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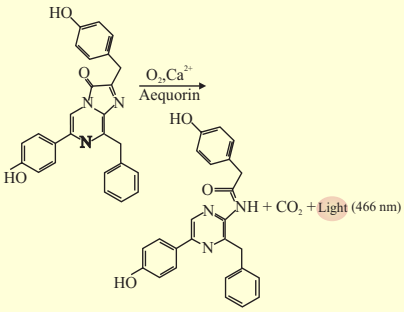


The activity of an enzyme is responsible for the glow of the luminescent jellyfish at left shown above

The enzyme aequorin catalyzes the oxidation of a compound by oxygen in the presence of calcium to release CO_2 and light.

[*Fred Bavendam/Peter Arnold*]

CHAPTER

16

Enzymes-I

Nomenclature and Classification

IMPORTANCE

Life is an intricate meshwork involving a perfect coordination of a vast majority of chemical reactions. Some of these reactions result in synthesizing large molecules, others in cleaving large molecules and all of them either utilize energy or liberate energy. All these reactions occur very slowly at the low temperatures and the atmospheric pressures—the conditions under which living cells carry on their life processes. Yet in the living cells these reactions proceed at extremely high rates. This is due to the presence of some catalysts produced and synthesized inside the body of the organisms. The term 'enzyme' was coined in 1878 by Friedrich Wilhelm Kuhne to designate these **'biological catalysts'** that had previously been called 'ferments'. As they quicken most of the chemical reactions occurring in the body, the enzymes have been designated as the "*manifestations of nature's impatience*".

In fact, Kuhne intended for the name **enzyme** to apply to both yeast ferment and the extracellular catalysts of more complex animals, *e.g., pepsin and trypsin*. Prophetically, his definition specifically implied that lower and higher forms of life are not so fundamentally different.

The name **'enzyme'** (en^G = in ; $zyme^G$ = yeast) literally means 'in yeast'. This was referred to denote one of the most noteworthy reactions wherein the production of ethyl alcohol and carbon dioxide through the agency of an enzyme, the

zymase, present in yeast takes place. This reaction is most popularly known as *alcoholic fermentation*. Sumner and Myrbäck (1950) have beautifully defined the enzymes as 'simple or combined proteins acting as specific catalysts'. They are soluble, colloidal molecules which are produced by living cells. All enzymes are globular proteins with a complex 3-'D' structure, capable of binding substrate molecules to a part of their surface. They affect the life of an organism to such an extent that life has aptly been called as "an orderly function of enzymes."

As an analogy, if one considers a living cell as a factory, the individual enzymes might be compared to the machines that work together to cause the transformation of a raw material (like steel) into parts of a finished product (like an automobile).

Difference from catalysts. Like catalysts, the enzymes do not alter the chemical equilibrium point of a reversible reaction but only the speed of the reaction is changed. They, however, differ from catalysts in being the biological products, *i.e*., produced from the living cells. Moreover, the enzymes are all protein and, unlike catalysts, cannot last indefinitely in a reaction system since they, being colloidal in nature, often become damaged or inactivated by the reactions they catalyze. Henceforth, they must be replaced constantly by further synthesis in the body. Furthermore, unlike catalysts, most individual enzymes are very specific in that they act either on a single or at the most on some structurally-related substrates.

Difference from vitamins. All enzymes are proteinaceous in nature and differ from vitamins in the fact that the latter are not synthesized by the animal cells, in contrast to the former.

Endoenyzmes and exoenyzmes. Most of the enzymes usually act within the cells in which they are produced and hence are called **intracellular enzymes** or **endoenzymes**, *e.g*. most of the plant enzymes. As these enzymes catalyze the metabolic reactions of the cell, they are also referred to as *metabolic enzymes*. On the other hand, certain enzymes which are liberated by living cells, catalyze useful reactions outside the cell in its environment and hence are known as **extracellular enzymes** or **exoenzymes**, *e.g*., enzymes found in bacteria, fungi and some insectivores like *Drosera* and *Nepenthes*. They act chiefly as digestive enzymes, catalyzing the breakdown of complex substances to simpler ones which can readily be absorbed by the cell.

LOUIS PASTEUR (LT, 1822–1895)

Louis Pasteur, a French chemist and biologist, was born on December 27,1822 in Dole France into a family of poor tanners. His father Joseph Pasteur was a sergent-major in Nepoleon's army and his valour had earned him the Legion of Honour. Louis did not seem to be particularly outstanding at school but was an excellent artist and made numerous portraits of his family. He studied at the Paris school, Ecole Normale Superieure, a school founded by Napoleon to train professors, and graduated as a Ph.D. in Sciences. In 1848, he achieved distinction in organic chemistry for his discovery that tartaric acid, a C-4 compound, forms two different types of crystals and successfully separated the two forms while looking through a microscope. He formulated a fundamental law : asymmetry differentiates the organic world from the mineral world. His work became the basis of a new science– **stereochemistry**. In January 1849, he became an Assistant Professor of Chemistry at the University of Strasborough and soon after in May, 1849 married Marie Laurent, daughter of the University principal. She often discussed his work, spurred his thinking and was one of the best scientific collaborators. The couple had 5 children, but only 2 survived to adulthood. And in September 1854, at the age of 32, he was appointed full-fledged Professor of Chemistry and Dean at the University of Lille in Northern France.

Three years later (*i.e*., in 1857), he was called back and appointed as Research Director at the Ecole Normal on the Rue d'Ulm in Paris. As a researcher, he showed

remarkable observational skill which allowed him to discover what other people looked at but did not see.

The same year, 1857, he unfolded the mystery of why local wines were turning sour. The prevailing theory held that wine fermentation results from the chemical breakdown of grape juice to alcohol. Pasteur, however, saw yeast cells under microscope and believed that yeasts played a major role in fermentation. In a classic series of experiments, he classified the role of **yeasts** in fermentation and showed that the sticks and rod-like structures (now known as bacteria) were responsible for making the wine sour. Pasteur's work also indicated that the bacteria could be a cause of disease, for if they could sour the wine, perhaps they could also make the body ill or diseased. He, henceforth, held the view that microorganisms are responsible for infectious diseases. He, thus, set down the foundation for the **germ theory of disease** and also founded the science of **Bacteriology.**

Pasteur also recommended a practical solution to the sour wine problem. He suggested that grape juice be heated to 55°C for several minutes to destroy all the evidence of life, after which yeasts could be added to begin the fermentation. Acceptance of this technique, known as **pasteurization** (which is also applied to milk), gradually ended the problem. Pasteur's elation was tempered with sadness as his daughter Jeanne died of typhoid fever in 1859.

