mouse embryonic development [11]) [1, 11]

K_m-Value (mM)

0.0028 <6> (deoxyadenosine, <6> pH 8.0, 20°C, recombinant dAK [13]) [13]
0.0049 <3> (deoxyadenosine, <3> pH 7.4, 37°C, CCRF-CEM leukemia cells [14]) [14]
0.0075 <6> (deoxyadenosine, <6> pH 8.0, 20°C, R79K mutant dAK [13]) [13]
0.011 <8> (ATP, <8> pH 7.6, 37°C, recombinant enzyme [15]) [15]
0.012 <8> (deoxyadenosine, <8> pH 7.6, 37°C, recombinant enzyme [15]) [15]
0.021 <8> (deoxyguanosine, <8> pH 7.6, 37°C, recombinant enzyme [15]) [15]
0.022 <8> (CTP, <8> pH 7.6, 37°C, recombinant enzyme [15]) [15]

0.029 <3> (deoxyadenosine, <3> pH 7.4, 37°C, erythrocyte [14]) [14]
0.063 <8> (TTP, <8> pH 7.6, 37°C, recombinant enzyme [15]) [15]
0.11 <6> ($MgATP^{2-}$, <6> pH 8.0, 20°C, R79K:dGK mutant dAK [13]) [13]
0.15 <6> ($MgATP^{2-}$, <6> pH 8.0, 20°C, R79K mutant dAK [13]) [13]
0.18 <8> (UTP, <8> pH 7.6, 37°C, recombinant enzyme [15]) [15]
0.18 <8> (deoxycytidine, <8> pH 7.6, 37°C, recombinant enzyme [15]) [15]
0.44 <6> ($MgATP^{2-}$, <6> pH 8.0, 20°C, recombinant dAK [13]) [13]
0.5 <2> (deoxyadenosine, <2> pH 7.5, 37°C [3]) [3]
3.2 <2> (ribavirin, <2> pH 7.5, 37°C [3]) [3]
5 <1> (deoxyadenosine, <1> pH 7.8, 37°C [1]) [1]
Additional information <3, 4> (<3,4> kinetic study [5,9]) [5, 9]

K_i-Value (mM)

0.001 <6> (dATP, <6> pH 8.0, 20°C, competitive vs. ATP [13]) [13]
0.0014 <6> (dATP, <6> pH 8.0, 20°C, noncompetitive vs. deoxadenosine [13]) [13]
0.035 <2> (adenosine, <2> pH 7.5, 37°C, substrate deoxyadenosine [3]) [3]
0.057 <2> (adenosine, <2> pH 7.5, 37°C, substrate ribavirin [3]) [3]
0.97 <2> (deoxyadenosine, <2> pH 7.5, 37°C, substrate ribavirin [3]) [3]
2.5 <2> (ribavirin, <2> pH 7.5, 37°C, substrate deoxyadenosine [3]) [3]

pH-Optimum

7.5 <1, 2> (<1> broad optimum, 10% difference in activity between pH 6.5 and pH 8.5 [8]; <2> with deoxyadenosine and ribavirin as substrates [3]) [3, 8]

pH-Range

5.7-8.8 <1> (<1> approx. half-maximal activity at pH 5.7, approx. 80% of maximal activity at pH 8.8 [8]) [8]
6-9 <2> (<2> approx. 30% of maximal activity at pH 6.0 and pH 9.0, respectively [3]) [3]

Temperature optimum (°C)

20 <4> (<4> assay at [5,6]) [5, 6]
37 <1, 2> (<1,2> assay at [1,3,4,8]) [1, 3, 4, 8]
44 <3> [9]

Temperature range (°C)

27-50 <3> (<3> approx. half-maximal activity at 27°C and 50°C [9]) [9]

4 Enzyme Structure

Molecular weight

50000 <8> (<8> gel filtration [15]) [15]
63000 <1> (<1> gel filtration [8]) [8]

Subunits

dimer <4, 8> (<4> 1 * 27200 + 1 * 29000, heterodimers in quarternary structure, SDS-PAGE [6]; <8> 2 * 27000, His-tagged enzyme, SDS-PAGE [15]) [6, 15]
Additional information <4, 6> (<4> both kinase complex I, i.e. dCyd kinase/dAdo kinase I, and kinase complex II, i.e. dGuo kinase/dAdo kinase II, are heterodimers carrying distinct phosphorylation sites for two deoxynucleotide substrates on separate subunits [6]; <6> dGK/dAK is composed of 2 nonidentical subunits of approx. 26000 Da [12]) [6, 12]

5 Isolation/Preparation/Mutation/Application

Source/tissue

CCRF-CEM cell <3> [14]
brain <3> (<3> low activity [10]) [10]
embryo <5> [11]
erythrocyte <3> (<3> very low activity [14]) [14]
granulocyte <3> (<3> peripheral from adult, very low activity [10]) [10]
heart <3> (<3> low activity [10]) [10]
kidney <3> (<3> low activity [10]) [10]
liver <2, 3> [2, 3, 10]
lung <3> (<3> low activity [10]) [10]
lymphocyte <3> (<3> peripheral from adult [10]) [10]
small intestine <3> (<3> low activity [10]) [10]
spleen <3> (<3> leukemic [9]) [9, 10]
thymus <1, 3> (<3> highest activity [10]) [1, 4, 8, 10]

Localization

soluble <2> [3]

Purification

<1> (streptomycin sulfate, protamine sulfate, ammonium sulfate, Sephadex G-150 [1,8]; affinity chromatography on Cibacron Blue F3G-A [4]) [1, 4, 8]
<2> (ammonium sulfate, Sephadex G-100, DEAE-cellulose, Sephadex G-75 [3]) [3]
<4> (affinity chromatography [6,7]) [6, 7]
<6> (recombinant dGK/dAK, dATP-Sepharose affinity chromatography [12]) [12]
<8> (recombinant His-tagged enzyme, metal affinity chromatography [15]) [15]

Cloning

<6> (expression of dGK/dAK in Escherichia coli [12]) [12]
<8> (expression in Escherichia coli [15]) [15]

Engineering

D78A <6> (<6> mutation on both subunits of dGK/dAK, 0.2% of wild-type dAK activity [13]) [13]
D78E <6> (<6> mutation on both subunits of dGK/dAK, 0.2% of wild-type dAK activity [13]) [13]
D78N <6> (<6> mutation on both subunits of dGK/dAK, 0.2% of wild-type dAK activity [13]) [13]
D84A <6> (<6> mutation in dGK of dAK/dGK, increase of dAK activity [16]) [16]
D84E <6> (<6> mutation in dGK of dAK/dGK, increase of dAK activity [16]) [16]
D84N <6> (<6> mutation in dGK of dAK/dGK, increase of dAK activity [16]) [16]
R79K <6> (<6> 2 types of mutants, one bears the mutation on both the dAK and dGK subunits while the other, called R79K:dGK, has the mutation on the dGK subunit only, strong increase in dAK activity, activation of dAK by deoxyguanosine is nearly eliminated in the case of R79K:dGK, while for the tandem mutated R79K mutant a 30% inhibition is observed, inhibition by dATP is reduced [13]) [13]

6 Stability

Temperature stability

4 <3> (<3> half-life of deoxyadenosine kinase activity: 1.5 days [9]) [9]
37 <3> (<3> $t_{1/2}$: 2 h, 1 mM ATP, 0.5 mM dTTP, 1 mM deoxyadenosine or 0.25 3-[(3-cholamidopropyl)dimethylamino]-1-propanesulfonate stabilize [9]) [9]
42 <2> (<2> first-order rate constant of inactivation: in the absence of dithiothreitol, 14.2/min, in the presence of 10 mM dithiothreitol, 6.82, substrate ribavirin [3]) [3]
48 <3> (<3> inactivation of deoxyadenosine kinase activity [9]) [9]
50 <3> (<3> 80% loss of activity [9]) [9]
Additional information <2, 3> (<3> temperature stability of deoxyadenosine kinase activity differs appreciably from deoxycytidine kinase activity [9]; <2> dithiothreitol protects against heat inactivation [3]) [3, 9]

General stability information

<1>, glycerol, 50%, or dithiothreitol stabilizes [1]
<2>, dithiothreitol protects against heat inactivation, glycerol and dithiothreitol increase stability during storage [3]
<3>, stability of deoxyadenosine kinase activity differs appreciably from deoxycytidine kinase activity [9]
<4>, dATP stabilizes [6]

Storage stability

<1>, -20°C, 60% glycerol, at least 3 months, slight loss of activity [8]
<3>, 4°C, $t_{1/2}$: 84 h with and $t_{1/2}$: 32 h without addition of bovine serum albumin [9]

<3>, ATP, dTTP, deoxyadenosine or CHAPS stabilizes at 4°C [9]
<4>, dATP stabilizes during storage [6]

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Nucleoside phosphotransferase 2.7.1.77

1 Nomenclature

EC number
2.7.1.77

Systematic name
nucleotide:nucleoside 5'-phosphotransferase

Recommended name
nucleoside phosphotransferase

Synonyms
NPT
nonspecific nucleoside phosphotransferase
nucleotide:3'-deoxynucleoside 5'-phosphotransferase
phosphotransferase, nucleoside

CAS registry number
9055-37-2

2 Source Organism

<1> *Daucus carota* [1, 2, 3]
<2> *Erwinia herbicola* [4]
<3> *Hordeum vulgare* [5, 6, 7]
<4> *Tetrahymena pyriformis* (ST [8]) [8]
<5> *Gallus gallus* [9, 10, 11]
<6> *Lupinus luteus* [12]
<7> *Morganella morganii* (NCIMB10466 [13]) [13]
<8> *Leishmania tropica* [14]

3 Reaction and Specificity

Catalyzed reaction
a nucleotide + a 2'-deoxynucleoside = a nucleoside + a 2'-deoxynucleoside 5'-monophosphate (<3> ping pong kinetics [5])

Reaction type
phospho group transfer

Substrates and products

- **S** (2',3')-uridylic acid + uridine <1> (<1> about 10% of the activity with phenyl phosphate [1]) (Reversibility: ? <1> [1]) [1]
- **P** uridine + UMP
- **S** 3'-AMP + adenosine <6> (Reversibility: ? <6> [12]) [12]
- **P** AMP + adenosine
- **S** 3'-AMP + cytidine <6> (Reversibility: ? <6> [12]) [12]
- **P** CMP + adenosine
- **S** 3'-AMP + guanosine <6> (Reversibility: ? <6> [12]) [12]
- **P** GMP + adenosine
- **S** 3'-AMP + inosine <6> (Reversibility: ? <6> [12]) [12]
- **P** IMP + adenosine
- **S** 3'-AMP + uridine <1, 6> (<1> 70% of the activity with phenyl phosphate [1]) (Reversibility: ? <1, 6> [1, 12]) [1, 12]
- **P** adenosine + UMP
- **S** 3'-CMP + thymidine <2> (<2> 20% of the activity with 5'-dAMP or 5'-dTMP [4]) (Reversibility: ? <2> [4]) [4]
- **P** cytosine + TMP
- **S** 3'-TMP + thymidine <2> (<2> 19% of the activity with 5'-dAMP or 5'-dTMP [4]) (Reversibility: ? <2> [4]) [4]
- **P** thymidine + TMP
- **S** 3'-dAMP + thymidine <2> (<2> 56% of the activity with 5'-dAMP or 5'-dTMP [4]) (Reversibility: ? <2> [4]) [4]
- **P** TMP + 2'-deoxyadenosine
- **S** 4-nitrophenyl phosphate + 1-(β-D-ribofuranosyl)-2(1H)pyridone <3> (Reversibility: ? <3> [6]) [6]
- **P** 4-nitrophenol + 1-(β-D-ribofuranosyl)-2(1H)pyridone 5'-phosphate
- **S** 4-nitrophenyl phosphate + 1-(β-D-ribofuranosyl)-4(1H)pyridone <3> (Reversibility: ? <3> [6]) [6]
- **P** 4-nitrophenol + 1-(β-D-ribofuranosyl)-4(1H)pyridone 5'-phosphate
- **S** 4-nitrophenyl phosphate + 2'-deoxy-2'-methyluridine <3> (Reversibility: ? <3> [6]) [6]
- **P** 4-nitrophenol + 2'-deoxy-2'-methyluridine 5'phosphate
- **S** 4-nitrophenyl phosphate + 2'-O-methylcytidine <3> (Reversibility: ? <3> [6]) [6]
- **P** 4-nitrophenol + 2'-O-methylcytidine 5'-phosphate
- **S** 4-nitrophenyl phosphate + 2'-bromo-2'-deoxyuridine <3> (Reversibility: ? <3> [6]) [6]
- **P** 4-nitrophenol + 2'-bromo-2'-deoxyuridine 5'-phosphate
- **S** 4-nitrophenyl phosphate + 2'-deoxyadenosine <2, 8> (Reversibility: ? <2,8> [4,14]) [4, 14]
- **P** 5'-dAMP + 3'-dAMP + nitrophenol <2> (<2> 5'- and 3'-nucleotide products in the approximate ratio of 2:1 [4]) [4]

S 4-nitrophenyl phosphate + 2'-deoxyadenosine <3> (Reversibility: ? <3> [6]) [6]
P 4-nitrophenol + 2'-deoxyadenosine 5'-phosphate
S 4-nitrophenyl phosphate + 2'-deoxycytidine <8> (Reversibility: ? <8> [14]) [14]
P 4-nitrophenol + 2'-deoxycytidine 5'-phosphate
S 4-nitrophenyl phosphate + 2'-deoxyguanosine <8> (Reversibility: ? <8> [14]) [14]
P 4-nitrophenol + 2'-deoxyguanosine 5'-phosphate
S 4-nitrophenyl phosphate + 2'-deoxyinosine <8> (Reversibility: ? <8> [14]) [14]
P 4-nitrophenol + 2'-deoxyinosine 5'-phosphate
S 4-nitrophenyl phosphate + 2-thiouridine <3> (Reversibility: ? <3> [6]) [6]
P 4-nitrophenol + 2-thiouridine 5'phosphate
S 4-nitrophenyl phosphate + 3'-deoxy-3'-methyluridine <3> (Reversibility: ? <3> [6]) [6]
P 4-nitrophenol + 3'-deoxy-3'-methyluridine 5'-phosphate
S 4-nitrophenyl phosphate + 3'-O-methylcytidine <3> (Reversibility: ? <3> [6]) [6]
P 4-nitrophenol + 3'-O-methylcytidine 5'phosphate
S 4-nitrophenyl phosphate + 3'-deoxyadenosine <8> (Reversibility: ? <8> [14]) [14]
P 4-nitrophenol + 3'-deoxyadenosine 5'-phosphate
S 4-nitrophenyl phosphate + 3'-deoxyadenosine <3> (Reversibility: ? <3> [6]) [6]
P 4-nitrophenol + 3'-deoxyadenosine 5'phosphate
S 4-nitrophenyl phosphate + 3'-deoxyinosine <8> (Reversibility: ? <8> [14]) [14]
P 4-nitrophenol + 3'-deoxyinosine 5'-phosphate
S 4-nitrophenyl phosphate + 3'-fluoroinosine <8> (Reversibility: ? <8> [14]) [14]
P 4-nitrophenol + 3'-fluoro-IMP
S 4-nitrophenyl phosphate + 3-(β-D-ribofuranosyl)uric acid <3> (Reversibility: ? <3> [6]) [6]
P 4-nitrophenol + 3-(β-D-ribofuranosyl)uric acid 5'-phosphate
S 4-nitrophenyl phosphate + 5-bromouridine <3> (Reversibility: ? <3> [6]) [6]
P 4-nitrophenol + 5-bromouridine 5'phosphate
S 4-nitrophenyl phosphate + 5-chlorouridine <3> (Reversibility: ? <3> [6]) [6]
P 4-nitrophenol + 5-chlorouridine 5'-phosphate
S 4-nitrophenyl phosphate + 5-hydroxyuridine <3> (Reversibility: ? <3> [6]) [6]
P 4-nitrophenol + 5-hydroxyuridine 5'phosphate

S 4-nitrophenyl phosphate + 5-iodouridine <3> (Reversibility: ? <3> [6]) [6]
P 4-nitrophenol + 5-iodouridine 5'phosphate
S 4-nitrophenyl phosphate + 6-chloro-9-(β-D-ribofuranosyl)purine <3> (Reversibility: ? <3> [6]) [6]
P 4-nitrophenol + 6-chloro-9-(β-D-ribofuranosyl)purine 5'-phosphate
S 4-nitrophenyl phosphate + 7-methylguanosine <3> (Reversibility: ? <3> [6]) [6]
P 4-nitrophenol + 7-methylguanosine 5'-phosphate
S 4-nitrophenyl phosphate + 9-(β-D-ribofuranosyl)purine <3> (Reversibility: ? <3> [6]) [6]
P 4-nitrophenol + 9-(β-D-ribofuranosyl)purine 5'-phosphate
S 4-nitrophenyl phosphate + adenosine <2, 3, 6, 8> (Reversibility: ? <2, 3, 6, 8> [4, 6, 12, 14]) [4, 6, 12, 14]
P 5'-AMP + 4-nitrophenol <2> [4]
S 4-nitrophenyl phosphate + β-pseudouridine <3> (Reversibility: ? <3> [6]) [6]
P 4-nitrophenol + β-pseudouridine 5'-phosphate
S 4-nitrophenyl phosphate + cytidine <8> (Reversibility: ? <8> [14]) [14]
P 4-nitrophenol + CMP
S 4-nitrophenyl phosphate + deazauridine <3> (Reversibility: ? <3> [6]) [6]
P 4-nitrophenol + deazauridine 5'-phosphate
S 4-nitrophenyl phosphate + formycin B <3> (Reversibility: ? <3> [6]) [6]
P 4-nitrophenol + ?
S 4-nitrophenyl phosphate + guanosine <8> (Reversibility: ? <8> [14]) [14]
P 4-nitrophenol + GMP
S 4-nitrophenyl phosphate + inosine <3, 6, 8> (Reversibility: ? <3, 6, 8> [6, 12, 14]) [6, 12, 14]
P 4-nitrophenol + IMP
S 4-nitrophenyl phosphate + thymidine <8> (Reversibility: ? <8> [14]) [14]
P 4-nitrophenol + TMP
S 4-nitrophenyl phosphate + thymidine <2> (<2> 24% of the activity with 5'-dAMP or 5'-dTMP [4]) (Reversibility: ? <2> [4]) [4]
P 4-nitrophenol + TMP
S 4-nitrophenyl phosphate + thymidine 3'-[(4-nitrophenyl)-phosphate] <3> (Reversibility: ? <3> [6]) [6]
P 4-nitrophenol + thymidine 3'-[(4-nitrophenyl)-phosphate] 5'-phosphate
S 4-nitrophenyl phosphate + uridine <3, 6, 7, 8> (<7> 115% of the activity with diphosphate [13]) (Reversibility: ? <3, 6, 7, 8> [6, 12, 13, 14]) [6, 12, 13, 14]
P 4-nitrophenol + 5'-UMP <3> [6]
S 4-nitrophenyl phosphate + xanthosine <3> (Reversibility: ? <3> [6]) [6]
P 4-nitrophenol + XMP
S 5'-AMP + adenosine <6> (Reversibility: ? <6> [12]) [12]
P AMP + adenosine
S 5'-AMP + cytidine <6> (Reversibility: ? <6> [12]) [12]
P CMP + adenosine

S 5'-AMP + guanosine <6> (Reversibility: ? <6> [12]) [12]
P GMP + adenosine
S 5'-AMP + inosine <6> (Reversibility: ? <6> [12]) [12]
P IMP + adenosine
S 5'-AMP + thymidine <2> (<2> 72% of the activity with 5'-dAMP or 5'-dTMP [4]) (Reversibility: ? <2> [4]) [4]
P adenosine + TMP
S 5'-AMP + uridine <6> (Reversibility: ? <6> [12]) [12]
P UMP + adenosine
S 5'-CMP + adenosine <6> (Reversibility: ? <6> [12]) [12]
P AMP + cytidine
S 5'-CMP + cytidine <6> (Reversibility: ? <6> [12]) [12]
P CMP + cytidine
S 5'-CMP + guanosine <6> (Reversibility: ? <6> [12]) [12]
P GMP + cytidine
S 5'-CMP + inosine <6> (Reversibility: ? <6> [12]) [12]
P IMP + cytidine
S 5'-CMP + thymidine <2> (<2> 92% of the activity with 5'-dAMP or 5'-dTMP [4]) (Reversibility: ? <2> [4]) [4]
P cytidine + TMP
S 5'-CMP + uridine <6> (Reversibility: ? <6> [12]) [12]
P UMP + cytidine
S 5'-GMP + adenosine <6> (Reversibility: ? <6> [12]) [12]
P AMP + guanosine
S 5'-GMP + cytidine <6> (Reversibility: ? <6> [12]) [12]
P CMP + guanosine
S 5'-GMP + inosine <6> (Reversibility: ? <6> [12]) [12]
P IMP + guanosine
S 5'-GMP + thymidine <2> (<2> 61% of the activity with 5'-dAMP or 5'-dTMP [4]) (Reversibility: ? <2> [4]) [4]
P TMP + guanosine
S 5'-GMP + uridine <6> (Reversibility: ? <6> [12]) [12]
P UMP + guanosine
S 5'-IMP + adenosine <6> (Reversibility: ? <6> [12]) [12]
P AMP + inosine
S 5'-IMP + cytidine <6> (Reversibility: ? <6> [12]) [12]
P CMP + inosine
S 5'-IMP + guanosine <6> (Reversibility: ? <6> [12]) [12]
P GMP + inosine
S 5'-IMP + inosine <6> (Reversibility: ? <6> [12]) [12]
P IMP + inosine
S 5'-IMP + uridine <6> (Reversibility: ? <6> [12]) [12]
P UMP + inosine
S 5'-UMP + adenosine <6> (Reversibility: ? <6> [12]) [12]
P AMP + uridine

S 5'-UMP + cytidine <6> (Reversibility: ? <6> [12]) [12]
P CMP + uridine
S 5'-UMP + guanosine <6> (Reversibility: ? <6> [12]) [12]
P GMP + uridine
S 5'-UMP + inosine <6> (Reversibility: ? <6> [12]) [12]
P IMP + uridine
S 5'-UMP + uridine <6> (Reversibility: ? <6> [12]) [12]
P UMP + uridine
S 5'-adenylic acid + uridine <1> (<1> 70% of the activity with phenyl phosphate [1]) (Reversibility: ? <1> [1]) [1]
P adenosine + UMP
S 5'-dAMP + thymidine <2> (Reversibility: ? <2> [4]) [4]
P 2'-deoxyadenosine + TMP
S 5'-dCMP + thymidine <2> (<2> 46% of the activity with 5'-dAMP or 5'-dTMP [4]) (Reversibility: ? <2> [4]) [4]
P 2'-deoxycytidine + TMP
S 5'-dGMP + thymidine <2> (<2> 65% of the activity with 5'-dAMP or 5'-dTMP [4]) (Reversibility: ? <2> [4]) [4]
P TMP + 2'-deoxyguanosine
S 5'-dTMP + thymidine <2> (Reversibility: ? <2> [4]) [4]
P 2'-deoxythymidine + TMP
S ADP + inosine <7> (<7> 26% of the activity with diphosphate [13]) (Reversibility: ? <7> [13]) [13]
P AMP + IMP
S ADP + thymidine <2> (<2> 50% of the activity with 5'-dAMP or 5'-dTMP [4]) (Reversibility: ? <2> [4]) [4]
P TMP + AMP
S AMP + inosine <7> (<7> 62% of the activity with diphosphate [13]) (Reversibility: ? <7> [13]) [13]
P adenosine + IMP
S AMP + thymidine <4> (Reversibility: ? <4> [8]) [8]
P adenosine + TMP
S ATP + inosine <7> (<7> 13% of the activity with diphosphate [13]) (Reversibility: ? <7> [13]) [13]
P ADP + IMP
S ATP + thymidine <2> (<2> 18% of the activity with 5'-dAMP or 5'-dTMP [4]) (Reversibility: ? <2> [4]) [4]
P ADP + TMP
S acetylphosphate + inosine <7> (<7> 233% of the activity with diphosphate [13]) (Reversibility: ? <7> [13]) [13]
P acetate + IMP
S adenosine 5'-phosphorothionate + H_2O <3> (Reversibility: ? <3> [6]) [6]
P ?
S β-glycerophosphate + uridine <1> (<1> about 15% of the activity with phenyl phosphate [1]) (Reversibility: ? <1> [1]) [1]
P glycerol + UMP

S carbamoylphosphate + inosine <7> (<7> 276% of the activity with diphosphate [13]) (Reversibility: ? <7> [13]) [13]
P carbamate + IMP
S diphosphate + adenosine <7> (<7> 35% of the activity with inosine [13]) (Reversibility: ? <7> [13]) [13]
P phosphate + 5'-AMP <7> [13]
S diphosphate + cytidine <7> (<7> 29% of the activity with inosine [13]) (Reversibility: ? <7> [13]) [13]
P phosphate + 5'-AMP <7> [13]
S diphosphate + guanosine <7> (<7> 35% of the activity with inosine [13]) (Reversibility: ? <7> [13]) [13]
P phosphate + 5'-GMP <7> [13]
S diphosphate + inosine <7> (Reversibility: ? <7> [13]) [13]
P phosphate + 5'-IMP <7> [13]
S diphosphate + uridine <7> (<7> 47% of the activity with inosine [13]) (Reversibility: ? <7> [13]) [13]
P phosphate + 5'-UMP <7> [13]
S diphosphate + xanthosine <7> (<7> 54% of the activity with inosine [13]) (Reversibility: ? <7> [13]) [13]
P phosphate + 5'-XMP <7> [13]
S glucose 6-phosphate + inosine <7> (<7> 95% of the activity with diphosphate [13]) (Reversibility: ? <7> [13]) [13]
P D-glucose + IMP
S *p*-nitrophenylphosphate + inosine <7> (<7> 115% of the activity with diphosphate [13]) (Reversibility: ? <7> [13]) [13]
P ? + IMP p-nitrophenol
S pAp + thymidine <2> (<2> 30% of the activity with 5'-dAMP or 5'-dTMP [4]) (Reversibility: ? <2> [4]) [4]
P adenosine 3'-phosphate + TMP
S phenyl phosphate + adenosine <1> (Reversibility: ? <1> [1,2]) [1, 2]
P AMP + phenol
S phenyl phosphate + cytidine <1> (Reversibility: ? <1> [1,2]) [1, 2]
P CMP + phenol
S phenyl phosphate + deoxyadenosine <1> (Reversibility: ? <1> [1,2]) [1, 2]
P dAMP + phenol
S phenyl phosphate + deoxycytidine <1> (Reversibility: ? <1> [1,2]) [1, 2]
P dCMP + phenol
S phenyl phosphate + deoxyguanosine <1> (Reversibility: ? <1> [1,2]) [1, 2]
P dGMP + phenol
S phenyl phosphate + deoxyuridine <1> (Reversibility: ? <1> [1,2]) [1, 2]
P dUMP + phenol
S phenyl phosphate + guanosine <1> (Reversibility: ? <1> [1,2]) [1, 2]
P GMP + phenol
S phenyl phosphate + thymidine <1> (Reversibility: ? <1> [1,2]) [1, 2]
P TMP + phenol

S phenyl phosphate + uridine <1> (Reversibility: ? <1> [1,2]) [1, 2]
P 5'-UMP + phenol
S phenylphosphate + inosine <7> (<7> 164% of the activity with diphosphate [13]) (Reversibility: ? <7> [13]) [13]
P IMP + phenol
S D-ribose 5'-phosphate + thymidine <2> (<2> 13% of the activity with 5'-dAMP or 5'-dTMP [4]) (Reversibility: ? <2> [4]) [4]
P D-ribose + TMP
S D-ribose 5-phosphate + uridine <1> (<1> about 30% of the activity with phenyl phosphate [1]) (Reversibility: ? <1> [1]) [1]
P D-ribose + UMP
S tripolyphosphate + inosine <7> (<7> 13% of the activity with diphosphate [13]) (Reversibility: ? <7> [13]) [13]
P ? + IMP
S Additional information <1, 3, 5> (<1>, the enzyme also exhibits phosphatase activity [1]; <1,5> protein carries both transfer and hydrolytic functions [1,11]) [1, 2, 6, 11]
P ?

Inhibitors

2,4-dinitrofluorobenzene <1> [3]
Ag^+ <7> [13]
AgCl <7> (<7> 1 mM, 91% inhibition [13]) [13]
Ca^{2+} <1, 2, 3> (<3> 2 mM, at least 80% inhibition of transphosphorylation [5]) [1, 2, 4, 5]
Cd^{2+} <3> (<3> 2 mM, at least 80% inhibition of transphosphorylation [5]) [5]
Co^{2+} <2> [4]
Cu^{2+} <2, 3, 7> (<3> 2 mM, at least 80% inhibition of transphosphorylation [5]) [4, 5, 13]
$CuSO_4$ <7> (<7> 1 mM, 23% inhibition [13]) [13]
EDTA <1, 2, 3> (<1> 0.625 mM, inhibition is reversed by 5 mM $MgCl_2$ [1]; <2> 1 mM, 53% inhibition of transferase activity [4]; <3> 2 mM, 85% inhibition. 95% inhibition at 10 mM [5]; <3> cAMP and arsenate can reduce the rate of inactivation [7]) [1, 2, 4, 5, 7]
Fe^{2+} <1> [1, 2]
Fe^{3+} <1> [1, 2]
Hg^{2+} <3, 7> (<3> 2 mM, at least 80% inhibition of transphosphorylation [5]; <7> 1 mM $HgCl_2$, 18% inhibition [13]) [5, 13]
Mn^{2+} <1> [1, 2]
NaF <1> (<1> 0.25 M, phosphatase is inhibited by less than 10% [2]) [2]
PCMB <7> (<7> 1 mM, 17% inhibition [13]) [13]
Pb^{2+} <1> [1, 2]
Zn^{2+} <2, 3> (<3> 2 mM, 20% inhibition of transphosphorylation [5]) [4, 5]
arsenate <3> (<3> strong competitive inhibitor [7]) [7]
cAMP <3> (<3> strong competitive inhibitor [7]) [7]

cysteine <1> (<1> 2.5 mM, depresses both transferase and phosphatase activity by about 25% [1]) [1, 2]
glutathione <1> (<1> 2.5 mM, depresses both transferase and phosphatase activity by about 25% [1]) [1, 2]
iodine <1> [2, 3]
iodoacetate <7> (<7> 1 mM, 41% inhibition [13]) [13]
phosphate <5> (<5> competitive inhibitor of the associated enzyme form [11]) [11]
urea <1> (<1> 6 M, complete inactivation [3]) [3]
uridine <1> (<1> inhibitor of phosphatase reaction [2]) [2]

Activating compounds

NaF <1> (<1> 0.25 M, enhances activity by about 45% [2]) [2]
acetone <3> (<3> 25%, 2.45fold stimulation [7]) [7]
acetonitrile <3> (<3> 8%, 2.4fold stimulation [7]) [7]
dimethyl formamide <3> (<3> 5%, 1.8fold, stimulation [7]) [7]
dimethyl sulfoxide <3> (<3> 20%, 1.8fold stimulation [7]) [7]
glycerol <3> (<3> 60%, 1.7fold stimulation [7]) [7]
methanol <3> (<3> 30%, 2.3fold stimulation [7]) [7]

Metals, ions

Ba^{2+} <3> (<3> 2 mM, activity with AMP and deoxythymidine is enhanced about 50%, no absolute requirement [5]) [5]
Co^{2+} <1, 3, 5> (<1> enhances phosphatase activity considerably, but transferase activity to a small extent [1]; <3> 2 mM, activity with AMP and deoxythymidine is enhanced about 50%, no absolute requirement [5]; <3> can restore activity of the metal-free apoenzyme [7]; <5> Mg^{2+}, Mn^{2+} or Co^{2+} required [11]) [1, 5, 7, 11]
Cu^{2+} <1> (<1> enhances phosphatase activity considerably, but transferase activity to a small extent [1]) [1]
Cu^{2+} <3> (<3> can restore activity of the metal-free apoenzyme to a minor extent [7]) [7]
Fe^{2+} <3> (<3> can restore activity of the metal-free apoenzyme to a minor extent [7]) [7]
Mg^{2+} <1, 2, 3, 5> (<1> enhances phosphatase activity considerably, but transferase activity to a small extent [1]; <3> 2 mM, with 0.5 mM AMP and deoxythymidine, 50% stimulation [5]; <2> 10 mM $MgCl_2$, 20% enhancement of transferase activity with *p*-nitrophenol as the donor, no absolute requirement for metal ion [4]; <3> 2 mM, activity with AMP and deoxythymidine is enhanced about 50%, no absolute requirement [5]; <3> contains one Mg^{2+} per dimeric enzyme molecule, the metal-free apoenzyme is inactive [7]; <5> Mg^{2+}, Mn^{2+} or Co^{2+} required [11]) [1, 4, 5, 7, 11]
Mn^{2+} <5> (<5> Mg^{2+}, Mn^{2+} or Co^{2+} required [11]) [11]
Ni^{2+} <3> (<3> can restore activity of the metal-free apoenzyme to a minor extent [7]) [7]
Zn^{2+} <3> (<3> can restore activity of the metal-free apoenzyme to a minor extent [7]) [7]

Turnover number (min^{-1})

24 <3> (adenosine-5'-phosphorothionate, <3> pH 5.5, phosphohydrolase activity [6]) [6]
156 <3> (1-naphthyl phosphate, <3> pH 5.5, phosphohydrolase activity [6]) [6]
408 <3> (3'-AMP, <3> pH 5.5, phosphohydrolase activity [6]) [6]
720 <3> (3'-UMP, <3> pH 5.5 [6]) [6]
1800 <3> (2-naphthyl phosphate, <3> pH 5.5, phosphohydrolase activity [6]) [6]
2460 <3> (5'-AMP, <3> pH 5.5, phosphohydrolase activity [6]) [6]
4020 <3> (5'-UMP, <3> pH 5.5, phosphohydrolase activity [6]) [6]
4620 <3> (4-nitrophenyl phosphate, <3> pH 5.5, phosphohydrolase activity [6]) [6]
Additional information <3> [5]

Specific activity (U/mg)

0.0021 <5> [9]
1.1 <6> [12]
4.7 <7> [13]
12 <2> [4]
32 <1> [2]
123 <3> [6]
130 <8> [14]
180 <3> [5]
Additional information <1, 5> [1, 3, 11]

K_m-Value (mM)

0.0057 <8> (2'-deoxyinosine, <8> pH 7.5, 37°C, reaction with 4-nitrophenyl phosphate [14]) [14]
0.0059 <8> (2'-deoxyadenosine, <8> pH 7.5, 37°C, reaction with 4-nitrophenyl phosphate [14]) [14]
0.0059 <8> (inosine, <8> pH 7.5, 37°C, reaction with 4-nitrophenyl phosphate [14]) [14]
0.0061 <8> (adenosine, <8> pH 7.5, 37°C, reaction with 4-nitrophenyl phosphate [14]) [14]
0.0063 <8> (2'-deoxyguanosine, <8> pH 7.5, 37°C, reaction with 4-nitrophenyl phosphate [14]) [14]
0.0064 <8> (guanosine, <8> pH 7.5, 37°C, reaction with 4-nitrophenyl phosphate [14]) [14]
0.0066 <8> (3'-fluoroinosine, <8> pH 7.5, 37°C, reaction with 4-nitrophenyl phosphate [14]) [14]
0.0071 <8> (3'-deoxyadenosine, <8> pH 7.5, 37°C, reaction with 4-nitrophenyl phosphate [14]) [14]
0.0084 <8> (3'-deoxyinosine, <8> pH 7.5, 37°C, reaction with 4-nitrophenyl phosphate [14]) [14]
0.014 <8> (thymidine, <8> pH 7.5, 37°C, reaction with 4-nitrophenyl phosphate [14]) [14]

0.0153 <8> (2'-deoxycytidine, <8> pH 7.5, 37°C, reaction with 4-nitrophenyl phosphate [14]) [14]
0.0167 <8> (uridine, <8> pH 7.5, 37°C, reaction with 4-nitrophenyl phosphate [14]) [14]
0.0169 <8> (cytidine, <8> pH 7.5, 37°C, reaction with 4-nitrophenyl phosphate [14]) [14]
0.018 <3> (5'-AMP, <3> pH 5.5 [6]) [6]
0.023 <3> (3'-AMP, <3> pH 5.5 [6]) [6]
0.087 <3> (deoxyadenosine, <3> pH 5.0, reaction with AMP [5]) [5]
0.09 <3> (5'-UMP, <3> pH 5.5 [6]) [6]
0.092 <3> (3'-UMP, <3> pH 5.5 [6]) [6]
0.22 <3> (adenosine 5'-phosphorothionate, <3> pH 5.5 [6]) [6]
0.26 <3> (2-naphthyl phosphate, <3> pH 5.5 [6]) [6]
0.28 <3> (4-nitrophenyl phosphate, <3> pH 5.5 [6]) [6]
0.4 <6> (5'-AMP, <6> pH 8.0, 30°C [12]) [12]
0.4 <6> (inosine, <6> pH 8.0, 30°C [12]) [12]
0.412 <3> (deoxyguanosine, <3> pH 5.0, reaction with AMP [5]) [5]
0.44 <3> (1-naphthyl phosphate) [6]
0.73 <7> (diphosphate, <7> pH 5.0 [13]) [13]
0.89 <3> (deoxythymidine, <3> pH 5.0, reaction with AMP [5]) [5]
2.13 <3> (guanosine, <3> pH 5.0, reaction with AMP [5]) [5]
3.5 <1> (phenylphosphate, <1> phosphotransferase reaction with uridine [2]) [2]
3.5 <1> (uridine, <1> phosphotransferase reaction with phenyl phosphate [2]) [2]
3.93 <3> (uridine, <3> pH 5.0, reaction with AMP [5]) [5]
4.15 <3> (deoxycytidine, <3> pH 5.0, reaction with AMP [5]) [5]
6.39 <3> (cytidine, <3> pH 5.0, reaction with AMP [5]) [5]
122 <7> (inosine, <7>, pH 5.0 [13]) [13]

K_i-Value (mM)

Additional information <3> (<3> inhibitors of phosphohydrolase activity [6]) [6]

pH-Optimum

5 <1> (<1> reaction with phenylphosphate and uridine or deoxycytidine [1]) [1, 2, 3]
5.2 <7> (<7> phosphotransferase activity [13]) [13]
5.5 <3> (<3> phosphatase activity [5]) [5, 7]
6 <7> (<7> dephosphorylation [13]) [13]
6.5 <3> (<3> transferase activity [5]) [5]
8 <6> [12]
8.8 <5> (<5> associated and dissociated enzyme form [11]) [11]

pH-Range

4-6.5 <7> (<7> pH 4.0: 30% of maximal activity, pH 6.5: about 60% of maximal activity, phosphotransferase activity [13]) [13]
4-7 <1> (<1> pH 4.0: about 25% of maximal activity in the reaction with phenyl phosphate and uridine, about 40% of maximal activity with phenyl phosphate and deoxycytidine, pH 7.0: about 20% of maximal activity in the reaction with phenyl phosphate and uridine, about 50% of maximal activity with phenyl phosphate and deoxycytidine [1]) [1]
4.5-8.5 <3> (<3> pH 4.5: about 65% of maximal activity, pH 8.5: about 50% of maximal activity [5]) [5]

Temperature optimum (°C)

30 <7> [13]

4 Enzyme Structure

Molecular weight

40000 <8> (<8> gel filtration [14]) [14]
44000 <1> (<1> gel filtration, equilibrium sedimentation, sucrose density gradient centrifugation [2,3]) [2, 3]
53400 <3> (<3> non-denaturing PAGE [5]) [5]
55000 <3> (<3> gel filtration [6]) [6]
68000 <2> (<2> sucrose density gradient sedimentation [4]) [4]
72000 <6> (<6> gel filtration [12]) [12]
160000 <7> (<7> gel filtration [13]) [13]
Additional information <1, 2> (<1> the particle weight of the enzyme as determined by gel filtration varies with pH. At pH 5.0 one species of 45000 Da is detected, at pH 9.5 two species of 44000 Da and 63000 Da are observed [1]; <2> the purified enzyme shows two separable bands in SDS-PAGE: 70000 Da and 24000 Da [4]) [1, 4]

Subunits

? <3> (<3> x * 26300, SDS-PAGE [6]) [6]
dimer <1, 3> (<1> 1 * 21500-25000 + 1 * 21500-25000, subunits differ substantially in amino acid composition , SDS-PAGE, sedimentation equilibrium ultracentrifugation or sucrose density gradient centrifugation in presence of 6 M guanidine HCl [3]; <3> 2 * 50000, SDS-PAGE [5]) [3, 5]
hexamer <7> (<7> 6 * 25000, SDS-PAGE [13]) [13]
monomer <6> (<6> 1 * 72000, SDS-PAGE [12]) [12]
Additional information <5> (<5> multisubunit protein at different degrees of association, it can dissociate into a component with a marked fall in catalytic activity [11]) [11]

Posttranslational modification

glycoprotein <3> (<3> carrying a variable amount of polysaccharide [5]; <3> 1.2-1.4% carbohydrate, protein contains 3-4 carbohydrate residues [6]) [5, 6]

5 Isolation/Preparation/Mutation/Application

Source/tissue
cotyledon <6> [12]
embryo <5> [9, 10]
erythrocyte <5> [9, 10]
intestinal mucosa <5> [11]
root <1> [1, 2]
seedling <3, 6> (<6> cotyledon [12]) [5, 12]
sprout <3> [6, 7]

Localization
cytoplasm <4, 8> [8, 14]
cytosol <5> [9]
membrane <2, 8> (<2> bound to [4]) [4, 14]
mitochondrion <5> [9]
nucleus <5> [9]

Purification
<1> [1, 2]
<2> [4]
<3> [5, 6]
<5> [11]
<6> [12]
<7> [13]

Crystallization
<7> [13]

6 Stability

pH-Stability
3-12 <7> (<7> stable [13]) [13]
9.5 <1> (<1> without protecting agent, about one-third of the initial transfer activity is retained whereas the hydrolase function is almost entirely lost after 22 h [2,3]) [2, 3]

Temperature stability
30 <7> (<7> pH 5.0, 30 min, stable [13]) [13]
50 <2> (<2> 5 min, stable [4]) [4]
63 <6> (<6> pH 8.0, 3 min, 50 mM Tris-HCl buffer, 50% loss of activity [12]) [12]
68 <6> (<6> pH 8.0, 3 min, 50 mM Tris-HCl buffer containing 10% glycerol, 50% loss of activity [12]) [12]
72 <6> (<6> pH 8.0, 3 min, 50 mM potassium phosphate buffer containing 10% glycerol, pH 6.8, 50% loss of activity [12]) [12]

Additional information <3, 5, 7> (<3> thermal stability is reduced when metal is removed but can be restored by addition of Mg^{2+} [7]; <5> unstable to heat, protected from inactivation by a large number of nucleotides [10,11]; <5> probably the free apoenzyme is converted by means of cooperative interactions between regulatory sites into an enzyme-nucleotide complex which is particularly stable at 37°C [10]; <5> addition of aliphatic alcohols, 0.2 M butanol, propanol or ethanol or urea induces a much more rapid inactivation at 37°C of associated forms [11]) [7, 10, 11, 13]

General stability information

<1>, dialysis first against EDTA or oxime and then against water produces an inhibition which results in the recovery of 36% of the initial phosphotransferase activity and 65% of the initial phosphatase activity [1]
<1>, no loss of activity after dialysis overnight against distilled water [1]
<2>, does not lose acttivity on lyophilization [4]
<2>, not stable to SDS [4]
<2>, stable in presence of 2 M urea, but not 4 M urea [4]
<2>, the enzyme can be isolated and handled only in the presence of detergents [4]
<2>, tolerates repeated freezing and thawing [4]
<3>, storage in 20% acetone for 1 week without loss of activity [7]
<3>, withstands repeated freezing and thawing if the protein concentration is less than 0.5 mg/ml [5]
<6>, the enzyme tolerates repeated freeze-thawing [12]

Storage stability

<1>, -20°C, stable for at least 1 months [1, 2]
<1>, 4°C, 1 week, 80% of the transferase activity is retained, no loss of hydrolase activity [1]
<2>, 4°C, 10% glycerol, pH 7.4, stable for at least 2 months [4]
<3>, -20°C, stable for at least 6 months [5]
<3>, 0°C, 0.01% Triton X-100 or 0.25 M sodium acetate, pH 5, 44 h, phosphotransferase activity decreases 20% and 50% respectively [5]

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Polynucleotide 5'-hydroxy-kinase 2.7.1.78

1 Nomenclature

EC number
2.7.1.78

Systematic name
ATP:5'-dephosphopolynucleotide 5'-phosphotransferase

Recommended name
polynucleotide 5'-hydroxy-kinase

Synonyms
5'-hydroxyl RNA kinase
5'-hydroxyl polynucleotide kinase
5'-hydroxyl polyribonucleotide kinase
ATP:5'-dephosphopolynucleotide 5'-phosphatase
DNA 5'-hydroxyl kinase
DNA kinase
PNK
kinase (phosphorylating), polynucleotide 5'-hydroxyl
polynucleotide 5'-hydroxyl kinase (phosphorylating)
polynucleotide 5'-hydroxyl-kinase
polynucleotide kinase
Additional information <2, 4, 5, 8> (<2, 4, 5, 8> enzyme also possesses a 3'-phosphatase activity [1, 13, 15, 16, 23-25, 27]) [1, 13, 15, 16, 23-25, 27]

CAS registry number
37211-65-7

2 Source Organism

<-3> no activity in *bacteriophage T5* [2]
<-2> no activity in *bacteriophage T1* [2]
<-1> no activity in *Escherichia coli* [1]
<1> *bacteriophage T2* (from infected Escherichia coli [1-3]) [1-3]
<2> *bacteriophage T4* (from infected Escherichia coli [1, 2, 5, 10, 15, 25]; gene pseT [1, 15]; mutant forms, genes pseT 1 and pseT 47 [15]) [1, 2, 5, 6, 8, 10, 11, 15, 25-27]
<3> *bacteriophage T6* [1]
<4> *Homo sapiens* (HeLa cells [1,18]) [1, 18, 24]
<5> *Bos taurus* (calf [1,9,20,22,23]) [1, 9, 20, 22, 23]

<6> *Mesocricetus auratus* (chinese hamster lung cells [1]) [1]
<7> *Mus musculus* [14]
<8> *Rattus norvegicus* (enzyme also has DNA 3'-phosphatase activity [13,16]) [1, 4, 7, 12, 13, 16, 17, 19, 23]
<9> *synthetic ribozyme* (class I polynucleotide kinase [21]) [21]
<10> *mammalia* [27]

3 Reaction and Specificity

Catalyzed reaction

ATP + 5'-dephospho-DNA = ADP + 5'-phospho-DNA (also acts on 5'-dephospho-RNA 3'-mononucleotides; <8> random reaction mechanism [4]; <8> random order sequential mechanism [12]; <2, 9> reaction mechanism [21, 27]; <2> 5 amino acids, Lys15, Ser116, Asp35, Arg38, and Arg126, comprise the 5'-kinase active site, structure-activity relationships [25]; <2> structure of the active sites for 5'-polynucleotide kinase and 3'phosphatase activity of the bifunctional enzyme, substrate binding mechanism [27])

Reaction type

phospho group transfer

Natural substrates and products

S ATP + 5'-dephospho-DNA <1-5, 8, 10> (<10> involved in repair of chromosomal DNA strand breaks that arise continously, but at low frequency, and are potentially lethal [27]; <4, 5> involved in repair of DNA single strand breaks [23,24]) (Reversibility: r <2, 8> [1, 6, 12, 19]; ? <1, 3-5, 8, 10> [1-7, 9-13, 15, 17-20, 23, 24, 27]) [1-7, 9-13, 15, 17-20, 23, 24, 27]
P ADP + 5'-phospho-DNA <1-5, 8, 10> [1, 2, 7, 9, 20, 23, 27]
S ATP + 5'-dephospho-RNA <2> (<2> involved in repair of broken RNA termini [25,27]) (Reversibility: ? <2> [25, 27]) [25, 27]
P ADP + 5'-phospho-RNA <2> [25, 27]
S Additional information <2, 10> (<10> enzyme interacts with XRCC1, a scaffold protein that helps recruit repair enzymes at sites of single-strand breakage [27]; <2> in vivo role is possibly in maintaining DNA or RNA in the 5'-phosphorylated 3'-hydroxylated state which is the substrate for many reactions such as ligation and packaging [1]) [1, 27]
P ?

Substrates and products

S ATP + 2'(3')-ribonucleotides <1> (<1> no activity with (2')-AMP [3]; <1> no activity with 2'-AMP and 2'-CMP [2]) (Reversibility: ? <1> [3]) [3]
P ?
S ATP + 5'-dephospho-DNA <1-8> (<5, 8> specific for DNA [23]; <2, 5, 8> single stranded DNA [1, 10, 19, 20, 23]; <2, 5, 8> double stranded DNA [1, 6, 19, 20]; <8> native and heat denatured DNA [13, 19]; <8> nuclease-treated calf thymus DNA [17]; <8> native and denatured DNA are sub-

strates for the reverse reaction [12]; <1, 2, 5> micrococcal-nuclease-treated calf thymus DNA [2, 10, 23]; <7> micrococcal-nuclease-treated calf thymus DNA is not effectively phosphorylated [14]; <5, 8> minimal length of the substrate is 7-8 nucleotides, optimal size is more than 18 nucleotides in length [23]; <2, 8> large DNA fragments released by nuclease treatment [1, 19]; <7> low activity [14]; <2> preference for single stranded termini due to the narrow tunnel formation of the active site that can only bind slim molecules [27]; <10> preference for recessed termini within duplex DNA [27]; <5,10> no preference for overhanging 5'-ends [23, 27]) (Reversibility: r <2, 8> [1, 6, 11, 12, 19]; ? <1-5, 8> [1-5, 7-10, 12-18, 20, 22-24, 26, 27]) [1-20, 22-24, 26, 27]

P ADP + 5'-phospho-DNA <1-8> (<2> excess ADP will cause the reverse reaction to be favored [1]; <8> reverse reaction is promoted by a variety of other nucleoside diphosphates but not by ATP [12]; <8> native and heat denatured DNA serve as substrates for the reverse reaction [12]; <8> UDP, ADP, GDP and CDP support the reverse reaction [19]) [1-20, 22-24, 26, 27]

S ATP + 5'-dephospho-RNA <1-5, 7, 8> (<4, 7> much more efficient as substrate than DNA [14, 18]; <8> enzyme cannot act on RNA less than 10 bases in length [1]; <8> solely RNA-specific [1]; <1> soluble and ribosomal RNA of Escherichia coli [2]; <1> synthetic polynucleotides [2]; <5> low activity [1]; <4-6,8> no activity [1, 9, 12, 19]; <8> 5'-HO-tRNA is a very poor substrate [17]; <5> phosphorylated at a much lower rate than DNA [20]) (Reversibility: ? <1-5, 7, 8> [1-3, 10, 14, 18, 20, 25, 27]) [1-3, 10, 14, 18, 20, 25, 27]

P ADP + 5'-phospho-RNA <1, 2, 4, 7> [1-3, 10, 14, 18, 25, 27]

S ATP + deoxynucleoside 3'-monophosphate <2> (Reversibility: ? <2> [1,8]) [1, 8]

P ADP + deoxynucleoside-3',5'-diphosphate

S ATP + nucleoside-3'-monophosphate <1, 2, 8> (<5> no activity with thymidine 3'-monophosphate [22]; <8> no activity [12]) (Reversibility: ? <1, 2, 8> [1, 3, 13]) [1, 3, 13]

P ADP + nucleoside-3',5'-diphosphate

S ATP + synthetic oligonucleotide <2, 4, 5, 7, 8> (<9> substrate 5'-OH-(ribonucleotide)$_7$ [21]; <8> oligodeoxynucleotides of chain length above 10-12 residues [1,19]; <8> no activity with low molecular weight oligonucleotides [12,19]; <2> oligodeoxynucleotides of chain length less than approximately 10-12 residues [11]; <5> no activity with poly(A) [9]; <4> low activity with 5'-hydroxyl poly(I) [18]; <4, 7> 5'-hydroxyl poly(A) [14, 18]; <4> 5'-hydroxyl poly(C) [18]; <4> 5'-hydroxyl poly(dA), at 6% of 5'-hydroxyl poly(A) [18]; <5> oligo (dT)25 [22]; <2> oligo dpT(pT)$_9$ [11]; <8> (dT)$_{10}$ [17]; <5> low activity with homopolymers such as oligo(dA)$_{24}$ and oligo(dT)$_{24}$ [23]) (Reversibility: ? <2, 4, 5, 7, 8> [1, 11, 14, 17-19, 21, 22, 25]) [1, 11, 14, 17-19, 22, 23, 25]

P ADP + oligonucleotide 5'-phosphate

S CTP + 5'-dephospho-DNA <1, 4-6, 8> (<8> UDP, ADP, GDP and CDP support the reverse reaction [19]; <4> CTP gives 15% of the activity with ATP [18]) (Reversibility: r <8> [19]; ? <1, 4-6, 8> [1,3,18]) [1, 3, 18, 19]
P CDP + 5'-phospho-DNA <1, 4-6, 8> [1, 3, 18, 19]
S CTP + 5'-dephospho-RNA <4> (<4> less effective than ATP [18]) (Reversibility: ? <4> [18]) [18]
P CDP + 5'-phospho-RNA <4> [18]
S GTP + 5'-dephospho-DNA <1, 4-6, 8> (<8> UDP, ADP, GDP and CDP support the reverse reaction [19]; <4> GTP gives 15% of the activity with ATP [18]) (Reversibility: r <8> [19]; ? <1,4-6,8> [1,3,18]) [1, 3, 18, 19]
P GDP + 5'-phospho-DNA <1, 4-6, 8> [1, 3, 18, 19]
S TTP + 5'-dephospho-DNA <2> (Reversibility: ? <2> [1]) [1]
P TDP + 5'-phospho-DNA <2> [1]
S UTP + 5'-dephospho-DNA <1, 2, 8> (<8> UDP, ADP, GDP and CDP support the reverse reaction [19]) (Reversibility: r <8> [19]; ? <1,2> [1,3]) [1, 3, 19]
P UDP + 5'-phospho-DNA <1, 2, 8> [1, 3, 19]
S [γ-S]ATP + 5'-OH-(ribonucleotide)$_7$ <9> (<9> best phosphate donor [21]; <9> very low activity with [γ-S]GTP [21]) (Reversibility: ? <9> [21]) [21]
P [γ-S]ADP + 5'-phospho-(ribonucleotide)$_7$ <9> [21]
S [γ-S]ATP + 5'-dephospho-RNA <7> (Reversibility: ? <7> [14]) [14]
P [γ-S]ADP + 5'-phospho-RNA <7> [14]
S [γ-S]GTP + 5'-dephospho-RNA <4, 7> (<4> less effective than ATP [18]) (Reversibility: ? <4,7> [14,18]) [14, 18]
P [γ-S]GDP + 5'-phospho-RNA <4, 7> [14, 18]
S β,γ-imidoadenylyl 5'-diphosphate + 5'-phospho-DNA <2> (<2> ATP analog, inhibitory, competitive against ATP [11]; <2> is no substrate in the forward reaction but can replace ADP and ATP in the reverse reaction [11]) (Reversibility: ir <2> [11]) [11]
P β,γ-imidoadenylyl 5'-triphosphate + 5'-dephospho-DNA
S dATP + 5'-dephospho-DNA <2> (Reversibility: ? <2> [1,3]) [1, 3]
P dADP + 5'-phospho-DNA <2> [1, 3]
S Additional information <1, 2, 4, 5, 8, 9> (<1, 2, 5, 9> substrate specificity [3, 6, 21-23]; <2> poorly active on recessed termini within duplex DNA [27]; <9> binding constants of the reaction steps [21]; <5> no 3'-phosphatase activity [22]; <5> no activity with thymidine 3'-monophosphate [22]; <2, 4, 5, 8> enzyme also has DNA 3'-phosphatase activity [1, 13, 15, 16, 23-25]; <1> no activity with adenosine, nucleotides, and 5'-mononucleotides [3]) [1, 3, 6, 13, 15, 16, 21-25, 27]
P ?

Inhibitors

5'-AMP <4> (<4> weak [18]) [18]
5'-hydroxyl poly(I) <4> (<4> in combination with 5'-hydroxyl poly(A) or poly(C) [18]) [18]
ADP <4, 8, 9> [18, 19, 21]

$AgNO_3$ <5, 8> (<5> complete inhibition at 0.3 mM [20]) [19, 20]
CTP <5> (<5> more than 90% inhibition at 0.3 mM [20]) [20]
Ca^{2+} <1> (<1> inhibits in combination with $MgCl_2$, stimulates without $MgCl_2$ [3]) [3]
Cibacron Blue F3GA <2> (<2> chromophore of blue dextran, inhibition is competitive to single stranded DNA, noncompetitive with respect to ATP [10]) [10]
Cu^{2+} <1, 4, 8> (<4,8> inhibits in presence of $MgCl_2$ [18,19]; <1> inhibits in presence of $CuCl_2$ [3]) [3, 18, 19]
EDTA <5, 8> (<5> complete inhibition at 25 mM [22]) [13, 22]
GTP <5> (<5> more than 90% inhibition at 0.3 mM [20]) [20]
KCl <2, 4, 5> (<5> 50% inhibition at 71 mM [22]; <4> weak inhibition, 50% at about 0.3 M [18]; <2> KCl stimulates at low concentrations, inhibits at high concentrations [1]) [1, 18, 22]
Mn^{2+} <4, 7> (<4,7> inhibition above 1 mM [14,18]) [14, 18]
N-ethylmaleimide <4> [18]
NH_4^+ <2, 4, 7> (<4> 50% at about 0.3 M [18]; <4,7> weak inhibition [14,18]) [1, 14, 18]
NaCl <2, 4, 5, 8> (<5> 50% inhibition at 67 mM [22]; <4> weak inhibition, 50% at about 0.3 M [18]; <8> maximum activity in presence of 0.1-0.15 M NaCl, higher concentrations inhibit [4]; <2> stimulates activity towards single stranded substrates, inhibitory with some duplexes [1]) [1, 18, 22]
Ni^{2+} <1> (<1> inhibits in combination with $MgCl_2$, stimulates without $MgCl_2$ [3]) [3]
UTP <5> (<5> more than 90% inhibition at 0.3 mM [20]) [20]
Zn^{2+} <4, 7> (<4,7> inhibition in presence of Mg^{2+} [14,18]) [14, 18]
ammonium sulfate <5> (<5> 50% inhibition at 8.5 mM [22]; <5> about 75% inhibition at 10 mM [9]; <5> 80% inhibition at 30 mM [20]) [9, 20, 22]
β,γ-imidoadenylyl 5'-diphosphate <2> (<2> ATP analog, competitive against ATP, noncompetitive against 5'-OH-DNA, serves as substrate for the reverse reaction only [11]) [11]
chloramphenicol <1, 2> (<1,2> inhibits the formation of enzyme in cells co-transfected with bacteriophage and chloramphenicol [2]) [2]
dATP <5> (<5> more than 95% inhibition at 0.3 mM [20]) [20]
dCTP <5> (<5> 80% inhibition at 0.3 mM [20]) [20]
dGTP <5> (<5> more than 95% inhibition at 0.3 mM [20]) [20]
dTTP <5> (<5> more than 95% inhibition at 0.3 mM [20]) [20]
deoxyribonucleoside triphosphates <8> [19]
dextran sulfate <5, 8> (<8> forward and reverse reaction at similar amounts [12]; <8> strong inhibition [19]; <8> strong inhibition, competitive to ATP and DNA [12]) [12, 19, 20]
diphosphate <1, 2, 4, 5, 8> (<5> 50% inhibition at 2.2 mM [22]; <5> 97% inhibition at 20 mM [20]; <5> weak [9]) [1, 3, 7, 9, 13, 16-18, 20, 25]
heparin <5, 8> (<8> strong inhibition [19]) [12, 19, 20]
iodoacetate <5, 8> (<5> complete inhibition at 0.3 mM [20]) [19, 20]
maleate <1> [3]

p-chloromercuribenzoate <5, 8> (<5> reversible [9]; <8> reversible by 2-mercaptoethanol [7]) [7, 9, 19]
p-hydroxymercuribenzoate <8> (<8> 2-mercaptoethanol prevents inhibition [13]) [13]
phosphate <1, 2, 4, 5, 8> (<5> 50% inhibition at 11 mM [22]; <4,8> weak [7,18]) [1, 3, 7, 13, 16-18, 22]
ribonucleoside 3'-phosphates <4> (<4> weak [18]) [18]
ribonucleoside triphosphates <8> [19]
spermine <8> (<8> 1 mM enhances activity 3times, inhibition above 1 mM [17]) [17]
sulfate <4, 5, 7, 8> (<5> 50% inhibition at 5.7 mM [22]; <4,5,7> weak [9,14,18]; <8> competitive to ATP, noncompetitive to DNA, less sensitive in the reverse reaction [12]) [7, 9, 12-14, 16-20, 22]
sulfhydryl antagonists <5> [20]
Additional information <5, 8> (<5> no inhibition by agar-agar [20]; <8> no inhibition by chondroitin sulfates A and C, and by dextran [12]) [12, 20]

Cofactors/prosthetic groups

ADP <2> (<2> reverse reaction [1,6]) [1, 6]
ATP <1-9> (<4> best at 2 mM [18]; <2> stabilizes the enzyme in an oligomeric state [8]; <1-6, 8> dependent on [1-13, 15-20, 22, 23]) [1-22, 23]
CDP <1, 4-6, 8> [1, 3, 18, 19]
CTP <1, 4-6, 8> [1, 3, 18, 19]
GDP <1, 4-6, 8> [1, 3, 18, 19]
GTP <1, 4-6, 8> [1, 3, 18, 19]
TDP <2> [1]
TTP <2> [1]
UDP <1, 2, 8> [1, 3, 19]
UTP <1, 2, 8> [1, 3, 19]
[γ-S]ATP} <7, 9> (<9> best phosphate-donor [21]) [14, 21]
[γ-S]GTP} <7> [14]
dATP <2> [1, 3]

Activating compounds

bovine serum albumin <4, 8> [18, 19]
dithiothreitol <4, 5> (<5> 54% remaining activity without DTT [22]) [18, 22]
polyamines <2, 5> (<5> stimulate at suboptimal Mg^{2+} concentration [9]; <1-6,8> stimulate [1]) [1, 9]
polyethylene glycol <2> (<2> 4-10%, stimulates [1]) [1]
spermidine <2, 5, 8> (<5> 3-4fold activation, activates by lowering the K_m for Mg^{2+} and enhancing V_{max}, optimal at 10-15 mM [9]; <2> stimulates [1]; <8> little influence [19]) [1, 9, 19]
spermine <2, 5, 8> (<5> activates 3-4fold at 2 mM [9]; <2> stabilizes the enzyme in an oligomeric state [8]; <2,8> little influence [6,19]; <8> 1 mM enhances activity 3times [17]; <8> inhibition above 1 mM [17]) [6, 8, 9, 17, 19]
sulfhydryl reagents <2, 4> (<2> essential for activity, maximal activity with 5 mM DTT, less stimulation with 2-mercaptoethanol and glutathione [1]) [1, 18]

Metals, ions

Ca^{2+} <1, 4, 7, 8> (<1,4> divalent cation required, slight activation in absence of $MgCl_2$ [3,18]; <4> about 17% of the activity with Mn^{2+}, synergistic to Mn^{2+} [18]; <7> about 17% of the activity with Mg^{2+}, synergistic to Mg^{2+} [14]; <1> inhibition in combination with $MgCl_2$ [3]; <8> Mg^{2+}, Mn^{2+}, Co^{2+}, Zn^{2+}, Ni^{2+} or Ca^{2+} are effective [19]) [3, 7, 14, 18, 19]

Co^{2+} <1, 8> (<8> less effective [7]; <1> Mg^{2+}, Mn^{2+}, Zn^{2+} and Co^{2+} are equally effective in activation [3]; <8> Mg^{2+}, Mn^{2+}, Co^{2+}, Zn^{2+}, Ni^{2+} or Ca^{2+} are effective [19]) [3, 7, 19]

K^+ <2, 5, 8> (<5> KCl slightly stimulates [9]; <2> KCl stimulates at low concentrations, inhibits at high concentrations [1]; <8> stimulates, optimal concentration: 0.1 M [17]) [1, 9, 17]

La^{2+} <8> (<8> Mn^{2+} or La^{2+} effective in activation [7]) [7]

Mg^{2+} <1, 2, 4, 5, 7-9> (<2, 5, 7, 9> required [1, 2, 9, 14, 21]; <2, 5> optimal concentration is 10 mM [1, 9]; <5> best at 1 mM [22]; <1> Mg^{2+}, Mn^{2+}, Zn^{2+} and Co^{2+} are equally effective [3]; <4, 5> Mg^{2+} or Mn^{2+} effective in activation [18, 20]; <4> 86% of the activity with Mn^{2+} [18]; <8> Mg^{2+}, Mn^{2+}, Co^{2+}, Zn^{2+}, Ni^{2+} or Ca^{2+} are effective [19]; <2> optimal concentration at pH optimum 7.6 is 10 mM [1,17,20]; <5,8> stimulates [13,22]) [1-3, 7, 9-23]

Mn^{2+} <1, 2, 4, 5, 8, 9> (<8> most effective divalent cation in activation, best at 1 mM [13]; <1> Mg^{2+}, Mn^{2+}, Zn^{2+} and Co^{2+} are equally effective [3]; <4, 5> Mg^{2+} or Mn^{2+} effective in activation [18,20]; <8> Mn^{2+} or La^{2+} effective [7]; <8> Mg^{2+}, Mn^{2+}, Co^{2+}, Zn^{2+}, Ni^{2+} or Ca^{2+} are effective [19]; <4> maximal activation at 0.6-1.0 mM, inhibition above 1 mM [18]; <5> optimal concentration: 10 mM [20]; <2, 4, 8> can partially replace Mg^{2+} in activation [1, 7, 17]; <2> at 3.3 mM 50% of the activity with 10 mM Mg^{2+} [1]; <7> no activation, 50% inhibition at 1 mM [14]) [1, 3, 7, 13, 17-21]

NH_4^+ <8> (<8> stimulates, optimal concentration 0.1 M [17]; <7> slightly inhibitory [14]) [17]

Na^+ <2, 8> (<8> maximal activity in presence of 0.1-0.15 M NaCl, higher concentrations inhibit [4]; <2> stimulates activity towards single stranded substrates, inhibitory to some duplexes [1]; <8> stimulates, optimal concentration: 0.1 M [17]) [1, 4, 17]

Ni^{2+} <1, 8> (<1> slight activation in absence of $MgCl_2$, inhibition in combination with $MgCl_2$ [3]; <8> Mg^{2+}, Mn^{2+}, Co^{2+}, Zn^{2+}, Ni^{2+} or Ca^{2+} are effective in activation [19]) [3, 19]

Zn^{2+} <1, 5, 8> (<5, 8> less effective [7, 13, 20]; <1> Mg^{2+}, Mn^{2+}, Zn^{2+} and Co^{2+} are equally effective in activation [3]; <8> Mg^{2+}, Mn^{2+}, Co^{2+}, Zn^{2+}, Ni^{2+} or Ca^{2+} are effective [19]; <4,7> no activation, inhibition in presence of Mg^{2+} [14, 18]) [3, 7, 13, 19, 20]

divalent cations <1, 4, 5, 8> (<1, 4, 5, 8> absolutely required [3, 7, 13, 17-20]; <1> Mg^{2+}, Mn^{2+}, Zn^{2+}, Co^{2+} are equally effective [3]; <8> Mg^{2+}, Mn^{2+}, Co^{2+}, Zn^{2+}, Ni^{2+} or Ca^{2+} are effective, descending order [19]) [3, 7, 13, 17-20]

Additional information <8> (<8> maximal activity at ionic strength of 135 mM [13]) [13]

Turnover number (min^{-1})

0.003 <9> (ATP, <9> pH-independent, 30°C [21]) [21]
0.17 <9> ([γ-S]ATP, <9> pH-independent, 30°C [21]) [21]

Specific activity (U/mg)

0.0002 <5> (<5> purified, polymin P precipitable enzyme, pH 5.5, 37°C [22]) [22]
0.0007 <7> (<7> partially purified enzyme, substrate RNA [14]) [14]
0.0012 <5> (<5> purified enzyme [23]) [23]
0.0028 <8> (<8> purified enzyme [23]) [23]
0.0043 <5> (<5> purified enzyme [20]) [20]
0.005 <8> (<8> above, purified enzyme, 30°C [17]) [17]
0.006 <8> (<8> partially purified enzyme [19]) [19]
0.0167 <5> (<5> purified enzyme [9]) [9]
0.0418 <8> (<8> purified enzyme [7]) [7]
0.125 <4> (<4> purified enzyme, substrate RNA [18]) [18]
0.393 <2> (<2> purified enzyme [10]) [10]
0.53 <2> (<2> purified enzyme [2]) [2]
5.67 <2> (<2> purified wild-type [15]) [15]
7 <2> (<2> purified mutant pseT 1 [15]) [15]
30-40 <2> (<2> commercial preparation [1]) [1]
Additional information <8> [13]

K_m-Value (mM)

0.00069 <8> (ATP, <8> pH 5.5, 37°C [13]) [13]
0.002 <8> (ATP, <8> pH 5.5, 37°C [19]) [19]
0.0025 <4> (5'-hydroxyl poly(A), <4> pH 8.4, 37°C [18]) [18]
0.0027 <2> (5'-OH-DNA, <2> wild-type, pH 7.6, 37°C [15]) [15]
0.0031 <2> (5'-OH-DNA, <2> mutant pseT 1, pH 7.6, 37°C [15]) [15]
0.0034 <4> (5'-hydroxyl poly(C), <4> pH 8.4, 37°C [18]) [18]
0.0036 <7> (poly(A), <7> pH 8.4, 37°C [14]) [14]
0.0039 <8> (5'-OH-DNA, <8> pH 5.5, 37°C [13]) [13]
0.004 <2, 5> (ATP, <5> pH 5.5, 37°C [9,20]; <2> phosphate exchange reaction between nucleotides, forward reaction, pH 7.6, 37°C [1]) [1, 9, 20]
0.0045 <2> (5'-OH-DNA, <2> mutant pseT 47, pH 7.6, 37°C [15]) [15]
0.0065 <2> (5'-OH-DNA, <2> pH 7.4, 37°C [10]) [10]
0.0076 <2> (5'-OH-DNA, <2> large DNA fragments released by nuclease treatment, pH 7.6, 37°C [1]) [1]
0.008 <5> (oligo (dT)25, <5> polymin P precipitable enzyme, pH 7.5, 37°C [22]) [22]
0.013-0.14 <2> (ATP, <2> depending on DNA acceptor, pH 7.6, 37°C [1]) [1]
0.014 <1> (ATP) [1]
0.015 <1> (UTP) [1]
0.016 <8> (ATP, <8> pH 5.5 [4]) [4]
0.0222-0.143 <2> (oligonucleotides, <2> depending on the 5'-base and the length of the oligonucleotide, pH 7.6, 37°C [1]) [1]
0.025 <1> (CTP) [1]
0.033 <1> (GTP) [1]

0.046 <8> (5'-OH-DNA, <8> pH 5.5 [4]) [4]
0.052 <5> (ATP, <5> polymin P precipitable enzyme, pH 7.5, 37°C [22]) [22]
0.065 <2> (ATP, <2> pH 7.4, 37°C [10]) [10]
0.1 <1> (Mn^{2+}, <1> pH 6.0, 37°C [3]) [3]
0.152 <2> (ATP, <2> mutant pseT 1, pH 7.6, 37°C [15]) [15]
0.171 <2> (ATP, <2> mutant pseT 47, pH 7.6, 37°C [15]) [15]
0.188 <2> (ATP, <2> wild-type, pH 7.6, 37°C [15]) [15]
0.2 <2> (ADP, <2> dephosphorylation of single-stranded oligonucleotides, pH 7.6, 37°C [1]; <2> phosphate exchange reaction between nucleotides, reverse reaction, pH 7.6, 37°C [1]) [1]
0.22 <7> ([γ-S]ATP, <7> pH 8.4, 37°C [14]) [14]
0.5 <4> (ATP, <4> pH 8.4, 37°C [18]) [18]
0.5 <1> (Mg^{2+}, <1> pH 6.0, 37°C [3]) [3]
0.53 <7> ([γ-S]GTP, <7> pH 8.4, 37°C [14]) [14]
2.5 <5> (Mg^{2+}, <5> pH 5.5, 37°C [9]) [9]
3.1 <9> ([γ-S]ATP, <9> pH-independent in the range pH 6.0-8.5, 30°C [21]) [21]
3.4 <9> (ATP, <9> pH-independent in the range pH 6.0-8.5, 30°C [21]) [21]
Additional information <2, 9> (<9> detailed reaction kinetics, pH- and temperature-in-/dependence [21]) [1, 21]

K_i-Value (mM)

0.2 <8> (diphosphate, <8> pH 5.5, 37°C [7]) [7]
0.2 <8> (sulfate, <8> competitive versus ATP, pH 5.5, 37°C [12]) [12]
0.5 <8> (sulfate, <8> pH 5.5, 37°C [7]) [7]
3 <2> (β,γ-imidoadenylyl 5'-diphosphate, <2> versus ATP, pH 8.0, 35°C, independent of pH-value [11]) [11]
3.8 <9> (ADP, <9> pH-independent in the range pH 6.0-8.5, 30°C [21]) [21]
5 <5> (diphosphate, <5> pH 5.5, 25°C [9]) [9]
7 <5> (sulfate, <5> pH 5.5, 25°C [9]) [9]
11 <2> (β,γ-imidoadenylyl 5'-diphosphate, <2> versus 5'-OH^-DNA, pH 8.0, 35°C, independent of pH-value [11]) [11]
Additional information <9> [21]

pH-Optimum

5.3 <8> [17]
5.5 <4, 5, 7, 8> (<4,7> substrate DNA [14,18]; <4,5,8> assay at [12,13,18,23]; <5,8> sharp maximum [9,19]) [4, 7, 9, 12-14, 18-20, 23]
6 <1, 2, 5> (<1,2> assay at [2]) [2, 23]
6-9 <1> (<1> broad optimum [3]) [3]
6.2 <2> (<2> imidazole buffer [1]) [1]
7-9 <5> (<5> polymin P precipitable enzyme [22]) [22]
7.4 <2> (<2> assay at [10]) [10]
7.6 <2> (<2> assay at [15]; <2> Tris buffer [1]) [1, 15]
7.9-8.9 <4> [18]
8 <2, 8> (<2,8> assay at [11,16]) [11, 16]
8.4 <4> (<4> assay at, substrate RNA [18]) [18]
9 <7> (<7> substrate RNA [14]) [14]

Additional information <8> (<9> detailed reaction kinetics, pH- and temperature-dependence [21]; <8> pH-optimum of 3'-phosphatase activity is 6.0 [13]) [13, 21]

pH-Range

4.5-7 <8> (<8> about 80-86% of maximal activity at pH 7.0 and pH 4.5 [7]) [7]
5-6.5 <8> (<8> pH 5: about 50% of activity maximum, pH 6.5: about 35% of activity maximum [19]) [19]
5-7 <5> (<5> pH 5: about 5% of activity maximum, pH 7: about 30% of activity maximum [20]) [20]
5-9.5 <1> (<1> about 50% of activity maximum at pH 5.0 and pH 9.5 [3]) [3]
6.5-8.9 <4> (<4> pH 6.5: about 50% of activity maximum, pH 7.9-8.9: activity maximum, substrate RNA [18]) [18]
7.4-8 <2> (<2> forward reaction, optimal range [1]) [1]
7.5-9.4 <7> (<7> pH 7.5: about 50% of activity maximum, pH 8.4 and pH 9.4: about 85% of activity maximum [14]) [14]

Temperature optimum (°C)

25-30 <5> [9]
35 <2> (<2> assay at [11]) [11]
37 <1, 2, 4, 5, 7, 8> (<1,2,4,5,7,8> assay at [3,7,10,12-16,18,20,22,23]) [1, 3, 7, 10, 12-16, 18, 20, 22, 23]
38 <1, 2> (<1,2> assay at [2]) [2]
Additional information <9> (<9> detailed reaction kinetics, pH- and temperature-dependence [21]) [21]

Temperature range (°C)

0-37 <2> (<2> 0°C: about 7% of activity maximum, 37°C: activity maximum, bacteriophage T4 enzyme [1]) [1]

4 Enzyme Structure

Molecular weight

38000 <8> (<8> gel filtration [17]) [17]
56000 <5> (<5> gel filtration [9]) [9]
70000 <5> (<5> gel filtration, sucrose density gradient centrifugation [20]) [20]
72000 <5> (<5> sucrose density gradient centrifugation [22]) [22]
79000 <8> (<8> gel filtration [13]) [13]
80000 <8> (<8> gel filtration [1,7,19]) [1, 7, 19]
96000 <7> (<7> gel filtration, native PAGE [14]) [14]
140000 <2> (<2> gel filtration [1,5]) [1, 5]

Subunits

? <5, 8> (<8> x * 60000, SDS-PAGE [23]; <5> x * 61000-62000, SDS-PAGE [23]) [23]
monomer <5, 7, 8, 10> (<8> 1 * 40000, SDS-PAGE [17]; <5> 1 * 54000, SDS-PAGE [9]; <5> 1 * 70000-75000, SDS-PAGE [20]; <7> 1 * 93000, SDS-PAGE [14]) [9, 14, 17, 20, 27]
tetramer <2> (<2> 4 * 33000, SDS-PAGE [1,5]; <2> 4 * 33000, sedimentation analysis and amino acid sequence determination [8]; <2> 4 * 33200, analytical ultracentrifugation [1]) [1, 5, 8, 27]
Additional information <2, 4, 9> (<2> enzyme consists of 2 domains of approximately equal size, separated by a flexible loop into N-terminal half, harboring the 5'-polynucleotide kinase activity, and C-terminal half, harboring the 3'-phosphatase activity, quartenary structure [27]; <4> epitope-mapping using specific monoclonal antibody [24]; <9> secondary structure of the ribozyme [21]; <2> high ionic strength, e.g. 0.1 M KCl, spermine, ATP and thymidine 3'-monophosphate are essential to stabilize the enzyme in an oligomeric state [8]; <2> each monomer contains 2 SH-groups, one of which is more buried [8]) [8, 21, 24, 27]

5 Isolation/Preparation/Mutation/Application

Source/tissue

HeLa cell <4> [1, 18]
L-cell <7> [14]
cell culture <4, 6> (<4> HeLa cells [1, 18]; <6> chinese hamster lung cells [1]) [1, 18]
cell suspension culture <7> (L-cells [14]) [14]
liver <4-6, 8> [1, 4, 7, 12, 13, 16, 19, 23]
lung <6> [1]
testis <8> [17]
thymus <5> [1, 9, 20, 22, 23]

Localization

chromatin <8> [13, 16]
nucleus <4, 8> [4, 7, 12, 18, 19, 23]

Purification

<1> [2]
<2> (to near homogeneity [10]; 2 mutant forms pseT 1 and pseT 47 [15]) [5, 10, 15]
<4> (recombinant His-tagged full length enzyme and recombinant truncated enzyme from Escherichia coli [24]; 431fold [18]) [18, 24]
<5> (polymin P precipitable enzyme, 1570fold [22]; 1500fold [20]; 6717fold, to near homogeneity [23]; to homogeneity [9]) [9, 20, 22, 23]
<7> (partial, 35fold [14]) [14]
<8> (10860fold, to near homogeneity [23]; over 1875fold, to homogeneity [17]; 39fold [13]; 261fold [7]; partial, 960fold [19]) [4, 7, 12, 13, 16, 17, 19, 23]

Cloning

<4> (expression of truncated enzyme, residue Met140 to C-terminus, in Escherichia coli [24]; expression as soluble His-tagged protein in Escherichia coli [24]) [24]

Engineering

D165A <2> (<2> site-directed mutagenesis, increased activity compared to the wild-type, no 3'-phosphatase activity remaining [25]) [25]
D180A <2> (<2> site-directed mutagenesis, slightly higher activity than the wild-type, reduced 3'-phosphatase activity [25]) [25]
D250A <2> (<2> site-directed mutagenesis, increased activity compared to the wild-type , reduced 3'-phosphatase activity [25]) [25]
D254A <2> (<2> site-directed mutagenesis, increased activity compared to the wild-type, nearly no 3'-phosphatase activity remaining [25]) [25]
D278A <2> (<2> site-directed mutagenesis, increased activity compared to the wild-type, no 3'-phosphatase activity remaining [25]) [25]
K125A <2> (<2> site-directed mutagenesis, slightly higher activity than the wild-type [25]) [25]
R122A <2> (<2> site-directed mutagenesis, slightly higher activity than the wild-type, reduced 3'-phosphatase activity [25]) [25]
R126A <2> (<2> site-directed mutagenesis, highly reduced activity [25]) [25]
R176A <2> (<2> site-directed mutagenesis, increased activity compared to the wild-type, highly reduced 3'-phosphatase activity [25]) [25]
R213A <2> (<2> site-directed mutagenesis, increased activity compared to the wild-type, no 3'-phosphatase activity remaining [25]) [25]
R246A <2> (<2> site-directed mutagenesis, increased activity compared to the wild-type, highly reduced 3'-phosphatase activity [25]) [25]
R279A <2> (<2> site-directed mutagenesis, increased activity compared to the wild-type, highly reduced 3'-phosphatase activity [25]) [25]
R36A <2> (<2> site-directed mutagenesis, similar activity than the wild-type [25]) [25]
R38A <2> (<2> site-directed mutagenesis, highly reduced activity, reduced 3'-phosphatase activity [25]) [25]
S84A <2> (<2> site-directed mutagenesis, slightly lower activity than the wild-type, reduced 3'-phosphatase activity [25]) [25]
Additional information <2> (<2> natural mutants pseT 1 and pseT 47, the first shows no 3'-phosphatase activity, but normal polynucleotide kinase activity, the second shows very little 3'-phosphatase activity, but no polynucleotide kinase activity, expression in Escherichia coli [15]) [15]

Application

biotechnology <2> (<2> application in DNA and RNA sequencing [8]) [8]
synthesis <2, 9> (<2> synthesis of photoreactive oligonucleotides, containing active groups at the 5'-end phosphate, as tools for photoaffinity modification of DNA-metabolizing enzymes and factors, utilization of γ-substituted ATP derivatives [26]; <9> construction of a catalytically useful ribozyme [21]; <2> production of β,γ-imidoadenylyl 5'-tetraphosphate by using β,γ-imidoadenylyl 5'-triphosphate as substrate in the reverse reaction [11]; <2> enzyme

is an important tool in the synthesis of genes corresponding to yeast alanine tRNAand to precursor tyrosine tRNA of Escherichia coli [8]; <2, 8> due to reversibility of the reaction, the bacteriophage can be utilized for exchange of labeled phosphate groups between 2 substrates [1,12]) [1, 8, 11, 12, 21, 26]

6 Stability

pH-Stability

5.5 <8> (<8> 37°C, unstable below [19]) [19]

Temperature stability

25 <1-6, 8> (<1-6,8> marked temperature lability in absence of substrate [1,2]) [1, 2]

37 <1, 2> (<1> activity is completely lost without substrates [3]; <2> 15 min, 77% loss of activity [1,2]) [1-3]

45 <2, 4> (<2> 5 min, loss of 94% activity with substrate DNA and 91% activity with substrate RNA [2]; <2> 5 min, 92% loss of activity [1]; <4> 57% loss of RNA-dependent activity, 34% of DNA-dependent activity [18]) [1, 2, 18]

55 <4> (<4> 5 min, complete loss of activity [18]) [18]

65 <8> (<8> quick inactivation [19]) [19]

Additional information <1, 8> (<1> protection against heat inactivation by substrates, e.g. calf thymus DNA and ribosomal RNA, both micrococcal nuclease-treated, and 3'-AMP [3]; <8> protection against heat inactivation by ATP or 5'-OH-DNA, not 5'-phospho-DNA, at 0.1-0.15 M NaCl [4]) [3, 4]

General stability information

<1>, greatest protection from heat inactivation with substrates for the kinase, e.g. thymus DNA and ribosomal RNA, both micrococcal nuclease-treated and 3'-AMP [3]

<2>, high ionic strength, 0.1 M KCl, spermine, ATP and thymidine 3'-monophosphate stabilize the oligomeric structure [8]

<2>, high ionic strength, e.g. 0.1 M KCl, spermine, ATP and thymidine 3'-monophosphate are essential to stabilize the enzyme in an oligomeric state [8]

<4>, bovine serum albumin and dithiothreitol stabilize the enzyme during activity assay [18]

<8>, ATP protects against heat denaturation and tryptic digestion [13]

<8>, protection against heat inactivation by ATP or 5'-OH-DNA at low and moderately high NaCl concentrations [4]

<1, 2>, freezing and thawing reduces activity after 1 cycle by 60% [1, 2]

Storage stability

<1>, 0°C, partially purified ammonium sulfate fraction, stable for more than 1 year [2]

<1>, 0°C, purified protein, concentration 0.014 mg/ml, stable for 5 months [2]

<2>, -20°C, 50 mM Tris-HCl, pH 7.5, 25 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.001 mM ATP, 50% glycerol, stable for up to 18 months [1]
<4>, 0°C, 10% loss of activity after 2 months [18]
<5>, 0°C, partially purified enzyme, 50 mM Tris-HCl, pH 7.5, 30 mM NaCl, 0.5 mM DTT, 0.5 mM EDTA, on ice, stable for several weeks [22]
<5>, 0°C, stable for at least 1 month [9]
<8>, -20°C, 10 mM Na_2HPO_4, pH 7.4, 50% v/v glycerol, 1 mM DTT, 0.001 mM ATP, stable for 2 months [17]
<8>, 0-4°C, stable for at least 1 week [7]

References

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[2] Novogrodsky, A.; Hurwitz, J.: The enzymatic phosphorylation of ribonucleic acid and deoxyribonucleic acid. I. Phosphorylation at 5-hydroxyl termini. J. Biol. Chem., **241**, 2923-2932 (1966)
[3] Novogrodsky, A.; Tal, M.; Traub, A.; Hurwitz, J.: The enzymatic phosphorylation of ribonucleic acid and deoxyribonucleic acid. II. Further properties of the 5-hydroxyl polynucleotide kinase. J. Biol. Chem., **241**, 2933-2943 (1966)
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Diphosphate-glycerol phosphotransferase 2.7.1.79

1 Nomenclature

EC number
2.7.1.79

Systematic name
diphosphate:glycerol 1-phosphotransferase

Recommended name
diphosphate-glycerol phosphotransferase

Synonyms
PPi-glycerol phosphotransferase
phosphotransferase, pyrophosphate-glycerol
pyrophosphate-glycerol phosphotransferase

CAS registry number
37278-13-0

2 Source Organism

<1> *Rattus norvegicus* (enzyme concentration decreases from fed over fasted to diabetic animals [1]) [1]

3 Reaction and Specificity

Catalyzed reaction
diphosphate + glycerol = phosphate + glycerol 1-phosphate (<1> may be identical with EC 3.1.3.9 [1])

Reaction type
phospho group transfer

Substrates and products
S diphosphate + glycerol <1> (<1> may be identical with EC 3.1.3.9 [1]) (Reversibility: ? <1> [1]) [1]
P phosphate + glycerol 1-phosphate <1> [1]

5 Isolation/Preparation/Mutation/Application

Source/tissue

kidney <1> [1]
liver <1> (<1> enzyme concentration decreases from fed over fasted to diabetic animals [1]) [1]

Localization

microsome <1> [1]

References

[1] Stetten, M.R.: Enzymatic synthesis of glycerol I-phosphate. Elevation in diabetic and fasted animals, compared with glucose-6-phosphatase and related enzyme activities. Biochim. Biophys. Acta, **208**, 394-403 (1970)

Diphosphate-serine phosphotransferase 2.7.1.80

1 Nomenclature

EC number
2.7.1.80

Systematic name
diphosphate:L-serine O-phosphotransferase

Recommended name
diphosphate-serine phosphotransferase

Synonyms
phosphotransferase, pyrophosphate-serine
pyrophosphate-L-serine phosphotransferase
pyrophosphate-serine phosphotransferase
pyrophosphate:L-serine O-phosphotransferase
pyrophosphate:L-serine phosphotransferase

CAS registry number
37205-58-6

2 Source Organism

<1> *Propionibacterium shermanii* (strain ATCC 9614 [1]) [1]

3 Reaction and Specificity

Catalyzed reaction
diphosphate + L-serine = phosphate + O-phospho-L-serine (<1> binary or ping-pong mechanism [1])

Reaction type
phospho group transfer

Natural substrates and products
S diphosphate + L-serine <1> (Reversibility: ? <1> [1]) [1]
P phosphate + O-phospho-L-serine <1> [1]

Substrates and products
S diphosphate + DL-homoserine <1> (<1> 7% activity compared to L-serine [1]) (Reversibility: ? <1> [1]) [1]
P phosphate + O-phospho-DL-homoserine
S diphosphate + L-serine <1> (Reversibility: ? <1> [1]) [1]

P phosphate + O-phospho-L-serine <1> [1]
S diphosphate + L-threonine <1> (<1> 8% activity compared to L-serine [1]) (Reversibility: ? <1> [1]) [1]
P phosphate + O-phospho-L-threonine
S diphosphate + N-chloroacetyl-L-serine <1> (<1> 19% activity compared to L-serine [1]) (Reversibility: ? <1> [1]) [1]
P phosphate + N-chloroacetyl-O-phospho-L-serine
S diphosphate + α-methylserine <1> (<1> 7% activity compared to L-serine [1]) (Reversibility: ? <1> [1]) [1]
P phosphate + α-methyl-O-phospho-L-serine
S diphosphate + glycyl-L-serine <1> (<1> 16% activity compared to L-serine [1]) (Reversibility: ? <1> [1]) [1]
P phosphate + glycyl-O-phospho-L-serine
S Additional information <1> (<1> substrate specificity [1]; <1> no activity with D-serine and L-cysteine, tripolyphosphate can substitute to some extent for diphosphate [1]) [1]
P ?