Finally, in an elegant series of experiments using swan-neck flasks, Pasteur successfully performed his meticulous experiments in public for the Academy of Science and silenced all but the ardent supporters of spontaneous generation hypothesis. By now, Pasteur became a national celebrity. And the theory of spontaneous generation was given a burial after holding sway for 20 centuries! In 1862, he was elected to the Academy. Once again, tragedy struck him as his two-year-old daughter Camille developed a tumour and died of blood poisoning in September 1865. Pasteur, thus, realized that he was no closure to solving the riddle of disease.

The same year (*i.e.*, 1865) **cholera** engulfed Paris, killing about 200 people daily! Pasteur tried to capture the responsible bacterium by filtering the hospital air and trapping the bacterium in cotton. Unfortunately, he was unable to cultivate one bacterium apart from the others as he was using broth (Later, Robert Koch used solid culture media, instead of broth media).

In order to help the ailing French industry, Pasteur turned to pebrine, the disease of silkworms. Late in 1865, he identified a protozoan infesting the sick silkworms and the mulberry leaves fed to them. Then, he separated the healthy silkworms from the diseased ones and their food, and he managed to check the spread of disease. His success further endorsed the germ theory of disease. It was again a terrible blow to Pasteur when twelve-year-old Cecille, another of his daughters died of typhoid fever in May 1866. This diverted his attention again to the study of human diseases, and he worked still harder. He himself suffered a brain hemorrhage which left him paralyzed on the left side of his body. But all this could not keep him down for long. In 1873, he was elected to the Academy of Medicine. Later, he developed **vaccines** against chicken cholera in poultry (1880), anthrax in sheep (1881) and most notably rabies in dogs (1885). He also discovered 3 bacteria responsible for human illnesses : *Staphyllococcus, Streptococcus* and *Pneumococcus*.

In fact, Pasteur's genius reached a peak with his development of a vaccine for rabies ('rage' or 'madness' in Latin). To prepare the vaccine, he inoculated rabbits with the brain tissue of rabid animals. He then removed the spinal cords and dried them. Next, he inoculated experimental animals with 15-day-old cord tissue and followed this up the next day with 14-day-old cord tissue and so on for two weeks. Animals so treated did not develop rabies.

Pasteur was a sturdy and unitiring worker who drove himself and his subordinates

mercilessly. At heart, however, he was quite sentimental as the following event displayed. On July 6, 1885, a 9-year-old boy named Joseph Meister was brought to Pasteur. Only two days ago, the boy had suffered as many as 18 bites from a rabid dog, and physicians assured Pasteur of the imminent horrible death, to the boy. The boy's only hope was the vaccine. But Pasteur's vaccine had never been tested on humans, and the scientist, now 63, could not bring himself to make an immediate decision. In his writing, he recalls the incident :

"The child's death appeared inevitable. I decided not without acute and harrowing anxiety, as may be imagined, to apply to Joseph Meister the method I had found consistently successful with dogs."

The next day, Pasteur began the treatment. He turned the first sample of vaccine over to two physicians from the Academy of Medicine and watched as they gave 12 successive injections in 10 days of the vaccine to the terrified, crying child. Each day his concern lessened as the injections proceeded smoothly. The vaccine appeared to be working. Meister survived his ordeal and the scientist heaved a sigh of relief. When Meister left Paris for his village, Pasteur gave him stamped envelopes so that he could write often. Pasteur's viewpoint in dealing with a disease was well known. In his own words :

"When meditating over a disease, I never think of finding a remedy for it, but, instead, a means of preventing it."

The defeat of rabies was Pasteur's crowing glory. After Pasteur saved the boy Joseph Meister from the dreaded rabies in 1885, French government on November 4,1888, established the **Pasteur Institute** in Paris to treat cases of rabies, one of the world's foremost scientific establishments. The institute was inaugurated by the then President of France Sadi Carnot. Many monetary rewards were floated towards the institute, including a generous gift from the Russian government after Pasteur immunized 20 Russians against rabies. Louis continued working till 1887, when he suffered another paralytic stroke which prevented him from personally doing experimental work but his dialogue with his pupils and his collaborators never ceased. Pasteur presided over the Institute until his death on September 28, 1895 in Villeneuvel Etang. He was given a State Funeral at the Cathedral of Notre Dame and his body placed in a permanent crypt at the Pasteur Institute. He would teach his disciples :

"Do not put forward anything that you cannot prove by experimentation."

For nearly half a century, he had dominated the scientific world; for a quarter of a century, he had surged ahead despite a half-paralyzed body. Such was his genius! In later years, a grateful Meister returned to Paris as caretaker of the Pasteur Institute and in 1940 preferred commiting suicide rather than obey the demands of some occupying Hitler's Nazi soldiers to open Pasteur's crypt.

Pasteur was perhaps best known to the French nation as the **'saviour of the wine industry'** because his pasteurization salvaged an ailing industry beset with problems of microbial contamination. He is truly called as the **'father of microbiology'**.

The life of Pasteur stands as a supreme testimony to the fact that the best and most far-reaching applications of science come from passionate studies of seemingly esoteric subjects. He evolved into a prophet whose vision accelerated the progress of science. He was a true **benefactor of mankind**.

Pasteur's famous quote reads as follows :

"Dans les champs de l'observation, le hasard ne favorise que les esprits prepares."

The English transliteration is :

"In the field of observation, chance favours only prepared minds."

HISTORICAL RESUME

The history of enzymes may be regarded as commencing with the work of **Dubrunfaut** (1830) who prepared malt extract from germinating barley seeds. This extract possessed the power of converting starch into sugar. Later in 1833, **Payen** and **Persoz** prepared an enzyme, the *diastase* (now known as *amylase*), from malt extract by precipitation from alcohol. The same year, **Horace de Saussure** prepared a substance from germinating wheat which acted like diastase, *i.e*., converted starch into sugar. Within the next few years, **Theodor Schwann** succeeded in extracting *pepsin*, which digests meat (protein), from gastric juice and he later identified *trypsin*, a peptidase in digestive fluids. Hence, the notion of diastases (the early name for enzymes) was soon extended to animals. By 1837, the famous chemist **Jönes Jacob Berzelius** recognized with remarkable foresight the catalytic nature of these biological diastases.

Anselme Payen, a chemist-industrialist was the owner of the sugar factory in Paris. When he and Jean-Francois Persoz called the first known biological catalyst "diastase", they introduced the now historical use of the suffix **-ase** in the naming of most enzymes.

Later, **Pasteur** (1857) demonstrated that alcoholic fermentation was brought about by the action of living yeast cells. It, thus, became apparent that such catalytic actions could be induced by the action of living cells (as in alcoholic fermentation) or by nonliving substances (*as diastase* and *emulsin*). Obviously, such catalysts or ferments were regarded as forming two classess : **organized ferments** (like yeasts and certain bacteria) which were living cells and **unorganized ferments** (like diastase and emulsin) which acted independently of living cells.

In fact, the term 'enzyme' was later proposed for the unorganized ferments by **Kuhne**. On the contrary, **Eduard Buchner** and his brother **Hans Buchner** (1897) showed that, besides the living yeast cells, even their extract (which Eduard named as *zymase*) could bring about alcoholic fermentation. In fact, the Buchners discovered cell-free fermentation when they attempted to preserve their yeast

EDUARD BUCHNER (LT, 1860–1917)

A German biochemist, studied under Prof. Adolf von Baeyer and later became his assistant. He received the **Nobel Prize in Chemistry in 1907** "for his biochemical researches and his discovery of cell-less fermentation." In fact, Buchner, in 1897, reported that he had prepared from brewer's yeast, a cell-free press juice that caused CO_2 and ethyl alcohol to form in solutions of various sugars (sucrose, glucose, fructose, maltose). And he concluded :

"the initiation of the fermentation process does not require so complicated an apparatus as is represented by the yeast cell. The agent responsible for the fermenting action of the press juice is rather to be regarded as a dissolved substance, doubtless a protein; this will be denoted **zymase.**"

In fact, his discovery of zymase was the first proof that fermentation was caused by enzymes and did not require the presence of living cells. Edward, a major in the German army, died in action on the Romanian front (1917) during World War I.

JAMES BATCHELLER SUMNER (LT, 1887-1955)

After Sumner lost his left arm as a result of hunting at age 17, he was discouraged by his teachers from pursuing a career in Chemistry. They felt that Sumner was too handicapped for the profession. However, his illustrious career belied all this. He is truly called as the **'father of modern enzymology'.** Sumner received the **1946 Nobel Prize in Chemistry** for crystallizing the first enzymes, along with his compatriots John H. Northrop and W.M. Stancey for their work on preparation of enzymes and virus proteins in pure form.

extracts with sugar, the preservative of jellies and jams. The words—ferment and enzyme—thus became synonym and the latter is now frequently used in the literature. The use of the term ferment (*fermentare*^{L} = to agitate) is, however, misleading as it also denotes the fermenting microorganisms.

James B. Sumner (1926), at Cornell University, for the first time isolated and purified an enzyme, the *urease*, in crystalline form from jack bean (*Canavalia ensiformis*), thus confirming the proteinaceous nature of the enzymes. Since then, some 250 enzymes have been obtained in pure crystalline form such as :

(*a*) Trypsin from beef pancreas by John H. Northrop and Kunitz (1936)
(*b*) Catalase from beef liver by Sumner and Dounce (1937)
(*c*) RNase from beef pancreas by Kunitz (1940)
(*d*) Pepsin from swine stomach by John H. Northrop (1946)
(*e*) DNase from beef pancreas by Kunitz (1950)

NOMENCLATURE AND CLASSIFICATION

With the continuous increase in our knowledge of enzymology, various systems have evolved to name and classify the enzymes, using one or the other criterion as the basis. However, many of the enzymes were known before these systems of naming enzymes were adopted. The names of such enzymes were not changed under the new systems. In this category belong : bromelin, chymotrypsin, diastase, emulsin, papain, pepsin, ptyalin, rennin, trypsin etc.

A. Substrate acted upon by the enzyme. The substance upon which an enzyme acts is called the substrate. Duclaux (1883) named the enzymes by adding the suffix *-ase* in the name of the substrate catalyzed. For example, enzymes acting upon carbohydrates were named as *carbohydrases*, upon proteins as *proteinases*, upon lipids as *lipases*, upon nucleic acids as *nucleases* and so on. A few of the names were even more specific like *maltase* (acting upon maltose), *sucrase* (upon sucrose), *urease* (upon urea), *lecithinase* (upon lecithin), *tyrosinase* (upon tyrosine) etc.

B. Type of reaction catalyzed. The enzymes are highly specific as to the reaction they catalyze. Hence, this has necessitated their naming by adding the suffix-*ase* in the name of the reaction; for example *hydrolases* (catalyzing hydrolysis), *isomerases* (isomerization), *oxidases* (oxidation), *dehydrogenases* (dehydrogenation), *transaminases* (transamination), *transaldolases* (transaldolation), *transketolases* (transketolation), *phosphorylases* (phosphorylation) etc.

In fact, the consensus at that time in Europe was that enzymes were not proteins, because of the findings of the influential German chemist **Richard Willstätter** (Nobel Laureate, 1915), who, in 1920s, reported that he could not detect protein in purified enzyme preparations from yeast. With hindsight, it is realized that the protein assays used in that era were not sensitive enough to detect the small amounts present in Willstätter's purified preparations. The nonprotein nature of enzyme was so entrenched in scientific thinking that decades passed before their polypeptidyl composition was unequivocally accepted.

Although these two systems are quite simple and easy to follow, there are certain discrepancies present in them. The former system does not take into account the type of the reaction catalyzed, whereas in the latter system no idea can be derived regarding the nature of the substrate acted upon by the enzyme.