Inhibitors

Ca^{2+} <1> (<1> 50% inhibition at 1 mM [1]) [1]
Cd^{2+} <1> (<1> 65% inhibition at 1 mM [1]) [1]
D-serine <1> (<1> 20 mM, weak inhibition [1]) [1]
DL-homoserine <1> (<1> 100 mM, weak inhibition [1]) [1]
EDTA <1> (<1> inpresence of phosphate [1]) [1]
Hg^{2+} <1> (<1> 55% inhibition at 1 mM [1]) [1]
L-alanine <1> (<1> 35% inhibition at 10 mM [1]) [1]
L-threonine <1> (<1> 100 mM, weak inhibition [1]) [1]
La^{3+} <1> (<1> 25% inhibition at 1 mM [1]) [1]
Sr^{2+} <1> (<1> weak inhibition [1]) [1]
Tris <1> (<1> 0.6 M, weak inhibition [1]) [1]
Zn^{2+} <1> (<1> 85% inhibition at 10 mM [1]) [1]
glycerol <1> (<1> 100 mM, weak inhibition [1]) [1]
glycine <1> (<1> 20% inhibition at 10 mM [1]) [1]
glycyl-L-serine <1> (<1> weak inhibition [1]) [1]
phosphate <1> (<1> competitive [1]) [1]

Metals, ions

Ba^{2+} <1> (<1> slight stimulation, about 14% of activity compared to Mg^{2+} [1]) [1]
Co^{2+} <1> (<1> slight stimulation, about 42% of activity compared to Mg^{2+} [1]) [1]
Cu^{2+} <1> (<1> slight stimulation, about 14% of activity compared to Mg^{2+} [1]) [1]
Mg^{2+} <1> (<1> high stimulation [1]) [1]
Mn^{2+} <1> (<1> slight stimulation, about 34% of activity compared to Mg^{2+} [1]) [1]
Zn^{2+} <1> (<1> slight stimulation, about 14% of activity compared to Mg^{2+} [1]) [1]

Specific activity (U/mg)
9.5 <1> (<1> partially purified enzyme [1]) [1]

K_m-Value (mM)
0.1 <1> (diphosphate, <1> pH 7.8, 37°C [1]) [1]
0.13 <1> (Mg^{2+}, <1> 1 mM diphosphate, pH 7.8, 37°C [1]) [1]
1.9 <1> (L-serine, <1> pH 7.8, 37°C [1]) [1]

K_i-Value (mM)
0.8 <1> (glycine, <1> pH 7.8, 37°C [1]) [1]

pH-Optimum
7 <1> [1]

pH-Range
5.4-7.8 <1> (<1> 60% of maximal activity at pH 5.4 and pH 7.8 [1]) [1]

Temperature optimum (°C)
37 <1> (<1> assay at [1]) [1]

4 Enzyme Structure

Molecular weight
65000 <1> (<1> gel filtration [1]) [1]

5 Isolation/Preparation/Mutation/Application

Purification
<1> (partial, 102fold [1]) [1]

6 Stability

Temperature stability
30 <1> (<1> stable at least 12 h [1]) [1]
37 <1> (<1> stable at least 1 h [1]) [1]
65 <1> (<1> 15 min, 46% remaining activity [1]) [1]

Storage stability
<1>, 4°C or -15°C, 0.02 M Tris buffer, pH 7.8, stable for several weeks [1]

References

[1] Cagen, L.M.; Friedmann, H.C.: Enzymatic phosphorylation of serine. J. Biol. Chem., **247**, 3382-3392 (1972)

Hydroxylysine kinase 2.7.1.81

1 Nomenclature

EC number
2.7.1.81

Systematic name
GTP:5-hydroxy-L-lysine O-phosphotransferase

Recommended name
hydroxylysine kinase

Synonyms
guanosine triphosphate:5-hydroxy-L-lysine O-phosphotransferase
hydroxylysine kinase (phosphorylating)
kinase, hydroxylysine (phosphorylating)

CAS registry number
9073-58-9

2 Source Organism

<-1> no activity in *Homo sapiens* (no activity in liver and kidney [1]) [1]
<1> *Rattus norvegicus* [1, 2]
<2> *Gallus gallus* [1]
<3> *Oryctolagus cuniculus* [1]
<4> *Mus musculus* [1]
<5> *Bos taurus* [1]
<6> *Cebus albifrons* [1]
<7> *Cercopithecus aethiops* [1]

3 Reaction and Specificity

Catalyzed reaction
GTP + 5-hydroxy-L-lysine = GDP + 5-phosphonooxy-L-lysine

Reaction type
phospho group transfer

Natural substrates and products
S GTP + 5-hydroxy-L-lysine <1> (<1> erythro-5-hydroxy-L8-lysine [1]) (Reversibility: ? <1> [1, 2]) [1, 2]
P GDP + O-phosphohydroxy-L-lysine <1> [1, 2]

Substrates and products

S GTP + 5-hydroxy-L-lysine <1> (<1> erythro-5-hydroxy-L8-lysine [1]) (Reversibility: ? <1> [1,2]) [1, 2]
P GDP + O-phosphohydroxy-L-lysine <1> [1, 2]
S GTP + allohydroxy-L-lysine <1> (Reversibility: ? <1> [1]) [1]
P ?
S Additional information <1> (<1> no activity with threonine, serine, or hydroxyproline [2]; <1> no activity with D-isomers, ITP can partially substitute for GTP [1]) [1, 2]
P ?

Inhibitors

2-amino-5-hydroxyadipate <1> (<1> 4 isomers, 40 mM, strong inhibition [1]) [1]
4,5-dehydro-(trans)-L-lysine <1> [1]
D-lysine <1> [1, 2]
D-ornithine <1> [1, 2]
L-2,4-diamino-n-butyrate <1> [1]
L-homoserine <1> (<1> 40 mM, weak inhibition [1]) [1]
L-lysine <1> (<1> higher inhibitory effect than with D-lysine [1]) [1, 2]
L-ornithine <1> (<1> higher inhibitory effect than with D-ornithine [1]) [1, 2]
Mn^{2+} <1> (<1> at 1 mM, in presence of 1 mM Mg^{2+}, strong inhibition, but activation in absence of Mg^{2+} [1]) [1]
S-aminoethyl-L-cysteine <1> [1]
Zn^{2+} <1> (<1> at 1 mM, in presence of 1 mM Mg^{2+}, strong inhibition [1]) [1]
carnitine <1> (<1> 2 isomers [1]) [1]
choline <1> (40 mM, weak inhibition [1]) [1, 2]
ethanolamine <1> (<1> 40 mM, weak inhibition [1]) [1, 2]
hydroxy-L-pipecolate <1> [1]
hydroxy-L-proline <1> (<1> 40 mM, strong [1]) [1]
Additional information <1> (<1> no inhibition by serine, threonine, and hydroxyproline [2]) [2]

Metals, ions

Ca^{2+} <1> (<1> requirement of a divalent metal ion, 1 mM, 13% of activity compared to Mg^{2+} [1]) [1]
Co^{2+} <1> (<1> requirement of a divalent metal ion, 1 mM, 13% of activity compared to Mg^{2+} [1]) [1]
Cu^{2+} <1> (<1> requirement of a divalent metal ion, 1 mM, 13% of activity compared to Mg^{2+} [1]) [1]
Fe^{2+} <1> (<1> requirement of a divalent metal ion, 1 mM, 13% of activity compared to Mg^{2+} [1]) [1]
Hg^{2+} <1> (<1> requirement of a divalent metal ion, 1 mM, 13% of activity compared to Mg^{2+} [1]) [1]
Mg^{2+} <1> (<1> requirement of a divalent metal ion, Mg^{2+} yields highest activity [1]; <1> required [2]) [1, 2]

Mn^{2+} <1> (<1> requirement of a divalent metal ion, 1 mM, 13% of activity compared to Mg^{2+}, strong inhibition in presence of 1 mM Mg^{2+} [1]) [1]
Additional information <1> (<1> K^+, Li^+ have no influence) [1]

Specific activity (U/mg)
0.114 <1> [1]

K_m-Value (mM)
0.0056 <1> (hydroxy-L-lysine, <1> pH 8.0, 37°C [1]) [1]
0.0062 <1> (allohydroxy-L-lysine, <1> pH 8.0, 37°C [1]) [1]
0.0238 <1> (5-hydroxy-lysine) [2]

pH-Optimum
7.7 <1> [1]
8 <1> [2]

pH-Range
6-9 <1> (<1> 25% of maximal activity at pH 6.0, 35% of maximal activity at pH 9.0 [1]) [1]

Temperature optimum (°C)
37 <1> (<1> assay at [1]) [1]

5 Isolation/Preparation/Mutation/Application

Source/tissue
kidney <1, 3, 4, 6, 7> (<1> cortex [2]) [1, 2]
liver <1-6> [1]

Purification
<1> (1200fold [1]; partial [1,2]) [1, 2]

6 Stability

Storage stability
<1>, frozen, little loss of activity for weeks [1]

References

[1] Hiles, R.A.; Henderson, LaVell M.: The partial purification and properties of hydroxylysine kinase from rat liver. J. Biol. Chem., **247**, 646-651 (1972)
[2] Chang, A.Y.: Guanosine triphosphate: 5-hydroxylysine phosphotransferase in rat kidney cortex. Enzyme, **22**, 230-234 (1977)

Ethanolamine kinase 2.7.1.82

1 Nomenclature

EC number
2.7.1.82

Systematic name
ATP:ethanolamine O-phosphotransferase

Recommended name
ethanolamine kinase

Synonyms
EKI
EKI1
EtnK
ethanolamine phosphokinase
kinase, ethanolamine (phosphorylating)
Additional information <1-8, 10, 11, 14, 17> (<14> choline kinase and ethanolamine kinase activities are mediated by 2 distinct active sites, possibly on a single protein [7]; <17> choline kinase and ethanolamine kinase may not have a common active site in a single enzyme protein [8]; <1-8, 10, 11, 17> choline kinase and ethanolamine kinase are 2 distinct enzymes [9, 10, 12, 14]; <17> ethanolamine kinase II and choline kinase do not use a common active site [11]; cf. EC 2.7.1.32) [7-12, 14]

CAS registry number
9075-78-9

2 Source Organism

<1> *Capra hircus* [9]
<2> *Heteropneustes fossilis* (catfish [9]) [9]
<3> *Rana tigrina* (frog [9]) [9]
<4> *Bufo malanostictus* (toad [9]) [9]
<5> *Hemidactylus sp.* (lizard [9]) [9]
<6> *Gallus gallus* (chicken [9]) [9]
<7> *Columba livia* (pigeon [9]) [9]
<8> *Cavia porcellus* [9]
<9> *Culex pipiens fatigans* [13]
<10> *Phormia regina* [14]

<11> *Mus musculus* (Ehrlich ascites cells [1]; α isoform, liver, inducible by CCl_4 [15]) [1, 9, 15]
<12> *Flavobacterium rhenanum* (NCIB 9157, enzyme formed only when amino alcohol serves as a substrate and when activated by ADP [2]) [2]
<13> *Flavobacterium arborescens* (NCIB 8185, enzyme formed only when amino alcohol serves as a substrate and when activated by ADP [2]) [2]
<14> *Bos taurus* [7]
<15> *Achromobacter sp.* (NCIB 9205 [2]) [2]
<16> *Homo sapiens* [5, 20]
<17> *Rattus norvegicus* [3, 8-12, 16, 20, 21]
<18> *Glycine max* [4]
<19> *Spinacia oleracea* [6]
<20> *Homo sapiens* (SwissProt-ID: Q9HBU6) [17]
<21> *Saccharomyces cerevisiae* [18, 20]
<22> *Drosophila melanogaster* [19]
<23> *Mus musculus* (isozyme α, several exxons [22]) [22]
<24> *Mus musculus* (isozyme β [22]) [22]

3 Reaction and Specificity

Catalyzed reaction

ATP + ethanolamine = ADP + O-phosphoethanolamine (<14> choline kinase and ethanolamine kinase activities are mediated by 2 distinct active sites, possibly on a single protein [7]; <17> choline kinase and ethanolamine kinase may not have a common active site in a single enzyme protein [8]; <1-8, 10, 11, 17> choline kinase and ethanolamine kinase are 2 distinct enzymes [9, 10, 12, 14]; <17> ethanolamine kinase II and choline kinase do not use a common active site [11]; <17> major part of choline kinase and ethanolamine kinase activities are catalyzed by the same enzyme [20])

Reaction type

phospho group transfer

Natural substrates and products

S ATP + ethanolamine <12, 13, 15, 20> (Reversibility: ? <12, 13, 15, 20> [2, 17]) [2, 17]
P ADP + O-phosphoethanolamine
S Additional information <12, 13, 15, 20> (<12, 13, 15> bacteria: enzyme functions in a biodegradative mode, higher organisms: biosynthetic function [2]; <20> rate-controlling step in phosphatidylethanolamine biosynthesis [17]) [2, 17]
P ?

Substrates and products

S ATP + DL-1-aminopropan-2-ol <12, 13, 15> (Reversibility: ? <12, 13, 15> [2]) [2]
P ADP + DL-1-aminopropane 2-phosphate

S ATP + choline <20, 21> (<20> very little activity [17]) (Reversibility: ? <20, 21> [17, 18]) [17, 18]
P ADP + ?
S ATP + ethanolamine <1-22> (Reversibility: ? <1-22> [1-14, 17-19]) [1-14, 17-19]
P ADP + O-phosphoethanolamine <12, 13, 15> [2]
S GTP + ethanolamine <9> (<9> 13% of the activity with ATP [13]) [13]
P GDP + O-phosphoethanolamine
S ITP + ethanolamine <9> (<9> 45% of the activity with ATP [13]) [13]
P IDP + O-phosphoethanolamine
S choline + ATP <17> (<18> not [4]; <17> ethanolamine kinase II, not ethanolamine kinase I [11]) (Reversibility: ? <17> [11]) [11]
P ADP + O-phosphocholine

Inhibitors

1,10-phenanthroline <19> [6]
2,3-dimercaptopropanol <11> [1]
ADP <9> [13]
AMP <9> [13]
ATP <9, 17> (<17> concentration exceeding that of Mg^{2+} [8]; <9> above 8 mM [13]; <17> free [3]) [3, 8, 13]
CTP <17> [10]
Ca^{2+} <9, 11, 18> (<9> inhibits activating effect of Mg^{2+} [13]; <18> in presence of Mg^{2+} [4]) [1, 4, 13]
Cu^{2+} <9, 10> (<9> inhibits activating effect of Mg^{2+} [13]) [13, 14]
EDTA <18, 19> [4, 6]
EGTA <19> (<19> stimulation at 0.4-0.9 mM, inhibition at 1.8 mM [6]) [6]
KCl <9> (<9> at 10 mM [13]) [13]
$MgATP^{2-}$ <19> (<19> substrate inhibition [6]) [6]
Mn^{2+} <9, 11, 14, 17, 18> (<18> in presence of Mg^{2+} [4]; <9> presence of Mn^{2+} at lower concentrations of Mg^{2+} has an additive stimulating effect, at higher concentrations of Mg^{2+}, Mn^{2+} inhibits [13]) [1, 3, 4, 7, 8, 10, 13]
N,N-dimethylethanolamine <17, 19> [3, 6]
N-ethylmaleimide <9> [13]
N-methyl-N-isopropylethanolamine <9> [13]
N-methylethanolamine <17> [3]
Ni^{2+} <9> (<9> inhibits activating effect of Mg^{2+} [13]) [13]
acetyl-β-methylcholine chloride <11> [1]
acetylcholine <11> [1]
adenosine <9> [13]
betaine <17> (<11, 17> not [1,3]) [10]
butyrylcholine iodide <11> [1]
butyrylthiocholine iodide <11> [1]
choline <11, 14, 17> (<17> ethanolamine kinase I not inhibited, ethanolamine kinase II strongly inhibited [11]; <17> very strong [8]; <17> mixed type [10]; <9, 18, 19> not [4, 6, 13]) [1, 3, 7, 8, 10, 11]
choline phosphate <17> [10]

cysteine <11> [1]
ethanolaminephosphate <17> [10]
hemicholinium <17> [10, 12]
methane sulfonylcholine chloride <11> [1]
monomethylethanolamine <19> [6]
p-chloromercuribenzoate <9> (<11> not [1]) [13]
phosphorylcholine <11> (<9,17> not [3,13]) [1]
phosphorylethanolamine <9, 11> (not, <17> [3]) [1, 13]
propionylcholine iodide <11> [1]
stearoyl-CoA <17> [10]
succinylcholine chloride <11> [1]
Additional information <9-11, 17> (<17> not inhibitory: CDP-choline [3]; <17> not inhibitory: citrate [10]; <9> not inhibitory: serine [13]; <9> not inhibitory: N-methyl-N,N-diisopropylethanolamine [13]; <11> not inhibitory: sodium arsenite [1]; <9> not inhibitory: N-methyl-N-butylethanolamine [13]; <9> not inhibitory: N,N,N-triethanolamine [13]; <9> not inhibitory: N-ethyl-N,N-dibutylethanolamine [13]; <9> not inhibitory: N,N-diisopropylethanolamine [13]; <10> criterion for an effective inhibitor is the presence of only one N-alkyl group (except dimethylethanol) and a distance of 2 carbon atoms between the N atom and the OH group [14]) [1, 3, 10, 13, 14]

Cofactors/prosthetic groups

ADP <12> (<12> stimulates [2]) [2]

Activating compounds

2-mercaptoethanol <9> (<9> stimulates [13]) [13]
EGTA <19> (<19> stimulation at 0.4-0.9 mM, inhibition at 1.8 mM [6]) [6]
cysteine <9> (<9> stimulates [13]) [13]
glutathione <9> (<9> stimulates [13]) [13]

Metals, ions

Ca^{2+} <17> (<17> can substitute for Mg^{2+} [11]; <11> neither Ca^{2+} nor Mn^{2+} can substitute for Mg^{2+} [1]) [11]
Mg^{2+} <9, 11-13, 15-19> (<19> free Mg^{2+} essential for maximal velocity [6]; <11-13, 15, 16> Mg^{2+} required [1, 2, 5]; <19> K_m $MgATP^{2-}$: 0.063 mM [6]; <12, 13, 15> maximum concentration equal to that of ATP [2]; <17-19> Mg-ATP complex is the substrate [3, 4, 6]; <17> 1.25-2.5 mM ATP: reaction requires free Mg^{2+} rather than an ATP-Mg^{2+} complex for maximal velocity [8]; <17> ATP concentration exceeding that of Mg^{2+}: strong inhibition [8]; <17> K_m $MgATP^{2-}$: 10 mM, in presence of equivalent amounts of ATP and Mg^{2+} [8]; <17> 1.5 mM, in 1.5-fold higher concentration of Mg^{2+} than ATP [8]; <17> ethanolamine kinase I: uses $MgATP^{2-}$ as substrate but also has an additional requirement for Mg^{2+} [11]; <17> maximal activation at 16 mM $MgATP^{2-}$, inhibition above [11]; <17> K_m $MgATP^{2-}$: 14 mM [11]; <17> ethanolamine kinase II: requires $MgATP^{2-}$ as substrate, maximal activation with 10 mM of Mg^{2+} and ATP [11]; <17> requires equimolar concentrations of Mg^{2+} and ATP (30 mM) for optimal activation [12]; <9> absolute requirement for Mg^{2+} and Mn^{2+}, Mg^{2+} is the most active divalent cation at concen-

trations above 5 mM, optimum: 18 mM, Mn^{2+} has a greater stimulatory effect on the enzyme than Mg^{2+} at levels less than 5 mM, presence of Mn^{2+} at lower concentrations of Mg^{2+} has an additive effect, at higher concentrations of Mg^{2+}, Mn^{2+} inhibits [13]) [1-6, 8, 11-13]
Mn^{2+} <9> (<9> absolute requirement for Mg^{2+} and Mn^{2+}, Mg^{2+} is the most active divalent cation at concentrations above 5 mM, optimum: 18 mM, Mn^{2+} has a greater stimulatory effect on the enzyme than Mg^{2+} at levels less than 5 mM, presence of Mn^{2+} at lower concentrations of Mg^{2+} has an additive effect, at higher concentrations of Mg^{2+}, Mn^{2+} inhibits [13]; <11> inhibition [1]) [13]

Turnover number (min^{-1})

0.0096 <16> (ethanolamine) [5]

Specific activity (U/mg)

1.398 <17> (<17> pH 8.5 [8]) [8]
10.73 <18> (<18> pH 8.5 [4]) [4]
Additional information <9, 16> [5, 13]

K_m-Value (mM)

0.008 <18> (ethanolamine, <18> pH 8.5 [4]) [4]
0.02 <21> (choline) [20]
0.041 <17> (ethanolamine, <17> 30°C, pH 9.0 [21]) [21]
0.042 <19> (ethanolamine) [6]
0.1 <17> (ethanolamine, <17> 37°C, pH 8.5 [3]) [3]
0.171 <21> (ethanolamine, <21> 30°C, pH 8.5 [18]) [18]
0.25 <16> (ethanolamine, <16> 37°C, pH 8.5 [5]) [5]
0.275 <21> (choline, <21> 30°C, pH 8.5 [18]) [18]
0.36 <12> (DL-1-aminopropan-2-ol) [2]
0.5 <12, 13, 15> (ATP) [2]
0.53 <12> (ethanolamine) [2]
1.5 <17> ($MgATP^{2-}$, <17> with 1.5-fold higher concentration of Mg^{2+} than ATP, pH 8.5 [8]) [8]
7.7 <17> (ethanolamine) [10]
10 <17> ($MgATP^{2-}$, <17> in presence of equivalent amounts of ATP and Mg^{2+}, pH 8.5 [8]) [8]
10 <21> (ethanolamine) [20]
Additional information <9, 14, 17> [7, 8, 10, 12, 13]

pH-Optimum

7.5-8.5 <17> (<17> ethanolamine kinase I [11]) [11]
8 <12, 14> [2, 7]
8-9 <17> [8]
8-9.5 <17> (<17> ethanolamine kinase II [11]) [11]
8.5 <17, 18> [3, 4]
8.5-10.5 <11> [1]

pH-Range

7.4-10 <17> [12]
8-9 <17> (<17> 80% of maximum activity at pH 8.0 and pH 9.0 [3]) [3]

Temperature optimum (°C)

30 <19> (<19> assay at [6]) [6]
37 <9, 11> (<11> assay at [1]) [1, 13]

4 Enzyme Structure

Molecular weight

17000-19000 <18> (<18> gel filtration, monomeric form [4]) [4]
36000 <17> (<17> ethanolamine kinase I, gel filtration [11]) [11]
36000-37000 <18> (<18> dimeric form, gel filtration [4]) [4]
44000 <9> (<9> gel filtration [13]) [13]
87000 <16> (<16> gel filtration [5,20]) [5, 20]
90000 <17> (<17> gel filtration [20]) [20]
110000 <19> (<19> gel filtration [6]) [6]
160000 <17> (<17> ethanolamine kinase II, gel filtration [11]) [11]
190000 <17> (<17> gel filtration [21]) [21]

Subunits

dimer <16-18> (<18> 2 * 17000-19000, SDS-PAGE, enzyme exists in monomeric and dimeric form [4]; <16> 2 * 44000, SDS-PAGE [5]; <17> 2 * 42000, SDS-PAGE [20]; <16> 2 * 44000, SDS-PAGE [20]; <17> 2 * 86000, SDS-PAGE [21]) [4, 5, 20, 21]
monomer <18> (<18> 1 * 17000-19000, SDS-PAGE, gel filtration, enzyme exists in monomeric and dimeric form [4]) [4]
oligomer <11> (<11> x * 47000, or x-y * 47000 + y *43000, or x * 43000, active form consitsts of oligomers of α/β isoforms, with relatively small parts of α/α or β/β homo-oligomers, SDS-PAGE [15]) [15]

5 Isolation/Preparation/Mutation/Application

Source/tissue

Ehrlich ascites carcinoma cell <11> [1]
brain <17> (<17> nerve endings from forebrain [12]) [9, 12]
head <22> [19]
kidney <17> [8, 20]
larva <9, 10> [13, 14]
leaf <19> [6]
lens <17> [16]
liver <11, 16, 17> (<11> α isozyme [15]) [3, 5, 9-11, 15, 20, 21]
mammary gland <14> [7]
seed <18> (<18> germinating [4]) [4]
testis <11> (<11> α isozyme [15]) [15]

Additional information <11> (<11> β isozyme, almost equal distribution in all tissues examined [15]) [15]

Localization

cytoplasm <17> [12]
cytosol <1-8, 11, 17> [8-10]
Additional information <11, 14, 17> (<17> a small percentage is membrane associated [10]; <11, 14, 17> supernatant fraction [1, 3, 7]; <14> high speed supernatant [7]) [1, 3, 7, 10]

Purification

<16> [5]
<17> (copurification of choline kinase and ethanolamine kinase [8]; ethanolamine kinase I and II [11]) [8, 11]
<18> [4]
<19> (partial [6]) [6]

Cloning

<20> [17]
<21> [18]
<16, 17, 21> (overview [20]) [20]
<23, 24> [22]

Engineering

Additional information <21, 22> (<21> eki1δ, main enzyme responsible for phosphoethanolamine synthesis eki1δ cki1δ, no residual enzymic activity [18]; <22> easily shocked mutation, significantly reduced levels of O-phosphoethanolamine and phosphatidylserine [19]) [18, 19]

Application

medicine <17> (<17> galactosemic cataractogenesis changes substrate specificity of enzyme [16]) [16]

6 Stability

Temperature stability

60 <9> (<9> 20 min, complete inactivation [13]) [13]

General stability information

<9>, inactivation by repeated freezing and thawing [13]

Storage stability

<9>, -20°C, 10 days, stable [13]
<11>, -20°C or 0-4°C, crude cell extract, appreciable loss of activity [1]
<17>, -20°C, 0.02 M phosphate buffer, pH 7.0, 2.9 mM DTT, protein concentration 1.0 mg/ml, ethanolamine kinase I, 2 days, 40% loss of activity [11]
<17>, 4°C, 1 week, ethanolamine kinase I, 50% loss of activity [11]
<19>, 0-4°C or-20°C, partially purified enzyme, 10-14 days, 50% loss of activity [6]

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Pseudouridine kinase 2.7.1.83

1 Nomenclature

EC number
2.7.1.83

Systematic name
ATP:pseudouridine 5'-phosphotransferase

Recommended name
pseudouridine kinase

Synonyms
kinase, pseudouridine (phosphorylating)

CAS registry number
62213-40-5

2 Source Organism

<1> *Escherichia coli* (strain Bu- and B5Ru [1]) [1]

3 Reaction and Specificity

Catalyzed reaction
ATP + pseudouridine = ADP + pseudouridine 5'-phosphate

Reaction type
phospho group transfer

Natural substrates and products
S ATP + pseudouridine <1> (<1> involved in utilization of pseudouridine as pyrimidine source by E. coli pyrimidine auxotrophs [1]) [1]
P ADP + pseudouridine-5'-monophosphate

Substrates and products
S ATP + pseudouridine <1> (Reversibility: ? <1> [1]) [1]
P ADP + pseudouridine-5'-monophosphate <1> (<1> i.e. pseudouridylate [1]) [1]

Metals, ions
K^+ <1> (<1> requirement [1]) [1]
Mg^{2+} <1> (<1> requirement [1]) [1]

NH_4^+ <1> (<1> can partially substitute for K^+ [1]) [1]
Additional information <1> (<1> Li^+, Na^+ are ineffective [1]) [1]

K_m-Value (mM)
0.18 <1> (pseudouridine, <1> pH 7.0 [1]) [1]
2.9 <1> (ATP, <1> pH 7.0, 37°C [1]) [1]
3.3 <1> (K^+, <1> pH 7.0, 37°C [1]) [1]
40 <1> (NH_4^+, <1> pH 7.0, 37°C [1]) [1]

pH-Optimum
7 <1> [1]

Temperature optimum (°C)
37 <1> (<1> assay at [1]) [1]

5 Isolation/Preparation/Mutation/Application

Purification
<1> (ammonium sulfate, partially purified [1]) [1]

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Alkylglycerone kinase 2.7.1.84

1 Nomenclature

EC number
2.7.1.84

Systematic name
ATP:O-alkylglycerone phosphotransferase

Recommended name
alkylglycerone kinase

Synonyms
alkyldihydroxyacetone (phosphorylating) kinase
alkyldihydroxyacetone kinase
kinase, alkyldihydroxyacetone (phosphorylating)

CAS registry number
52227-80-2

2 Source Organism

<1> *Mus musculus* (C57BL/6mice [1]) [1]

3 Reaction and Specificity

Catalyzed reaction
ATP + O-alkylglycerone = ADP + O-alkylglycerone phosphate

Reaction type
phospho group transfer

Natural substrates and products
S ATP + O-alkylglycerone <1> (Reversibility: ? <1> [1]) [1]
P ADP + O-alkylglycerone phosphate <1> [1]
S ATP + octadecyldihydroxyacetone <1> (<1> involved in biosynthesis and degradation of complex ether lipids [1]) (Reversibility: ir <1> [1]) [1]
P ADP + O-octadecyldihydroxyacetone phosphate <1> [1]

Substrates and products
S ATP + O-alkylglycerone <1> (Reversibility: ? <1> [1]) [1]
P ADP + O-alkylglycerone phosphate <1> [1]

S ATP + octadecyldihydroxyacetone <1> (<1> reverse reaction is catalysed by a different phosphatase [1]) (Reversibility: ir <1> [1]) [1]
P ADP + O-octadecyldihydroxyacetone phosphate <1> [1]

Cofactors/prosthetic groups
ATP <1> (<1> required [1]) [1]

Metals, ions
Mg^{2+} <1> (<1> requirement [1]) [1]

K_m-Value (mM)
3.6 <1> (ATP, <1> pH 7.0, 37°C [1]) [1]

pH-Optimum
7 <1> (<1> assay at [1]) [1]

Temperature optimum (°C)
37 <1> (<1> assay at [1]) [1]

5 Isolation/Preparation/Mutation/Application

Source/tissue
preputial gland tumor <1> (<1> ESR-586, grown subcutaneously [1]) [1]

Localization
microsome <1> [1]

Purification
<1> (C57BL/6mice, partial [1]) [1]

References

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β-Glucoside kinase 2.7.1.85

1 Nomenclature

EC number
2.7.1.85

Systematic name
ATP:cellobiose 6-phosphotransferase

Recommended name
β-glucoside kinase

Synonyms
BglK
β-glucoside (cellobiose):phosphotransferase
β-glucoside kinase
kinase, β-D-glucoside (phosphorylating)

CAS registry number
37205-53-1

2 Source Organism

<1> *Aerobacter aerogenes* [1, 2]
<2> *Klebsiella pneumonia* (ATCC 23357 [3]) [3]
<3> *Listeria monocytogenes* (EGD-e [3]) [3]
<4> *Listeria innocua* (CLIP 11262 [3]) [3]

3 Reaction and Specificity

Catalyzed reaction
ATP + cellobiose = ADP + 6-phospho-β-D-glucosyl-(1,4)-D-glucose

Reaction type
phospho group transfer

Natural substrates and products
S ATP + cellobiose <1> (<1> inducible enzyme that functions in metabolism of cellobiose and gentiobiose [2]; <1> first reaction in metabolism of cellobiose, inducible enzyme [1]) (Reversibility: ? <1> [1, 2]) [1, 2]
P ADP + 6-phospho-β-D-glucosyl-(1,4)-D-glucose

S ATP + gentiobiose <1> (<1> inducible enzyme that functions in metabolism of cellobiose and gentiobiose [2]) (Reversibility: ? <1> [1]) [2]
P ADP + 6-phospho-β-D-glucosyl-(1,6)-D-glucose

Substrates and products

S ATP + D-glucose <2> (Reversibility: ? <2> [3]) [3]
P ADP + D-glucose 6-phosphate
S ATP + amygdalin <1, 2> (<1> 21% of the activity with cellobiose [1]) (Reversibility: ? <1,2> [1,2,3]) [1, 2, 3]
P ADP + amygdalin 6-phosphate
S ATP + arbutin <1, 2> (<1> 36% of the activity with cellobiose [1]) (Reversibility: ? <1,2> [1,2,3]) [1, 2, 3]
P ADP + arbutin 6-phosphate
S ATP + cellobiitol <1, 2> (<1> 25% of the activity with cellobiose [1]) (Reversibility: ? <1,2> [1,2,3]) [1, 2, 3]
P ?
S ATP + cellobiose <1, 2, 3, 4> (Reversibility: ? <1,2,3,4> [1,2,3]) [1, 2, 3]
P ADP + 6-phospho-β-D-glucosyl-(1,4)-D-glucose <1> [1]
S ATP + cellotetraose <1> (<1> 33% of the activity with cellobiose [1]) (Reversibility: ? <1> [1,2]) [1, 2]
P ADP + 6-phospho-β-glucosyl-(1,4)-β-D-glucosyl-(1,4)-β-D-glucosyl-(1,4)-D-glucose
S ATP + cellotriose <1> (<1> 47% of the activity with cellobiose [1]) (Reversibility: ? <1> [1]) [1]
P ADP + 6-phospho-β-D-glucosyl-(1,4)-β-D-glucosyl-(1,4)-D-glucose
S ATP + gentiobiose <1, 2> (<1> 70% of the activity with cellobiose [1]) (Reversibility: ? <1,2> [1,2,3]) [1, 2, 3]
P ADP + 6-phospho-β-D-glucosyl-(1,6)-D-glucose <1> [2]
S ATP + isopropyl β-D-thioglucopyranoside <2> (Reversibility: ? <2> [3]) [3]
P ?
S ATP + isopropyl β-D-thioglucopyranoside <2> (Reversibility: ? <2> [3]) [3]
P ADP + isopropyl β-D-thioglucopyranoside 6-phosphate
S ATP + laminaribiose <2> (Reversibility: ? <2> [3]) [3]
P ADP + 6-phospho-β-D-glucopyranosyl-(1,3)-D-glucose
S ATP + methyl β-D-glucoside <1, 2> (<1> as active as cellobiose [1]) (Reversibility: ? <1,2> [1,2,3]) [1, 2, 3]
P ADP + methyl β-D-glucoside 6-phosphate
S ATP + n-octyl-β-D-glucopyranoside <2> (Reversibility: ? <2> [3]) [3]
P ADP + n-octyl-β-D-glucopyranoside 6-phosphate
S ATP + phenyl β-D-glucoside <1, 2> (<1> 70% of the activity with cellobiose [1]) (Reversibility: ? <1,2> [1,2,3]) [1, 2, 3]
P ADP + phenyl β-D-glucoside 6-phosphate
S ATP + salicin <1, 2> (<1> 87% of the activity with cellobiose [1]) (Reversibility: ? <1,2> [1,2,3]) [1, 2, 3]
P ADP + salicin 6-phosphate

S ATP + sophorose <1, 2> (<1> 19% of the activity with cellobiose [1]) (Reversibility: ? <1,2> [1,2,3]) [1, 2, 3]
P ADP + 6-phospho-β-D-glucopyranosyl-(1,2)-D-glucose
S ATP + thiocellobiose <2> (Reversibility: ? <2> [3]) [3]
P ?
S ATP + thiocellobiose <2> (Reversibility: ? <2> [3]) [3]
P ?
S CTP + cellobiose <1> (<1> 12% of the activity with ATP [1]) [1]
P CDP + 6-phospho-β-D-glucosyl-(1,4)-D-glucose
S CTP + cellobiose <1> (<1> 12% of the activity with ATP [1]) (Reversibility: ? <1> [1]) [1]
P CDP + 6-phospho-β-D-glucosyl-(1,4)-D-glucose <1> [1]
S GTP + cellobiose <1> (<1> 15% of the activity with ATP [1]) (Reversibility: ? <1> [1]) [1]
P GDP + 6-phospho-β-D-glucosyl-(1,4)-D-glucose
S GTP + cellobiose <1> (<1> 15% of the activity with ATP [1]) (Reversibility: ? <1> [1]) [1]
P GDP + 6-phospho-β-D-glucosyl-(1,4)-D-glucose <1> [1]
S ITP + cellobiose <1> (<1> 7% of the activity with ATP [1]) (Reversibility: ? <1> [1]) [1]
P IDP + 6-phospho-β-D-glucosyl-(1,4)-D-glucose
S ITP + cellobiose <1> (<1> 7% of the activity with ATP [1]) (Reversibility: ? <1> [1]) [1]
P IDP + 6-phospho-β-D-glucosyl-(1,4)-D-glucose <1> [1]
S UTP + cellobiose <1> (<1> 3% of the activity with ATP [1]) (Reversibility: ? <1> [1]) [1]
P UDP + 6-phospho-β-D-glucosyl-(1,4)-D-glucose
S Additional information <1> (<1> not: ADP, acetyl phosphate, α-glycerophosphate, D-fructose, 1,6-diphosphate, phosphoramidate, choline phosphate, creatine phosphate, 3-phosphoglycerate, phenylphosphate, phosphoenolpyruvate [1]) [1]
P ?

Inhibitors

NEM <2> (<2> considerable protection [3]) [3]

Specific activity (U/mg)

7.6 <1> [2]
24 <2> [3]
45 <1> [1]

K_m-Value (mM)

0.11 <2> (n-octyl β-D-glucopyranoside, <2> pH 7.5 [3]) [3]
0.18 <2> (salicin, <2> pH 7.5 [3]) [3]
0.21 <2> (arbutin, <2> pH 7.5 [3]) [3]
0.24 <2> (ATP, <2> pH 7.5 [3]) [3]
0.47 <2> (amygdalin, <2> pH 7.5 [3]) [3]
0.47 <2> (cellobiose, <2> pH 7.5 [3]) [3]

0.56 <2> (gentiobiose, <2> pH 7.5 [3]) [3]
0.6 <1> (arbutin, <1> pH 7.5 [1]) [1]
0.63 <2> (laminaribiose, <2> pH 7.5 [3]) [3]
0.65 <2> (phenyl β-D-glucopyranoside, <2> pH 7.5 [3]) [3]
1 <1> (cellobiose, <1> pH 7.5 [1]) [1]
1.5 <1> (gentiobiose, <1> pH 7.5 [1]) [1]
1.56 <2> (cellobiitol, <2> pH 7.5 [3]) [3]
1.7 <1> (ATP, <1> pH 7.5 [1]) [1]
2.03 <2> (isopropyl β-D-thioglucopyranoside, <2> pH 7.5 [3]) [3]
2.23 <2> (methyl β-D-glucopyranoside, <2> pH 7.5 [3]) [3]
2.5 <1> (phenyl β-D-glucoside, <1> pH 7.5 [1]) [1]
2.5 <1> (salicin, <1> pH 7.5 [1]) [1]
3.1 <1> (amygdalin, <1> pH 7.5 [1]) [1]
3.1 <1> (sophorose, <1> pH 7.5 [1]) [1]
3.15 <2> (sophorose, <2> pH 7.5 [3]) [3]
4 <1> (cellobiitol, <1> pH 7.5 [1]) [1]
6.3 <1> (cellotetraose, <1> pH 7.5 [1]) [1]
6.7 <1> (cellotriose, <1> pH 7.5 [1]) [1]
10.54 <2> (thiocellobiose, <2> pH 7.5 [3]) [3]
13.1 <1> (methyl β-D-glucoside, <1> pH 7.5 [1]) [1]
40.29 <2> (D-glucose, <2> pH 7.5 [3]) [3]

pH-Optimum

7.2 <1> (<1> glycylglycine buffer [2]) [2]
7.3 <1> (<1> MES buffer [1]) [1]

pH-Range

6.2-8.2 <1> (<1> pH 6.2: about 60% of maximal activity in Mes buffer, pH 8.2: reaction in PIPES buffer, about 70% of maximal activity in MES buffer [1]) [1]

4 Enzyme Structure

Molecular weight

150000 <1> (<1> sucrose density gradient centrifugation [1,2]) [1, 2]

Subunits

? <3, 4> (<3,4> x * 32000, SDS-PAGE [3]) [3]
tetramer <2> (<2> 4 * 32697, calculation from nucleotide sequence [3]; <2> 4 * 33000, non-covalently linked subunits, SDS-PAGE [3]) [3]

5 Isolation/Preparation/Mutation/Application

Purification

<1> [1, 2]
<2> (recombinant enzyme [3]) [3]

Cloning

<2> (cloning and high expression of His6BglK in Escherichia coli TOP10 [3]) [3]

Engineering

D103G <2> (<2> catalytically inactive protein [3]) [3]
D7G <2> (<2> catalytically inactive protein [3]) [3]
G131A <2> (<2> catalytically inactive protein [3]) [3]
G133A <2> (<2> catalytically inactive protein [3]) [3]
G9A <2> (<2> catalytically inactive protein [3]) [3]

6 Stability

Temperature stability

45 <1> (<1> half-life: 10 min [1]) [1]

Storage stability

<1>, 4°C, 35% loss of activity after 2 months [1, 2]
<1>, frozen, 50% loss of activity after 2 weeks [1]

References

[1] Palmer, R.E.; Anderson, R.L.: Cellobiose metabolism in Aerobacter aerogenes. II. Phosphorylation of cellobiose with adenosine 5-triphosphate by α-glucoside kinase. J. Biol. Chem., **247**, 3415-3419 (1972)
[2] Anderson, R.L.; Palmer, R.E.: Cellobiose metabolism in Aerobacter aerogenes. Methods Enzymol., **42 C**, 3-6 (1975)
[3] Thompson, J.; Lichtenthaler, F.W.; Peters, S.; Pikis, A.: β-Glucoside kinase (BglK) from Klebsiella pneumoniae. Purification, properties, and preparative synthesis of 6-phospho-β-D-glucosides. J. Biol. Chem., **277**, 34310-34321 (2002)

NADH Kinase 2.7.1.86

1 Nomenclature

EC number
2.7.1.86

Systematic name
ATP:NADH 2'-phosphotransferase

Recommended name
NADH kinase

Synonyms
ATP:NADH 2'-phosphotransferase
ATP:$NADH_2$ 2'-phosphotransferase
DPNH kinase
NADH kinase
$NADH_2$ kinase
kinase, reduced nicotinamide adenine dinucleotide (phosphorylating)
reduced diphosphopyridine nucleotide kinase
reduced nicotinamide adenine dinucleotide kinase (phosphorylating)

CAS registry number
62213-39-2

2 Source Organism

<1> *Saccharomyces cerevisiae* (strain X2181-1A [3,4,5]) [1, 3, 4, 5]
<2> *Pichia membranifaciens* [2]

3 Reaction and Specificity

Catalyzed reaction
ATP + NADH = ADP + NADPH

Reaction type
phospho group transfer

Natural substrates and products
S ATP + NADH <1> (<1>, may be involved in maintaining the level of triphosphopyridine nucleotide in the mitochondria [1]) (Reversibility: ? <1> [1]) [1]
P ADP + NADPH <1> [1]

Substrates and products

S ATP + NADH <1, 2> (<1, 2>, specific for NADH [1, 2, 5]; <1>, NAD^+ completely ineffective [5]) (Reversibility: ? <1,2> [1-5]) [1-5]
P ADP + NADPH <1, 2> [1-5]
S CTP + NADH <1> (<1>, 67% of activity with ATP [1]) (Reversibility: ? <1> [1]) [1]
P CDP + NADPH
S GTP + NADH <1> (<1>, 45% of activity with ATP [1]) (Reversibility: ? <1> [1]) [1]
P GDP + NADPH
S ITP + NADH <1> (<1>, 61% of activity with ATP [1]) (Reversibility: ? <1> [1]) [1]
P IDP + NADPH
S UTP + NADH <1> (<1>, 50% of activity with ATP [1]) (Reversibility: ? <1> [1]) [1]
P UDP + NADPH

Inhibitors

3-acetyl-NAD^+ <1> (<1>, 1 mM, 15% inhibition [5]) [5]
ATP <1> (<1> above 6 mM [5]; <1>, above 5 mM [1]) [1, 5]
Mg^{2+} <1> (<1>, at 10 mM or above [1]) [1]
Mn^{2+} <1> (<1>, at 1 mM or above [1]) [1]
NEM <1> (<1>, 5 mM, 77% inhibition, NADH protects, ATP not [5]) [5]
PCMB <1> (<1>, 5 mM, 49% inhibition [5]) [3, 5]
eosin-5-maleimide <1> [3]
iodoacetic acid <1> (<1>, 5 mM, 87% inhibition, NADH protects [5]) [5]
phosphoenolpyruvate <1> (<1>, 20 mM, 63% inhibition, reconstituted enzyme. Solubilized enzyme is inhibited 55% by 30 mM [4]; <1>, 30 mM, 57% inhibition [5]) [4, 5]

Activating compounds

acetate <1> (<1>, activates [5]; <1>, high concentrations of carboxylic acid, e.g. acetate required for maximal activity [1]; <1>, maximal enhancement of activity, about 7-fold is observed with 100 mM acetate, enzyme reconstituted in liposomes, maximal enhancement of solubilized enzyme with 400 mM acetate [4]) [1, 4, 5]

Metals, ions

Mg^{2+} <1> (<1> either Mn^{2+} or Mg^{2+} activate at low concentrations. K_m: 1.0 mM [1]) [1]
Mn^{2+} <1> (<1> either Mn^{2+} or Mg^{2+} activate at low concentrations [1]) [1]

Specific activity (U/mg)

0.279 <1> [1]
729 <1> [5]
Additional information <2> (<2>, amplification of NADH without the influence of coexisting NAD^+, by phosphorylating NADH into NADPH and putting this NAPH through an $NADP^+$-NADPH cycling system [2]) [2]

K_m-Value (mM)

0.027 <1> (NADH, <1>, NADH kinase reconstituted in liposomes [4]) [4]
0.042 <1> (NADH, <1>, pH 7.8, 30°C, in presence of 0.2 M sodium acetate [1]) [1]
0.105 <1> (NADH, <1>, pH 7.4, 30°C [5]; <1>, solubilized enzyme form [4]) [4, 5]
0.133 <1> (ATP, <1>, NADH kinase reconstituted in liposomes [4]) [4]
1 <1> (ATP, <1>, pH 7.8, 30°C, in presence of 0.2 M sodium acetate [1]) [1]
2.1 <1> (ATP, <1>, pH 7.4, 30°C [5]; <1>, solubilized enzyme form [4]) [4, 5]

pH-Optimum

7.9-8.6 <1> [1]
8 <1> (<1>, NADH kinase reconstituted in liposomes [4]) [4]
8.5 <1> [5]

pH-Range

6.2-9.9 <1> (<1>, pH 6.2: about 35% of maximal activity, pH 9.9: about 50% of maximal activity [1]) [1]

4 Enzyme Structure

Molecular weight

160000 <1> (<1>, gel filtration [5]) [5]

Subunits

Additional information <1> (<1>, presence of 2 or more subunits of different size [5]) [5]

5 Isolation/Preparation/Mutation/Application

Localization

cytosol <1> [1]
mitochondrion <1, 2> (<1>, the enzyme is localized and functions at the intermembrane space side of the inner membrane [3]; <1>, localized exclusively in mitochondria [5]) [1, 2, 3, 5]

Purification

<1> [1, 5]

Application

analysis <2> (<2>, the enzyme is useful for amplification of NADH in presence of excess NAD^+ and is applicable to sensitive measurement of NAD^+ dependent dehydrogenase or ist substrate [2]) [2]

6 Stability

Temperature stability

30 <2> (<2>, 10 min, stable [2]) [2]
35 <1, 2> (<2>, 10 min, 17% loss of activity [2]; <1>, 5 min, 35% loss of activity [5]) [2, 5]
40 <2> (<2>, 10 min, 40% loss of activity [2]) [2]
55 <1> (<1>, 5 min, 95% loss of activity [5]) [5]

General stability information

<1>, ammonium sulfate, 0.2 M protects against denaturation [1]

Storage stability

<1>, -30°C, 20 mM Tris-HCl buffer, pH 7.8, 0.2 M ammonium sulfate, 20 mM $MgCl_2$, 2 mM EDTA, stable for 1 month or more [5]
<1>, 0-5°C, 20 mM Tris-HCl buffer, pH 7.8, 0.2 M ammonium sulfate, 20 mM $MgCl_2$, 2 mM EDTA, stable for 7 days [5]
<1>, 4°C or -20°C, 95% loss of activity after overnight storage [1]

References

[1] Griffiths, M.M.; Bernofsky, C.: Purification and properties of reduced diphosphopyridine nucleotide kinase from yeast mitochondria. J. Biol. Chem., **247**, 1473-1478 (1972)
[2] Ohno, T.; Suzuki, T.; Horiuchi, T.: Specific amplification of NADH using NADH kinase in a reaction mixture containing excess NAD^+. Biosci. Biotechnol. Biochem., **58**, 976-977 (1994)
[3] Iwahashi, Y.; Nakamura, T.: Localization of the NADH kinase in the inner membrane of yeast mitochondria. J. Biochem., **105**, 916-921 (1989)
[4] Iwahashi, Y.; Nakamura, T.: Orientation and reactivity of NADH kinase in proteoliposomes. J. Biochem., **105**, 922-926 (1989)
[5] Iwahashi, Y.; Hitoshio, A.; Tajima, N.; Nakamura, T.: Characterization of NADH kinase from Saccharomyces cerevisiae. J. Biochem., **105**, 588-593 (1989)

Streptomycin 3"-kinase 2.7.1.87

1 Nomenclature

EC number
2.7.1.87

Systematic name
ATP:streptomycin 3"-phosphotransferase

Recommended name
streptomycin 3"-kinase

Synonyms
SM 3"-phosphotransferase
SPH
streptomycin 6-kinase
streptomycin 6-phosphotransferase
kinase, streptomycin 3"- (phosphorylating)
streptomycin 3"-phosphotransferase

CAS registry number
39391-15-6

2 Source Organism

<1> *Streptomyces griseus* (ATCC 10971 [1,2]; N^1-3-11 [3]) [1-3]

3 Reaction and Specificity

Catalyzed reaction
ATP + streptomycin = ADP + streptomycin 3"-phosphate

Reaction type
phospho group transfer

Substrates and products
S ATP + 3'-deoxydihydrostreptomycin <1> (Reversibility: ? <1> [2]) [2]
P ADP + 3'-deoxydihydrostreptomycin 3"-phosphate
S ATP + 3-deoxydihydrostreptomycin 6-phosphate <1> (Reversibility: ? <1> [1]) [1]
P ADP + 3-deoxydihydrostreptomycin 3",6-diphosphate
S ATP + dihydrostreptomycin <1> (Reversibility: ? <1> [2]) [2]

P ADP + dihydrostreptomycin 3"-phosphate
S ATP + dihydrostreptomycin 6-phosphate <1> (Reversibility: ? <1> [1]) [1]
P ADP + dihydrostreptomycin 3",6-bisphosphate
S ATP + streptomycin <1> (Reversibility: ? <1> [1-3]) [1-3]
P ADP + streptomycin 3"-phosphate <1> [1]
S ATP + streptomycin 6-phosphate <1> (Reversibility: ? <1> [1]) [1]
P ADP + streptomycin 3",6-bisphosphate
S Additional information <1> (<1>, no activity with dihydrostreptomycin 3'α,6-diphosphate, dihydrostreptomycin 3'α-phosphate [1]) [1]
P ?

4 Enzyme Structure

Subunits

? <1> (<1>, 29000, calculation from nucleotide sequence [3]) [3]

5 Isolation/Preparation/Mutation/Application

Source/tissue

mycelium <1> [1]

Cloning

<1> [3]

6 Stability

Temperature stability

2 <1> (<1>, 8 h, almost complete inactivation, crude enzyme extract [1]) [1]

Storage stability

<1>, stable in frozen mycelial pads or in frozen extracts [1]

References

[1] Walker, J.B.; Walker, M.S.: ATP:streptomycin 3"-phosphotransferase. Methods Enzymol., **43**, 632-634 (1975)
[2] Walker, J.B.; Skovarga, M.: Phosphorylation of streptomycin and dihydrostreptomycin by Streptomyces. Enzymatic synthesis of different diphosphorylated derivatives. J. Biol. Chem., **248**, 2435-2440 (1973)
[3] Heinzel, P.; Werbitzky, O.; Distler, J.; Piepersberg, W.: A second streptomycin resistance gene from Streptomyces griseus codes for streptomycin-3-phosphotransferase. Relationships between antibiotic and protein kinases. Arch. Microbiol., **150**, 184-192 (1988)

Dihydrostreptomycin-6-phosphate 3'α-kinase 2.7.1.88

1 Nomenclature

EC number
2.7.1.88

Systematic name
ATP:dihydrostreptomycin-6-phosphate 3'α-phosphotransferase

Recommended name
dihydrostreptomycin-6-phosphate 3'α-kinase

Synonyms
ATP:dihydrostreptomycin-6-P 3'α-phosphotransferase
dihydrostreptomycin 6-phosphate kinase (phosphorylating)
kinase (phosphorylating), dihydrostreptomycin 6-phosphate

CAS registry number
39391-14-5

2 Source Organism

<1> *Streptomyces bikiniensis* (ATCC 1062 [1,2]) [1, 2]

3 Reaction and Specificity

Catalyzed reaction
ATP + dihydrostreptomycin 6-phosphate = ADP + dihydrostreptomycin 3'α,6-bisphosphate

Reaction type
phospho group transfer

Natural substrates and products
S ATP + dihydrostreptomycin 6-phosphate <1> (Reversibility: ? <1> [1, 2]) [1, 2]
P ADP + dihydrostreptomycin 3'α,6-bisphosphate <1> [1, 2]

Substrates and products
S ATP + dihydrostreptomycin 6-phosphate <1> (Reversibility: ? <1> [1,2]) [1, 2]
P ADP + dihydrostreptomycin 3'α,6-bisphosphate <1> [1, 2]

S Additional information <1> (<1> not: streptomycin derivatives containing a 3'-α-aldehyde group [1]; <1> not: streptomycin 6-phosphate, UTP, GTP, CTP, streptomycin, dihydrostreptomycin, dihydrostreptomycin-3"-phosphate, dihydrostreptobiosamine [2]) [1, 2]

P ?

Temperature optimum (°C)

35 <1> (<1> assay at [2]) [2]

5 Isolation/Preparation/Mutation/Application

Source/tissue

mycelium <1> (<1> mature mycelias [2]) [2]

6 Stability

Temperature stability

2 <1> (<1> 6 h, crude enzyme extract, little activity remains [2]) [2]

General stability information

<1>, labile enzyme [2]

Storage stability

<1>, 2°C, crude enzyme extract, 6 h, little activity remains [2]

<1>, 4°C, 20fold diluted extract, 30% glycerol, overnight, significant activity retained [2]

<1>, frozen, extract or in intact mycelial pads, months, stable [2]

References

[1] Walker, J.B.; Skorvaga, M.: Phosphorylation of streptomycin and dihydrostreptomycin by Streptomyces. Enzymatic synthesis of different diphosphorylated derivatives. J. Biol. Chem., **248**, 2435-2440 (1973)

[2] Walker, J.B.; Walker, M.S.: ATP-:dihydrostreptomycin-6-P 3α-phosphotransferase. Methods Enzymol., **43**, 634-637 (1975)

Thiamine kinase 2.7.1.89

1 Nomenclature

EC number
2.7.1.89

Systematic name
ATP:thiamine phosphotransferase

Recommended name
thiamine kinase

Synonyms
ATP:thiamin phosphotransferase
kinase, thiamin (phosphorylating)
thiamin kinase
thiamin kinase (phosphorylating)
thiamin phosphokinase

CAS registry number
62213-38-1

2 Source Organism

<1> *Escherichia coli* (K12 [1]) [1]

3 Reaction and Specificity

Catalyzed reaction
ATP + thiamine = ADP + thiamine phosphate

Reaction type
phospho group transfer

Natural substrates and products
S ATP + thiamine <1> (Reversibility: ? <1> [1]) [1]
P ADP + thiamine monophosphate <1> [1]

Substrates and products
S ATP + thiamine <1> (Reversibility: ? <1> [1]) [1]
P ADP + thiamine monophosphate <1> [1]

Activating compounds
ATP <1> (<1> dependent on the presence of [1]) [1]

Metals, ions
K^+ <1> (<1> marked stimulation [1]) [1]
Mg^{2+} <1> (<1> absolute requirement [1]) [1]
NH_4^+ <1> (<1> marked stimulation [1]) [1]

K_m-Value (mM)
0.0028 <1> (thiamine, <1> at 37°C, pH 7.5 [1]) [1]

pH-Optimum
7.5 <1> (<1> assay at [1]) [1]

Temperature optimum (°C)
37 <1> (<1> assay at [1]) [1]

5 Isolation/Preparation/Mutation/Application

Localization
soluble <1> (<1> in the soluble fraction [1]) [1]

Purification
<1> (partial [1]) [1]

References

[1] Iwashima, A.; Nishino, H.; Nose, Y.: Conversion of thiamine to thiamine monophosphate by cell-free extracts of Escherichia coli. Biochim. Biophys. Acta, **258**, 333-336 (1972)

Diphosphate-fructose-6-phosphate 1-phosphotransferase 2.7.1.90

1 Nomenclature

EC number
2.7.1.90

Systematic name
diphosphate:D-fructose-6-phosphate 1-phosphotransferase

Recommended name
diphosphate-fructose-6-phosphate 1-phosphotransferase

Synonyms
6-phosphofructokinase (pyrophosphate)
FBPase <42> [45]
PFP <7, 21, 23, 35, 36, 40, 42> [22, 25, 26, 34, 37, 41, 43, 45, 48]
PPi-PFK <1, 30, 35, 37-39, 41, 44> [31-33, 35, 36, 38-40, 44, 47]
PPi-dependent phosphofructokinase <1, 35, 37, 39, 40> [33, 35, 39-41]
diphosphate-D-fructose-6-phosphate 1-phosphotransferase
fructose 1,6-bisphosphatase <42> [45]
inorganic pyrophosphate-dependent phosphofructokinase
inorganic pyrophosphate-phosphofructokinase
phosphotransferase, pyrophosphate-D-fructose 6-phosphate 1-
pyrophosphate D-fructose-6-phosphate 1-phosphotransferase
pyrophosphate-D-fructose 6-phosphate 1-phosphotransferase
pyrophosphate-D-fructose 6-phosphate 1-phosphotransferase
pyrophosphate-D-fructose 6-phosphate phosphotransferase
pyrophosphate-dependent phosphofructo-1-kinase
pyrophosphate-dependent phosphofructokinase
pyrophosphate-dependent phosphofructokinase <1, 35, 37, 39, 40, 44> [33, 35, 39-41, 47]
pyrophosphate:D-fructose-6-phosphate 1-phosphotransferase

CAS registry number
55326-40-4

2 Source Organism

<-7> no activity in *Rhodopseudomonas viridis* [11]
<-6> no activity in *Rhodopseudomonas palustris strain 1a1* [11]
<-5> no activity in *Rhodopseudomonas sphaeroides* [11]
<-4> no activity in *Rhodopseudomonas capsulata strain Kb1* [11]

<-3> no activity in *Mycoplasma gallisepticum S6* [8]
<-2> no activity in *Spiroplasma floricola 23-6* [8]
<-1> no activity in *Acanthamoeba castellani, NEFF⁻ strain* [3]
<1> *Entamoeba histolytica* (strain H200 [1]; K-9 [28]; DKB-strain [2]) [1, 2, 28, 38, 40]
<2> *Naegleria fowleri* (KVL-strain [3]) [3]
<3> *Naegleria gruberi* (B G-6-strain [3]) [3]
<4> *Toxoplasma gondii* [4]
<5> *Acer saccharum* [20]
<6> *Allium cepa* [20]
<7> *Ananas comosus* [15, 37]
<8> *Brassica oleracea* (var. botrytis [20]) [20]
<9> *Cucumis sativus* [6]
<10> *Daucus carota* [20, 21]
<11> *Kalanchoe blossfeldiana* [20]
<12> *Lycopersicon esculentum* (cv. Super First [24]) [24]
<13> *Glycine max* (cv. Mandarin [18]) [18]
<14> *Oryza sativa* (Japonica cv. Koshihikari [23]) [23]
<15> *Persea americana* [20]
<16> *Phaseolus coccineus* (2 enzyme forms [5]) [5]
<17> *Phaseolus vulgaris* (cv. Top Crop [19]) [19]
<18> *Pisum sativum* [20]
<19> *Ricinus communis* (var. Hale [14,17,20]) [14, 17, 20]
<20> *Sansevieria trifasciata* [5, 7]
<21> *Solanum tuberosum* (var. Russett [49]; cv. Danshaku [42]; cv. Maris Piper [16]; cv. Record [22]) [16, 20, 22, 26, 42, 49]
<22> *Spinacia oleracea* [16, 20, 43]
<23> *Triticum aestivum* (cv. WH-157 [25]) [20, 25]
<24> *Vigna radiata* (mung bean [12,13,20,27]; i.e. Phaseolus aureus [12]; commercial product [12]; i.e. Phaseolus mungo or Vigna mungo [13]) [12, 13, 20, 27]
<25> *Zea mays* [20]
<26> *Acholeplasma laidlawii* (strain B-PG9 [8]) [8]
<27> *Acholeplasma florum* (strain L1 [8]) [8]
<28> *Alcaligenes sp.* [29]
<29> *Bacteroides fragilis* [30]
<30> *Propionibacterium freudenreichii* [9, 31]
<31> *Propionibacterium shermanii* (subspecies of Propionibacterium freudenreichii [12]; commercial product [12]) [10, 12]
<32> *Pseudomonas marina* [30]
<33> *Rhodospirillum rubrum* (strain S_1 [11]) [11]
<34> *Rhodopseudomonas gelatinosa* [11]
<35> *Giardia lamblia* (strain Portland-1, ATCC 30888 [32,33]) [32, 33, 48]
<36> *Brassica nigra* [34]
<37> *Entamoeba histolytica* [35]
<38> *Hexamita inflata* [36]
<39> *Dictyoglomus thermophilum* (strain Rt46 B.1 [39]) [39]

<40> *Citrus sinensis* (cv. Shamouti [41]) [41]
<41> *Amycolatopsis methanolica* (enzyme is completely replaced by an ATP-dependent phosphofructokinase, when cells grow on only one C1-carbon source, e.g. methanol [44]) [44]
<42> *Porphyromonas gingivalis* [45]
<43> *Treponema pallidum* [46]
<44> *Thermoproteus tenax* [47]

3 Reaction and Specificity

Catalyzed reaction

diphosphate + D-fructose 6-phosphate = phosphate + D-fructose 1,6-bisphosphate (<1> random bi bi mechanism [2]; <31> rapid equilibrium random mechanism [12]; <30> random terreactant or rapid equilibrium random biter mechanism [9]; <17> mechanism [19]; <1> Met249 plays a critical role in the binding of fructose 6-phosphate and the stabilization of the transition state [38]; <1> Arg423 and Tyr420 are involved in substrate binding and catalytic mechanism [40])

Reaction type

phospho group transfer

Natural substrates and products

S diphosphate + D-fructose 6-phosphate <1-35, 39> (<35> physiological role of the enzyme [48]; <7, 21> regulatory role in glucose metabolism [37, 42]) (Reversibility: r <7, 13, 14, 17, 19, 21-23, 30, 31, 33-36, 38, 39> [10-12, 15-17, 19, 23, 25, 32-34, 36, 37, 39, 42, 48]; ? <1-6, 8-12, 15, 16, 18, 20, 22, 25-29, 32> [1-9, 13, 14, 20-22, 24, 26-31]) [1-32, 37, 39, 42, 48]
P phosphate + D-fructose 1,6-bisphosphate <1-35, 39> [1-32, 37, 39, 42]

Substrates and products

S $(polyphosphate)_n$ + D-fructose 6-phosphate <39> (<39> n: 12-18 [39]; <39> 75% of activity with diphosphate [39]) (Reversibility: r <39> [39]) [39]
P $(polyphosphate)_{n-1}$ + D-fructose 1,6-bisphosphate
S arsenate + D-fructose 1,6-bisphosphate <1, 26, 31> (<26> specific for phosphate or arsenate in the reverse reaction [8]; <31> no substrates are SO_4^{2-}, MoO_4^{2-}, SeO_4^{2-} or ribulose 1,6-bisphosphate [10]) (Reversibility: r <1, 26, 31> [2, 8, 10, 28]) [2, 8, 10, 28]
P ?
S diphosphate + 2,5-anhydro-D-mannitol 6-phosphate <1, 31> (Reversibility: r <1, 31> [12, 28]) [12, 28]
P phosphate + 2,5-anhydro-D-mannitol 1,6-bisphosphate <1, 31> [12, 28]
S diphosphate + D-fructose 6-phosphate <1-44> (<1-4, 7, 10, 14, 19, 20-24, 26, 27, 30, 31, 33-38, 41-44> specific for diphosphate as phosphor donor [1-38, 40, 43-47]; <31, 33, 34, 39, 44> specific for D-fructose 6-phosphate and D-fructose 1,6-bisphosphate [10, 11, 39, 47]; <42> equilibrium lies in

reverse direction [45]; <39> equilibrium lies in forward direction [39]; <35> substrates are Mg^{2+}-chelated complexes of phosphate and diphosphate, respectively, not uncomplexed ligands [33]; <7> substrate Mg-diphosphate [37]; <31, 24> favored anomeric, epimeric and tautomeric form of sugar substrate is the β-D-fructofuranose [12]; <26, 27> phosphate cannot replace diphosphate and vice versa [8]; <1> no phosphate group exchange reaction between phosphate and diphosphate, or D-fructose 6-phosphate and D-fructose 1,6-bisphosphate in absence of a third substrate [2]; <10> ratio forward/reverse reaction: 7:1 without D-fructose 2,6-bisphosphate and 1:1 in the presence of this effector [21]) (Reversibility: r <1, 4-26, 30, 31, 33-36, 38-44> [2, 4-12, 15-21, 23-28, 32-34, 36, 37, 39-48]; ? <1-3, 12, 27-29, 32, 37> [1, 3, 13, 14, 22, 24, 29-31, 35, 38]) [1-48]

P phosphate + D-fructose 1,6-bisphosphate <1-44> (<1, 26> arsenate can replace phosphate in the reverse reaction [2, 8]) [1-48]

S diphosphate + D-sedoheptulose 7-phosphate <1, 31> (Reversibility: r <1, 31> [12, 28]) [12, 28]

P phosphate + D-sedoheptulose 1,7-bisphosphate <1, 31> [12, 28]

S phosphate + D-glucose 1,6-bisphosphate <33> (<33> reaction at about 70% of the rate with D-fructose 1,6-bisphosphate [11]; <31> no activity [10]) (Reversibility: ? <33> [11]) [11]

P ?

S triphosphate + D-fructose 6-phosphate <39> (<39> 82% of activity with diphosphate [39]) (Reversibility: r <39> [39]) [39]

P diphosphate + D-fructose 1,6-bisphosphate

Inhibitors

1,n-alkanediol biphosphates <24, 31> (<24, 31> n: 2-9, weak [12]) [12]

2,3-O-isopropylidene-α-L-sorbofuranose-1-phosphate <24, 31> (<24, 31> weak [12]) [12]

2,5-anhydro-D-glucitol-6-phosphate <24> [12]

2,5-anhydro-D-mannitol 1,6-bisphosphate <30> (<30> D-fructose 1,6-bisphosphate-analog, kinetics [9]) [9]

3-[N-(2-phenylthioethyl)-N-methylamino]-1-hydroxypropylidene-1,1-bisphosphonate <37> (<37> i.e. CGP 48048, bisphosphonate, synthetic diphosphate analogue [35]; <37> inhibition of growth [35]) [35]

3-phospho-D-glycerate <19, 22> (<22> diminishes the activating effect of fructose 2,6-bisphosphate [43]; <19> predominantly a competitive inhibitor [14]; <36> no inhibition [34]) [14, 43]

ADP <1, 19, 33, 36> (<36> forward reaction, as Mg^{2+}-complex [34]; <1> weak [2]; <33> kinetics [11]; <26, 44> no inhibition [8, 47]) [2, 11, 14, 34]

AMP <19, 20, 33> (<33, 34> kinetics [11]; <19> weak [14]; <26, 27, 31, 36, 38, 44> no inhibition [8, 10, 34, 36, 47]) [7, 11, 14]

ATP <19, 22, 33, 36> (<22> diminishes the activating effect of fructose 2,6-bisphosphate [43]; <36> forward reaction, as Mg^{2+}-complex [34]; <33> only at low concentrations of $MgCl_2$, reverse reaction [11]; <2, 4, 31, 44> no inhibition [3, 4, 10, 47]) [11, 14, 34, 43]

Ca^{2+} <1, 12, 19, 43> (<43> complete inhibition at 1 mM [46]; <12> complete inhibition in the forward reaction, slightly inhibitory in the reverse reaction [24]) [2, 17, 24, 28, 46]
Cl^- <12, 19, 23, 40> (<19> at high concentrations [17]) [17, 24, 25, 41]
Co^{2+} <12, 26> (<26> inhibitory at high concentration [8]; <12> complete inhibition in the forward reaction, strong inhibition in the reverse reaction [24]) [8, 24]
Cu^{2+} <39, 43> (<43> 85% inhibition at 0.2 mM [46]; <39> complete inhibition at 1 mM [39]) [39, 43, 46]
D-arabinose-5-phosphate <24, 31> (<24, 31> weak [12]) [12]
D-fructose 1,6-bisphosphate <19, 30, 35> (<30, 35> forward reaction, product inhibition, kinetics [9, 33]) [9, 17, 33]
D-fructose 2,6-bisphosphate <31, 35> (<31> at high concentration [12]; <31, 35> competitive versus D-fructose 1,6-bisphosphate, reverse reaction [12,32]) [12, 32]
D-fructose 6-phosphate <1, 17, 19, 21, 22, 30, 35> (<22> reverse reaction, diminishes the activating effect of fructose 2,6-bisphosphate [43]; <1> competitive versus D-fructose 1,6-bisphosphate and phosphate [2]; <21, 30, 35> reverse reaction, product inhibition, kinetics [9, 16, 33]) [2, 9, 16, 17, 19, 33, 43]
D-glucitol-6-phosphate <24, 31> (<24, 31> weak [12]) [12]
D-glucose 6-phosphate <33, 34> (<33, 34> weak [11]; <19, 36> no inhibition [14,34]) [11]
D-tagatose 6-phosphate <30> (<30> kinetics, dead-end substrate [9]) [9]
EDTA <1, 12, 37, 44> (<37> complete inhibition of growth at 0.005 mM [35]) [2, 24, 35, 47]
Fe^{3+} <39> (<39> 84% inhibition at 0.1 mM [39]) [39]
KCl <12> (<12> complete inhibition in the forward reaction, slightly inhibitory in the reverse reaction [24]) [24]
KSCN <2> [3]
L-sorbose-6-phosphate <24, 31> (<24, 31> weak [12]) [12]
Mg^{2+} <19, 24> (<19,24> at high concentrations [13, 17]) [13, 17]
Mn^{2+} <12, 26, 39, 43> (<39> 46% inhibition at 0.1 mM [39]; <12> inhibitory in the forward reaction only, slightly activating in the reverse reaction [24]; <26> inhibitory at high concentration [8]) [8, 24, 39, 46]
NH_4^+ <12, 19> (<19> weak [17]; <12> complete inhibition in the forward reaction, slightly inhibitory in the reverse reaction [24]) [17, 24]
NO_3^- <19, 21> (<19> at high concentrations [17]; <23> no inhibition [25]) [17, 26]
NaCl <12, 21> (<12> complete inhibition in the forward reaction, slightly inhibitory in the reverse reaction [24]) [24, 26]
Ni^{2+} <1, 19> [2, 17, 28]
SO_4^{2-} <12, 19, 30> (<30> phosphate analog, kinetics [9]; <12> gluconeogentic reaction [24]; <19> at high concentrations [17]; <23> no inhibition [25]) [9, 17, 24]
Se^{2+} <39> (<39> 75% inhibition at 0.1 mM [39]) [39]
Zn^{2+} <1, 19, 43> [2, 17, 28, 46]

alendronate <37> (<37> bisphosphonate, synthetic diphosphate analogue [35]; <37> inhibition of growth [35]) [35]
bicarbonate <23> [25]
citrate <19, 33> (<19> not relieved by Mg^{2+} [14]; <24, 26, 27, 31, 36, 44> no inhibition [8, 10, 13, 34, 47]) [11, 14]
clodronate <37> (<37> bisphosphonate, synthetic diphosphate analogue [35]; <37> inhibition of initial growth after inoculation [35]) [35]
diethylene glycol-monophosphate <24, 31> (<31,24> weak [12]) [12]
diphosphate <1, 12, 13, 17, 19, 21, 22, 33, 35, 36> (<22> reverse reaction, diminishes the activating effect of fructose 2,6-bisphosphate [43]; <21> substrate-substrate interaction between phosphate and diphosphate in forward and reverse reaction [26]; <21, 33, 35, 36> reverse reaction [11, 16, 33, 34]; <21, 35> kinetics [16, 26, 33]) [2, 11, 16-19, 24, 26, 33, 34, 43]
etidronate <37> (<37> bisphosphonate, synthetic diphosphate analogue [35]; <37> inhibition of growth [35]) [35]
imidodiphosphate <21, 22> (<22> inhibition in leaves in vivo when fed [16]; <21> mixed inhibitor with respect to both fructose 6-phosphate and diphosphate [16]) [16]
increased ionic strength <24> (<24> e.g. 30 mM KCl, NH_4Cl, 10 mM Na_2SO_4, $(NH_4)_2SO_4$ or 5 mM sodium phosphate [27]) [27]
magnesium diphosphate <30> (<30> reverse reaction, product inhibition, kinetics [9]) [9]
malate <21> (<19> no inhibition [14]) [26]
methylenediphosphonate <20, 30> (<30> kinetics, diphosphate analog [9]) [7, 9]
nucleotides <19> [14]
pamidronate <37> (<37> bisphosphonate, synthetic diphosphate analogue [35]; <37> inhibition of growth [35]) [35]
phosphate <1, 9, 13, 14, 17, 19, 21-24, 30, 35, 36> (<22> forward reaction, diminishes the activating effect of fructose 2,6-bisphosphate [43]; <21> substrate-substrate interaction between phosphate and diphosphate in forward and reverse reaction [26]; <19> inhibitory in the reverse reaction [17]; <14, 19, 23> in the forward reaction only in presence of fructose 2,6-bisphosphate [17, 23, 25]; <1> competitive versus diphosphate [2]; <9> sequential ternary complex mechanism [6]; <9> noncompetitive versus diphosphate and D-fructose 6-phosphate [6]; <21, 30, 35, 36> forward reaction, product inhibition, kinetics [9, 26, 33, 34]) [2, 6, 9, 13, 14, 17-19, 23, 25, 26, 33, 34, 43]
phosphoenolpyruvate <19, 20, 22, 33, 36> (<22> diminishes the activating effect of fructose 2,6-bisphosphate [43]; <36> forward reaction [34]; <33> weak, reverse reaction [11]; <24, 26, 31, 44> no inhibition [8, 10, 13, 47]) [7, 11, 14, 34, 43]
phosphoglycolate <19> [14]
risedronate <37> (<37> bisphosphonate, synthetic diphosphate analogue [35]; <37> inhibition of growth [35]) [35]
sulfate <19> (<19> weak [17]) [17]
sulfite <23> [25]
tetrapolyphosphate <30> [9]

thiosulfate <23> [25]
tripolyphosphate <30> [9]
tungstate <23> [25]
zoledronate <37> (<37> bisphosphonate, synthetic diphosphate analogue [35]; <37> inhibition of initial growth after inoculation [35]) [35]
Additional information <2, 4, 19, 26, 31, 33, 36, 44> (<44> no effect of glucose, pyruvate, fructose 2,6-bisphosphate [47]; <36> no inhibition by 2-phosphoglycerate [34]; <31> not affected by ATP, AMP, citrate, pyruvate, and malate [10]; <26> no inhibition by ammonium sulfate, D-fructose 2,6-bisphosphate, GDP [8]; <33> no inhibition by cAMP [11]; <4,26> no inhibition by nucleoside triphosphates [4,8]; <19> no inhibition by pyruvate, malate [14]; <19> no inhibition by glucose, glucose 1-phosphate, sucrose, D-fructose, glycolate, succinate, UDPglucose [14]; <23> no inhibition by acetate, carbonate [25]; <2> no inhibition by polyphosphate [3]) [3, 4, 8, 10, 11, 14, 34, 47]

Activating compounds

AMP <2> (<2> activation, Triton X-100 enhances stimulation by AMP [3]; <44> no effect [47]) [3]
ATP <33> (<33> slight activation of forward reaction [11]; <44> no effect [47]) [11]
CMP <2> (<2> slight activation [3]) [3]
D-fructose 2,6-bisphosphate <7, 9, 10, 12-14, 17, 19, 21-24, 26, 31, 36, 40> (<21> pH-independent in forward and reverse reaction [49]; <22> activating effect is greatly influenced by substrates of both directions, phosphoenolpyruvate, 3-phosphoglycerate, and ATP, kinetics [43]; <13> dependent on substrate concentrations [18]; <40> dependent on buffer type [41]; <7> pH-dependent activation [37]; <7> only weak activation in the reverse reaction [37]; <14, 36> shifts pH-optimum for both reaction directions to slightly acidic [23, 34]; <12> 11fold [24]; <10, 12> forward reaction only [21, 24]; <19> 20fold activation of forward reaction, 10fold increase in affinity for the substrates of both reaction directions [17]; <36> 60fold increase in affinity for fructose 6-phosphate and 3-5fold for fructose 1,6-bisohosphate [34]; <17, 21> kinetics [19, 49]; <2, 4, 26, 31, 35, 38, 44> no activation [3, 4, 8, 12, 32, 36, 47]) [6, 8, 12-14, 17-19, 21-27, 34, 37, 41-43, 49]
D-glucose <12> (<12> forward reaction [24]; <44> no effect [47]) [24]
D-glucose 1,6-bisphosphate <24> [27]
D-glucose 1-phosphate <12> (<12> reverse reaction [24]; <19> no activation [14]) [24]
GMP <2> (<2> activation, about 35% as efficient as AMP [3]) [3]
IMP <2> (<2> activation, about 35% as efficient as AMP [3]) [3]
$MgADP^-$ <2> (<2> activation, about 15% as efficient as AMP [3]) [3]
$MgATP^{2-}$ <2> (<2> activation, about 15% as efficient as AMP [3]) [3]
NAD^+ <2> (<2> slight activation [3]) [3]
UMP <2> (<2> slight activation [3]) [3]
XMP <2> (<2> slight activation [3]) [3]
adenosine <2> (<2> slight activation [3]) [3]

aminoimidazolecarboxamide riboside <2> (<2> about 25% as efficient as AMP [3]) [3]
cAMP <2, 33> (<2> slight activation [3]; <33> forward reaction [11]) [3, 11]
citrate <33> (<33> slight activation of forward reaction [11]; <26> no activation [8]) [11]
dAMP <2> (<2> activation, about 25% as efficient as AMP [3]) [3]
phosphate <2> (<2> slight activation [3]) [3]
polyethylene glycol <2> (<2> stimulation, synergistic with AMP [3]) [3]
Additional information <2, 4, 19, 21, 26, 31, 44> (<44> no effect of ADP, pyruvate, citrate, phosphoenolpyruvate [47]; <21> wounding of tubers increases the respiratory oxygen uptake significantly [42]; <31> not affected by ATP, AMP, citrate, pyruvate, and malate [10]; <19> no activation by glucose 6-phosphate, UDPglucose, glucose, D-fructose, sucrose, pyruvate, malate, succinate or glycolate [14]; <2-4, 26> no activation by ATP [3, 4, 8]; <4, 26> no activation by polyphosphate, nucleoside triphosphates [4, 8]; <26> no activation by or phosphoenolpyruvate, AMP, GDP, citrate, and ammonium sulfate [8]) [3, 4, 8, 10, 14, 42, 47]

Metals, ions

Ca^{2+} <39> (<39> activates 120% at 0.1 mM [39]) [39]
Co^{2+} <1, 9, 17, 19, 24, 26, 39> (<1, 26> less effective than Mn^{2+} [2, 8]; <1, 9, 19, 26> less effective than Mg^{2+} [2, 6, 8, 17]; <12, 23> no activation of forward reaction [24, 25]; <12, 23, 33> no activitaion [11, 24, 25]; <26> inhibitory at high concentration [8]) [2, 6, 8, 9, 17, 19, 24, 27, 39]
Fe^{2+} <39> (<39> activates 120% at 0.1 mM [39]) [39]
K^{+} <12, 39> (<39> activates 140% at 10 mM [39]; <12> slightly activating in forward reaction [24]) [24, 39]
Mg^{2+} <1-27, 30, 31, 33-36, 39, 43, 44> (<1-27, 30, 31, 33-38, 44> requirement [1-14, 16-28, 31, 33-37, 47, 49]; <35> substrates are Mg^{2+}-chelated complexes of phosphate and diphosphate, respectively, not uncomplexed ligands [33]; <39> optimal at 0.5-3.5 mM [39]; <1,12> less effective than Mn^{2+} [2, 24]; <30> mainly required for the forward reaction [9]; <30> magnesium diphosphate is the actual substrate of forward reaction [9, 31]; <30> product inhibition of reverse reaction, kinetics [9]; <19, 24> inhibitory at high concentration [13, 17]; <1> enzyme requires 0.5 mM in the forward reaction, 0.0008 mM in the reverse reaction [2]) [1-28, 31-37, 39, 40, 43, 46, 47, 49]
Mn^{2+} <1-3, 9, 12, 17, 19, 24, 26, 31> (<1-3, 9, 17, 19, 24, 26, 27, 31> requirement [2, 3, 6, 8, 10, 17, 19, 27]; <12> inhibitory in the forward reaction only, slightly activating in the reverse reaction [24]; <9, 19, 24, 26> less effective than Mg^{2+} [6, 8, 17, 27]; <33> not required [11]; <26> inhibitory at high concentration [8]) [2, 3, 6, 8, 10, 17, 19, 24, 27]
Na^{+} <39> (<39> activates 135% at 10 mM [39]) [39]
divalent cations <1-3, 9, 17, 19, 24, 26, 44> (<1-3, 9, 17, 19, 24, 26, 44> required [2, 3, 6, 8, 17, 19, 27, 28, 47]; <1-3, 9, 19, 24, 26> Mn^{2+} or Mg^{2+} [3, 6, 8, 17, 27, 28]; <1, 17> desecending order Mn^{2+}, Mg^{2+}, Co^{2+} [2, 19]; <9, 17, 26> descending order Mg^{2+}, Mn^{2+} and Co^{2+} [6, 8, 19]; <39> activates 120% at 0.1 mM [39]) [2, 3, 6, 8, 17, 19, 24, 26-28, 39, 47]

Additional information <1, 17, 23, 26, 33> (<26> no activation by $(NH_4)_2SO_4$ [8]; <1, 17, 26, 33> no activation by Zn^{2+} [2, 8, 11, 19]; <17> no activation by Fe^{2+} [19]; <1> no activation by Ni^{2+} [2]; <17, 23> no activation by Cu^{2+} [19, 25]; <23> no activation by Ba^{2+}, Sn^{2+}, Sr^{2+} [25]) [2, 8, 11, 19, 25]

Turnover number (min^{-1})

3.6 <1> (D-fructose 6-phosphate, <1> mutant M249I, pH 7.2, 30°C [38]) [38]
25.2 <1> (D-fructose 6-phosphate, <1> mutant M249L, pH 7.2, 30°C [38]) [38]
4980 <35> (diphosphate, <35> both reaction directions, pH 7.4, 30°C [33]; <35> substrate is a Mg^{2+}-chelated complexe of diphosphate, not uncomplexed ligand [33]) [33]
4980 <35> (phosphate, <35> both reaction directions; pH 7.4, 30°C [33]; <35> substrate is a Mg^{2+}-chelated complexe of phosphate, not uncomplexed ligand [33]) [33]
5520 <1> (D-fructose 6-phosphate, <1> mutant M249A, pH 7.2, 30°C [38]) [38]
19500 <1> (D-fructose 6-phosphate, <1> wild-type enzyme, pH 7.2, 30°C [38]) [38]
19800 <1> (D-fructose 6-phosphate, <1> wild-type, pH 7.2, 30°C [40]) [40]
19800 <1> (diphosphate, <1> wild-type, pH 7.2, 30°C [40]) [40]
Additional information <1, 24> (<1> k_{cat} values of mutant enzymes [40]) [13, 28, 40]

Specific activity (U/mg)

0.00016 <27> (<27> crude enzyme extract, phosphor donor in the forward reaction is diphosphate [8]) [8]
0.00021 <27> (<27> crude enzyme extract, phosphor donor in the forward reaction is ATP [8]) [8]
0.095 <21> (<21> freshly sliced tissue samples, i.e. wounded, in presence of fructose 2,6-bisphosphate [42]) [42]
0.101 <21> (<21> sliced tissue samples, i.e. wounded, after 24 h, in presence of fructose 2,6-bisphosphate [42]) [42]
0.136 <37> (<37> purified recombinant His-tagged enzyme [35]) [35]
0.2-0.6 <7> (<7> partially purified enzyme [15]) [15]
0.24 <37> (<37> partially purified native enzyme [35]) [35]
1.5 <23> (<23> partially purified enzyme [25]) [25]
1.72 <12> (<12> partially purified enzyme [24]) [24]
2 <10> (<10> purified enzyme [21]) [21]
3.5 <44> (<44> purified enzyme, assay temperature 50°C is not the temperature optimum [47]) [47]
5.2 <9> (<9> purified enzyme, in presence of D-fructose 2,6-bisphosphate at 0.001 mM [6]) [6]
6.5 <17> (<17> partially purified enzyme [19]) [19]
8.25 <19> (<19> purified enzyme [17]) [17]
8.4 <20> (<20> partially purified enzyme [7]) [7]
8.47 <13> (<13> partially purified enzyme [18]) [18]

8.9 <7> (<7> reverse reaction, purified enzyme, pH 7.7, in presence of fructose 2,6-bisphosphate [37]) [37]
10 <21> (<21> purified enzyme [20]) [20]
12 <24, 26> (<24> purified enzyme [27]; <26> partially purified enzyme [8]) [8, 27]
13.6 <21> (<21> partially purified enzyme [16]) [16]
24.2 <33> (<33> partially purified enzyme [11]) [11]
24.4 <39> (<39> purified enzyme [39]) [39]
26 <36> (<36> purified enzyme [34]) [34]
30.2 <21> (<21> purified enzyme [49]) [49]
36.3 <22> (<22> purified enzyme [43]) [43]
40 <1> (<1> partially purified enzyme [2]) [2]
43.8 <7> (<7> forward reaction, purified enzyme, pH 7.7, in presence of fructose 2,6-bisphosphate [37]) [37]
45.7 <1> (<1> purified enzyme [1]) [1]
48.6 <14> (<14> purified enzyme [23]) [23]
65 <2> (<2> purified enzyme [3]) [3]
74-82 <24> (<24> purified enzyme [12]) [12]
146 <35> (<35> purified enzyme [32]) [32]
245 <31> (<31> purified enzyme [10]) [10]
278 <42> (<42> purified recombinant enzyme [45]) [45]

K_m-Value (mM)

0.005 <30> (diphosphate, <30> as Mg^{2+} complex, pH 8.0, 25°C [9]) [9]
0.006 <1> (diphosphate, <1> pH 7.0 [2]) [2]
0.008 <1> (Mg^{2+}, <1> forward reaction, pH 7.0, 30°C [2]) [2]
0.009 <1> (D-fructose 1,6-bisphosphate, <1> pH 7.0, 30°C [2]) [2]
0.01 <2> (D-fructose 6-phosphate, <2> pH 7.0, 30°C [3]) [3]
0.011 <7, 38> (diphosphate, <7> pH 7.7, 25°C, in presence of fructose 2,6-bisphosphate [37]) [37, 36]
0.014-0.017 <1-3, 10, 19, 21, 36, 37> (diphosphate, <37> native enzyme, pH 7.0, 25°C [35]; <36> pH 6.8, 25°C, in presence of fructose 2,6-bisphosphate [34]; <19> pH 7.5, 25°C [17]; <10,21> pH 8.0, 25°C [16,21]; <2> pH 7.0, 30°C [3]) [1, 3, 16, 17, 21, 28, 34, 35]
0.018 <37> (diphosphate, <37> recombinant His-tagged enzyme, pH 7.0, 25°C [35]) [35]
0.018-0.023 <1, 33, 38> (D-fructose 1,6-bisphosphate, <33> pH 8.6, 30°C [11]) [1, 11, 28, 36]
0.022 <39> (diphosphate, <39> pH 6.0, 50°C [39]) [39]
0.023 <19> (D-fructose 1,6-bisphosphate, <19> reverse reaction, pH 7.8, 25°C [17]) [17]
0.025-0.033 <4, 23, 33> (diphosphate, <33> pH 7.2, 30°C [11]) [4, 11, 25]
0.03 <30> (D-fructose 1,6-bisphosphate, <30> reverse reaction, pH 8.0, 25°C [9]) [9]
0.035 <2> (D-fructose 1,6-bisphosphate, <2> pH 7.0, 30°C [3]) [3]
0.038 <1> (D-fructose 6-phosphate) [1, 28]
0.039 <35> (diphosphate, <35> pH 7.4, 30°C [32]) [32]

0.04 <37> (D-fructose 6-phosphate, <37> native enzyme, pH 7.0, 25°C [35]) [35]
0.04 <1> (diphosphate, <1> wild-type, pH 7.2, 30°C [38,40]) [38, 40]
0.042 <43> (diphosphate, <43> pH 7.0, 37°C [46]) [46]
0.045 <37> (D-fructose 6-phosphate, <37> recombinant His-tagged enzyme, pH 7.0, 25°C [35]) [35]
0.05 <36> (D-fructose 6-phosphate, <36> pH 6.8, 25°C, in presence of fructose 2,6-bisphosphate [34]) [34]
0.051 <31> (D-fructose 1,6-bisphosphate, <31> pH 7.4, 25°C [10]) [10]
0.06 <1> (sedoheptulose 7-phosphate) [28]
0.064 <31> (Mn^{2+}, <31> pH 7.4, 25°C [10]) [10]
0.069 <31> (diphosphate, <31> pH 7.4, 25°C [10]) [10]
0.083 <31> (Mg^{2+}, <31> pH 7.4, 25°C [10]) [10]
0.085 <1> (D-fructose 6-phosphate, <1> wild-type, pH 7.2, 30°C [38,40]) [38, 40]
0.09 <36> (D-fructose 1,6-bisphosphate, <36> pH 6.8, 25°C, in presence of fructose 2,6-bisphosphate [34]) [34]
0.094 <7> (D-fructose 1,6-bisphosphate, <7> pH 7.7, 25°C, in presence of fructose 2,6-bisphosphate [37]) [37]
0.1 <17, 30> (Mg^{2+}, <17> pH 7.5, 25°C [19]; <30> reverse reaction, pH 8.0, 25°C [9]) [9, 19]
0.11 <42> (D-fructose 1,6-bisphosphate, <42> 37°C [45]) [45]
0.129 <23> (phosphate) [25]
0.13 <10, 30> (D-fructose 6-phosphate, <10,30> pH 8.0, 25°C [9,21]) [9, 21]
0.139-0.21 <14, 23> (D-fructose 1,6-bisphosphate) [23, 25]
0.14 <20> (phosphate, <20> pH 7.3, in presence of 2 mM Mg^{2+} [7]) [7]
0.15 <7> (phosphate, <7> pH 7.7, 25°C, in presence of fructose 2,6-bisphosphate [37]) [37]
0.17 <1> (D-fructose 6-phosphate, <1> mutant M249I, pH 7.2, 30°C [38]) [38]
0.23 <24, 39> (D-fructose 6-phosphate, <39> pH 6.0, 50°C [39]; <24> pH 8.0, 25°C [12]) [12, 39]
0.24 <31> (D-fructose 6-phosphate, <31> pH 8.0, 25°C [12]) [12]
0.25 <35> (D-fructose 6-phosphate, <35> pH 7.4, 30°C [32]) [32]
0.25 <36> (phosphate, <36> pH 6.8, 25°C, in presence of fructose 2,6-bisphosphate [34]) [34]
0.27 <4> (D-fructose 6-phosphate, <4> pH 7.5, 30°C [4]) [4]
0.27 <43> (D-fructose1,6-bisphosphate, <43> pH 8.0, 37°C [46]) [46]
0.29 <24> (diphosphate) [13]
0.3 <19> (D-fructose 6-phosphate, <19> forward reaction, pH 7.5, 25°C [17]) [17]
0.3-0.38 <12, 23, 33> (D-fructose 6-phosphate, <33> pH 7.2, 30°C [11]) [11, 24, 25]
0.38 <31> (sedoheptulose 7-phosphate, <31> pH 8.0, 25°C [12]) [12]
0.49 <24> (sedoheptulose 7-phosphate, <24> pH 8.0, 25°C [12]) [12]
0.5 <1, 10> (Mg^{2+}, <10> forward reaction, pH 8.0, 25°C [21]; <1> reverse reaction, pH 7.0, 30°C [2]) [2, 21]

0.53 <21, 43> (D-fructose 6-phosphate, <43> pH 7.0, 37°C [46]; <21> pH 8.0, 25°C [16]) [16, 46]
0.56 <30> (phosphate, <30> pH 8.0, 25°C [9]) [9]
0.58 <20> (diphosphate, <20> pH 7.8, in presence of 1.5 mM Mg^{2+} [7]) [7]
0.59 <2> (phosphate, <2> pH 7.0, 30°C [3]) [3]
0.59-1 <1, 3, 14, 33> (phosphate, <33> pH 8.6, 30°C [11]) [1-3, 11, 23, 28]
0.63 <7> (Mg^{2+}, <7> pH 7.7, 25°C [37]) [37]
0.63 <19> (phosphate, <19> pH 7.8, 25°C [17]) [17]
0.8 <20, 38> (D-fructose 6-phosphate, <20> pH 7.8, in presence of 1.5 mM Mg^{2+} [7]) [7, 36]
0.83 <20> (D-fructose 1,6-bisphosphate, <20> pH 7.3, reverse reaction, in presence of 2 mM Mg^{2+} [7]) [7]
0.89 <7> (D-fructose 6-phosphate, <7> pH 7.7, 25°C, in presence of fructose 2,6-bisphosphate [37]) [37]
0.92 <24> (2,5-anhydro-D-mannitol-6-phosphate, <24> pH 8.0, 25°C [12]) [12]
0.97 <31> (2,5-anhydro-D-mannitol-6-phosphate, <31> pH 8.0, 25°C [12]) [12]
1 <10> (D-fructose 1,6-bisphosphate, <10> reverse reaction, pH 8.0, 25°C [21]) [21]
1.14 <38> (phosphate) [36]
1.39 <43> (phosphate, <43> pH 8.0, 37°C [46]) [46]
1.7 <10> (phosphate, <10> reverse reaction, pH 8.0, 25°C [21]) [21]
1.9 <10> (Mg^{2+}, <10> reverse reaction, pH 8.0, 25°C [21]) [21]
2.2 <42> (D-fructose 6-phosphate, <42> 37°C [45]) [45]
2.9 <39> (D-fructose 1,6-bisphosphate, <39> pH 7.2, 50°C [39]) [39]
3.6 <1> (arsenate, <1> pH 7.0, 30°C [2]) [2]
3.6 <1> (arsenate, <1> with D-fructose 1,6-bisphosphate [2,28]) [2, 28]
3.8-10.6 <13, 14, 24> (D-fructose 6-phosphate, <13> pH 7.5, 25°C [18]; <13,14,24> with diphosphate [13,18,23]) [13, 18, 23]
4.3 <39> (phosphate, <39> pH 7.2, 50°C [39]) [39]
6.8 <1> (D-fructose 6-phosphate, <1> mutant M249A, pH 7.2, 30°C [38]) [38]
4600 <1> (D-fructose 6-phosphate, <1> mutant M249L, pH 7.2, 30°C [38]) [38]
Additional information <1, 7, 10, 13, 14, 17, 19, 21, 22, 24, 26, 30, 35, 40> (<7, 17, 21, 22, 26, 30, 35> kinetics [8, 9, 19, 33, 37, 43, 49]; <21> reaction kinetics are pH-dependent [49]; <1> K_m values for substrates of mutant enzymes [40]; <10, 13, 14, 40> kinetic parameters, in the absence or presence of D-fructose 2,6-bisphosphate [18, 21, 23, 41]) [8, 9, 14, 18, 19, 21, 23, 27, 33, 37, 40, 41, 43, 49]

K_i-Value (mM)

0.0025 <19> (diphosphate, <19> forward reaction, pH 7.5, 25°C [17]) [17]
0.013 <19> (D-fructose 1,6-bisphosphate, <19> reverse reaction, pH 7.8, 25°C [17]) [17]
0.023 <30> (diphosphate, <30> versus phosphate, pH 8.0, 25°C [9]) [9]

0.05 <37> (3-[N-(2-phenylthioethyl)-N-methylamino]-1-hydroxypropylidene-1,1-bisphosphonate, <37> pH 7.0, 25°C [35]) [35]
0.05 <37> (zoledronate, <37> pH 7.0, 25°C [35]) [35]
0.086 <30> (D-fructose 1,6-bisphosphate, <30> versus D-fructose 6-phosphate, pH 8.0, 25°C [9]) [9]
0.113 <19> (D-fructose 6-phosphate, <19> forward reaction, pH 7.5, 25°C [17]) [17]
0.115 <1> (D-fructose 6-phosphate, <1> versus diphosphate, pH 7.0, 30°C [2]) [2]
0.16 <1> (D-fructose 6-phosphate, <1> versus D-fructose 1,6-bisphosphate, pH 7.0, 30°C [2]) [2]
0.25 <19> (3-phosphoglycerate, <19> pH 7.5 [14]) [14]
0.3 <37> (risedronate, <37> pH 7.0, 25°C [35]) [35]
0.32 <31> (2,5-anhydro-D-glucitol-6-phosphate, <31> forward reaction, pH 8.0, 25°C [12]) [12]
0.39 <19> (phosphate, <19> reverse reaction, pH 7.8, 25°C [17]) [17]
0.4 <37> (alendronate, <37> pH 7.0, 25°C [35]) [35]
0.56 <37> (etidronate, <37> pH 7.0, 25°C [35]) [35]
0.62 <30> (D-fructose 6-phosphate, <30> versus D-fructose 1,6-bisphosphate, pH 8.0, 25°C [9]) [9]
0.78 <23> (phosphate, <23> versus fructose 6-phosphate, pH 7.5, 25°C, in presence of fructose 2,6-bisphosphate at saturating concentration [25]) [25]
0.85 <1> (phosphate, <1> versus diphosphate, pH 7.0, 30°C [2]) [2]
1.2 <23> (phosphate, <23> versus diphosphate, pH 7.5, 25°C, in presence of fructose 2,6-bisphosphate at saturating concentration [25]) [25]
1.3 <12> (diphosphate, <12> pH 8.0, 25°C [24]) [24]
1.46 <14> (phosphate, <14> versus fructose 6-phosphate, pH 7.5, 25°C, in presence of fructose 2,6-bisphosphate at saturating concentration [23]) [23]
1.49 <31> (diethylene glycol-monophosphate, <31> forward reaction, pH 8.0, 25°C [12]) [12]
1.52 <24> (2,5-anhydro-D-glucitol-6-phosphate, <24> forward reaction, pH 8.0, 25°C [12]) [12]
1.53 <14> (phosphate, <14> versus diphosphate, pH 7.5, 25°C, in presence of fructose 2,6-bisphosphate at saturating concentration [23]) [23]
2.05 <31> (L-sorbose-6-phosphate, <31> forward reaction, pH 8.0, 25°C [12]) [12]
2.16 <31> (D-glucitol-6-phosphate, <31> forward reaction, pH 8.0, 25°C [12]) [12]
2.22 <31> (1,6-hexanediol-monophosphate, <31> forward reaction, pH 8.0, 25°C [12]) [12]
2.37 <31> (2,3-O-isopropylidene-α-L-sorbofuranose-1-phosphate, <31> forward reaction, pH 8.0, 25°C [12]) [12]
2.39 <24> (L-sorbose-6-phosphate, <24> forward reaction, pH 8.0, 25°C [12]) [12]
2.41 <24> (D-tagatose-6-phosphate, <24> forward reaction, pH 8.0, 25°C [12]) [12]

2.56 <31> (D-tagatose-6-phosphate, <31> forward reaction, pH 8.0, 25°C [12]) [12]
3 <1> (phosphate, <1> versus D-fructose 1,6-bisphosphate, pH 7.0, 30°C [2]) [2]
3.24 <35> (D-fructose 2,6-bisphosphate, <35> versus fructose 1,6-bisphosphate, competitive, pH 7.4, 30°C [33]) [33]
3.62 <24> (D-arabinose-5-phosphate, <24> forward reaction, pH 8.0, 25°C [12]) [12]
3.9 <31> (D-fructose 2,6-bisphosphate, <31> pH 8.0, 25°C [12]) [12]
5.07 <24> (D-glucitol-6-phosphate, <24> forward reaction, pH 8.0, 25°C [12]) [12]
6.7 <24> (2,3-O-isopropylidene-α-L-sorbofuranose-1-phosphate, <24> forward reaction, pH 8.0, 25°C [12]) [12]
7.36 <35> (D-fructose 2,6-bisphosphate, <35> versus phosphate, pH 7.4, 30°C [33]) [33]
10.3 <30> (phosphate, <30> versus diphosphate, pH 8.0, 25°C [9]) [9]
10.87 <24> (diethylene glycol-monophosphate, <24> forward reaction, pH 8.0, 25°C [12]) [12]
20.2 <40> (Cl^-, <40> forward reaction, HEPES-NaOH buffer + NaCl, pH 7.2, 25°C [41]) [41]
21.7 <40> (Cl^-, <40> forward reaction, Tris-HCl buffer, pH 7.2, 25°C [41]) [41]
56.9 <40> (Cl^-, <40> reverse reaction, pH 8.0, 25°C [41]) [41]
85 <37> (clodronate, <37> pH 7.0, 25°C [35]) [35]
Additional information <17, 21, 24, 30, 31, 35> (<21> substrate-substrate interaction between phosphate and diphosphate in forward and reverse reaction [26]; <21, 35> kinetic product inhibition [16, 26, 33]; <24, 31> kinetics, various inhibitors, forward and reverse reaction [12]; <30> inhibition kinetics: inhibition by product and inhibitors [9]) [9, 12, 16, 19, 26, 33]

pH-Optimum

5.7-6.3 <39> (<39> 50°C, forward reaction [39]) [39]
6 <2> (<2> forward reaction [3]) [3]
6.7-8.7 <7> (<7> reverse reaction, pH-independent, broad optimum [37]) [37]
6.8 <36> (<36> forward reaction in presence of fructose 2,6-bisphosphate [34]) [34]
7 <1, 35, 37, 44> (<35> reverse reaction [32]; <1, 37, 44> assay at [2, 35, 47]) [2, 32, 35, 47]
7-7.5 <39> (<39> 50°C, reverse reaction [39]) [39]
7.2 <1, 21, 33, 35> (<1,21> assay at [40, 42]; <21, 33, 35> forward reaction [11, 32, 42]) [11, 32, 40, 42]
7.3 <20, 21> (<21> assay at [26]; <20> assay at, reverse reaction [7]) [7, 26]
7.3-7.7 <19> (<19> forward reaction [17]) [17]
7.4 <14, 24, 26, 31> (<24> assay at [13]; <31> imidazole-HCl buffer, forward and reverse reaction [10]; <14> in the presence of D-fructose 2,6-bisphosphate [23]) [8, 10, 13, 23]

7.5 <2, 13, 14, 19, 23, 40> (<19, 40> assay at [14, 41]; <2,40> reverse reaction [3, 41]; <14> in the presence of D-fructose 2,6-bisphosphate [23]; <23> forward reaction, D-fructose 2,6-bisphosphate has no effect on pH-optimum [25]) [3, 14, 18, 23, 25, 41]
7.5-7.8 <9> (<9> forward and reverse reaction [6]) [6]
7.5-8.5 <43> (<43> forward reaction [46]) [46]
7.6 <22, 36> (<36> reverse reaction, broad optimum, in both presence and absence of fructose 2,6-bisphosphate [34]; <22> assay at [43]; <36> forward reaction in absence of fructose 2,6-bisphosphate [34]) [34, 43]
7.7 <21> (<21> both reaction directions [49]) [49]
7.7-7.9 <7> (<7> forward reaction, strongly pH-dependent [37]) [37]
7.8 <19, 20, 24> (<19> reverse reaction [17]; <20> assay at, forward reaction [7]; <24> HEPES-NaOH buffer [27]) [7, 17, 27]
7.8-8.3 <43> (<43> reverse reaction [46]) [46]
7.9 <14> (<14> reverse reaction, in absence of D-fructose 2,6-bisphosphate [23]) [23]
8 <7, 10, 12, 17, 21, 30, 31, 24, 40> (<10, 12, 21, 30, 31, 24, 40> assay at [9, 12, 21, 22, 24, 41]; <40> forward reaction [41]) [9, 12, 15, 19, 21, 22, 24, 41]
8.1 <14> (<14> forward reaction, in absence of D-fructose 2,6-bisphosphate [23]) [23]
8.6 <33> (<33> reverse reaction [11]) [11]

pH-Range

3.5-10.3 <26> (<26> smooth hyperbolic pH-value/activity curve [8]) [8]
6.3-8.5 <35> (<35> half-maximal activity at pH 6.3 and pH 8.5 [32]) [32]
6.8-7.6 <36> (<36> reverse reaction, 96% activity at pH 6.8, maximum at pH 7.6 [34]) [34]

Temperature optimum (°C)

21-22 <1, 26> (<1, 26> assay at [1,8]) [1, 8]
25 <1, 7, 10, 12, 13, 17, 19, 21, 22, 30, 31, 36, 37, 40> (<1, 7, 10, 12, 13, 17, 19, 21, 22, 30, 31, 36, 37, 40> assay at [9, 10, 16-22, 24, 34, 35, 37, 40, 41, 43]) [9, 10, 16-22, 24, 34, 35, 37, 40, 41, 43]
30 <1, 2, 14, 24, 30, 33, 35> (<1, 2, 14, 24, 30, 33, 35> assay at [2, 3, 11, 23, 27, 31, 32]) [2, 3, 11, 23, 27, 31, 32]
37 <42, 43> (<42,43> assay at [45,46]) [45, 46]
50 <39, 44> (<39,44> assay at [39,47]) [39, 47]
86 <44> [47]
Additional information <7> (<7> temperature-independent [37]) [37]

4 Enzyme Structure

Molecular weight

61000-62000 <12> (<12> PAGE, gel filtration [24]) [24]
64000-67000 <35> (<35> native PAGE, gel filtration [32]) [32]
65000 <39> (<39> gel filtration [39]) [39]
70000 <38> (<38> gel filtration [36]) [36]

74000 <26> (<26> sucrose density gradient centrifugation [8]) [8]
81000 <26> (<26> PAGE [8]) [8]
83000 <1> (<1> gel filtration [2,28]) [2, 28]
95000 <31, 33> (<31> high speed meniscus depletion ultracentrifugation [10]; <33> gel filtration [11]) [10, 11]
97000 <7> (<7> gel filtration [37]) [37]
100000 <4, 44> (<4,44> gel filtration [4,47]) [4, 47]
103000 <14> (<14> gel filtration [23]) [23]
115000 <20> [5]
123000-136000 <12> (<12> dimer, PAGE, gel filtration [24]) [24]
125000 <20> (<20> gel filtration [7]) [7]
128000 <13> (<13> sucrose density gradient centrifugation [18]) [18]
170000 <23> (<23> gel filtration [25]) [25]
180000 <2> (<2> gel filtration [3]) [3]
183000 <13> (<13> gel filtration [18]) [18]
190000 <24> (<24> gel filtration [12]) [12]
220000 <16> (<16> two enzyme forms [5]) [5]
243000-262000 <12> (<12> predominant tetrameric form, PAGE, gel filtration, in the presence of diphosphate the oligomer dissociates into monomers, D-fructose 2,6-bisphosphate does not reassociate [24]) [24]
265000 <21> (<21> gel filtration, in the presence of diphosphate the enzyme dissociates into a 130000 dimer, D-fructose 2,6-bisphosphate prevents or reassociates [20]) [20]
294000 <10> (<10> gel filtration, enzyme does not dissociate in the presence of D-fructose 2,6-bisphosphate [21]) [21]
425000 <12> (<12> hexamer, PAGE, in the presence of diphosphate the oligomer dissociates into monomers, D-fructose 2,6-bisphosphate does not reassociate [24]) [24]
450000 <16> (<16> two enzyme forms [5]) [5]
460000 <36> (<36> gel filtration [34]) [34]
520000 <36> (<36> native PAGE [34]) [34]

Subunits

? <1, 30, 37> (<30> x * 43243, deduced amino acid sequence [31]; <37> x * 47580, DNA sequence determination [35]; <1> * 60000, recombinant mutants, SDS-PAGE [38]) [31, 35, 38]
dimer <4, 7, 23, 31, 39> (<39> 2 * 37000, SDS-PAGE [39]; <4> 2 * 45000, SDS-PAGE [4]; <31> 2 * 48000, SDS-PAGE [10]; <7> 2 * 61500, SDS-PAGE [37]; <23> 1 * 80000 + 1 * 90000, SDS-PAGE [25]) [4, 10, 25, 37, 39]
monomer <14, 35, 38> (<35> 1 * 64000, SDS-PAGE [32]; <38> 1 * 67000, SDS-PAGE [36]; <14> 1 * 100000, SDS-PAGE [23]) [23, 32, 36]
octamer <36> (<36> 4 * 60000 + 4 * 66000, SDS-PAGE [34]) [34]
oligomer <12, 21> (<21> x * 55700 + x * 58000, urea-SDS-PAGE [22]; <12> x * 64000 + x * 68000, SDS-PAGE [24]) [22, 24]

tetramer <2, 10, 21, 44> (<44> 4 * 37000, SDS-PAGE [47]; <2> 4 * 51000, SDS-PAGE [3]; <21> 2 * 65000 + 2 * 60000, SDS-PAGE [20]; <10> 2 * 60000 + 2 * 65000-67000, α_2-β_2, SDS-PAGE [21]) [3, 20, 21, 47]
Additional information <7> (<7> dimer-monomer mixture might exist [37]) [37]

5 Isolation/Preparation/Mutation/Application

Source/tissue

amoeba <1-3, 37> [1-3, 28, 35]
bulb <6> [20]
cell culture <35> [32, 33]
cell suspension culture <13, 36> [18, 34]
cotyledon <5, 18> [20]
endosperm <19, 23> [14, 17, 20, 25]
floral primordium <8> (<8> differentiated [20]) [20]
fruit <12> (<12> mature green [24]) [24]
hypocotyl <24> [20]
leaf <7, 11, 19, 20, 22, 23, 25> [5, 7, 15, 16, 20, 37, 43]
mesocarp <15> [20]
radicle <18> [20]
root <10> (<10> tap root [20]) [20, 21]
seed <9, 17, 19, 23, 24> (<9, 17, 19, 23, 24> germinating [6, 13, 14, 17, 19, 25, 27]) [6, 13, 14, 17, 19, 25, 27]
seedling <14> [23]
stem <16> [5]
trophozoite <37> [35]
tuber <21> (<21> mature [16,42]) [16, 20, 22, 26, 42]
Additional information <41> (<41> enzyme is completely replaced by an ATP-dependent phosphofructokinase, when cells grow on only one C1-carbon source, e.g. methanol [44]) [44]

Localization

cytosol <1, 13, 20, 21, 26, 39> (<20> exclusively [7]) [1, 7, 8, 16, 18, 39]

Purification

<1> (recombinant wild-type and mutant [38]; partial [2]) [1, 2, 28, 38]
<2> (1124fold to near homogeneity [3]; inactivation by gel filtration, reactivation by AMP [3]) [3]
<4> (to near homogeneity [4]) [4]
<7> (partial, approximately 10fold [15]; 40fold, to homogeneity [37]) [15, 37]
<9> (more than 700fold [6]) [6]
<10> (167fold [21]) [21]
<12> (partial, 569fold [24]) [24]
<13> (partial, 102fold [18]) [18]
<14> (852fold, to apparent homogeneity [23]) [23]
<16> (partial [5]) [5]

<17> (partial [19]) [19]
<19> (573fold [17]; over 500fold [14]; partial [14,17]) [14, 17]
<20> (partial [5,7]) [5, 7]
<21> (to homogeneity [20,22]; approximately 700fold [16]; partial [20,26]) [16, 20, 22, 26]
<22> (1066fold, to near homogeneity [43]) [43]
<23> (partial, 127fold [25]) [25]
<24> (partial [13]; purification to homogeneity of a commercial lyophilized product [12]) [12, 13, 27]
<26> (partial, 430fold [8]) [8]
<30> (to apparent homogeneity [9]) [9]
<31> (purification to homogeneity from a commercial lyophilized product [12]; 631fold [10]) [10, 12]
<33> (partial 83.4fold [11]) [11]
<35> (2 different methods, method 1: 292fold to homogeneity [32]) [32]
<36> (184fold [34]) [34]
<37> (native, partial 146fold, further purification leads to inactivation [35]; recombinant His-tagged protein from Escherichia coli [35]) [35]
<38> (over 650fold, to homogeneity [36]) [36]
<39> (231fold, to homogeneity [39]) [39]
<42> (recombinant from Escherichia coli, 32fold to homogeneity [45]) [45]
<44> (74fold, to homogeneity [47]) [47]

Cloning

<1> (expression of wild-type and mutants in Escherichia coli DF1020 [40]; expression of wild-type and mutants in Escherichia coli [38]) [38, 40]
<30> (DNA and amino acid sequence determination, expression in Escherichia coli [31]) [31]
<35> (functional overexpression in transgenic tabacco Nicotiana tabacum cv. Petit Havanna SR1 leaves, transformation via Agrobacterium tumefaciens infection [48]) [48]
<37> (DNA sequence determination and analysis, functional expression in Escherichia coli BL21(DE3) as His-tagged protein [35]) [35]
<42> (expression in Escherichia coli BL21(DE3) [45]) [45]
<43> (gene TP0542, DNA sequence determination, expression in Escherichia coli DH5α [46]) [46]

Engineering

H415A <1> (<1> site-directed mutagenesis, reduced activity, increased K_m for fructose 6-phosphate and decreased K_m for diphosphate [40]) [40]
H416A <1> (<1> site-directed mutagenesis, reduced activity, slightly increased K_m for fructose 6-phosphate [40]) [40]
K408A <1> (<1> site-directed mutagenesis, activity , decreased K_m for diphosphate [40]) [40]
M249A <1> (<1> site-directed mutagenesis, reduced activity and increased K_m-value for fructose 6-phosphate compared to wild-type [38]) [38]
M249I <1> (<1> site-directed mutagenesis, highly reduced activity, increased K_m-value for fructose 6-phosphate compared to wild-type [38]) [38]

M249L <1> (<1> site-directed mutagenesis, highly reduced activity and increased K_m-value for fructose 6-phosphate compared to wild-type [38]) [38]
R377A <1> (<1> site-directed mutagenesis, increased K_m values for fructose 6-phosphate and diphosphate [40]) [40]
R405A <1> (<1> site-directed mutagenesis, increased activity, kinetic properties similar to the wild-type [40]) [40]
R423A <1> (<1> site-directed mutagenesis, decrease of k_{cat} by 10000fold, 10fold increased K_m for diphosphate, 126fold increased K_m for fructose 6-phosphate [40]) [40]
S392A <1> (<1> site-directed mutagenesis, activity and kinetic properties similar to the wild-type [40]) [40]
Y420A <1> (<1> site-directed mutagenesis, reduced activity, increased K_m values for fructose 6-phosphate and diphosphate [40]) [40]
Y420F <1> (<1> site-directed mutagenesis, reduced activity, increased K_m for fructose 6-phosphate, decreased K_m for diphosphate [40]) [40]
Y420H <1> (<1> site-directed mutagenesis, reduced activity, increased K_m values for fructose 6-phosphate and diphosphate [40]) [40]
Additional information <35> (<35> construction of transgenic tabacco plants, functionally overexpressing the recombinant enzyme, show decrease in biomass formation, but no dramatic physiological or morphological alterations, and no significant reduction of seed production, endogenous plant enzyme activity EC 2.7.1.11 is not reduced, increased usage of storage starch [48]) [48]

6 Stability

pH-Stability

5.3-8 <35> (<35> 1 h, 30°C, stable [32]) [32]

Temperature stability

0 <2> (<2> slight inactivation [3]) [3]
30 <2, 3, 23> (<23> 15 min stable [25]; <2,3> at enzyme concentrations of more than 10-20 units/ml, partial activation, enhanced by AMP, crude cell extract: $t_{1/2}$: 20 min, AMP stabilizes, 25% loss of activity within 2 h [3]) [3, 25]
50 <23> (<23> $t_{1/2}$: 15 min [25]) [25]
60 <23> (<23> above, complete inactivation [25]) [25]
62 <33> (<33> 10 min stable in the presence of 5 mM $MgCl_2$ [11]) [11]
70 <26> (<26> 2 min, about 60% loss of activity, inactivation within 10 min [8]) [8]
80 <39> (<39> half-life: 150 min [39]) [39]
90 <39> (<39> half-life: 10 min [39]) [39]

Oxidation stability

<39>, not sensitive to O_2 [39]

General stability information

<1>, enzyme is stable if whole cells are lyophilized or vigorously sonicated [1]
<1>, unstable to freezing in 20 mM imidazole buffer [28]
<2>, gel filtration inactivates, AMP restores activity, more rapidly at 30°C than at 0°C [3]
<19>, Mg^{2+} stabilizes [17]
<19>, glycerol essential for stability during purification [17]
<20>, 2-mercaptoethanol stabilizes [7]
<23>, ethylene glycol stabilizes during purification, almost complete loss of activity without [25]
<33>, PAGE or dialysis against Mg-free Tris-HCl buffer, pH 7.5, inactivates [11]
<36>, chymostatin and leupeptin stabilize the enzyme and prevent degradation during purification [34]
<44>, dialysis in absence of KCl or $MgCl_2$ results in total loss of enzyme activity [47]

Storage stability

<1>, -20°C, in salt-containing solution, 25% glycerol, $t_{1/2}$: 1 year [28]
<1>, -20°C, partially purified enzyme, loss of 25% activity after 1 month [2]
<7>, -80°C, purified enzyme, 25 mM Tris-HCl, pH 7.0, 20% glycerol v/v, 2 mM 2-mercaptoethanol, stable for at least 6 months [37]
<13>, -90°C, quick frozen in a dry-ice and methanol bath, at least 6 months [18]
<13>, 0°C, in HEPES-glycol buffer, several days [18]
<14>, 4°C, 10% loss of activity within 2 weeks [23]
<17>, -20°C, in 10 mM HEPES-NaOH buffer, pH 7.5, 10 mM 2-mercaptoethanol, 30% v/v glycerol, several months [19]
<19>, 4°C, in glycerol, 20% w/v, 20% loss of activity within 1 week [17]
<23>, -20°C, in 50 mM HEPES buffer, pH 7.5, 10 mM 2-mercaptoethanol, 0.5 mM EDTA, 1 mM $MgCl_2$, 5 mM KCl, 30% ethylene glycol, more than 1 month [25]
<24>, -20°C, in 10 mM Tris-acetate buffer, pH 7.3, 0.1 mM EDTA, 0.5 mM DTT, 17 mM KCl, plus 20% v/v glycerol, 6 months [27]
<24>, -80°C, in 10 mM Tris-acetate buffer, pH 7.3, 0.1 mM EDTA, 0.5 mM DTT, 17 mM KCl, 6 months [27]
<24>, 4°C, without glycerol, 1 week, then slow decrease of activity over a period of weeks [27]
<26>, -20°C, in κ-buffer with glycerol, 10% v/v, about 25% loss of activity within 6 months [8]

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Sphinganine kinase 2.7.1.91

1 Nomenclature

EC number
2.7.1.91

Systematic name
ATP:sphinganine 1-phosphotransferase

Recommended name
sphinganine kinase

Synonyms
SK
SPHK
dihydrosphingosine kinase
kinase, dihydrosphingosine (phosphorylating)
kinase, sphingosine (phosphorylating)
sphingoid base kinase

CAS registry number
50864-48-7

2 Source Organism

<1> *Homo sapiens* (Swiss 3T3 cell culture [18, 22]; SPHK1 [21, 25]; recombinant SPHK1, SPHK2 [23]; recombinant enzyme [28]) [1, 3, 9, 13, 18, 19, 21, 22, 23, 25, 27, 28]
<2> *Rattus norvegicus* (mast-cell line RBL-2H3 [26]) [5, 8, 16, 26]
<3> *Bos taurus* [7]
<4> *Tetrahymena pyriformis* [2, 6]
<5> *Mus musculus* [4, 12, 14]
<6> *Sus scrofa* [1, 3]
<7> *Saccharomyces cerevisiae* [10, 20, 24]
<8> *Homo sapiens* [11]
<9> *Homo sapiens* (SPHK2 [15]) [15]
<10> *Mus musculus* (SPHK2 [15]) [15]
<11> *mammalia* [17, 24]
<12> *yeast* [17]
<13> *plant* [17]

3 Reaction and Specificity

Catalyzed reaction

ATP + sphinganine = ADP + sphinganine 1-phosphate (<11-13> overview on regulation [17])

Reaction type

phospho group transfer

Natural substrates and products

S ATP + sphinganine <1, 2, 4, 5, 11-13> (<2> D-erythro-sphinganine, sphingosine metabolism [5]; <5> involved in regulation of GTP cyclohydrolase I and 6(r)-5,6,7,8-tetrahydrobiopterin [14]; <7,11-13> overview on cellular functions [17,24]; <1> involved in signal transduction in the nucleus [18]; <2> calcium mobilization through antigen receptors utilizes enzyme [26]) [2, 5, 14, 17, 18, 26]

P ADP + sphinganine 1-phosphate

Inhibitors

ADP <1> [9]
B-5354c <1> (<1> SPHK1, SPHK2, noncompetitive to sphingosine [23]) [23]
Cutsum <4> (<4> detergent, required for sphinganine suspension, 0.05 mg/ml gives optimal rates, inhibition above 1 mg/ml [6]) [6]
D(+)threo-sphinganine <1, 2> [3, 5]
DL-threo-dihydrosphingosine <1, 2> (<2> competitive [26]) [9, 26]
EDTA <1> [3, 9]
F-12509A <1> (<1> SPHK1, SPHK2, competitive to sphingosine [23]) [23]
KCl <10> (<10> IC50 200 mM [15]) [15]
L(-)erythro-sphinganine <1> [3]
L(-)threo-sphinganine <1, 2> [3, 5]
N,N,N-trimethylsphingosine <1> [9]
N,N-dimethylsphingosine <1, 2, 9, 10> [9, 15, 16, 21]
N-ethylmaleimide <1> [3]
NaCl <10> (<10> IC50 200 mM [15]) [15]
Triton X-100 <10> (<10> above 0.005% [15]) [15]
dihydrosphingosine <1, 2> (<2> L-erythro-, L-threo- and D-threo-isomer [5]; <1> DL-threo-isomers [21]) [5, 9, 21]
p-chloromercuribenzoate <1> [3]
Additional information <7> (<7> not inhibitory: DL-threo-dihydrosphingosine [10]) [10]

Activating compounds

Cutsum <4> (<4> detergent, required for sphinganine suspension, 0.05 mg/ml gives optimal rates, inhibition above 1 mg/ml [6]) [6]

Metals, ions

Ca^{2+} <4> (<4> 30% of the stimulation with Mg^{2+} [6]; <1> less stimulating than Mg^{2+}, Mn^{2+} [9]) [6, 9]

Mg^{2+} <1-4> (<3,4> required [6,7]; <4> ratio Mg^{2+}:ATP is 5:1 [6]; <3> ATP:Mg^{2+} ratio 2:1 [7]; <1> stimulation at concentration equimolar to ATP [3]; <3> optimum concentration: 10 mM [7]; <1> highest activity with [9]; <2> maximal activity at 5-10 mM [16]) [3, 6, 7, 9, 16]
Mn^{2+} <1, 4> (<1> stimulation at concentration equimolar to ATP [3]; <4> 40% of the stimulation with Mg^{2+} [6]; <1> less stimulating than Mg^{2+} [9]) [3, 6, 9]

Specific activity (U/mg)
0.00375 <3> [7]

K_m-Value (mM)
0.0034 <10> (D(+)erythro-sphinganine) [15]
0.005 <2> (D(+)erythro-sphinganine) [16]
0.016 <1> (D-erythrosphinganine, <1> as BSA-complex [9]) [9]
0.02 <1> (DL-erythro-dihydrosphingosine) [9]
0.072 <1> (D(+)erythro-4t-sphinganine) [3]
0.077 <1> (ATP) [9]
0.09 <4> (sphinganine) [2]
0.093 <2> (ATP) [16]
0.1 <4> (D(+)erythro-sphinganine) [2]
1 <1> (L(-)threo-sphinganine) [3]
Additional information <1, 6> (<1,6> simple quantitative radio-assay [1]; <1> assay for measurement of intracellular levels of free sphingoid bases [22]) [1, 15, 22]

K_i-Value (mM)
0.001 <1> (ADP) [9]
0.0022 <1> (B-5354c, <1> SPHK2 [23]) [23]
0.003 <1> (N,N-dimethylsphingosine, <1> SPHK1 [23]) [23]
0.004 <1> (B-5354c, <1> SPHK1 [23]) [23]
0.004 <1> (F-12509A, <1> SPHK1 [23]) [23]
0.005 <2> (DL-threo-dihydrosphingosine) [16]
0.005-0.018 <2> (DL-threo-dihydrosphingosine) [26]
0.0055 <1> (F-12509A, <1> SPHK2 [23]) [23]
0.006 <1> (DL-threo-dihydrosphingosine) [9]
0.008 <1> (N,N-dimethylsphingosine) [9]
0.0086 <1> (N,N-dimethylsphingosine, <1> SPHK2 [23]) [23]
0.01 <2> (N,N-dimethylsphingosine) [16]
0.012 <10> (N,N-dimethylsphingosine) [15]

pH-Optimum
6.6-7.5 <2> [16]
7 <1, 4, 6> [3, 6]
7.4 <1> [9]
7.5 <10> [15]
7.5-8.5 <4> [2]

pH-Range

6-8.5 <1> (<1> pH 6: about 55% of maximum activity, pH 8.5: about 40% of maximum activity [3]) [3]
6-9 <4> (<4> pH 6: about 35% of maximum activity, pH 9: about 70% of maximum activity [2]) [2]
6.5-8 <10> [15]
6.8-7.4 <1> (<1> greater than 60% of maximum activity [9]) [9]

Temperature optimum (°C)

43 <7> [10]

4 Enzyme Structure

Molecular weight

49000 <2> (<2> gel filtration [16]) [16]
190000 <3> (<3> gel filtration [7]) [7]

Subunits

monomer <2> (<2> 1 * 49000, SDS-PAGE [16]) [16]
Additional information <1> (<1> relationship to diacylglyceride kinases, NAD^+ kinases and 6-phosphofructokinases [27]) [27]

5 Isolation/Preparation/Mutation/Application

Source/tissue

brain <1-3> [5, 7, 19]
cell culture <5> (<5> melanoma B16 cells, variant F10, Swiss 3T3, balb/c 3T3 clone A31 [4]) [4]
endothelial cell <5> [12]
kidney <1, 2> [16, 19]
liver <2> [8]
placenta <1> [9]
thrombocyte <1, 6> (<1,6> platelets [1,19]) [1, 3, 19]
Additional information <1, 10> (<1,10> tissue distribution [15,19,21,25]) [15, 19, 21, 25]

Localization

Golgi apparatus <7> (<7> enzymes cycles between trans-Golgi network and late endosomes, facing the cytosol [20]) [20]
cytoplasm <1, 6> [3]
cytosol <7> [10]
endosome <7> (<7> enzymes cycles between trans-Golgi network and late endosomes, facing the cytosol [20]) [20]
extracellular <5> (<5> enzyme is constitutively exported [12]) [12]
microsome <2, 4> [2, 5]
nucleus <1> (<1> both nuclear envelope and nucleoplasm [18]) [18]
soluble <1, 4> [3, 6]

Purification

<1> [9]
<2> (partial) [5, 8, 16]
<3> (partial, multiple forms [7]) [7]

Cloning

<1> (SPHK1 [21,25]) [9, 21, 25]
<9> [15]
<10> [15]
<11-13> (<11-13> overview [17]; <11> overview [24]) [17, 24]

Engineering

G113A <1> (<1> increased catalytic activity [28]) [28]
Additional information <1, 7> (<7> spontaneous mutants with reduced enzyme activity [10]; <1> deletion mutants in highly conserved regions, truncation mutants [13]) [10, 13]

Application

medicine <8> (<8> involved in signal transduction by TRAF2, tumor necrosis factor-α receptor-associated factor 2 [11]) [11]

6 Stability

Temperature stability

37 <1> (<1> 10% glycerol, 0.05% Triton X-100, 0.5 M NaCl, stable for 30 min [9]) [9]

General stability information

<3>, extensive dialysis or dilution in potassium phosphate buffer, 100 mM, pH 7.4, results especially during the early stages of purification in rapid loss of activity [7]
<3>, repeated freezing and thawing results in 20-30% loss of activity [7]

Storage stability

<1>, -18°C, stable for at least 3 weeks [3]
<3>, -20°C, 100 mM phosphate buffer, pH 7.4, 1 mM 2-mercaptoethanol, 1 mM EDTA, 20% glycerol, stable for several months [7]

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5-Dehydro-2-deoxygluconokinase 2.7.1.92

1 Nomenclature

EC number
2.7.1.92

Systematic name
ATP:5-dehydro-2-deoxy-D-gluconate 6-phosphotransferase

Recommended name
5-dehydro-2-deoxygluconokinase

Synonyms
5-keto-2-deoxyglucono kinase (phosphorylating)
5-keto-2-deoxygluconokinase
DKH kinase
kinase, 5-keto-2-deoxyglucono- (phosphorylating)

CAS registry number
62213-35-8

2 Source Organism

<1> *Aerobacter aerogenes* (C1, constitutive strain [1]) [1]

3 Reaction and Specificity

Catalyzed reaction
ATP + 5-dehydro-2-deoxy-D-gluconate = ADP + 6-phospho-5-dehydro-2-deoxy-D-gluconate

Reaction type
phospho group transfer

Natural substrates and products
S ATP + 2-deoxy-5-keto-D-gluconic acid <1> (<1> involved in myo-inositol degradation [1]) (Reversibility: ? <1> [1]) [1]
P ADP + 2-deoxy-5-keto-D-gluconic acid 6-phosphate

Substrates and products
S ATP + 2-deoxy-5-keto-D-gluconic acid <1> (Reversibility: ? <1> [1]) [1]
P ADP + 2-deoxy-5-keto-D-gluconic acid 6-phosphate <1> [1]

Activating compounds
Additional information <1> (<1> enzyme is induced by growth on myo-inositol [1]) [1]

Metals, ions
Mg^{2+} <1> (<1> $MgCl_2$ is included in assay mixture on the assumption that it is required [1]) [1]

Specific activity (U/mg)
13.6 <1> [1]

K_m-Value (mM)
0.097 <1> (2-deoxy-5-keto-D-gluconic acid) [1]
0.22 <1> (ATP) [1]

pH-Optimum
7-8.5 <1> (<1> broad, Tris-chloride buffer [1]) [1]
8 <1> (<1> assay at [1]) [1]

Temperature optimum (°C)
30 <1> (<1> assay at [1]) [1]

4 Enzyme Structure

Molecular weight
225000 <1> (<1> sucrose density gradient centrifugation [1]) [1]

5 Isolation/Preparation/Mutation/Application

Purification
<1> (partial, 20fold, not seperated from 2-deoxy-5-keto-D-gluconic acid 6-phosphate aldolase [1]) [1]

6 Stability

General stability information
<1>, purified enzyme is unstable to repeated freezing and thawing in 20 mM potassium phosphate buffer, pH 7, 5 mM EDTA, 10 mM 2-mercaptoethanol [1]

Storage stability
<1>, 0°C, 20 mM potassium phosphate buffer, pH 7, 5 mM EDTA, 10 mM 2-mercaptoethanol, 1 month or more, stable [1]
<1>, 4°C, enzyme of DEAE-Sephadex fractions, 20 mM potassium phosphate buffer, pH 7, 5 mM EDTA, 10 mM 2-mercaptoethanol, 0.2 M KCl, 5 months, stable [1]

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Alkylglycerol kinase 2.7.1.93

1 Nomenclature

EC number
2.7.1.93

Systematic name
ATP:1-O-alkyl-sn-glycerol 3-phosphotransferase

Recommended name
alkylglycerol kinase

Synonyms
1-alkylglycerol kinase (phosphorylating)
ATP-alkylglycerol phosphotransferase
ATP:1-alkyl-sn-glycerol phosphotransferase
alkylglycerol phosphotransferase
kinase, 1-alkylglycerol (phosphorylating)

CAS registry number
55354-37-5

2 Source Organism

<1> *Oryctolagus cuniculus* (adult male New Zealand white rabbit [1]) [1, 2]

3 Reaction and Specificity

Catalyzed reaction
ATP + 1-O-alkyl-sn-glycerol = ADP + 1-O-alkyl-sn-glycerol 3-phosphate

Reaction type
phospho group transfer

Natural substrates and products
S ATP + 1-alkyl-sn-glycerol <1> (<1> involved in the biosynthesis of complex ether-linked glycerolipids [1]) (Reversibility: ? <1> [1]) [1]
P ADP + 1-alkyl-sn-glycero-3-phosphate <1> (<1> the product 1-alkyl-2-lyso-sn-glycero-3-phosphate is the branch point intermediate in the de novo biosynthesis of ether-linked glycerolipids, plasmanylcholine, i.e. ethanolamine, and corresponding plasmalogenic analogs in membranes or platelet-activating factor PAF [2]) [1, 2]

Substrates and products

- **S** ATP + 1-O-hexadecyl-sn-glycerol <1> (Reversibility: ? <1> [1,2]) [1, 2]
- **P** ADP + 1-O-hexadecyl-sn-glycero-3-phosphate <1> [1, 2]
- **S** ATP + 1-alkyl-sn-glycerol <1> (<1> stereoselective transfer of the terminal phosphate of ATP to position 3 of the substrate, ATP is an absolute requirement for enzymatic activity [1]; <1> stereospecific for the substrates possessing an sn-1 alkyl chain [2]) (Reversibility: ? <1> [1,2]) [1, 2]
- **P** ADP + 1-alkyl-sn-glycero-3-phosphate <1> (<1> formation of 1-alkyl-2-lyso-sn-glycero-3-phosphate [2]) [1, 2]
- **S** ATP + 1-octadecyl-sn-glycerol <1> (Reversibility: ? <1> [1,2]) [1, 2]
- **P** ADP + 1-octadecyl-sn-glycero-3-phosphate <1> [1]
- **S** Additional information <1> (<1> not: alkylethylene glycols, only trace activity with 1-thio-hexadecyl-2,3-propanediol [1,2]; <1> not: S-alkyl-alkylglycerols, 2-hexadecylglycerol, 3-hexadecyl-sn-glycerol, 1-hexadecylethylene glycol [1]; <1> not: 3-alkyl-sn-glycerols [2]) [1, 2]
- **P** ?

Inhibitors

Additional information <1> (<1> not inhibited by rac-1-hexadecanoylglycerol, rac-1,2-dihexadecanoylglycerol, rac-1-hexadecyl-2-hexadecanoylglycerol or 2-hexadecylglycerol [1]) [1]

Metals, ions

Mg^{2+} <1> (<1> absolute requirement for a divalent metal ion, Mg^{2+} is most effective [1]) [1]

Mn^{2+} <1> (<1> about 50% as effective as Mg^{2+} in activation [1]) [1]

Additional information <1> (<1> little activity with Zn^{2+}, Ba^{2+}, Cu^{2+}, Ca^{2+}, Hg^{2+}, Sn^{2+}, Sr^{2+}, Ni^{2+}, Fe^{2+}, NaCl and KCl [1]) [1]

K_m-Value (mM)

1.6 <1> (ATP) [1]

pH-Optimum

7.1 <1> (<1> 0.1 M Tris-maleate buffer [1]; <1> no sharp pH optimum [1,2]) [1, 2]

pH-Range

6.1-8.1 <1> (<1> about 66% of maximal activity at pH 6.1 and 8.1 [1,2]) [1, 2]

Temperature optimum (°C)

37 <1> (<1> assay at [1,2]) [1, 2]

5 Isolation/Preparation/Mutation/Application

Source/tissue

harderian gland <1> (<1> pink portions of [1]) [1, 2]

Localization
microsome <1> [1, 2]

Purification
<1> (partial [1,2]) [1, 2]

Application
nutrition <1> (<1> enzyme plays a very important role in nutritional experiments, both in vivo and in cell culture, where the intent is either to restore or to increase the cellular levels of ether-linked phospholipids [2]) [2]

6 Stability

Storage stability
<1>, -20°C, whole microsomal preparation, 0.25 M sucrose, 5 mM dithiothreitol, at least 2 months, stable [1]
<1>, -20°C, whole microsomal preparation, at least 2 months, stable [2]

References

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Acylglycerol kinase 2.7.1.94

1 Nomenclature

EC number
2.7.1.94

Systematic name
ATP:acylglycerol 3-phosphotransferase

Recommended name
acylglycerol kinase

Synonyms
MGK
kinase, monoacylglycerol (phosphorylating)
monoacylglycerol kinase
monoglyceride kinase
monoglyceride phosphokinase
sn-2-monoacylglycerol kinase

CAS registry number
62213-37-0

2 Source Organism

<1> *Cavia porcellus* [1]
<2> *Bos taurus* (calf [1]) [1, 3]
<3> *Escherichia coli* (strain B [2]) [2]
<4> *Rattus norvegicus* (a single enzyme may function as diacylglycerol kinase and as monoacylglycerol kinase [4]) [4]
<5> *Oryctolagus cuniculus* (identity of diacylglycerol kinase and monoacylglycerol kinase is established by employing purified enzyme and immunospecific antibody [5]) [5]

3 Reaction and Specificity

Catalyzed reaction
ATP + acylglycerol = ADP + acyl-sn-glycerol 3-phosphate

Reaction type
phospho group transfer

Substrates and products

- **S** ATP + 1,2-dipalmitoylglycerol <3> (Reversibility: ? <3> [2]) [2]
- **P** ADP + 1,2-dipalmitoylglycerol 3-phosphate <3> (product identified as α-phosphatidic acid) [2]
- **S** ATP + 1-arachidonoylglycerol <2> (Reversibility: ? <2> [3]) [3]
- **P** ADP + 1-arachidonoylglycerol 3-phosphate
- **S** ATP + 1-arachidoylglycerol <2> (Reversibility: ? <2> [3]) [3]
- **P** ADP + 1-arachidoylglycerol 3-phosphate
- **S** ATP + 1-caproylglycerol <2> (Reversibility: ? <2> [3]) [3]
- **P** ADP + 1-caproylglycerol 3-phosphate
- **S** ATP + 1-capryloylglycerol <2> (Reversibility: ? <2> [3]) [3]
- **P** ADP + 1-capryloylglycerol 3-phosphate
- **S** ATP + 1-lauroylglycerol <2> (Reversibility: ? <2> [3]) [3]
- **P** ADP + 1-lauroylglycerol 3-phosphate
- **S** ATP + 1-linolenoylglycerol <2> (Reversibility: ? <2> [3]) [3]
- **P** ADP + 1-linolenoylglycerol 3-phosphate
- **S** ATP + 1-linoleoylglycerol <2> (Reversibility: ? <2> [3]) [3]
- **P** ADP + 1-linoleoylglycerol 3-phosphate
- **S** ATP + 1-monoolein <2> (i.e. 1-monooleolylglyerol) (Reversibility: ? <2> [1,3]) [1, 3]
- **P** ADP + 1-oleoylglycerol 3-phosphate
- **S** ATP + 1-myristoylglycerol <2> (Reversibility: ? <2> [3]) [3]
- **P** ADP + 1-myristoylglycerol 3-phosphate
- **S** ATP + 1-palmitoleoylglycerol <2> (Reversibility: ? <2> [3]) [3]
- **P** ADP + 1-palmitoleoylglycerol 3-phosphate
- **S** ATP + 1-palmitoylglycerol <2, 3> (Reversibility: ? <2,3> [1-3]) [1-3]
- **P** ADP + 1-palmitoylglycerol 3-phosphate <3> [2]
- **S** ATP + 1-stearoylglycerol <2> (Reversibility: ? <2> [3]) [3]
- **P** ADP + 1-stearoylglycerol 3-phosphate
- **S** ATP + 2-arachidonoylglycerol <2> (Reversibility: ? <2> [3]) [3]
- **P** ADP + 2-arachidonoylglycerol 3-phosphate
- **S** ATP + 2-arachidoylglycerol <2> (Reversibility: ? <2> [3]) [3]
- **P** ADP + 2-arachidoylglycerol 3-phosphate
- **S** ATP + 2-caproylglycerol <2> (Reversibility: ? <2> [3]) [3]
- **P** ADP + 2-caproylglycerol 3-phosphate
- **S** ATP + 2-lauroylglycerol <2> (Reversibility: ? <2> [3]) [3]
- **P** ADP + 2-lauroylglycerol 3-phosphate
- **S** ATP + 2-linolenoylglycerol <2> (Reversibility: ? <2> [3]) [3]
- **P** ADP + 2-linolenoylglycerol 3-phosphate
- **S** ATP + 2-linoleoylglycerol <2> (Reversibility: ? <2> [3]) [3]
- **P** ADP + 2-linoleoylglycerol 3-phosphate
- **S** ATP + 2-monoolein <2> (i.e. 2-monooleolylglyerol) (Reversibility: ? <2> [3]) [3]
- **P** ADP + 2-oleoyoglycerol 3-phosphate
- **S** ATP + 2-palmitoleoylglycerol <2> (Reversibility: ? <2> [3]) [3]
- **P** ADP + 2-palmitoleoylglycerol 3-phosphate
- **S** ATP + 2-palmitoylglycerol <2> (Reversibility: ? <2> [1,3]) [1, 3]

P ADP + 2-palmitoylglycerol 3-phosphate
S ATP + 2-stearoylglycerol <2> (Reversibility: ? <2> [3]) [3]
P ADP + 2-stearoylglycerol 3-phosphate
S Additional information <2, 4> (<2> 1-monostearin amongst the 1-acylglycerols and 2-monoarachidonin amongst the 2-acylglycerols give the highest activity [3]; <2> preference for substrates with unsaturated fatty acids except for 1- and 2-monostearins [3]; <4> increasing activity in the order: 1-monoacylglycerol, 2-monoacylglycerol, 1,2-diacylglycerol [4]; <4> when saturated fatty acids are present the order of decreasing activity varies directly with increasing chain length for C_{10} to C_{20} [4]; <4> a single enzyme may function as diacylglycerol and monoacylglycerol kinase [4]) [3, 4]
P ?

Inhibitors

5,5'-dithiobis(2-nitrobenzoic acid) <2> (<2> DTT prevents inhibition [3]) [3]
NEM <2> (<2> DTT prevents inhibition [3]) [3]
p-chloromercuriphenylsulfonate <2> (<2> DTT prevents inhibition [3]) [1, 3]
phosphatidylcholine <2> (<2> inhibition alone, synergistic activation with the protein peak eluted from hydroxylapatite by 25 mM phosphate [3]) [3]
phosphatidylethanolamine <2> (<2> inhibition alone, synergistic activation with the protein peak eluted from hydroxylapatite by 25 mM phosphate [3]) [3]
sphingomyelin <2> (<2> inhibition alone, synergistic activation with the protein peak eluted from hydroxylapatite by 25 mM phosphate [3]) [3]

Activating compounds

Cutsum <3> (<3> no absolute requirement, optimal concentration: 0.1% [2]) [2]
glutathione <2> (<2> stimulates [1]) [1]
phospholipids <2> (<2> e.g. phosphatidylcholine, phosphatidylethanolamine or sphingomyelin, inhibition alone, synergistic activation with the protein peak eluted from hydroxylapatite by 25 mM phosphate [3]) [3]
Additional information <2> (<2> protein peak eluted from hydroxylapatite by 25 mM phosphate activates and stabilizes [3]) [3]

Metals, ions

Ca^{2+} <2, 3> (<2> 5 mM, 58% as effective as Mg^{2+} in activation [3]; <3> requirement for divalent cation is best fulfilled by Mg^{2+}. Mn^{2+}, Zn^{2+} and Ca^{2+}, in that order are fair to poor substitutes for Mg^{2+}) [2, 3]
Co^{2+} <2> (<2> 5 mM, 60% as effective as Mg^{2+} in activation [3]) [3]
Cu^{2+} <2> (<2> 5 mM, 51% as effective as Mg^{2+} in activation [3]) [3]
Fe^{2+} <2> (<2> 5 mM, 60% as effective as Mg^{2+} in activation [3]) [3]
Mg^{2+} <2, 3> (<3> requirement for divalent cation is best fulfilled by Mg^{2+}. Mn^{2+}, Zn^{2+} and Ca^{2+}, in that order are fair to poor substitutes for Mg^{2+}, op-

timal concentration of Mg^{2+} is 0.03 M [2]; <2> optimal concentration is 10 mM [3]; <2,3> required [2, 3]) [2, 3]
Mn^{2+} <2, 3> (<2> 5 mM, as effective as Mg^{2+} in activation [3]; <3> requirement for divalent cation is best fulfilled by Mg^{2+}. Mn^{2+}, Zn^{2+} and Ca^{2+}, in that order are fair to poor substitutes for Mg^{2+} [2]) [2, 3]
Ni^{2+} <2> (<2> 5 mM, 60% as effective as Mg^{2+} in activation [3]) [3]
Zn^{2+} <2, 3> (<2> 5 mM, 48% as effective as Mg^{2+} in activation [3]; <3> requirement for divalent cation is best fulfilled by Mg^{2+}. Mn^{2+}, Zn^{2+} and Ca^{2+}, in that order are fair to poor substitutes for Mg^{2+} [2]) [2, 3]

Specific activity (U/mg)

Additional information <2> [3]

pH-Optimum

7 <3> [2]
7.4-7.5 <4> [4]

pH-Range

6.5-7.5 <3> (<3> about 35% of maximal activity at pH 6.5 and pH 7.5 [2]) [2]

4 Enzyme Structure

Subunits

? <2> (<2> x * 72000, bovine, SDS-PAGE, enzyme exists as a complex that is stabilized by 0.5 M NaCl and, on complete dissociation yields a major protein of MW 72000 [3]) [3]

5 Isolation/Preparation/Mutation/Application

Source/tissue

brain <1, 2, 4, 5> [1, 3, 4, 5]

Localization

cytoplasm <2> [1]
cytosol <2, 5> [3, 5]
microsome <1, 2, 5> [1, 5]
particle-bound <3> [2]

Purification

<2> [3]

6 Stability

General stability information

<2>, DTT, EDTA or ATP stabilizes during purification [3]

<2>, protein peak eluted from hydroxylapatite by 25 mM phosphate activates and stabilizes [3]

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Kanamycin kinase 2.7.1.95

1 Nomenclature

EC number
2.7.1.95

Systematic name
ATP:kanamycin 3'-O-phosphotransferase

Recommended name
kanamycin kinase

Synonyms
aminoglycoside phosphotransferase 3'-IIIa, i.e. APH3'-IIIa <3> [7]
kinase, kanamycin (phosphorylating)
neomycin phosphotransferase
neomycin-kanamycin phosphotransferase

CAS registry number
62213-36-9

2 Source Organism

<1> *Pseudomonas aeruginosa* (ten strains: H1-H9 and A3 [1]; strain Ps49, neomycin phosphotransferase II [5]) [1, 5]
<2> *Staphylococcus aureus* (multiple drug-resistant strain B294 [2,5]; drug-resistant strain B295 [2]) [2, 5]
<3> *Escherichia coli* (R-factor strain JR35 [3]; strains JR35/W677, JR39/W677, JR66/W677, neomycin phosphotransferase II, ML1629, neomycin phosphotransferase I [5]; pkm2-transformed C-600 cells [4]; Escherichia coli strain transformed with plasmid encoding kanamycin kinase, higher antibiotica resistance in bacteria cultvated in the presence of putrescine [6]) [3-11]
<4> *monkey* [4]
<5> *Mus musculus* [4]

3 Reaction and Specificity

Catalyzed reaction
ATP + kanamycin = ADP + kanamycin 3'-phosphate (<3> isoenzyme APH3'-IIIa follows a Theorell-Chance mechanism [7])

Reaction type

phospho group transfer

Substrates and products

S ATP + kanamycin <1-5> (<1> phosphorylates the C-3 hydroxyl group of 6-amino-6-deoxy-D-glucose moiety of kanamycin B or C [1]; <1-3> specific for ATP [1,5]; <1> no activity with ADP [1]; <1,2> no activity with streptomycin [1,2]; <3> isoenzyme APH3'-IIIa [7]) (Reversibility: ? <1-5> [1-5]; r <3> [7]) [1-7]
P ADP + kanamycin 3'-phosphate <1-5> [1-7]
S ATP + neamine <1> (Reversibility: ? <1> [1]) [1]
P ADP + neamine phosphate <1> [1]
S ATP + neomycin <1-3> (Reversibility: ? <1-3> [1-3]) [1-3]
P ADP + neomyin phosphate <1-3> [1-3]
S ATP + paromomycin <1> (Reversibility: ? <1> [1]) [1]
P ADP + paromomycin phosphate <1> [1]

Inhibitors

gentamicin 1a <1, 3> (<1,3> with neomycin as substrate, no inhibition in E. coli strain JR66/W677 or Pseudomonas aeruginosa strain Ps49 [5]) [5]
kanamycin <2> (<2> substrate inhibition at 2 mM [5]) [5]
tobramycin <1-3> [5]

Metals, ions

Co^{2+} <1> (<1> activation at 1 mM, can replace Mg^{2+} [5]) [5]
Mg^{2+} <1, 3> (<1> activation [1]; <3> required for activity [3,5]) [1, 3, 5]
Mn^{2+} <1> (<1> activation at 1 mM, can replace Mg^{2+} [5]) [5]
Zn^{2+} <1> (<1> activation at 1 mM, can replace Mg^{2+} [5]) [5]

Turnover number (min^{-1})

6.6 <3> (ATP, <3> pH 7.5, 37°C, isoenzyme APH3'-IIIa [7]) [7]
105.6 <3> (ATP, <3> pH 7.5, 37°C, isoenzyme APH3'-IIIa [7]) [7]
107.4 <3> (kanamycin A, <3> pH 7.5, 37°C, isoenzyme APH3'-IIIa [7]) [7]

K_m-Value (mM)

0.004 <3> (kanamycin A, <3> pH 6.5, 37°C, isoenzyme APH3'-IIIa [7]) [7]
0.013 <3> (kanamycin A, <3> pH 7.5, 37°C, isoenzyme APH3'-IIIa [7]) [7]
0.022 <3> (ADP, <3> pH 7.5, 37°C, isoenzyme APH3'-IIIa [7]) [7]
0.028 <3> (ATP, <3> pH 7.5, 37°C, isoenzyme APH3'-IIIa [7]) [7]
0.3 <1> (kanamycin, <1> pH 7.5, 35°C [5]) [5]
1.32 <3> (3'-phosphokanamycin, <3> pH 7.5, 37°C, isoenzyme APH3'-IIIa [7]) [7]

pH-Optimum

5.5-8 <1, 3> (<1,3> broad, no marked optimum [5]) [5]
7-7.5 <2> [2, 5]
7.5 <1> (<1> with kanamycin [5]) [5]

pH-Range

6-8 <2> (<2> approx. half-maximal activity at pH 6.0 and pH 8.0 [2]) [2]
6.5-8 <1> (<1> approx. half-maximal activity at pH 6.5 and pH 8.0 [1]) [1]

Temperature optimum (°C)

20 <3-5> (<3-5> assay at [4]) [4]
35-37 <1-3> (<1-3> assay at [1,2,5]) [1, 2, 5]

5 Isolation/Preparation/Mutation/Application

Source/tissue

3T3 cell <5> (<5> transformed with pAG60 [4]) [4]
COS-1 cell <4> (<4> transfected with plasmid pSV2neo [4]) [4]

Localization

periplasm <3> [3]
soluble <1> [1]

Purification

<1> (ammonium sulfate, Sephadex G 50, Sephadex G 100, partial purification [1]) [1]

Cloning

<3> (overexpression of aminoglycoside phosphotransferase-IIIa, i.e. APH3'-IIIa in Escherichia coli [7]; expression of neoR gene in Trypanosoma cruzi [8]; expression of kanaR gene in Escherichia coli [9]; expression of neomycin phosphotransferase II in Nicotiana tobacco [10]; expression of neomycin phosphotransferase II-glycophorin A fusion protein in Escherichia coli and CHO cells [11]) [7, 8, 9, 10, 11]

6 Stability

pH-Stability

7.5-9 <1> (<1> stable [5]) [5]

Temperature stability

45 <3> (<3> strain ML1629, 5 min, inactivation [5]) [5]
55 <1> (<1> strain H-9, 20 min stable [5]) [5]
65 <1> (<1> strain H-9, 5 min stable [5]) [5]
75 <1> (<1> strain H-9, inactivation [5]) [5]

General stability information

<3>, ammonium chloride or neomycin B stabilizes, E. coli JR39 enzyme [5]
<1-3>, dithiothreitol stabilizes and restores activity of denatured enzyme [5]

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NADH Kinase 2.7.1.96

1 Nomenclature

EC number
2.7.1.96 (deleted, included in EC 2.7.1.86)

Recommended name
NADH kinase

Opsin kinase 2.7.1.97

1 Nomenclature

EC number
2.7.1.97 (deleted, included in EC 2.7.1.125)

Recommended name
opsin kinase

Phosphoenolpyruvate-fructose phosphotransferase 2.7.1.98

1 Nomenclature

EC number
2.7.1.98 (deleted)

Recommended name
phosphoenolpyruvate-fructose phosphotransferase

[Pyruvate dehydrogenase (lipoamide)] kinase 2.7.1.99

1 Nomenclature

EC number
2.7.1.99 (Protein kinases are in a state of review by the NC-IUBMB. This EC class will presumably be transferred to EC 2.7.11.2)

Systematic name
ATP:[pyruvate dehydrogenase (lipoamide)] phosphotransferase

Recommended name
[pyruvate dehydrogenase (lipoamide)] kinase

Synonyms
PDH kinase <4> [27]
PDHK <4> [27]
PDK <1, 4, 6-8, 11, 15, 16-26> [19, 28-35]
kinase (phosphorylating), pyruvate dehydrogenase
pyruvate dehydrogenase kinase
pyruvate dehydrogenase kinase (phosphorylating)
pyruvate dehydrogenase kinase activator protein (i.e. free pyruvate dehydrogenase kinase)
Additional information <4, 7, 8, 11> (<4> enzyme possibly belongs to the ATPase/kinase family [24]; <7, 8, 11> PDK isozymes and the related branched-chain dehydrogenase kinase form a unique family of serine kinases [30, 36]) [24, 30, 36]

CAS registry number
9074-01-5

2 Source Organism

<1> *Bos taurus* [1, 2, 4-14, 20, 30]
<2> *Sus scrofa* [11, 16]
<3> *Oryctolagus cuniculus* [18]
<4> *Rattus norvegicus* (female Wistar rats [27,34]; 4 different isozymes: PDK1, PDK2, PDK3, PDK4 [35]; isozymes PDK2 and PDK4 [34]; isozymes PDK1 and PDK2 [29]; selective increase in amount of PDHK4 protein in both hyperthyroidism and high-fat feeding [27]; 3 isozymes PDHK1, PDHK2, PDHK4 [27,31]; isozyme PDK2 [21,24,33]; two kinases: pyruvate dehydrogenase-intrinsic activity and free pyruvate dehydrogenase kinase

[17]; 4.5fold higher specific activity in starved than in fed rats [17]) [3, 17, 21, 23, 24, 27, 29-31, 33-35]
<5> *Pisum sativum* (var. Alaskan Marvel [15]) [15]
<6> *Homo sapiens* (isozyme PDK4 [19]) [19]
<7> *mammalia* (at least 4 different isozymes: PDK1, PDK2, PDK3, PDK4 [30, 31, 35]) [30, 31, 35]
<8> *Homo sapiens* (isozyme PDK3 [31]; isozymes PDK2 and PDK3 [28]; 4 different isozymes: PDK1, PDK2, PDK3, PDK4 [30,35]; isozyme development via gene duplication, gene locations are: PDK1 in chromosome 2, PDK2 in chromosome 17, PDK3 in chromosome Xp22, PDK4 in chromosome 7q21q22 [30]) [28, 30, 31, 35]
<9> *Zea mays* (at least 2 isozymes [30]) [30]
<10> *Arabidopsis thaliana* [26, 30]
<11> *Rattus norvegicus* (isozyme PDK1 [33]; from heart [36]) [33, 36]
<12> *Ascaris suum* [22, 25]
<13> *Mus musculus* (ob/ob mouse, animal model for diabetes [23]) [23]
<14> *Caenorhabditis elegans* [25]
<15> *Rattus norvegicus* (isozyme PDK2 [32]) [32]

3 Reaction and Specificity

Catalyzed reaction

ATP + [pyruvate dehydrogenase (lipoamide)] = ADP + [pyruvate dehydrogenase (lipoamide)] phosphate (A mitochondrial enzyme associated with the pyruvate dehydrogenase complex, phosphorylation inactivtes pyruvate dehydrogenase (lipoamide), EC 1.2.4.1; <1,10,15> mechanism [6,14,26,32]; <15> structure of the nucleotide binding pocket with responsible His115 residue and of the catalytic site [32]; <4> molecular modeling of the catalytic domain, structure [24]; <10> His121 is involved in the catalytic reaction [26])

Reaction type

phospho group transfer

Natural substrates and products

S ATP + [pyruvate dehydrogenase (lipoamide)] <1-15> (<4> high-fat feeding increases the expression of isozyme PDK2, but not of PDK4, hyperthyroidism increases the expression of both isozymes, physiological implications [34,35]; <7> isozyme PDK3 has a putative regulatory role of the pyruvate dehydrogenase complex in sperm [30]; <4> enzyme regulation in the heart depends on thyroid hormone and lipid status [27]; <4> enzyme regulates glucose oxidation by pyruvate dehydrogenase complex, isozyme PDHK1 is of more potential importance in adult heart than the other isozymes [27]; <4, 7, 8, 10> the enzyme is the primary regulator of flux through the mitochondrial pyruvate dehydrogenase complex [26, 35]; <1-8, 11-14> catalyzes inactivation through phosphorylation of pyruvate dehydrogenase complex EC 1.2.4.1 [1-36]; <4, 7, 8> tissue-specific regulation of the pyruvate dehydrogenase complex in order to adjust glu-

cose consumption [30, 34, 35]; <5> involved in regulation of mitochondrial pyruvate dehydrogenase complex [15]; <1> regulatory role [2, 4]; <1> β-subunit harbors a regulatory role [9]) (Reversibility: ir <1-26> [1-36]) [1-36]

P ADP + [pyruvate dehydrogenase (lipoamide)] phosphate <1-15> [1-36]

S Additional information <7> (<7> model of specific interactions and signal translation within the pyruvate dehydrogenase complex and between pyruvate dehydrogenase kinase and subunits, differences between the isozymes, mechanisms [30]) [30]

P ?