C. Substrate acted upon and type of reaction catalyzed. The names of some enzymes give clue of both the substrate utilized and the type of reaction catalyzed. For example, the enzyme *succinic dehydrogenase* catalyzes the dehydrogenation of the substrate succinic acid. Similarly, *L-glutamic dehydrogenase* indicates an enzyme catalyzing a dehydrogenation reaction involving *L-glutamic* acid.

D. Substance that is synthesized. A few enzymes have been named by adding the suffix *-ase* to the name of the substance synthesized, *viz*., *rhodonase* that forms rhodonate irreversibly from hydrocyanic acid and sodium thiosulphate, and also *fumarase* that forms fumarate irreversibly from L-malate.

E. Chemical composition of the enzyme. Based on their chemical composition, the enzymes have been classified into following three categories :

1. Enzyme molecule consisting of protein only— *e.g.*, pepsin, trypsin, urease, papain, amylase etc.
2. Enzyme molecule containing a protein and a cation— *e.g.*, carbonic anhydrase (containing Zn^{2+} as cation), arginase (Mn^{2+}), tyrosinase (Cu^{2+}) etc.
3. Enzyme molecule containing a protein and a nonprotein organic compound known as prosthetic group—Tauber (1950) has further subdivided them, on the basis of the nature of prosthetic group involved :
   (*a*) Iron prophyrin enzymes— catalase, cytochrome *c* peroxidase I and II.
   (*b*) Flavoprotein enzymes— glycine oxidase, pyruvate oxidase, histamine.
   (*c*) Diphosphothiamin enzymes — β-carboxylase, pyruvate mutase.
   (*d*) Enzymes requiring other coenzymes— phosphorylase, amino acid decarboxylase.

F. Substance hydrolyzed and the group involved.

1. Carbohydrate-hydrolyzing enzymes
   (*a*) Glycosidases—cellulase, amylase, sucrase, lactase, maltase
   (*b*) β-glucorinidase
2. Protein-hydrolyzing enzymes
   (*a*) Peptide bonds
      I. Endopeptidases
         Animals— pepsin, trypsin, rennin
         Plants—papain ficin, bromolin
      II. Exopeptidases—dipeptidase, tripeptidase
   (*b*) Nonpeptide C—N linkages (amidases)
      urease, arginase, glutaminase
3. Lipid-hyrolyzing enzymes
   lipases, esterases, lecithinases
4. Other ester-hydrolyzing enzymes
   (*a*) Phosphatases
   (*b*) Cholinesterases
   (*c*) Chlorophyllases
   (*d*) Sulfatases
   (*e*) Pectinesterases
   (*f*) Methylases
5. Oxidation-reduction enzymes
   hydrases, mutases, oxidases, dehydrogenases, peroxidases
6. Miscellaneous enzymes
   catalase, carboxylase, carbonic anhydrase, thiaminase, transpeptidase

G. Over-all chemical reaction taken into consideration. The chemical reaction catalyzed is the specific property which distinguishes one enzyme from another. In 1961, *International Union of Biochemistry* (*I.U.B.*) used this criterion as a basis for

The I.U.B. system is based on the report of the Commission on Enzymes, International Union of Biochemistry Symposium, Vol. 20, Pergamon Press, New York, 1961. In fact, the international symbolic language of chemistry was originally developed by **Jönes Jacob Berzelius**, who proposed that an element be identified by the initial letter or first two letters of its Latin name.

the classification and naming of enzymes. Although complicated, the I.U.B. system is precise, descriptive and informative.

The major features of this system of classification of enzymes are as follows :

(*a*) The reactions and the enzymes catalyzing them are divided into 6 major classes, each with 4 to 13 subclasses.

(*b*) Each enzyme name has 2 parts—the first part is the name of the substrate(s) and the second part which ends in the suffix *-ase*, indicates the type of reaction catalyzed.

(*c*) Additional information regarding the nature of the reaction, if needed, is given in parenthesis. For example, the enzyme *malate dehydrogenase* catalyzes the following reaction :

$$\text{L-malate} + NAD^+ \longrightarrow \text{Pyruvate} + CO_2 + NADH + H^+$$

This enzyme has now been designated as L-malate : NAD oxidoreductase (decarboxylating).

(*d*) Each enzyme has been alloted a systemic code number called Enzyme Commission (E.C.) number. The E.C. number for each enzyme consists of a series of numbers at 4 places : the first place numbers representing the major class to which the enzyme belongs, the two median numbers denoting the subclass and the sub-subclass of the enzyme within the major class. The last place number or the fourth digit represents the serial number of the enzyme within the sub-subclass. Thus E.C. 2.7.1.1 represents class 2 (a transferase), subclass 7 (transfer of phosphate), sub-subclass 1 (an alcohol group as phosphate acceptor). The final digit denotes the enzyme, *hexokinase* or *ATP*: *D-hexose-6-phosphotransferase*. This enzyme catalyzes the transfer of phosphate from ATP to the hydroxyl group on carbon 6 of glucose.

$$\text{ATP} + \text{D-hexose} \xrightarrow{\text{Hexokinase}} \text{ADP} + \text{Hexose-6-phosphate}$$

(*e*) Where no specific category has been created for an enzyme, it is listed with a final figure of 99 in order to leave space for new subdivisions. For example, 4.2.99 refers to "other carbon-oxygen lyases."