Substrates and products

S ATP + Ac-YHGHSMSDPGVSYR <4> (<4> recombinant enzyme [29]; <4> synthetic peptide substrate [29]) (Reversibility: ? <4> [29]) [29]

P ADP + [Ac-YHGHSMSDPGVSYR]phosphate

S ATP + [pyruvate dehydrogenase (lipoamide)] <1-15> (<7> isozyme PDK2 can phosphorylate free pyruvate dehydrogenase complex but bound dihydrolipoyl transacetylase enhances the rate up to 5000fold [35]; <4,11> recombinant hybrid enzyme of PDK1 and PDK2 phosphorylates site 3 with lower activity than the PDK1 homodimer [33]; <4,11> serine phosphorylation site 3 of subunit E1 is exclusively phosphorylated by isozyme PDK1, not by PDK2, which prefers site 1 over site 2 [33]; <4,8> the 3 serine phosphorylation sites of the E1 subunit are specifically and with different activity phosphorylated by the 4 isozymes, overview: site 1 is preferably utilized by PDK2, site 2 by PDK3, and site 3 is exclusively utilized by PDK1 [31]; <4,7,8> pyruvate dehydrogenase complex substrate is inactivated by ATP-dependent phosphorylation of 3 serine residues on the E1 subunit [29, 31, 35]; <10> substrate is kinase-depleted pyruvate dehydrogenase complex from Zea mays [26]; <1> highly specific for the substrate [1, 7-9]; <1, 2> incorporates γ-phosphate from ATP into E1-component of pyruvate dehydrogenase-complex α-subunit [7, 8, 11, 20]; <1> phosphorylation sites are 3 Ser-residues in the α-subunit, i.e. E1, MW 41000, of pyruvate dehydrogenase [1, 7, 8]; <2> optimum activity within a small range of ionic strength of 0.03-0.05 M [16]) (Reversibility: ir <1-15> [1-36]) [1-36]

P ADP + [pyruvate dehydrogenase (lipoamide)] phosphate <1-15> [1-36]

S ATP + casein <1> (<1> kidney enzyme, low activity [4]) (Reversibility: ? <1> [4]) [4]

P ADP + casein phosphate <1> [4]

S Additional information <1, 4, 8, 10-12, 14> (<4, 11> binding of homodimers of PDK1 and PDK2, respectively and the heterodimer of PDK1+PDK2 to the pyruvate dehydrogenase complex via dihydrolipoyl transacetylase [33]; <4, 8> activity depends on the buffer system, the reduction status of the lipoyl groups and on the serine phosphorylation site of the E1 subunit of the pyruvate dehydrogenase complex used as substrate [31]; <4> performs autophosphorylation [29]; <10> performs pH-dependent autophosphorylation on serine residues [26]; <12, 14> no autophosphorylation [22, 25]; <1> little, if any activity with casein of bovine kidney [9]; <1> no activity with histones of calf thymus type II-A [4]; <1> no activity with histones of calf thymus type II-A, VI-S and VIII-S [9]; <1> no activity with glycogen synthase a and rabbit skeletal muscle phosphorylase b [1, 7-9]) [1, 4, 7-9, 22, 25, 26, 29, 31, 33]

P ?

Inhibitors

(R)-3,3,3-trifluoro-2-hydroxy-2-methylpropioamide <4> (<4> strong inhibition, isozymes PDK1 and PDK2, inhibition mechanism [29]) [29]

2-chloroisohexanoate <3> (<3> weak [18]) [18]

2-oxobutyrate <1> [4]

ADP <1, 4, 5, 7, 8, 11> (<4, 11> recombinant homodimers of PDK1 and PDK2 and heterodimers of PDK1 + PDK2, synergism with dichloroacetate [33]; <7> isozyme PDK2: synergistic with phosphate [35]; <7> isozyme PDK3: synergistic with phosphate [30]; <7> isozyme PDK4: K^+ and dichloroacetate increase the inhibitory effect [30]; <8> 50-60% inhibition of isozyme PDK3, in presence of dihydrolipoyl transacetylase 70% [28]; <4> isozyme PDK2, wild-type and mutants G284A and G319A [24]; <1, 4, 5> competitive to ATP [4, 5, 7, 8, 15, 24]; <4, 5, 7, 8> synergism with pyruvate [15, 30, 35]; <1> inhibition only in the presence of monovalent cations [1, 5, 7, 8]; <5> inhibition only together with pyruvate, kinetics [15]; <1> Mg^{2+} does not protect [5]) [1, 4, 5, 7, 8, 15, 24, 28, 30, 33, 35]

ATP <1> (<1> above 0.5 mM, substrate inhibition, only in the presence of K^+, Mg^{2+} does not protect [5]) [5]

$CaCl_2$ <1> (<1> no inhibition [4]) [13]

Cl^- <2> (<2> 40% inhibition at 80 mM, K^+-independent inhibition [16]) [16]

CoA <7> [30]

DTNB <1> (<1> most potent at 0.001 mM [8]) [1, 8]

HPO_4^{2-}<1, 2> (<1> enhances inhibition by pyruvate or dichloroacetate [6]; <2> within physiological range, only in the presence of K^+, not in its absence [16]; <2> noncompetitive to ATP in the range of 1-10 mM [16]) [6, 16]

K^+ <1> (<1> synergism with ADP [5]; <1> pyruvate or dichloroacetate [6]) [5, 6]

$MnCl_2$ <1> [13]

NAD^+ <7> [30]

NEM <1> [1, 8]

Na^+ <1> (<1> above 50 mM, alone and synergism with ADP [5]) [5]
SO_4^{2-} <2> (<2> with the same effect as HPO_4^{2-} [16]) [16]
adenosine 5'-[β,γ,imido]triphosphate <4> (<4> isozymes PDK1 and PDK2, inhibition mechanism [29]) [29]
butyryl-CoA <1> (<1> at high concentrations [10]) [10]
chymotrypsin <1> (<1> proteolysis of kinase α-, not β-subunit, no inactivation by trypsin-mediated proteolysis of β-subunit [9]) [9]
decanoyl-CoA <1> [10]
dichloroacetate <1, 4, 7, 8, 11> (<4,11> recombinant homodimers of PDK1 and PDK2 and heterodimers of PDK1 + PDK2, synergism with dichloroacetate [33]; <7> isozyme PDK2: ADP and K^+ increase the inhibitory effect [30]; <7> isozyme PDK4: ADP, K^+ and Cl^- increase the inhibitory effect [30]; <8> inhibition of isozyme PDK3 is independent of dihydrolipoyl transacetylase, while isozyme PDK2 is more sensitive to inhibition when bound to it [28]; <11> highly specific [36]; <4> potent and highly specific synthetic allosteric inhibitor mimicking pyruvate, inhibition mechanism [21]; <1> noncompetitive [6]; <1> pyruvate analog, synergism with ADP, K^+ or phosphate, kinetics [6]) [3, 6, 21, 28, 29, 30, 33, 36]
dichloroacetophenone <4> (<4> isozymes PDK1 and PDK2, inhibition mechanism [29]) [29]
disulfides <1> (<1> thiols reverse [1,8]) [1, 8]
hexanoyl-CoA <1> [10]
insulin <7> (<7> blockage of the expression of isozyme PDK4 via insulin-activated pathway [35]) [35]
lactone derivative of dichloroacetophenone <4> (<4> isozymes PDK1 and PDK2, inhibition mechanism [29]) [29]
linolenoyl-CoA <1> [10]
linoleoyl-CoA <1> [10]
myristoyl-CoA <1> [10]
oleoyl-CoA <1> [10]
oximes of triterpenes <4, 13> (<4,13> with 17β hydroxyl and abietane derivatives, several, overview [23]) [23]
phosphate <7> (<7> isozyme PDK2, synergistically with ADP and pyruvate [35]; <7> enhances inhibition by pyruvate [30]) [30, 35]
pyruvate <1, 2, 4, 5, 7, 8> (<7> isozyme PDK3; very weak inhibition [35]; <7> isozyme PDK2: synergistic with phosphate [35]; <7> isozyme PDK2: ADP and K^+ increase the inhibitory effect [30]; <4> isozyme PDHK4 is less sensitive than PDHK1 and PDHK2 [27]; <7> inhibits at concentrations above 0.1 mM, activates below 0.05 mM [30]; <1,5> noncompetitive to ATP [6, 15]; <1, 5, 7> synergism with ADP [4, 6, 7, 8, 15, 30]; <1> synergism with K^+ or phosphate [6]; <5> dead-end inhibitor [15]; <1, 5> kinetics [6, 15]) [1, 3, 4, 6-8, 11, 15, 27, 30, 35]
stearoyl-CoA <1> [10]
thiamine diphosphate <1, 5, 8> (<1> non- or uncompetitively inhibition of K^+-stimulated activity [13, 14]; <1> 2-oxoisopentanoate protects, not pyruvate [13]; <5> in the presence of pyruvate [15]; <1> kinetics [14]) [3, 8, 13-15, 28]

triterpenes <4> (<4> isozymes PDK1 and PDK2, inhibition mechanism [29]) [29]
trypsin <12, 14> [25]
Additional information <1, 2, 7, 8> (<7> isozyme PDK3 undergoes self-association in absence of dihydrolipoyl transacetylase domain L2 leading to a decrease in activity [35]; <7> starvation and diabetes reduce the expression of isozyme PDK2 [35]; <7> model of specific interactions and signal translation within the pyruvate dehydrogenase complex and between pyruvate dehydrogenase kinase and subunits, differences between the isozymes, mechanisms [30]; <8> carnitine, acetylcarnitine, malate, spermine, and calcium have no effect on isozyme PDK3 in presence of dihydrolipoyl transacetylase [28]; <7> the effects of mono- and divalent ions vary greatly between the isozymes [30]; <1> not affected by calmodulin with or without Ca^{2+} [9]; <2> increase of ionic strength inhibits, changes of osmolarity of assay medium do not affect activity [16]; <1> no inhibition by 2-oxoglutarate [4]; <1> no inhibition by cGMP [7-9]; <1> no inhibition by cAMP [4, 7-9]; <1> no inhibition by succinyl-CoA, tiglyl-CoA, crotonyl-CoA, glutaryl-CoA, DL-3-hydroxy-3-methylglutaryl-CoA, acetylcarnitine or 3-hydroxybutyryl-CoA [10]) [4, 7-10, 16, 28, 30, 35]

Cofactors/prosthetic groups

ATP <1-8, 10-12, 14> (<1-8, 10-12, 14> dependent on [1-36]) [1-36]
Additional information <1> (<1> no activation by Ca^{2+}/calmodulin or calmodulin alone [9]; <1> no activation by cAMP [4, 7-9]; <1> no activation by cGMP [7-9]; <1> no activation by succinyl-CoA, tiglyl-CoA, crotonyl-CoA, glutaryl-CoA, DL-3-hydroxy-3-methylglutaryl-CoA, acetylcarnitine or 3-hydroxybutyryl-CoA [10]) [4, 7-10]

Activating compounds

2-oxoisopentanoate <1> (<1> only in the presence of K^+ and thiamine diphosphate, kinetics [14]) [1, 7, 8, 10, 14]
L-methylmalonyl-CoA <1> (<1> slight activation [10]) [10]
NADH <1, 4, 7, 8, 12, 14> (<7> activates the enzyme, especially isozyme PDK2 [35]; <7> isozyme PDK2: addition of K^+ and Cl^- required, phosphate increases the stimulating effect [30]; <7, 8> domain-specific binding, isozymes PDK2 and PDK3 [28, 35]; <12,14> kinase bound to transacetylase core [22, 25]; <8, 12, 14> synergism with acetyl-CoA [21, 22, 25, 28]; <1, 4> involved in the regulation of enzyme activity regulating pyruvate dehydrogenase complex [20, 21]; <1> activation, in the presence of K^+ [1, 7, 8, 10]; <1> activation, in the presence of NH_4^+ [1, 7, 8]) [1, 7, 8, 10, 20-22, 25, 28, 30, 35]
WY-14,643 <4> (<4> activator of peroxisome proliferator-activated receptor-α [30, 34]; <4> in liver, specifically increases PDK4 expression [34]; <4> in gastrocnemius muscle, specifically increases PDK4 expression [30]) [30, 34]
acetoacetyl-CoA <1> (<1> slight activation [10]) [10]
acetyl-CoA <1, 4, 7, 8, 12, 14> (<7> activates the enzyme, especially isozyme PDK2 [35]; <7> isozyme PDK2: addition of K^+ and Cl^- required, phosphate increases the stimulating effect [30]; <7,8> domain-specific binding, isozymes PDK2 and PDK3 [28, 35]; <12,14> kinase bound to transacetylase core

[22, 25]; <1, 7, 8, 12, 14> synergism with NADH [10, 21, 22, 25, 28, 30]; <1> high stimulation through acetylation of the transacetylase-catalyzing inner core portion of the dihydrolipoyl acetyltransferase [20]; <1, 4> involved in the regulation of enzyme activity regulating pyruvate dehydrogenase complex [20, 21]) [1, 7, 8, 10, 20-22, 25, 28, 30, 35]
benzoyl-CoA <1> (<1> slight activation [10]) [10]
dibutyryl cAMP <4> (<4> in muscle, reversed by insulin [30]) [30]
dihydrolipoyl transacetylase <1, 2, 4, 7, 8, 12, 14, 15> (<7> domaine-specific binding, isozymes PDK2 and PDK3, the latter binding more tightly to the L2 domain [35]; <7> isozyme PDK2 can phosphorylate free pyruvate dehydrogenase complex but bound dihydrolipoyl transacetylase enhances the rate up to 5000fold [35]; <4, 7, 8> dynamic, effector-modified interactions of the regulatory isozymes with the flexibly held outer domains of the core-forming dihydrolipoyl acetyl transferase component of pyruvate dehydrogenase complex to adapt the complex activity, regulatory mechanism [35]; <15> isozyme PDK2, activation mechanism, binding structure [32]; <4, 8> activation depends on the buffer system, the isozyme and the reduction status of the lipoyl groups [31]; <4, 8> activation in presence of a binding protein, referred to as dihydrolipoamide dehydrogenase-binding protein [31]; <4> requirement [29]; <8> lipoylation is required for binding, structural mutants stimulate less [28]; <8> stimulation rates in different buffers, stimulating domains for the isozyme PDK2 and PDK3 differ, overview [28]; <1, 7> binding and activation mechanism [20, 35]; <1> involved in the regulation of enzyme activity regulating pyruvate dehydrogenase complex [20]; <4, 7, 8> degree of interaction and mechanism differ for the 4 different isozymes [30, 31, 35]; <7> acts as a direct allosteric agent in altering the regulatory kinase activity, serves as an anchoring scaffold [30]; <1, 12, 14> pyruvate dehydrogenase-complex transacetylase core [12, 22, 25]; <2> rate-limiting in the holo-complex [11]; <1> 3-5fold stimulation [7]) [4, 7-9, 11, 12, 20-22, 25, 28-32, 35]
free fatty acids <7> (<7> leads to overexpression of isozyme PDK4 via mechanism involving peroxisome proliferator-activated receptor-α [35]) [35]
glucocorticoids <7> (<7> leads to overexpression of isozyme PDK4 via mechanism involving peroxisome proliferator-activated receptor-α [35]) [35]
lipid <4> (<4> enhance expression of hepatic isozyme PDK2 during high-fat feeding [34]; <4> in muscle, reversed by insulin [30]; <4> selective increase in amount of isozyme PDHK4 protein in both hyperthyroidism and high-fat feeding [27]) [27, 30, 34]
malonyl-CoA <1> [10]
octanoate <4> (<4> in muscle, reversed by insulin [30]) [30]
peroxisome proliferator-activated receptor-α <4> (<4> involved in mechanism to enhance expression of isozyme PDK4, but not isozyme PDK2, during starvation [34]) [34]
phosphate <8> (<8> isozyme PDK43, direct inhibition and elevation of the K_m for ATP [28]) [28]

propionyl-CoA <1> (<1> slight activation at low concentrations, synergism with NADH plus NAD^+ [10]) [10]
pyruvate <1, 7> (<1> at low concentration, with the thiamine diphosphate containing pyruvate dehydrogenase complex, stimulation mechanism [20]; <7> inhibits at concentrations above 0.1 mM, activates below 0.05 mM, dependent on thiamine diphosphate [30]) [20, 30]
thyroid hormones <4> (<4> selective increase in amount of isozyme PDHK4 protein in both hyperthyroidism and high-fat feeding [27]) [27]
Additional information <4, 7> (<4> during hyperthyroidism, the expression of hepatic isozymes PDK2 and PDK4 is increased [34]; <4,7> starvation increases expression of isozyme PDK4 [34,35]; <7> model of specific interactions and signal translation within the pyruvate dehydrogenase complex and between pyruvate dehydrogenase kinase and subunits, differences between the isozymes, mechanisms [30]) [30, 34, 35]

Metals, ions

K^+ <1, 2, 6> (<1> inhibits in presence of ADP [5]; <2> 2.2fold activation at 20 mM K^+, not pH- and buffer concentration-dependent [16]; <1> K^+-dependent activation, inhibited by thiamine diphosphate [13]; <6> activation [19]) [5, 13, 16, 19]
Mg^{2+} <1, 2, 4-8, 10-12, 14> (<1, 2, 4, 5, 7, 12> requirement [1, 4-17, 22, 24, 30]; <1, 2, 12> actual substrate: $MgATP^{2-}$ [4, 5, 7, 8, 11, 22]) [1, 4-17, 19, 21, 22, 24-26, 28, 30, 31, 33, 36]
Mn^{2+} <1> (<1> requirement [1, 4, 7, 8]; <1> can replace Mg^{2+} to some extent [1, 4, 7, 8]) [1, 4, 7, 8]
NH_4^+ <1> (<1> activation in the absence of ADP, inhibits in presence of ADP [5]) [5]
divalent cations <4> (<4> requirement [3]) [3]
Additional information <1, 2> (<1> no activation by Ca^{2+} [4]; <2> no activation by Na^+ [16]; <1> no activation by NaCl, LiCl [13]) [4, 13, 16]

Turnover number (min^{-1})

2.3 <4> ([pyruvate dehydrogenase (lipoamide)], <4> phosphorylation site 3 of subunit E1, isozyme PDK1, pH 7.0, 30°C [31]) [31]
5.6 <4> ([pyruvate dehydrogenase (lipoamide)], <4> phosphorylation site 2 of subunit E1, isozyme PDK3, pH 7.0, 30°C [31]) [31]
6.5 <4> ([pyruvate dehydrogenase (lipoamide)], <4> phosphorylation site 2 of subunit E1, isozyme PDK4, pH 7.0, 30°C [31]) [31]
6.6 <4> ([pyruvate dehydrogenase (lipoamide)], <4> phosphorylation site 1 of subunit E1, isozyme PDK1, pH 7.0, 30°C [31]) [31]
16.6 <4> ([pyruvate dehydrogenase (lipoamide)], <4> phosphorylation site 1 of subunit E1, isozyme PDK2, pH 7.0, 30°C [31]) [31]
30.7 <1> (ATP) [12]
32 <1> (ATP) [1]

Specific activity (U/mg)

0.008 <6> (<6> purified recombinant enzyme [19]) [19]
0.018 <1> (<1> purified dihydrolipoyl transacetylase-protein X-pyruvate dehydrogenase kinase subcomplex [12]) [12]
0.276 <4> (<4> fed rat, purified enzyme [17]) [17]
0.33 <1> [8, 9]
0.332 <1> (<1> purified enzyme, in presence of dihydrolipoyl transacetylase [7,8]) [7, 8]
0.44 <8> (<8> isozyme PDK3, pH 7.3, 30°C [28]) [28]
0.69 <8> (<8> isozyme PDK2, pH 7.3, 30°C [28]) [28]
0.74 <14> (<14> purified recombinant enzyme, in presence of NADH and acetyl-CoA [25]) [25]
0.92 <12> (<12> purified recombinant enzyme, in presence of NADH and acetyl-CoA [25]) [25]
1.24 <4> (<4> starved rat, purified enzyme [17]) [17]
1.9-2.7 <2> (<2> purified enzyme, in presence of dihydrolipoyl transacetylase, reconstituted enzyme complex [11]) [11]
Additional information <4, 8, 12> (<4,8> activity depends on the buffer system, the reduction status of the lipoyl groups and on the serine phosphorylation site of the E1 subunit of the pyruvate dehydrogenase complex used as substrate [31]) [22, 27, 31]

K_m-Value (mM)

0.0006 <1> (pyruvate dehydrogenase, <1> pH 7.5, 30°C [4]; <1> pH 7.0, 30°C [7,8]; <1> in presence of dihydrolipoyl transacetylase [4,7,8]) [4, 7, 8]
0.006 <8> (ATP, <8> isozyme PDK2, 30°C, pH 7.3, MOPS-K^+ buffer [28]; <8> isozyme PDK3, 30°C, pH 7.3, Tris-HEPES buffer [28]) [28]
0.007 <4> (ATP, <4> recombinant His-tagged isozyme PDK2, 37°C, pH 7.8 [33]) [33]
0.01 <2> (ATP, <2> at 0.2 M buffer concentration [16]) [16]
0.013 <8> (ATP, <8> isozyme PDK3, 30°C, pH 7.3, MOPS-K^+ buffer [28]) [28]
0.016 <4> (ATP, <4> 37°C, recombinant isozyme PDK2 wild-type [24]) [24]
0.02 <1, 5> (ATP, <5> pH 7.5, 25°C [15]; <1> pH 7.5, 30°C [4]; <1> pH 7.0, 30°C [7,8]; <1> kidney enzyme [4,7,8]; <1> pyruvate dehydrogenase complex [4,7,8]) [4, 7, 8, 15]
0.02 <1> (Mg^{2+}, <1> pH 7.5, 30°C [4]; <1> pH 7.0, 30°C [7,8]; <1> kidney enzyme [4,7,8]; <1> pyruvate dehydrogenase complex [4,7,8]) [1, 4, 7, 8]
0.02 <1> (pyruvate dehydrogenase, <1> pH 7.5, 30°C [4]; <1> pH 7.0, 30°C [7,8]; <1> in absence of dihydrolipoyl transacetylase [4,7,8]) [4, 7, 8]
0.023 <8> (ATP, <8> isozyme PDK2, 30°C, pH 7.3, Tris-HEPES buffer [28]) [28]
0.025 <1, 2> (ATP, <2> at 0.04 M buffer concentration [16]) [6, 16]
0.026 <5> (ATP) [15]
0.028 <4> (ATP, <4> 37°C, recombinant mutant G284A of isozyme PDK2 [24]) [24]

0.029 <8> (ATP, <8> isozyme PDK3, 30°C, pH 7.3, phosphate buffer [28]) [28]
0.034 <11> (ATP, <11> recombinant His-tagged isozyme PDK1, 37°C, pH 7.8 [33]) [33]
0.04 <8> (ATP, <8> isozyme PDK2, 30°C, pH 7.3, phosphate buffer [28]) [28]
1.5 <4> (ATP, <4> 37°C, recombinant mutant G319A of isozyme PDK2 [24]) [24]
Additional information <1, 2> (<1> the K_m value for ADP depends on the presence and concentration of K^+, effect of K^+ on kinetic parameters [5]; <2> changes in ionic strength [16]) [5, 16]

K_i-Value (mM)

0.1 <1> (ADP, <1> pH 7.5, 30°C [4]) [4]
0.2 <4> (ADP, <4> 37°C, recombinant isozyme PDK2 wild-type [24]) [24]
0.2 <4> (dichloroacetate, <4> pH 7.4, 37°C [21]) [21]
0.21 <5> (ADP, <5> pH 7.5, 25°C [15]) [15]
0.27 <1> (dichloroacetate, <1> pH 7.2, 30°C [6]; <1> kinetic constant from binding study [6]) [6]
0.27 <1> (pyruvate, <1> pH 7.2, 30°C [6]; <1> kinetic constant from binding study [6]) [6]
0.3 <4> (ADP, <4> 37°C, recombinant mutant G284A of isozyme PDK2 [24]) [24]
0.325 <5> (pyruvate, <5> pH 7.5, 25°C [15]) [15]
1.6 <4> (ADP, <4> 37°C, recombinant mutant G319A of isozyme PDK2 [24]) [24]
10 <2> (HPO_4^{2-}, <2> pH 7.8, 30°C [16]) [16]
Additional information <1, 7> (<7> isozyme PDK4: K^+ reduces the K_i for ADP and therefor enhances the inhibitory affect [30]; <1> inhibition kinetics for thiamine diphosphate [14]; <1> Ki value for ADP depends on the presence and concentration of monovalent cations, e.g. NH_4^+ or K^+ [5]) [5, 14, 30]

pH-Optimum

7 <1, 2> (<1,2> assay at [7-9,11,12]) [7-9, 11, 12]
7-7.2 <1> (<1> in presence of Mg^{2+} or Mn^{2+} [4]) [4]
7-7.4 <4, 8> (<4,8> assay at [27,28]) [27, 28]
7.2 <1> (<1> assay at [6]) [6]
7.2-8 <2> (<2> broad, at 0.15 M buffer concentration [16]) [16]
7.3 <1, 10> (<10> autophosphorylation [26]; <1> assay at [10]) [10, 26]
7.4 <4, 11, 12, 14> (<4,11,12,14> assay at [21,22,25,36]) [21, 22, 25, 36]
7.5 <5, 6> (<5, 6> assay at [15, 19]) [15, 19]
7.8 <4, 11> (<4,11> assay at [33]) [33]
Additional information <2, 4, 8> (<4,8> different buffer systems [31]; <2> optimal activity within a range of 0.03 M and 0.05 M buffer concentrations [16]) [16, 31]

pH-Range

5.5-8.5 <1> (<1> about 50% or 60% of maximal activity at pH 5.5 and about 65% or 50% of maximal activity at pH 8.5, in the presence of Mg^{2+} or Mn^{2+}, respectively [4]) [4]
6.2-9 <2> (<2> about half-maximal activity at pH 6.2 and 9 [16]) [16]

Temperature optimum (°C)

22 <6> (<6> room temperature, assay at [19]) [19]
25 <5, 10> (<5,10> assay at [15,26]) [15, 26]
30 <1, 2, 4, 8, 12> (<1, 2, 4, 8, 12> assay at [4-14, 16, 22, 27, 28, 31]; <8> phosphate buffer [28]) [4-14, 16, 22, 27, 28, 31]
37 <3, 4, 11, 12, 14> (<3, 4, 11, 12, 14> assay at [18, 21, 24, 25, 33]) [18, 21, 24, 25, 33]

4 Enzyme Structure

Molecular weight

76000 <14> (<14> recombinant wild-type, gel filtration [25]) [25]
80000 <12> (<12> about, recombinant enzyme, gel filtration [22]) [22]
86000 <10> (<10> approximately, recombinant enzyme with maltose-binding protein cleaved off, gel filtration [26]) [26]
92000 <4, 11> (<4,11> recombinant hybrid dimer of PDK1 and PDK2, gel filtration [33]) [33]
100000 <1> [1]
136000 <2> (<2> pyruvate dehydrogenase, gel filtration [11]) [11]

Subunits

? <4, 6, 8, 11> (<11> x * 36000, recombinant enzyme, SDS-PAGE [36]; <8> x * 45066, isozyme PDK2, DNA sequence determination [30]; <8> x * 45806, recombinant isozyme PDK2, detagged, amino acid determination [28]; <8> x * 45883, isozyme PDK3, DNA sequence determination [30]; <8> x * 46230, isozyme PDK4, DNA sequence determination [30]; <8> x * 46504, recombinant isozyme PDK3, detagged, amino acid determination [28]; <8> x * 48391, isozyme PDK1, DNA sequence determination [30]; <6,16-26> x * 47500, recombinant His-tagged enzyme, SDS-PAGE [19]; <4> x * 45000, free pyruvate dehydrogenase kinase, SDS-PAGE [17]; <11> x * 48000, native enzyme, SDS-PAGE [36]) [17, 19, 28, 30, 36]
dimer <1, 12, 14, 15> (<15> dimer of monomers built of 2 different domains, crystal structure [32]; <12> 2 * 43000-43470, mature protein, SDS-PAGE and DNA sequence determination [22]; <10> 2 * 44000, recombinant enzyme with maltose-binding protein cleaved off, SDS-PAGE [26]; <14> 2 * 45000, recombinant wild-type, SDS-PAGE [25]; <1> 1 * 48000, α + 1 * 45000, β, SDS-PAGE [1,7-9]) [1, 7-9, 22, 25, 26, 32]
Additional information <1, 2, 7> (<1,2,7> subunit composition and complex structure [11,12,30]; <1> kinase activity resides in α-subunit [1,9]) [1, 9, 11, 12, 30]

5 Isolation/Preparation/Mutation/Application

Source/tissue

brain <4, 7> (<7> isozymes PDK2 and PDK3 [30]; <4> higher activity in cerebral cortex and hippocampus than in hypothalamus, pons, medulla and olfactory bulbs [3]) [3, 30]
brown adipose tissue <7> (<7> isozyme PDK2 [30]) [30]
cerebral cortex <4> [3]
gastrocnemius muscle <4> (<4> overexpression of isozyme PDK4 during starvation or diabetes, reversible by insulin [30]) [30]
heart <1, 2, 4, 7, 11, 13> (<7> appearance of isozyme PDK1 is limited to the heart, isozyme PDK2 [30]; <4> developing and adult heart, the latter contains 3 isozymes: PDHK1, PDHK2 and PDHK4, clear differences in protein expression patterns of the isoforms dependent on developmental stage, overview [27]) [1, 2, 4, 7, 11, 13, 14, 23, 27, 30, 35, 36]
hippocampus <4> [3]
kidney <1, 2, 4, 7> (<1> PDK2-like isozyme very tightly bound to dihydrolipoyl transacetylase, possibly 2 forms of isozyme PDK2 exist [30]; <7> isozymes PDK2, PDK3 and PDK4 [30]; <2> cortex [16]) [1, 2, 4-12, 16, 20, 30, 35]
leaf <5> (<5> green leaf tissue [15]) [15]
liver <3, 4, 7> (<4> a complex signalling mechanism mediates the regulation of the isozymes PDK2 and PDK4 in response to the feeding status of the organism, overview [34]; <7> isozymes PDK2 and PDK4 are both overexpressed under conditions of starvation, diabetes, or high-fat feeding, low-carbohydrate diet, or due to artificially elevated cAMP or 3,5,3'-triidothyronine levels, reversible by insulin or high-carbohydrate diet [30]) [17, 18, 30, 34]
lung <7> (<7> isozymes PDK3 and PDK4, low levels of isozyme PDK2 [30]) [30]
mammary gland <7> (<7> lactating, isozyme PDK2 [30]) [30]
muscle <4, 12> (<12> adult [22]) [22, 23, 25]
seedling <5> [15]
skeletal muscle <7> (<7> isozyme PDK2 [30]) [30]
sperm <7> (<7> isozyme PDK3 with unique E1 subunit probably encoded on the Y-chromosome in contrary to the normal E1 subunit which is encoded on the X-chromosome, regulatory role [30]) [30]
spleen <7> (<7> isozyme PDK2, low level [30]) [30]
white adipose tissue <7> (<7> isozyme PDK2 [30]) [30]
Additional information <4, 7> (<7> isozyme PDK4 is overexpressed in many tissues during diabetes and starvation, particularly important in skeletal and heart muscle [30]; <4,7,8> tissue-specific expression, regulation mechanisms [30,31]; <4> selective increase in amount of isozyme PDHK4 protein in both hyperthyroidism and high-fat feeding [27]) [27, 30, 31]

Localization

mitochondrial inner membrane <1> [8]
mitochondrial matrix <1> [8]

mitochondrion <1, 2, 4, 5, 7, 8, 10-12, 15> (<12> enzyme contains a 18-amino acid mitochondrial import signal sequence [22]; <1> inner membrane matrix compartment [1,8]; <1> tightly bound to dihydrolipoamide acetyltransferase of pyruvate dehydrogenase complex [1,2,7,8]; <1> purified pyruvate dehydrogenase-complex contains 3 molecules of kinase, but one molecule of dihydrolipoyl transacetylase-protein x-subcomplex of pyruvate dehydrogenase activates more than 15 molecules of kinase [12]; <1> protein x serves to anchor the kinase to the core of the complex [12]) [1, 2, 4, 7-9, 11, 12, 15, 17, 22, 26, 27, 32, 33, 35]

Purification

<1> (2700fold to homogeneity [9]; kidney, from highly purifed pyruvate dehydrogenase-complex [1,7-9,11,12]; removed from the dihydrolipoyl transacetylase by treatment with *p*-hydroxymercuriphenylsulfonate [11,12]) [1, 2, 7-9, 11-13]
<2> [11]
<4> (recombinant wild-type and mutants as His-tagged proteins from Escherichia coli BL21(DE3) [24]; recombinant His-tagged isozymes PDK1 and PDK2 expressed in Escherichia coli BL21(DE3) [29]; recombinant His-tagged PDK2 expressed in Escherichia coli [21,33]; free pyruvate dehydrogenase kinase, separable from pyruvate dehydrogenase complex by gel filtration, to homogeneity [17]) [17, 21, 24, 29, 33]
<8> (recombinant His-tagged isozymes PDK2 and PDK3 from Escherichia coli BL21(DE3) [28]) [28]
<10> (recombinant from Escherichia coli as a fusion protein with the maltose-binding protein, to near homogeneity [26]) [26]
<11> (recombinant from Escherichia coli as His-tagged isozyme PDK1 [33]; recombinant from Escherichia coli, large scale [36]) [33, 36]
<12> (recombinant from Escherichia coli as His-tagged protein to homogeneity [22,25]) [22, 25]
<14> (recombinant wild-type and truncated form from Escherichia coli as His-tagged and maltose-binding fusion protein, respectively, to homogeneity [25]) [25]
<15> (recombinant from Escherichia coli BL21(DE3) as His-tagged protein, to homogeneity [32]) [32]
<4, 11> (recombinant hybrid dimer of T7-tagged PDK1 and His-tagged PDK2 [33]) [33]
<6> (recombinant His-tagged protein from Sf9 insect cells, to near homogeneity [19]) [19]

Renaturation

<11> (reconstitution after solubilization with 6 M urea [36]) [36]

Crystallization

<1> (phosphorylated and nonphosphorylated components of the pyruvate dehydrogenase complex from heart and kidney [2]) [2]
<15> (vapor diffusion method, protein 10 mg/ml in 100 mM imidazole or 100 mM MES, pH 6.5-7.0, 0.5 M KCl, 3.5 mM 3-iodopropionic acid, 3.5 mM

ADP, 7 mM $MgCl_2$, 6.5% polyethylene glycol 6000, 1.0% ethylene glycol, 1 week, cryoprotection with 25% glycerol, X-ray diffraction structure determination [32]) [32]

Cloning

<4> (expression of His-tagged isozymes PDK1 and PDK2 in Escherichia coli BL21(DE3) [29]; expression of wild-type isozyme PDK2 and mutants in Escherichia coli as His-tagged enzymes [24]; overexpression of isozymes PDK1, PDK2, and PDK4 in Escherichia coli BL21(DE3) as His-tagged proteins [31]; expression of isozyme PDK2 as T7-tagged protein in Escherichia coli [33]; expression of isozyme PDK2 in Escherichia coli as His-tagged enzyme [21,33]) [21, 24, 29, 31, 33]

<7> (4 isozymes: PDK1, PDK2, PDK3, PDK4 [30]) [30]

<8> (expression of isozyme PDK4 in Escherichia coli, unmodified and modified enzyme [35]; overexpression of isozyme PDK3 in Escherichia coli BL21(DE3) as His-tagged protein [31]; isozymes PDK2 and PDK3, expression in Escherichia coli BL21(DE3) as His-tagged proteins [28]) [28, 31, 35]

<10> (functional expression as maltose-binding protein fusion protein in Escherichia coli [26]) [26]

<11> (expression of T7-tagged and His-tagged isozyme PDK1 in Escherichia coli [33]; DNA and amino acid sequence determination, functional expression as soluble protein in Escherichia coli strain HMS 174 (DE3) [36]) [33, 36]

<12> (DNA sequence determination and analysis [22]; functional expression in Escherichia coli strain BL21(DE3) as His-tagged protein [22,25]) [22, 25]

<14> (expression of truncated enzyme forms, comprising residues 284-402 and 1-334, respectively, as maltose-binding-protein fusion proteins in Escherichia coli JM109 [25]; functional expression in Escherichia coli as His-tagged protein [25]) [25]

<15> (expression of isozyme PDK2 in Escherichia coli as His-tagged protein [32]) [32]

<4, 11> (coexpression of T7-tagged PDK1 and His-tagged PDK2 polypeptides in Escherichia coli, formation of a hybrid dimer [33]) [33]

<6> (gene pdk4, DNA and amino sequence determination and analysis, genomic organisation, functional expression of the His-tagged enzyme in Spodoptera frugiperda Sf9 cells via bacuovirus infection system [19]) [19]

Engineering

D282A <4> (<4> isozyme PDK2, site-directed mutagenesis, mutation of conserved amino acid, no activity, not able to bind ATP but the protein substrate [24]) [24]

G284A <4> (<4> isozyme PDK2, site-directed mutagenesis, mutation of conserved amino acid, properties similar to wild-type [24]) [24]

G286A <4> (<4> isozyme PDK2, site-directed mutagenesis, mutation of conserved amino acid, no activity, not able to bind ATP but the protein substrate [24]) [24]

G319A <4> (<4> isozyme PDK2, site-directed mutagenesis, mutation of conserved amino acid, catalytically active, but very poor binding of ATP [24]) [24]
H121A <10> (<10> site-directed mutagenesis, 50% decreased trans- and autophosphorylation activity [26]) [26]
H121Q <10> (<10> site-directed mutagenesis, 50% decreased trans- and autophosphorylation activity [26]) [26]
N247A <4> (<4> isozyme PDK2, site-directed mutagenesis, mutation of conserved amino acid, no activity, not able to bind ATP but the protein substrate [24]) [24]
Additional information <14> (<14> construction of truncated enzyme forms, the N-terminally truncated form, residues 284-402, is catalytically inactive, the C-terminally reduced form, residues 1-334, shows reduced activity [25]) [25]

Application

pharmacology <7> (<7> target for development of specific inhibitors of PDK isozymes to regulate glucose levels in the blood [30]) [30]

6 Stability

General stability information

<1>, kinase has tendency to aggregate in other buffers than 0.01 M imidazole-asparagine, pH 7.3, 0.1 mM $MgCl_2$, 0.01 M EDTA [7]
<1>, labile to freeze-thawing [8, 9]

Storage stability

<1>, 4°C, 0.02% NaN_3, $t_{1/2}$: 1 month [8, 9]

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S-Methyl-5-thioribose kinase 2.7.1.100

1 Nomenclature

EC number
2.7.1.100

Systematic name
ATP:S-methyl-5-thio-D-ribose 1-phosphotransferase

Recommended name
S-methyl-5-thioribose kinase

Synonyms
ATP:S-methylthioribose kinase
MtnK <9> [8]
kinase, 5-methylthioribose (phosphorylating)
methylthioribose kinase

CAS registry number
68247-56-3

2 Source Organism

<1> *Lupinus luteus* (yellow lupin [1]) [1]
<2> *Enterobacter aerogenes* [2, 3]
<3> *Klebsiella pneumoniae* [4, 7]
<4> *Lycopersicon esculentum* (Mill cv Rutgers [5]) [5, 6]
<5> *Persea americana* [6]
<6> *Pyrus communis* (d'Anjou [6]) [6]
<7> *Fragaria x ananassa* (red [6]) [6]
<8> *Malus sp.* (appletree, Golden delicious [6]) [6]
<9> *Bacillus subtilis* (enzyme expression is induced by sulfur, nitrogen or carbon starvation [8]) [8]

3 Reaction and Specificity

Catalyzed reaction
ATP + S-methyl-5-thio-β-D-ribose = ADP + S-methyl-5-thio-D-ribose 1-phosphate

Reaction type
phospho group transfer

Natural substrates and products

- **S** ATP + 5-methylthioribose <1-8> (<1> enzyme may be involved in an alternative pathway of methionine synthesis in plant tissues [1]; <2> may be a primary enzyme involved in the recycling of the methylthio group of 5-methylthioribose back into methionine [2,3]; <3> key step in recycling of methionine from 5'-methylthioadenosine a co-product of polyamine biosynthesis, expression of methylthioribose kinase may be under control of the methionine regulon [4]) (Reversibility: ? <1-8> [1-4, 6]) [1-4, 6, 7]
- **P** ADP + 5-methylthioribose 1-phosphate <1-8> [1-4, 6, 7]

Substrates and products

- **S** ATP + 5-butylthioribose <2> (Reversibility: ? <2> [3]) [3]
- **P** ADP + 5-butylthioribose 1-phosphate <2> [3]
- **S** ATP + 5-ethylthioribose <2> (Reversibility: ? <2> [3]) [3]
- **P** ADP + 5-ethylthioribose 1-phosphate <2> [3]
- **S** ATP + 5-isobutylthioribose <1, 2> (Reversibility: ? <1,2> [1,3]) [1, 3]
- **P** ADP + 5-isobutylthioribose 1-phosphate <1, 2> [1, 3]
- **S** ATP + 5-isopropylthioribose <2> (Reversibility: ? <2> [3]) [3]
- **P** ADP + 5-isopropylthioribose 1-phosphate <2> [3]
- **S** ATP + 5-methylthioribose <1-8> (<1> no activity with AMP, ADP and α,β-CH_2-ATP [1]; <2> no activity with GTP and UTP [2,3]) (Reversibility: ? <1-8> [1-6]) [1-6]
- **P** ADP + 5-methylthioribose 1-phosphate <1-8> [1-6]
- **S** ATP + 5-propylthioribose <2> (Reversibility: ? <2> [3]) [3]
- **P** ADP + 5-propylthioribose 1-phosphate <2> [3]
- **S** CTP + 5-methylthioribose <2> (<2> 20% of activity with ATP [2]) (Reversibility: ? <2> [2]) [2]
- **P** CDP + 5-methylthioribose 1-phosphate <2> [2]
- **S** αATP + 5-methylthioribose <1> (Reversibility: ? <1> [1]) [1]
- **P** αADP + 5-methylthioribose 1-phosphate <1> [1]
- **S** dATP + 5-methylthioribose <1> (<1> 120% of activity with ATP [1]) (Reversibility: ? <1> [1]) [1]
- **P** dADP + 5-methylthioribose 1-phosphate <1> [1]

Inhibitors

2-oxo-4-methylthiobutyrate <1> (<1> 1 mM, 10% inhibition [1]) [1]
5-ethylthioribose <4> (<4> weak inhibition [5]) [5]
5-isobutylthioribose <1, 3, 4> (<1> competitive vs. 1-phospho-5-methylthioribose [1]; <4> 41% inhibition [5]) [1, 4, 5]
5-isopropylthioribose <4> (<4> weak inhibition [5]) [5]
ADP <1, 2> (<1> 1 mM, 66% inhibition [1]; <2> 52% inhibition at equimolar concentrations with ATP [2]) [1-3]
CTP <2> (<2> in the presence of ATP [2]) [2]
S-adenosylhomocysteine <1> (<1> 1 mM, 36% inhibition [1]) [1]
adenine <1> (<1> 1 mM, 25% inhbition [1]) [1]
p-chloromercuribenzoate <1> [1]

Activating compounds

2-mercaptoethanol <2> (<2> 20 mM, 70% of activity with dithiothreitol [3]) [3]
dithiothreitol <2> (<2> required for activity, maximal activity at 20 mM [2,3]; <2> can partially be replaced by 20 mM 2-mercaptoethanol or glutathione [3]) [2, 3]
glutathione <2> (<2> 20 mM, 44% of activity with dithiothreitol [3]) [3]

Metals, ions

Mg^{2+} <1, 2> (<1,2> divalent metal ion is strictely required for activity, Mg^{2+} is most effective [1-3]; <2> maximal activity at 5 mM [2,3]) [1-3]
Mn^{2+} <1, 2> (<1> divalent cation is strictley required for activity [1]; <2> 20% of activity with Mg^{2+} [3]; <1> 12% of activity with Mg^{2+} [1]) [1, 3]

Specific activity (U/mg)

0.0007 <4> (<4> activity in fruit at breaker stage of ripening [5]) [5]
0.028 <2> [2, 3]
0.1425 <1> [1]

K_m-Value (mM)

0.0043 <1> (5-methylthioribose, <1> pH 9.7, 35°C [1]) [1]
0.0081 <2> (5-methylthioribose, <2> pH 7.3, 37°C [2]) [2, 3]
0.0083 <1> (ATP, <1> pH 9.7, 35°C [1]) [1]
0.012 <3> (5-methylthioribose, <3> pH 9.5, 37°C [7]) [7]
0.06 <9> (5-methylthioribose, <9> 37°C, approx. value [8]) [8]
0.074 <2> (ATP, <2> pH 7.3, 37°C [2]) [2, 3]

K_i-Value (mM)

0.0014 <1> (5-isobutylthioribose) [1]

pH-Optimum

7.3 <2> [2, 3]
10-10.5 <1> [1]

pH-Range

7-10.5 <1> (<1> approx. 25% of maximal activity at pH 7.0 [1]) [1]

Temperature optimum (°C)

30 <5> [6]
35 <1> (<1> assay at [1]) [1]
37 <2> (<2> assay at [3]) [3]

4 Enzyme Structure

Molecular weight

50000 <3> (<3> gel filtration [7]) [7]
70000 <1> (<1> gel filtration [1]) [1]

Subunits

monomer <3> (<3> 1 * 46000, SDS-PAGE [7]) [7]

5 Isolation/Preparation/Mutation/Application

Source/tissue

fruit <4-8> (<4> highest activity at the breaker stage [5]) [5, 6]
seed <1> [1]

Purification

<1> (ammonium sulfate, aminohexyl-Sepharose, Sephadex G-200, hydroxyapatite Bio-Gel [1]) [1]
<2> (ammonium sulfate, Sephadex G-200, DEAE-cellulose [2,3]) [2, 3]
<3> (ammonium sulfate, DEAE-Sepharose, 5-(*p*-aminophenyl)thioribose affinity chromatography [7]) [7]
<5> (ammonium sulfate, partial purified [6]) [6]

6 Stability

Storage stability

<2>, -20°C, 20% glycerol, at least 5 mg/ml, stable [2, 3]

References

[1] Guranowski, A.: Plant 5-methylthioribose kinase. Plant Physiol., **71**, 932-935 (1983)
[2] Ferro, A.J.; Barrett, A.; Shapiro, S.K.: 5-Methylthioribose kinase. A new enzyme involved in the formation of methionine from 5-methylthioribose. J. Biol. Chem., **253**, 6021-6025 (1978)
[3] Ferro, A.J.; Marchitto, K.S.: 5-Methylthioribose kinase (Enterobacter aerogenes). Methods Enzymol., **94**, 361-364 (1983)
[4] Tower, P.A.; Alexander, D.B.; Johnson, L.L.; Riscoe, M.K.: Regulation of methylthioribose kinase by methionine in Klebsiella pneumoniae. J. Gen. Microbiol., **139**, 1027-1031 (1993)
[5] Kushad, M.M.; Richardson, D.G.; Ferro, A.J.: 5'-Methylthioadenosine nucleosidase and 5-methylthioribose kinase activities and ethylene production during tomato fruit development and ripening. Plant Physiol., **79**, 525-529 (1985)
[6] Kushad, M.M.; Richardson, D.G.; Ferro, A.J.: 5-Methylthioribose kinase activity in plants. Biochem. Biophys. Res. Commun., **108**, 167-173 (1982)
[7] Cornell, K.A.; Winter, R.W.; Tower, P.A.; Riscoe, M.K.: Affinity purification of 5-methylthioribose kinase and 5-methylthioadenosine/S-adenosylhomocysteine nucleosidase from Klebsiella pneumoniae [corrected]. Biochem. J., **317**, 285-290 (1996)
[8] Sekowska, A.; Mulard, L.; Krogh, S.; Tse, J.K.; Danchin, A.: MtnK, methylthioribose kinase, is a starvation-induced protein in Bacillus subtilis. BMC Microbiol., 1, 15 (2001)

Tagatose kinase 2.7.1.101

1 Nomenclature

EC number
2.7.1.101

Systematic name
ATP:D-tagatose 6-phosphotransferase

Recommended name
tagatose kinase

Synonyms
D-tagatose 6-phosphate kinase
kinase, tagatose 6-phosphate (phosphorylating)
tagatose 6-phosphate kinase (phosphorylating)
tagatose-6-phosphate kinase

CAS registry number
39434-00-9

2 Source Organism

<1> *Mycobacterium butyricum* (dulcitol-grown [1]) [1]

3 Reaction and Specificity

Catalyzed reaction
ATP + D-tagatose = ADP + D-tagatose 6-phosphate

Reaction type
phospho group transfer

Natural substrates and products
S ATP + D-tagatose <1> (<1> part of the dulcitol degradation pathway in saprophytic mycobacteria [1]) (Reversibility: ? <1> [1]) [1]
P ADP + D-tagatose 6-phosphate

Substrates and products
S ATP + D-tagatose <1> (<1> highly specific for D-tagatose, phosphorylates D-tagatose at C-6 [1]) (Reversibility: ir <1> [1]) [1]
P ADP + D-tagatose 6-phosphate <1> [1]

S Additional information <1> (<1> no substrates: D-glucose, D-mannose, D-galactose, D-fructose, D-galactosamine, D-2-deoxygalactose, dulcitol, L-sorbose, L-rhamnose, D-ribose, D-xylose, D-lyxose, D-xylulose or glycerol [1]) [1]

P ?

Inhibitors

$CuSO_4$ <1> (<1> 1 mM, 50% inhibition [1]) [1]
$HgCl_2$ <1> (<1> 2 mM, 32% inhibition [1]) [1]
$MgCl_2$ <1> (<1> above 3 mM [1]) [1]
$ZnSO_4$ <1> (<1> 1 mM, 38% inhibition [1]) [1]
p-chloromercuribenzoate <1> (<1> 2 mM, 15% inhibition [1]) [1]
p-chloromercuriphenylsulfate <1> (<1> 2 mM, 21% inhibition [1]) [1]
Additional information <1> (<1> not inhibited by 1 mM N-ethylmaleimide, 1 mM iodoacetate, 1 mM NaF, 1 mM EDTA or 1 mM 1,10-phenanthroline [1]) [1]

Metals, ions

Fe^{2+} <1> (<1> 2.5 mM $FeSO_4$, activates, can replace Mg^{2+} with 24% efficiency [1]) [1]
Mg^{2+} <1> (<1> requirement, maximum activity in the presence of 3 mM $MgCl_2$, inhibitory above 3 mM [1]) [1]
Mn^{2+} <1> (<1> 2.5 mM $MnCl_2$, activates, can replace Mg^{2+} with 63% efficiency [1]) [1]
Additional information <1> (<1> not activated by $CaCl_2$, $CoSO_4$, $NiSO_4$, $CdSO_4$ or $ZnSO_4$ [1]) [1]

Specific activity (U/mg)

3.92 <1> (<1> at 37°C, pH 7.5 [1]) [1]

K_m-Value (mM)

0.8 <1> (D-tagatose, <1> at 22°C, pH 7.5 [1]) [1]
1 <1> (ATP, <1> at 22°C, pH 7.5 [1]) [1]

pH-Optimum

7.5 <1> (<1> 22°C [1]) [1]

pH-Range

6.4-8.4 <1> (<1> about half-maximal activity at pH 6.4 and 8.4 [1]) [1]

Temperature optimum (°C)

22 <1> (<1> assay at, spectrophotometric method, measurement of ADP formation [1]) [1]
37 <1> (<1> assay at, measurement of the rate of disappearance of tagatose [1]) [1]

4 Enzyme Structure

Molecular weight
63000 <1> (<1> gel filtration [1]) [1]

5 Isolation/Preparation/Mutation/Application

Purification
<1> (106fold, partial [1]) [1]

6 Stability

Temperature stability
48 <1> (<1> 1 min, stable, rapid inactivation above [1]) [1]
50 <1> (<1> rapidly loses activity on heating above [1]) [1]
56 <1> (<1> $t_{1/2}$: 1 min [1]) [1]

Storage stability
<1>, room temperature, phosphate buffer, pH 6.3-8, 1 day, stable [1]

References

[1] Szumilo, T.: A novel enzyme, tagatose kinase, from Mycobacterium butyricum. Biochim. Biophys. Acta, **660**, 366-370 (1981)

Hamamelose kinase 2.7.1.102

1 Nomenclature

EC number
2.7.1.102

Systematic name
ATP:D-hamamelose 2'-phosphotransferase

Recommended name
hamamelose kinase

Synonyms
ATP/hamamelose 2'-phosphotransferase
hamamelose kinase (phosphorylating)
hamamelosekinase (ATP:hamamelose 2'-phosphotransferase)
kinase, hamamelose (phosphorylating)

CAS registry number
74506-53-9

2 Source Organism

<1> *Kluyvera citrophila* (strain 627, grown on D-hamamelose [1]) [1]

3 Reaction and Specificity

Catalyzed reaction
ATP + D-hamamelose = ADP + D-hamamelose 2'-phosphate

Reaction type
phospho group transfer

Natural substrates and products
S ATP + D-hamamelose <1> (<1> initial step of the catabolic reaction sequence for dissimilation of hamamelose [1]) (Reversibility: ? <1> [1]) [1]
P ADP + hamamelose 2'-phosphate <1> [1]

Substrates and products
S ATP + D-hamamelitol <1> (<1> poor substrate [1]) (Reversibility: ? <1> [1]) [1]
P ADP + D-hamamelitol 2'-phosphate

S ATP + D-hamamelose <1> (<1> specific for hamamelose [1]) (Reversibility: ? <1> [1]) [1]
P ADP + hamamelose 2'-phosphate <1> [1]
S Additional information <1> (<1> no substrates: D-glucose, D-glucosamine, D-fructose, D-mannose, D-galactose, D-arabinose, D-xylose [1]) [1]
P ?

Inhibitors

ATP <1> (<1> above 7 mM: significant substrate inhibition [1]) [1]
hamamelose <1> (<1> above 5 mM: significant substrate inhibition [1]) [1]

Activating compounds

Additional information <1> (<1> enzyme is induced by growing on D-hamamelose [1]) [1]

Specific activity (U/mg)

2.6-6.6 <1> (<1> at 30°C, pH 7.5 [1]) [1]

K_m-Value (mM)

2.5 <1> (ATP, <1> at 30°C, pH 7.5 [1]) [1]
3 <1> (D-hamamelose, <1> at 30°C, pH 7.5 [1]) [1]

pH-Optimum

7.5 <1> [1]

pH-Range

6.6-8.5 <1> (<1> 10% of maximal activity at pH 6.6, 80% of maximal activity at pH 8.5 [1]) [1]

Temperature optimum (°C)

30 <1> (<1> assay at [1]) [1]

4 Enzyme Structure

Molecular weight

21000 <1> (<1> gel filtration [1]) [1]

5 Isolation/Preparation/Mutation/Application

Purification

<1> (partial, 51fold [1]) [1]

6 Stability

pH-Stability

6.4 <1> (<1> optimal enzyme stability in the crude extract at [1]) [1]

General stability information

<1>, no stabilization by dithioerythritol, mercaptoethanol, glycerol or bovine serum albumin [1]

<1>, purified enzyme is very labile, 4 mM D-hamamelose, added to storage buffer, stabilizes [1]

Storage stability

<1>, -20°C, purified enzyme, within 10 days, complete loss of activity [1]

<1>, low temperatures, 4 mM hamamelose, protein concentration not lower than 0.2 mg/ml, stable [1]

References

[1] Beck, E.; Wieczorek, J.; Reinecke, W.: Purification and properties of hamamelosekinase. Eur. J. Biochem., **107**, 485-489 (1980)

Viomycin kinase 2.7.1.103

1 Nomenclature

EC number
2.7.1.103

Systematic name
ATP:viomycin O-phosphotransferase

Recommended name
viomycin kinase

Synonyms
capreomycin phosphotransferase
viomycin phosphotransferase

CAS registry number
77000-11-4
77000-12-5

2 Source Organism

<-1> no activity in *Streptomyces coelicolor* (strain A, standard reference actinomycete, sensitive to viomycin and capreomycin [1]) [1]
<1> *Streptomyces capreolus* (NCIB 9801 [1]) [1]
<2> *Streptomyces lividans* [2]
<3> *Streptomyces vinaceus* (NCIB 8852 [1]) [1]

3 Reaction and Specificity

Catalyzed reaction
ATP + viomycin = ADP + O-phosphoviomycin

Reaction type
phospho group transfer

Natural substrates and products
S ATP + viomycin <1-3> (<1,3> antibiotic-inactivating enzymes, involved in self-defence mechanism [1]) (Reversibility: ? <1-3> [1, 2]) [1, 2]
P ADP + O-phosphoviomycin <1, 3> [1]

Substrates and products

- **S** ATP + capreomycin IA <1, 3> (<1,3> capreomycin IB poor substrate [1]) (Reversibility: ? <1,3> [1]) [1]
- **P** ADP + O-phosphocapreomycin IA <1, 3> [1]
- **S** ATP + capreomycin IIA <1, 3> (Reversibility: ? <1,3> [1]) [1]
- **P** ADP + O-phosphocapreomycin IIA
- **S** ATP + viomycin <1-3> (Reversibility: ? <1-3> [1,2]) [1, 2]
- **P** ADP + O-phosphoviomycin <1-3> [1, 2]
- **S** Additional information <1, 3> (<1,3> a serine residue acts as phosphate-acceptor [1]; <3> capreomycin IIB is no substrate [1]) [1]
- **P** ?

5 Isolation/Preparation/Mutation/Application

Localization

ribosome <1, 3> (<1,3> postribosomal supernatant (S100-fraction), intracellular or cytoplasmic membrane associated [1]) [1]

Cloning

<2> (vph gene of plasmid pVE138 is expressed and confers resistance to viomycin in Streptomyces spp. and Salmonella typhimurium MJ1 [2]) [2]

<3> (shotgun cloning, resistance to viomycin is cloned into Streptomyces lividans, recombinant clones are cross resistant to capreomycin IA, but not to capreomycin IB [1]) [1]

Application

medicine <1, 3> (<1,3> antibiotic-inactivating enzyme [1]) [1]

References

[1] Skinner, R.H.; Cundliffe, E.: Resistance to the antibiotics viomycin and capreomycin in the Streptomyces species which produce them. J. Gen. Microbiol., **120**, 95-104 (1980)

[2] Paradiso, M.J.; Roberts, G.; Streicher, S.L.; Goldberg, R.B.: Characterization of suppressible mutations in the viomycin phosphotransferase gene of the Streptomyces enteric plasmid pVE138. J. Bacteriol., **169**, 1325-1327 (1987)

Diphosphate-protein phosphotransferase 2.7.1.104

1 Nomenclature

EC number
2.7.1.104 (Protein kinases are in a state of review by the NC-IUBMB. This EC class will presumably be transferred to EC 2.7.99.1)

Systematic name
diphosphate:microsomal-membrane-protein O-phosphotransferase

Recommended name
diphosphate-protein phosphotransferase

Synonyms
phosphotransferase, pyrophosphate-protein
pyrophosphate-protein phosphotransferase
pyrophosphate:protein phosphotransferase

CAS registry number
74092-32-3

2 Source Organism

<1> *Rattus norvegicus* [1]

3 Reaction and Specificity

Catalyzed reaction
diphosphate + microsomal-membrane protein = phosphate + O-phospho-microsomal-membrane protein

Reaction type
phospho group transfer

Natural substrates and products
S diphosphate + microsomal polypeptides <1> (Reversibility: ? <1> [1]) [1]
P phosphate + phosphorylated microsomal polypeptides

Substrates and products
S diphosphate + microsomal polypeptides <1> (<1> intrinsic membrane polypeptides of rat liver with molecular weights of 145000 Da and 130000 Da [1]) (Reversibility: ? <1> [1]) [1]
P phosphate + phosphorylated microsomal polypeptides <1> [1]

Inhibitors
ATP <1> (<1> 0.025 mM, weak [1]) [1]
Mg^{2+} <1> (<1> 1 mM, strong [1]) [1]
NaF <1> (<1> 5 mM, strong [1]) [1]

Metals, ions
Mg^{2+} <1> (<1> micromolar levels required [1]) [1]

pH-Optimum
6.5-7.5 <1> [1]

5 Isolation/Preparation/Mutation/Application

Source/tissue
liver <1> [1]

Localization
microsome <1> (<1> membrane-bound [1]) [1]

Purification
<1> (partial [1]) [1]

References

[1] Lam, K.S.; Kasper, C.B.: Pyrophosphate:protein phosphotransferase: a membrane-bound enzyme of endoplasmic reticulum. Proc. Natl. Acad. Sci. USA, 77, 1927-1931 (1980)

6-Phosphofructo-2-kinase 2.7.1.105

1 Nomenclature

EC number
2.7.1.105

Systematic name
ATP:β-D-fructose-6-phosphate 2-phosphotransferase

Recommended name
6-phosphofructo-2-kinase

Synonyms
6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase
6-phosphofructose 2-kinase
ATP:D-fructose-6-phosphate 2-phosphotransferase
fructose 6-phosphate 2-kinase
kinase, 6-phosphofructo-2-(phosphorylating)
phosphofructokinase 2
Additional information (cf. EC 3.1.3.46, not identical with EC 2.7.1.11)

CAS registry number
78689-77-7

2 Source Organism

<1> *Rattus norvegicus* (foetal and adult [12]) [1-18, 20, 22, 23, 25-27, 44-51, 55, 56, 60, 62, 64]
<2> *Canis familiaris* [16]
<3> *Bos taurus* [16, 18-24, 26, 46, 50, 63]
<4> *Homo sapiens* (human enzyme is 95% identical with rat or bovine enzyme [25]) [25, 52-55, 66]
<5> *Gallus gallus* [27-29, 59, 60]
<6> *Columba sp.* [26, 28]
<7> *Rana esculenta* (frog [31]) [31]
<8> *Sparus aurata* (teleost fish [30,61]) [30, 61]
<9> *Mytilus galloprovincialis* (marine mussle [32]) [32, 34]
<10> *Helianthus tuberosus* (Jerusalem artichoke [35]) [35]
<11> *Ricinus communis* (castor bean [35]) [35]
<12> *Spinacia oleracea* [8, 35-37]
<13> *Aspergillus niger* [38]

<14> *Saccharomyces cerevisiae* (baker's yeast, haploid strain X2180 [39]) [8, 33, 39-42]
<15> *Escherichia coli* (strains DF903 and DF905 (mutant) [43]) [43, 45, 67]
<16> *Mus musculus* [56]
<17> *Solanum tuberosum* (potato plant, enzyme of Solanum tuberosum is 83% identical with enzyme of Zea mays and 72% identical with enzyme of Pinus taeda [57]) [57]
<18> *Arabidopsis thaliana* [58]
<19> *Spinacia oleracea* [65]
<20> *Orconectes limosus* (crayfish [66]) [66]

3 Reaction and Specificity

Catalyzed reaction

ATP + β-D-fructose 6-phosphate = ADP + β-D-fructose 2,6-bisphosphate (<1, 3, 4, 8, 15-18> bifunctional protein: 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase, reverse reaction catalysed by fructose 2,6-bisphosphatase: EC 3.1.3.46 [3, 5-7, 9-11, 15, 18-30, 44-62, 64, 65]; <1, 4> high 6-phosphofructo-2-kinase activity compared to the fructose 2,6-bisphosphatase [46, 53]; <1> mechanism [6, 51]; <4> random mechanism [52]; <1> kinetic mechanism [64])

Reaction type

phospho group transfer

Natural substrates and products

S ATP + β-D-fructose 6-phosphate <1, 3-5, 6, 8, 12, 17-19> (<1, 3, 6, 19> responsible for regulation of fructose 2,6-bisphosphate-concentration [13, 18, 26, 46, 47, 51, 57-59, 65]; <1, 12> enzyme plays an important role in the regulation of hepatic carbohydrate metabolism [16, 36]; <1> enzyme plays an indirect yet key role in the regulation of glucose metabolism [47, 51-53, 55, 56]; <4> the inducible enzyme is an important regulator of glycolysis that may be responsible for sustaining the high glycolytic flux of rapidly proliferating leukemia cells [66]) (Reversibility: r <1, 19> [36, 65]; ? <1, 3-5, 6, 8, 12, 17> [13, 16, 26, 30, 47, 51-53, 55-59]) [13, 16, 18, 26, 30, 36, 47, 51-53, 55-59, 65, 66]
P ADP + β-D-fructose 2,6-bisphosphate <1, 3-5, 6, 8, 12, 17-19> [13, 16, 18, 26, 30, 36, 47, 51-53, 55-59, 65, 66]

Substrates and products

S ADP + β-D-fructose 2,6-bisphosphate <1> (<1> reverse reaction, at 50% the rate of forward reaction [7]; <1> not [6]) (Reversibility: r <1> [7]) [6, 7]
P ATP + β-D-fructose 6-phosphate <1> [7]
S ATP + D-psicose 6-phosphate <1> (Reversibility: ? <1> [17]) [17]
P ?
S ATP + D-tagatose 6-phosphate <1> (Reversibility: ? <1> [17]) [17]

P ?
S ATP + L-sorbose 6-phosphate <1> (Reversibility: ? <1> [17]) [17]
P ?
S ATP + β-D-fructose 6-phosphate <1-19> (<1> transfers γ-phosphoryl group of ATP to hydroxyl group at C-2 of fructose 6-phosphate [1,2]; <1> sugar phosphate specificity [17]; <1> no substrates are diphosphate, glucose 6-phosphate [2]; <1> not: 1-O-methyl-D-fructose 6-phosphate, α-/β-methyl-D-fructofuranoside 6-phosphate, 2,5-anhydro-D-mannitol 6-phosphate, D-arabinose 5-phosphate [17]; <1> not: ribose 5-phosphate [2,17]) (Reversibility: r <1, 3, 5, 6, 8, 15-19> [3, 5-7, 9-11, 15, 18-30, 44-62, 64, 65]; ? <2, 4, 7, 9-14> [1, 2, 4, 8, 12-14, 16, 17, 31-43, 63, 66, 67]) [1-67]
P ADP + β-D-fructose 2,6-bisphosphate <1-3, 8, 12, 14-19> (<1, 3, 8> via phosphorylenzyme intermediate [6, 15, 20, 30]) [1-8, 10, 15, 16, 18-20, 30, 44-67]
S CTP + D-fructose 6-phosphate <15> (Reversibility: ? <15> [43]) [43]
P CDP + β-D-fructose 2,6-bisphosphate
S GTP + β-D-fructose 6-phosphate <1, 15> (<1> less effective than ATP []) (Reversibility: ? <1, 15> [2, 43]) [2, 43]
P GDP + β-D-fructose 2,6-bisphosphate
S ITP + β-D-fructose 6-phosphate <15> (Reversibility: ? <15> [43]) [43]
P IDP + β-D-fructose 2,6-bisphosphate
S UTP + D-fructose 6-phosphate <15> (Reversibility: ? <15> [43]) [43]
P UDP + β-D-fructose 2,6-bisphosphate
S Additional information <1-9, 15-18> (<1> allosteric bifunctional enzyme, not heart [16]; <1, 3, 6> with discrete catalytic sites [6, 10, 26]; <-1, 1-9, 15-18> also catalyses the degradation of fructose 2,6-bisphosphate (EC 3.1.3.46) [3, 5-7, 9-11, 15-28, 30, 31, 34, 44-62, 64, 65]; <1> isotope exchange reaction between ATP and ADP in the absence of fructose 6-phosphate [6, 7]; <1> isotope exchange between fructose 6-phosphate and fructose 2,6-bisphosphate in the absence of adenine nucleotides [6]) [3, 5-7, 9-11, 15-28, 30, 31, 34, 44-62, 64, 65]
P ?