The 6 major classes of enzymes with some important examples from some subclasses are described below :

1. Oxidoreductases. This class comprises the enzymes which were earlier called dehydrogenases, oxidases, peroxidases, hydroxylases, oxygenases etc. The group, in fact, includes those enzymes which bring about oxidation-reduction reactions between two substrates, S and S′.

$$S_{reduced} + S'_{oxidized} \longrightarrow S_{oxidized} + S'_{reduced}$$

More precisely, they catalyze electron transfer reactions. In this class are included the enzymes catalyzing oxidoreductions of CH—OH, C═O, CH—CH, CH—NH_2 and CH═NH groups. Some important subclasses are :

1.1 Enzymes acting on CH—OH group of electron donor. For example :

1.1.1.1 Alcohol : NAD oxidoreductase

[common or trivial name, Alcohol dehydrogenase]

This enzyme catalyzes the following reaction :

$$\text{Alcohol} + NAD \longrightarrow \text{Aldehyde or Ketone} + NADH + H^+$$

1.3 Enzymes acting on CH—CH group of electron donor. For example :

1.3.2.2 Acyl-CoA : cytochrome c oxidoreductase

[Acyl-CoA dehydrogenase]

Acyl-CoA + oxidized cytochrome c ⟶ 2,3-dehydroacyl-CoA + reduced cytochrome c

1.9 Enzymes acting on the heme groups of electron donors. For example :

1.9.3.1 Cytochrome c : O_2 oxidoreductase

[Cytochrome oxidase]

4. reduced cytochrome + O_2 + $4H^+$ ⟶ 4 oxidized cytochrome c + $2H_2O$

1.11 Enzymes acting on H_2O_2 as electron acceptor. For example :

1.11.1.6 H_2O_2: H_2O_2 oxidoreductase

[Catalase]

$$H_2O_2 + H_2O_2 \rightleftharpoons 2H_2O + O_2$$

Although not an oxidizing enzyme, **catalase** is usually classified with oxidases because its action is closely connected with physiological oxidation.

2. Transferases. Enzymes which catalyze the transfer of a group, G (other than hydrogen) between a pair of substrates, S and S′ are called transferases.

$$S—G + S' \longrightarrow S + S'—G$$

In these are included the enzymes catalyzing the transfer of one-carbon groups, aldehydic or ketonic residues and acyl, glycosyl, alkyl, phosphorus or sulfur-containing groups. Some important subclasses are :

2.3 Acyltransferases. For example :

2.3.1.6 Acetyl-CoA : choline O-acetyltransferase

[Choline acetyltransferase]

Acetyl-CoA + choline ⟶ CoA + O-acetylcholine

2.4 Glycosyltransferases. For example :

2.4.1.1 α-1, 4-Glucan : orthophosphate glycosyl transferase

[Phosphorylase]

$(\alpha\text{-1, 4-Glucosyl})_n$ + orthophosphate ⟶ $(\alpha\text{-1, 4-Glycosyl})_{n-1}$ + α-D-glucose-1-phosphate

2.7 Enzymes catalyzing the transfer of phosphorus-containing groups. For example :

2.7.1.1 ATP : D-hexose-6-phosphotransferase

[Hexokinase]

ATP + D-hexose ⟶ ADP + D-hexose-6-phosphate

3. Hydrolases. These catalyze the hydrolysis of their substrates by adding constituents of water across the bond they split. The substrates include ester, glycosyl, ether, peptide, acid-anhydride, C—C, halide and P—N bonds. Representative subclasses are :

3.1 Enzymes acting on ester bonds. For example :

3.1.1.3 Glycerol ester hydrolase

[Lipase]

A triglyceride + H_2O ⟶ A diglyceride + a fatty acid

3.2 Enzymes acting on glycosyl compounds. For example :

3.2.1.23 β-D-galactoside galactohydrolase

[β-galactosidase]

A β-D-galactoside + H_2O ⟶ An alcohol + D-galactose

3.4. Enzymes acting on peptide bonds

Here the classical trivial names (pepsin, trypsin, thrombin, plasmin etc.) have been largely retained due to their consistent long usage and also due to dubious specificities which make systematic nomenclature almost impractical at this time.

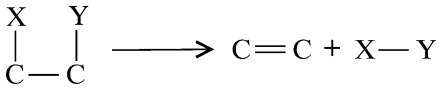
3.5 Enzymes acting on C—N bonds, other than peptide bonds. For example :

3.5.3.1 L-arginine ureohydrolase

[Ariginase]

L-arginine + H_2O ⟶ L-ornithine + urea

4. Lyases (= Desmolases). These are those enzymes which catalyze the removal of groups from substrates by mechanisms other than hydrolysis, leaving double bonds.



In these are included the enzymes acting on C—C, C—O, C—N, C—S and C—halide bonds. Important subclasses include :

4.1 Carbon-carbon lyases. For example :

4.1.2.7 Ketose-1-phosphate aldehyde-lyase

[Aldolase]

A ketose-1-phosphate ⟶ Dihydroxyacetone phosphate + an aldehyde

4.2 Carbon-oxygen lyases. For example :

4.2.1.2 L-malate hydro-lyase

[Fumarase]

L-malate ⟶ Fumarate + H_2O

4.3 Carbon-nitrogen lyases. For example :

4.3.1.3 L-histidine ammonia-lyase

[Histidase]

L-histidine ⟶ Urocanate + NH_3

5. Isomerases. These catalyze interconversions of optical, geometric or positional isomers by intramolecular rearrangement of atoms or groups. Important subclasses are :

5.1 Racemases and epimerases. For example :

5.1.1.1 Alanine racemase

L-alanine ⟶ D-alanine

5.2 *Cis-trans* isomerases. For example :

5.2.1.3 *All trans*-retinene 11-*cis-trans* isomerase

[Retinene isomerase]

All *trans*-retinene ⟶ 11-*cis*-retinene

5.3 Intramolecular oxidoreductases. For example :

5.3.1.9 D-glucose-6-phosphate keto-isomerase

[Glucosephosphate isomerase]

D-glucose-6-phosphate ⟶ D-fructose-6-phosphate

6. Ligases (*ligare*^{L} = to bind) **or Synthetases.** These are the enzymes catalyzing the linking together of two compounds utilizing the energy made available due to simultaneous breaking of a pyrophosphate bond in ATP or a similar compound. This category includes enzymes catalyzing reactions forming C—O, C—S, C—N and C—C bonds. Important subsclasses are :

6.2 Enzymes catalyzing formation of C—S bonds. For example :

6.2.1.1 Acetate : CoA ligase (AMP)

[Acetyl-CoA synthetase]

ATP + acetate + CoA ⟶ AMP + pyrophosphate + acetyl-CoA

6.3 Enzymes catalyzing formation of C—N bonds. For example :

6.3.1.2 L-glutamate : ammonia ligase (ADP)

[Glutamine synthetase]

ATP + L-glutamate + NH_3 ⟶ ADP + orthophosphate + L-glutamine

6.4 Enzymes catalyzing formation of C—C bonds. For example :

6.4.1.2 Acetyl-CoA : CO_2 ligase (ADP)

[Acetyl-CoA carboxylase]

$$ATP + \text{acetyl-CoA} + CO_2 + H_2O \longrightarrow ADP + \text{orthophosphate} + \text{malonyl-CoA}$$

To date, over 2,000 different enzymes are known, of which the oxidoreductases, transferases and hydrolases predominate. Because official names are often lengthy, the trivial names of enzymes are generally used after initial identification.

ISOENZYMES OR ISOZYMES

Many enzymes occur in more than one molecular form in the same species, in the same tissue or even in the same cell. In such cases, the different forms of the enzyme catalyze the same reaction but since they possess different kinetic properties and different amino acid composition, they can be separated by appropriate techniques such as electrophoresis. Such multiple forms of the enzymes are called **isoenzymes** or **isozymes**. Isozymes are of widespread nature. Over a hundred enzymes are now known to be of isozymic nature and consequently occur in two or more molecular forms. *Lactic dehydrogenase*, LDH (E.C. No 1.1.1.27), for example, is an enzyme wihch exists in 5 possible forms in various organs of most vertebrates. LDH catalyzes the reversible oxidation-reduction reaction :

Isoenzymes is in fact, the preferable spelling according to the International Union of Biochemistry.

$$\text{Lactate} + NAD^+ \rightleftharpoons \text{Pyruvate} + NADH + H^+$$

Two basically-different types of LDH are found :

(*a*) heart *LDH*. This predominates in the heart and is active at low levels of pyruvate. This has 4 identical subunits called *H* subunits (H for heart).

(*b*) *muscle LDH*. This is characteristic of many skeletal muscles and maintains its activity in much higher concentrations of pyruvate. This also has 4 identical subunits called M *subunits* (*M* for muscle) which are enzymatically inactive.

The two types of subunits, H and M, have the same molecular weight (35,000) but differ in amino acid composition and in immunological properties. The two subunits are produced by two separate genes. LDH can be formed from two types of polypeptide chains designated as H and M subunits to yield a pure H tetramer and a pure M tetramer. Combinations of H and M subunits will, however, produce 3 additional types of hybrid enzymes, thus making the total number of possible forms as five (refer Fig. 16-1). This is confirmed by the fact that when the 2 subunits are mixed in equal proportions, a sequence of 5 bands is obtained by electrophoresis. The 5 different LDH enzyme forms are designated as H_4, H_3M, H_2M_2, HM_3 and M_4. The various isozyme forms of LDH differ significantly in the maximum activities V_{max}, in the Michaelis constant Km for their substrates, especially for pyruvate and in the degree of their allosteric inhibition by pyruvate.

The relative concentrations of the LDH enzymes differ from one type of tissue to another, and the relative composition is characteristic of the tissue. For example, LDH-1 plus LDH-2 makes up about 60% of the LDH of cardiac muscle, while LDH-4 plus LDH-5 constitutes about 80% of the LDH of liver. Thus, a diagnosis based on elevated serum LDH can be made more specific with respect to the organ involved by an electrophoretic analysis of the LDH isozymes. For example, the elevated serum level of LDH in a myocardial infarct is primarily due to an increase in LDH-1 and LDH-2; while in liver disease the elevated serum LDH is primarily due to increases in LDH-4 and LDH-5.

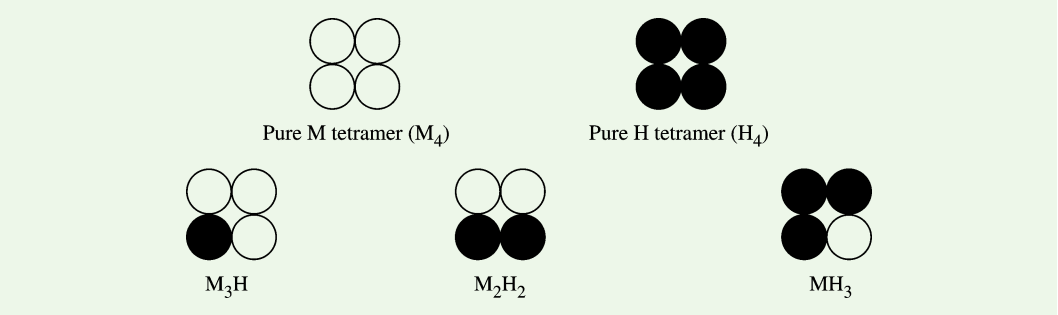


Fig. 16-1. Possible combinations of H and M subunits of lactic dehydrogenase

Damages to tissues and cell death both result in a release of intracellular enzymes from damaged cells into the blood. Therefore, the concentrations of these enzymes increase in the blood serum and can serve as a valuable diagnostic aid in a number of diseases, including myocardial infarction, pancreatitis, liver disease and prostatic cancer.

The serum concentrations of 3 enzymes are often used in diagnosis of myocardial infarction (MI). These are creatinine phosphokinase (CPK), glutamate oxaloacetate transaminase (GOT), and lactate dehydrogenase (LDH). In more than 95% of MI patients, serum levels of GOT rise rapidly and then return to normal in 4—5 days. The serum levels of CPK characteristically rise and fall even more rapidly than GOT levels. However, the serum levels of LDH typically rise and fall more slowly, returning to normal levels in about 10 days.

There seems to be an interesting relation between LDH and the physiological role of flight in birds. In those birds grouped as *short flyers*, over 90% muscle LDH is present in breast muscle. In *long flyers*, breast muscle contains over 95% heart LDH which suggests a possible relation to sustained heart contraction. Those birds classed as *intermediate flyers* contain mixtures of both heart LDH and muscle LDH.

Other noteworthy examples of isozymes are *malic dehydrogenase* (*MDH*), *hexokinase, esterase and glycol dehydrogenase*.

MULTIENZYME SYSTEMS

A few examples of complex enzyme systems are known to exist. These are not independent molecules but occur as aggregates in a mosaic pattern involving several different enzymes. *Pyruvic acid dehydrogenase* of *E. coli* is one such example. This complex molecule has a molecular weight 4,800,000 and consists of 3 enzymes : 24 moles of pyruvate decarboxylase (90,000), 24 moles of pyruvate decarboxylase (90,000), 24 moles of dihydrolipoic dehydrogenase (55,000) and 8 subunits of lipoyl reductase transacetylase (120,000). Each component of this complex enzyme is so arranged as to provide an efficient coupling of the individual reactions catalyzed by these enzymes. In other words, the product of the first enzyme becomes the substrate of the second and so on.