Inhibitors

2,5-anhydro-D-mannitol 6-phosphate <1> [17]
3-phosphoglycerate <19> [65]
ADP <1, 8, 18> (<1> product inhibition [6]; <1> kinetics [4, 7]) [4, 6, 7, 30, 58]
AMP <1, 13, 14, 18> (<13> weak [38]; <1, 14, 18> not [6, 41, 58]) [6, 38, 41, 58]
ATP <14, 15, 18> (<15> free form [43]; <14> at low concentrations of Mg^{2+} and fructose 6-phosphate [33]; <18> not [58]) [33, 43, 58]
Mg^{2+} <5> (<5> MgATP [27]) [27]
$MgNTP^{2-}$ <15> (<15> strain DF903, substrate inhibition, most effective: $MgATP^{2-}$ at low fructose concentration [43]) [43]
MoO_4^{2-} <1> [10]

N-(1-pyrenil)maleimide <15> (<15> complete loss of catalytic activity, but modified enzyme is able to bind β-D-fructose 6-phosphate, the presence of $MgATP^{2-}$ completely protects the enzyme activity, the modified enzyme elutes as a monomer [67]) [67]
N-bromoacetylethanolamine <1, 16> (<1> specific active site-directed inactivator of enzyme, in vitro and in vivo [56]; <16> repetitive administration affects inhibition of glycolysis and lipid metabolism, causing suppression of body weight gain [56]) [56]
SO_4^{2-} <1> [10]
SeO_4^{2-} <1> [10]
VO_4^{2-} <1> [10]
WO_4^{2-} <1> [10]
β-D-fructose 2,6-bisphosphate <1> (<1> product inhibition [6]; <1> kinetics [7]) [6-8]
citrate <1, 3, 5-9, 12-14> (<7> strong [31]; <13> weak [38]; <3> at physiological concentrations [24]; <8> phosphorylation enhances sensitivity [30]; <1,3> heart enzyme is more sensitive than liver enzyme [18]; <1,6> skeletal muscle enzyme is more sensitive than liver enzyme [26]; <1,14> not [6,41]) [1, 6, 8, 13, 14, 18, 24, 26, 28, 30-32, 34, 38, 39, 41, 44, 46, 63, 65]
dihydroxyacetone phosphate <12, 18, 19> (<12> weak [35]) [35, 58, 65]
diphosphate <1, 12, 13, 14, 18, 19> (<13, 14, 18, 19> not phosphate [38, 41, 58, 65]) [10, 35, 38, 41, 58, 65]
glycerate 2-phosphate <12, 18, 19> [35, 58, 65]
glycerol 2-phosphate <14> [39]
glycolate 2-phosphate <12> [35]
guanidine <1> (<1> inactivation, unfolding [48]) [48]
m-periodate <1> (<1> strong, DTT protects or reverses [10]) [10]
o-phthalaldehyde <5> (<5> kinetics, DTT or substrates do not protect [29]) [29]
phosphoenolpyruvate <1, 3, 9, 12-14, 18, 19> (<1> kinetics [4]; <7> strong [31]; <13> weak [38]; <8> mixed-type inhibitory effect, phosphorylation enhances sensitivity [30]; <3> not [24]) [1, 4, 8, 13, 14, 26, 28, 30-35, 38, 39, 41, 44, 46, 50, 58, 65]
sn-glycerol 3-phosphate <1, 3, 7, 8, 12, 18, 19> (<1> 75% decrease in activity of liver enzyme but not hepatoma cells [13]; <3> i.e. α-glycerol phosphate [6,20]; <8> most potent inhibitor of phosphorylated liver enzyme, phosphorylation enhances sensitivity [30,61]; <8> skeletal muscle enzyme is not sensitive to inhibition [61]; <1> liver enzyme [26]; <1,3> heart enzyme is less sensitive than liver [18,26]; <1> stimulates phosphatase activity [12]; <3, 7> not [24, 31]) [6, 12, 13, 18, 20, 24, 26, 30, 31, 33, 35, 41, 58, 65]
Additional information <1, 3, 5-8, 12, 13, 15> (<8> no inhibition by lactate, glyceraldehyde 3-phosphate, β-D-fructose 1,6-bisphosphate [30]; <15> not: ITP, GTP, UTP, CTP, strain DF905 [43]; <1> not: cAMP or protein kinase alone [14]; <7> no inhibition by phosphorylation with Ca^{2+}/calmodulin dependent protein kinase [31]; <1, 3, 6, 7, 13> no inhibition by protein kinase C [26, 31, 38]; <1, 3, 5, 6> phosphorylation by cAMP-dependent protein kinase causes inactivation of liver enzyme [3, 5, 8, 12-16, 18, 26, 28, 44, 59]; <15>

phosphorylation by cAMP-dependent protein kinase causes inactivation [43, 45]; <1,4> phosphorylation site: Ser-32 [11, 45, 52]; <1> phosphorylation of foetal liver enzyme by protein kinase C, but no effect on adult liver cells [12]; <1> phosphorylation at lower pH-values [8]; <1> phosphorylation at pH 6.6, not at pH 8 [14]; <1> phosphorylation by cAMP-dependent protein kinase causes 80% decrease in activity of the liver cells but not of hepatoma cells [13]; <1> phosphorylation by cAMP-dependent protein kinase of liver enzyme, not of skeletal muscle enzyme, since the phosphorylation site target Ser-32 of the liver isozyme is replaced by Ala in the muscle isozyme [15]; <1> phosphorylation by cAMP-dependent protein kinase causes inactivation of liver enzyme, not of kidney, testis and heart enzyme [16]; <1,3> rat liver enzyme is inhibited by phosphorylation by cAMP-dependent protein kinase, but not rat skeletal muscle, bovine and rat heart enzyme [18]; <1,3> rat liver enzyme is inhibited by phosphorylation by cAMP-dependent protein kinase but not rat kidney, testis and skeletal muscle enzyme and bovine and rat heart enzyme [26]; <1> phosphorylation by cAMP-dependent protein kinase causes inactivation of liver enzyme, not of heart and skeletal muscle enzyme [44]; <11,13> phosphorylation by cAMP-dependent protein kinase, but no inhibition [35,38]; <8> phosphorylation by cAMP-dependent protein kinase causes inactivation of liver enzyme, not skeletal muscle enzyme [61]; <1> loss of phosphorylation-dependent reduction of enzyme by deletion of the N-terminal residues of enzyme. The deletion of 7-N-terminal amino acids causes a 75% decrease in activity [45]; <1> the islet enzyme lacks protein kinase A and C phosphorylation sites [46]; <1> phosphorylation by cAMP-dependent protein kinase causes 35% inactivation of isozyme L of adipose tissue, not isozyme M [62]; <1> kinetic of phosphorylation by cAMP-dependent protein kinase [64]) [3, 5, 8, 11-16, 18, 26, 28, 30, 31, 35, 38, 43-46, 52, 59, 61, 62, 64]

Cofactors/prosthetic groups

AMP <1, 3, 6, 12, 14> (<1,12,14> activation, 0.025 mM [8]) [1, 8, 26]
GMP <1> (<1> activation [8]) [8]
IMP <1> (<1> activation [8]) [8]

Activating compounds

ATP <1, 5> (<1,5> the binding of ATP to the fructose 2,6-bisphosphatase domain of chicken liver 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase leads to activation of enzyme, but no activation of rat liver enzyme [59,60]) [59, 60]
glucose <1> (<1> inhibition by N-bromoacetylethanolamine [56]) [56]
insulin <3> (<3> activation of transfected heart enzyme in human embryonic kidney 293 cells by inducing the phosphorylation of Ser466 and Ser483, activation is mediated by 3-phosphoinositide-dependent kinase-1, protein kinase B is in vivo not essential, activation is sensitive to LY294002 and wortmannin and insensitive to rapaycin and PD98059 [63]) [63]
iodoacetamide <1> (<1> activation, kidney and liver, not heart or testis enzyme [16]) [12, 16]
pyruvate <18> [58]

Additional information <12> (<12> one enzyme form is activated dramatically as result of modification by MgATP during preincubation [36]) [36]

Metals, ions

AsO_4^{3-} <1> (<1> activation [10]; <1> requirement [8]; <1> can replace phosphate [8]) [8, 10]
Mg^{2+} <1, 3, 5, 6, 12-15> (<15> requirement, active substrate: MgNTP [43]; <1, 3, 5, 15> $MgATP^{2-}$ [1, 2, 6, 7, 12, 13, 18, 20, 29, 43]; <1, 15> $MgGTP^{2-}$ [2, 43]; <5> inhibition [27]; <15> inhibits at higher concentrations, strain DF903 [43]; <1> K51 is essential for binding of Mg(mantATP) [49]; <5> inhibition of enzyme at 2 mM, but no effect on activation by ATP [60]) [1, 2, 6-8, 12-16, 18, 20, 26, 29, 33, 35-44, 49, 60]
phosphate <1, 3-6, 9, 12-14, 18, 19> (<1, 12, 19> activation [1, 6, 10, 13, 14, 35, 65]; <1, 9, 12-14> requirement [8, 32, 38, 39]; <1> liver enzyme [26]) [1, 6, 8, 10, 13, 14, 20, 25, 26, 28, 32, 35, 38-40, 58, 65]

Turnover number (min^{-1})

Additional information <1, 3> (<1, 3> comparison of k_{cat}/K_m values of wild-type and mutant enzymes [49, 50]) [49, 50]

Specific activity (U/mg)

0.00031 <1> (<1> skeletal muscle [44]) [44]
0.0005 <1> [2]
0.00295 <1> (<1> heart [44]) [44]
0.0076 <1> [1]
0.0104-0.0159 <19> [65]
0.021 <1> (<1> liver [44]) [44]
0.04 <3> [22]
0.042 <3> [20]
0.046 <1, 4> [25]
0.055 <1> (<1> adult [12]) [12]
0.057 <1> [5]
0.06 <1> [20]
0.066 <1> [15]
0.068 <8> [30]
0.075 <3> [23]
0.08 <1> (<1> foetal [12]) [12]
0.092 <3> [24]
0.11 <6> [28]
0.142 <4> [53]
0.55 <14> [39]
0.718 <12> [35]
1.3 <14> [33]
Additional information <1, 3, 8, 13, 14> [1, 3, 18, 38, 40, 42, 61]

K_m-Value (mM)

0.006 <1> (β-D-fructose 6-phosphate, <1> pH 7.5, 30°C and heating at 90°C for 10 min, with ATP, in presence of phosphate [10]) [10]
0.0072 <6> ($MgATP^{2-}$, <6> pH 7.1, 30°C, phosphorylated enzyme [28]) [28]

0.0095-0.012 <3, 6> ($MgATP^{2-}$, <3> pH 7.5, 30°C [20]; <6> pH 7.1, 30°C, native pigeon enzyme [28]) [20, 28]
0.016 <1> (β-D-fructose 6-phosphate, <1> pH 7.5, 30°C, kinetic study [7]; <1> pH 7.5, 25°C, liver [46]) [7, 46]
0.017 <1> (β-D-fructose 6-phosphate, <1> pH 7.5, 25°C, islet [46]) [46]
0.018 <8> (ATP, <8> pH 7.5, 30°C, in presence of phosphate [30]) [30]
0.019-0.021 <1, 4> (β-D-fructose 6-phosphate, <1, 4> pH 7.8, in presence of phosphate [25]) [25]
0.02 <1> (β-D-fructose 2,6-bisphosphate, <1> pH 7.5, 30°C [3]) [3]
0.02-0.03 <1, 5> (β-D-fructose 6-phosphate, <1, 5> pH 7.5, 30°C, in presence of phosphate [27]) [27]
0.022 <5> (β-D-fructose 6-phosphate, <5> pH 7.5, 30°C, comparison of K_m of wild-type, mutant and mutant phosphorylated enzyme [59]) [59]
0.023 <3> (β-D-fructose 6-phosphate, <3> pH 7.5, 25°C, heart [46, 50]; <1, 3> comparison of K_m of wild-type, mutant and mutant phosphorylated enzyme [50]) [46, 50]
0.025 <1> (β-D-fructose 6-phosphate, <1> pH 7.5, 30°C, liver, comparison of K_m of wild-type, mutant and mutant phosphorylated enzyme [50]) [50]
0.027 <3> (β-D-fructose 6-phosphate, <3> pH 7.1, 30°C [24]) [24]
0.032 <4> (β-D-fructose 6-phosphate, <4> pH 7.5, 30°C [53]) [53]
0.035 <1> (β-D-fructose 6-phosphate, <1> pH 7.5, 30°C [17]) [17]
0.04 <1> (β-D-fructose 6-phosphate, <1> pH 7.5, 25°C, testis [46]; <1> pH 7.5, 30°C, wild-type enzyme, testis [49, 50]) [46, 49, 50]
0.044 <1> (β-D-fructose 6-phosphate, <1> pH 8.5, 30°C, foetal liver enzyme [12]) [12]
0.047 <1> (β-D-fructose 6-phosphate, <1> pH 8.5, 30°C, adult liver enzyme [12]) [12]
0.048 <1> (ATP, <1> pH 7.5, 30°C, comparison of K_m of skeletal muscle and liver enzyme [15]; <1> pH 7.5, 25°C, heart [46]) [15, 46]
0.05 <1, 3, 5> (β-D-fructose 6-phosphate, <1> pH 7.5, 30°C, with ATP, in presence of phosphate [6]; <3> pH 7.5, 30°C [18]; <5> pH 7.1, 30°C, $MgATP^{2-}$, phosphorylated chicken enzyme [28]) [6, 18, 28]
0.055 <3> (ATP, <3> pH 7.1, 30°C [24]) [24]
0.056 <1> (β-D-fructose 6-phosphate, <1> pH 7.5, 30°C, comparison of K_m of skeletal muscle and liver enzyme [15]; <1> pH 7.5, 25°C, muscle [46]) [15, 46]
0.06 <15> (ATP, <15> strain DF903 [43]) [43]
0.061 <1> (ATP, <1> pH 7.5, 30°C, liver [50]) [50]
0.07 <3> (β-D-fructose 6-phosphate, <3> pH 7.5, 30°C [23]) [23]
0.083 <3> (ATP, <3> pH 7.5, 25°C, heart [46, 50]) [46, 50]
0.09 <1> (β-D-fructose 6-phosphate, <1> pH 6.6, 25°C [4]) [4]
0.1 <1, 3, 5> (ATP, <1> pH 7.5, 30°C [6]; <1,3> pH 7.5, 30°C, MgATP [18]; <5> pH 7.5, 30°C, in absence of phosphate [27]; <1> pH 7.5, 25°C, testis [46]; <1> pH 7.5, 30°C, testis [49,50]) [6, 18, 27, 46, 49, 50]
0.1 <1, 5> (β-D-fructose 6-phosphate, <1,5> pH 7.5, 30°C [3,27]) [3, 27]
0.11 <5> (ATP, <5> pH 7.5, 30°C [59]) [59]
0.12 <1> (β-D-fructose 6-phosphate, <1> pH 7.8 [25]) [25]

0.12-0.36 <1, 5, 15> (ATP, <1> pH 7.8 [25]; <1,5> pH 7.5, 30°C, kinetic study [7, 27]; <15> pH 8.2, 30°C, strain DF905 [43]) [7, 25, 27, 43]
0.14 <1> (β-D-fructose 6-phosphate, <1> pH 7.5, 30°C [18]) [18]
0.156 <8> (β-D-fructose 6-phosphate, <8> pH 7.5, 30°C, with ATP, in presence of phosphate [30]) [30]
0.175 <1> (L-sorbose 6-phosphate, <1> pH 7.5, 30°C [17]) [17]
0.19 <1, 18> (ATP, <1> pH 8.5, 30°C, foetal enzyme [12]; <18> pH 6.0, 25°C [58]) [12, 58]
0.195 <1> (ATP, <1> pH 8.5, 30°C, adult enzyme [12]) [12]
0.2 <1> (ATP, <1> pH 7.5, 22°C [2]) [2]
0.21 <8> (β-D-fructose 2,6-bisphosphate, <8> pH 7.4, 30°C, in presence of phosphate [61]) [61]
0.22 <4> (ATP, <4> pH 7.5, 30°C [53]) [53]
0.223 <8> (ATP, <8> pH 7.4, 30°C, presence of phosphate [61]) [61]
0.226 <8> (ATP, <8> pH 7.5, 30°C, absence of phosphate [30]) [30]
0.26 <3> (ATP, <1> pH 7.5, 30°C [23]) [23]
0.29 <5> ($MgATP^{2-}$, <5> pH 7.1, 30°C, native chicken enzyme [28]) [28]
0.29-0.38 <14> (ATP, <14> pH 7.1, 30°C, kinetic data of various enzyme forms [41]) [41]
0.3 <15> (ITP, <15> pH 8.2, 30°C, strain DF903 [43]) [43]
0.31 <1> (ATP, <1> pH 7.5, 25°C, liver [46]) [46]
0.328 <1> (ATP, <1> pH 7.5, 25°C, islet [46]) [46]
0.33-0.4 <14> (β-D-fructose 6-phosphate, <14> pH 7.1, 30°C, kinetic data of various enzyme forms [41]) [41]
0.35 <15> (GTP, <15> pH 8.2, 30°C, strain DF903 [43]) [43]
0.37 <9> (β-D-fructose 6-phosphate) [32]
0.4 <1, 4> (ATP, <1> pH 6.6, 25°C [4]; <4> pH 7.8 [25]) [4, 25]
0.4 <1> (β-D-fructose 6-phosphate, <1> pH 7.1, 37°C [1]) [1]
0.5 <12> (ATP, <12> pH 8.0, three forms [37]) [37]
0.5 <1, 14> ($MgATP^{2-}$, <1,14> pH 7.5, 30°C [2,6,40]) [1, 6, 40]
0.5 <1, 18> (β-D-fructose 6-phosphate, <1> pH 7.5, 22°C [2]; <18> pH 6.0, 25°C [58]) [2, 58]
0.5-1 <12> (β-D-fructose 6-phosphate, <12> pH 8.0, forms 1-3, kinetic data of various enzyme forms [37]) [37]
0.6 <13> (β-D-fructose 6-phosphate) [38]
0.62-0.7 <9> (ATP) [32]
0.7 <13, 14> (ATP, <14> pH 7.5, 30°C, isozyme I [40]) [38, 40]
0.78 <15> (UTP, <15> pH 8.2, 30°C, strain DF903 [43]) [43]
0.9 <15> (CTP, <15> pH 8.2, 30°C, strain DF903 [43]) [43]
0.94 <8> (β-D-fructose 6-phosphate, <8> pH 7.4, 30°C, presence of phosphate [61]) [61]
1-1.1 <1, 4, 15> (β-D-fructose 6-phosphate, <1,4> pH 7.8, absence of phosphate [25]; <1> pH 7.5, 30°C, in absence of phosphate [27]; <15> pH 8.2, 30°C, ITP, strain DF905 [43]) [25, 27, 43]
1.32 <19> (ATP, <19> pH 7.8, 25°C, effect of phosphate on K_m [65]) [65]

1.4 <8, 19> (β-D-fructose 6-phosphate, <8> pH 7.5, 30°C, with ATP, absence of phosphate [30]; <19> pH 7.8, 25°C, effect of phosphate on K_m [65]) [30, 65]
1.5 <15> (GTP, <15> pH 8.2, 30°C, strain DF905 [43]) [43]
2 <15> (CTP, <15> pH 8.2, 30°C, strain DF905 [43]) [43]
2.3 <15> (UTP, <15> pH 8.2, 30°C, strain DF905 [43]) [43]
7.4 <1> (D-psicose 6-phosphate, <1> pH 7.5, 30°C [17]) [17]
15 <1> (D-tagatose 6-phosphate, <1> pH 7.5, 30°C [17]) [17]
Additional information <1, 3, 5, 8, 9, 12, 14, 18> (<1, 8, 9, 12, 14> kinetic data of native and phosphorylated enzyme [5, 30, 34, 35, 39]; <1> kinetic study [14]; <1> comparison of K_m of hepatoma cells and liver enzyme [13]; <1, 3, 5, 18> comparison of K_m of wild-type and mutant enzyme [48, 58, 60, 63]) [5-7, 14, 30, 34, 35, 39, 48, 49, 58, 60, 62, 63]

K_i-Value (mM)

0.008 <15> (ATP, <15> pH 8.2, 30°C, mutant strain DF905 [43]) [43]
0.01 <15> (ATP, <15> pH 8.2, 30°C, mutant strain DF903 [43]) [43]
0.014 <1> (phosphoenolpyruvate, <1> pH 7.5, 30°C, testis [50]) [50]
0.016 <12> (glycerate 2-phosphate, <12> L form, pH 7.8, 30°C [35]) [35]
0.018 <12> (phosphoenolpyruvate, <12> L form, pH 7.8, 30°C [35]) [35]
0.018 <12> (sn-glycerol 3-phosphate, <12> L form, pH 7.8, 30°C [35]) [35]
0.02 <12> (glycolate 2-phosphate, <12> L form, pH 7.8, 30°C [35]) [35]
0.025 <1> (citrate, <1> hepatoma cells [13]) [13]
0.026 <1> (citrate, <1> liver [44]) [44]
0.026 <12> (diphosphate, <12> L form, pH 7.8, 30°C [35]) [35]
0.028 <5> (*o*-phthalaldehyde, <5> pH 7.4, room temperature [29]) [29]
0.029 <19> (glycerate 2-phosphate, <19> pH 7.8, 25°C [65]) [65]
0.035 <1> (citrate) [13]
0.045 <19> (phosphoenolpyruvate, <19> pH 7.8, 25°C [65]) [65]
0.08 <1> (phosphoenolpyruvate, <1> pH 7.5, 25°C, islet [46]) [46]
0.08 <1> (phosphoenolpyruvate, <1> pH 7.5, 30°C, liver [50]) [50]
0.083 <6> (citrate, <6> pH 7.1, 30°C, native enzyme [28]) [28]
0.084 <6> (citrate, <6> pH 7.1, 30°C, phosphorylated enzyme [28]) [28]
0.084 <19> (glycerate 3-phosphate, <19> pH 7.8, 25°C [65]) [65]
0.095 <1> (2,5-anhydro-D-mannitol 6-phosphate, <1> pH 7.5, 30°C [17]) [17]
0.1 <3> (phosphoenolpyruvate, <3> pH 7.5, 30°C, heart [50]) [50]
0.106 <19> (diphosphate, <19> pH 7.8, 25°C [65]) [65]
0.12 <8> (citrate, <8> pH 7.4, 30°C [61]) [61]
0.12 <1> (phosphoenolpyruvate, <1> hepatoma cells [13]) [13]
0.13 <5> (citrate, <5> pH 7.1, 30°C, phosphorylated enzyme [28]) [28]
0.13 <18> (phosphoenolpyruvate, <18> pH 6.0, 25°C [58]) [58]
0.14 <1> (citrate, <1> skeletal muscle [44]) [44]
0.14 <12> (glycerate 2-phosphate, <12> H form, pH 7.8, 30°C [35]) [35]
0.15 <1> (citrate, <1> heart [44]) [44]
0.15 <12> (phosphoenolpyruvate, <12> H form, pH 7.8, 30°C [35]) [35]
0.15 <1> (sn-glycerol 3-phosphate, <1> pH 7.1 [12]) [12]
0.16 <1> (ADP, <1> pH 6.6, 25°C [4]) [4]

0.16 <12> (diphosphate, <12> H form, pH 7.8, 30°C [35]) [35]
0.16 <12> (sn-glycerol 3-phosphate, <12> H form, pH 7.8, 30°C [35]) [35]
0.17 <8> (phosphoenolpyruvate, <8> noncompetitive to ATP, pH 7.5, 30°C [30]) [30]
0.18 <12> (glycolate 2-phosphate, <12> H form, pH 7.8, 30°C [35]) [35]
0.2 <1> (citrate, <1> liver [13]) [13]
0.2 <1> (phosphoenolpyruvate, <1> liver [13]) [13]
0.23 <8> (phosphoenolpyruvate, <8> pH 7.4, 30°C [61]) [61]
0.25 <12> (dihydroxyacetone phosphate, <12> L form, pH 7.8, 30°C [35]) [35]
0.35 <18> (sn-glycerol 3-phosphate, <18> pH 6.0, 25°C [58]) [58]
0.45 <18> (glycerate 2-phosphate, <18> pH 6.0, 25°C [58]) [58]
0.49 <5> (citrate, <5> pH 7.1, 30°C, native enzyme [28]) [28]
0.53 <8> (phosphoenolpyruvate, <8> noncompetitive to fructose 6-phosphate, pH 7.5, 30°C [30]) [30]
0.55 <8> (citrate, <8> pH 7.5, 30°C [30]) [30]
0.737 <19> (dihydroxyacetone phosphate, <19> pH 7.8, 25°C [65]) [65]
1.35 <18> (dihydroxyacetone phosphate, <18> pH 6.0, 25°C [58]) [58]
1.9 <12> (dihydroxyacetone phosphate, <12> H form, pH 7.8, 30°C [35]) [35]
2 <1> (N-bromoacetylethanolamine, <1> pH 7.5, 30°C, recombinant testis enzyme [56]) [56]
2 <14> (phosphoenolpyruvate, <14> 30°C [39]) [39]
2.3 <1> (phosphoenolpyruvate, <1> pH 6.6, 25°C [4]) [4]
6 <14> (citrate, <14> 30°C [39]) [39]
8.07 <19> (sn-glycerol 3-phosphate, <19> pH 7.8, 25°C [65]) [65]
15 <16> (N-bromoacetylethanolamine, <16> pH 7.5, 30°C, crude liver extracts [56]) [56]
Additional information <1, 15> (<1> kinetic study [7]; <15> comparison of K_i for $MgATP^{2-}$ inhibition at different free Mg^{2+} concentrations [43]; <1> guanidine-inhibition of wild-type and mutant enzymes [48]; <1> comparison of K_i of wild type and mutant enzymes [50]) [7, 10, 43, 48, 50]

pH-Optimum

6 <3> (<3> of 54000 Da and 58000 Da isozymes [23]) [23]
6.6 <3> [18]
6.6 <1> (<1> in the presence of phosphate [14]) [14]
7.1 <1> [18]
7.3-8.4 <1> (<1> wild-type enzyme [49]) [49]
7.4-7.9 <12> [36]
7.5 <1, 12> (<1> native enzyme, absence of phosphate [14]) [14, 35]
7.5 <14> (<14> 25 mM phosphate [40]) [40]
7.5-8 <9> (<9> native enzyme [34]) [34]
7.5-8.5 <9> [34]
7.6 <12> [36]
8 <1> (<1> rat [20]; <1> after incubation with cAMP-dependent protein kinase [14]) [14, 20]
8.4 <14> (<14> 5 mM phosphate [40]) [40]

8.5 <1, 3> (<1> skeletal muscle [15]; <3> bovine [20]) [15, 20]
9.3 <7> (<7> muscle-type isozyme [31]) [31]
10 <5, 6> [28]
Additional information <1> (<1> biphasic pH profile with two optima at pH 6.8 and 10.0 and a minimum at 8.5, comparison of pH optima of N- and C-deletion mutants of enzyme, comparison of kinetic properties of wild-type and deletion mutants at pH 6.8 and pH 8.2 [45]; <1> comparison of pH-profiles of wild-type and mutant enzymes [49]) [45, 49]

pH-Range

5.5-8.5 <9> (<9> continuous increase of activity from pH 5.5 with 70% of maximal activity to maximal activity from pH 7.5 to 8.5, phosphorylated enzyme [34]) [34]
6-10 <5, 8> (<5> about 80% of maximal activity at pH 6.0 and 9.5 [28]) [28, 30]
6.6-8.5 <9> (<9> about half-maximal activity at pH 6.6 and about 90% of maximal activity at pH 8.5, native enzyme [34]) [34]
7-8.2 <12> (<12> about half-maximal activity at pH 7.0 and 8.2, peak II enzyme [36]; <12> about 60% of maximal activity at pH 7.0 and about 40% of maximal activity at pH 8.2 [37]) [36, 37]
7.1-8.3 <12> (<12> about half-maximal activity at pH 7.1 and 8.3, peak 1 enzyme [36]) [36]
7.3-9.5 <3> (<3> about half-maximal activity at pH 7.3 and 9.5 [20]) [20]
7.5-10 <6> (<6> linear increase up to pH 10 from 10% of maximal activity at pH 7.5 [28]) [28]
7.8-8 <12> (<12> MgATP-activated enzyme form [37]) [37]

Temperature optimum (°C)

22 <1> (<1> assay at [2]) [2]
25 <1, 3, 12, 18, 19> (<1, 3, 12, 18, 19> assay at [4, 36, 46, 50, 58, 65]) [4, 36, 46, 50, 58, 65]
30 <1, 3-6, 8, 10-12, 14> (<1, 3-6, 8, 10-12, 14> assay at [5-8, 10, 14, 15, 18, 23, 24, 28, 30, 35, 39, 40, 49, 53, 59, 61]) [5-8, 10, 14, 15, 18, 23, 24, 28, 30, 35, 39, 40, 49, 53, 59, 61]
37 <1> (<1> assay at [1]) [1]

4 Enzyme Structure

Molecular weight

85000-90000 <1> (<1> gel filtration [2]) [2]
89100 <1> (<1> foetal liver, gel filtration [12]) [12]
97000 <3> (<3> gel filtration [18]) [18]
98000 <8> (<8> gel filtration [30]) [30]
100000 <1, 9> (<1> adult liver, gel filtration [12]; <1> gel filtration [15]) [12, 15, 32]
101000 <1> (<1> high speed sedimentation equilibrium [9]) [9]
102000 <3> (<3> gel filtration [20]) [20]

107000-109000 <1> (<1> equilibrium sedimentation, sedimentation velocity analysis [16]) [16]
110000 <1, 5, 6> (<1, 5, 6> gel filtration [20,28]) [20, 28]
112500 <1> (<1> gel filtration [18]) [18]
118000 <3> (<3> gel filtration [23]) [23]
120000 <4, 14> (<4,14> gel filtration [41,53]) [41, 53]
132000 <12> (<12> L-form, gel filtration [35]) [35]
225000 <3> (<3> gel filtration [24]) [24]
250000 <14> (<14> gel filtration [40]) [40]
320000 <19> (<19> gel filtration [65]) [65]
370000 <18> (<18> gel filtration [58]) [58]
390000 <12> (<12> H-form, gel filtration [35]) [35]
600000 <14> (<14> gel filtration [39]) [39]
Additional information <1, 4, 17> (<1, 17> amino acid composition [3, 9, 11, 15, 57]; <1,4> primary structures of human, rat and bovine liver enzyme [25]; <17> amino acid composition [57]) [3, 9, 11, 15, 25, 57]

Subunits

? <3> (<3> x * 61000, SDS-PAGE [63]) [63]
dimer <1-9> (<3> 2 * 49000, SDS-PAGE [20]; <3> 2 * 52000, SDS-PAGE [18]; <6> 2 * 53000, SDS-PAGE, with a minor constituent of MW 54000 [28]; <9> 2 * 53000, SDS-PAGE [32]; <7> 2 * 53900, muscle-type isozyme, SDS-PAGE [31]; <1> 2 * 54000, SDS-PAGE, amino acid sequence [15]; <5,8> 2 * 54000, SDS-PAGE [28,30]; <1> 2 * 54760, calculated from amino acid sequence [11]; <1> 2 * 55000, SDS-PAGE [3, 9, 10, 12, 16, 20, 22, 23, 25, 47]; <1> 2 * 55000, high speed sedimentation equilibrium in 6 M guanidinium chloride, amino acid sequence [9]; <7> 2 * 55800, liver isoenzyme, SDS-PAGE [31]; <1,4> 2 * 58000, SDS-PAGE [18, 53]; <3> 2 * 120000, SDS-PAGE [24]) [3, 9-12, 15, 16, 18, 20-25, 28, 30-32, 47, 53]
tetramer <10-12, 18, 19> (<10-12> 4 * 90000, H-form, SDS-PAGE [35]; <18> 4 * 83000 [58]; <19> 4 * 90800, SDS-PAGE [65]) [35, 58, 65]
Additional information <1, 3> (<3> two isozymes: 58000 Da and 54000 Da, SDS-PAGE [21-23]; <1> two isozymes: 55000 Da and 52000 Da [62]) [21-23, 62]

Posttranslational modification

phosphoprotein <1, 3-9, 12-15> (<1, 3, 4, 9, 14> phophorylation by cAMP-dependent protein kinase causes activation [1, 19, 21-23, 34, 39, 41, 53]; <3, 4> phosphorylation by protein kinase C causes activation [19, 21, 23, 53]; <9> at acid pH-values [34]; <3> MW 58000 enzyme form [23]; <3> phosphorylation site [19, 22, 23]; <3> brain enzyme is phosphorylated, but not activated [24]; <1, 3, 5, 6> phosphorylation by cAMP-dependent protein kinase causes inactivation of liver enzyme [3, 5, 8, 12-16, 18, 26, 28, 44, 59]; <15> phosphorylation by cAMP-dependent protein kinase causes inactivation [43, 45]; <1,4> phosphorylation site: Ser-32 [11, 45, 52]; <1> phosphorylation of foetal liver enzyme by protein kinase C, but no effect on adult liver cells [12]; <1> phosphorylation at lower pH-values [8]; <1> phosphorylation at pH 6.6, not at pH 8 [14]; <1> phosphorylation by cAMP-dependent protein

kinase causes 80% decrease in activity of the liver cells but not of hepatoma cells [13]; <1> phosphorylation by cAMP-dependent protein kinase of liver enzyme, not of skeletal muscle enzyme, since the phosphorylation site target Ser-32 of the liver isozyme is replaced by Ala in the muscle isozyme [15]; <1> phosphorylation by cAMP-dependent protein kinase causes inactivation of liver enzyme, not of kidney, testis and heart enzyme [16]; <1, 3> rat liver enzyme is inhibited by phosphorylation by cAMP-dependent protein kinase, but not rat skeletal muscle, bovine and rat heart enzyme [18]; <1, 3> rat liver enzyme is inhibited by phosphorylation by cAMP-dependent protein kinase but not rat kidney, testis and skeletal muscle enzyme and bovine and rat heart enzyme [26]; <1> phosphorylation by cAMP-dependent protein kinase causes inactivation of liver enzyme, not of heart and skeletal muscle enzyme [44]; <11,13> phosphorylation by cAMP-dependent protein kinase, but no inhibition [35,38]; <8> phosphorylation by cAMP-dependent protein kinase causes inactivation of liver enzyme, not skeletal muscle enzyme [61]; <1> loss of phosphorylation-dependent reduction of enzyme by deletion of the N-terminal residues of enzyme, The deletion of 7 N-terminal amino acids causes a 75% decrease in activity [45]; <1> the islet enzyme lacks protein kinase A and C phosphorylation sites [46]; <1> phosphorylation by cAMP-dependent protein kinase causes 35% inactivation of isozyme L of adipose tissue, not isozyme M [62]; <1> kinetic of phosphorylation by cAMP-dependent protein kinase [64]) [1, 3, 5, 8, 11-16, 18, 19, 21-23, 26, 28, 30, 31, 34, 35, 38, , 39, 41, 43-46, 52, 53, 59, 61, 62, 64]
side-chain modification <15> (<15> specific modification by N-(1-pyrenyl)-maleimide, the results demonstrate the presence of SH residue in the interface of enzyme subunits critical for interactions between them and that conformational changes occurring through dimers are essential for catalytic activity [67]) [67]

5 Isolation/Preparation/Mutation/Application

Source/tissue

Kupffer cell <1> (placenta-type isozyme is expressed in Kupffer cells of the liver [55]) [55]
adipose tissue <1> (<1> equal amounts of isozyme L and M [62]) [62]
brain <3> (<3> cortex [24]) [24]
endosperm <11> [35]
heart <1, 3> (<3> two isozymic forms [21, 26]; <3> 2 isozymes: result of alternative splitting of the same primary transcript [22]) [16, 18, 19, 21-23, 26, 44, 46, 50, 63]
hepatocyte <1> [1, 14]
hepatoma <1> (<1> resembles muscle enzyme [13]) [13]
kidney <1> [16]
leaf <12, 17, 19> [8, 35-37, 57, 65]
liver <1, 2, 3, 4, 5, 16> [1-13, 15-18, 20, 22, 25-28, 44-46, 50, 52, 56, 59, 60, 61]
lung <1> [16]

mantle tissue <9> [32, 34]
midgut <20> (<20> gland [66]) [66]
muscle <6> [28]
mycelium <13> [38]
pancreatic islet <1> [46]
placenta <4> [53-55]
skeletal muscle <1, 6> [15, 16, 26, 31, 44-46, 61]
testis <1> [16, 46-51, 56]
tuber <10> [35]
Additional information <1> (<1> tissue distribution, in extrahepatic tissue only 10% or less of activity in liver [16]; <1> POROS-HQ column chromatography followed by Western blot analysis of extracts from various rat tissues show that proteins of placenta-type isozyme are expressed in placenta, brain, testis, liver, spleen, heart and lung, but not in kidney and skeletal muscle [55]) [16, 55]

Localization

cytosol <1> [1, 2, 14]

Purification

<1> (partial [1,2]; liver [18]; recombinant enzyme [46]; mutant enzyme [49, 50]; adipose tissue [62]) [1-3, 5, 9, 11-13, 15, 16, 18, 46, 49, 50, 62]
<3> (heart [18,22]; 2 enzyme forms [22]; mutant enzyme [50]; wild-type and mutant enzyme [63]) [18, 20, 22-24, 50, 63]
<4> (recombinant enzyme [25, 53, 54]) [25, 53, 54]
<5> [28]
<6> [28]
<7> (2 isozymes, liver (L) and muscle (M) type [31]) [31]
<8> (liver [30]; skeletal muscle [61]) [31, 30, 61]
<9> [32]
<12> (partial [36,37]; multiple molecular forms, predominant: H-(heavy) and L-(light)form [35]; 2 distinct molecular forms [36,37]) [35-37]
<13> [38]
<14> (2 isozymes, partial [40,41]) [33, 39-42]
<19> (recombinant enzymes [65]) [65]

Crystallization

<1> (the crystal structure of H256A to a resolution of 2.4 A by molecular replacement [51]) [51]
<1> (the crystal structure of mutant enzyme, the two subunits in the homodimer are arranged in a head-to-head manner, each monomer consists of independent kinase and phosphatase domains, the kinase domains are in close contact, forming an extended hydrophobic core between them, while the phosphatase domains are essentially independent of one another, enzyme is related to the nucleotide monophosphate kinases, and the catalytic domain of G proteins [47]) [47]
<4> (the crystal structure of liver enzyme: a head-to-head homodimer, depending on the liganding conditions each subunit contains an ATPγS, ADP or

P molecule to the kinase domain, and two phosphates, regardless of the liganding conditions, bound to phosphatase domain, structure of enzyme very similar to that of the rat testis isoform [52]) [52]

Cloning

<3> [19]
<3> (wild-type enzyme, S466E and S466E/S483E mutants are expressed in Escherichia coli BL21(DE3)pLysE and S483E mutant is expressed in BL21(DE3)pLysS, human embryonic kidney 293 cells are transiently transfected with vectors expressing wild-type heart enzyme [63]) [63]
<4> (isoform of enzyme that is induced by proinflammatory stimuli and that is distinguished by the presence of multiple copies of the AUUUA instability motif in its 3' untranslated region is identified and the complete cDNA is cloned and sequenced, expression in eigth human tumor cell lines [66]) [66]
<16> (using of recombinant testis enzyme [56]) [56]
<18> (expression in Saccharomyces cerevisiae [58]) [58]
<19> (expression in Spodoptera frugiperda cells infected with recombinant baculovirus and Escherichia coli [65]) [65]
<1, 3-5, 17> (expression in Escherichia coli [27, 59]) [27, 45, 46, 49, 50, 53, 57, 59]
<1, 4> (mutant expressed in Escherichia coli BL21(DL3) [25]) [25]

Engineering

H256A <1> (<1> crystal structure [51]) [51]
K172A <1> (<1> increase in K_m of β-D-fructose 6-phosphate [49]) [49]
K172E <1> (<1> increase in K_m of $MgATP^{2-}$, increase in K_m of β-D-fructose 6-phosphate [49]) [49]
K172H <1> (<1> increase in K_m of $MgATP^{2-}$ [49]) [49]
K172R <1> (<1> increase in K_m of β-D-fructose 6-phosphate [49]) [49]
K51A <1> (<1> increase in K_m of β-D-fructose 6-phosphate [49]) [49]
K51H <1> (<1> increase in K_m of $MgATP^{2-}$, increase in K_m of β-D-fructose 6-phosphate [49]) [49]
P2R <1, 4> (<1,4> kinetic properties of human liver mutant and rat wild-type enzyme are very similar [25]) [25]
R136K <1> (<1> increase in K_m of $MgATP^{2-}$ [49]) [49]
R136L <1> (<1> increase in K_m of β-D-fructose 6-phosphate [49]) [49]
R193H <1> (<1> increase in K_m of $MgATP^{2-}$, increase in K_m of β-D-fructose 6-phosphate [49]) [49]
R193L <1> (<1> increase in K_m of β-D-fructose 6-phosphate [49]) [49]
R279A <5> (<5> mutation eliminates both the binding of ATP to the bisphosphatase domain of the bifunctional enzyme and the activation of enzyme by ATP [60]) [60]
R359A <5> (<5> mutation eliminates both the binding of ATP to the bisphosphatase domain of the bifunctional enzyme and the activation of enzyme by ATP [60]) [60]
R78H <1> (<1> increase in K_m of $MgATP^{2-}$ [49]) [49]
R78L <1> (<1> increase in K_m of $MgATP^{2-}$ [49]) [49]
R79H <1> (<1> increase in K_m of $MgATP^{2-}$ [49]) [49]

R79L <1> (<1> increase in K_m of $MgATP^{2-}$ [49]) [49]
S466E <3> (<3> mutant is phosphorylated by protein kinase B with a stoichiometry to about half that of wild-type enzyme in vitro [63]) [63]
S466E/S483E <3> (<3> the double mutant is not phosphorylated by protein kinase B, mutation decreases K_m of β-D-fructose 6-phosphate in vitro [63]) [63]
S483E <3> (<3> mutant is phosphorylated by protein kinase B with a stoichiometry to about half that of wild-type enzyme and mutation decreases citrate inhibition in vitro [63]) [63]
Additional information <1, 3, 5, 18> (<1> effects of N- and C-terminal deletions of skeletal muscle and liver enzyme, e.g. ND4, ND7, ND12, ND23, CD30, comparison of the kinetic properties of deletion mutants [45]; <1> mutant enzyme, in which the four tryptophan residues in the isoenzyme are mutated to phenylalanine, structure [47]; <1> enzyme contains four tryptophan residues: Trp15, Trp64, Trp299 and Trp320, mutant enzymes contain either no Trp or a single Trp at each location, the other Trp residues having been converted to Phe, mutations do not cause large change in protein conformation and function, Trp299 and Trp320 in mutant enzymes quenched by iodide to a small extent can indicate an altered conformation, Trp64 is not accessible [48]; <1> ATP binding and the effect of pH on the kinetics are characterized [49]; <1,3> mutant enzymes of rat testis are constructed, in which its terminal peptides are replaced with those of the liver or the heart enzyme [50]; <18> the deletion of N-terminal 318 amino acids abolishes the Michaelis-Menten kinetics of enzyme, K_m of β-D-fructose 6-phosphate is increased, whereas K_m of ATP is not significantly altered. When the first 66 amino acids are deleted, the activity ratio between 6-phosphofructo-2-kinase and β-D-fructose 2,6-bisphosphatase is halved. The deletion of 125, 179, 249 and 318 amino acids results in progressive further decreases in the activity ratio and the activity ratio is reduced 4fold when N-terminus is deleted completely. The full-length enzyme is eluted as a tetramer, whereas the truncated enzymes are eluted as monomers [58]; <5> a series of C-terminal deletion mutants are generated: 15, 20, 25 and 30 amino acids, the deletion of the C-terminal 25 or 30 residues of enzyme increases K_m of β-D-fructose 6-phosphate by approximately 2fold. The mutations E446A, H444A, H444K, H444E, R445E, R445L prove the importance of His444 and Arg445.The C-terminal region I involves in the activation of enzyme by ATP. [59]; <5> the chicken enzyme in which the C-termini tail are replaced with that of rat enzyme is not activated by ATP [60]) [45, 47, 49, 50, 58, 59, 60]

6 Stability

Temperature stability

4 <18> (<18> 10 min, enzyme is stable, both in the absence and in the presence of phosphate [58]) [58]
25 <18> (<18> about 76% of enzyme activity remains after 10 min, both in the absence and in the presence of phosphate [58]) [58]

42 <18> (<18> 10 min, activation of enzyme and deletion mutant decreases rapidly, but the presence of phosphate protectes the enzyme activities from inactivation and 75% of enzyme activity remains after 15 min [58]) [58]
57 <1> (<1> 10 min, pH 7.1, progressive inactivation, phosphate does not protect [8]) [8]

General stability information

<1>, repeated freeze-thawing inactivates [5]
<12>, unstable in crude homogenate, iodoacetate, antipain, chymostatin or leupeptin stabilizes, not pepstatin, PMSF or soybean trypsin inhibitor [35]
<13>, very unstable upon purification [38]

Storage stability

<1>, -80°C to 0°C, at least 1 month [5]
<12>, -80°C, partially purified, less than 10% loss of activity within 1 month [36]
<12>, 0°C, L-form, at least 2 h in the absence of protease inhibitor [35]
<12>, 0°C, in 100 mM potassium acetate, 5 mM magnesium acetate, 2.5 mM DTT, 1 mM iodoacetate, 1 mg/ml antipain, 50 mM Tris-acetate, pH 7.8, several h [35]
<13>, 4°C, $t_{1/2}$: 4 h [38]
<14>, -20°C, pH 6.8, partially purified, isozyme I, 1 month [40]

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Glucose-1,6-bisphosphate synthase 2.7.1.106

1 Nomenclature

EC number
2.7.1.106

Systematic name
3-phospho-D-glyceroyl-phosphate:α-D-glucose-1-phosphate 6-phosphotransferase

Recommended name
glucose-1,6-bisphosphate synthase

Synonyms
glucose 1,6-diphosphate synthase
glucose-1,6-bisphosphate synthetase

CAS registry number
56214-39-2

2 Source Organism

<1> *Mus musculus* [1]
<2> *Bos taurus* [2, 3]

3 Reaction and Specificity

Catalyzed reaction
3-phospho-D-glyceroyl phosphate + α-D-glucose 1-phosphate = 3-phospho-D-glycerate + α-D-glucose 1,6-bisphosphate

Reaction type
phospho group transfer

Natural substrates and products
S 3-phospho-D-glyceroyl phosphate + D-glucose 1-phosphate <1, 2> (<1> responsible for glucose 1,6-diphosphate biosynthesis in brain [1]) (Reversibility: r <1, 2> [1-3]) [1, 2]
P 3-phospho-D-glycerate + D-glucose 1,6-bisphosphate

Substrates and products
S 3-phospho-D-glyceroyl phosphate + D-glucose 1-phosphate <1, 2> (<1,2> i.e. 1,3-diphosphoglycerate [1,2]; <2> specific for 3-phospho-D-glyceroyl

phosphate [2]; <1> no phosphoryl donors are acetyl phosphate, ATP, phosphoenolpyruvate, creatine phosphate, fructose 1,6-diphosphate [1]; <1> little or no acceptor activity: glucose, glucose 6-sulfate, glucosamine 6-phosphate, 2-deoxy-glucose 6-phosphate, 3-phosphoglycerate, galactose or fructose monophosphates [1]; <1,2> no acceptor activity mannose 6-phosphate [1,2]) (Reversibility: r <1,2> [1-3]) [1-3]

P 3-phospho-D-glycerate + D-glucose 1,6-bisphosphate

S 3-phospho-D-glyceroyl phosphate + D-glucose 6-phosphate <1, 2> (<1> phosphorylation at 56% the rate of glucose 1-phosphate [1]) (Reversibility: ? <1,2> [1,2]) [1, 2]

P 3-phospho-D-glycerate + D-glucose 1,6-bisphosphate

S 3-phospho-D-glyceroyl phosphate + D-mannose 1-phosphate <1, 2> (<1> phosphorylation at 81% the rate of glucose 1-phosphate [1]) (Reversibility: ? <1,2> [1,2]) [1, 2]

P 3-phospho-D-glycerate + D-mannose 1,6-bisphosphate

Inhibitors

D-fructose 1,6-diphosphate <1, 2> (<2> strong, liver [2]) [1-3]
D-glucose 1,6-diphosphate <1, 2> [1, 3]
K^+ <2> (<2> weak [3]) [3]
L-malate <2> (<2> weak [3]) [3]
Li^+ <2> (<2> strong, dilution, Co^{2+} or Zn^{2+} protects, not Ca^{2+}, Mg^{2+} or Mn^{2+} [3]) [3]
Na^+ <2> (<2> weak [3]) [3]
acetyl-CoA <1> [1]
cis/trans-aconitate <2> [3]
citrate <2> [3]
glutarate <2> (<2> weak [3]) [3]
glycerate 2,3-diphosphate <1, 2> (<2> strong, liver [2]) [1-3]
glycerate 3-phosphate <1, 2> (<1> weak [1]) [1, 3]
imidazole/EDTA <1> [1]
isocitrate <2> [3]
phosphate <1, 2> [1, 3]
phosphoenolpyruvate <1, 2> (<1,2> strong [1-3]) [1-3]
tricarballylate <2> [3]
Additional information <2> (<2> no inhibition by adipate or succinate [3]) [3]

Metals, ions

Ca^{2+} <1> (<1> requirement, can be replaced by Mg^{2+}, Mn^{2+}, Zn^{2+}, Ni^{2+}, Co^{2+}, Cd^{2+} [1]) [1]
Cd^{2+} <1> (<1> requirement, can be replaced by Mg^{2+}, Mn^{2+}, Ca^{2+}, Zn^{2+}, Ni^{2+}, Co^{2+} [1]) [1]
Co^{2+} <1, 2> (<1> requirement, can be replaced by Mg^{2+}, Mn^{2+}, Ca^{2+}, Zn^{2+}, Ni^{2+}, Cd^{2+} [1]; <2> tightly bound, can replace Zn^{2+} [3]) [1, 3]
Mg^{2+} <1, 2> (<1> requirement, can be replaced by Mn^{2+}, Ca^{2+}, Zn^{2+}, Ni^{2+}, Co^{2+}, Cd^{2+} [1]; <2> activation, easily dissociates from enzyme protein [3]; <2> K_m-value: 1 mM [3]; <2> no activation of enzyme in Zn-form [3]) [1-3]

Mn^{2+} <1> (<1> requirement, can be replaced by Mg^{2+}, Ca^{2+}, Zn^{2+}, Ni^{2+}, Co^{2+}, Cd^{2+} [1]) [1]
Ni^{2+} <1> (<1> requirement, can be replaced by Mg^{2+}, Mn^{2+}, Ca^{2+}, Zn^{2+}, Co^{2+}, Cd^{2+} [1]) [1]
Zn^{2+} <1, 2> (<1> requirement, can be replaced by Mg^{2+}, Mn^{2+}, Ca^{2+}, Ni^{2+}, Co^{2+}, Cd^{2+} [1]; <2> Zn-form of the enzyme: tightly bound, about 65% of brain enzyme in Zn-form [3]) [1, 3]

Specific activity (U/mg)

0.0045 <2> (<2> liver enzyme [2]) [2]
0.136 <2> (<2> erythrocyte enzyme [2]) [2]

K_m-Value (mM)

0.000087 <2> (3-phospho-D-glyceroyl 1-phosphate, <2> 23°C, pH 8, Mg^{2+}-enzyme in brain [3]) [3]
0.000091 <2> (3-phospho-D-glyceroyl 1-phosphate, <2> 23°C, pH 8, Zn^{2+}-enzyme in brain [3]) [3]
0.00023 <2> (3-phospho-D-glyceroyl 1-phosphate, <2> 25°C, pH 7.5, liver enzyme peak I [2]) [2]
0.00028 <2> (3-phospho-D-glyceroyl 1-phosphate, <2> 25°C, pH 7.5, liver enzyme peak II [2]) [2]
0.00045 <2> (3-phospho-D-glyceroyl 1-phosphate, <2> 25°C, pH 7.5, erythrocyte enzyme [2]) [2]
0.009 <2> (D-glucose 1-phosphate, <2> 25°C, pH 7.5, liver enzyme peak I [2]) [2]
0.015 <2> (D-glucose 6-phosphate, <2> 25°C, pH 7.5, liver enzyme peak I [2]) [2]
0.059 <2> (D-glucose 1-phosphate, <2> 25°C, pH 7.5, liver enzyme peak II [2]) [2]
0.061 <2> (D-glucose 6-phosphate, <2> 25°C, pH 7.5, liver enzyme peak II [2]) [2]
0.09 <2> (D-glucose 1-phosphate, <2> 23°C, pH 8, Mg^{2+}- and Zn^{2+}-enzyme in brain [3]) [3]
0.16 <2> (D-glucose 1-phosphate, <2> 25°C, pH 7.5, erythrocyte enzyme [2]) [2]

pH-Optimum

8 <1> [1]

pH-Range

7.3-8.7 <1> (<1> about half-maximal activity at pH 7.3 and 8.7 [1]) [1]

Temperature optimum (°C)

25 <1, 2> (<1,2> assay at [1-3]) [1-3]

5 Isolation/Preparation/Mutation/Application

Source/tissue
brain <1, 2> [1, 3]
erythrocyte <2> [2]
liver <2> [2]

Localization
soluble <1, 2> [1, 2]

Purification
<1> (partial [1]) [1]
<2> (liver: 2 isozymes [2]) [2]

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Diacylglycerol kinase 2.7.1.107

1 Nomenclature

EC number
2.7.1.107

Systematic name
ATP:1,2-diacylglycerol 3-phosphotransferase

Recommended name
diacylglycerol kinase

Synonyms
1,2-diacylglycerol kinase
ATP:diacylglycerol phosphotransferase
DAGK
DAGKα
DG kinase
DGK
DGK-α
DGK-θ
DGKβ
DGKδ
DGKγ
DGKι
DGKξ
adenosine 5'-triphosphate:1,2-diacylglycerol 3-phosphotransferase
arachidonoyl-specific diacylglycerol kinase
diacylglycerol kinase
diacylglycerol:ATP kinase
diglyceride kinase
kinase (phosphorylating), 1,2-diacylglycerol
kinase, 1,2-diacylglycerol (phosphorylating)
sn-1,2-diacylglycerol kinase

CAS registry number
60382-71-0

2 Source Organism

<1> *Gallus gallus* [1]
<2> *Escherichia coli* (K 12 [11]) [2, 6, 7, 10, 11, 12, 21, 30, 37]

<3> *Sus scrofa* (DGKα [35]) [3, 20, 28, 35]
<4> *Catharanthus roseus* [4, 5, 29]
<5> *Homo sapiens* (DGKγ [23,35]; DGK-θ [26]; DGKβ [32]; DGKη1 and DGKη2 [39]) [7, 13, 15, 23, 24, 26, 28, 32, 33, 35, 36, 39]
<6> *Drosophila melanogaster* [8]
<7> *Rattus norvegicus* (DGK-I, DGK-II and DGK-III [25]; DGK-θ [26]; DGKβ [35]) [9, 14, 16, 18, 19, 22, 25, 26, 28, 35]
<8> *Triticum aestivum* [17]
<9> *Mus musculus* [27, 38]
<10> *Mesocricetus auratus* (DGKη [11]; DGKθ and DGKδ [34]) [28, 31, 34]

3 Reaction and Specificity

Catalyzed reaction

ATP + 1,2-diacyllycerol = ADP + 1,2-diacyl-sn-glycerol 3-phosphate (<2> random equilibrium mechanism [37])

Reaction type

phospho group transfer

Natural substrates and products

S ATP + 1,2-diacyl-sn-glycerol <2, 3> (<2>, the enzyme functions to recycle diacylglycerol which is generated largely as a by-product of membrane-derived oligosaccharide biosynthesis [2]; <3>, the enzyme may regulate the intracellular concentration of diacylglycerol [3]) (Reversibility: ? <2, 3> [2, 3]) [2, 3]
P ADP + 1,2-diacyl-sn-glycerol 3-phosphate
S ATP + 1,2-diacyl-sn-glycerol <3, 5, 6, 7, 9, 10> (<3>, DGK-Iα is involved in IL-2-mediated lymphocyte proliferation [28]; <3>, the 80000 Da and the 150000 Da enzyme form do not possess specificity towards diacylglycerol molecular species [3]; <6>, the enzyme may have an important function in the adult nervous system and muscle and during the development of the embryonic nervous system [8]; <5>, DGKγ negatively regulates macrophage differentiation through its catalytic action operating on the cytoskeleton [23]; <7>, the enzyme is involved in resynthesis of phosphatidylinositol by converting a second messenger diacylglycerol to phosphatidic acid [25]; <9> high level expression of DGKα is induced following a signal transmitted through the pre-T-cell-receptor and the protein tyrosine kinase lck. Activity of DGKα contributes to survival in $CD4^+$ 8^+ double positive thymocytes as pharmacological inhibition of DGK activity results in death of this cell population both in cell suspension and thymic explants. DGKα promotes survival in theses thymocytes through a Bcl-regulated pathway [27]; <10> the enzyme plays a role in cellular processes by regulating the intracellular concentration of the second messenger diacylglycerol. DGKη may play a more general role in regulating cellular diacylglycerol levels [31]; <10>, nuclear DGK-θ is activated in response to α-thrombin [34]; <5>, DGKι may have important cellular func-

tions in retina and brain [36]; <9>, DAGKα is stimulated vby Src-like kinase-dependent phosphoinositide 3 kinase activation in lymphocytes. In vivo the increase in cellular levels of Src-like kinase-dependent phosphoinositide 3 kinase products is sufficient to induce DAGKα activation, allowing DAGKα relocation to the intact lymphocyte [38]; <5>, the expression of DGKη2 is suppressed by glucocorticoid in contrast to the marked induction of DGKη1 [39]) (Reversibility: ? <3, 5, 6, 7, 9, 10> [3, 8, 23, 25, 27, 28, 31, 34, 36, 38, 39]) [3, 8, 23, 25, 27, 28, 31, 34, 36, 38, 39]

P ADP + 1,2-diacyl-sn-glycerol 3-phosphate

S ATP + 1-stearoyl-2-arachidonoyl-sn-glycerol <5> (<5>, since diacylglycerol kinase is an enzyme of the phosphatidylinositol cycle, its natural substrate could be 1-stearoyl-2-arachidonoyl-sn-glycerol, thought to be the main diacylglycerol analog generated from phosphoinositide [7]) (Reversibility: ? <5> [7]) [7]

P ADP + 1-stearoyl-2-arachidonoyl-sn-glycerol 3-phosphate

Substrates and products

S 1,2-dipalmitoyl-sn-glycerol + GTP <1, 2> (Reversibility: ? <1,2> [1,10]) [1, 10]

P GDP + 1,2-dipalmitoyl-sn-glycerol 3-phosphate

S 2'-deoxy-ATP + sn-1,2-dihexanoylglycerol <2> (Reversibility: ? <2> [37]) [37]

P 2'-deoxy-ADP + sn-1,2-dihexanoylglycerol 3-phosphate

S ADP + sn-1,2-dihexanoylglycerol <2> (<2>, $MgADP^-$ is a very poor phosphoryl donor [37]) (Reversibility: ? <2> [37]) [37]

P AMP + sn-1,2-dihexanoylglycerol 3-phosphate

S ATP + 1,2-diarachidonoyl-glycerol <7> (Reversibility: ? <7> [14]) [14]

P ADP + 1,2-diarachidonoyl-glycerol 3-phosphate

S ATP + 1,2-dicapryl-sn-glycerol <5> (<5>, about 140% of the activity with sn-1,2-dioleoylglycerol [33]) (Reversibility: ? <5> [33]) [33]

P ADP + 1,2-dicapryl-sn-glycerol 3-phosphate

S ATP + 1,2-didecanoylglycerol <7> (<7>, enzyme type I: activity is 157% of the activity with rac-1,2-dioleoylglycerol, enzyme type II: activity is 141% of the activity with rac-1,2-dioleoylglycerol [16]) (Reversibility: ? <7> [16]) [16]

P ADP + 1,2-didecanoyl 3-phosphate

S ATP + 1,2-didodecanoyl <7> (<7>, enzyme type I: activity is 107% of the activity with rac-1,2-dioleoylglycerol, enzyme type II: activity is 227% of the activity with rac-1,2-dioleoylglycerol [16]) [16]

P ADP + 1,2-didodecanoyl 3-phosphate

S ATP + 1,2-dihexadecanoylglycerol <7> (<7>, enzyme type I: activity is 198% of the activity with rac-1,2-dioleoylglycerol, enzyme type II: activity is 231% of the activity with rac-1,2-dioleoylglycerol [16]) (Reversibility: ? <7> [16]) [16]

P ADP + 1,2-dihexadecanoylglycerol 3-phosphate

S ATP + 1,2-dioctanoylglycerol <7> (<7>, enzyme type I: activity is 149% of the activity with rac-1,2-dioleoylglycerol, enzyme type II: activity is 114% of the activity with rac-1,2-dioleoylglycerol [16]) (Reversibility: ? <7> [16,19]) [16, 19]
P ADP + 1,2-dioctanoylglycerol 3-phosphate
S ATP + 1,2-dipalmitate <2> (Reversibility: ? <2> [11]) [11]
P ADP + ?
S ATP + 1,2-dipalmitoyl-sn-glycerol <1> (Reversibility: ? <1> [1]) [1]
P ADP + 1,2-dipalmitoyl-sn-glycerol 3-phosphate
S ATP + 1,2-dipalmitoyl-sn-glycerol <5> (<5>, 15% of the activity with 1-stearoyl-2-arachidonyl-sn-glycerol [7]) (Reversibility: ? <5> [7]) [7]
P ADP + 1,2-dipalmitoyl-sn-glycerol 3-phosphate
S ATP + 1,2-ditetradecanoylglycerol <7> (<7>, enzyme type I: activity is 200% of the activity with rac-1,2-dioleoylglycerol, enzyme type II: activity is 262% of the activity with rac-1,2-dioleoylglycerol [16]) [16]
P ADP + 1,2-ditetradecanoyl 3-phosphate
S ATP + 1-O-hexadecyl-2-oleoyl-sn-glycerol <5> (<5>, 45.4% of the activity with 1-stearoyl-2-arachidonyl-sn-glycerol [7]) (Reversibility: ? <5> [7]) [7]
P ADP + 1-O-hexadecyl-2-oleoyl-sn-glycerol 3-phosphate
S ATP + 1-oleoyl-2-palmitoyl-sn-glycerol <5> (<5>, about 85% of the activity with sn-1,2-dioleoylglycerol, DGKξ [33]) (Reversibility: ? <5> [33]) [33]
P ADP + 1-palmitoyl-2-oleoyl-sn-glycerol 3-phosphate
S ATP + 1-palmitoyl-2-oleoyl-sn-glycerol <7> (<7>, enzyme type I: activity is 125% of the activity with rac-1,2-dioleoylglycerol, enzyme type II: activity is 101% of the activity with rac-1,2-dioleoylglycerol [16]) (Reversibility: ? <7> [16]) [16]
P ADP + 1-palmitol-2-oleoyl-sn-glycerol 3-phosphate
S ATP + 1-palmitoyl-2-arachidonoyl-sn-glycerol <7> (<7>, enzyme type I: activity is 181% of the activity with rac-1,2-dioleoylglycerol, enzyme type II: activity is 116% of the activity with rac-1,2-dioleoylglycerol [16]) (Reversibility: ? <7> [16]) [16]
P ADP + 1-palmitoyl-2-arachidonoyl-sn-glycerol 3-phosphate
S ATP + 1-palmitoyl-2-linoleoyl-sn-glycerol <7> (<7>, enzyme type I: activity is 116% of the activity with rac-1,2-dioleoylglycerol, enzyme type II: activity is 86% of the activity with rac-1,2-dioleoylglycerol [16]) (Reversibility: ? <7> [16]) [16]
P ADP + 1-palmitoyl-2-linoleoyl-sn-glycerol 3-phosphate
S ATP + 1-palmitoyl-2-oleoyl-sn-glycerol <5, 7> (<5>, about 80% of the activity with sn-1,2-dioleoylglycerol, DGKξ [33]) (Reversibility: ? <5,7> [22,33]) [22, 33]
P ADP + 1-palmitoyl-2-oleoyl-sn-glycerol 3-phosphate
S ATP + 1-palmitoyl-2-oleoyl-sn-glycerol <5, 7> (<7>, about 60% of the activity with 1-palmitoyl-2-arachidonoyl-sn-glycerol [22]; <5> about 80% of the activity with sn-1,2-dioleoylglycerol [33]; <5>, 96.5% of the activity

with 1-stearoyl-2-arachidonoyl-sn-glycerol [7]) (Reversibility: ? <5, 7> [7, 22, 33]) [7, 22, 33]
P ADP + 1-palmitoyl-2-oleoyl-sn-glycerol 3-phosphate
S ATP + 1-stearoyl-2-arachidonoyl-sn-glycerol <5, 7> (<5>, 108% of the activity with sn-1,2-dioleoylglycerol [33]) (Reversibility: ? <5, 7> [7, 14, 33]) [7, 14, 33]
P ADP + 1-stearoyl-2-arachidonoyl-sn-glycerol 3-phosphate
S ATP + 2-monooleoyl-rac-glycerol <5> (<5>, 10.7% of the activity with 1-stearoyl-2-arachidonoyl-sn-glycerol [7]) (Reversibility: ? <5> [7]) [7]
P ADP + 2-monooleoyl-rac-glycerol 3-phosphate
S ATP + ceramide <2> (<7>, hardly utilized [18]; <7>, no activity [9]) (Reversibility: ? <2> [2,11,12]) [2, 11, 12]
P ADP + ceramide 3-phosphate
S ATP + rac-1,2-dioleoylglycerol <7> (Reversibility: ? <7> [16]) [16]
P ADP + rac-1,2-dioleoylglycerol 3-phosphate
S ATP + sn-1,2-dihexanoylglycerol <2> (Reversibility: ? <2> [37]) [37]
P ADP + sn-1,2-dihexanoylglycerol 3-phosphate
S ATP + sn-1,2-dioctanoylglycerol <2> (Reversibility: ? <2> [6]) [6]
P ADP + sn-1,2-dioctanoylglycerol 3-phosphate
S ATP + sn-1,2-dioleoylglycerol <2, 3, 4, 5, 7> (<5>, recombinant DGKξ [33]; <7>, enzyme type I: activity is 18% of the activity with rac-1,2-dioleoylglycerol, enzyme type II: activity is 19% of the activity with rac-1,2-dioleoylglycerol [16]) (Reversibility: ? <2, 3, 4, 5, 7> [2, 3, 5, 6, 7, 14, 16, 19, 21, 33]) [2, 3, 5, 6, 7, 14, 16, 19, 21, 33]
P ADP + sn-1,2-dioleoylglycerol 3-phosphate
S ATP + sn-1,3-dioleoylglycerol <5> (<5>, about 10% of the activity with sn-1,2-dioleoylglycerol [33]) (Reversibility: ? <5> [33]) [33]
P ADP + ?
S GTP + dioleoylglycerol <4> (Reversibility: ? <4> [5]) [5]
P GDP + dioleoylglycerol 3-phosphate
S GTP + sn-1,2-dihexanoylglycerol <2> (Reversibility: ? <2> [37]) [37]
P GDP + sn-1,2-dihexanoylglycerol 3-phosphate
S ITP + sn-1,2-dihexanoylglycerol <2> (Reversibility: ? <2> [37]) [37]
P IDP + sn-1,2-dihexanoylglycerol 3-phosphate
S Additional information <2, 7> (<2>, sn-1,3-dioleoylglycerol is not a substrate [6]; <2>, no activity with ficaprenol [12]; <7> enzyme form I and II show a preference for diacylglycerol substrates with saturated acyl chains of 10-12 carbon atoms [16]; <7>, diacylglycerol emulsion [18]; <7>, the enzyme is active in mixed micelles containing octyl glucoside and dioleoylglycerol [19]) [6, 12, 16, 18, 19]
P ?

Inhibitors

ADP <4, 7> (<7>, 0.5 mM, 77% inhibition of DGK I and 66% inhibition of DGK IV [14]) [5, 14]
AMP <7> (<7>, 0.5 mM, 18% inhibition of DGK I and 14% inhibition of DGK IV [14]) [14]

ATP <4> [5]
ATPγS <4, 7> (<7>, 0.5 mM, 93% inhibition of DGK I and 71% inhibition of DGK IV [14]) [5, 14]
CDP <4, 7> (<7>, 0.5 mM, 43% inhibition of DGK I and 28% inhibition of DGK IV [14]) [5, 14]
CTP <4, 7> (<7>, 0.5 mM, 42% inhibition of DGK I and 9% inhibition of DGK IV [14]) [5, 14]
Ca^{2+} <2, 4> [2, 4]
Co^{2+} <4> [4]
GDP <4, 7> (<7>, 0.5 mM, 74% inhibition of DGK I and 72% inhibition of DGK IV [14]) [5, 14]
GDPβS <4> [5]
GTP <1, 4, 7> (<7>, 0.5 mM, 74% inhibition of DGK I and 56% inhibition of DGK IV [14]) [1, 5, 18]
GTPγS <7> (<7>, 0.5 mM, 37% inhibition of DGK I and 32% inhibition of DGK IV [14]) [14]
IDP <4> [5]
ITP <4> [5]
Nonidet <1> (<1> 0.5%, 45% inhibition [1]) [1]
R59022 <3, 5, 8> (<3> inhibits the 80000 Da enzyme form but not the 150000 Da enzyme form [3]; <5>, inhibits DGK-II and to a lesser extent DGK-III, but little affects DGK-I [15]) [3, 15, 17, 24]
R59949 <9> (<9>, DGKα [27]) [27]
Triton N-101 <1, 5, 8> (<1> 0.5%, 45% inhibition [1]; <5>, enzyme form DGK-I, DGK-II and DGK-III [15]) [1, 15, 17]
Triton X-100 <3, 4> (<3>, extremely inhibitory to the 80000 Da enzyme and the 150000 Da enzyme form [3]; <4>, 0.5 mM, 50% inhibition [4]) [3, 4]
UDP <4> [5]
UTP <4> [5]
adenosine <7> (<7>, 0.5 mM, 5% inhibition of DGK I and 28% inhibition of DGK IV [14]) [14]
adenosine 5'-tetraphosphoryl-3-O-(1,2-dihexanoyl)-sn-glycerol <2> [37]
cAMP <7> (<7>, 0.5 mM, 20% inhibition of DGK I and 15% inhibition of DGK IV [14]) [14]
ceramide <7> [9]
deoxycholate <1> (<1>, 0.5%, 30% inhibition [1]) [1]
deoxycholate <4> (<4> 1 mM, about 50% inhibition [4]) [4]
diacylglycerol <2> (<2>, above 3.4 mol%, substrate inhibition [2]) [2]
dioctanoylglycerol <7> (<7>, above 3.4 mol% [19]) [19]
dioleoylglycerol <7> (<7>, above 3.4 mol% [19]) [19]
ethanol <1> (<1>, 0.5%, 20% inhibition [1]) [1]
octyl glucoside <5> (<5>, enzyme form DGK-I, DGK-II and DGK-III [15]) [15]
phosphate <4> [5]
phosphatidate <3> (<3>, moderate [20]) [20]

phosphatidylcholine <5> (<5>, enzyme form DGK-I, DGK-II and DGK-III [15]) [15]
phosphatidylglycerol <3> (<3>, inhibits phosphatidylcholine-dependent kinase activity [20]) [20]
phosphatidylinositol <3> (<3>, 2.5 mol% results in 50% inhibition of the phosphatidylcholine-dependent kinase activity [20]) [20]
quercetin <7> (<7>, 0.1 mM, 41% inhibition of DGK I and 15% inhibition of DGK IV [14]) [14]
sphingosine <3> (<3>, inhibits the 150000 Da enzyme [3]) [3]

Activating compounds

1,3-dioleoylglycerol <2> (<2>, activates [6]) [6]
1-O-alkylphosphatidylcholine <2> (<2>, half-maximal activation at 21.9 mol% [6]) [6]
1-monooleoylglycerol <2> (<2> activates [11]) [11]
1-palmitoyl-2-oleoylglycerophosphocholine <2> (<2> activates [11]) [11]
Span-20 <2> (<2>, activates [12]) [12]
Triton X-100 <2> (<2>, activates [2]) [2]
bis-phosphatidic acid <2> (<2>, half-maximal activation at 3.9 mol% [6]) [6]
cardiolipin <2, 7> (<2>, good activator [11]; <2> activates [12]; <7> half-maximal activation by 1 mol% [19]; <2>, mitochondrial, half-maximal activation at 2.3 mol% [6]) [6, 11, 12, 19]
cholesterol 3-sulfate <2> (<2>, activates [2]) [2]
deoxycholate <3, 4, 5> (<4>, stimulation [5]; <4> purified enzyme is completely devoid of activity without addition of phospholipid or deoxycholate [4]; <5>, the enzyme shows optimal activity in presence of phosphatidylserine or deoxycholate. Lower activity in presence of phosphatidylcholine. Diacylglycerol analogs containing an unsaturated fatty acid at the sn-2 position give optimal enzyme activity irrespective of the presence of deoxycholate [7]; <5>, enhances activity of enzyme form DGK-II and DGK-III, enzyme form DGK-I is not much affected [15]; <3>, enhances activity [20]) [4, 5, 7, 15, 20]
detergent <7> (<7>, no activity in absence of detergent [14]) [14]
di-O-hexadecylphosphatidylcholine <2> (<2>, half-maximal activation at 13.5 mol% [6]) [6]
diacylglycerol 3-phosphate <2> (<2>, the enzyme apoprotein is attributed to a novel feedback activation involving diacylglycerol 3-phosphate [10]) [10]
dilauroyl-N,N-dimethylglycerophosphoethanolamine <2> (<2> activates [11]) [11]
dilauroyl-N-methylglycerophosphoethanolamine <2> (<2> activates [11]) [11]
dilauroylglycerophosphocholine <2> (<2> activates [11]) [11]
dilauroylglycerophosphoethanolamine <2> (<2> activates [11]) [11]
dilauroylphosphatidylcholine <2> (<2>, half-maximal activation at 11.9 mol% [6]) [6]
dimethylmyristamide <2> (<2>, activates [6]) [6]
dioleoyl ethylene glycol <2> (<2>, activates [6]) [6]

dioleoylphosphatidylcholine <2> (<2>, half-maximal activation at 10.4 mol% [6]) [6]
dioleoylphosphatidylglycerol <2> (<2>, half-maximal activation at 6.3 mol% [6]) [6]
dipalmitoylphosphatidic acid <2> (<2>, activates only in presence of Triton X-100 [11]) [11]
hexadecanol <2> (<2>, activates [6]) [6]
hexadecyl phosphorylcholine <2> (<2>, half-maximal activation at 17.3 mol% [6]) [6]
hexadecylphosphorylcholine <2> (<2>, activates [2]) [2]
lauryl maltoside <2> (<2>, activates in presence of 11 mM Triton X-100 [10]) [10]
lipid <2> (<2>, purified enzyme is completely inactive unless a lipid is added to the assay buffer containing Triton X-100 [12]) [12]
lysophosphatidylcholine <3> (<3>, activation of phospholipids in the order of decreasing efficiency: phosphatidylcholine, lysophosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, sphingomyelin [20]) [20]
lysophosphatidylethanolamine <2> (<2>, activates [11]) [11]
methyl myristate <2> (<2>, activates [6]) [6]
myristoylcholine chloride <2> (<2>, activates [6]) [6]
myristyl acetate <2> (<2>, activates [6]) [6]
n-hexyl β-D-glucoside <2> (<2>, activates in presence of 11 mM Triton X-100 [10]) [10]
nitrododecane <2> (<2>, activates [6]) [6]
octyl acetate <2> (<2>, activates [6]) [6]
octyl β-glucoside <2> (<2>, activates in presence of 11 mM Triton X-100 [10]) [10]
oleic acid <2> (<2>, activates only in presence of Triton X-100 [11]) [11]
oleoylcholine chloride <2> (<2>, activates [6]) [6]
palmitic acid <2> (<2>, activates only in presence of Triton X-100 [11]) [11]
phosphatidic acid <2, 7> (<7>, good activator of DGK IV, no effect on DGK I activity [14]; <2>, activates only in presence of Triton X-100 [11]) [11, 14]
phosphatidyl glycerol <2> (<2>, good activator [11]) [11]
phosphatidylcholine <2, 3, 5, 7> (<7>, moderate enhancement of DGK IV, no effect on DGK I activity [14]; <5>, the enzyme shows optimal activity in presence of phosphatidylserine or deoxycholate. Lower activity in presence of phosphatidylcholine [7]; <2>, activates [11]; <7>, enzyme type II has a prefrence for phosphatidylcholine as cofactor, enzyme type I can utilize both phosphatidylserine and phosphatidylinositol, but has a lower preference for phosphatidylcholine [16]; <3>, activation of phospholipids in the order of decreasing efficiency: phosphatidylcholine, lysophosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, sphingomyelin [20]) [7, 11, 14, 16, 20]
phosphatidylcholine plasmalogen <2> (<2>, half-maximal activation at 7.3 mol% [6]) [6]
phosphatidylethanolamine <2, 3> (<2>, activates only in presence of Triton X-100 [11]; <2>, plus cardiolipin, activates [12]; <3>, activation of phospho-

lipids in the order of decreasing efficiency: phosphatidylcholine, lysophosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, sphingomyelin [20]) [11, 12, 20]
phosphatidylglycerol <4> (<4>, effective stimulation [5]) [5]
phosphatidylinositol <4, 7> (<4>, effective stimulation [5]; <7>, enhances activity of DGK I and DGK IV [14]; <7>, enzyme type II has a preference for phosphatidylcholine as cofactor, enzyme type I can utilize both phosphatidylserine and phosphatidylinositol, but has a lower preference for phosphatidylcholine [16]) [5, 14, 16]
phosphatidylserine <2, 3, 5, 7> (<2, 3>, activates [3, 6]; <5>, the enzyme shows optimal activity in presence of phosphatidylserine or deoxycholate. Lower activity in presence of phosphatidylcholine [7]; <2>, good activator [11]; <7>, enhances activity of DGK I and DGK IV [14]; <7>, enzyme type II has a prefrence for phosphatidylcholine as cofactor, enzyme type I can utilize both phosphatidylserine and phosphatidylinositol, but has a lower preference for phosphatidylcholine [16]; <3>, activation of phospholipids in the order of decreasing efficiency: phosphatidylcholine, lysophosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, sphingomyelin [20]) [3, 6, 7, 11, 14, 16, 20]
phospholipid <3, 7> (<7>, activation by phospholipid is not stereospecific and is mimicked partially by fatty acids [19]; <3>, enhances activity. Activation of phospholipids in the order of decreasing efficiency: phosphatidylcholine, lysophosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, sphingomyelin [20]; <4> purified enzyme is completely devoid of activity without addition of phospholipid or deoxycholate [4]; <4> a combination of diacylglycerol and phospholipid exclusively leads to full activation [29]) [4, 19, 20, 29]
platelet-activating factor <2> (<2>, half-maximal activation at 22.4 mol% [6]) [6]
sn-1,2-dioleoylglycerol <2> (<2>, activates [6]) [6]
sn-1,3-dioleoylglycerol <2> (<2>, activates [6]) [6]
sodium cholate <7> (<7>, enhances activity of DGK I and DGK IV [14]) [14]
sodium deoxycholate <7> (<7>, enhances activity of DGK I and DGK IV [14]) [14]
sodium dodecyl sulfate <2> (<2>, activates [2]) [2]
sodium hexadecyl sulfate <2> (<2>, half-maximal activation at 9.8 mol% [6]) [6]
sphingomyelin <3> (<3>, activation of phospholipids in the order of decreasing efficiency: phosphatidylcholine, lysophosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, sphingomyelin [20]) [20]
sphingosine <3> (<3>, potently activates the 80000 Da enzyme [3]) [3]
stearic acid <2> (<2>, activates only in presence of Triton X-100 [11]) [11]
stearoyllysophosphatidylcholine <2> (<2>, half-maximal activation at 15.8 mol% [6]) [6]
sulfatidic acid <2> (<2>, half-maximal activation at 2.7 mol% [6]) [6]

Metals, ions

Ca^{2+} <1, 3, 5, 7> (<1>, absolute requirement for a divalent ion. Mg^{2+} is more effective than Mn^{2+} or Ca^{2+} [1]; <3>, 2 mol of Ca^{2+} per mol of enzyme. Ca^{2+} plus phosphatidylserine markedly activates by reducing the K_m value for ATP [3]; <7>, DGK I: good activator, DGK IV: activation with 1 mM $CaCl_2$ is comparable to that obtained on presence of $MgCl_2$ [14]; <3>, plus phosphatidylserine, activates enzyme DGK-Iα [28]; <7>, plus phosphatidylserine, activates enzyme DGK-Iβ [28]; <5>, plus phosphatidylserine, activates enzyme DGK-Iγ [28]; <3,5,7>, enzyme can bind approximately 2 mol of Ca^{2+} per mol [35]; <3>, enzyme requires Ca^{2+}, dissociation constant is 0.0099 mM, hydrophobic region of the enzyme is masked by addition of Ca^{2+} [35]; <5>, activity is independent of Ca^{2+}, dissociation constant is 0.00044 mM, hydrophobic region of the enzyme is masked by addition of Ca^{2+} [35]; <7>, activity is independent of Ca^{2+}, dissociation constant is 0.00089 mM, binding of Ca^{2+} results in exposure of hydrophobic amino acids [35]) [1, 3, 14, 28, 35]

Cd^{2+} <2, 7> (<2>, enzyme requires a free divalent metal cation: Mg^{2+}, Mn^{2+}, Co^{2+}, Cd^{2+} or Zn^{2+} [2]; <7>, when Mg^{2+} is excluded from the assay, Cd^{2+} supports activity to lesser extent [19]) [2, 19]

Co^{2+} <2, 7> (<2>, enzyme requires a free divalent metal cation: Mg^{2+}, Mn^{2+}, Co^{2+}, Cd^{2+} or Zn^{2+} [2]; <7>, when Mg^{2+} is excluded from the assay, Co^{2+} supports activity to lesser extent [19]) [2, 19]

Mg^{2+} <1, 2, 4, 5, 7, 8> (<1>, absolute requirement for a divalent ion. Mg^{2+} is more effective than Mn^{2+} or Ca^{2+}. 50% stimulation by 0.4 mM Mg^{2+}, maximal activity at 5-40 mM [1]; <2>, enzyme requires a free divalent metal cation: Mg^{2+}, Mn^{2+}, Co^{2+}, Cd^{2+} or Zn^{2+} [2]; <4>, divalent cation is required for activity. Mg^{2+} is most effective, maximal activation at about 10 mM [4]; <7>, the enzyme is dependent on [9]; <5>, required [13]; <7>, DGK I: maximal activation at 20 mM. DGK IV: maximal activation at 5 mM [14]; <5>, required, optimal activity at 2.5 mM for DGK-I, 5.0 mM for DGK-II and 10.0 mM for DGK-III [15]; <8>, required, maximal activity at 3 mM $MgCl_2$, half-maximal activity at around 1 mM [17]; <7>, required, optimal activity of microsomal enzyme at 2 mM, optimal activity of soluble enzyme at 10 mM [18]; <7>, a second Mg^{2+} in addition to MgATP is required for activity [19]) [1, 2, 4, 9, 13, 14, 15, 17, 18, 19, 20]

Mn^{2+} <1, 2, 7, 8> (<1>, absolute requirement for a divalent ion. Mg^{2+} is more effective than Mn^{2+} or Ca^{2+}. Mn^{2+} is more effective on the enzyme from microtubule than on the enzyme from supernatant [1]; <2>, enzyme requires a free divalent metal cation: Mg^{2+}, Mn^{2+}, Co^{2+}, Cd^{2+} or Zn^{2+}. Ka for Mg^{2+} is 3.4 mM [2]; <4>, slight activation at 5 mM [4]; <7>, DGK I: activation is not so great as with $MgCl_2$. Maximal stimulation at 0.1 mM. DGK IV: activation is 10fold less than with $MgCl_2$ [14]; <8>, Mg^{2+} requirement can be partially substituted by Mn^{2+} [17]; <7>, when Mg^{2+} is excluded from the assay, Mn^{2+} supports activity to lesser extent [19]) [1, 2, 4, 14, 17, 19]

Zn^{2+} <2, 7> (<2>, enzyme requires a free divalent metal cation: Mg^{2+}, Mn^{2+}, Co^{2+}, Cd^{2+} or Zn^{2+} [2]; <7>, when Mg^{2+} is excluded from the assay, Zn^{2+} supports activity to lesser extent [19]) [2, 19]

zinc <5> (<5>, the enzyme contains two zinc fingers [33]) [33]

Specific activity (U/mg)

0.231 <4> [4]
2.207 <5> (<5>, enzyme form DGK-II [15]) [15]
3.767 <2> [10]
4.74 <5> (<5>, enzyme form DGK-I [15]) [15]
5.187 <7> (<7>, enzyme form DGK IV [14]) [14]
6.753 <5> (<5>, enzyme form DGK-III [15]) [15]
6.913 <5> [7]
7.7 <3> (<3>, 1500000 Da enzyme [3]) [3]
8 <3> [20]
11.5 <2, 7> (<7>, enzyne form DGK I [14]) [14, 21]
15 <3> (<3>, 80000 Da enzyme [3]) [3]
Additional information <5> (<5>, the enzyme is assayed by using endogenous substrate [13]) [13]

K_m-Value (mM)

0.03 <1> (GTP, <1> pH 6.6, 30°C [1]) [1]
0.05 <5> (diolein, <5>, pH 7.4, 30°C, enzyme form DGK-I [15]) [15]
0.065 <5> (diolein, <5>, pH 7.4, 30°C, enzyme form DGK-II [15]) [15]
0.07 <7> (1-stearoyl-2-arachidonoyl-glycerol, <7>, pH 7.4, 30°C, enzyme form DGK I [14]) [14]
0.08 <5> (diolein, <5>, pH 7.4, 30°C, enzyme form DGK-III [15]) [15]
0.09 <7> (1,2-diarachidonoyl-glycerol, <7>, pH 7.4, 30°C, enzyme form DGK I [14]) [14]
0.09 <7> (1-stearoyl-2-arachidonoyl-glycerol, <7>, pH 7.4, 30°C, enzyme form DGK IV [14]) [14]
0.1 <4, 8> (ATP, <4> pH 7.5, 25°C [4]) [4, 17]
0.1 <7> (sn-1,2-dioleoylglycerol, <7>, pH 7.4, 30°C, enzyme form DGK IV [14]) [14]
0.102 <4> (ATP, <4>, pH 7.5, 25°C [5]) [5]
0.104 <4> (GTP, <4>, pH 7.5, 25°C [5]) [5]
0.105 <7> (ATP, <7>, pG 7.4, 30°C, enzyme type I [16]) [16]
0.12 <2> ($MgATP^{2-}$, <2> wild-type enzyme [30]) [30]
0.125 <7> (ATP, <7>, pH 7.4, 30°C, in presence of 1,2-dioleoylglycerol, enzyme form DGK I [14]) [14]
0.125 <7> (sn-1,2-dioleoylglycerol, <7>, pH 7.4, 30°C, enzyme form DGK I [14]) [14]
0.14 <7> (1,2-diarachidonoyl-glycerol, <7>, pH 7.4, 30°C, enzyme form DGK IV [14]) [14]
0.14 <2> ($MgATP^{2-}$, <2>, mutant enzyme E76L [30]) [30]
0.15 <5> (ATP, <5>, pH 7.4, 30°C, enzyme form DGK-I [15]) [15]
0.16 <1> (ATP, <1> pH 6.6, 30°C [1]) [1]
0.16 <7> (ATP, <7>, pH 7.0, 37°C, microsomal enzyme [18]) [18]
0.17 <7> (ATP, <7>, pH 7.0, 37°C, soluble enzyme [18]) [18]
0.23 <2> (ceramide, <2>, pH 6.6, 25°C [12]) [12]
0.245 <5> (ATP, <5>, pH 7.4, 30°C, enzyme form DGK-II [15]) [15]

0.25 <7> (ATP, <7>, pH 7.4, 30°C, in presence of 1,2-dioleoylglycerol, enzyme form DGK IV [14]) [14]
0.25 <4> (diacylglycerol, <4>, pH 7.5, 25°C [4]) [4]
0.3 <3> (ATP, <3>, pH 7.4, 30°C [20]) [20]
0.33 <2> ($MgATP^{2-}$, <2>, mutant enzyme E69C [30]) [30]
0.42 <7> (GTP, <7>, pH 7.0, 37°C, soluble enzyme [18]) [18]
0.44 <2> ($MgATP^{2-}$, <2>, mutant enzyme N72S [30]) [30]
0.45 <5> (1,2-diolein, <5>, pH 7.5, 30°C [7]) [7]
0.45 <5> (ATP, <5>, pH 7.4, 30°C, enzyme form DGK-III [15]) [15]
0.5 <5> (ATP, <5>, pH 7.5, 30°C, without deoxycholate [7]) [7]
0.506 <7> (ATP, <7>, pG 7.4, 30°C, enzyme type II [16]) [16]
0.63 <7> (GTP, <7>, pH 7.0, 37°C, microsomal enzyme [18]) [18]
0.9 <4> (dioleoylglycerol, <4>, pH 7.5, 25°C [5]) [5]
0.91 <2> ($MgATP^{2-}$, <2>, mutant enzyme A14Q [30]) [30]
1 <2> (ADP, <2> about, pH 6.8, 30°C, reaction with sn-2,3-dihexanoylglycerol [37]) [37]
1.4 <2> (ATP, <2>, pH 6.6, 25°C [12]) [12]
1.5 <2> ($MgATP^{2-}$, <2>, mutant enzyme K94V [30]) [30]
1.6 <5> (ATP, <5>, pH 7.5, 30°C, with 1 mM deoxycholate [7]) [7]
2.1 <2> ($MgATP^{2-}$, <2>, mutant enzyme D95N [30]) [30]
2.6 <2> (ATP, <2> pH 6.8, 30°C, reaction with sn-2,3-dihexanoylglycerol [37]) [37]
4.2 <2> (2'-deoxy-ATP, <2> pH 6.8, 30°C, reaction with sn-2,3-dihexanoylglycerol [37]) [37]
5.9 <2> (ITP, <2> pH 6.8, 30°C, reaction with sn-2,3-dihexanoylglycerol [37]) [37]
8.7 <2> (GTP, <2> pH 6.8, 30°C, reaction with sn-2,3-dihexanoylglycerol [37]) [37]
Additional information <2, 4, 7> (<2>, K_m for dioleoylglycerol is 0.92 mol% [2]; <4>, in presence of Triton X-100, used for purification, a biphasic dependency upon diacylglycerol is observed and the apparent Michaelis constant values for diacylglycerol decreases with decreasing Triton concentration [5]; <7> K_m-values are 0.92 mol% for dioleoylglycerol and 3.6 mol% for dioctanoylglycerol [19]) [2, 5, 18, 19, 30]

K_i-Value (mM)

0.038 <4> (IDP, <4>, pH 7.5, 25°C [5]) [5]
0.043 <4> (UDP, <4>, pH 7.5, 25°C [5]) [5]
0.058 <4> (GDP, <4>, pH 7.5, 25°C [5]) [5]
0.06 <4> (CDP, <4>, pH 7.5, 25°C [5]) [5]
0.081 <4> (GTP, <4>, pH 7.5, 25°C [5]) [5]
0.095 <4> (ADP, <4>, pH 7.5, 25°C [5]) [5]
0.102 <4> (ATP, <4>, pH 7.5, 25°C [5]) [5]
0.123 <4> (GDPβS, <4>, pH 7.5, 25°C [5]) [5]
0.26 <4> (UTP, <4>, pH 7.5, 25°C [5]) [5]
0.48 <4> (CTP, <4>, pH 7.5, 25°C [5]) [5]

0.51 <4> (ITP, <4>, pH 7.5, 25°C [5]) [5]
2.5 <4> (phosphate, <4>, pH 7.5, 25°C [5]) [5]
Additional information <2> (<2> K_i-value for adenosine 5'-tetraphosphoryl-3-O-(1,2-dihexanoyl)-sn-glycerol is 0.036 mol% [37]) [37]

pH-Optimum

6.1 <1> (<1>, enzyme from supernantant fraction [1]) [1]
6.3-8.3 <2> [12]
6.4 <1> (<1>, enzyme from microtubule [1]) [1]
7 <4, 5, 7, 8> (<5>, and a second optimum at pH 8 [13]; <8>, broad around, enzyme in plasma membrane vesicles from shoots [17]) [4, 13, 17, 18]
7.4 <3, 7> (<7> DGK I and DGK IV [14]; <3>, phosphatidylcholine-dependent activity [20]) [14, 20]
8 <3, 5> (<5>, and a second optimum at pH 7 [13]; <3>, broad, deoxycholate-dependent or phosphatidylcholine-dependent activity [20]) [13, 20]

pH-Range

4.5-7.5 <1> (<1>, pH 4.5: about 50% of maximal activity, pH 7.5: about 45% of maximal activity, enzyme from supernatant. pH 4.5: about 55% of maximal activity, pH 7.5: about 45% of maximal activity, enzyme from microtubuli [1]) [1]
6-8.5 <8> (<8>, pH 6.0: about 40% of maximal activity, pH 8.5: about 50% of maximal activity [17]) [17]
6-9 <5> (<5>, about 50% of maximal activity at pH 6.0 and 9.0 [13]) [13]
6.5-7 <4> (<4>, rapid decrease of activity below pH 6.5, pH 7: optimum [4]) [4]
6.5-8.5 <7> (<7>, pH 6.5: about 45% of maximal activity, pH 8.5: about 45% of maximal activity [14]) [14]
6.5-9 <3> (<3> pH 6.5: about 35% of maximal activity, pH 9.0: about 65% of maximal activity, phosphatidylcholine-dependent activity [20]) [20]
6.8-9 <7> (<7>, pH 6.5: about about 85% of maximal activity pH 9.0: about 50% of maximal activity, enzyme form DGK I [14]) [14]

Temperature optimum (°C)

35 <5> [13]

4 Enzyme Structure

Molecular weight

15400 <2> (<2>, gel filtration [12]) [12]
68000 <4> (<4>, less than, sucrose density gradient centrifugation [4]) [4]
75000 <1, 5> (<1> microtubular enzyme, sucrose density gradient centrifugation [1]; <5>, enzyme form DGK-II, gel filtration [15]) [1, 15]
76000 <3> (<3>, gel filtration [20]) [20]
86000 <5> (<5>, gel filtration [7]) [7]
95000 <7> (<7>, gel filtration [16]) [16]
250000 <4> (<4>, gel filtration [4]) [4]

Additional information <1> (<1>, the molecular weight of the peak fraction of enzyme from supernatant is 125000 Da and the average molecular weight is 160000 Da, suggesting that more than one species might be present [1]) [1]

Subunits

? <2, 3, 5, 7, 10> (<2> x * 13245, calculation from nucleotide sequence [21]; <2>, x * 14000 Da, SDS-PAGE [10]; <2>, x * 14300, SDS-PAGE [21]; <5>, x * 58000, enzyme form DGK-III, SDS-PAGE [15]; <5>, x * 63900, DGK-IIIε, calculation from amino acid sequence [28]; <7>, x * 82200, DGK-Iα, calculation from amino acid sequence [28]; <3>, x * 82600, DGK-Iα, calculation from amino acid sequence [28]; <5>, x * 82700, DGK-Iα, calculation from amino acid sequence [28]; <7>, x * 88500, DGK-Iγ, calculation from amino acid sequence [28]; <5>, x * 89000, DGK-Iγ, calculation from amino acid sequence [28]; <7>, x * 90300, DGK-Iβ, calculation from amino acid sequence [28]; <5>, x * 101400, DGK-Vθ, calculation from amino acid sequence [28]; <5>, x * 103900, calculation from nucleotide sequence [33]; <5,7>, x * 104000, DGK-IVξ, calculation from amino acid sequence [28]; <5>, x * 108000, enzyme from Hela cells, SDS-PAGE [26]; <5>, x * 110000, enzyme from cell lines MDA-MB-453 and MCF-7, SDS-PAGE [26]; <7>, 1 * 110000, enzyme from cell line PC12, SDS-PAGE [26]; <10>, x * 126800, DGK-IIη, calculation from amino acid sequence [28]; <5>, x * 128000 DGKη1, calculation from nucleotide sequence [39]; <5>, x * 130000, DGK-IIδ, calculation from amino acid sequence [28]; <5>, x * 135000, DGKη2, calculation from nucleotide sequence [39]; <5>, x * 152000, enzyme form DGK-I, SDS-PAGE [15]) [10, 15, 21, 26, 28, 33, 39]

monomer <2, 3, 4, 5> (<2>, 1 * 15000, SDS-PAGE [12]; <4>, 1 * 51000, SDS-PAGE [4]; <3>, 1 * 78000, SDS-PAGE [20]; <5>, 1 * 86000, SDS-PAGE [7]) [4, 7, 12, 20]

Posttranslational modification

phosphoprotein <3> (<3>, the 80000 Da enzyme form is phosphorylated by an unidentified protein kinase [3]) [3]

5 Isolation/Preparation/Mutation/Application

Source/tissue

3T3 cell <7> [16]

HL-60 cell <5> (<5>, level of DGKγ is rapidly and markedly decreased upon cellular differentiation into macrophages [23]) [23]

HeLa cell <5> [26]

Hep-G2 cell <5> (<5>, DGK-IIδ [28]) [28]

IIC9 cell <10> (<10>, a subclone of embryo fibroblasts [34]) [34]

MCF$^-$7 cell <5> (<5> mammary carcinoma [26]) [26]

MDA-MB-453 cell <5> (<5> mammary carcinoma [26]) [26]

PC-12 cell <7> [26]

T-cell <9> (<9>, DGKα [27]) [27]

T-lymphocyte <3, 5, 7> (<7>, DGK-I [25]; <3>, DGK-Iα [28]) [7, 25, 28]

U937 cell <5> (<5>, level of DGKγ is rapidly and markedly decreased upon cellular differentiation into macrophages [23]) [23]
adrenal gland <7> (<7>, DGK-Iβ [28]) [28]
brain <3, 5, 7, 9, 10> (<9>, DGKα [27]; <7>, microvessel [22]; <5>, DGK-Iα [28]; <7>, oligodendrocyte, DGK-Iα [28]; <7>, neuron, DGK-Iβ [28]; <7>, Purkinje cells, DGK-Iγ [28]; <10>, DGK-IIη [28]; <7>, DGK-IVξ [28]; <5>, DGK-Vθ, cerebellar cortex and hippocampus [28]; <5>, DGKξ mRNA is expressed at high level [33]; <5>, the enzyme is highly expressed in hippocampus, caudate nucleus, occipital pole, cerebral cortex and cerebellum [36]; <5>, DGKη1 [39]) [3, 9, 14, 16, 20, 22, 25, 27, 28, 33, 36, 39]
caudate nucleus <5> [36]
caudate putamen <7> (<7>, neuron, DGK-II [25]) [25]
central nervous system <5> (<5>, DGKβ [32]) [32]
cerebellar Purkinje cell <7> (<7>, DGK-III [25]; <7>, DGK-Iγ [28]) [25, 28]
cerebellum <5> [36]
cerebral cortex <5, 6> [8, 36]
colon <5> (<5>, DGKη1 and DGKη2 [39]) [39]
embryo <1> (<1> muscle [1]) [1]
endothelium <5> [33]
erythrocyte <5> [13]
eye <6> [8]
fibrillar flight muscle <6> [8]
fibroblast <7> (<7> ras-transformed [16]) [16]
granule cell <7> (<7>, DGK-III [25]) [25]
granulocyte <5> (<5>, enzyme expression remains almost unchanged in granulocytic differentiation pathway [23]) [23]
heart <7> (<7> low activity [14]) [14]
hippocampus <5> [36]
kidney <5, 7> (<7> low activity [14]; <5>, DGKη1 and DGKη2 [39]) [14, 39]
leukocyte <5> [7]
liver <5, 7> (<7>, low activity [14]; <5>, DGKη1 [39]) [14, 18, 39]
lung <7, 10> (<10>, DGK-IIη [28]) [14, 28]
lymphocyte <5> [24, 30]
lymphocyte <9> [38]
lymphoid tissue <3> [3]
muscle <1, 6> (<1>, embryonic [1]) [1, 8]
muscle <6> (<6> tubular muscle [8]) [8]
nervous system <6> [8]
neuron <7> (<7>, of accumbens nucleus, caudate-putamen and olfactory tubercle, DGK-II [25]; <7>, of the brain, DGK-Iβ [28]) [25, 28]
neutrophil <5> [24]
nucleus accumbens <7> (<7>, DGK-II [25]) [25]
occipital pole <5> [36]
olfactory tubercle <7> (<7>, DGK-II [25]) [25]

oligodendrocyte <7> (<7>, DGK-I [25]; <7>, of the brain, DGK-Iα [28]) [25, 28]
ovary <5> (<5>, DGKη1 [39]) [39]
pancreas <5> (<5>, DGKη1 [39]) [39]
peripheral blood <5> (<5>, DGKη1 [39]) [24, 30]
platelet <5> (<5>, isoenzyme DGK-I, DGK-II and DGK-II [15]) [15]
prostate <5> (<5>, DGKη1 [39]) [39]
retina <5> (<5>, DGK-Iγ [28]) [28, 36]
root <8> (<8>, dark-grown [17]) [17]
shoot <8> (<8>, dark-grown [17]) [17]
skeletal muscle <5, 7> (<5>, DGK-IIδ [28];< 7>, DGK-IVξ [28]) [28]
small intestine <5> (<5>, DGKη1 [39]) [39]
smooth muscle <10> [31]
spleen <5, 7> (<7>, DGK-I [25]; <5>, DGKη1 [39]) [14, 25, 39]
suspension culture <4> [4]
testis <5, 10> (<5>, DGK-IIδ and DGK-IIIε [28]; <10>, DGK-IIη [28]; <5>, DGKη1 and DGKη2 [39]) [28, 39]
thymocyte <9> (<9>, DGKα [27]) [27]
thymus <5, 7> (<7>, DGK-I [25]; <5>, DGK-IVξ [28]; <5>, DGKη1 [39]) [14, 25, 28, 39]
uterus <5> (<5>, DGKβ [32]; <7> low activity [14]) [14, 32]
vascular system <7> [22]
Additional information <5> (<5>, DGKη1 is ubiquitously distributed in various tissues, DGKη2 is detected only in testis, kidney and colon [39]) [39]

Localization

cytoplasm <5> (<5> prior to cell attachment, phorbol ester induce translocation of DGKγ from the cytoplasm to the cell periphery [23]; <5>, one variant of DGKβ is associated with the plasma membrane, the other isoform is predominantly localized within the cytoplasm [32]; <5>, DGKι [36]; <5>, DGKη1 and DGKη2 are rapidly translocated from the cytoplasm to endosomes in response to stress stimuli. DGKη1 is rapidly relocated back to the cytoplasm upon removal of stress stimuli. DGKη2 exhibits sustained endosomal association [39]) [23, 32, 36, 39]
cytoplasmic vesicle <7> (<7> DGK-II [25]) [25]
cytosol <3, 5, 7> (<4>, no activity in cytosol [5]; <7>, DGK I [14]; <5>, isoenzyme DGK-I, DGK-II and DGK-II [15]; <5>, in lymphocytes the basal activity is 1.6fold higher in the membrane fraction than in cytosol. In neutrophils the basal activity is identical in cytosol and mambrane-fraction [24]) [3, 12, 15, 16, 18, 24]
endosome <5> (<5>, DGKη1 and DGKη2 are rapidly translocated from the cytoplasm to endosomes in response to stress stimuli. DGKη1 is rapidly relocated back to the cytoplasm upon removal of stress stimuli. DGKη2 exhibits sustained endosomal association. Oligomerization of DGKη2 mediated by its SAM domain is largely responsible for its sustained endosomal localization [39]) [39]

extracellular <5> (<5> prior to cell attachment, phorbol ester induce translocation of DGKγ from the cytoplasm to the cell periphery [23]) [23]
membrane <2, 4, 5, 7> (<2,4,7> bound to [4,7,14]; <4>, intrinsic membrane-protein [5]; <7>, DGK IV [14]; <2>, tightly associated with the inner membrane [2]; <7>, integral membrane protein [19]; <2>, bound to [21]; <5>, in lymphocytes the basal activity is 1.6fold higher in the membrane fraction than in cytosol. In neutrophils the basal activity is identical in cytosol and membrane-fraction [24]) [2, 4, 5, 7, 11, 12, 13, 14, 19, 21, 24, 29, 30, 37]
microsome <7> [18]
microtubule <1> [1]
nucleus <5, 7, 10> (<5,7>, DGK-θ is localized in the speckle domain of the nucleus [26]; <10>, agonist-induced activity of nuclear DGKθ activity, nuclear localization of DGKδ [34]; <5> DGKι [36]) [26, 34, 36]
particle-bound <7> (<7>, activity of DGK-I is recovered dominantly in the soluble fraction, that for DGK-II in the particulate fraction and that for DGK-III in soluble and particulate fraction [25]) [25]
plasma membrane <5, 8, 9> (<8>, active site of the enzyme is localized to the inner cytoplasmic surface [17]; <5>, one variant of DGKβ is associated with the plasma membrane, the other isoform is predominantly localized within the cytoplasm [32]; <9>, activation and relocalization to plasma membrane of DGKα is a direct consequence of PI3K activation [38]) [17, 32, 38]
soluble <1, 7> (<7>, activity of DGK-I is recovered dominantly in the soluble fraction, that for DGK-II in the particulate fraction and that for DGK-III in soluble and particulate fraction [25]) [1, 18, 25]

Purification

<2> (apoprotein [11]) [10, 11, 12, 21, 37]
<3> (a heat-labile 80000 Da enzyme and a heat-stable 150000 Da enzyme [3]) [3, 20]
<4> [4, 29]
<5> (isoenzyme DGK-I and DGK-II, DGK-II only partially purified [15]) [7, 15, 36]
<7> (DGK I and DGK IV [14]) [14]

Cloning

<2> (a 100fold overproduction is obtained when dgkA is placed on a hybrid plasmid under control of the lambdapl promoter [21]) [21]
<3> (cDNA subcloned into the EcoRI site of the simian virus 40-based expression vector pSRE [35]) [35]
<5> (subcloning into the expression vector pMT-2 and transfection in COS-7 cells results in a 6-7fold increase in diacylglycerol kinase activity [7]; the DGKβ gene can generate several enzyme isoforms which can display different expression levels and subcellular localization but similar enzymatic activities in vitro [32]; COS-7 cells transfected with DGKξ cDNA express a 117000 Da and a 114000 Da protein. The transfected cells also express increased diacylglycerol kinase activity, which is not altered in the presence of R59949 [33]; cDNA subcloned into the EcoRI site of the simian virus 40-based expression vector pSRE [35]; COS-7 transfection [36]) [7, 32, 33, 35, 36]

<6> [8]
<7> (high kinase activity is shown in COS cells transfected with either one of the three cDNAs for DGK-I, DGK-II or DGK-III [25]; cDNA subcloned into the EcoRI site of the simian virus 40-based expression vector pSRE [35]) [25, 35]
<10> (insect cells infected with recombinant baculovirus containing the cDNA [31]) [31]

Engineering

A14Q <2> (<2>, significantly impaired catalytic function, without evidence of gross structural alterations, subunit mixing experiments of mutant enzymes, subunit mixing experiments of mutant enzymes [30]) [30]
D95N <2> (<2>, significantly impaired catalytic function, without evidence of gross structural alterations. K_m-value for $MgATP^{2-}$ raises 18fold, subunit mixing experiments of mutant enzymes [30]) [30]
E69C <2> (<2>, mutant enzyme has an altered structure even in SDS [30]) [30]
E76L <2> (<2>, significantly impaired catalytic function, without evidence of gross structural alterations, subunit mixing experiments of mutant enzymes [30]) [30]
K94L <2> (<2>, significantly impaired catalytic function, without evidence of gross structural alterations. K_m-value for $MgATP^{2-}$ raises 13fold, subunit mixing experiments of mutant enzymes [30]) [30]
N72S <2> (<2>, significantly impaired catalytic function, without evidence of gross structural alterations, subunit mixing experiments of mutant enzymes [30]) [30]

6 Stability

Temperature stability

42 <5> (<5>, 5 min, complete loss of activity of DGK-II and DGK-III, about 10% loss of activity of enzyme form DGK-I [15]) [15]
43-45 <1> (<1>, preincubation of microtubules, 10 min, 50% loss of activity [1]) [1]
57 <2> (<2>, Triton-solubilized preparation, $t_{1/2}$: 12 min [2]) [2]
100 <2> (<2>, enzyme activity of membranes, $t_{1/2}$: 20 min [2]; <2>, the membrane preparation shows a threefold stimulation by heating for 5 min followed by a gradual loss of activity, half-life: about 1 h. Butane-1-ol-dissolved enzyme has a half-life of about 45 min [10]) [2, 10]
Additional information <2> (<2>, addition of phospholipids provides some protection from thermal inactivation [2]) [2]

General stability information

<1>, enzyme activity is destroyed by trypsin, no protection by substrate [1]
<2>, lipid activators stabilize the enzyme against inactivation induced by diacylglycerol. Mg^{2+} and Mn^{2+} show only a small stabilization effect both in presence and in absence of 10 mol% phosphatidylglycerol [6]

<2>, when cosolubilized with diacylglycerol in octylglucoside micelles, the enzyme undergoes rapid irreversible inactivation [2]
<4>, enzyme is unstable in membrane extract, upon storage overnight at 4°C 90% of the activity is lost [4]
<7>, microsomal activity is more unstable on storage at 0°C than the soluble enzyme [18]

Storage stability

<3>, 4°C, enzyme concentrated by polyethyleneglycol 6000, stable for 1 week [20]
<3>, 4°C, purified enzyme, 50% loss of activity after 3 days [20]
<3>, the 150000 Da enzyme is very unstable but can be stored at -80°C in presence of bovine serum albumin [3]
<7>, -20°C, in presence of 1 mM dithiothreitol, microsomal and soluble enzyme stable for about 1 week [18]
<10>, 4°C, -20°C or -70°C, more than 50% of the activity is lost after overnight storage in presence of 10 or 20% glycerol [31]

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Dolichol kinase 2.7.1.108

1 Nomenclature

EC number
2.7.1.108

Systematic name
CTP:dolichol O-phosphotransferase

Recommended name
dolichol kinase

Synonyms
DK <4, 7> [12]
dolichol phosphokinase

CAS registry number
71768-07-5

2 Source Organism

<1> *Ascaris suum* [8]
<2> *Bos taurus* (calf [1,6,7]; bovine [3]) [1, 3, 6, 7]
<3> *Glycine max* (soybean [9]) [9]
<4> *Homo sapiens* (clone KIAA1094 from a human brain library [12]) [12]
<5> *Onchocerca volvulus* [8]
<6> *Rattus norvegicus* (rat [2,4]; Wistar [2]; Sprague-Dawley [4,6,10]; cultured C-6 glial cells [6]) [2, 4, 6, 10]
<7> *Saccharomyces cerevisiae* (yeast, SEC59 gene [12]) [12]
<8> *Secale cereale* (rye [11]) [11]
<9> *Tetrahymena pyriformis* [5]

3 Reaction and Specificity

Catalyzed reaction
CTP + dolichol = CDP + dolichyl phosphate

Reaction type
phospho group transfer

Natural substrates and products

- **S** CTP + dolichol <2-4, 6, 7, 9> (<6> enzyme can function in augmenting the steady-state levels of dolichyl phosphate by phosphorylating preexisting dolichol molecules [4]; <2> newly formed dolichyl monophosphate, phosphorylated via CTP is available for biosynthesis of mannosylphosphoryldolichol and N-acetylglucosaminylpyrophosphoryldolichol [1]; <4,7> CTP mediated phosphorylation of dolichol, the terminal step in dolichyl monophospate biosynthesis de novo [12]) (Reversibility: ? <2-4, 6, 7, 9> [1-7, 9, 10, 12]) [1-7, 9, 10, 12]
- **P** CDP + dolichyl phosphate <2-4, 6, 7, 9> [1-7, 9, 10, 12]

Substrates and products

- **S** CTP + dolichol <2-4, 6, 7, 9> (<6> pig liver dolichol as substrate, (R,S)-dolichol-11, dolichol-16, dolichol-19 [4]) (Reversibility: ? <2-4, 6, 7, 9> [1-7, 9, 10, 12]) [1-7, 9, 10, 12]
- **P** CDP + dolichyl phosphate <2, 3, 6, 9> [1-7, 9, 10]
- **S** CTP + ficaprenol <6> (Reversibility: ? <6> [4]) [4]
- **P** CDP + ficaprenyl phosphate
- **S** CTP + polyprenol <6> (<6> polyprenol-16, α-trans-polyprenol-16, polyprenol-19 [4]) (Reversibility: ? <6> [4]) [4]
- **P** CDP + polyprenyl phosphate
- **S** dCTP + dolichol <2> (Reversibility: ? <2> [7]) [7]
- **P** dCDP + dolichyl phosphate <2> [7]
- **S** Additional information <2, 3, 6, 9> (<6> ATP cannot replace CTP [2]; <6> microsomal enzyme shows a marked preference for saturation of the α-isoprene, dolichol-16 and dolichol-19 are 2.5fold more active than the corresponding polyprenols, the enzyme is twice as active against naturally occurring polyprenol-16 (α-cis-isoprene) compared to synthetic α-trans-polyprenol-16, all-trans-2,3-dihydrosolanesol and solanesol are not substrates [4]; <6> requires CTP as phosphoryl donor [10]; <2> ATP and GTP are not effective substrates compared to CTP [1]; <9> ATP, UTP and GTP are ineffective [5]; <3> CTP or other phosphate group donors like ATP, GTP or UTP are not required for maximal activity [9]) [1, 2, 4, 5, 9, 10]
- **P** ?

Inhibitors

ATP <6> [10]
CDP <1-3, 5, 6> (<2> end product inhibition [1]) [1, 2, 7-9]
CTP <3> [9]
Ca^{2+} <2> (<2> above 6 mM [3]) [3]
Co^{2+} <2> (<2> above 6 mM [3]) [3]
EDTA <2, 6> [2, 7]
GTP <6> [10]
Mn^{2+} <2> (<2> above 6 mM [3]) [3]
Triton X-100 <6> (<6> above 0.5% w/v, activation below [2]) [2]
Zn^{2+} <2> [3]
chlorpromazine <9> [5]

dCDP <2> [7]
trifluoperazine <9> [5]
Additional information <2, 6> (<6> product of the reaction, dolichyl phosphate, is noninhibitory, no inhibition by dithiothreitol [2]; <2> not inhibited by CMP, ADP, GDP or UDP [7]) [2, 7]
Additional information <2, 6> (<6> trifluoperazine and chlorpromazine are no inhibitors [6]; <2> Mg^{2+} is not inhibitory [3]) [3, 6]

Activating compounds

2-mercaptoethanol <2> (<2> slight stimulation [1]) [1]
R-dolichol (C95) <2> (<2> markedly stimulated by addition, K_m 0.009 mM [7]) [7]
S-dolichol (C95) <2> (<2> markedly stimulated by addition, K_m 0.009 mM [7]) [7]
Triton X-100 <1-3, 5, 6, 8, 9> (<8> required for optimum activity [11]) [2, 3, 5, 8-11]
dimyristoylphosphatidylcholine <2> (<2> stimulatory in 1:1 ratio with deoxycholate [3]) [3]
dithiothreitol <6> (<6> slight stimulation of activity [2]) [2]
monothioglycerol <2> (<2> slight stimulation [1]) [1]
phosphatidylcholine <2> (<2> stimulated by after dialysis [7]) [7]
phosphatidylethanolamine <2> (<2> stimulated by after dialysis [7]) [7]
Additional information <2> (<2> sodium dodecylsulfate is ineffective in stimulating enzymic activity [3]) [3]

Metals, ions

Ca^{2+} <1, 2, 5, 6, 9> (<1,5> less effective [8]; <6> activated almost equally well by Ca^{2+}, Zn^{2+} or Mg^{2+} [10]; <9> required for maxiumum activity [5]) [1-3, 5-8, 10]
Cd^{2+} <6> (<6> slight activation [6]) [6]
Co^{2+} <2, 6> (<2> divalent cation required for activity, Mg^{2+}, Ca^{2+} and Co^{2+} are equally effective [3]) [3, 6]
Fe^{2+} <6> (<6> slight activation [6]) [6]
Mg^{2+} <1-3, 5, 6, 9> (<1,2,5> enzyme is dependent on divalent cations [3,8]; <6> activated almost equally well by Ca^{2+}, Zn^{2+} or Mg^{2+} [10]; <9> required for maxiumum activity [5]; <3> low level of stimulation noted, 80% of maximal activity is observed in absence of divalent k_{cat}ions) [1-3, 5, 6, 8-10]
Mn^{2+} <1, 2, 5, 6, 9> (<1,5> less effective [8]; <9> activated by [5]) [1, 2, 5, 8]
Ni^{2+} <6> (<6> slight activation [6]) [6]
Sr^{2+} <6> (<6> slight activation [6]) [6]
Zn^{2+} <2, 6> (<6> most effective cation for activation of brain enzyme [6]; <2> markedly stimulated by [7]) [6, 7, 10]
Additional information <2, 6> (<6> skeletal muscle kinase is not activated by Mn^{2+} [10]; <2> Zn^{2+} is not effective [3]; <6> not activated by Cu^{2+} [6]) [3, 6, 10]

Specific activity (U/mg)

0.15 <6> (<6> relative specific activity in the soluble fraction [4]) [4]
0.21 <6> (<6> relative specific activity in the mitochondrial lysosomal fraction [4]) [4]
0.52 <6> (<6> relative specific activity in the nuclear fraction [4]) [4]
3.8 <6> (<6> relative specific activity in the microsomal fraction, rough and smooth endoplasmic reticulum are found to have higher specific activities than the Golgi fraction [4]) [4]

K_m-Value (mM)

0.0065 <2> (CTP, <2> pH 7.0, 37°C [1]) [1]
0.0077 <2> (CTP, <2> pH 7.2, 37°C [7]) [7]
0.0091 <2> (dCTP, <2> pH 7.2, 37°C [7]) [7]
0.023 <6> (dolichol, <6> pH 7.4, 37°C [10]) [10]
0.05 <6> (dolichol, <6> pH 7.4, 37°C [2]) [2]
0.055 <3> (dolichol, <3> pH 7.4, 37°C [9]) [9]
0.07 <9> (dolichol, <9> pH 7.4, 37°C [5]) [5]
0.075 <6> (dolichol, <6> pH 7.4, 37°C, brain enzyme [6]) [6]
0.15 <2> (CTP, <2> pH 7.5, 35°C, in deoxycholate/dimyristoylphosphatidylcholine 1:1 [3]) [3]
0.55 <2> (CTP, <2> pH 7.5, 35°C, in Triton X-100/dimyristoylphosphatidylcholine 1:1 [3]) [3]
0.75 <5> (CTP, <5> pH 7.4, 37°C [8]) [8]
0.85 <1> (CTP, <1> pH 7.4, 37°C [8]) [8]
1.5 <6> (CTP, <6> pH 7.4, 37°C, skeletal muscle enzyme [10]) [10]
1.62 <6> (CTP, <6> pH 7.4, 37°C, brain enzyme [6]) [6]
3 <9> (CTP, <9> pH 7.4, 37°C [5]) [5]
4 <6> (CTP, <6> pH 7.4, 37°C [2]) [2]
Additional information <1, 5> (<1> K_m for dolichol 0.0075 mg/ml [8]; <5> K_m for dolichol 0.009 mg/ml [8]) [8]

K_i-Value (mM)

0.121 <2> (CDP, <2> pH 7.2, 37°C [7]) [7]

pH-Optimum

7 <2> [1]
7-7.5 <6> [2]
7-8 <3> (<3> second optimum at pH 9.5 [9]) [9]
7.4 <1, 5, 9> (<9> Tris/maleate buffer, second optimum at pH 9.0 [5]) [5, 8]
7.4-7.7 <6> [10]
7.5 <2> [7]
8 <8> (<8> shoulder at pH 6.5 [11]) [11]
8.5 <2> [3]
9 <9> (<9> glycine buffer, second optimum at pH 7.4 [5]) [5]
9.5 <3> (<3> second optimum at pH 7.0-8.0 [9]) [9]

pH-Range

4-10 <6> (<6> about half-maximal activity at pH 6.0 and 8.0 [2]) [2]
4-11 <3> (<3> no second optimum with Tris-HCl, activity increases fairly linearly between pH 8.0 and 10.0, activity decreases about 25% between pH 9.5 and 10.0 in CHAPS buffer [9]) [9]
6.6-9.6 <9> (<9> 2 optima, about half-maximal activity at pH 6.6, pH 8.5 and pH 9.6 [5]) [5]
6.6-10.2 <2> (<2> broad pH-activity profile with good activity observed from pH 7.0-10.0, about half-maximal activity at pH 6.6 and 10.2 [3]) [3]

Temperature optimum (°C)

30-37 <1, 5> [8]

4 Enzyme Structure

Molecular weight

59000-60000 <4> (<4> SDS-PAGE [12]) [12]
59270 <4> (<4> predicted from cDNA open reading frame [12]) [12]

5 Isolation/Preparation/Mutation/Application

Source/tissue

brain <2, 4, 6> [1, 6, 7, 10, 12]
liver <2, 6> [2-4, 10]
muscle <6> (<6> leg muscle [10]) [10]
seedling <3, 8> [9, 11]
skeletal muscle <6> [10]

Localization

cytoplasm <6> [4]
endoplasmic reticulum <6> [4]
lysosome <6> [4]
membrane <2-4, 6, 7> [1, 3, 4, 9, 10, 12]
microsome <1-8> [2-4, 6-12]
mitochondrion <6> [4]
nucleus <6> [4]
sarcoplasmic reticulum <6> [10]

Purification

<2> (partially [3]) [3]
<6> [4]

Cloning

<4> (human cDNA complements defect in dolichol kinase activity in Saccharomyces cerevisiae sec59-1 mutant, human SEC59 gene also expressed in Sf-9 cells [12]) [12]
<7> [12]

6 Stability

Storage stability

<2>, -17°C, 65% of original activity retains when stored for 6 weeks [7]
<2>, -17°C, stable for at least 1 month [1]
<2>, -20°C, stored frozen as pellet for months with little loss of activity [3]
<2>, 4°C, no detectable loss of activity when solubilized extract is kept for 24 h [7]
<9>, -20°C, stable for at least 15 days [5]

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[Hydroxymethylglutaryl-CoA reductase (NADPH)] kinase 2.7.1.109

1 Nomenclature

EC number
2.7.1.109 (Protein kinases are in a state of review by the NC-IUBMB.)

Systematic name
ATP:[hydroxymethylglutaryl-CoA reductase (NADPH)] phosphotransferase

Recommended name
[hydroxymethylglutaryl-CoA reductase (NADPH)] kinase

Synonyms
3-hydroxy-3-methylglutaryl coenzyme A reductase kinase
3-hydroxy-3-methylglutaryl-CoA reductase kinase
AMP-activated protein kinase
AMPK
[hydroxymethylglutaryl-CoA reductase ($NADPH_2$)] kinase
β-hydroxy-β-methylglutaryl-CoA reductase kinase
hydroxymethylglutaryl coenzyme A reductase kinase
hydroxymethylglutaryl coenzyme A reductase kinase (phosphorylating)
reductase kinase

CAS registry number
172522-01-9

2 Source Organism

<1> *Brassica oleracea* (cauliflower, var. botrytis [10,11]) [10, 11]
<2> *Homo sapiens* (human [14,17-19,24,36]; colorectal carcinoma RKO cells [17]; IDH4, IMr90 and WI38 fibroblasts [19]; HEK-293 cell culture [35]; HAEC cell culture [36]) [14, 17-19, 24, 30, 33, 35, 36]
<3> *Mus musculus* (mouse, H-2K cells [15]) [15, 38]
<4> *Rattus norvegicus* (Sprague-Dawley [4, 7, 16, 29, 34]; Wistar [9]) [1-10, 12-14, 16, 20-23, 25-29, 32, 34, 37]
<5> *Sus scrofa* (porcine [14]) [14]
<6> *Cricetulus griseus* (chinese hamster, cell line CHO-G4myc [31]) [31]

3 Reaction and Specificity

Catalyzed reaction

ATP + [hydroxymethylglutaryl-CoA reductase (NADPH)] = ADP + [hydroxymethylglutaryl-CoA reductase (NADPH)] phosphate (EC 1.1.1.34 hydroxymethylglutaryl-CoA reductase (NADPH) is inactivated by the phosphorylation of the enzyme protein. Histones can also act as acceptors.)

Reaction type

phospho group transfer

Natural substrates and products

S ATP + [hydroxymethylglutaryl-CoA reductase (NADPH)] <2, 4> (<1, 4> inactivates EC 1.1.1.34 by phosphorylation [1-9, 11, 12]; <4> bicyclic phosporylation system, enzyme is believed to be involved in protecting cells against ATP depletion due to environmental stress by inactivating several key biosynthetic enzymes [25]; <4> activated AMPK acts to down-regulate ATP-consuming pathways such as fatty acid synthesis by phosphorylating and inactivating acetyl-CoA carboxylase and protein synthesis by promoting the phosphorylationof eukaryotic elongation factor-2, in heart AMPK activation stimulates glycolysis by increasing glucose uptake [37]) (Reversibility: ? <2, 4> [1-12, 14, 25-27, 32, 37]) [1-12, 14, 25, 32, 37]

P ADP + [hydroxymethylglutaryl-CoA reductase (NADPH)] phosphate <2, 4> [1-12, 14, 25-27, 32, 37]

Substrates and products

S ATP + HGRSAMSGLHLVKRR <1> (<1> SAMS-containing peptide as substrate [11]) (Reversibility: ? <1> [11]) [11]

P ADP + ?

S ATP + HMGSAMSGLHLVKRR <1> (<1> SAMS-containing peptide as substrate [11]) (Reversibility: ? <1> [11]) [11]

P ADP + ?

S ATP + HMKSAMSGLHLVKRR <1> (<1> synthetic SAMS-containing peptide as substrate [11]) (Reversibility: ? <1> [11]) [11]

P ADP + ?

S ATP + HMRSAGSGLHLVKRR <1> (<1> SAMS-containing peptide as substrate [11]) (Reversibility: ? <1> [11]) [11]

P ADP + ?

S ATP + HMRSAMSGLHGGKRR <1> (<1> SAMS-containing peptide as substrate [11]) (Reversibility: ? <1> [11]) [11]

P ADP + ?

S ATP + HMRSAMSGLHGVKRR <1> (<1> SAMS-containing peptide as substrate [11]) (Reversibility: ? <1> [11]) [11]

P ADP + ?

S ATP + HMRSAMSGLHLGKRR <1> (<1> SAMS-containing peptide as substrate [11]) (Reversibility: ? <1> [11]) [11]

P ADP + ?

S ATP + HMRSAMSGLHLVKRR <1, 3> (<1> SAMS-containing peptide as substrate [11]) (Reversibility: ? <1,3> [11,15]) [11, 15]
P ADP + ?
S ATP + HMRSAMSGLHLVKRR <2> (<2> synthetic SAMS-containing peptide as substrate [35]) (Reversibility: ? <2> [35]) [35]
P ADP + ?
S ATP + HMRSAMTGLHLVKRR <1> (<1> SAMS-containing peptide as substrate [11]) (Reversibility: ? <1> [11]) [11]
P ADP + ?
S ATP + HMRSAMYGLHLVKRR <1> (<1> SAMS-containing peptide as substrate [11]) (Reversibility: ? <1> [11]) [11]
P ADP + ?
S ATP + MAP-2 <4> (<4> relative kinase activity for low-MW kinase 14%, high MW-kinase 566% [7]) (Reversibility: ? <4> [7]) [7]
P ADP + MAP-2 phosphate
S ATP + RNA-binding protein HUR <2> (<2> inhibits the protein by phosporylation [17,19]) (Reversibility: ? <2> [17,19]) [19]
P ADP + ?
S ATP + [hydroxymethylglutaryl-CoA reductase (NADPH)] <1, 2, 4> (<1> 2 isoforms, major form A and minor form B, both phosphorylates mammalian HMG-CoA reductase [11]) (Reversibility: ? <1,2,4> [1-12,14,20-27]) [1-12, 14, 20-27]
P ADP + [hydroxymethylglutaryl-CoA reductase (NADPH)] phosphate <1, 4> [1-12]
S ATP + [malonylCoAdecarboxylase] <4> (Reversibility: ? <4> [16]) [16]
P ADP + [malonylCoAdecarboxylase]phosphate
S ATP + [sn-glycerol-3-phosphate acyltransferase] <4> (Reversibility: ? <4> [16]) [16]
P ADP + [sn-glycerol-3-phosphate acyltransferase]phosphate
S ATP + [sn-glycerol-3-phosphate acyltransferase] <4> (Reversibility: ? <4> [29]) [29]
P ADP + [sn-glycerol-3-phosphate acyltransferase]phosphate
S ATP + acetyl-CoA carboxylase <2> (Reversibility: ? <2> [30,33]) [30, 33]
P ADP + [acetyl-CoA carboxylase] phosphate <2> [30, 33]
S ATP + acetyl-CoA carboxylase <2, 4> (<4> substrate Rattus norvegicus hepatic acetyl-CoA carboxylase, enzyme phosphorylates Ser-residues 79, 1200 and 1215 [10]; <2> AMPK plays an important role in regulating malonyl-CoA levels through the phosphorylation of acetyl-CoA carboxylase [18]) (Reversibility: ? <2,4> [10,14,16,18]) [10, 14, 16, 18]
P ADP + [acetyl-CoA carboxylase] phosphate <2, 4> [10, 14, 16, 18]
S ATP + adipose hormone-sensitive lipase <2, 4> (Reversibility: ? <2,4> [10,14]) [10, 14]
P ADP + [adipose hormone-sensitive lipase] phosphate
S ATP + adipose hormone-sensitive lipase <2> (Reversibility: ? <2> [30]) [30]
P ADP + adipose hormone-sensitive lipase phosphate

S ATP + α,β-tubulin <4> (<4> relative kinase activity high MW-kinase 15% [7]) (Reversibility: ? <4> [7]) [7]
P ADP + [α,β-tubulin] phosphate
S ATP + bovine serum albumin <4> (<4> fraction V [5]) (Reversibility: ? <4> [5]) [5]
P ADP + [bovine serum albumin] phosphate
S ATP + casein <4> (<4> relative kinase activity for low-MW kinase 8%, high MW-kinase 48% [7]) (Reversibility: ? <4> [7]) [7]
P ADP + casein phosphate
S ATP + glycogen synthase <4> (<4> relative kinase activity for low-MW kinase 7%, high MW-kinase 87% [7]) (Reversibility: ? <4> [7]) [7]
P ADP + [glycogen synthase] phosphate
S ATP + heavy meromyosin <4> (<4> relative kinase activity for low-MW kinase 2%, high MW-kinase 100% [7]) (Reversibility: ? <4> [7]) [7]
P ADP + [heavy meromyosin] phosphate
S ATP + histone 2A <4> [37]
P ?
S ATP + histone H1 (IIIS) <4> (<4> histones are better substrates for high-MW kinase than hydroxymethylglutaryl-CoA reductase, relative kinase activity for low-MW kinase 275%, high MW-kinase 103% [7]) (Reversibility: ? <4> [7]) [7]
P ADP + [histone H1 (IIIS)] phosphate
S ATP + histone II-S <4> (<4> relative kinase activity for low-MW kinase 38%, high MW-kinase 159% [7]) (Reversibility: ? <4> [7]) [7]
P ADP + [histone II-S] phosphate
S ATP + histone VIIIS <4> (<4> relative kinase activity for low-MW kinase 65%, high MW-kinase 141% [7]) (Reversibility: ? <4> [7]) [7]
P ADP + [histone VIIIS] phosphate
S ATP + myelin basic protein <4> (<4> moderate substrate for low-MW kinase, better than hydroxymethylglutaryl-CoA reductase for high-MW kinase, relative kinase activity for low-MW kinase 36%, high MW-kinase 238% [7]) (Reversibility: ? <4> [7]) [7]
P ADP + [myelin basic protein] phosphate
S ATP + myosin mixed light chains <4> (<4> relative kinase activity for low-MW kinase 4%, high MW-kinase 27% [7]) (Reversibility: ? <4> [7]) [7]
P ADP + [myosin mixed light chains] phosphate
S ATP + phosphorylase B <4> (<4> relative kinase activity high MW-kinase 12% [7]) (Reversibility: ? <4> [7]) [7]
P ADP + [phosphorylase B] phosphate
S ATP + phosvitin <4> (<4> relative kinase activity for low-MW kinase 2%, high MW-kinase 2% [7]) (Reversibility: ? <4> [7]) [7]
P ADP + phosvitin phosphate
S ATP + protamine <4> (<4> relative kinase activity for low-MW kinase 24%, high MW-kinase 38% [7]) (Reversibility: ? <4> [7]) [7]
P ADP + protamine phosphate

S ATP + rabbit muscle glycogen synthase <4> (<4> rabbit muscle glycogen synthase [10]) (Reversibility: ? <4> [10]) [10]
P ADP + [rabbit muscle glycogen synthase] phosphate
S ATP + synapsin 1 <4> (<4> as good substrate as hydroxymethylglutaryl-CoA reductase, relative kinase activity for low-MW kinase 151%, high MW-kinase 103% [7]) (Reversibility: ? <4> [7]) [7]
P ADP + [synapsin 1] phosphate
S CTP + [hydroxymethylglutaryl-CoA reductase (NADPH)] <4> (Reversibility: ? <4> [8]) [8]
P CDP + [hydroxymethylglutaryl-CoA reductase (NADPH)] phosphate
S GTP + [hydroxymethylglutaryl-CoA reductase (NADPH)] <4> (<4> phosphorylation at about 30% the rate of ATP [5]) (Reversibility: ? <4> [5,8]) [5, 8]
P GDP + [hydroxymethylglutaryl-CoA reductase (NADPH)] phosphate
S ITP + [hydroxymethylglutaryl-CoA reductase (NADPH)] <4> (<4> phosphorylation at about 10% the rate of ATP [5]) (Reversibility: ? <4> [5,8]) [5, 8]
P IDP + [hydroxymethylglutaryl-CoA reductase (NADPH)] phosphate
S UTP + [hydroxymethylglutaryl-CoA reductase (NADPH)] <4> (<4> phosphorylation at about 5% the rate of ATP [5]) (Reversibility: ? <4> [5,8]) [5, 8]
P UDP + [hydroxymethylglutaryl-CoA reductase (NADPH)] phosphate
S dATP + [hydroxymethylglutaryl-CoA reductase (NADPH)] <4> (<4> phosphorylation at about 90% the rate of ATP [5]) (Reversibility: ? <4> [5]) [5]
P dADP + [hydroxymethylglutaryl-CoA reductase (NADPH)] phosphate
S Additional information <2, 4> (<4> autophosphorylation in absence of substrate [4,7]; <4> incorporates 0.5 mol phosphate/mol MW 53000 enzyme substrate fragment, 2 mol phosphate/mol native enzyme substrate [7]; <4> protein kinase C and Ca^{2+}/calmodulin dependent reductase kinases are no substrates [8]; acetyl-CoA carboxylase kinase EC 2.7.1.128 and hydroxymethylglutaryl-CoA reductase kinase activity are catalyzed by the same enzyme [9]; <2> conditions that elevate the AMP:ATP ratio in cells, such as growth factor depletion, hypoglycemia, ischemia in heart muscle, exercise in skeletal muscle, as well as treatment with arsenite, azide, oxidative agents and the pharmacological agent AICAR, which mimics the effect of AMP can cause activation of AMPK [19]) [4, 7-9, 19]
P ?
S Additional information <2, 6> (<6> enzyme functions as a metabolic sensor that monitors cellular AMP and ATP levels [31]; <2> phosphorylates key target proteins that control flux through metabolic pathways of hepatic ketogenesis, cholesterol synthesis, adipocyte lipolysis and skeletal muscle fatty acid oxidation [30]; <4> regulates triacylglycerolsynthesis and fatty acid oxidation in liver and muscle reciprocally [29]) [29-31]
P ?

Inhibitors

2'-deoxy-ATP <4> [13]
5'-fluorosulfonylbenzoyladenosine <1, 4> [9, 11]
8-bromo-AMP <4> [34]
ADP <4> [9]
EGTA <4> [7]
adenine-9-β-D-arabinofuranoside <4> [34]
adenosine <4> [9]
adenosine-5'-tetraphospho-5'-adenosine <4> (i.e. Ap4A, inhibits in the presence of AMP [21]) [21]
ara-ATP <4> [13]
glycerol <4> (<4> 25% v/v, reversible inhibition [4]) [4]
hydroxymethylglutaryl-CoA <4> (<4> only with hydroxymethylglutaryl-CoA reductase as substrate [12]) [12]
inhibitor W-7 <4> (<4> specific Ca^{2+}/calmodulin-dependent kinase inhibitor [7]) [7]
iodotubercidin <4> [34]
mammalian protein phosphatase 2C <1> [11]
protein phosphatase <2> [24]
protein phosphatase C <4> [23]
trifluperazine <4> (<4> specific Ca^{2+}/calmodulin-dependent kinase inhibitor [7]) [7]
Additional information <4> (<4> no inhibition by adenosine-5'-pentaphospho-5'-adenosine [8]) [8]

Cofactors/prosthetic groups

2'-dAMP <4> (<4> activation, can replace AMP or ADP [8]) [8]
calmodulin <4> (<4> requirement, Ca^{2+}/calmodulin dependent kinase, no phosphorylation of substrate observed in absence [7]) [7]

Activating compounds

2'-AMP <4> [6]
3'-AMP <4> [6, 8]
5'-AMP <2-6> (<4> regulated by allosteric activation [25]) [6, 8-10, 13, 14, 16-19, 25-27, 31, 33, 34, 36-38]
5-amino-4-imidazolecarboxamide ribonucleoside <2, 4, 6> [31, 34, 36, 37]
5-amino-4-imidazolecarboxamide ribotide <2-4> [13, 15, 19]
ADP <4> (<4> requirement [5]) [5, 6, 8]
CDP <4> (<4> allosteric activator [5,8]) [5, 8]
CMP <4> [6, 8]
GMP <4> [6, 8]
IDP <4> [8]
IMP <4> [8]
UDP <4> (<4> allosteric activator [5,8]) [5, 8]
UMP <4> [6, 8]
adenosine <4> [8]
adiponectin <4> [37]

α,β-methylene-ADP <4> (<4> allosteric activator, can replace ADP, with 66% efficiency with bovine serum albumin as substrate [5]) [5]
cAMP <4> [8]
leptin <4> [37]
metformin <4> (<4> antidiabetic drug [37]) [37]
reductase kinase kinase <2, 4> (activation, i.e. EC 2.7.1.110, in the presence of $MgATP^{2-}$ [2, 20-24]) [2, 20-24]
ribose 5'-phosphate <4> [8]
rosiglitazone <4> (<4> antidiabetic drug [37]) [37]
Additional information <4> (<4> AMPK can also be activated by hyperosmotic stress [37]) [37]
Additional information <4> (<4> not activated by cAMP [5,9, 20, 23]) [5, 9, 20, 23]
Additional information <4> (activated by phosphorylation by upstream protein kinases AMPKK and CaMKIK [25]) [25]
Additional information <4> (no activation by cGMP, <4> [23]) [23]
Additional information <4> (no activation by cIMP, cCMP [23]) [23]

Metals, ions

Ca^{2+} <4> [7]
Mg^{2+} <2, 4> (<2,4> requirement, actual substrate: $MgATP^{2-}$ [2,20-24]) [2, 4, 8, 20-24]

Specific activity (U/mg)

0.0102 <4> [12]
0.069 <4> [9]
0.292 <4> [7]
3.281 <1> (<1> major form B [11]) [11]
13.1 <1> (<1> major form A [11]) [11]
Additional information <4> (<4> 3.130 nU/mg [5]) [5]

K_m-Value (mM)

0.00085 <4> (hydroxymethylglutaryl-CoA reductase, <4> pH 6.5, 30°C, low-MW kinase [7]) [7]
0.0029 <4> (histone H1, <4> pH 6.5, 30°C, low-MW kinase [7]) [7]
0.013 <1> (HMRSAMSGLHGVKRR, <1> pH 7.0, 30°C, kinase A [11]) [11]
0.028 <4> (ATP, <4> pH 6.5, 30°C, low-MW kinase [7]) [7]
0.034 <1> (HMRSAMTGLHGVKRR, <1> pH 7.0, 30°C, kinase A [11]) [11]
0.038 <1> (HMRSAMSGLHLGKRR, <1> pH 7.0, 30°C, kinase A [11]) [11]
0.0404 <1> (HGRSAMSGLHLVKRR, <1> pH 7.0, 30°C, kinase A [11]) [11]
0.042 <1> (HMRSAMSGLHLGKRR, <1> pH 7.0, 30°C, kinase B [11]) [11]
0.046 <4> (ATP, <4> in absence of AMP [13]) [13]
0.049 <1> (HMRSAMSGLHGGKRR, <1> pH 7.0, 30°C, kinase A [11]) [11]
0.0498 <1> (HMRSAMSGLHLVKRR, <1> pH 7.0, 30°C, kinase A [11]) [11]
0.065 <1> (HMRSAMTGLHGVKRR, <1> pH 7.0, 30°C, kinase B [11]) [11]
0.069 <1> (HMRSAGSGLHLVKRR, <1> pH 7.0, 30°C, kinase B [11]) [11]
0.07 <1> (HMRSAGSGLHLVKRR, <1> pH 7.0, 30°C, kinase A [11]) [11]

0.091 <1> (HMRSAMSGLHLVKRR, <1> pH 7.0, 30°C, kinase B [11]) [11]
0.096 <1> (HMRSAMSGLHGVKRR, <1> pH 7.0, 30°C, kinase B [11]) [11]
0.111 <1> (HMKSAMSGLHLVKRR, <1> pH 7.0, 30°C, kinase B [11]) [11]
0.117 <4> (ATP, <4> in presence of AMP [13]) [13]
0.118 <1> (HMHSAMSGLHLVKRR, <1> pH 7.0, 30°C, kinase A [11]) [11]
0.123 <4> (ATP, <4> in presence of AMP [13]) [13]
0.133 <1> (HMKSAMSGLHLVKRR, <1> pH 7.0, 30°C, kinase A [11]) [11]
0.14 <4> (ATP, <4> pH 7.5, 37°C [5]) [5]
0.295 <4> (ATP, <4> pH 7.5, 37°C [8]) [8]
0.315 <4> (ATP, <4> pH 7.5, 37°C, in presence of AMP [8]) [8]
0.428 <1> (HMHSAMSGLHLVKRR, <1> pH 7.0, 30°C, kinase B [11]) [11]
0.573 <1> (HMGSAMSGLHLVKRR, <1> pH 7.0, 30°C, kinase A [11]) [11]
2.316 <1> (HMGSAMSGLHLVKRR, <1> pH 7.0, 30°C, kinase B [11]) [11]

pH-Optimum

6.5 <4> (<4> low-MW kinase [7]) [7]
Additional information <4> (<4> pI: 5.6 [20]) [20]

pH-Range

5.5-7 <4> (<4> about half-maximal activity at pH 5.5 and 7.0, low-MW kinase [7]) [7]

Temperature optimum (°C)

30 <2, 4> (<2,4> assay at [21,24]) [21, 24]
37 <4> (<4> assay at [20,22,23]) [20, 22, 23]

4 Enzyme Structure

Molecular weight

30000 <4> (<4> β-subunit, predicted protein sequence [27]) [27]
45000 <1> (<1> major form B, gel filtration [11]) [11]
110000-125000 <4> (<4> low-MW kinase, gel filtration [7]) [7]
150000 <1> (<1> major form A, electrophoresis in non-denaturing gels [11]) [11]
160000 <1> (<1> major form A, PAGE [11]) [11]
190000 <4> [26]
200000 <1> (<1> major form A, gel filtration [11]) [11]
205000 <4> (<4> gel filtration [4]) [4]
380000 <4> (rat, gel filtration) [20]
560000-600000 <4> (<4> high-MW kinase, gel filtration [7]) [7]

Subunits

? <4> (<4> x * 56000, SDS-PAGE [5]; <1> x * 58000, catalytic subunit [11]) [5, 11]
? <4> (x * 58000, rat, SDS-PAGE) [20]
dimer <4> (<4> 2 * 105000, SDS-PAGE [4]) [4]

heterotrimer <2-4, 6> (<4> 1 * 63000 + 1 * 38000 + 1 * 35000, 3 subunits α, β and γ, SDS-PAGE [26]; <4> 1 * 63000 + 1 * 40000 + ?, α and β subunit, SDS-PAGE [37]; <4> 1 * 63000 * ? + 1 * 38000 + 1 * 36000 , α, β, γ, SDS-PAGE [27]) [26-34, 36-38]
Additional information <2-4, 6> (<2,4,6> heterotrimeric serine/threonine kinase consists of a catalytic α-subunit and 2 regulatory subunits β and γ, each subunit exists as multiple isoforms, α_1, α_2, β_1, β_2, γ_1, γ_2 and γ3, giving 12 different possible combinantions of holoenzyme with different tissue distribution and subcellular localization [31,33,37,38]) [31, 33, 37, 38]

5 Isolation/Preparation/Mutation/Application

Source/tissue

HAEC cell <2> [36]
HEK-293 cell <2> [35]
IDH4 cell <2> [19]
IMr90 cell <2> [19]
RKO cell <2> [17]
WI38 cell <2> [19]
adipose tissue <4> [16]
aorta <2> [36]
brain <4, 5> [7, 14]
endothelium <2> [36]
fibroblast <2> [19]
heart <2-5> [14, 18, 38]
hepatocyte <4> [13, 29]
inflorescence <1> [10, 11]
kidney <4, 5> [14]
liver <2, 4> [1-6, 8-10, 13, 14, 16, 20-24, 27, 29, 30, 32, 37, 38]
lung <4, 5> [14]
ovary <6> [31]
pancreas <2> [30]
skeletal muscle <2-4> [14-16, 30, 34, 38]

Localization

cytoplasm <2> [17]
cytosol <2, 4> (<4> predominant [23]) [2-8, 20-24]
microsome <2, 4> [1-4, 8, 9, 20, 22, 24]

Purification

<1> (2 isoformes, major form A and major form B [11]) [11]
<2> (partial) [24]
<4> (partial [2, 9, 20-22, 26, 27]; low-MW kinase [7]; catalytic subunit α [14]; wild-type and mutant enzymes, expressed in bacteria [37]) [2-5, 7-9, 13, 14, 16, 20-22, 26, 27, 32, 37]

Cloning

<2> (AMPK heterotrimer expressed in COS7 cells [33]) [33]
<2> (cDNA identified with porcine cDNA [14]) [14]
<3> (transgenic mouse constructed by injection of Rattus norvegicus α_2 subunit cDNA [38]) [38]
<4> (cDNA identified with porcine cDNA [14]; sequence analysis of cDNA clones encoding the subunits [27]) [14, 27]
<4> (transfection of CCL13 cells [37]; transfections of COS7 cells [28]) [28, 37]
<5> (cDNA encoding porcine AMPK α_1 isolated [14]) [14]

Engineering

D157A <4> (<4> site-directed mutagenesis [32]) [32]
R70Q <2> (<2> site-directed mutagenesis, marked increase in activity, largely AMP-independent [33]) [33]
S108A <2> (<2> site-directed mutagenesis, reduces enzyme activity by 60% [35]) [35]
S182A <2> (<2> site-directed mutagenesis, no effect on enzyme activity [17]) [17]
S485A <4> (<4> site-directed mutagenesis, non-phosphorylatable mutant [37]) [37]
S485D <4> (<4> site-directed mutagenesis [37]) [37]
T172A <4> (<4> site-directed mutagenesis [28,32]) [28, 32]
T172D <4> (<4> site-directed mutagenesis [32]) [32]
T172E <4> (<4> site-directed mutagenesis [37]) [37]
T258A <4> (<4> site-directed mutagenesis, non-phosphorylatable mutant [37]) [37]
T258D <4> (<4> site-directed mutagenesis [37]) [37]

Application

medicine <2, 3> (<2> decreasing the ischaemic-induced activation of AMPK my be a therapeutic approach to treating ischaemic heart disease, AMPK my be an important pharmacological target for improving cardiac efficiency following ischaemia [18]; <3> compounds that would cause activation of enzyme in skeletal muscle promise to be attractive agents for therapeutic intervention [15]) [15, 18]
medicine <2> (<2> defects or disuse of the AMPK signaling system would be predicted to result in many of the metabolic perturbations observed in type 2 diabetes mellitus [30]) [30]

6 Stability

Temperature stability

37 <4> (<4> 2 h, inactivation, $MgATP^{2-}$ in a low, not high salt buffer restores activity, not cAMP or in phosphate buffer [2]) [2]

General stability information

<4>, about 10% decrease of activity after each freeze-thawing [4]
<4>, highly labile enzyme [3, 7]
<4>, protease inhibitors stabilizes enzyme [7]
<4>, very stable at either 4°C or -20°C when in microsomes [4]

Storage stability

<1>, -70°C, 0.5 mg protein/ml, frozen in liquid nitrogen, stored with no loss of activity [11]
<4>, -196°C, stored in liquid nitrogen, stable for several months [5]
<4>, -20°C, can be stored in buffer containing 50% glycerol for up to a month [26]
<4>, -20°C, in 0.05 M Tris-HCl, pH 7.5, 0.05 M NaF, 0.005 M diphosphate, 1 mM EDTA, 1 mM EGTA and 1 mM DTT, 0.1 mM PMSF, soybean trypsin inhibitor, benzamidine, Brij-35, 50% w/v glycerol, stable for at least 2 months [9]
<4>, -80°C, in 0.05 M Tris-HCl buffer, pH 7.4, 0.05 M NaF, 0.003 M EDTA, 0.002 M EGTA, 0.005 M DTT, 0.5 mM PMSF, 10% v/v glycerol, remains stable for at least 3 months [4]
<4>, -80°C, partially purified preparation, stable to freezing [2]
<4>, 4°C, unstable when solubilized, loses 90% activity within 3 days [4]

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Dephospho-[reductase kinase] kinase 2.7.1.110

1 Nomenclature

EC number
2.7.1.110 (Protein kinases are in a state of review by the NC-IUBMB. This EC class will presumably be transferred to EC 2.7.11.3)

Systematic name
ATP:dephospho[[hydroxymethylglutaryl-CoA reductase (NADPH)] kinase] phosphotransferase

Recommended name
dephospho-[reductase kinase] kinase

Synonyms
AMP-activated protein kinase kinase
hydroxymethylglutaryl coenzyme A reductase kinase kinase
hydroxymethylglutaryl coenzyme A reductase kinase kinase (phosphorylating)
reductase kinase kinase

CAS registry number
72060-33-4

2 Source Organism

<1> *Rattus norvegicus* (Sprague-Dawley [4]; Wistar [5]) [1-5, 7-8, 9]
<2> *Homo sapiens* [6]

3 Reaction and Specificity

Catalyzed reaction
ATP + dephospho-[[hydroxymethylglutaryl-CoA reductase (NADPH)] kinase] = ADP + [[hydroxymethylglutaryl-CoA reductase (NADPH)] kinase] (phosphorylates and activates EC 2.7.1.109 [hydroxymethylglutaryl-CoA reductase (NADPH)]kinase that has been inactivated by EC 3.1.3.16 phosphoprotein phosphatase.)

Reaction type
phospho group transfer

Natural substrates and products

- **S** ATP + dephospho-[[hydroxymethylglutaryl-CoA reductase (NADPH)] kinase] <1, 2> (<1,2> phosphorylation activates EC 2.7.1.109 [1-8]; <1> involved in regulation cascade of hydroxymethylglutaryl-CoA reductase, EC 1.1.1.34 [2]; <1> important for the responses of cells to metabolic stresses such as lack of cell nutrients, hypoxia, ischemia and muscular exercise [9]; <1> bicyclic phosporylation system, enzyme is believed to be involved in protecting cells against ATP depletion due to environmental stress by inactivating several key biosynthetic enzymes [7]) [1-9]
- **P** ADP + [[hydroxymethylglutaryl-CoA reductase (NADPH)] kinase] <1> [1-3, 9]

Substrates and products

- **S** ATP + casein <1> [2]
- **P** ?
- **S** ATP + dephospho-[[hydroxymethylglutaryl-CoA reductase (NADPH)] kinase] <1, 2> (<1> i.e. AMP-activated protein kinase [3]; <1> phosphorylates catalytic subunit of EC 2.7.1.109 [3]; <1> No substrate is hydroxymethylglutaryl-CoA reductase (NADPH) [2]) [1-9]
- **P** ADP + [[hydroxymethylglutaryl-CoA reductase (NADPH)] kinase] <1> [3, 9]
- **S** ATP + histone 2A <1> (poor substrate) [2]
- **P** ?
- **S** ATP + phosvitin <1> (slight activity) [2]
- **P** ?

Inhibitors

adenosine(5')tetraphospho(5')adenosine <1> (i.e. Ap4A, inhibits in the presence of AMP) [3]
poly(Arg) <1> (casein as substrate) [2]
Additional information <1> (no inhibition by inhibitor of cAMP-dependent protein kinase) [5]

Cofactors/prosthetic groups

AMP <1> (<1> allosteric activator, the allosteric effect and the promotion of phosphorylation and activation by the kinase kinase are due to binding of AMP to a single site on the kinase [3]) [3]

Activating compounds

5'-AMP <1> [8]
8-aza-9-deaza-AMP <1> (activation, i.e. formycin A-5'-monophosphate, can replace AMP) [3]
8-aza-9-deaza-IMP <1> (slight activation, i.e. formycin B-5'-monophosphate) [3]
mevalonolactone <1> (activation, in vitro and in vivo) [4]
Additional information <1> (cAMP-independent enzyme, <1> [2,5]; no activation by formycin A or B, <1> [3]) [2, 3, 5]

Metals, ions
Mg^{2+} <1, 2> (requirement, actual substrate: $MgATP^{2-}$) [2-6]

Specific activity (U/mg)
101 <1> [8]

K_m-Value (mM)
0.2 <1> (ATP, <1> pH 7.4, 37°C [1]) [1]

K_i-Value (mM)
3-4 <1> (sodium fluoride, <1> pH 7.4, 37°C [1]) [1]

pH-Optimum
7 <1> (<1> assay at [2,4]) [2, 4]
7.4 <1, 2> (<1,2> assay at [5,6]) [5, 6]

Temperature optimum (°C)
30 <1> (assay at [2]) [3]
37 <1, 2> (assay at [2]) [2, 4, 6]

4 Enzyme Structure

Molecular weight
196000 <1> [8]
380000 <1> (<1> gel filtration [2]) [2]

Subunits
? <1> (<1> x * 58000 + ?, catalytic α subunit, SDS-PAGE [8]) [8]

5 Isolation/Preparation/Mutation/Application

Source/tissue
liver <1, 2> [1-8]

Localization
cytosol <1> (<1> predominant [4,5]) [2-5]
microsome <1, 2> [1, 2, 6]
Additional information <1> (subcellular distribution) [5]

Purification
<1> (partial [2-5,8]; <1> cytosolic enzyme [2]) [2-5]
<2> (partial) [6]

Cloning
<1> (bacterially expressed recombinant α_1 subunit proteins) [9]

Engineering
T172D <1> (<1> site-directed mutagenesis [9]) [9]

6 Stability

Storage stability

<1>, -20°C, can be stored in buffer containing 50% glycerol for up to a month [8]

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Acetyl-CoA carboxylase] kinase 2.7.1.111

1 Nomenclature

EC number
2.7.1.111 (transferred to EC 2.7.1.128)

Recommended name
acetyl-CoA carboxylase] kinase

Protein-tyrosine kinase 2.7.1.112

1 Nomenclature

EC number

2.7.1.112 (Protein kinases are in a state of review by the NC-IUBMB. This EC class will presumably be split up into EC 2.7.10.1 and EC 2.1.10.2. For detailed data see EC 2.7.1.112.ETK, EC 2.7.1.112.TK, EC 2.7.1.112DUAL)

Systematic name

ATP:protein-tyrosine O-phosphotransferase

Recommended name

protein-tyrosine kinase

Synonyms

70 kDa ζ-associated protein
ATK
AXL oncogene
Abl tyrosine kinase <1> (present in 95% of patients with chronic myelogenous leukemia, CML [39]) [39]
adhesion-related kinase
agammaglobulinaemia tyrosine kinase
amylovoran biosynthesis membrane-associated protein amsA
anaplastic lymphoma kinase
B cell progenitor kinase
B lymphocyte kinase
B-cell/myeloid kinase
BCR-ABL tyrosine kinase
BMK
BPK
bone marrow kinase BMX
brain-specific kinase
breast tumor kinase
breathless protein
Bruton's tyrosine kinase
C-FES
C-SRC
C-SRC kinase
C-YES
C-mer
C-ret
CADTK
CAK β

CD115 antigen
CD117 antigen
CD135 antigen
CD136 antigen
CD140a antigen
CD140b antigen
CD167a antigen
CD202b antigen
CD220 antigen
CD221 antigen
CD246 antigen
CDW136
CEK4
CER
cadherin 96Ca
calcium-dependent tyrosine kinase
cell adhesion kinase
Cell adhesion kinase β
D-ash
DFGF-R1
DFer
DRT
derailed protein
discoidin receptor tyrosine kinase
doughnut protein
dror
Drosophila relative of ERBB
Dsrc28C
EBK
EGF receptor tyrosine kinase
EGFr TK
ELK
EPH-and ELK-related kinase
EPS I polysaccharide export protein epsB
ERK
ETK
Egfr
egg laying defective protein 15
embryonic brain kinase
endothelial kinase receptor EK1
epidermal growth factor receptor-related protein
epithelial and endothelial tyrosine kinase
epithelial cell kinase
ErbB2 kinase
Fms proto-oncogene
GCTK
GP145-TrkB

GP145-TrkB/GP95-TrkB
GP145-TrkC
Gurken receptor
HEK
HEK3
HEK4
HEK6
HGF receptor
HGF-SF receptor
HYK
Heartless protein
Hematopoietic consensus tyrosine-lacking kinase
Hemopoietic cell kinase
Heparin-binding growth factor receptor
IL-2-inducible T-cell kinase
ILP receptor
IR-related receptor
IRK
IRR
Itk (tec kinase)
JAK protein tyrosine kinase
keratinocyte growth factor receptor
kinase EMB
kinase EMT
kinase NYK
kinase TLK
kinase VIK
kinase insert domain receptor
L-JAK
LSK
Lck Tyrosine kinase
leukocyte janus kinase
Linotte protein
MEK4
MFR
MIR
MLN 19
MSP receptor
Met proto-oncogene tyrosine kinase
Met-related kinase
NET
NEU proto-oncogene
NRTK
NTK38
NUK
neurospecific receptor tyrosine kinase
nuclear tyrosine protein kinase RAK

ORF6
P140 TEK
P55-FGR
P56-LCK
P57-STK
P59-FYN
P60-SRC
P61-YES
PDGF-R-α
PDGF-R-β
PDGFR kinase
PP125FAK
PTK
PTK-RL-18
PTK70
Pagliaccio
protein kinase (tyrosine-phosphorylating)
protein kinase BATK
protein kinase HYL
protein kinase Lck
protein kinase NTK
protein kinase p56-LCK
protein kinase p56lck
protein p56c-lck kinase
protein p56lck tyrosine kinase
protein tyrosine kinase
protein tyrosine kinase lck
protein tyrosine kinase p56lck
protein tyrosine kinase pp56lck
protein-tyrosine kinase C-TKL
protein-tyrosine kinase CYL
protein-tyrosine kinase byk
protein-tyrosine kinase receptor MPK-11
proto-oncogene tyrosine-protein kinase Kit
Quek1
RTK
receptor protein-tyrosine kinase HEK11
receptor protein-tyrosine kinase HEK5
receptor protein-tyrosine kinase HEK7
receptor protein-tyrosine kinase HEK8
receptor protein-tyrosine kinase TKT
receptor tyrosine kinase MerTK
receptor-activated Janus kinase
related adhesion focal tyrosine kinase
resting lymphocyte kinase
Rlk/Txk (tec kinase)
SCFR

SLK
SRC-related intestinal kinase
SYN
slow nerve growth factor receptor
spleen tyrosine kinase
stem cell-derived tyrosine kinase
Syk-related tyrosine kinase
T cell-specific protein-tyrosine kinase
T-cell-specific kinase
TRK1 transforming tyrosine kinase protein
Torpedo protein
TrkB tyrosine kinase
TrkC tyrosine kinase
Tunica interna endothelial cell kinase
tyrosine kinase
tyrosine kinase ARG
tyrosine kinase CEK6 receptor
tyrosine kinase DDR
tyrosine kinase lck
tyrosine kinase p56lck
tyrosine kinase receptor HD-14
tyrosine kinase-type cell surface receptor HER2
tyrosine kinase-type cell surface receptor HER3
tyrosine kinase-type cell surface receptor HER4
tyrosine phosphokinase
tyrosine protein kinase
tyrosine protein kinase p56lck
tyrosine-protein kinase CAK
tyrosine-protein kinase CEK9
tyrosine-protein kinase CTK
tyrosine-protein kinase DTK
tyrosine-protein kinase FLT3
tyrosine-protein kinase FRT
tyrosine-protein kinase Lyk
tyrosine-protein kinase RSE
tyrosine-protein kinase SKY
tyrosine-protein kinase TYRO 10
tyrosine-protein kinase brk
tyrosine-protein kinase receptor CEK10
tyrosine-protein kinase receptor CEK11
tyrosine-protein kinase receptor CEK5
tyrosine-protein kinase receptor CEK7
tyrosine-protein kinase receptor CEK8
tyrosine-protein kinase receptor CEPHA7
tyrosine-protein kinase receptor ECK
tyrosine-protein kinase receptor EEK
tyrosine-protein kinase receptor EPH

tyrosine-protein kinase receptor ESK
tyrosine-protein kinase receptor ETK1
tyrosine-protein kinase receptor FLT
tyrosine-protein kinase receptor FLT3
tyrosine-protein kinase receptor FLT4
tyrosine-protein kinase receptor HTK
tyrosine-protein kinase receptor PAG
tyrosine-protein kinase receptor QEK5
tyrosine-protein kinase receptor REK4
tyrosine-protein kinase receptor SEK
tyrosine-protein kinase receptor TCK
tyrosine-protein kinase receptor TEK
tyrosine-protein kinase receptor XEK
tyrosine-protein kinase receptor XELK
tyrosine-protein kinase receptor ZEK1
tyrosine-protein kinase receptor ZEK2
tyrosine-protein kinase receptor ZEK3
tyrosine-specific protein kinase
tyrosylprotein kinase
Vascular permeability factor receptor
WEE1hu
YES related kinase
c-ABL
c-FER
c-Kit tyrosine kinase
c-erbB3
c-fgr
c-fms
c-kit
c-met
ectoprotein kinase
erbB tyrosine kinase
gene lck protein kinase
gene lck tyrosine kinase
hydroxyaryl-protein kinase
insulin receptor protein-tyrosine kinase
kinase, protein (phosphorylating tyrosine)
kinase, protein p56lck (phosphorylating)
mROR1
mROR2
nonreceptor protein tyrosine kinase
p140-TrkA
p150
p180erbB4
p185-Ron
p185erbB2
p55-BLK

p56-HCK
p56-HCK/p59-HCK
p56lck kinase
p56lck protein kinase
p56lck protein tyrosine kinase
p56lck tyrosine kinase
p59-HCK/p60-HCK
p60-YRK
p94-FER
phosphotyrosyl-protein kinase
src-kinase
v-fps Protein-tyrosine kinase
Additional information <1, 2> (this group of enzymes is under review by NC-IUBMB, recommendation for a nomenclature system based on acceptor amino acid specificity rather than on protein substrate. In accordance with this system protein-tyrosine kinases would belong to EC 2.7.11.X, <1,2> [1]; The present data set is restricted to a literature review and does not contain a complete description of kinases. Classification system based on kinase domain phylogeny revealing families of enzymes with related substrate specificities, <1,2> [3]) [1, 3]

CAS registry number
114051-78-4 (p56lck protein kinase)
80449-02-1 (protein-tyrosine kinase)

2 Source Organism

<1> *eukaryota* (overview [1]; e.g. plants, fungi, protozoa, vertebrates [3, 12]; ubiquitous [11]; Schizosaccharomyces pombe [11]; pig [30]) [1, 3, 5, 11, 12, 30, 39, 40-47]
<2> *prokaryote* (overview [1]) [1, 3, 5]
<3> *cellular organism* [2, 4, 6-10, 13-29, 31-38]

3 Reaction and Specificity

Catalyzed reaction
ATP + protein tyrosine = ADP + protein tyrosine phosphate (<1> regulation of mitosis, differentiation, migration, neovascularization, and apoptosis [40]; <1> PTKs are closely related with cell groth, proliferation, differentiation and signalling of the immune system)

Reaction type
phospho group transfer

Natural substrates and products

S ATP + protein tyrosine <3> (regulation of cellular function, <3> [2]; signalling, <3> [2, 22, 26]; role in development of cancer, diabetes, <3> [2]; role in cytokine signalling, <3> [19]) [2, 19, 22, 26]
P ADP + protein tyrosine phosphate

Substrates and products

S ATP + protein tyrosine <3, 1, 2> (some kinases are able to phosphorylate both serine/threonine and tyrosine, <1> [1]; specificity, <3> [16]; structure-function relationship, <3> [17]; in vitro specificity, <3> [23]; <1> overview of the best studied protein targets of src kinases, functions [42]) [1-3, 14, 16, 17, 19, 22-24, 26, 42]
P ADP + protein tyrosine phosphate

Inhibitors

5'-(*p*-fluorosulfonylbenzoyl)-2'-methylanthranoyl adenosine <1> (<1> irreversible inhibitor of EGFr tyrosine kinase [46]) [46]
5'-(*p*-fluorosulfonylbenzoyl)-3'-methylanthranoyl adenosine <1> (<1> irreversible inhibitor of EGFr tyrosine kinase [46]) [46]
5'-*p*-fluorosulfonylbenzoyl adenosine <1> (<1> irreversible inhibitor of EGFr tyrosine kinase [46]) [46]
AG 1517 <1> (<1> potently inhibits ligand-induced autophosphorylation og EGF-R, downstream signal transduction events, DNA replication and cell cycle progression at micromolar concentrations [45]) [45]
CI-1033 <1> (<1> irreversible inhibitor [43]; <1> PD 183805 [46]) [43, 46]
CL-387,785 <1> (<1> irreversible inhibitor of EGFr tyrosine kinase [46]) [46]
CP-358,778 <1> (<1> reversible inhibitors of EGFr TK [46]) [46]
CPG 59326 <1> (<1> reversible inhibitors of EGFr TK [46]) [46]
EKB-569 <1> (<1> irreversible inhibitor [43]) [43]
GW 2016 <1> [43]
Iressa <1> [43]
PD 158780 <1> (<1> reversible inhibitors of EGFr TK [46]) [46]
PD 160678 <1> (<1> irreversible inhibitor of EGFr tyrosine kinase [46]) [46]
PD 168393 <1> (<1> irreversible inhibitor of EGFr tyrosine kinase [46]) [46]
PD 183805 <1> (<1> irreversible inhibitor of EGFr tyrosine kinase, CI^-1033 [46]) [46]
PKI 166 <1> (<1>, a pyrrolo[2,3-d]pyrimidine derivative, a dual inhibitor of both the EGFR and the ErbB2 kinases, in vivo antitumor activity [39]) [39]
STI 571 <1> (<1>, a phenylaminopyrimidine derivative, a potent inhibitor of the Abl tyrosine kinase, PDGFR kinases and c-Kit tyrosine kinases [39]; <1> designed to inhibit ABL and BCR-ABL tyrosine kinases, inhibition through competitive ATP-binding pocket interactions [40]; <1> use is second-line therapy of small-cell lung cancer, inhibits c-kit kinase, resulting in an metabolic change of tumor cells [40]) [39, 40]
Tarceva <1> [43]
ZD 1839 <1> (<1> reversible inhibitors of EGFr TK [46]) [46]
autoinhibition <3> (discussion) [6]
erbstatin <3, 1, 2> [5, 10]

genistein <3, 1, 2> [5, 8]
herbimycin <3, 1, 2> [5, 9]
imidazolequinazolines <1> (<1> reversible inhibitors of EGFr TK, bind to a narrow hydrophobic pocket, which corresponds to the binding site for the adenine base of ATP [46]) [46]
lavendustin <1, 2> [5]
pyrazoloquinazolines <1> (<1> reversible inhibitors of EGFr TK, bind to a narrow hydrophobic pocket, which corresponds to the binding site for the adenine base of ATP [46]) [46]
pyrroloquinazolines <1> (<1> reversible inhibitors of EGFr TK, to a narrow hydrophobic pocket, which corresponds to the binding site for the adenine base of ATP [46]) [46]
sulfhydryl-specific reagents <1> (<1> irreversible inhibitor of EGFr tyrosine kinase, modification of certain cysteine residues [46]) [46]
tyrphostins <3, 1, 2> (AG 126, AG 1288, <1,2> [5]; <1>, the PTK inhibitors block EGF-dependent cell proliferation [45]) [5, 7, 45]
Additional information <1> (<1> overview describing research progress in erbB family tyrosine kinase inhibition, anti-cancer activity in clinical trials, irreversible inhibition and dual inhibitors are discussed [41]; <1> clinically active inhibitors of the erbB kinases are most readily attained with compounds at the ATP site [43]; <1> PTK inhibitors in the treatment of psoriasis [45]) [41, 43, 45]

Activating compounds

T-cell receptor <1> (<1> TCR activates both Rlk/Txk and Itk of the tec kinase family [44]) [44]
Additional information <1> (<1> can be activated by phosphorylation by src family kinases [44]; <1> autophosphorylation of RTKs phosphorylation of tyrosines [47]; autophosphorylation of Tyr-416 in the src activation loop is required for maximal activity [47]) [44, 47]

Specific activity (U/mg)

Additional information <3> (assay methods) [27-29]

K_m-Value (mM)

Additional information <1> (<1>, K_m values for ATP of most kinases is usually in the micromolar range [43]) [43]

4 Enzyme Structure

Molecular weight

52000-62000 <1> (<1> range of MW of proteins of the src group, comprising six distinct functional domains [42]) [42]

5 Isolation/Preparation/Mutation/Application

Source/tissue

T-lymphocyte <1> (<1> Itk and Rlk/Txk, Tec kinases are expressed in T-cells [44]) [44]
Additional information <3> (immunohistochemical localization in the nervous system) [20]

Localization

cytoplasm <3, 1> (<1> non receptor tyrosine kinases NRTKs lack an extracellular ligand-binding domain and a transmembrane-spanning region and are localized in the cytoplasm [47]) [6, 11, 47]
membrane <3> [18]
nucleus <3, 1> [11, 13]
plasma membrane <1> (<1>, the ligand binding domain and the protein kinase activity are separated by the plasma membrane [45]) [11, 45]

Purification

<1> [30]
<3> (affinity purification) [31]

Crystallization

<1> (SH_2 and SH_3 domains play a key role in regulation of catalytic activity of src kinases. Their intramolecular interactions stabilize the inactive conformational structure, the SH_3 domain interacts with the catalytic domain and linker sequences located between SH_2 and catalytic domains. The SH_2 domain interacts with phosphotyrosine at position 527 localized in the C-terminal region of the protein [42]) [42]
<1> (all receptor tyrosine kinases share a similar structure, a hydrophobic transmembrane doamin, an extracellular region, and an intracelluar, cytoplasmic region. The cytoplasmic and the catalytic domain include regulatory sites [42]) [42]
<1> (crystallization confirmed that the ATP-binding domain is an attractive target for drug design [39]) [39]
<1> (in vertebrates the proteins of the src family have similar structures [42]) [42]
<1> (most protein kinases cocrystalized with ATP or an ATP-competitive inhibitor, 60 structures have been solved so far [43]) [43]
<1> (the overall architecture of the tyrosine kinase is similar to that of the serine/threonine kinases: an amino-terminal lobe comprising a five-stranded β sheet and one α helix, and a larger carboxy-terminal lobe that is mainly α-helical [47]) [47]
<3> (comparison of structures [4]; structure of IRK i.e. fragment of cytoplasmic kinase domain of insulin receptor β-chain [6]) [4, 6]

Cloning

<3> (expression in Escherichia coli [36]; use of Baculovirus [37]; mutant of v-fps protein-tyrosine kinase [38]) [32-38]

Application

medicine <1> (<1> crystallization confirmed that the ATP-binding domain is an attractive target for drug design [39]; <1> their spectrum and association with specific malignancies offer multiple targets for therapeutic intervention [40]; <1> useful drug development to block the enhanced PTK activity in many nonmalignat diseases, such as psoriasis, papilloma, restenosis and pulmonary fibrosis [45]) [39, 40, 45]

Additional information <1> (<1>, possible clinical use of STI 571 as a potent inhibitor of PDGFR and c-Kit tyrosine kinases in solid tumors [39]) [39]

6 Stability

General stability information

<1>, ligand-binding stabilizes a dimeric configuration of the extracellular domains [47]

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Protein kinase (dual specificity kinase) 2.7.1.112.DUA

1 Nomenclature

EC number

2.7.1.112.DUA (Protein kinases are in a state of review by the NC-IUBMB. This EC class will presumably be transferred to EC 2.7.12.1)

Systematic name

ATP:protein phosphotransferase

Recommended name

protein kinase (dual specificity kinase)

Synonyms

Clk/Sty protein kinase <13> [20]
DYRK <25> [41]
DYRK1A <29> [15]
DYRK1B <6, 33> [53]
Dyrk <29> [47]
Esk kinase <192> [363]
MNB protein <25> [42]
RPK1 <228> [417]
STY protein <13> [23]
TTK <186> [350]
Yak1p protein kinase <12> [17]
cdc2/CDC28-like protein kinase <16> [27]
dual specificity protein kinase TTK <186, 192> [350, 351, 363]
dual-specificity tyrosine-phosphorylation regulated kinase 1A <29> [15, 47]
dual-specificity tyrosine-phosphorylation regulated kinase 1A <25> [39, 40, 41, 42]
dual-specificity tyrosine-phosphorylation regulated kinase 1A <26> [41, 44]
dual-specificity tyrosine-phosphorylation regulated kinase 1B <6, 33> [53]
dual-specificity tyrosine-phosphorylation regulated kinase 2 <30> [15]
dual-specificity tyrosine-phosphorylation regulated kinase 3 <11> [14, 15]
protein kinase CLK1 <13, 16> [20-24, 27]
protein kinase CLK2 <8, 17> [13, 22, 28, 29]
protein kinase CLK3 <9, 18, 27> [13, 22, 45]
protein kinase CLK4 <10> [13]
protein kinase KNS1 <3> [3]
protein kinase YAK1 <12> [16, 17, 18, 19]
protein kinase gene DYRK3 <11> [14]

protein kinase lkh1 <1> [1, 3]
serine/threonine protein kinase MPS1 <228> [415, 416, 417]
serine/threonine protein kinase minibrain <15> [26]

2 Source Organism

<1> *Schizosaccharomyces pombe* [1, 3]
<3> *Saccharomyces cerevisiae* [3, 8, 10, 11]
<6> *Homo sapiens* [53]
<8> *Mus musculus* [13]
<9> *Mus musculus* [13]
<10> *Mus musculus* [13]
<11> *Homo sapiens* [14, 15]
<12> *Saccharomyces cerevisiae* [16, 17, 18, 19]
<13> *Mus musculus* [20, 21, 22, 23, 24]
<15> *Drosophila melanogaster* [26]
<16> *Homo sapiens* [22, 27]
<17> *Homo sapiens* [22, 28, 29]
<18> *Homo sapiens* [22]
<25> *Homo sapiens* [39, 40, 41, 42, 43]
<26> *Mus musculus* [41, 44]
<27> *Rattus norvegicus* [45]
<29> *Rattus norvegicus* [15, 47]
<30> *Homo sapiens* [15]
<33> *Homo sapiens* [53]
<186> *Homo sapiens* [350, 351]
<192> *Mus musculus* [363]
<228> *Saccharomyces cerevisiae* [415, 416, 417]

3 Reaction and Specificity

Catalyzed reaction

ATP + a protein = ADP + a phosphoprotein

Reaction type

phospho group transfer

Natural substrates and products

S ATP + SR protein <6, 8, 9, 10, 12, 13, 15, 25, 26, 27, 29, 33> (<8, 9, 10>, enzyme may be constituent of a network of regulatory mechanisms that enable SR proteins to control RNA splicing [13]; <12>, Yak1p and Pop2p are part of a novel glucose-sensing system in yeast that is involved in growth control in response to glucose availability [16]; <12>, functions as a negative regulator of the cell cycle in Saccharomyces cerevisiae, acting

downstream of the cAMP-dependent protein kinase [17]; <12>, Yak1 acts downstream from, or on a parallel pathway to, the kinase step in the Ras/cAMP pathway [19]; <13>, the enzyme phosphorylates SR splicing factors and regulates their intranuclear distribution [20]; <13>, enzyme may be involved in cell cycle control [24]; <15>, the enzyme is required in distinct neuroblast proliferation centers during postembryonic neurogenesis [26]; <25>, DYRK may be involved in the abnormal neurogenesis found in Down syndrome [41]; <25>, MNB protein may play a significant role in a signaling pathway regulating nuclear functions of neuronal cell proliferation, contributing to certain features of Down syndrome [42,43]; <26>, enzyme is a good candidate to mediate some of the pleiotropic effects of Down syndrome [44]; <27>, enzyme regulates a predominately testicular function [45]; <29>, might be a component of a signaling pathway regulating nuclear functions [47]; <6,33>, enzyme is involved in the regulation of nuclear functions [53]) (Reversibility: ? <6, 8, 9, 10, 12, 13, 15, 25, 26, 27, 29, 33> [13, 16, 17, 19, 20, 24, 26, 40, 41, 42, 43, 44, 45, 47, 53]) [13, 16, 17, 19, 20, 24, 26, 40, 41, 42, 43, 44, 45, 47, 53]

P ADP + ?