BIOLOGICAL ROLES OF ENZYMES

The enzymes find many applications in our daily life. Enzymatic processes such as baking, brewing and tanning have been known from antiquity. The manifold applications of the enzymes are described below :

1. **Wine manufacturing.** Much of the early interest in enzymology was developed by scientists like Pasteur, Payen and Persoz, who were associated with food, wine, and beer industries. Pasteur was perhaps best known to the French nation as the "*saviour of the wine industry*" because his pasteurization process salvaged an ailing industry beset with problems of microbial contamination. *Papain* is used in brewing industry as a stabilizer for chill-proof beer, because it removes small amounts of protein that cause turbidity in chilled beer.

2. **Cheese making.** Since long the animal rennin (or *rennet*) is employed in making cheese. The enzyme rennet is obtained on a commercial scale from the fourth or true stomach of the unweaned calves which are specifically slaughtered for this purpose. One calf produces only 5 to 10 gm of rennet. The enzyme helps in coagulating the casein of milk. Certain preservatives (boric acid, benzoic acid or sodium chloride) are, sometimes, added to prevent decomposition of the enzyme preparations by bacteria. An enzyme *lipase* is added to cheese for imparting flavour to it. Many vegetarians are unaware that the cheese made in india contains animal rennet. However, an international charitable trust concerned with the welfare of animals, the Beauty Without Cruelty (BWC) has, with the help of Aurey Dairy, Mumbai, undertaken successful experimental trials in cheese making using nonanimal rennet.
3. **Candy making.** An enzyme, *invertase* helps preventing granulation of sugars in soft-centred candies. Another enzyme, *lactase* prevents formation of lactose crystals in ice cream which would otherwise not allow the product seem sandy in texture.
4. **Bread whitening.** *Lipoxygenase* is used for whitening the bread.
5. **Clarifying fruit-juices.** The enzymes are being used in processing of fruit juices such as apple juice and grape juice. The juices are clarified by adding a mixture of *pectic enzymes* which hydrolyze the pectic substances causing turbidity.
6. **Tenderizing meat.** Because hydroxyprolyl residues create bends in collagen helices, which contribute to the tough and rubbery texture often associated with cooked meat, treating the meat with a protease (*bromelain* or *papain*) prior to its cooking hydrolyzes peptide bonds, and thus tenderizes it.
7. **Desizing fabrics.** The woven fabrics are sized by applying starch to the warp (lengthwise) threads to strengthen the yarn before weaving. But when these fabrics are printed or dyed, the sizing should be removed. Desizing may be done by acids, alkalies or enzymes. Enzymatic desizing is, however, preferred as it does not weaken the fabrics. Enzymes for this purpose are obtained from a variety of sources including bacteria, fungi and malt.
8. **Destaining fabrics.** In drycleaning, the stains due to glue, gelatin or starch are removed by employing certain enzymes, such as *alcalase*.
9. **Dehairing hide.** In the manufacture of leather, the hide is made free from hair. This is done by *employing pancreatic enzymes* which hydrolyze the proteins of the hair follicles, thus freeing the hair so that it may be easily scraped off from the hide.
10. **Recovering silver.** *Pepsin* is used to digest gelatin in the process of recovering silver from photographic films.
11. **Correcting digestion.** When the enzymes are present insufficiently in the body, certain digestive disorders come up. These may be cured by supplying the lacking enzymes. *Pepsin, papain* and *amylases* aid digestion in the stomach while *pancreatic enzymes* act in the duodenum.
12. **Wound healing.** *Proteolytic enzymes* from pig pancreas are used to alleviate skin diseases, bed sores and sloughing wounds. These enzymes act by destroying proteolytic enzymes of man, that prevent the healing of such wounds. The enzymes commonly used for wound debridement are the proteases such as *streptodornase*, *ficin* and *trypsin.*

Rennin is a milk-curdling enzyme found in the stomach of ruminant mammals and is probably widely distributed among other mammals. By clotting and precipitating milk proteins, it apparently slows the movement of milk through the stomach. Human infants, who lack rennin, digest milk proteins with acidic pepsin, just as adults do. Rennin should not be confused with another phonetically similar enzyme called **renin** which is produced by the kidney. Renin catalyzes the synthesis of angitensins which cause vasoconstriction in the kidneys, thereby causing electrolyte and water retention in the body.

13. **Analyzing biochemicals.** Certain enzymes are used in clinical analysis. For example, *uricase* and *urease* are employed in the determination of uric acid and urea respectively in blood. Besides, sucrose and raffinose contents in sugar mixtures are determined by polarimetry before and after treating the solutions with the enzymes, *sucrase* and *melibiase*.
14. **Dissolving blood clot.** The enzyme *urokinase*, which is manufactured from urine, is being used effectively in Japan in the treatment of blood clot in brain, artery and other circulatory diseases. A team of Soviet scientists led by Yevgeni Chazov, Director of the National Cardiological Research Centre, Moscow, have, in 1982, developed an effective enzyme *streptodekase*, which can dissolve blood clots in vessels. The new enzyme is particularly useful in preventing heart attacks as clots are responsible for 9 out of 10 fatal cases of cardiac arrests.
15. **Changing the blood type.** In 1981, Prof. Ken Furukawa and his associates of the Gunma University's Medical School, Japan have successfully employed several types of specific enzymes in an epoch-making experiment to freely change human blood types. They found that the composition of polysaccharide on the surface of blood corpuscles determines each person's type of blood. Different kinds of sugar characteristics of each blood type form on the surface of RBCs due to the function of a synthetic enzyme. If the sugar is separated from the surface of RBCs by using a specific decomposition enzyme, type A blood and type B blood can be reverted to type O, the prototype of the two blood types. If this breakthrough can be put to practical use, it will fulfill a long-cherished dream of doctors to administer blood transfusions irrespective of the type of blood a patient has by merely changing the patient's blood type to match the blood available.
16. **Diagnosing hypertension.** A new method called radio immunoassay procedure for diagnosing cases of hypertension has been developed by Bhabha Atomic Research Centre (BARC). In it, the activity of *renin*, a proteolytic enzyme secreted by the kidneys, is calculated indirectly by measuring angiotensin-I which is formed by the action of renin. Renin acts as part of a complex feedback mechanism for regulating blood volume and pressure.
17. **Augmenting surgery.** A technique using the enzyme *trypsin* as an adjunct to cataract surgery has been developed in 1980 by Dr. Joseph Spina Jr. of Philadelphia. With older techniques it required an incision about 2.5 cm long in the white of the eye to remove the clouded lens. Modern microsurgery has, however, reduced this cut to only 0.3 cm. But Dr. Spina's method involves a still smaller cut wide enough for a needle 0.025 cm wide. The hollow needle is used to inject a microscopic amount of trypsin, a digestive enzyme secreted by the pancreas. Trypsin digests and liquefies the semisolid interior of the lens without harming other parts of the eye. Once the enzyme liquefies the lens—which takes from a few hours to overnight—the lens is removed by suction through the same hollow needle. This eliminates the necessity of intervention in the eye, the constant passing in and out of the instruments and suturing. The lesser the tissue is wounded, the quicker it recovers. This enzyme surgery for cataracts could be done as an outpatient operation. The patients would come in one day to have the enzyme injected and return the next day to have the cataract removed.