S Additional information <186> (<186>, the enzyme is associated with cell proliferation [350]) [350]

P ?

S Additional information <192> (<192>, Esk kinase may play some role in the control of cell proliferation or differentiation [363]) [363]

P ?

S Additional information <228> (<228>, may function in a checkpoint control which couples DNA replication to mitosis. The level of the RPK1 transcript is extremely low and constant throughout the mitotic cycle. However it is regulated during cellular differentiation, being decreased in α-factor-treated cells and increased late in meiosis in α diploids. Rpk1 is involved in a pathway that coordinates cell proliferation and differentiation [417]) [417]

P ?

S Additional information <3> (<3>, negative regulation of filamentous growth and flocculation [3]; <3>, the enzyme is not essential for cell growth and a variety of other cellular processes in yeast [11]) [3, 11]

P ?

Substrates and products

S ATP + Pop2p <12> (Reversibility: ? <12> [16]) [16]

P ADP + phosphorylated Pop2p

S ATP + SR protein <8, 9, 10> (<8,9,10>, i.e. serine-rich and arginine-rich proteins [13]) (Reversibility: ? <8,9,10> [13]) [13]

P ADP + hyperphosphorylated SR protein <8, 9, 10> [13]

S ATP + Ser/Arg-rich splicing factors <13> (Reversibility: ? <13> [20]) [20]

P ADP + phosphorylated Ser/Arg-rich splicing factor

S ATP + histone <29> (<29>, recombinant glutathione S-transferase-Dyrk/fusion protein catalyzes histone phosphorylation on tyrosine and Ser/Thr residues [47]) (Reversibility: ? <29> [47]) [47]
P ADP + phosphorylated histone
S ATP + myelin basic protein <12> (<12>, phosphorylation on a C-terminal Ser residue [17]) (Reversibility: ? <12> [17]) [17]
P ADP + phosphorylated myelin basic protein
S ATP + myelin basic protein <192> (<192>, phosphorylation on serine, threonine, and tyrosine residues [363]) (Reversibility: ? <192> [363]) [363]
P ADP + phosphorylated myelin basic protein
S ATP + protein <186> (<186>, can phosphorylate serine, threonine, and tyrosine hydroxyamino acids) (Reversibility: ? <186> [350]) [350]
P ADP + phosphoprotein
S ATP + protein <192> (<192>, autophosphorylation on serine, threonine, and tyrosine residues [363]) [363]
P ADP + phosphoprotein
S ATP + protein <228> (<228>, kinase can phosphorylate serine, threonine and tyrosine residues [416]) (Reversibility: ? <228> [416]) [416]
P ADP + phosphoprotein
S ATP + protein <8-10, 12, 17, 27, 30> (<8-10, 12, 29, 30>, autophosphorylation [13, 15, 17, 29, 47]; <12>, autophosphorylation on Tyr residues [17]; <17>, autophosphorylates on Ser/Thr and Tyr residues [29]; <29, 30>, when expressed in E. coli the enzyme catalyzes autophosphorylation on Tyr residues [15]; <27>, recombinant glutathione S-transferase-Dyrk fusion protein catalyzed autophosphorylation on tyrosine and serine/threonine residues [45]) (Reversibility: ? <8-10, 12, 17, 27, 30> [13, 15, 17, 29, 45]) [13, 15, 17, 29, 45]
P ADP + phosphoprotein
S Additional information <27, 29> (<27>, a glutathione S-transferase fusion protein of Clk3 catalyzes autophosphorylation of the kinase but not phosphorylation of the exogenous substrates histone or casein [45]; <29>, activity is dependent on tyrosine residues between subdomains VII and VIII [47]) [45, 47]
P ?
S Additional information <3> (<3>, autophosphorylation [10]) [10]
P ?

K_m-Value (mM)
Additional information <29> [47]

4 Enzyme Structure

Posttranslational modification
phosphoprotein <192> (<192>, autophosphorylation on serine, threonine, and tyrosine residues [363]) [363]

phosphoprotein <8, 9, 10, 12, 29, 30> (<8, 9, 10, 12, 29, 30>, autophosphorylation [13, 15, 17, 29, 47]; <29>, dual specificity protein kinase that is regulated by tyrosine phosphorylation in the activation loop [47]) [13, 15, 17, 25, 29, 47]

5 Isolation/Preparation/Mutation/Application

Source/tissue

brain <25, 26, 29> (<25>, expressed in the neuronal regions affected in Down syndrome [43]; <25>, overexpression in Down syndrome [39]; <25>, fetal and adult [42]; <26>, expression pattern in frontal brain nuclei during murine embryogenesis [44]) [39, 42, 43, 44, 47]
cell culture <186> (<186>, most malignant tumors assessed express TTK mRNA, as well, all rapidly proliferating cell lines tested express TTK mRNA [350]) [350]
cell culture <192> (<192>, embryonal carcinoma cell line [363]) [363]
embryonic carcinoma cell line <13> [23]
erythroleukemia cell <13> [24]
muscle <6, 11, 33> (<6, 33>, predominately expressed in muscle and testis [53]) [14, 53]
neuroblast <15> [26]
testis <6, 11, 27, 30, 33, 186> (<27, 30>, predominately expressed in testis [15, 45]; <6, 33>, predominately expressed in muscle and testis [53]) [14, 15, 45, 53, 350]
thymus <186> [350]

Localization

membrane <192> [363]
nucleus <25, 26> (<25,26>, enzyme contains a nuclear targeting signal sequence [41]) [41]
Additional information <11, 13> (<13>, STY protein contains a putative nuclear localization signal [23]; <11>, nuclear localization of DYRK1A is mediated by its nuclear targeting signal, amino acids 105-139, but ist characteristic subnuclear distribution depends on additional N-terminal elements, amino acids 1-104 [15]) [15, 23]

Cloning

<3> [3]
<6> (green fluorescent protein fusion protein of DYRK1B is found mainly in the nucleus of transfected COS-7 cells [53]) [53]
<8> [13]
<9> [13]
<10> [13]
<11> [14]
<13> [23]
<16> [27]
<17> [22]

<18> [22]
<25> [41, 42]
<27> [45]
<29> (fusion protein of DYRK1A accumulates in the nucleus of transfected COS-7 and HEK293 cells, expression in Escherichia coli [15]) [15, 47]
<33> (green fluorescent protein fusion protein of DYRK1B is found mainly in the nucleus of transfected COS-7 cells [53]) [53]

Engineering

Additional information <29> (<29>, exchange of two Tyr residues in the activation loop between subdomains VII and VIII for Phe almost completely suppresses the activity and Tyr autophosphorylation of Dyrk. Tyr autophosphorylation is also reduced by exchange of Tyr219 in a tyrosine phosphorylation consensus motif [47]) [47]

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Protein-tyrosine kinase (ETK, WZC) 2.7.1.112.ETK

1 Nomenclature

EC number
2.7.1.112.ETK (Protein kinases are in a state of review by the NC-IUBMB. This EC class will presumably be transferred to EC 2.7.10.1)

Systematic name
ATP:protein-tyrosine O-phosphotransferase

Recommended name
protein-tyrosine kinase (ETK, WZC)

Synonyms
tyrosine-protein kinase Etk <5, 6, 7> [2, 5, 6, 9, 12, 14, 17, 18]
tyrosine-protein kinase Ptk <8> [10, 11, 16]
tyrosine-protein kinase Wzc <1, 2, 3, 4> [1, 2, 3, 5, 6, 7, 8, 12, 13, 15]

2 Source Organism

<1> *Escherichia coli* [1, 6, 8, 12, 13, 15]
<2> *Escherichia coli* (strain O157 [5]) [2, 5]
<3> *Salmonella typhimurium* [3]
<4> *Salmonella typhimurium* [4, 7]
<5> *Escherichia coli* [2, 5]
<6> *Escherichia coli* [6, 12, 14, 17, 18]
<7> *Escherichia coli* [9]
<8> *Acinetobacter johnsonii* [10, 11, 16]

3 Reaction and Specificity

Catalyzed reaction
tyrosine-protein + ATP = phosphotyrosine-protein + ADP ???

Reaction type
phospho group transfer

Natural substrates and products
S Additional information <1, 7, 8> (<1>, enzyme is involved in signal transduction [1]; <1>, phosphorylation of Wzc, as regulated by Wzb, is directly connected with the production of a particular capsular polysaccharide, colanic acid. Thus, when Wzc is phosphorylated on tyrosine, no

colanic acid is synthesised by bacteria, but when dephosphorylated by Wzb, colanic acid is produced [6]; <1>, phosphotyrosine-protein phosphatase Wzb is able to dephosphorylate previously autophosphorylated Wzc. Reversible protein phosphorylation on tyrosine may be part of the cascade of reactions that determine the pathogenicity of bacteria [8]; <7>, involved in exopolysaccharide production and virulence [9]; <8>, possible involvement of the enzyme in cell recognition and bacterial pathogenicity [11]; <1>, enzyme is involved in the production of the extracellular polysaccharide colanic acid [13]) [1, 6, 8, 9, 11, 13]

P ?

Substrates and products

S ATP + poly(Glu:Tyr) <7> (Reversibility: ? <7> [9]) [9]

P ADP + phosphorylated poly(Glu:Tyr)

S ATP + tyrosine-protein kinase Etp <6> (Reversibility: ? <6> [6]) [6]

P ADP + phosphotyrosine-protein kinase Etp

S ATP + tyrosine-protein kinase Wcz <1, 7, 8> (<1>, The C-terminal domain alone can undergo autophosphorylation and thus appears to harbor the protein-tyrosine kinase activity. By contrast, the N-terminal domain is not phosphorylated when incubated either alone or in the presence of the C-domain, and does not influence the extent of phosphorylation of the C-domain. The C-domain contains six different sites of phosphorylation. Among these, five are located at the C-terminal end of the molecule in the form of a tyrosine cluster Tyr708, Tyr710, Tyr711, Tyr713, and Tyr715, and one site is located upstream, at Tyr569. The Tyr569 residue can autophosphorylate through an intramolecular process, whereas the tyrosine cluster cannot. The phosphorylation of Tyr569 results in an increased protein kinase activity of Wzc, which can, in turn, phosphorylate the five terminal tyrosines through an intermolecular process. It is concluded that protein Wzc autophosphorylates by using a cooperative two-step mechanism that involves both intraphosphorylation and interphosphorylation [1]; <1, 7, 8>, autophosphorylation [6, 9, 10, 11]; <8>, autophosphorylation at several tyrosine residues [16]; <1>, efficient autophosphorylation in presence of ATP [8]; <8>, presence of at least five isoforms, all phosphorylated exclusively at tyrosine supports the concept that autophosphorylation occurs at multiple sites within the protein [11]) (Reversibility: ? <1, 7, 8> [1, 6, 8, 9, 10, 11, 16]) [1, 6, 8, 9, 10, 11, 16]

P ADP + phosphotyrosine-protein kinase Wcz

S Additional information <8> (<8>, no phosphorylation of synthetic substrates such as poly(Glu80 Tyr20) or angiotensin II [11]) [11]

P ?

Inhibitors

Additional information <8> (<8>, no inhibition by genestein [11]) [11]

4 Enzyme Structure

Molecular weight
81000 <8> [16]

Subunits
? <8> (<8>, x * 82373, calculation from nucleotide sequence [11]) [11]

5 Isolation/Preparation/Mutation/Application

Localization
membrane <7, 8> (<7>, inner membrane protein [9]; <8>, located in the inner-membrane fraction [16]) [9, 16]

Purification
<1> [8]
<8> [10, 16]

Cloning
<8> [11]

Application
medicine <7> (<7>, may serve as a new target for the development of new antibiotics [9]) [9]

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Protein-tyrosine kinase (PTK, not ETK, WZC) 2.7.1.112.TK

1 Nomenclature

EC number

2.7.1.112.TK (Protein kinases are in a state of review by the NC-IUBMB. This EC class will presumably be split up into EC 2.7.10.1 and EC 2.1.10.2)

Systematic name

ATP:protein-tyrosine O-phosphotransferase

Recommended name

protein-tyrosine kinase (PTK, not ETK, WZC)

Synonyms

70 kDa zeta-associated protein
A-type platelet-derived growth factor receptor <80> [232, 233, 234]
ALK tyrosine kinase receptor <185, 301> [474, 633]
ATK
AXL oncogene
Abl kinase <10> [22]
Abl protein tyrosine kinase <10> [15, 19]
Abl tyrosine kinase <322> (<322> present in 95% of patients with chronic myelogenous leukemia, CML [705]) [705]
adhesion-related kinase
agammaglobulinaemia tyrosine kinase
amylovoran biosynthesis membrane-associated protein amsA
anaplastic lymphoma kinase
B cell progenitor kinase
B cell progenitor kinase <137> [374]
B lymphocyte kinase
B-cell/myeloid kinase
BCR-ABL tyrosine kinase
BDNF/NT-3 growth factors receptor <77, 233, 260, 273, 291> [124, 207, 221, 222, 223, 224, 569, 570, 571, 572, 614, 615, 628, 633]
BEK/FGFR-2 receptor <93> [273]
BMK
BPK
Batk <143> [392]
Bcr-Abl kinase <10> [18]
bone marrow kinase BMX
brain-specific kinase

breast tumor kinase
breathless protein
Bruton's tyrosine kinase
Bruton's tyrosine kinase <137> [372]
Btk <137> [372]
C-FES
C-SRC
C-SRC kinase
C-YES
C-mer
C-ret
CADTK
CAK β
CD115 antigen
CD117 antigen
CD135 antigen
CD136 antigen
CD140a antigen
CD140b antigen
CD167a antigen
CD202b antigen
CD220 antigen
CD221 antigen
CD246 antigen
CDW136
CEK4
CER
Cadherin 96Ca
Cak I receptor <202> [514]
calcium-dependent tyrosine kinase
Cek5 <112> [325]
cell adhesion kinase
cell adhesion kinase β
Ctk <142> [391]
D-ash
DDR2 <253> [606]
DFGF-R1
DFer
DRT
DTK <181> [464]
DTK receptor tyrosine kinase <212> [534]
derailed protein
Dfak65 <305> [635]
discoidin receptor tyrosine kinase
doughnut protein

Drl RTK <237> [576]
Dror
Dror protein <235> [574]
Drosophila relative of ERBB
Dsrc28C
EBK
EGF receptor <102> [312]
EGF receptor tyrosine kinase
EGF-R <190> [482]
EGFr TK
ELK
EPH-and ELK-related kinase
EPHA1 receptor tyrosine kinase <91> [251]
EPS I polysaccharide export protein epsB
ERK
ETK
Ebk <247> [593]
Eek receptor <3> [3]
Egfr
Egg laying defective protein 15
Elk tyrosine kinase <54> [164, 165, 166, 167]
EmRK2 <136> [369]
embryonic brain kinase
endothelial kinase receptor EK1
Eph homologous kinase 3 <176> [458]
Eph-like kinase1 <4> [4]
Eph-related receptor tyrosine kinase Cek9 <220> [545]
epidermal growth factor receptor-related protein
epithelial and endothelial tyrosine kinase
epithelial cell kinase
ErbB-4 receptor <230> [566]
ErbB2 kinase
FAK <305, 307> [635, 637]
FER tyrosine kinase <82> [226, 237, 238, 239]
FGF receptor <60> [184]
FGFR-4 <97> [285]
FGFR1K <60> [192]
FGFR2 <92> [252, 255]
FGFR3 <98> [287, 288, 289, 290, 291, 292, 293, 303]
FGFR3 <248> [594]
FGFR4 <200> [512]
FL cytokine receptor <138, 186> [84, 86, 376, 377, 378, 379, 380, 381, 382, 475-477]
FLT3/FLK2 receptor tyrosine kinase <186> [475]
FLT4 <133> [358]
FLT4 receptor tyrosine kinase <133> [360]
Flk-1 <135> [362, 366]

Fms proto-oncogene
GCTK
GCTK <144> [393]
GP145-TrkB
GP145-TrkB/GP95-TrkB
GP145-TrkC
Gurken receptor
HCK <47> [132]
HEK
HEK 2 <171> [451]
HEK3
HEK4
HEK6
HER3/ERRB3 <95> [281]
HGF receptor
HGF-SF receptor
HTK <177> [459]
HTL protein <274> [124, 556]
HYK
HYL <145> [397]
heartless protein
hematopoietic consensus tyrosine-lacking kinase
hemopoietic cell kinase
heparin-binding growth factor receptor
IL-2-inducible T-cell kinase
ILP receptor
ILP receptor <1> [1]
IR-related receptor
IRK
IRR
IRR <72> [215]
IRR-protein tyrosine kinase <276> [630]
Itk (tec kinase)
JAK protein tyrosine kinase
JAK1 kinase <226> [558]
JAK2 protein tyrosine kinase <255> [608]
Jak-3 Janus kinase <250> [600]
Jak2 protein <6> [6]
Janus family kinase JAK3 <164> [431]
Janus kinase 2 <6> [8]
KGF receptor <93> [274]
KIT <9> [11]
Keratinocyte growth factor receptor
kinase EMB
kinase EMT
kinase NYK
kinase TLK

kinase VIK
kinase insert domain receptor
Kit protein <221> [546]
Kiz-1 <168> [445]
L-JAK
LIM domain kinase 1 <167, 168, 169> [441, 442, 443, 444, 445, 446, 447, 448, 449]
LIMK <167> [443]
LSK
Lck Tyrosine kinase
Let-23 receptor protein-tyrosine kinase <102> [309, 310, 311, 312]
leukocyte janus kinase
Linotte protein
MATK <145> [394]
MDK1 <247> [592]
MEK4
MFR
MIL proto-oncogene serine/threonine-protein kinase <35> [77]
MIR
MLN 19
MSP receptor
Met proto-oncogene tyrosine kinase
Met-related kinase
Met/hepatocyte growth factor receptor tyrosine kinase <45> [121]
NET
NET <179> [463]
NEU proto-oncogene
NRTK
NT-3 growth factor receptor <104, 203, 232, 280> [316, 515, 516, 517, 568, 569]
NTK38
NUK
NYK/FLK-1 <135> [364]
neurospecific receptor tyrosine kinase
Ntk <142> [390]
nuclear tyrosine protein kinase RAK
ORF6
P140 TEK
P55-FGR
P56-LCK
P57-STK
P59-FYN
P60-SRC
P61-YES
PDGF A <108> [322]

PDGF β-receptor <53> [158]
PDGF-R-α
PDGF-R-β
PDGFR kinase
PP125FAK
PTK
PTK-RL-18
PTK70
Pag <266> [621]
Pagliaccio
protein kinase (tyrosine-phosphorylating)
protein kinase BATK
protein kinase HYL
protein kinase Lck
protein kinase NTK
protein kinase p56-LCK
protein kinase p56lck
protein p56c-lck kinase
protein p56lck tyrosine kinase
protein tyrosine kinase
protein tyrosine kinase lck
protein tyrosine kinase p56lck
protein tyrosine kinase pp56lck
protein-tyrosine kinase C-TKL
protein-tyrosine kinase CYL
protein-tyrosine kinase byk
protein-tyrosine kinase receptor MPK-11
proto-oncogene tyrosine-protein kinase Kit
Pyk2 <304> [634]
Quek1
Quek2 <165> [438]
RET oncogene protein <275> [124, 629]
RMIL serine/threonine-protein kinase <128> [350]
RMIL serine/threonine-protein kinase <209> [531]
RTK
RTK <197> [503]
Raftk <304> [640]
Rak tyrosine kinase <151> [406]
receptor protein-tyrosine kinase HEK11
receptor protein-tyrosine kinase HEK5
receptor protein-tyrosine kinase HEK7
receptor protein-tyrosine kinase HEK8
receptor protein-tyrosine kinase TKT
receptor tyrosine kinase MerTK
receptor-activated Janus kinase

related adhesion focal tyrosine kinase
resting lymphocyte kinase
Rlk <148> [402]
Rlk/Txk (tec kinase)
Ron tyrosine kinase receptor <208> [528]
Ron/Stk receptor tyrosine kinase <251> [602]
SCFR
SLK
SRC-related intestinal kinase
STK-1 <138> [378]
SYN
Sek-1 receptor tyrosine kinase <269> [624]
Sky receptor <182> [468]
slow nerve growth factor receptor
spleen tyrosine kinase
Src64 <46> [126]
Srcasm <138> [380]
stem cell-derived tyrosine kinase
Syk-related tyrosine kinase
T cell-specific protein-tyrosine kinase
T-cell-specific kinase <205> [521]
TRK1 transforming tyrosine kinase protein
Tec family kinase EMT/ITK/TSK <205> [523]
Tek receptor tyrosine kinase <197> [503]
Tie2 <195, 197> [497, 498, 508]
Torpedo protein
TrkB receptor <260> [615]
TrkB tyrosine kinase
TrkC <203> [517]
TrkC <232> [568]
TrkC receptor tyrosine kinases <203> [517]
TrkC tyrosine kinase
Tsk <205> [521]
Tunica interna endothelial cell kinase
Tyro 10 receptor tyrosine kinase <253> [573, 605, 606]
tyrosine kinase
tyrosine kinase ARG
tyrosine kinase CEK6 receptor
tyrosine kinase DDR
tyrosine kinase lck
tyrosine kinase p56lck
tyrosine kinase receptor HD-14
tyrosine kinase-type cell surface receptor HER2
tyrosine kinase-type cell surface receptor HER3
tyrosine kinase-type cell surface receptor HER4

tyrosine phosphokinase
tyrosine protein kinase
tyrosine protein kinase p56lck
tyrosine-protein kinase CAK
tyrosine-protein kinase CEK9
tyrosine-protein kinase CTK
tyrosine-protein kinase DTK
tyrosine-protein kinase FLT3
tyrosine-protein kinase FRT
tyrosine-protein kinase Lyk
tyrosine-protein kinase RSE
tyrosine-protein kinase SKY
tyrosine-protein kinase TYRO 10
tyrosine-protein kinase brk
tyrosine-protein kinase receptor CEK10
tyrosine-protein kinase receptor CEK11
tyrosine-protein kinase receptor CEK5
tyrosine-protein kinase receptor CEK7
tyrosine-protein kinase receptor CEK8
tyrosine-protein kinase receptor CEPHA7
tyrosine-protein kinase receptor ECK
tyrosine-protein kinase receptor EEK
tyrosine-protein kinase receptor EPH
tyrosine-protein kinase receptor ESK
tyrosine-protein kinase receptor ETK1
tyrosine-protein kinase receptor FLT
tyrosine-protein kinase receptor FLT3
tyrosine-protein kinase receptor FLT4
tyrosine-protein kinase receptor HTK
tyrosine-protein kinase receptor PAG
tyrosine-protein kinase receptor QEK5
tyrosine-protein kinase receptor REK4
tyrosine-protein kinase receptor SEK
tyrosine-protein kinase receptor TCK
tyrosine-protein kinase receptor TEK
tyrosine-protein kinase receptor XEK
tyrosine-protein kinase receptor XELK
tyrosine-protein kinase receptor ZEK1
tyrosine-protein kinase receptor ZEK2
tyrosine-protein kinase receptor ZEK3
tyrosine-specific protein kinase
tyrosylprotein kinase
VAB-1 Eph receptor tyrosine kinase <7> [9]
VEGF receptor 2 <165> [439]

VEGF receptor-1 <136> [369]
vascular permeability factor receptor
WEE1hu
Xek <265> [620]
Xenopus Elk-like kinase <265> [620]
YES related kinase
Yes-related kinase <198> [509]
adhesion-related kinase <189> [481]
α platelet-derived growth factor receptor <80, 89, 107, 108> [232, 247, 248, 320, 321, 322]
angiopoietin 1 receptor <8> [10]
angiopoietin 1 receptor <8, 195, 197> [10, 497, 498, 499, 503, 504, 505, 506, 507, 508]
angiopoietin 1 receptor <215> [507]
ark <189> [481]
basic fibroblast growth factor receptor 1 <60> [131, 182, 183, 184, 185, 186, 187, 189, 190, 191, 192, 193, 194, 195]
basic fibroblast growth factor receptor 1 <79, 94, 206> [227, 228, 229, 230, 231, 277, 278, 525]
basic-FGF receptor <60> [183]
β platelet-derived growth factor receptor <34, 53> [76, 158, 159, 160, 161, 162]
brain specific kinase <243> [587]
c-ABL
c-FER
c-Kit tyrosine kinase
c-Met <184> [471]
c-erbB3
c-fgr
c-fms
c-kit
c-kitR tyrosine kinase <57> [172]
c-met
c-mil protein <35> [77]
chicken embryo kinase 5 <112> [325]
class II receptor tyrosine kinase <144> [393]
cytoplasmic tyrosine-protein kinase BMX <162> [426, 427]
discoidin domain receptor 2 <234, 253> [573, 606]
discoidin receptor tyrosine kinase <222> [551]
ectoprotein kinase
embryo brain kinase <247> [593]
embryonic receptor kinase <136> [369]
ephrin receptor 1 <7> [9]
ephrin type-A receptor 1 <91> [250, 251]

ephrin type-A receptor 2 <113, 201> [327, 452, 513]
ephrin type-A receptor 3 <2, 4, 114-116> [2, 4, 328-330]
ephrin type-A receptor 4 <180, 219> [326, 333, 544]
ephrin type-A receptor 4 <199> [510]
ephrin type-A receptor 4A <269> [624]
ephrin type-A receptor 4B <266> [621]
ephrin type-A receptor 5 <173, 174, 175, 243> [326, 333, 455, 456, 457, 587]
ephrin type-A receptor 6 <254> [607]
ephrin type-A receptor 7 <5, 176, 231, 247> [5, 333, 458, 592, 593]
ephrin type-A receptor 8 <3, 117> [3, 331, 332]
ephrin type-B receptor 1 <54, 179> [163, 164, 165, 462, 463]
ephrin type-B receptor 1A <265> [620]
ephrin type-B receptor 2 <112, 118, 264> [325, 326, 331, 333, 334, 335, 336, 337, 619]
ephrin type-B receptor 3 <171, 172> [451, 452, 453, 454]
ephrin type-B receptor 4 <177, 178> [453, 459, 460, 461]
ephrin type-B receptor 5 <220> [326, 545]
epidermal growth factor receptor <190, 296, 299> [482, 483, 633]
epidermal growth factor receptor 4 <230> [565, 566, 567]
epithelial cell kinase <113> [327]
epithelial discoidin domain receptor 1 <202, 222, 259> [165, 388, 514, 547, 548, 549, 550, 551, 613]
erbB tyrosine kinase
fetal liver kinase 1 <135> [362, 366]
fibroblast growth factor receptor 1 <60, 96> [192, 282]
fibroblast growth factor receptor 2 <92, 93, 190, 204> [182, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 272, 273, 274, 275, 276, 483, 484, 485, 518]
fibroblast growth factor receptor 3 <98, 248> [284, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 299, 300, 301, 302, 303, 594, 595, 596]
fibroblast growth factor receptor 4 <97, 200, 268> [283, 284, 285, 286, 388, 512, 623]
fibroblast growth factor receptor BFR-2 <191> [265]
fibroblast growth factor receptor homolog 1 <217> [541, 542]
fibroblast growth factor receptor homolog 2 <224> [542, 554, 555, 556]
flk-1 <165> [439]
focal adhesion kinase <305-307> [635, 636, 637]
focal adhesion kinase 1
focal adhesion kinase 2 <289> [633]
gene lck protein kinase
gene lck tyrosine kinase
gp145trkC <104> [316]
hFAK <309> [645]
hek <116> [329]

hematopoietic consensus tyrosine-lacking kinase <145> [397]
hepatocyte growth factor receptor <45, 78, 184> [113, 118, 119, 120, 121, 122, 123, 225, 226, 470, 471, 472, 473]
high affinity nerve growth factor receptor <132, 284> [356, 357, 633]
hydroxyaryl-protein kinase
hyk <197> [505]
insulin receptor <75, 76, 302> [218, 219, 220, 633]
insulin receptor protein-tyrosine kinase
insulin receptor-related protein <72, 73, 276> [215, 216, 630]
insulin receptor-related receptor <101> [308]
insulin-like growth factor I receptor <43, 101, 245> [111, 112, 113, 114, 226, 305, 307, 308]
insulin-like peptide receptor <1> [1]
insulin-like receptor <50, 270> [146, 147, 148, 149, 625]
keratinocyte growth factor receptor <93> [274]
kinase, protein (phosphorylating tyrosine)
kinase, protein p56lck (phosphorylating)
leukocyte Janus kinase <164> [432, 433, 434, 435, 436]
leukocyte tyrosine kinase receptor <49, 119> [141, 142, 143, 144, 145, 338, 339, 340]
ltk receptor tyrosine kinase <49> [142]
mROR1
mROR2
macrophage colony stimulating factor I receptor <40, 52, 65, 187> [103, 104, 105, 106, 107, 153, 154, 155, 156, 157, 208, 478]
macrophage colony-stimulating factor receptor <52> [157]
macrophage-stimulating protein receptor <208, 251> [528, 601, 602]
mast/stem cell growth factor receptor <57> [103, 171, 172, 173, 174, 175, 176, 177, 178, 179]
mast/stem cell growth factor receptor <9, 33, 71, 72, 73, 74, 75, 158, 221, 239, 240> [11, 420, 546, 583, 584]
megakaryocyte-associated tyrosine-protein kinase <142, 145> [389, 390, 391, 392, 394, 395, 396]
melanoma receptor protein-tyrosine kinase <66> [209]
mouse developmental kinase 1 <247> [592]
myoblast growth factor receptor egl-15 <286> [633]
neuregulin receptor ErbB-4 <230> [566]
neuronal proto-oncogene tyrosine-protein kinase SRC <32> [70]
non-receptor tyrosine kinase brk <229> [564]
non-receptor tyrosine kinase spore lysis A <85> [242, 243]
non-receptor tyrosine-protein kinase TYK2 <120, 287> [341, 633]
nonreceptor protein tyrosine kinase
nonreceptor tyrosine kinase Src <15> [45]
nonreceptor tyrosine kinase Srm <252> [604]

p135tyk2 tyrosine kinase <120> [284, 341, 342]
p140-TrkA
p145c-kit <57> [179]
p150
p180erbB4
p185-Ron
p185erbB2
p190MET kinase <45> [121]
p55-BLK
p55blk kinase <81> [236]
p56-HCK
p56-HCK/p59-HCK
p56lck <36> [78, 79]
p56lck kinase
p56lck protein kinase
p56lck protein tyrosine kinase
p56lck tyrosine kinase
p59-HCK/p60-HCK
p60-YRK
p72ITK/EMT <205> [520]
p94-FER
phosphotyrosyl-protein kinase
pp125FAK <307, 309, 311> [637, 641, 646]
pp56lck <36> [81]
protein kinase eck <113> [327]
protein receptor tyrosine kinase RTK 6 <222> [549]
protein tyrosine kinase <288> [633]
protein tyrosine kinase 2β
protein tyrosine kinase PTK70 <252> [603, 604]
protein-tyrosine kinase Brk <229> [562, 563, 564]
protein-tyrosine kinase ITK/EMT <205> [520]
proto-oncogene tyrosine-protein kinase ABL1 <10> [12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23]
proto-oncogene tyrosine-protein kinase ABL1 <295> [633]
proto-oncogene tyrosine-protein kinase FER <82> [237]
proto-oncogene tyrosine-protein kinase FES/FPS <39, 71> [101, 102, 214]
proto-oncogene tyrosine-protein kinase FGR <70> [212, 213]
proto-oncogene tyrosine-protein kinase FYN <37, 67> [25, 89, 90, 91, 92, 93, 94, 95, 96, 97, 210]
proto-oncogene tyrosine-protein kinase FYN <110, 211> [323, 509]
proto-oncogene tyrosine-protein kinase LCK <36, 149, 281> [78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 403, 404, 633]
proto-oncogene tyrosine-protein kinase MER <183, 228, 246> [469, 560, 561, 591]

proto-oncogene tyrosine-protein kinase ROS <48> [138, 139, 140]
proto-oncogene tyrosine-protein kinase SRC <14, 61, 300> [32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 196, 197, 198, 199, 200, 201, 633]
proto-oncogene tyrosine-protein kinase YES <41, 51, 58, 109, 207, 241> [108, 150, 151, 152, 180, 323, 526, 527, 585]
proto-oncogene tyrosine-protein kinase YRK <198> [509]
proto-oncogene tyrosine-protein kinase receptor ret <130, 285> [354, 633]
rceptor protein-tyrosine kinase erbB-4 <257> [100, 609, 611]
receptor protein tyrosine kinase RTK <234> [573]
receptor protein-tyrosine kinase erbB-2 <31, 38, 242> [66, 67, 68, 69, 98, 99, 100, 586]
receptor protein-tyrosine kinase erbB-3 <95> [279, 280, 281]
receptor protein-tyrosine kinase erbB-3 <256> [609, 610]
receptor protein-tyrosine kinase erbB-4 <230> [565]
receptor tyrosine kinase Cek8 <219> [544]
receptor tyrosine kinase Sek <199> [388, 510, 511]
receptor tyrosine kinase Sky <212> [536]
receptor-associated kinase JAK2 <6> [7]
receptor-like protein tyrosine kinase bsk <243> [587]
receptor-like protein-tyrosine kinase TK14 <92> [254]
receptor-like tyrosine-protein kinase kin-15 <126> [349]
receptor-like tyrosine-protein kinase kin-16 <127> [349]
receptor-type tyrosine kinase termed Rse <212> [466]
resting lymphocyte kinase <148> [402]
sam3 protein <248> [595]
serine/threonine-protein kinase CTR1 <210> [532, 533]
serine/threonine-protein kinase transforming protein Rmil <56, 111> [169, 170, 324]
serine/threonine-protein kinase transforming protein mil <21> [54, 55]
sevenless protein <64, 90> [124, 204, 205, 206, 207, 249]
sik <262> [617]
sky receptor tyrosine kinase <182> [468]
sponge receptor tyrosine kinase <238> [580, 581, 582]
src-kinase
src-related intestinal kinase <262> [617]
stem cell tyrosine kinase 1 <138> [378]
tlk <149> [403]
trkB <260> [614]
tyrosine kinase Emt/Itk <205> [521]
tyrosine kinase PTK6 <229> [563]
tyrosine kinase QEK5 <264> [619]
tyrosine kinase cyl <140> [387]
tyrosine kinase p59fyn <138> [381]
tyrosine kinase receptor CEK2 <86> [244]

tyrosine-protein kinase receptor TYRO3 <181, 182, 212> [464, 465, 466, 467, 468, 534, 535, 536, 537]
tyrosine-protein kinase receptor Tie-1 <131, 213, 214> [355, 508, 538, 539]
tyrosine-protein kinase receptor UFO <121, 189> [113, 343, 344, 480, 481]
tyrosine-protein kinase receptor torso <88> [245, 246]
tyrosine-protein kinase shark <294> [633]
tyrosine-protein kinase transforming protein ABL <12, 55> [16, 28, 168]
tyrosine-protein kinase transforming protein FES <25, 26> [60]
tyrosine-protein kinase transforming protein FGR <27> [61]
tyrosine-protein kinase transforming protein FPS <20, 24> [52, 53, 59]
tyrosine-protein kinase transforming protein RYK <125> [348]
tyrosine-protein kinase transforming protein SEA <99> [304]
tyrosine-protein kinase transforming protein SRC <68, 69, 74, 105, 122> [211, 217, 317, 345]
tyrosine-protein kinase transforming protein SRC <15, 16, 17> [38, 42, 43, 44, 45, 46, 47, 48, 49]
tyrosine-protein kinase transforming protein SRC <218> [543]
tyrosine-protein kinase transforming protein YES <18> [50]
tyrosine-protein kinase transforming protein erbB <22, 23, 59> [56, 57, 58, 181]
tyrosine-protein kinase transforming protein fms <28> [62, 63]
tyrosine-protein kinase transforming protein kit <30> [65]
tyrosine-protein kinase transforming protein ros <19> [51]
tyrosine-protein kinase transmembrane receptor ROR1 <193, 278> [491, 492, 632]
tyrosine-protein kinase transmembrane receptor ROR2 <194, 277> [491, 493, 494, 495, 496, 631, 632]
tyrosine-protein kinase transmembrane receptor Ror <235> [124, 207, 574]
v-fps Protein-tyrosine kinase
vascular endothelial growth factor receptor 1 <170, 283> [450, 633]
vascular endothelial growth factor receptor 2 <135, 136, 165, 282> [361, 362, 363, 364, 365, 366, 367, 368, 369, 437, 438, 439, 633]
vascular endothelial growth factor receptor 3 <133, 134> [358, 359, 360, 361]
vascular endothelial growth factor receptor-1 <136> [369]
yrk <198> [509]
Additional information <322, 323> (this group of enzymes is under review by NC-IUBMB, recommendation for a nomenclature system based on acceptor amino acid specificity rather than on protein substrate. In accordance with this system protein-tyrosine kinases would belong to EC 2.7.11.X, <322,323> [667]; The present data set is restricted to a literature review and does not contain a complete description of kinases. Classification system based on kinase domain phylogeny revealing families of enzymes with related substrate specificities, <323> [669]) [667, 669]

CAS registry number

114051-78-4 (p56lck protein kinase)
80449-02-1 (protein-tyrosine kinase)

2 Source Organism

<1> *Branchiostoma lanceolatum* [1]
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<4> *Brachydanio rerio* [4]
<5> *Gallus gallus* [5]
<6> *Homo sapiens* [6, 7, 8]
<7> *Caenorhabditis elegans* [9]
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<11> *Mus musculus* [12, 24, 25, 26, 27]
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<15> *Rous sarcoma virus* [38, 42, 43, 44, 45, 46, 47]
<16> *Avian sarcoma virus* [44, 48]
<17> *Rous sarcoma virus* (strain Prague C [44]) [44, 49]
<18> *Avian sarcoma virus* (strain Y73 [50]) [50]
<19> *Avian sarcoma virus* (strain UR2) [51]
<20> *Fujinami sarcoma virus* [52, 53]
<21> *Avian retrovirus MH2* [54, 55]
<22> *Avian leukosis virus* [56]
<23> *Avian erythroblastosis virus* (strain ES4 [57,58]) [57, 58]
<24> *Avian sarcoma virus* (strain PRCII [59]) [59]
<25> *Feline sarcoma virus* (strain Gardner-Arnstein [60]) [60]
<26> *Feline sarcoma virus* (strain Snyder-Theilen [60]) [60]
<27> *Feline sarcoma virus* (strain Gardner-Rasheed [61]) [61]
<28> *Feline sarcoma virus* (strain McDonough [62]) [62, 63]
<29> *Caenorhabditis elegans* [64]
<30> *Feline sarcoma virus* (strain Hardy-Zuckerman 4 [65]) [65]
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<56> *Avian retrovirus IC10* [169, 170]
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<86> *Gallus gallus* [244]

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<105> *Rous sarcoma virus* (strain H-19 [317]) [317]
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<122> *Rous sarcoma virus* [345]
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<125> *avian retrovirus RPL30* [348]
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<322> *eukaryota* (overview [667]; e.g. plants, fungi, protozoa, vertebrates [669, 678]; ubiquitous [677]; Schizosaccharomyces pombe [677]; pig [678]) [667, 669, 671, 677, 678, 696, 705, 706-713]
<323> *prokaryote* (overview [667]) [667, 669, 671]
<324> *cellular organism* [668, 670, 672-676, 679-695, 697-704]

3 Reaction and Specificity

Catalyzed reaction

ATP + protein tyrosine = ADP + protein tyrosine phosphate (<322> regulation of mitosis, differentiation, migration, neovascularization, and apoptosis [706]; PTKs are closely related with cell growth, proliferation, differentiation and signalling of the immune system)

Reaction type

phospho group transfer

Natural substrates and products

S ATP + Src protein <145> (<145>, MATK can phosphorylate the carboxyl-terminal conserved tyrosine of the Src protein [394]) (Reversibility: ? <145> [394]) [394]
P ADP + Src protein phosphate
S ATP + phospholipase C γ <60> (<60>, growth factor-induced tyrosine phosphorylation of PLC γ is essential for stimulation of phosphatidylinositol hydrolysis in vitro and in vivo [191]) [191]
P ADP + phosphorylated phospholipase C γ <60> [191]
S ATP + protein <139, 140, 167, 168> (<139>, phosphorylates the regulatory C-terminal tyrosine residue present on cytoplasmic tyrosine kinases of the Src family [383]; <140>, CSK phosphorylates other members of the src-family of tyrosine kinases at their regulatory carboxy-terminus [385]; <167>, phosphorylates and inactivates the actin binding/depolymerizing factor cofilin and induces actin cytoskeletal changes [441]; <168>, GST-Limk1-fusion protein can autophosphorylate on serine, tyrosine and threonine residues in vitro [448]) (Reversibility: ? <139, 140, 167, 168> [383, 385, 441, 448]) [383, 385, 441, 448]
P ADP + protein tyrosine phosphate
S ATP + protein <140> (<140>, CSK phosphorylates other members of the src-family of tyrosine kinases at their regulatory carboxy-terminus. By regulating the activity of these kinases, CSK may play an important role in cell growth and development [385]) (Reversibility: ? <140> [385]) [385]
P ?
S ATP + protein tyrosine <116, 120, 123> (<116>, autophosphorylated on tyrosine and also mediated tyrosine phosphorylation of casein [329]; <120>, p135tyk2 tyrosine kinase directly binds and tyrosine phosphorylates α subunit of the type I IFN receptor, IFN-R [341]; <123>, specifically phosphorylates Tyr527 of p60c-src from neonatal rat brain, specifically phosphorylates a negative regulatory site of p60c-src [346]) (Reversibility: ? <116, 120, 123> [329, 341, 346]) [329, 341, 346]
P ADP + protein tyrosine phosphate
S ATP + protein tyrosine <260> (<260>, phospholipase C-γ 1 is directly phosphorylated by TrkB [615]) (Reversibility: ? <260> [615]) [615]
P ADP + protein tyrosine phosphate <260> [615]

S ATP + protein tyrosine <260> (<260>, phospholipase C-γ 1 is directly phosphorylated by TrkB [615]) (Reversibility: ? <260> [615]) [615]
P ADP + protein tyrosine phosphate <260> [615]
S ATP + protein tyrosine <324> (regulation of cellular function, <324> [668]; signalling, <324> [668, 688, 692]; role in development of cancer, diabetes, <324> [668]; role in cytokine signalling, <324> [685]) [668, 685, 688, 692]
P ADP + protein tyrosine phosphate
S Additional information <10, 13, 14, 15, 23, 24, 31> (<13>, mutations in the gene encoding the Drosophila tyrosine kinase Abelson substantially enhanced the severity of the CNS phenotype of armadillo mutations, consistent with these proteins functioning co-operatively at adherens junctions in both the CNS and the epidermis [31]; <14>, enzyme plays a critical role in a variety of signal transduction pathways [40]; <15>, associated with breast cancer and osteoporosis [45]; <23>, the erB oncogene efficiently transforms erythroblasts [57]; <23>, transformation of fibroblasts and transformation of erythroid cells [58]; <24>, oncogene v-fps induces fibrosarcomas in birds [59]; <31>, amplification of the c-erb-B-2 gene in a salivary adenocarcinoma and a gastric cancer cell line MKN-7 suggests that its over-expression is sometimes involved in the neoplastic process [69]) [23, 29, 31, 40, 45, 57, 58, 59, 69]
P ?
S Additional information <155> (<155>, ZAP-70 that associates with T cell antigen receptor zeta chain and undergoes tyrosine phosphorylation following TCR stimulation [411]; <155>, tandem ZAP-70 SH_2 domains bind phosphorylated, but not nonphosphorylated, T cell antigen receptor zeta cyt. The NH_2-terminal ZAP-70 SH_2 domain also binds to T cell antigen receptor ζ cyt but with 100-fold lower affinity. No binding is observed with the COOH-terminal ZAP-70 SH_2 domain. Similar studies demonstrate that the ZAP-70 tandem SH_2 domain can bind a T cell antigen receptor ζ 3 TAM peptide in which both tyrosine residues are phosphorylated: Little or no binding is observed with peptides phosphorylated at only one tyrosine residue, or a nonphosphorylated peptide. Binding of the tandem SH_2 domains to the other two TCR ζ TAM peptides and to a CD3 ε TAM peptide is also observed. All four doubly tyrosine phosphorylated TAM peptides cross-compete with each other for binding to the tandem SH_2 domains of ZAP-70. The affinity of these peptides for the tandem SH_2 construct demonstrates a hierarchy of TAM ζ 1, TAM ζ 2, TAM ε, TAM ζ 3 [413]) [411, 413]
P ?
S Additional information <138, 142, 143, 145, 151, 155, 159, 161, 162, 163, 164, 167, 168, 172, 179, 181, 184, 185> (<184>, increased c-met expression indicates that this gene may participate in the healing process of gastric mucosa after injury [472]; <184>, tyrosine kinase receptor for hepatocyte growth factor [470]; <167>, LIMK may be involved in developmental or oncogenic processes through interactions with LIM-containing proteins [443]; <138>, involved in the proliferation of early progenitor/stem cells

[378]; <138>, enzyme is implicated in control of cell growth [379]; <138>, Srcasm may help promote Src family kinase signaling in cells [380]; <142>, Ntk may play an inhibitory role in the control of T-cell proliferation [390]; <142>, Ctk is involved in the regulation of neural function and differentiation of male germ cells through interactions with members of the Src family kinases [391]; <143>, Batk may function as a brain-specific regulator of kinases involved in the development and maintenance of the nervous system [392]; <145>, enzyme plays a pivotal role in cell signal transduction [395]; <145>, HYL plays a significant role in the signal transduction of hematopoietic cells [397]; <148>, Rlk has potential functions in intrathymic T cell development and mature T cell signaling [402]; <151>, may have a role in human cancer [406]; <155>, autosomal recessive form of severe combined immunodeficiency disease in which ZAP-70 is absent as a result of mutations in the ZAP-70 gene. This absence is associated with defects in TCR signal transduction. Integral role in T cell activation and differentiation [412];<156>, activating motif occurs in four discrete steps: binding of p59fyn, phosphorylation of the motif, binding of ZAP-70, and activation of ZAP-70 kinase activity [414]; <159>, perinatal lethality and blocked B-cell development in mice lacking the tyrosine kinase Syk. Syk-deficient radiation chimaeras fail to accumulate mature B cells, Syk-deficient mice show impaired development of thymocytes using the V γ 3 variable region gene, Syk is not required for signalling through the IL-2 and G-CSF receptors [422]; <161>, may play an important role in thymopoiesis, role in controlling cellular growth and differentiation [425]; <162>, effector of phosphatidylinositol 3'-kinase and is involved in interleukin 6-induced neuroendocrine differentiation of prostate cancer cells [426]; <162>, may play a role in the growth and differentiation of hematopoietic cells [427]; <163>, role for JAK1 kinase in the differentiation or function of a subset of CNS neurons [429]; <164>, JAK3 deficiency in a patient with severe combined immunodeficiency over 20 years after bone marrow transplantation: persistence of genetically and biochemically defective autologous B cells, associated with reconstitution of cellular and humoral immunity, suggests that integrity of the γc-JAK3 signalling pathway is not strictly required for immunoglobulin production [430]; <164>, mutations of the Janus family kinase JAK3 are responsible for autosomal recessive severe combined immunodeficiency. Abnormalities in tyrosine phosphorylation of JAK3 in response to interleukin-2 (IL-2) and IL-4 is present in all patients. In one patient carrying a single amino acid change, Glu481Gly, in the JH3 domain of JAK3, a partially conserved IL-2 responses is observed resulting in reduced but detectable levels of JAK3 and STAT5 phosphorylation. A single cysteine to arginine substitution, Cys759Arg, results in high basal levels of constitutive JAK3 tyrosine phosphorylation unresponsive to either downregulation by serum starvation or cytokine-mediated upregulation [431]; <164>, JAK3 splice isoforms are functional in JAK3 signaling and may enrich the complexity of the intracellular response functional in IL-2 or cytokine signaling [433]; <164>, mutations of Jak-3 gene in patients with autosomal severe com-

bined immune deficiency [434]; <164>, JAK3 deficiency in humans results in autosomal recessive T-B+ severe combined immunodeficiency disease [435]; <167>, LIM-kinase1 hemizygosity is implicated in impaired visuospatial constructive cognition [442]; <167>, LIMK1 may be particularly relevant when explaining cognitive defects observed in WS patients [444]; <168>, Kiz-1 may play distinct roles in dividing cells and in differentiated neurons [445]; <168>, Williams syndrome is a complex neurodevelopmental disorder arising from a microdeletion at Chr band 7q11.23, which results in a hemizygous condition for a number of genes, LIMK1, WBSCR1, and RFC_2 [447]; <172>, important role in gestational growth and differentiation [453]; <172>, possible role for repulsive B-class Eph receptor/ligand interactions in constraining the orientation of longitudinal axon projections at the ventral midline [454]; <179>, NET potentially plays important roles in human neurogenesis [463]; <181>, tyro3 may function as a novel neurotrophic factor receptor [467]; <185>, ALK plays an important role in the development of the brain [474]; <186>, receptor may be a key signal transducing component in the totipotent hematopoietic stem cell and its immediate self-renewing progeny [476]) [378, 379, 380, 390, 391, 392, 394, 397, 402, 406, 412, 414, 422, 425, 426, 429, 430, 431, 433, 434, 442, 443, 444, 445, 447, 453, 454, 463, 467, 470, 472, 474, 476]

P ?

S Additional information <187, 189, 190, 194, 195, 197, 202, 203, 205, 207, 208, 212, 216, 219, 220> (<187>, mrfms gene products may play a role in the normal and neoplastic growth of muscular cells [478]; <189>, ufo may function as a signal transducer between specific cell types of mesodermal origin [480]; <190>, the mouse waved-2 phenotype results from a point mutation in the EGF receptor tyrosine kinase [484]; <190>, expression of the EGF-R gene in mouse blastocysts is tightly regulated by maternal steroid hormonal status [485]; <194>, recessive Robinow syndrome, allelic to dominant brachydactyly type B, is caused by loss of ROR2 activity [493]; <194>, dominant mutations in ROR2, encoding an orphan receptor tyrosine kinase, cause brachydactyly type B [494]; <194>, distinct mutations in the receptor tyrosine kinase gene ROR2 cause brachydactyly type B [495]; <194>, mutation of the gene encoding the ROR2 tyrosine kinase causes autosomal recessive Robinow syndrome [496]; <195>, vascular dysmorphogenesis caused by an activating mutation in the receptor tyrosine kinase TIE2 [498]; <197>, tek receptor tyrosine kinase may be critically involved in the determination and/or maintenance of cells of the endothelial lineage [504]; <202>, primary function is likely to be in developmental regulation [514]; <203>, rat trkC locus encodes multiple neurogenic receptors that exhibit differential response to neurotrophin-3 in PC_{12} cells [516]; <205>, Emt/Itk is a protein tyrosine kinase required for T cell Ag receptor TCR-induced activation and development [521]; <205>, role for Tsk in early T-lymphocyte differentiation [522]; <205>, important role for this kinase in CD2 co-stimulation of T cell responses [523]; <205>, itk functions in a signal transduction pathway unique to T lym-

phocytes [524]; <207>, could be responsible for the tyrosine phosphorylation observed in a murine thymic medullary epithelial cell line E-5 following complex formation with thymocytes [526]; <208>, role for the Ron receptor in progression toward malignancy [528]; <208>, ron gene product is a specific cell-surface receptor for macrophage-stimulating protein [530]; <212>, Sky may be involved in cell adhesion processes, particularly in the central nervous system [536]; <216>, appear to play a role in B-cell-IgM and FcERI receptor signaling [109]; <219>, Cek8 suggests its involvement in cellular survival or cell-cell interactions for specific subpopulations of developing motoneurons [544]; <220>, Cek9 plays an active role in embryonic signal transduction pathways [545]) [109, 478, 480, 483, 485, 493, 494, 495, 498, 504, 514, 516, 521, 522, 523, 524, 526, 528, 530, 536, 544, 545]

P ?

S Additional information <222, 223, 224, 228, 229, 235, 236, 237, 246, 247, 250, 253, 256, 257, 259, 264, 265, 266, 269, 275, 276, 277> (<265>, may play a role in the development or function of the central nervous system [620]; <257>, receptor of neuregulin [611]; <250>, involvement of the Jak-3 Janus kinase in signalling by interleukins 2 and 4 in lymphoid and myeloid cells [600]; <235>, the neurotrophic receptor may function during early stages of neural development [574]; <222>, involved in cell-cell interactions [550]; <223>, transcriptionally induced in normal T cells by interleukin 2 stimulation, roles in T cell proliferation and differentiation [553]; <224>, required for directed tracheal cell extension [554]; <224>, essential for migration of tracheal and specific midline glial cells [555]; <228>, mutations in MERTK, the human orthologue of the RCS rat retinal dystrophy gene, cause retinitis pigmentosa [560]; <229>, sensitizes mammary epithelial cells to epidermal growth factor [562]; <229>, PTK6 may function as an intracellular signal transducer in specific tissues [563]; <236>, required maternally for the establishment of the normal array of embryonic segments [575]; <237>, essential for the pathfinding ability of expressing neurons, participates in a mechanism required for muscle attachment site selection [576]; <237>, receptor protein-tyrosine kinase involvement in key aspects of neuronal pathway recognition [577]; <237>, part of a novel signal transduction cascade involved in learning and memory [578]; <246>, tyrosine kinase may play an important function in the developing mouse [591]; <247>, important role in the formation of neuronal structures and possibly other morphogenic processes [592]; <250>, role for JAK3 in hematopoiesis and T- and B-cell development [598]; <250>, JAK3 kinase is associated with terminal differentiation of hematopoietic cells [599]; <253>, role for DDR2 in critical events during wound repair [606]; <256>, expression of neuregulins and their putative receptor ErbB3, is induced during Wallerian degeneration [609]; <259>, may play an important role in the generation of the mammalian nervous system [613]; <264>, may play a role in cell-cell interactions involved in retinotectal projections and differentiation of the central nervous system [619]; <266>, Pag may play a role in the differentiation of cranial neural crest

and other tissues [621]; <269>, Sek-1, perhaps with other Eph-related receptors, is required for interactions that regulate the segmental identity or movement of cells [624]; <273>, Dnrk may play an important role in neural development during Drosophila embryogenesis [628]; <275>, important role in neurogenesis [629]; <276>, receptor may play a role in the regulation of β-cell mass [630]; <277>, required for cartilage and growth plate development [631]) [550, 553, 555, 560, 563, 574, 575, 576, 577, 578, 591, 592, 598, 599, 600, 606, 609, 611, 613, 619, 620, 621, 624, 628, 629, 630, 631]

P ?

S Additional information <305, 306, 308> (<305>, plays important roles in cell adhesion, functions downstream of integrins, enzyme is involved in integrin-mediated cell adhesion signaling [635]; <306>, the enzyme transduces signals initiated by integrin engagement and G protein-coupled receptors. Several splice isoforms of FAK are preferentially expressed in rat brain, some of which have an increased autophosphorylation activity [636]; <308>, participate in signalling pathways activated in response to cell interaction, role for FAK in gastrulation [638]; <308>, is likely to participate in a variety of integrin-extracellular-matrix-dependent signaling events during morphogenesis [639]; <306>, activation of FadK may be an important early step in intracellular signal transduction pathways triggered in response to cell interactions with the extracellular matrix [647]) [635, 636, 638, 639, 647]

P ?

S Additional information <33, 36, 37, 38, 40, 43, 45> (<33>, the proto-oncogene c-kit is involved in signal transduction [75]; <36>, p56lck participates in lymphocyte-specific signal transduction pathways. Disturbances in lck expression may contribute to the pathogenesis of some human neoplastic diseases [78]; <36>, p56lck can positively regulate T-cell functions and it mediates at least some of the effects of CD4 and CD8 on T-cell activation [79]; <36>, enzyme is implicated in neoplastic transformation [82]; <36>, lskT-encoded protein-tyrosine kinase may aid in transducing proliferative or differentiative signals unique to lymphocytes [82]; <36>, enzyme is involved in pathway for signaling through glycosyl-phosphatidylinositol (GPI)-anchored membrane proteins [86]; <36>, elevated levels of p56tck can contribute to the malignant properties of LSTRA cells [88]; <37>, involved in signal transduction by association with a number of membrane receptors [92]; <38>, receptor for an as yet unidentified growth factor [98]; <40>, implicated in the control of cell growth and differentiation [103]; <40>, enzyme functions as the cell surface receptor for the macrophage colony stimulating factor [105]; <43>, plays an important role in cell growth control [111]; <45>, cell-surface receptor for an as-yet-unknown ligand [122]; <45>, missense mutations located in the MET proto-oncogene lead to constitutive activation of the MET protein and papillary renal carcinomas [123]; <46>, Drosophila Bruton's tyrosine kinase Btk homolog is required for adult survival and male genital formation [125]; <46>, Src64 is required for the proper growth and stability of

the ovarian ring canals [126]) [75, 78, 79, 82, 86, 88, 92, 98, 103, 105, 111, 122, 123, 125, 126]

P ?

S Additional information <47, 49, 50, 57, 58, 60> (<47>, Hck is involved in a number of cell signal transduction pathways, frequently in pathways associated with immune response [132]; <47>, enzyme may serve specialized functions in hemopoietic cells, it is possible that damage to HCK may contribute to the pathogenesis of some human leukemias [134]; <47>, participates in signal transduction events regulating the growth, differentiation and function of phagocytes [136]; <49>, Ltk is expressed at a very low level in only a few cell lines and tissues and may be the receptor for a pre-B lymphocyte growth or differentiation factor [142]; <49>, human ltk gene maps to chromosome 15, bands q13-21, a region containing the breakpoint of a recurring chromosomal abnormality in B-cell non-Hodgkin lymphomas [143]; <50>, activity of inr gene appears to be required in the embryonic epidermis and nervous system among others, since development of the cuticle, as well as the peripheral and central nervous systems are affected by inr mutations [146]; <54>, Elk tyrosine kinase may be involved in cell-cell interactions in the nervous system [164]; <57>, implicated in the control of cell growth and differentiation [103]; <57>, human piebald trait resulting from a dominant negative mutant allele of the c-kit membrane receptor gene [171]; <57>, mutations in the coding sequence of the proto-oncogene c-kit in a human mast cell leukemia cell line causing ligand-independent activation of c-kit product [172]; <57>, piebaldism results from mutations of the KIT proto-oncogene, which encodes the cellular receptor transmembrane tyrosine kinase for mast/stem cell growth factor [178]; <57>, functions as a cell surface receptor for an as yet unidentified ligand [179]; <58>, yes gene product is likely to play an important role in oogenesis or early development [180]; <60>, mutations in the fibroblast growth factor receptor-1 gene, which maps to 8p, cause one form of familial Pfeiffer syndrome. A C to G transversion in exon 5, predicting a proline to arginine substitution in the putative extracellular domain, is identified in all affected members of five unrelated PS families but not in any unaffected individuals [194]) [103, 132, 134, 136, 142, 143, 146, 164, 171, 172, 178, 179, 180, 194]

P ?

S Additional information <64, 66, 72, 73, 77, 82, 85, 88, 89, 90, 91, 92> (<64>, sevenless gene determines the fate of a single photoreceptor cell type in the eye of Drosophila [204]; <66>, malignant melanoma in Xiphophorus fish hybrids is caused by the activity of a dominant oncogene Tu which codes for a receptor tyrosine kinase [209]; <72,73>, IR-related protein is a receptor for insulin, IGF-I, IGF-II, or an as yet unidentified peptide hormone or growth factor belonging to the insulin family [216]; <77>, trkB may code for a cell surface receptor involved in neurogenesis [222]; <82>, it may be involved in key regulatory processes [237]; <85>, regulates the differentiation of spore cells [242]; <85>, a role for tyrosine phosphorylation in controlling Dictyostelium development [243]; <88>,

determination of anterior and posterior terminal structures of Drosophila embryos requires activation of two genes encoding putative protein kinases, torso and D-raf [246]; <89>, enzyme is involved in glial cell generation [248]; <90>, enzyme is required for normal eye development [249]; <91>, ephrin type-A receptor 1 may be involved in the neoplastic process of some tumors [250]; <92>, Jackson-Weiss syndrome and Crouzon syndrome are allelic with mutations in fibroblast growth factor receptor 2 [255]; <92>, FGFR2 mutations in Pfeiffer syndrome [257]) [204, 209, 216, 222, 237, 242, 243, 246, 248, 249, 250, 255, 257]

P ?

S Additional information <75, 101, 102, 103, 104, 107, 108, 109, 110, 116, 118, 119, 120, 121, 123, 126, 127, 129, 132, 133, 135, 137> (<75>, increased ERBB3 expression may play a role in some human malignancies [218]; <101>, inhibition of vascular smooth muscle cell growth through antisense transcription of a rat insulin-like growth factor I receptor cDNA [307]; <102>, receptor for the inductive signal required for vulval development [309]; <102>, receptor tyrosine kinase is necessary for the induction of a vulva, survival past the L1 stage, hermaphrodite fertility and for male spicule development [311]; <102>, acts through a conserved Ras/MAP kinase signaling pathway to induce vulval differentiation [312]; <103> may be specifically involved in the cell growth of hepatocytes or in the step of hepatocarcinogenesis [313]; <103>, participates in the signalling pathways of a broad range of cytokines [315]; <104>, gp145trkC may play an important role in mediating the neurotrophic effects of NT-3 [316]; <106>, may interact with the intracellular domain of cell surface receptors [318]; <107>, possibility that overexpression of PDGF-α receptor in high-metastatic clones may contribute to an increase in the capacity of tumor cells to generate metastases in the lung [320]; <108>, ectodermally produced PDGF A may act on the mesoderm during gastrulation and mesoderm induction establishes the tissue pattern of ligand and receptor expression [322]; <109,110>, possible function during secondary steps of tumor progression [323]; <114,115>, may play an important role during development and in signal transduction pathways [328]; <116>, may have a role in tumorigenesis [329]; <118>, plays a part in human neurogenesis, DRT gene may play a part in neuroblastoma and SCLC tumorigenesis [334]; <118>, gene plays important role in embryonic development and carcinogenesis of the stomach [335]; <119>, tk gene product likely functions as a cell surface receptor for an unidentified cellular growth factor [338]; <120>, p135tyk2 tyrosine kinase directly binds and tyrosine phosphorylates α subunit of the type I IFN receptor, IFN-R. Tyk2 protein functions as part of a receptor complex to initiate intracellular signaling in response to type I interferons [341]; <121>, element of the complex signaling network involved in the control of cell proliferation and differentiation [343]; <123>, specifically phosphorylates Tyr527 of p60c-src from neonatal rat brain. Specifically phosphorylates a negative regulatory site of p60c-src [346]; <123>, CSK is involved in regulation of src family kinases [347]; <126,127>, may be specifically involved in cell-

cell interactions regulating cell fusions that generate the hypodermis during postembryonic development [349]; <129>, may be involved in tumor progression of epithelial ovarian cancer [353]; <132>, signal transducing receptor for nerve growth factor [356]; <133>, receptors for at least two hematopoietic growth factors: the stem cell factor and the colony-stimulating factor 1 [358]; <135>, Flk-1 as a major regulator of vasculogenesis and angiogenesis [363]; <135>, may play a role in vascular development and regulation of vascular permeability [366]; <136>, enzyme is a receptor for vascular endothelial cell growth factor [368]; <137>, enzyme is crucial for B cell development. Loss of kinase activity results in the human immunodeficiency, X-linked agammaglobulinemia, characterized by a failure to produce B cells [372]; <137>, directly implicated in the pathogenesis of X-linked aγglobulinaemia [373]; <137>, deficient expression of a B cell cytoplasmic tyrosine kinase in human X-linked agammaglobulinemia [374]) [218, 280, 307, 309, 311, 312, 313, 316, 318, 320, 322, 323, 328, 329, 334, 335, 338, 341, 343, 346, 347, 349, 353, 356, 358, 363, 366, 368, 372, 373, 374]

P ?

S Additional information <97> (<97>, FGFR-4 binds acidic fibroblast growth factor with high affinity but does not bind basic fibroblast growth factor [285]; <97>, high affinity receptor for both acidic and basic fibroblast growth factor but not for keratinocyte growth factor [286]) [285, 286]

P ?

Substrates and products

S AEEEIYGEFEAKKKK + ATP <14> (Reversibility: ? <14> [39]) [39]

P ? + ADP

S ATP + phosphatidylinositol 3-kinase <57> (<57>, binding of c-kit ligand, stem cell factor SCF to c-kit receptor c-kitR is known to activate c-kitR tyrosine kinase, thereby leading to autophosphorylation of c-kitR on Tyr and to association of c-kitR with substrates such as phosphatidylinositol 3-kinase [172]) (Reversibility: ? <57> [172]) [172]

P ADP + phosphorylated phosphatidylinositol 3-kinase

S ATP + phospholipase C-γ <60> (Reversibility: ? <60> [191,195]) [191, 195]

P ADP + phosphorylated phospholipase C-γ <60> [191, 195]

S ATP + protein <199, 201, 220> (<199>, Y602 is a major site of in vitro autophosphorylation in Sek, Y596 is phosphorylated to a lower degree [510]; <201>, intrinsic autophosphorylation activity [513]; <220>, autophosphorylation [545]) (Reversibility: ? <199, 201, 220> [510, 513, 545]) [510, 513, 545]

P ADP + protein tyrosine phosphate

S ATP + protein tyrosine <222, 242, 276> (<222, 242, 260>, autophosphorylation [549, 586, 615]; <260>, phospholipase C-γ 1 is directly phosphorylated by TrkB [615]; <260>, major sites of autophosphorylation are in the region containing Tyr670, Tyr674, and Tyr675 [615]; <276>, IRS-1 and

IRS-2 proteins serve as substrates [630]) (Reversibility: ? <222, 242, 276> [549, 586, 615, 630]) [549, 586, 615, 630]
P ADP + protein tyrosine phosphate
S ATP + protein tyrosine <37, 40> (<37, 40>, protein kinase activity with specificity for tyrosine residues [89, 105]) (Reversibility: ? <37, 40> [89, 105]) [89, 105]
P ADP + protein tyrosine phosphate
S ATP + protein tyrosine <48, 57> (<48>, specific for tyrosine [139]; <57>, binding of c-kit ligand, stem cell factor SCF to c-kit receptor c-kitR is known to activate c-kitR tyrosine kinase, thereby leading to autophosphorylation of c-kitR on Tyr and to association of c-kitR with substrates such as phosphatidylinositol 3-kinase [172]) (Reversibility: ? <48, 57> [139, 172]) [139, 172]
P ADP + protein tyrosine phosphate
S ATP + protein tyrosine <76, 85> (<76>, insulin stimulates sequential autophosphorylation of Tyr1148, Tyr1152 and Tyr1153. Transition from the doubly to the triply phosphorylated forms is primarily responsible for the activation of substrate phosphorylation [219]; <85>, autophosphorylated on Ser and Thr residues [242]) (Reversibility: ? <76, 85> [219, 242]) [219, 242]
P ADP + protein tyrosine phosphate
S ATP + protein tyrosine <322-324> (some kinases are able to phosphorylate both serine/threonine and tyrosine, <322> [667]; specificity, <324> [682]; structure-function relationship, <324> [683]; in vitro specificity, <324> [689]; <322> overview of the best studied protein targets of src kinases, functions [708]) [667-669, 680, 682, 683, 685, 688-690, 692, 708]
P ADP + protein tyrosine phosphate
S denatured enolase + ATP <14> (Reversibility: ? <14> [39]) [39]
P phosphorylated denatured enolase + ADP
S Additional information <1, 4, 6, 7, 23> (<1>, autophosphorylation [1]; <4>, generalized function of the Zek1 receptor in neuronal cell ontogeny [4]; <6>, JAK2 plays a central role in non-protein tyrosine kinase receptor signaling pathways [7]; <7>, functions in neural and epithelial morphogenesis [9]; <14>, p60src, the transforming protein of Rous sarcoma virus, is a protein kinase that has a strict specificity for Tyr. The phosphorylation of cellular proteins by p60src results in transformation [36]; <14>, catalytic domain Src including the C-terminal tail autophosphorylates and efficiently phosphorylates substrate peptides and proteins. Autophosphorylation occurs by an intermolecular mechanism [39]; <23>, N-terminal portion of the enzyme is sufficient for the transformation of fibroblasts, one-third of the carboxy-terminal domain has a key role for the transformation of erythroid cells [58]) [1, 4, 7, 9, 36, 39, 58]
P ?
S Additional information <305> (<305>, in vitro autophosphorylation activity at tyrosine residues [635]) [635]
P ?

S Additional information <37, 45> (<45>, autophosphorylation [121]; <37>, interaction of the Fyn SH3 domain with the p85 subunit of PI3-kinase [96]) [96, 121]
P ?
S Additional information <53> (<53>, autophosphorylation [161]) [161]
P ?
S Additional information <92> (<92>, alternative splicing of the FGFR2 gene in the region encoding the carboxyl-terminal half of the third immunoglobulin domain determines the ligand specificity of this group of receptors [252]) [252]
P ?

Inhibitors

5'-(*p*-fluorosulfonylbenzoyl)-2'-methylanthranoyl adenosine <322> (<322> irreversible inhibitor of EGFr tyrosine kinase [712]) [712]
5'-(*p*-fluorosulfonylbenzoyl)-3'-methylanthranoyl adenosine <322> (<322> irreversible inhibitor of EGFr tyrosine kinase [712]) [712]
5'-*p*-fluorosulfonylbenzoyl adenosine <322> (<322> irreversible inhibitor of EGFr tyrosine kinase [712]) [712]
AG 1517 <322> (<322> potently inhibits ligand-induced autophosphorylation og EGF-R, downstream signal transduction events, DNA replication and cell cycle progression at micromolar concentrations [711]) [711]
CI-1033 <322> (<322> irreversible inhibitor [709]; <322> PD 183805 [712]) [709, 712]
CL-387,785 <322> (<322> irreversible inhibitor of EGFr tyrosine kinase [712]) [712]
CP-358,778 <322> (<322> reversible inhibitors of EGFr TK [712]) [712]
CPG 59326 <322> (<322> reversible inhibitors of EGFr TK [712]) [712]
EKB-569 <322> (<322> irreversible inhibitor [709]) [709]
GW 2016 <322> [709]
Iressa <322> [709]
PD 158780 <322> (<322> reversible inhibitors of EGFr TK [712]) [712]
PD 160678 <322> (<322> irreversible inhibitor of EGFr tyrosine kinase [712]) [712]
PD 168393 <322> (<322> irreversible inhibitor of EGFr tyrosine kinase [712]) [712]
PD 183805 <322> (<322> irreversible inhibitor of EGFr tyrosine kinase, CI-1033 [712]) [712]
PKI 166 <322> (<322>, a pyrrolo[2,3-d]pyrimidine derivative, a dual inhibitor of both the EGFR and the ErbB2 kinases, in vivo antitumor activity [705]) [705]
STI 571 <322> (<322>, a phenylaminopyrimidine derivative, a potent inhibitor of the Abl tyrosine kinase, PDGFR kinases and c-Kit tyrosine kinases [705]; <322> designed to inhibit ABL and BCR-ABL tyrosine kinases, inhibition through competitive ATP-binding pocket interactions [706]; <322> use un second-line therapy of small-cell lung cancer, inhibits c-kit kinase, resulting in an metabolic change of tumor cells [706]) [705, 706]

Tarceva <322> [709]
ZD 1839 <322> (<322> reversible inhibitors of EGFr TK [712]) [712]
autoinhibition <324> (discussion) [672]
erbstatin <322-324> [671, 676]
genistein <322-324> [671, 674]
herbimycin <322-324> [671, 675]
imidazolequinazolines <322> (<322> reversible inhibitors of EGFr TK, bind to a narrow hydrophobic pocket, which corresponds to the binding site for the adenine base of ATP [712]) [712]
lavendustin <322, 323> [671]
p-fluorosulfonylbenzoyl 5'-adenosine <14> (<14>, inactivates tyrosine protein kinase activity of p60src by reacting with lysine 295 [36]) [36]
pyrazoloquinazolines <322> (<322> reversible inhibitors of EGFr TK, bind to a narrow hydrophobic pocket, which corresponds to the binding site for the adenine base of ATP [712]) [712]
pyrroloquinazolines <322> (<322> reversible inhibitors of EGFr TK, to a narrow hydrophobic pocket, which corresponds to the binding site for the adenine base of ATP [712]) [712]
sulfhydryl-specific reagents <322> (<322> irreversible inhibitor of EGFr tyrosine kinase, modification of certain cysteine residues [712]) [712]
tyrphostins <322-324> (AG 126, AG 1288, <322, 323> [671, 673]; <322>, the PTK inhibitors block EGF-dependent cell proliferation [711]) [671, 673, 711]
Additional information <322> (<322> overview describing research progress in erbB family tyrosine kinase inhibition, anti-cancer activity in clinical trials, irreversible inhibition and dual inhibitors are discussed [707]; <322> clinically active inhibitors of the erbB kinases are most readily attained with compounds at the ATP site [709]; <322> PTK inhibitors in the treatment of psoriasis [711]) [707, 709, 711]
Additional information <60> (<60>, inhibitors based on an oxindole core, indolinones. Oxindole occupies the site in which theATP binds, whereas the moieties that extend from the oxindole contact residues in the hinge region between the two kinase lobes. The more specific inhibitor of FGFR1 induces a conformational change in the nucleotide-binding loop [193]) [193]
Additional information <9, 10, 14> (<9>, inhibitory role for the KIT juxtamembrane region in controlling the receptor kinase activity [11]; <10>, Abl-SH_3 domain is implicated in negative regulation of the Abl kinase by mediating protein-protein interactions [22]; <14>, completely inhibited by an excess of substrate peptide [39]) [11, 22, 39]

Activating compounds

(B1-Thr)ILP <1> (<1>, autophosphorylation of the expressed ILP receptor is half-maximally stimulated by a synthetic ILP analog, (B1-Thr)ILP, at a concentration of about 0.0005 mM [1]) [1]
IGF-I <1> (<1>, stimulates autophosphorylation of the ILP receptor [1]) [1]
T-cell receptor <322> (<322> TCR activates both Rlk/Txk and Itk of the tec kinase family [710]) [710]

betacellulin <230> (<230>, activates [565]) [565]
heparin-binding EGF-like growth factor <230> (<230>, activates [565]) [565]
neuregulins <230> (<230>, activates [565]) [565]
neurotrophin-3 <232> (<232>, activates [568]) [568]
Additional information <155> (<155>, tyrosine phosphorylation and association of ZAP-70 with zeta require the presence of src family PTKs [411]) [411]
Additional information <205> (<205>, protein-tyrosine kinase ITK/EMT is associated with CD28 and becomes tyrosine-phosphorylated and activated within seconds of CD28 ligation [520]) [520]
Additional information <250> (<250>, Jak-3 activation requires the serine-rich, membrane-proximal domain of the interleukin-2 receptor β-chain, but does not require the acidic domain that is required for association and activation of Src family kinases [600]) [600]
Additional information <3, 10, 28> (<3>, the Eek receptor can be activated by at least three different GPI-linked ligands [3]; <10>, Abl-SH_3 domain is implicated in negative regulation of the Abl kinase by mediating protein-protein interactions. High-affinity SH3 ligands can compete for these interactions and specifically activate the Abl kinase [22]; <28>, cellular counterpart of the viral v-fms oncogene product, the c-fms product, is the receptor for colony-stimulating factor 1 of macrophages, CSF-1. CSF-1 appeares to activate the serine/threonine kinase [63]) [3, 22, 63]
Additional information <322> (<322> can be activated by phosphorylation by src family kinases [710]; <322> autophosphorylation of RTKs phosphorylation of tyrosines [713]; autophosphorylation of Tyr-416 in the src activation loop is required for maximal activity [713]) [710, 713]
Additional information <93> (<93>, the BEK receptor, like FLG, also requires an interaction with heparan sulfate proteoglycans to facilitate binding to its ligands [273]) [273]

Turnover number (min^{-1})

Additional information <14> (<14>, the overall activity k_{cat} of the catalytic domain Src, Src-CD, including the C-terminal tail for two exogenous substrates, the Src substrate peptide AEEEIYGEFEAKKKK and denatured enolase is about 10 times higher than that of wild-type Src. The k_{cat} values for phosphorylation of the Src substrate peptide are similar for the unphosphorylated and monophosphorylated Src-CD, 50 min^{-1} [39]) [39]

Specific activity (U/mg)

Additional information <324> (assay methods) [693-695]

K_m-Value (mM)

Additional information <14> (<14>, the overall activity k_{cat} of the catalytic domain Src, Src-CD, including the C-terminal tail for two exogenous substrates, the Src substrate peptide AEEEIYGEFEAKKKK and denatured enolase is about 10 times higher than that of wild-type Src. The k_{cat} values for phosphorylation of the Src substrate peptide are similar for the unphosphorylated

and monophosphorylated Src-CD, 50 min^{-1}, but the apparent K_m values differ significantly, approximately 0.003 mM and 0.010 mM, respectively [39]) [39]
Additional information <322> (<322>, K_m values for ATP of most kinases is usually in the micromolar range [709]) [709]

4 Enzyme Structure

Molecular weight

52000-62000 <322> (<322> range of MW of proteins of the src group, comprising six distinct functional domains [708]) [708]
54000 <151> [406]
58000 <161> [425]
60630 <207> (<207>, calculation from nucleotide sequence [527]) [527]
68710 <144> [393]
70000 <155> [411]
72000 <159, 205> [422, 524]
73620 <146> [398]
125000 <164> [432]
130000 <100, 199, 201, 254> [306, 510, 513, 607]
140000 <195, 197, 222> [499, 503, 549]
145000 <104, 197> [316, 506]
170000 <133> [359]
180000 <230, 256> [567, 610]
Additional information <209> (<209>, c-Rmil gene encodes two proteins of 94000 Da and 95000 Da, resulting from an alternative splicing mechanism [531]) [531]
Additional information <14, 21, 24, 27, 28, 31> (<31>, nucleotide sequence analysis of the ERBB2 proton-oncogene [67]; <14>, secondary structure [40]; <21>, complete nucleotide sequence of v-mil [55]; <24>, v-fps domain in PRCII encodes a polypeptide with a molecular weight of ca. 60500 Da fused to a portion of the polyprotein encoded by the viral structural gene gag. The hybrid gag-fps polyprotein of PRCII has a molecular weight of about 98100 Da [59]; <27>, the v-fgr oncogene appears to have arisen as a result of recombinational events involving two distinct cellular genes, one coding for a structural protein and the other for a protein kinase [61]; <28>, sequence of the transforming polyprotein of the McDonough strain of feline sarcoma virus includes 231 nucleotides specifying a leader peptide, 1,377 nucleotides encoding most of the feline leukemia virus-derived gag gene, and 2,969 nucleotides representing the viral transforming gene v-fms [62]) [40, 55, 59, 61, 62, 67]
Additional information <47> (<47>, determination of secondary structure of the SH_2 domain for Hck [136]) [136]

Subunits

? <19, 23> (<19>, x * 61113, calculation from nucleotide sequence [51]; <23>, x * 67638, calculation from nucleotide sequence [58]) [51, 58]
? <241> (<241>, x * 60368, calculation from nucleotide sequence [585]) [585]
? <33, 35, 39, 41> (<41>, x * 60801, calculation from nucleotide sequence [108]; <35>, x * 73132, calculation from nucleotide sequence [77]; <39>, x * 93000, calculation from nucleotide sequence [101]; <39>, x * 93390, calculation from nucleotide sequence [101]; <33>, x * 109001, calculation from nucleotide sequence [73]) [73, 77, 101, 102, 108]
? <51, 62, 63> (<63>, x * 60000, calculation from nucleotide sequence [203]; <62>, x * 57000, calculation from nucleotide sequence [202]; <51>, x * 60911, calculation from nucleotide sequence [152]) [152, 202]
? <82> (<82>, x * 95000, calculation from nucleotide sequence [238]) [238]
dimer <208> (<208>, mature Ron protein is a heterodimer of disulfide-linked α and β chains, originated by proteolytic cleavage of a single-chain precursor of 185000 Da [528]) [528]
dimer <322> (<322> ligand binding to the extracellular portion of RTKs mediates the noncovalent oligomerization of monomeric receptors and stabilizes a dimeric configuration of the extracellular domains [713]) [713]
monomer <322> (<322> RTKs consist of an extracellular portion that binds polypeptide ligands, a transmembrane helix, and a cytoplasmic portion that posseses tyrosine kinase catalytic activity [713]) [713]
tetramer <72> (<72>, heterotetrameric receptor [215]) [215]
Additional information <270> (<270>, the recombinant receptor is not post-translationally processed into an α-sunbunit and a β-subunit [625]) [625]

Posttranslational modification

glycoprotein <40, 49, 57, 181, 184> [105, 143, 179, 465, 466, 470]
glycoprotein <98, 104, 133> (<133>, extracellular domain of FLT4 consists of 7 immunoglobulin-like loops, including 12 potential glycosylation sites [360]) [295, 316, 360]
glycoprotein <192, 197, 199, 201> (<192>, five potential N-linked glycosylation sites [486]) [486, 506, 510, 513]
glycoprotein <233> (<233>, glycosylation sites on the extracellular domain [571]) [571]
glycoprotein <28> (<28>, fourteen potential sites for glycosylation are predicted within the v-fms-encoded portion of the protein, primary translation product is rapidly glycosylated [62]) [62]
lipoprotein <138> (<138>, palmitoylation of the amino-terminal cysteine residue together with myristoylation of the amino-terminal glycine residue defines important motifs for the association of enzyme with GPI-anchored proteins [86]; <138>, Gly2, Cys3, Lys7, and Lys9 are required for efficient binding of p59fyn to the TCR and for efficient fatty acylation, myristoylation at Gly2 and palmitoylation at Cys3 [381]; <138>, palmitoylation of p59fyn is

reversible and sufficient for plasma membrane association [382]) [86, 381, 382]
phosphoprotein <100, 116> [306, 329]
phosphoprotein <155, 168> (<155>, ZAP-70 associates with T cell antigen receptor ζ chain and undergoes tyrosine phosphorylation following TCR stimulation [411]; <168>, GST-Limk1-fusion protein can autophosphorylate on serine, tyrosine and threonine residues in vitro [448]) [411, 448]
phosphoprotein <160, 167> (<160>, autophosphorylation [423]; <167>, phosphorylation is an essential regulatory feature of LIM kinase, threonine 508 and the adjacent basic insert sequences of the activation loop are required for this process [441]) [423, 441]
phosphoprotein <199, 201, 205, 220> (<199>, Y602 is a major site of in vitro autophosphorylation in Sek, Y596 is phosphorylated to a lower stoichiometry [510]; <201>, intrinsic autophosphorylation activity [513]; <205>, protein-tyrosine kinase ITK/EMT is associated with CD28 and becomes tyrosine-phosphorylated and activated within seconds of CD28 ligation [520]; <205>, CD2 stimulation also leads to tyrosine phosphorylation and activation of the Tec family kinase, EMT/ITK/TSK [523]; <220>, autophosphorylation [545]) [510, 513, 520, 523, 545]
phosphoprotein <222, 242, 249, 260> (<222>, autophosphorylation [549]; <249>, IL-3 stimulation results in the rapid and specific tyrosine phosphorylation of Jak2 [597]; <260>, major sites of autophosphorylation are in the region containing Tyr670, Tyr674, and Tyr675 [615]) [549, 586, 597, 615]
phosphoprotein <52, 53, 60> (<52>, CSF-1 receptor is phosphorylated on several different serine residues in vivo, stimulation with CSF-1 at 37°C results in rapid phosphorylation on Tyr at one major site, Tyr706, and one or two minor sites. CSF-1 receptors are capable of autophosphorylation at six to eight major sites in vitro [156]; <53>, two platelet-derived growth factor (PDGF)-dependent autophosphorylation sites in the β subunit of the human PDGF receptor (PDGF-R). The major site of phosphorylation is Tyr857. Tyr751 is a second in vivo site and the major in vitro site. Autophosphorylation in the kinase insert region triggers the binding of the activated PDGF-R to specific cellular proteins, including a PI kinase whose activity is known to be stimulated by PDGF [161]; <60>, phosphorylation of the conserved Tyr 766 of the FGF receptor is essential for phosphorylation of PLC γ and for hydrolysis of phosphatidylinositol [191]) [156, 161, 191]
phosphoprotein <76, 84, 85> (<76>, insulin stimulates sequential autophosphorylation of Tyr 1148, Tyr1152 and Tyr1153. Transition from the doubly to the triply phosphorylated forms is primarily responsible for the activation of substrate phosphorylation [219]; <84>, enzyme is likely myristoylated and regulated by phosphorylation [241]; <85>, autophosphorylated on serine and threonine residues [242]) [219, 241, 242]
proteolytic modification <208> (<208>, mature Ron protein is a heterodimer of disulfide-linked α and β chains, originated by proteolytic cleavage of a single-chain precursor of 185000 Da. Abnormal accumulation of an uncleaved single-chain protein δ-Ron of 165000 Da in gastric cancer cell line KATO-III, this molecule is encoded by a transcript differing from the full-length RON

mRNA by an in-frame deletion of 49 amino acids in the β-chain extracellular domain. The deleted transcript originates by an alternatively spliced cassette exon of 147 bp, flanked by two short introns [528]) [528]
proteolytic modification <50> (<50>, INR proreceptor of 280000 Da is processed proteolytically to generate an insulin-binding α subunit of 120000 Da and a β subunit of 170000 Da with protein tyrosine kinase domain [146]) [146]
side-chain modification <14> (<14>, pp60c-src is phosphorylated at Ser12 in vivo under certain conditions, probably by protein kinase C [35]; <14>, pp60-c-src is phosphorylated on Ser and Tyr. It is likely that these phosphorylations serve to regulate the function pp60c-src [37]; <14>, protein expressed in Schizosaccharomyces pombe is a mixture of unphosphorylated, 80%, and mono-phosphorylated, 20%, species. The mono-phosphorylated form is phosphorylated either at Tyr416 or at Tyr436 [39]) [35, 37, 39]
side-chain modification <36, 37> (<36>, palmitoylation may play an important role in the localization and function of Src family protein tyrosine kinases [84]; <36>, palmitoylation of an amino-terminal cysteine motif of protein tyrosine kinase mediates interaction with glycosyl-phosphatidylinositol-anchored proteins [86]; <37>, phosphorylation of Tyr531 in the carboxy-terminal chymotryptic peptide of the fyn protein [95]; <37>, activation by phosphorylation, p190MET kinase is constitutively phosphorylated on Tyr in a gastric carcinoma cell line GTL16 [95]) [84, 86, 95]
Additional information <142> (<142>, lacks the consensus tyrosine phosphorylation and myristoylation sites [390]) [390]
Additional information <262> (<262>, lacks a myristoylation site [617]) [617]

5 Isolation/Preparation/Mutation/Application

Source/tissue

B-cell <138, 157> [377, 416]
B-cell <49> (<49>, only one out of seven mature B cell lines expressed ltk and the in vitro maturation of pre-B into B cells is in one case accompanied by the inactivation of ltk expression [142]) [142]
B-cell <6> (<6>, levels of Jak2 protein expression increased significantly in mitogen- and anti-IgM-stimulated B cells and to a lesser degree in activated T cells [6]) [6]
B-lymphocyte <309> [641]
KATO-III cell <208> [528]
KATO-III cell <92> (<92>, the gene is amplified in stomach cancer-derived cell line KATO-III [253,256]) [253, 256]
LK63 cell <95, 107, 116, 118, 129> (<116>, pre-B cell acute lymphoblastic leukemia cell line LK63 [329]) [280, 320, 329, 335, 353]
T-cell cell <36, 223> [87, 88, 553]

T-cell cell <147, 155, 161> (<161>, expression of BLK in immature T cells [425]) [399, 411, 425]
T-cell cell <192, 205> (<192>, expression in CD3-T cells, CD4-T cells and CD8-T cells at a low level [489]) [489, 521, 524]
T-cell cell <53> (<53>, HTLV-I$^-$infected T-cells [158]) [158]
T-cell cell <6> (<6>, levels of Jak2 protein expression increased significantly in mitogen- and anti-IgM-stimulated B cells and to a lesser degree in activated T cells [6]) [6]
T-lymphocyte <205, 223, 309> (<205>, exclusively expressed in T lymphocytes [522]) [522, 552, 641]
T-lymphocyte <322> (<322> Itk and Rlk/Txk, Tec kinases are expressed in T-cells [710]) [710]
alimentary canal <262> (<262>, lining of the alimentary canal [617]) [617]
astrocyte <203> [517]
bladder <181> [464]
blastocyst <190> [485]
blastula <308> [638]
blood vessel <214> (<214>, endothelium of blood vessel [539]) [539]
bone marrow <212, 214, 250> [537, 538, 598]
bone marrow <138, 142> (<138>, selectively expressed in CD34$^+$ human bone marrow cells [378]) [378, 391]
brain <2, 4, 32, 33> [2, 4, 70, 73]
brain <104, 112> (<104>, preferentially expressed in brain, transcripts in the hippocampus, cerebral cortex, and the granular cell layer of the cerebellum [316]; <112>, expression of Cek5 in the brain gradually diminishes during embryonic development [325]) [316, 325]
brain <112, 114, 115, 118> (<112>, expression of Cek5 in the brain gradually diminishes during embryonic development [325]; <112>, Cek5 variant is expressed in the brain, but not in other tissues of the 10-day chick embryo [326]; <114,115>, of the embryo [328]; <118>, fetal [333,334]) [325, 326, 328, 333, 334]
brain <141, 142, 143, 145, 149, 168, 169, 171, 174, 175, 177, 179, 180, 181, 182, 186> (<143>, in adults primarily expressed in neurons, including those of the hippocampus and cortex. Embryos have markedly decreased expression levels. Batk may be upregulated at birth throughout the brain except in the cerebellum [392]; <168>, in adult brain Kiz-1 is expressed exclusively in neurons, not in astrocytes or oligodendrocytes. In the developing embryo, Kiz-1 is expressed in all tissues [445]; <174,179,180>, fetal brain [333,456,463]; <175>, gene is expressed by all neurons of the adult brain, but mostly in the hippocampus, cerebral cortex and large neurons of the deep cerebellar nuclei, as well as the Purkinje and granular cells of the cerebellum [457]; <177>, HTK appears to be expressed in fetal but not adult brain [459]; <181>, highest levels of tyro3 expression in neurons [467]; <182>, Sky receptor may play an important role in development, function, and maintenance of specific neuronal populations in the central nervous system [468]) [333, 389, 390, 391, 392, 397, 404, 445, 448, 449, 451, 456, 457, 459, 463, 464, 467, 468, 476]

brain <195, 197, 203, 206, 212, 220> (<195>, at a low level [499]; <203>, areas expressing high levels of trkB or trkC mRNAs included olfactory formations, neocortex, hippocampus, thalamic and hypothalamic nuclei, brainstem nuclei, cerebellum and spinal cord motoneurons [515]) [466, 499, 505, 515, 525, 534, 536, 545]
brain <221, 230, 231, 233, 243, 247, 248, 254, 257, 260, 265> (<231>, fetal brain [333]; <233>, fetal and adult [570]; <247>, expression in many parts of the developing mouse brain, in the adult brain it is expressed exclusively and abundantly in the hippocampus [593]; <257>, distinct and highly regionalized patterns of expression in the adult brain [611]) [333, 546, 567, 568, 570, 587, 592, 593, 595, 607, 611, 614, 620]
brain <306-309> (<306>, hippocamus [636]; <306>, several splice isoforms of FAK are preferentially expressed in rat brain [636]; <307>, high activity [637]) [636, 637, 639, 645]
brain <49, 54> (<49>, Ltk is expressed in adult, but not in embryonic brain, in neurons of the cerebral cortex and hippocampus, but not in the cerebellum [142]; <49>, lymphoid ltk uses a CUG translational start codon and has a 110 amino acid putative extracellular domain. The predominant ltk mRNA in brain is alternatively spliced and predicts a protein with a substantially larger extracellular part [143]) [142, 143, 144, 164]
brain <77, 79> (<77>, preferentially expressed in brain [222]) [222, 230]
breast cancer cell <31, 151, 159, 164> (<151>, breast cancer [407]) [67, 406, 407, 421, 433]
breast cancer cell <229> (<229>, breast carcinomas [562]; <229>, breast tumours [564]) [562, 564]
breast cancer cell line <151, 159, 164> (<159>, upregulated expression of syk is observed in aggressive, metastasizing mammary gland tumours but not in well differentiated, non-metastasizing tumors [421]) [406, 407, 421, 433]
breast cancer cell line <95, 107, 116, 118, 129> (<95>, markedly elevated ERBB3 mRNA levels are demonstrated in certain human mammary tumor cell lines [280]) [280, 320, 329, 335, 353]
carcinoma cell <151, 159, 164> (<151>, p54rak is overexpressed in subsets of primary human epithelial tumors [406]; <164>, hematopoietic and epithelial cancer cells [433]) [406, 407, 421, 433]
carcinoma cell line <95, 107, 116, 118, 129> (<107>, 3LL carcinoma and T10 sarcoma, overexpression of PDGF$^-\alpha$ receptor in high-metastatic clones [320]) [280, 320, 329, 335, 353]
cardiac myocyte <2> (<2>, neonatal [2]) [2]
cell culture <10, 31> (<10>, cell line K562 [23]; <31>, amplification of the c-erb-B-2 gene in gastric cancer cell line MKN-7 [69]) [23, 69]
cell culture <145, 146, 147, 148, 151, 157, 163, 164, 167, 171, 177, 179, 184, 186> (<186>, expressed in populations enriched for stem cells and primitive uncommitted progenitors [476]; <145>, expression of matk mRNA is predominantly found in cells of megakaryocytic lineage [395]; <145>, megakaryoblastic cell line UT-7 [397]; <145>, various myeloid cell lines [397]; <146>,

highly expressed in many hematopoietic cell lines [398]; <147>, T cells and some myeloid cell lines [399]; <148>, predominantly expressed within the T cell lineage [401]; <151>, cell lines of breast and colon origin [406]; <151>, hepatoma cell line Hep3B [408]; <157>, cells derived from multiple hematopoietic lineages [416]; <157>, basophilic leukemia cell line KU812 [419]; <163>, hemopoietic cell line FDC-P1 [428]; <164>, natural-killer-like cell line [432]; <164>, expressed at low levels in human umbilical vein endothelial cells, human aortic smooth muscle cells, A549, and DLD-1 human colon adenocarcinoma cells [436]; <167>, HepG2 cell [443]; <171> epidermoid carcinoma cell line A431 [451]; <177>, malignant cell lines [459]; <179>, several human tumor cell lines derived from neuroectoderm including primitive neuroectodermal tumor, small cell lung carcinoma, and neuroblastoma express NET transcripts [463]; <184>, mammary gland-derived epithelial cell line [470]) [395, 397, 398, 399, 402, 406, 408, 416, 428, 432, 436, 443, 451, 459, 463, 470, 476]
cell culture <212, 214> (<212>, premegakaryocytopoietic cell lines CMK11-5 and Dami [466]; <212>, hepatoma HepG2 [536]; <214>, acute myelogenic cell line [507]) [466, 507, 536]
cell culture <212, 208> (<212>, prominent expression in the embryonal carcinoma cell line NT2/D1 [534]; <208>, gastric cancer cell line KATO-III [528]) [528, 534]
cell culture <222, 230, 252, 254, 263> (<222>, trkE transcript is expressed at low levels by PC12 cells [547]; <222>, breast carcinoma cells [548]; <222>, epithelial ovarian cancer cell line SKOV-3 [549]; <230>, several breast carcinoma cell lines [567]; <252>, melanoma or fibroblast cell lines [603]; <263>, RBL-2H3 cells [618]) [547, 548, 549, 567, 603, 607, 608, 618]
cell culture <249> (<249>, hemopoietic cell line FDC-P1 [597]; <277,278>, induced upon neuronal differentiation of P19EC cells [632]) [597, 632]
cell culture <306, 307, 309> (<307>, astrocytes [637]; <309>, HeLa cells [645]; <309>, lymphoid cells [645]; <309>, neuroblastoma cells [645]; <307>, neurons [637]; <306>, BALB/c 3T3 cells [647]) [637, 645, 647]
cell culture <36, 44> (<36>, lymphoma cell line [82]; <36>, enzyme is expressed constitutively at a low level in normal T-cells and at an elevated level in the LSTRA and Thy19 Moloney murine leukemia virus-induced thymoma cell lines [87,88]; <44>, src-related gene is expressed in normal macrophages and in cell lines representing both the myeloid and lymphoid B-cell lineages [115]) [82, 87, 88, 115]
cell culture <48, 57> (<48>, glioblastoma cell line SW-1088 [139]; <57>, mast cell leukemia cell line HMC-1 [172]) [139, 172]
cell culture <77, 79, 92> (<77>, NIH 3T3 cells [224]; <79>, breast cancer cells SC-3 [228]; <92>, gene is amplified in stomach cancer-derived cell line, KATO-III [253,256]) [224, 228, 253, 256]
cell culture <95, 97, 106, 113, 116, 118, 120, 131, 132, 133, 137> (<95>, carcinoma cell line [281]; <95>, K-562 leukemia cells [281]; <95>, K562 erythroleukemia cells [281]; <97>, epithelial cell line B5/589 [286]; <106>, expressed

in myeloid and B-lymphoid lineage cells [319]; <113>, HeLa cells [327]; <116>, pre-B cell line LK63 [329,330]; <120>, widely expressed in hematopoietic and non-hematopoietic cell lines [342]; <120>, K-562 leukemia cells [284]; <131>, expression of the tie gene is restricted in some cell lines: large amounts of tie mRNA are detected in endothelial cell lines and in some myeloid leukemia cell lines with erythroid and megakaryoblastoid characteristics [355]; <132>, PC12 cells [356]; <133>, Wilms' tumor cell line, a retinoblastoma cell line, and a nondifferentiated teratocarcinoma cell line express FLT4. Differentiated teratocarcinoma cells are negative [360]; <137>, expressed in all stages of the B lineage and in myeloid cells [374]) [281, 284, 286, 319, 327, 329, 330, 335, 342, 355, 356, 360, 374]
central nervous system <77, 181, 305> (<181>, expression of the Tyro 3 gene is strongly up-regulated in neurons of the neocortex, cerebellum, and hippocampus after the day of birth, during periods of active synaptogenesis, and high expression is maintained in the adult CNS [465]) [222, 465, 635]
central nervous system <193> (<193>, fetal and adult human CNS, in human leukemia, lymphoma cell lines, and a variety of human cancers derived from neuroectoderm express a truncated Ror1 receptor tyrosine kinase, lacking both extracellular and transmembrane domains [492]) [492]
central nervous system <257> (<257>, expressed during central nervous system neurogenesis [100]) [100]
cerebellum <198, 230, 260> [509, 567, 614]
chondrocyte <277> [631]
cranial ganglion <77> [222]
ear <254> (<254>, inner ear, expressed in the developing and adult cochlea [607]) [607]
egg <308> (<308>, fertilized [639]) [639]
embryo <8, 96, 103, 108, 112, 114, 115, 118, 135, 136> (<103>, embryonic limb [315];<112>, expression of Cek5 in the brain gradually diminishes during embryonic development [325]; <112>, Cek5 variant is expressed in the brain, but not in other tissues of the 10-day chick embryo [326]; <114,115>, greatest levels of expression occurring in the brain [328]; <118>, embryo stomach [335]; <135>, mouse day 10 embryonic neuroepithelium and day 18 embryonic colon. Endothelial cells within the embryonic lung, spleen, liver and metanephros [364]; <135>, restricted to the vascular endothelium and the umbilical cord stroma [366]; <136>, embryonic stem cells [369]) [10, 282, 315, 322, 325, 326, 328, 335, 364, 366, 369]
embryo <141, 149, 165, 172, 173, 181> (<141>, hindbrain [388]; <149>, embryonic fibroblasts [404]; <165>, endothelium [438]; <165>, expressed during vasculogenesis and vascular differentiation in the quail embryo. flk-1 mRNA is present in the unincubated blastodisc at low levels and is largely upregulated during gastrulation at embryonic day 1. flk-1 mRNA is initially present in the entire mesoderm of day 1 embryos but from day 2 on is restricted to endothelial cells [439]; <172>, expression is not primarily re-

stricted to neuronal structures [453]; <181>, expressed during embryonic stem cell differentiation [464]; <181>, in mid-gestational embryos, DTK RNA is expressed in many tissues including brain, eye, thymus, lung, heart, gut, liver, testis and limbs [464]) [388, 404, 438, 439, 453, 455, 464]
embryo <192, 195, 197, 202, 204, 212, 214, 215, 217, 220> (<195>, strongly expressed in ES cells and later stages of embryos, but at low levels in midgestation embryos, also expressed at a low level in neural precursor cells from 10-day embryos, but at high levels in embryonic day 15 and neonatal brains [499]; <197>, expressed in early embryonic vascular system [507]; <204>, localized to the anterior neural plate in early neurula stage embryos. Later in development, XFGFR-2 expression is found in the eye anlagen, midbrain-hindbrain boundary and the otic vesicle [518]; <212>, in fetal tissues, transcripts for DTK are detected in brain, kidney, lung and heart. Prominent expression in embryonal carcinoma cell line NT2/D1 [534]; <214,215>, expressed in early embryonic vascular system [507]; <217>, in early embryos, DFR1 RNA expression, requiring both twist and snail proteins, is specific to mesodermal primordium and invaginated mesodermal cells. At later stages, putative muscle precursor cells and cells in the central nervous system express DFR1 [542]; <220>, at embryonic day 2, Cek9 immunoreactivity is concentrated in the eye, the brain, the posterior region of the neural tube, and the most recently formed somites. Later in development, Cek9 expression is widespread but particularly prominent in neural tissues [545]) [487, 499, 507, 514, 518, 534, 542, 545]
embryo <224, 235, 237, 243, 246, 259, 262, 265, 266, 272, 273, 275, 277, 278> (<224>, embryonic tracheal system [554]; <235>, expressed specifically in the developing nervous system [574]; <237>, embryonic muscles and neighboring epidermal cells [576]; <237>, expressed by a small subset of embryonic interneurons whose growth cones choose common pathways during development [577]; <243>, in 16.5 day mouse embryos, bsk is expressed predominantly in the primordial cortex of the telencephalon [587]; <246>, expressed during most, if not all, stages of embryological development beginning in the morula and blastocyst and progressing through the yolk sac and fetal liver stages. This early and consistent expression of c-mer is confirmed during in vitro differentiation of embryonic stem cells [591]; <259>, hippocampal neurons obtained from 17.5-day-old embryos [613]; <262>, sik mRNA is first detected at day 15.5 of gestation in the embryo, where it is expressed in the newly forming granular layer of the skin [617]; <265>, Xek mRNA is expressed at higher levels in the anterior and dorsal regions of embryonic stages 16, 24 and 37 [620]; <272>, dnt is expressed in dynamic patterns in the embryonic epidermis, at high level in epithelia adjacent to cells that are invaginating into the interior of the embryo, including ventral furrow, cephalic furrow, fore- and hindgut, optic lobe and tracheal pits [626]; <272>, expressed in invaginating cells during embryogenesis [627]; <273>, during embryogenesis, the Dnrk gene is expressed specifically in the developing nervous system [628]; <275>, expressed in embryonic neuronal precursor cells including neuroblasts and CNS cells [629]; <277>, mRor2 is detected mainly in the developing nervous system within broader regions and declines

after birth [632]; <278>, the mRor1 gene is expressed in the developing nervous system within restricted regions and in the developing lens epithelium. The expression of mRor1 is sustained in the nervous system and is also detected in non-neuronal tissues after birth [632]) [554, 574, 576, 577, 587, 591, 613, 617, 620, 621, 626, 627, 628, 629, 632]
embryo <305, 308, 310> (<305>, muscle-epidermis attachment site [635]; <308>, cleavage stage embryos [639]; <310>, localized to focal adhesion [642]) [635, 638, 639, 642]
embryo <41, 46> (<41>, fibroblasts [108]; <46>, pre-blastoderm embryos, maternal origin [129]) [108, 129]
embryo <50> (<50>, activity is greatest during embryogenesis [148]) [148]
embryo <77, 79, 86, 87, 88> (<77>, mid and late gestation embryos [222]; <79>, neuroepithelium of the neural tube of 10-day-old mouse embryos [230]; <88>, spatial restriction of torso activity results from a localized activation of the torso protein at the anterior and posterior egg pole [245]) [222, 230, 244, 245, 246]
embryonic stem cell <197> [505]
endothelial cell <8, 79> [10, 231]
endothelium <131, 135, 136> (<131>, endothelial cell surface receptor tyrosine kinase [355]; <135>, selectively expressed in vascular endothelium [366]) [355, 366, 367]
endothelium <162, 165> (<162>, preferentially expressed in epithelial and endothelial cells [426]) [426, 438]
endothelium <197, 214> (<197>, endothelial cells and their progenitors [503,504]; <197>, maternal decidual vascular endothelial cells [507]; <197>, gene is expressed specifically in the endothelial lineage, present in endothelial cell precursors, angioblasts, and also in endothelial cells of sprouting blood vessels [508]; <214>, endothelium of blood vessel [539]) [503, 504, 507, 508, 539]
epithelium <151, 162, 164, 166> (<162>, preferentially expressed in epithelial and endothelial cells [426]; <164>, epithelial cancer cells [433]) [406, 426, 433, 440]
epithelium <228, 262> (<228>, tissues of epithelial and reproductive origin [561]; <262>, expressed only in epithelial tissues, including the skin and lining of the alimentary canal [617]) [561, 617]
epithelium <97, 98, 102, 113> (<97>, epithelial cell line B5/589 [284]; <98>, colonic epithelium [295]; <102>, basolateral membrane domain of the epithelial vulval precursor cells [312]) [284, 295, 312, 327]
eye <163, 220> (<163>, retinal ganglion cell layer and the inner part of the inner nuclear layer. During retinal development, JAK1 protein is first detected in retinal ganglion cells and in their axons as early as embryonic day 14. Expression of JAK1 protein in amacrine cells and horizontal cells occurrs only postnatally [429]) [429, 545]
fetus <200> (<200>, expressed in definitive endoderm of the developing gut and extraembryonic endoderm of the yolk-sac from 8.5 to 14.5 days p.c. [512]) [512]

fetus <97, 133> (<97>, FGFR-4 is expressed as a 3.0 kb mRNA in the adrenal, lung, kidney, liver, pancreas, intestine, striated muscle and spleen tissues of human fetuses [285]; <133>, spleen, brain intermediate zone, and lung showing the highest levels of activity [360]) [285, 360]
fibroblast <41> [108]
forebrain <49, 266> [143, 621]
gastric cancer cell <95, 107, 116, 118, 129> (<118>, gastric cancer [335]) [280, 320, 329, 335, 353]
germ cell cancer cell <92> [253, 256]
glioblastoma cell <57> [179]
gonad <186> [475, 477]
granulocyte <47> [137]
heart <2, 6, 171, 193> [2, 8, 451, 492]
heart <230, 239> [567, 583]
heart <103, 133, 135> (<103>, faintly [313]) [313, 358, 364]
heart <309> (<309>, weak activity [645]) [645]
hematopoietic cell <70, 192, 205> [212, 375, 489]
hematopoietic cell <103, 106, 119, 135> (<135>, primitive and more mature hematopoietic cells [362]) [314, 319, 340, 362]
hematopoietic cell <138, 159, 163, 164, 186> (<163>, hemopoietic cell line FDC-P1 [428]) [378, 422, 428, 433, 475, 476]
hematopoietic cell <151, 159, 164> (<164>, hematopoietic and epithelial cancer cells [433]) [406, 407, 421, 433]
hematopoietic cell <246, 250, 251> (<246>, c-mer seems to be expressed predominantly if not exclusively in the monocytic lineage [591]; <250>, JAK3 is expressed at very low levels in immature hematopoietic cells, expression is dramatically up-regulated during terminal differentiation of these cells [599]; <251>, hematopoietic stem cells [601]) [591, 599, 601]
hematopoietic cell <47> (<47>, expression is prominent in the lymphoid and myeloid lineages of hemopoiesis. Expression in granulocytic and monocytic leukemia cells increases after the cells have been induced to differentiate [134]; <47>, primarily expressed in hematopoietic cells, particularly granulocytes [137]) [134, 135, 136, 137]
hematopoietic stem cell <192, 214> [490, 538]
hepatoma cell <103, 129> [313, 351]
hindbrain <141, 172, 199, 200, 201, 202, 269> [388, 452, 511, 624]
hippocampus <143> [392]
hypodermis <126, 127> (<126,127>, expressed during postembryonic development in the large hypodermal syncytium [349]) [349]
intestine <258, 262> (<262>, intestinal crypt cells [617]) [612, 617]
keratinocyte <199, 208, 222, 252> (<208>, foreskin keratinocytes [529]) [510, 529, 547, 603]
kidney <133, 135, 151, 171, 184, 193, 195, 212, 258> [358, 364, 406, 451, 470, 492, 499, 512, 534, 612]
kidney <101, 103> (<101>, preferentially expressed in kidney [308]; <103>, faintly expressed [313]) [308, 313, 315]

larva <46> (<46>, gene Dsrc28C is expressed predominantly during embryogenesis, in imaginal disks of third-instar larvae, and in adult females, in adult females is largely confined to nurse cells and developing oocytes [127]) [127]
leukemia cell <119, 120> (<119>, preferentially expressed in leukemias (10 out of 18 cases) with no cell lineage specificity, but none of 17 nonleukemic neoplasms expressed hltk gene [339]; <120>, K-562 leukemia cells [284]) [284, 339]
leukemia cell <140, 157> (<140>, K562 human leukemia cell line [387]; <157>, basophilic leukemia cell line KU812 [419]) [387, 419]
leukemia cell line <193, 206, 208> (<193>, fetal and adult human CNS, in human leukemia, lymphoma cell lines, and a variety of human cancers derived from neuroectoderm express a truncated Ror1 receptor tyrosine kinase, lacking both extracellular and transmembrane domains [492]; <206>, isoforms are products of abnormal alternative splicing in tumor cell lines [525]) [492, 525, 529]
leukocyte <6> (<6>, peripheral blood leukocytes [8]) [8]
limbic system <243> (<243>, expressed at high levels in the hippocampus, tenia tecta, indusium griseum, and the piriform cortex, major components of the limbic system [587]) [587]
liver <60, 151, 170, 171, 184, 186, 200> [186, 406, 450, 451, 470, 475, 512]
liver <103> (<103>, preferentially expressed [313]) [313, 315]
liver <250, 256> (<250>, fetal [598]) [598, 610]
lung <2, 77, 140, 145, 171, 184, 193, 195, 197, 198, 200, 208, 258> [2, 222, 386, 397, 451, 470, 492, 499, 506, 509, 512, 529, 612]
lung <133, 135> (<133>, bronchial epithelial cells of fetal lung [360]) [358, 360, 364]
lymphoblast <37> (<37>, IM-9 lymphoblast [95]) [95]
lymphocyte <81> [235]
lymphocyte <142, 149, 161> (<161>, B-lymphocytes [424]; <149>, expression in T lymphocytes but not in B lymphocytes [403]) [390, 403, 424, 425]
lymphocyte <34, 36> (<34>, lymphocyte-specific tyrosine protein kinase p56lck [76]) [76, 85]
lymphocyte <49> (<49>, lymphoid Ltk exhibits a dual Nexo/Ccyt and Ncyt/Cexo transmembrane topology in transfected cells [145]) [144, 145]
lymphoma cell <309> (<309> altered expression in human lymphocyte tumor cell lines may contribute to their transformed phenotype [641]) [641]
lymphoma cell line <193, 206, 208> (<193>, fetal and adult human CNS, in human leukemia, lymphoma cell lines, and a variety of human cancers derived from neuroectoderm express a truncated Ror1 receptor tyrosine kinase, lacking both extracellular and transmembrane domains [492]; <206>, isoforms are products of abnormal alternative splicing in tumor cell lines [525]) [492, 525, 529]
macrophage <44> [115]
macrophage <52> (<52>, BAC1.2F5 macrophage cell line [156]) [156]

mammary gland <178> (<178>, expression is induced during the proliferation of the mammary gland and down-regulated by its differentiation [461]) [461]
mast cell <9, 137, 205> [11, 375, 521]
megakaryocyte <160> [423]
melanocyte <212, 309> [537, 643]
melanocyte <100> (<100>, derived from the neural crest [306]) [306]
melanocyte <43> (<43>, derived from the neural crest [113]) [113]
melanoma cell <109, 110> (<109,110>, elevated levels of malignant cells [323]) [323]
mesangial cell <258> [612]
monocyte <60, 228> [184, 561]
monocyte <106> (<106>, bone marrow-derived [319]) [319]
monocyte <70> (<70>, monocyte tumor cell lines [212]; <70>, bone marrow-derived monocytic cells [213]) [212, 213]
mononuclear cell <54> (<54>, normal peripheral blood mononuclear cell [166]) [166]
motoneuron <219> [544]
muscle <77> [222]
muscle <112> (<112>, in the skeletal muscle of the thigh a sharp decrease in Cek5 expression is detected at the time of terminal muscle differentiation [325]) [325]
muscle <6, 135> (<6, 135>, skeletal muscle [8, 364]) [8, 364]
myeloid cell <192> (<192>, mature [489]) [489]
myeloid leukemia cell <121> [344]
myoblast <187> (<187>, proliferating L6 α 1 myoblasts, mrfms gene is transcriptionally active only in undifferentiated myoblasts [478]) [478]
myocyte <257> (<257>, adult ventricular myocyte [611]) [611]
natural killer cell <155, 164, 223> [411, 432, 552]
nerve <308> (<308>, cranial nerve [639]) [639]
nerve plexus <135> (<135>, which surrounds the developing neuroepithelium [364]) [364]
nervous system <38, 273> [100, 628]
nervous system <168, 185> (<168>, developing [446]; <168>, high expression levels in the spinal cord and the cranial nerve and dorsal root ganglia [448]) [446, 448, 474]
nervous system <5> (<5>, expression of chick EphA7 during segmentation of the central and peripheral nervous system EphA7 expression is restricted to prosomeres 1 and 2 in the diencephalon and all the rhombomeres in the hindbrain during segmentation stages. Later on, a superimposed pattern appears that correlates with the formation of several axonal tracts. In the somitic mesoderm, the expression correlates with segmentation and the guidance of both neural crest and motor axons through the sclerotomes [5]) [5]
neural tube <4> [4]
neural tube <220> (<220>, posterior region of neural tube [545]) [545]
neuroblastoma cell <49> (<49>, C1300 cells [144]) [144]

neuroepithelium <79> (<79>, of the neural tube of 10-day-old embryos [230]) [230]
neuron <49, 119, 143, 306, 307> [143, 340, 392, 636, 637]
neuron <212> (<212>, nuclear membrane of neurons [535]) [535]
nurse cell <46> (<46>, gene Dsrc28C is expressed predominantly during embryogenesis, in imaginal disks of third-instar larvae, and in adult females, in adult females is largely confined to nurse cells and developing oocytes [127]) [127]
olfactory bulb <89> [248]
olfactory epithelium <89> [248]
oocyte <67, 96> [210, 282]
oocyte <46> (<46>, gene Dsrc28C is expressed predominantly during embryogenesis, in imaginal disks of third-instar larvae, and in adult females, in adult females is largely confined to nurse cells and developing oocytes [127]) [127]
oocyte <62, 63> (<62,63>, gene is found in the maternal RNA pool of the oocyte [202,203]) [202, 203]
ovarian cancer cell <95, 107, 116, 118, 129> (<129>, epithelial ovarian cancer [353]) [280, 320, 329, 335, 353]
ovary <58, 77, 181, 212, 254, 265, 270> [180, 222, 464, 534, 607, 620, 625]
ovary <103> (<103>, faintly [313]) [313]
pancreas <171> [451]
pancreatic islet <276> [630]
peripheral nerve <203> [517]
peripheral nervous system <77> [222]
phagocyte <52> (<52>, the c-fms protooncogene, is selectively expressed in immature and mature mononuclear phagocytes and trophoblasts [157]) [157]
pituitary gland <230> [567]
placenta <60, 133, 171, 177, 186, 195> [187, 358, 451, 459, 475, 477, 499]
placenta <40, 42> (<40>, trophoblasts and monocytes [106]) [106, 110]
plasma cell <157> [416]
pre-B leukemia cell <6> [6]
pre-B-lymphocyte <49> (<49>, lymphoid ltk uses a CUG translational start codon and has a 110 amino acid putative extracellular domain. The predominant ltk mRNA in brain is alternatively spliced and predicts a protein with a substantially larger extracellular part [143]) [143]
pre-T-cell <192> [489]
retina <264> (<264>, QEK5 transcripts accumulate in a ventral to dorsal gradient within the retinal neuroepithelium, where its expression becomes restricted to the ganglion and bipolar cell layers. Within the tectum, QEK5 transcripts are detectable in a posterior to anterior gradient in the ventricular layer and newly formed superficial layers [619]) [619]
salivary gland <31> (<31>, 30-fold amplification of c-erbB-2 in adenocarcinoma of the salivary gland [68]; <31>, amplification of the c-erb-B-2 gene in a salivary adenocarcinoma [69]) [68, 69]

sarcoma cell line <95, 107, 116, 118, 129> (<107>, 3LL carcinoma and T10 sarcoma, overexpression of PDGF-α receptor in high-metastatic clones [320]) [280, 320, 329, 335, 353]
skeletal muscle <171, 230> [451, 567]
skeletal muscle <309> (<309>, weak activity [645]) [645]
skin <198> [509]
skin <252, 262> [603, 617]
small cell lung cancer cell <92> [253, 256]
spermatid <33> (<33>, postmeiotic male germ cells [74]) [74]
spinal cord <77, 168> [222, 448]
spinal cord <219> (<219>, brachial and lumbar segments which innervate limb muscles [544]) [544]
spinal ganglion <77> [222]
spleen <6, 149, 187, 198, 247, 250, 254, 258> [8, 404, 478, 509, 592, 598, 607, 612]
stomach <118> (<118>, embryo stomach and gastric cancer [335]) [335]
stomach <92> (<92>, gene is amplified in stomach cancer-derived cell line, KATO-III [253,256]) [253, 256]
tadpole <308> [638]
tadpole <265> (<265>, mRNA expression in the brain, brachial arches, trigeminal facial ganglion, and the retina of the swimming tadpole stage of development [620]) [620]
tail bud <308> [638]
teratocarcinoma cell <212> [537]
testis <6, 11, 54, 142, 181, 212, 247, 254> [8, 26, 164, 391, 464, 534, 592, 607]
thymocyte <250> (<250>, adult CD4-CD8-thymocytes [598]) [598]
thymus <148> (<148>, fetal thymus as early as day 13 of embryonic development as well as in adult thymus and mature resting peripheral T cells [402]) [402]
thymus <205, 207> (<205>, increase in thymus during development from neonate to adult. Tsk is expressed in day 14 fetal thymus [522]; <192>, thymic epithelial cells [489]; <207>, thymic medullary epithelial cell line E-5 [526]) [489, 522, 526]
thymus <250, 252, 254> (<250>, highest levels of JAK3 in adult, 2-week-old, and fetal thymus [598]) [598, 603, 607]
tonsil <309> (<309>, T cells and C cells [645]) [645]
trophoblast <168> (<168>, giant cell [446]) [446]
trophoblast <52> (<52>, the c-fms protooncogene, is selectively expressed in immature and mature mononuclear phagocytes and trophoblasts [157]) [157]
trunk <77> (<77>, paravertebral trunk of the sympathetic nervous system [222]) [222]
umbilical vein endothelium <195> [499]
vascular system <164> (<164>, expressed in primary human vascular cells, as well as other non-lymphoid and non-myeloid cell types [436]) [436]
vascular system <197> (<197>, early embryonic [507]) [507]
vascular system <214, 215> (<214, 215>, expressed in early embryonic vascular system [507]) [507]

vulva <102> (<102>, basolateral membrane domain of the epithelial vulval precursor cells [312]) [312]
Additional information <95, 103, 133> (<103>, tissue expression pattern of the two predominant transcripts, TecIII and TecIV: both TecIII and TecIV are expressed as early as embryonic day 10.5 in mouse development, as well as in adult and embryonic organs. The ratio of TecIV to TecIII expression is markedly reduced in adult liver and kidney tissues and d16 embryonic limb [315]; <95>, normal tissues of epithelial origin [280]) [280, 315, 360]
Additional information <146, 153, 164> (<146>, high expression of the tec gene in all of the three patients examined with myelodysplastic syndrome [398]; <153>, Spk-1 is expressed in both intact and regenerating organisms [410]; <164>, three splice variants are isolated from different mRNA sources: breast, spleen, and activated monocytes. JAK3 splice isoforms are functional in JAK3 signaling and may enrich the complexity of the intracellular responses functional in IL-2 or cytokine signaling [433]) [398, 410, 433]
Additional information <189, 189, 192, 197> (<189>, in adult animals the gene ufo is expressed in cells forming organ capsules as well as in connective tissue structures [480]; <189>, expressed in most cell lines and adult tissues examined except those of hematopoietic lineage. It is undetectable in undifferentiated teratocarcinoma cells, F9 and N Tera 2 [481]; <192>, no expression in myeloid precursors or B cell precursors [489]; <197>, in adult tissues it is expressed ubiquitously [505]; <197>, throughout development and in all organs and tissues so far examined. tie-2 is down-regulated in the adult [508]) [480, 481, 489, 505, 508]
Additional information <243> (<243>, no expression detected in adult heart, spleen, lung, liver, skeletal muscle, and kidney [587]) [587]
Additional information <307-309> (<307>, very low levels of activity in non-nervous tissue [637]; <308>, maternal FAK transcript is present in Xl eggs, with levels decreasing slightly through cleavage and early blastula stages. At early gastrulation, the FAK mRNA level becomes modestly elevated, followed by a steady decline through late gastrulation. The mRNA level undergoes a further drop at the neurula stage, then begins a steady increase through the tailbud and tadpole stages [638]; <308>, during gastrulation, FAK protein expression increases significantly and is detected in mesoderm, marginal zone ectoderm, and cells of the blastocoel roof [639]; <309>, of all organs tested the highest activity is detected in brain and the least in skeletal muscle and heart [645]) [637, 638, 639, 645]
Additional information <324> (immunohistochemical localization in the nervous system) [686]

Localization

cytoplasm <47, 82, 103, 137, 306, 307> [132, 237, 314, 315, 374, 636, 637]
cytoplasm <150, 163, 168> (<168>, in COS cells transfected with Kiz-1 complementary DNA and in the immortalized olfactory epithelial cells, Kiz-1 is found mainly in the cytoplasm, but in neurons of the adult brain, it resides also in the nucleus [445]) [405, 429, 445]

cytoplasm <322, 324> (<322> non receptor tyrosine kinases NRTKs lack an extracellular ligand-binding domain and a transmebrane-spanning region and are localized in the cytoplasm [713]) [672, 677, 713]
endoplasmic reticulum <28> (<28>, the presence of hydrophobic signal peptides within the amino-terminal leader sequence and in the middle of the v-fms-encoded moiety suggests that the transforming glycoprotein becomes oriented with its amino terminus within the lumen of the rough endoplasmic reticulum and its carboxyl terminus protruding across the membrane of the rough endoplasmic reticulum into the cytoplasm [62]) [62]
endoplasmic reticulum <49> (<49>, lymphoid Ltk exhibits a dual Nexo/Ccyt and Ncyt/Cexo transmembrane topology in transfected cells [145]) [144, 145]
intracellular <151> [408]
membrane <138, 144, 153, 165, 177, 181, 184, 185> (<138>, contains a VHS membrane association domain [380]; Gly2, Cys3, Lys7, and Lys9 are required for plasma membrane targeting of p59fyn [381]; <138>, palmitoylation of p59fyn is reversible and sufficient for plasma membrane association [382]; <153>, anchored to the plasma membrane [410]; <165, 177, 181, 184, 185>, transmembrane protein [437, 459, 464, 470, 474]) [379, 380, 381, 382, 393, 410, 437, 459, 464, 470, 474]
membrane <192, 193, 194, 197, 205, 208, 212> (<192, 193, 194, 197>, transmembrane protein [486, 487, 491, 506]; <205>, CD3ε chain induces the membrane colocalization of Emt/Itk with TCR/CD3 [521]; <212>, nuclear membrane of neurons [535]) [486, 487, 491, 493, 506, 521, 530, 535]
membrane <238, 240, 241, 242, 260, 275> (<238>, transmembrane protein [581]) [581, 584, 585, 586, 614, 629]
membrane <33, 36, 37, 40, 43, 45> (<33, 40, 43, 45>, transmembrane protein [74, 105, 111, 121]; <33>, transmembrane tyrosine protein kinase receptor [72, 73]; <33>, the transmembrane domain is encoded by the nucleotide residues 519-543 [73]) [72, 73, 74, 78, 95, 105, 111, 119, 121]
membrane <47, 48, 49, 54, 57> (<48, 49, 57>, transmembrane protein [138, 139, 140, 142, 143, 144, 179]; <47>, likely to be a peripheral membrane protein [134]) [134, 138, 139, 140, 141, 142, 143, 164, 179]
membrane <72, 78, 79, 90> (<72, 79, 90>, transmembrane enzyme [215, 231, 249]) [215, 225, 231, 249]
membrane <93, 94, 95, 98, 100, 112, 119, 126, 127, 129, 130, 135, 136> (<94, 95, 98, 112, 126, 127, 129, 130, 135, 136>, transmembrane protein [277, 278, 279, 280, 297, 325, 349, 351, 352, 354, 366, 369]; <100>, associated [306]; <119>, transmembrane protein containing a transmembrane domain but only a short, or virtually non-existent, extracellular domain [338]; <119>, unusual membrane protein lacking an extracellular domain [340]; <130>, the extracellular domain contains a cadherin-related sequence, important for Ca^{2+}-dependent homophilic binding of cadherins [354]; <131>, transmembrane protein with extracellular epidermal growth factor homology domains [355]) [273, 277, 278, 279, 280, 287, 306, 318, 325, 338, 340, 349, 351, 352, 354, 355, 366, 369]
nucleus <322, 324> [677, 679]

nucleus <168> (<168>, in neurons of the adult brain it resided in cytoplasm and also in the nucleus [445]) [445]
nucleus <82> (<82>, associated with the chromatin fraction [237]) [237]
plasma membrane <322> (<322>, the ligand binding domain and the protein kinase activity are separated by the plasma membrane [710]) [677, 711]
Additional information <47, 49, 60> (<47>, two isoforms of murine hck, generated by utilization of alternative translational initiation codons, exhibit different patterns of subcellular localization. Translation of murine p59hck initiates from a CTG codon located 21 codons 5' of an ATG that is utilized to generate p56hck. While p59hck and p56hck are associated with membranes of various murine B-lymphoid and myeloid cell lines, p59hck alone is also located in the cytosol [117]; <49>, two alternatively spliced mouse lymphocyte and brain ltk cDNAs predict small transmembrane tyrosine kinases that use CUG translational start codons and that differ upstream of their transmembrane segment. A human neuroblastoma ltk cDNA, includes a regular AUG start codon and predicts a more conventional receptor kinase with a larger N-terminal segment [144]; <47>, two isoforms of murine hck, generated by utilization of alternative translational initiation codons, exhibit different patterns of subcellular localization [117]; <60>, alternative splicing generates diverse FGF receptor isoforms [183]) [117, 144, 183]
Additional information <98, 119> (<98>, two alternative exons, IIIb and IIIc, encode the C-terminal half of Ig domain 3. The alternative splicing choice between IIIb and IIIc exons of the FGFR-3 is not strictly tissue-specific: epithelial cells show exclusively IIIb transcripts while fibroblastic cells show a mixture of IIIb and IIIc transcripts [297]; <119>, differently spliced cDNAs of human leukocyte tyrosine kinase receptor tyrosine kinase predict receptor proteins with and without a tyrosine kinase domain and a soluble receptor protein [340]) [297, 340]

Purification

<14> (catalytic domain Src including the C-terminal tail Src-CD, expressed in Schizosaccharomyces pombe [39]; crystal structure of chicken Src which is phosphorylated at Tyr527 [40]) [39, 40]
<94> [277]
<116> [329]
<230> [567]
<322> [696]
<324> (affinity purification) [697]

Crystallization

<10> (crystal structure of the Abl regulatory region containing the SH_3 and SH_2 domains [18]; crystal structure of the Abl-SH_3 domain in complex with the high-affinity peptide ligand p41 at 1.6 A resolution [22]) [18, 22]
<11> (high-resolution crystal structures of the complexes between the SH3 domains of Abl and Fyn tyrosine kinases, and two ten-residue proline-rich peptides derived from the SH_3-binding proteins 3BP-1 and 3BP-2 [25]) [25]
<15> (crystal structure of the phosphotyrosine recognition domain SH_2 of v-src complexed with tyrosine-phosphorylated peptides, X-ray crystallogra-

phy at resolutions of 1.5 and 2.0 A [46]; crystal structure of the Src SH_2 domain complexed with a high affinity 11-residue phosphopeptide, determined at 2.7 A resolution by X-ray diffraction. Crystal structure of Src SH_2 in the absence of peptide, determined at 2.5 A resolution [47]) [46, 47]
<37> (crystal structure of the conserved core of HIV-1 Nef in complex with the SH_3 domain of a mutant Fyn, tyrosine kinase [90]; high-resolution crystal structures of tyrosine kinase SH_3 domain complexed with proline-rich peptides [93]; crystal structure of the SH_3 domain [94]) [90, 93, 94]
<37> (solution structure [91]) [91]
<47> (crystal structure of the unbound SH_3 domain from hemopoietic cell kinase Hck [130]; crystal structure determined at 2.6/2.9 A resolution [135]) [130, 135]
<47> (solution structure of the human Hck SH_3 domain [132]) [132]
<60> (crystal structure of the tyrosine kinase domain of fibroblast growth factor receptor 1 determined in its unliganded form to 2.0 A resolution and in complex with with an ATP analog to 2.3 A resolution. A dimeric form of FGFR1K is observed in the crystal structure [192]; crystal structures of the tyrosine kinase domain of FGFR1 in complex with two inhibitors based on an oxindole core, indolinones [193]) [192, 193]
<61> (solution structure of the human pp60c-src SH_2 domain complexed with a phosphorylated tyrosine pentapeptide [200]) [200]
<61> (structure of a large fragment of the c-Src tyrosine kinase, comprising the regulatory and kinase domains and the carboxy-terminal tall, has been determined at 1.7 A resolution in a closed, inactive state [201]) [201]
<81> (determination of the three-dimensional solution structure of the SH_2 domain of blk kinase by nuclear magnetic resonance NMR spectroscopy [236]) [236]
<94> [277]
<116> [329]
<140> (crystal structure of the SH_3 domain of Csk (c-Src specific tyrosine kinase) has been refined at a resolution of 2.5 A, with an R-factor of 22.4% [384]) [384]
<157> (crystal structure of the tandem SH_2 domain of Syk complexed with a dually phosphorylated ITAM peptide. The structure is solved by multiple isomorphous replacement at 3.0 A resolution [415]) [415]
<157> (solution structure of the C-terminal SH_2 domain of the human tyrosine kinase Syk complexed with a phosphotyrosine pentapeptide [418]) [418]
<322> (SH_2 and SH_3 domains play a key role in regulation of catalytic activity of src kinases. Their intramolecular interactions stabilize the inactive conformational structure, the SH_3 domain interacts with the catalytic domain and linker sequences located between SH_2 and catalytic domains. The SH_2 domain interacts with phosphotyrosine at position 527 localized in the C-terminal region of the protein [708]) [708]
<322> (all receptor tyrosine kinases share a similar structure, a hydrophobic transmembrane doamin, an extracellular region, and an intracelluar, cytoplasmic region. The cytoplasmic and the catalytic domain include regulatory sites [708]) [708]

<322> (crystallization confirmed that the ATP-binding domain is an attractive target for drug design [705]) [705]
<322> (in vertebrates the proteins of the src family have similar structures [708]) [708]
<322> (most protein kinases cocrystalized with ATP or an ATP-competitive inhibitor, 60 structures have been solved so far [709]) [709]
<322> (the overall architecture of the tyrosine kinase is similar to that of the serine/threonine kinases: an amino-terminal lobe comprising a five-stranded β sheet and one α helix, and a larger carboxy-terminal lobe that is mainly α-helical [713]) [713]
<324> (comparison of structures [670]; structure of IRK i.e. fragment of cytoplasmic kinase domain of insulin receptor β-chain [6]) [670, 672]

Cloning

<3> (isolation of a full-length cDNA, encoding the mouse homologue of a previous by partially characterized Eek protein [3]) [3]
<6> [6, 7, 8]
<8> [10]
<10> (primary structure of normal abl protein is determined by sequencing the coding region of its cDNA [23]) [23]
<11> (testis-specific c-abl mRNAs arise as a result of 3' truncation, and the v-abl gene has arisen from its cellular homologue as a result of an extensive deletional/mutational process [26]) [26, 27]
<13> (abl gene is highly conserved through evolution [29]) [29]
<14> (catalytic domain Src including the C-terminal tail Src-CD, expressed in Schizosaccharomyces pombe [39]; analysis of cDNAs of the proto-oncogene c-src [33]) [33, 39]
<20> (determination of the entire nucleotide sequence of the molecularly cloned DNA of Fujinami sarcoma virus [53]) [53]
<31> (potential cell surface receptor of the tyrosine kinase gene family identified and characterized by molecular cloning [66]) [66, 69]
<32> (isolation of a c-src cDNA clone [70]) [70]
<33> (cloning of a novel c-kit mRNA of 3.2 kb expressed in postmeiotic male germ cells [74]; determination of the primary structure of murine c-kit from a DNA clone isolated from a brain cDNA library [73]) [73, 74]
<34> [76]
<36> (lskT gene is rearranged and overexpressed in the murine T cell lymphoma LSTRA [85]) [85, 88]
<37> (gene syn identified in the human genome on chromosome 6 and characterized by molecular cloning [97]) [97]
<39> (analysis of c-fes cDNA [101]; <39>, complete nucleotide sequence of a human DNA fragment of approximately 13 kbp, which contains the entire v-fes/fps cellular homolog [101]) [101]
<41> (c-yes cDNA clones are obtained from poly(A)+ RNA of human embryo fibroblasts [108]) [108]

<42> (cDNAs encoding two forms of the LYN protein tyrosine kinase, expressed in rat mast cells and human myeloid cells [109]; lyn gene is located on human chromosome 8 q13-qter [110]) [109, 110]
<44> (isolation of cDNA [115]; two isoforms of murine hck, generated by utilization of alternative translational initiation codons [117]) [115, 117]
<48> (isolation and characterization of human ROS1 cDNA [139]) [139]
<50> [146, 147]
<52> (the c-fms clone is inserted into a retroviral vector containing a neomycin resistance gene and cell lines resistant to G418 are isolated, that express the protein [155]) [155]
<53> (PDGF receptor expressed in CHO cells [159]; expression in baby hamster kidney cells [160]) [159, 160]
<54> [163]
<54> (isolation and sequencing of cDNA clones [165]) [165]
<54> (the gene for c-fgr is located on chromosome 1 [167]) [167]
<57> [174]
<57> (localized to chromosome 4 [179]) [179]
<58> (isolation of cDNA [180]) [180]
<60> (cloning of cDNA and expression of a biologically active extracellular domain in a baculovirus system [190]; isolation of cDNA [184,188]) [182, 184, 188, 190]
<62> (expression in yeast [202]) [202]
<63> (isolation of cDNA [203]) [203]
<70> [212, 213]
<72> (isolation of a full-length human IRR cDNA [215]) [215]
<75> [218]
<77> (leucine-rich motifs in TrkB are essential for ligand binding and signaling [223]) [223]
<78> (gene is located on chromosome 6 [225]) [225]
<79> (expression in CHO cells [229]) [228, 229]
<79> (fibroblast growth factor receptor 1 gene generates multiple messenger RNAs containing two open reading frames via alternative splicing [227]) [227]
<80> (PDGF receptor gene is localized to chromosome 4q11-4q12 [234]) [234]
<80> (expression in CHO cells [232,234]) [232, 234]
<82> (isolation and sequence analysis of the gene [238]) [238]
<83> (isolation and structural analysis of murine c-fes cDNA clones [240]) [240]
<89> [247, 248]
<91> [250]
<91> (gene contains 18 exons [251]) [251]
<92> (overexpression of the TK14 protein by transfection of COS-1 cells with the corresponding cDNA in a simian virus 40-based expression vector [254]; expression in COS cells [265]) [182, 253, 254, 265]

<92> (the K-sam gene expresses multiple sizes of mRNAs in brain tissue, the immature teratoma cell line NCC-IT, and KATO-III. There are at least four classes of K-sam mRNAs [256]) [256]
<93> (bek cDNA expression in receptor-negative Chinese hamster ovary cells and in 32D myeloid cells [273]) [273, 275]
<93> (expression cDNA cloning of the KGF receptor by creation of a transforming autocrine loop [274]) [274]
<93> (identification of the gene [272]) [272]
<94> [277]
<95> (expression in COS cells [281]) [281]
<97> (expression in COS cells [285]) [285]
<97> (genomic structure [283]) [283]
<98> (coronal synostosis is due to the mutation Pro250Arg in the fibroblast growth factor receptor 3 gene [284]) [284]
<98> (expression in COS cells [295]) [295]
<99> [304]
<102> [311]
<103> (Tec gene consists of 18 exons and spans more than 86 kb [315]) [315]
<103> (Tec gene is tightly linked to the c-Kit gene on chromosome 5 [314]) [314]
<106> (expression in COS cells [318]) [318, 319]
<108> (expression in COS cells [322]) [322]
<109> [323]
<113> [327]
<116> (subcloned into the expression vector CDM8 and transfected into COS cells [330]) [330]
<119> [340]
<120> (isolation and sequencing of a full length tyk2 cDNA clone [342]) [342]
<120> (mapping to chromosome 19p13.2 [342]) [342]
<121> (overexpression of axl cDNA in NIH 3T3 cells induces neoplastic transformation with the concomitant appearance of a 140-kDa axl tyrosine-phosphorylated protein. Expression of axl cDNA in the baculovirus system results in the expression of the appropriate recombinant protein that is recognized by antiphosphotyrosine antibodies [344]) [343, 344]
<123> [346]
<125> [348]
<128> (expression in COS cells [350]) [350]
<129> (cDNA cloned from an interleukin 1-stimulated hepatoma cDNA library [351]) [351]
<130> [354]
<131> [355]
<133> (cloning of a partial complementary DNA for FLT4 [358]; expression in COS-7 cells [359]) [358, 359]
<135> [362]
<136> [367]
<137> [374]

<139> [383]
<140> (isolation of cDNA encoding tyrosine kinase cyl [387]) [387]
<144> (isolation and characterization of cDNA [393]) [393]
<145> [394]
<145> (HYL gene is assigned to chromosome 19 [397]) [397]
<145> (MATK gene is located on chromosome 19 [394]) [394]
<146> (tec gene is mapped to chromosome 4p12 [398]) [398]
<147> (TXK is mapped to chromosome position 4p12 [399,400]) [399, 400]
<150> (is located at chromosome position 1q24-25 [405]) [405]
<151> [408]
<155> (isolation of a cDNA clone encoding ZAP-70 [411]) [411]
<157> (SYK locus is mapped to chromosome 9 at band q22 [416]) [416]
<157> (cloning of the cDNA for the deleted syk kinase homologous to ZAP-70 from basophilic leukemia cell line KU812, two different sized cDNA clones of syk, Syk11 and Syk41. Reverse transcribed polymerase chain reaction targeting this region shows that both forms of the polyA RNA are expressed in Jurkat cells, human peripheral leukocytes and also KU812 cells, the inserted form is dominant [419]) [419]
<157> (isolation of a full-length cDNA encoding the human homologue of Syk) [416]
<158> (sequence of a bovine c-kit proto-oncogene cDNA [420]) [420]
<161> [424, 425]
<161> (blk gene is mapped to chromosome 8 at p22-23 [424,425]) [424, 425]
<162> (COS cells transfected with a Bmx expression vector and NIH3T3 cells expressing a Bmx retrovirus contain a tyrosyl phosphorylated Bmx polypeptide [427]) [427]
<162> (the gene is located in chromosome Xp22.2 [427]) [427]
<164> [432]
<165> [437, 438, 439]
<166> (characterization of cDNA clones for the gene HTK16 [440]) [440]
<167> (identification of cDNA [443]) [443]
<168> (in COS cells transfected with Kiz-1 complementary DNA Kiz-1 is found mainly in the cytoplasm [445]) [445]
<168> (located at the distal end of mouse chromosome 5 [448]) [448]
<169> (isolation of cDNA [449]) [449]
<173> (identification of a complete Cek7 receptor protein tyrosine kinase coding sequence and cDNAs of alternatively spliced transcripts [455]) [455]
<174> [333]
<177> [459]
<177> (HTK gene is located on chromosome 7 [459]) [459]
<179> [463]
<181> (DTK gene maps to mouse chromosome 2, band F [464]) [464]
<181> (expression in NIH3T3 cells [466]) [464, 466]
<181> (isolation of mouse cDNA clones encoding Tyro 3 [465]) [465]
<182> [468]

<184> (cloning of the complete coding region of the rat HGF receptor [473]) [473]
<185> [474]
<186> (expression in COS-1 cells [475]) [475]
<187> (isolation of cDNA [478]) [478]
<188> (isolation of cDNA [478]) [479]
<189> [480, 481]
<190> (cloning and sequencing of the extracellular ligand binding domain of murine EGF-R [482]) [482]
<191> (expressed in COS cells [265]) [265]
<192> [488]
<192> (localized to chromosome 9 [487]) [487]
<195> (transfected into COS-7 cells [499]) [499]
<197> (cDNA introduced into COS cells [503]) [503, 507]
<197> (tek, which maps to mouse chromosome 4 between the brown and pmv-23 loci [504]) [504]
<198> (unique domain of the yrk protein expressed in bacteria [509]) [509]
<199> (bacterially expressed gst-Sek kinase domain fusion protein autophosphorylated exclusively on tyrosine residues [510]) [510]
<201> [513]
<203> [515]
<204> (expression of XFGFR-2 cDNA in COS1 cells [518]) [518]
<210> [532]
<212> (TYRO3 and TYRO3P are both assigned to chromosome 15q14-q25 [537]) [537]
<212> (TYRO3 gene processed pseudogene TYRO3P are cloned from human teratocarcinoma cell, bone marrow and melanocyte cDNA libraries [537]) [537]
<212> (gene for rse is localized to human chromosome 15 [466]) [466]
<214> [538, 539]
<214> (endothelial-specific gene expression directed by the tie gene promoter in vivo [539]) [539]
<216> (transfected into Chinese hamster ovary cells [540]) [540]
<217> (alternative splicing generates two distinct transcripts for the Drosophila melanogaster fibroblast growth factor receptor homolog [541]) [541]
<221> [546]
<222> (Cak gene is localized to chromosome 6 [550]) [547, 550]
<223> [552, 553]
<223> (localized to chromosome 5q31-32 [552]) [552]
<228> [561]
<229> (isolation and characterisation a cDNA [564]) [564]
<230> (isolation and recombinant expression of a cDNA clone encoding HER4 [567]) [567]
<231> [333]
<232> [568, 569]
<232> (TrkC is mapped to chromosome 15q24-q25 [568]) [568]

<233> (TRK-B gene is localized to chromosome 9q22.1 [571]) [571]
<233> (cloning of a non-catalytic form of human trkB [570]) [569, 570, 571]
<239> [583]
<240> [584]
<242> (activity is transformed in an NIH 3T3 assay [586]) [586]
<245> (application of the polymerase chain reaction to cloning [226]) [226]
<246> [591]
<247> (maps to chromosome 4 [593]) [593]
<248> [596]
<249> (application of the polymerase chain reaction to cloning [226]) [226]
<252> [604]
<252> (maps to the distal end of chromosome 2 [604]) [604]
<253> (isolation of cDNA [605]) [605]
<254> (translation of Jak2 mRNA in rabbit reticulocytes [607]) [607]
<256> (expressed in cultured NIH-3T3 mouse fibroblasts [610]) [610]
<260> [614]
<262> (expression in Escherichia coli [617]) [617]
<263> (expression in COS cells [618]) [618]
<264> [619]
<268> [623]
<270> [625]
<276> (a chimeric receptor consisting of the extracellular domain of insulin receptor and the intracellular domain of IRR is expressed in Chinese hamster ovary cells [630]) [630]
<305> [635]
<307> [637]
<308> [638, 639]
<309> [645]
<324> (expression in Escherichia coli [36]; use of Baculovirus [703]; mutant of v-fps protein-tyrosine kinase [704]) [698-704]
<310, 311> (isolation of cDNA [642,646]) [642, 646]
<67, 92> (isolation of cDNA [210,252]) [210, 252]

Engineering

E1025D <20> (<20>, the mutation causes a complete ts phenotype [52]) [52]
H559R <20> (<20>, mutation results in a partial temperature sensitivity [52]) [52]
K273R <36> (<36>, mutant protein is unable to transfer the γ-phosphate of ATP but able to bind 8-azido-ATP with an efficiency similar to that of wild-type pp56lck [81]) [81]
T508V <167> (<167>, activity is abolished [441]) [441]
Y766F <60> (<60>, the Y766F FGF receptor mutant is unable to associate with tyrosine-phosphorylate PLC γ or to stimulate hydrolysis of phosphatidylinositol. Nevertheless, the Y766F FGF receptor mutant can be autophosphorylated, and can phosphorylate several cellular proteins and stimulate DNA synthesis [191]; <60>, fibroblast growth factor receptor with the single point mutation fails to associate with PLC γ in response to FGF. The mutant

receptor also fails to mediate PtdIns hydrolysis and Ca^{2+} mobilization after FGF stimulation. However, the mutant receptor phosphorylats itself and several other cellular proteins, and it mediats mitogenesis in response to FGF [195]) [191, 195]
Additional information <167, 168, 183> (<167>, replacement of Thr508 with 2 glutamic acid residues results in a fully active enzyme [441]; <168>, mutation of residue D460 within the IHRDL motif abolishes kinase activity [448]; <183>, mutation of the receptor tyrosine kinase gene Mertk in the retinal dystrophic RCS rat [469]) [441, 448, 469]
Additional information <190, 193, 194, 195> (<190>, the mouse waved-2 phenotype results from a point mutation in the EGF receptor tyrosine kinase [484]; <193>, fetal and adult human CNS, in human leukemia, lymphoma cell lines, and a variety of human cancers derived from neuroectoderm express a truncated Ror1 receptor tyrosine kinase, lacking both extracellular and transmembrane domains [492]; <194>, recessive Robinow syndrome, allelic to dominant brachydactyly type B, is caused by loss of ROR2 activity. The identification of mutations in three distinct domains containing Frizzled-like, kringle and tyrosine kinase motifs indicates that these are all essential for ROR2 function [493]; <194>, dominant mutations in ROR2, encoding an orphan receptor tyrosine kinase, cause brachydactyly type B [494]; <194>, distinct mutations in the receptor tyrosine kinase gene ROR2 cause brachydactyly type B [495]; <194>, mutation of the gene encoding the ROR2 tyrosine kinase causes autosomal recessive Robinow syndrome [496]; <195>, R849W or the Y897S: the receptors containing either mutation show ligand-independent hyperphosphorylation, resulting in dominantly inherited venous malformations [497]) [484, 492, 493, 496, 497]
Additional information <33, 36, 37> (<33>, mutations at the W locus affect various aspects of hematopoiesis, the proliferation and migration of primordial germ cells and melanoblasts during development. The original W mutation and W37 are severe lethal mutations when homozygous. In the heterozygous state the W mutation has a weak phenotype while W37 has dominant characteristics. Wv and W41 are weak W mutations with dominant characteristics. W37, Wv and W41 are the result of missense mutations in the kinase domain of the c-kit coding sequence, E582K in W37, T660M in Wv, V831M in W41, which affect the c-kit associated tyrosine kinase to varying degrees [72]; <33>, the W42 mutation has a particularly severe effect in both the homozygous and the heterozygous states. The c-kit protein products in homozygous mutant mast cells are expressed normally but display a defective tyrosine kinase activity in vitro. Missense mutation D790N in the c-kit protein product, D790 is a conserved residue in all protein kinases [72]; <36>, generation of mutants of the protein-tyrosine kinase pp56lck that have single amino acid substitutions within the area surrounding the conserved residue Lys-273 in subdomain II. When compared with wild-type pp56lck, these mutants display profound reductions in their phosphotransfer efficiencies and small differences in their affinities for ATP [81]; <36>, temperature-sensitive mutants of the lck tyrosine protein kinase [83]; <37>, loss of the phosphor-

ylation site at Tyr531 may contribute to the transforming abilities of carboxy-terminal deletion mutants of the fyn protein [95]) [72, 75, 81, 83, 95]
Additional information <53, 57> (<53>, integration of proviral DNA into the PDGF β-receptor gene in HTLV-I-infected T-cells results in a novel tyrosine kinase product with transforming activity [158]; <57>, conversion of Asp-816 to Val in human c-kitR may be an activating mutation and responsible for the constitutive activation of c-kitR in HMC-1 cells [172]; <57>, human piebald trait resulting from a dominant negative mutant allele of the c-kit membrane receptor gene [171]; <57>, piebaldism is an autosomal dominant genetic disorder that results from Gly664Arg mutations within the tyrosine kinase domain of the c-Kit protooncogene [173]; <57>, c-kit gene mutations in three patients with piebaldism: a missense substitution Phe584-Leu, within the tyrosine kinase domain, is associated with a severe piebald phenotype, whereas two different frameshifts, within codons 561 and 642, are both associated with a variable and relatively mild piebald phenotype [177]; <57>, piebaldism results from mutations of the KIT proto-oncogene, which encodes the cellular receptor transmembrane tyrosine kinase for mast/stem cell growth factor [178]) [158, 171, 172, 173, 177, 178]
Additional information <64, 91, 92> (<64>, a single amino acid substitution in the ATP-binding site of the putative kinase domain results in the synthesis of an inactive sev protein unable to determine cell fate [204]; <91>, ephrin type-A receptor 1 may be involved in the neoplastic process of some tumors [250]; <92>, Jackson-Weiss syndrome and Crouzon syndrome are allelic with mutations in fibroblast growth factor receptor 2 [255]; <92>, FGFR2 mutations in Pfeiffer syndrome [257]; <92>, Pfeiffer's syndrome results from an S351C mutation in the fibroblast growth factor receptor-2 gene [258]; <92>, FGFR2 exon IIIa and IIIc mutations in Crouzon, Jackson-Weiss, and Pfeiffer syndromes, missense changes, insertions, and a deletion due to alternative RNA splicing [259]; <92>, Apert syndrome is characterised by syndactyly of the hands and feet, recurrent mutations of a serine-proline dipeptide, either Ser252Trp or Pro253Arg, in the linker between the IgII and IgIII extracellular immunoglobulin-like domains. A C to T mutation that predicts a Ser252Leu substitution, ascertained in a boy with mild Crouzon syndrome is also present in three clinically normal members of his family. A CG to TT mutation that predicts a Ser252Phe substitution results in a phenotype consistent with Apert syndrome. Finally, a CGC to TCT mutation that predicts a double amino acid substitution, Ser252Phe and Pro253Ser, causes a Pfeiffer syndrome variant with mild craniosynostosis, broad thumbs and big toes, fixed extension of several digits, and only minimal cutaneous syndactyly [260]; <92>, mutations in the third immunoglobulin domain of the fibroblast growth factor receptor-2 gene in Crouzon syndrome [261]; <92>, FGFR2 mutations associated with a spectrum of craniosyostosis phenotypes: tyrosine 105 to cysteine, glycine 338 to glutamic acid, serine 351 to cysteine and glycine 384 to arginine [262]; <92>, mutations in the fibroblast growth factor receptor 2 gene cause Crouzon syndrome [263]; <92>, identical mutations in the FGFR2 gene cause both Pfeiffer and Crouzon syndrome phenotypes [264]; <92>, A to G transition is found at position 886 in exon 5 of the fibroblast growth

factor receptor 2 in members of a family with Crouzon phenotype and plagiocephaly [266]; <92>, Crouzon syndrome: deletion, duplication, and point mutation within FGFR2 gene [267]; <92>, the mutations in FGFR2-associated craniosynostoses are clustered in five structural elements of immunoglobulin-like domain III of the receptor [268]; <92>, Trp290Cys mutation in exon IIIa of the fibroblast growth factor receptor 2 gene is associated with Pfeiffer syndrome [269]; <92>, mutations C934G and C937G of fibroblast growth factor receptor 2 gene in Chinese patients with Apert syndrome [270]; <92>, Apert syndrome results from localized mutations of FGFR2 and is allelic with Crouzon syndrome [271]) [204, 250, 255, 257, 258, 259, 260, 261, 262, 263, 264, 266, 267, 268, 269, 270, 271]
Additional information <98, 101, 102> (<98>, achondroplasia is defined by recurrent G380R mutations of FGFR3 [287]; <98>, a recurrent mutation in the tyrosine kinase domain of fibroblast growth factor receptor 3 causes hypochondroplasia. This mutation causes a C to A transversion at nucleotide 1620, resulting in an Asn540Lys substitution in the proximal tyrosine kinase domain [288]; <98>, frequent activating mutations of FGFR3 in human bladder and cervix carcinomas [289]; <98>, Asn540Thr substitution in the fibroblast growth factor receptor 3 tyrosine kinase domain causes hypochondroplasia [290]; <98>, G370C mutation in the FGFR3 gene in a Japanese patient with thanatophoric dysplasia [291]; <98>, FGFR3 transmembrane domain mutation, Ala391Glu, in three unrelated families with Crouzon syndrome and acanthosis nigricans, a specific skin disorder of hyperkeratosis and hyperpigmentation [294]; <98>, mutations in the gene encoding fibroblast growth factor receptor-3 in achondroplasia [296]; <98>, thanatophoric dwarfism (TD) is a sporadic lethal skeletal dysplasia with micromelic shortening of the limbs, macrocephaly, platyspondyly and reduced thoracic cavity. Identification of FGFR3 mutations in 25 of 26 thanatophoric dwarfism cases. Two novel missense mutations, Y373C and G370C, are detected in 8/26 and 1/26 thanatophoric dwarfism 1 cases respectively. Both mutations creat cysteine residues in the juxta extramembrane domain of the receptor. Sixteen cases carry the previously reported R248C mutation - 9/26 cases, S249C - 2/26 cases, or stop codon FGFR3 mutations, 5/26 cases [297]; <98>, Gly375Cys substitution in the transmembrane domain of the fibroblast growth factor receptor-3 in a newborn with achondroplasia [299]; <98>, mutation that results in the substitution of an unpaired cysteine residue in the extracellular domain of FGFR3 in thanatophoric dysplasia type I [300]; <98>, a sporadic mutation causing a Lys650Glu change in the tyrosine kinase domain of FGFR3 is found in 16 of 16 individuals with one type of thanatophoric dysplasia. Of 39 individuals with a second type of thanatophoric dysplasia, 22 have a mutation causing an Arg248Cys change and one has a Ser371Cys substitution, both in the extracellular region of the protein [301]; <98>, FGFR3 is a possible candidate for the Huntington disease gene [302]; <98>, constitutive activation of fibroblast growth factor receptor 3 by the transmembrane domain point mutation found in achondroplasia [303]; <101>, inhibition of vascular smooth muscle cell growth through antisense transcription of a rat insulin-like growth factor I receptor cDNA [307]; <102>, mutations in the

Caenorhabditis elegans let-23 EGFR-like gene [310]) [287, 288, 289, 290, 291, 294, 296, 297, 299, 300, 301, 302, 303, 307, 310]

Application

medicine <322> (<322> crystallization confirmed that the ATP-binding domain is an attractive target for drug design [705]; <322> spectrum and association with specific malignancies offer multiple targets for therapeutic intervention [706]; <322> useful drug development to block the enhanced PTK activity in many nonmalignat diseases, such as psoriasis, papilloma, restenosis and pulmonary fibrosis [711]) [705, 706, 711]

medicine <7> (<7>, may serve as a new target for the development of new antibiotics [9]) [9]

Additional information <322> (<322>, possible clinical use of STI 571 as a potent inhibitor of PDGFR and c-Kit tyrosine kinases in solid tumors [705]) [705]

6 Stability

General stability information

<322>, ligand-binding stabilizes a dimeric configuration of the extracellular domains [713]

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