CLINICAL IMPLICATIONS

A cataract is a clouding of eye lens which can cause blindness. It is the most common eye disease and comes with age. The lens of the eye is made of protein and as time goes by, there occurs denaturation process and the protein gets cloudy and one cannot see. India, at present, has 18 million blind people, out of which 12 million are cataract patients.

18. **Breaking down chemicals.** Recently, in 1993, a group of scientists from the Netherlands led by Han G. Brunner have found a tiny genetic defect that appears to predispose some men toward aggression, impulsiveness and violence. The afflicted persons often react to the most

mildly stressful occasions with aggressive outbursts, cursing or assaulting the persons they deem a threat. The researchers have linked the abnormal behaviours to mutations in the gene responsible for the body's production of *monamine oxidase*-a, an enzyme critical for breaking down chemicals that allow brain cells to communicate. It is proposed that lacking the enzyme, the brains of afflicted men end up with excess deposits of potential signalling molecules like serotonin, dopamine and noradrenaline. Those surplus neurotransmitters, in turn, stimulate often hostile conduct. The erratic behaviour is due to point mutation. The gene is on the X chromosome, which explains why only males, with their single copy of the X chromosome, can suffer from the enzyme deficiency. Women can serve as carriers of the genetic defect, but are themselves protected from its symptoms by their possession of a second, good copy of the gene, sitting on their second X chromosome. Although the number of persons afflicted with this disease is not known but based on other types of hereditary disorders, the researchers estimate that the illness is likely to be quite rare in the general population, *i.e.*, no more than one in 1,00,000 people.

19. **Destroying acids.** Sprouts are the natural health boosters. They are basically the young new plants and are, in fact, the organic answer to simple natural health. Almost any edible seed can be sprouted. Far from being the invention of food faddists, sprouting dates back to 2939 B.C. in China. Sprouts are found in all shapes and colours and it is best to choose these from legume plants. Sprouting greatly improves the safety and nutritional quality of all pulses, seeds and grains. The enzymes which go into action during sprouting not only neutralize trypsin-inhibiting factors but also destroy harmful acids like phytic acid. Phytic acid, an integral constituent of grains, tends to bind minerals, making them unavailable to the body.
20. **Syrup manufacturing**. An *immobilized enzyme* is one that is physically entrapped or covalently-bonded by chemical means to an insoluble matrix, *e.g.*, glass beads, polyacrylamide or cellulose. Immobilization of an enzyme often greatly enhances its stability, which makes its prolonged catalytic life a valuable industrial trait. These days, *immobilized glucose isomerase* is being successfully used in the production of high-fructose corn syrup, *esp.*, in the United States.

Table 16-1 outlines some of industrial applications of enzymes. This is a rapidly changing field, and new applications of enzyme technology appear all the time.

Table 16.1 Some Applications of Enzymes

Enzyme	*Reaction*	*Source of enzyme*	*Application*
Industrial applications			
α-amylase	breaks down starch	bacteria	converts starch to glucose in the food industry
Glucose isomerase	converts glucose to fructose	fungi	production of high fructose syrups
Proteases	digests protein	bacteria	washing powder
Rennin	clots milk protein	animal stomach linings; bacteria	cheese making
Catalase	splits hydrogen peroxide into $H_2O + O_2$	bacteria; animal livers	turns latex into foam rubber by producing gas
β-galactosidase	hydrolyses lactose	fungi	in dairy industry, hydrolyses lactose in milk or whey

Medical applications			
L-asparginase	removes L-asparagine from tissues - this nutrient is needed for tumour growth	bacteria (*E. coli*)	cancer chemotherapy- particularly leukaemia
Urokinase	breaks down blood clots	human urine	removes blood clots, e.g., in heart disease patients
Analytical applications			
Glucose oxidase	oxidises glucose	fungi	used to test for blood glucose, e.g., in Clinistix™ diabetics
Luciferase	produces light	marine bacteria; fireflies	binds to particular chemicals indicating their presence, e.g., used to detect bacterial contamination of food
Manipulative applications			
Lysozyme	breaks 1-4 glycosidic bonds	hen egg white	disrupts bacterial cell walls
Endonucleases	breaks DNA into fragments	bacteria	used in genetic manipulation techniques, e.g., gene transfer, DNA Finger printing.

REFERENCES

See list following Chapter 18 .

PROBLEM

1. Which of the reactions listed below are catalyzed by an isomerase, lyase, hydrolase or transferase ?

(*a*) Protein $\longrightarrow$ Amino acids

(*b*) Histidine $\longrightarrow$ Histamine + CO_2

(*c*) Glucose + ATP $\longrightarrow$ Glucose-6-phosphate + ADP

(*d*) Glucose-6-phosphate $\longrightarrow$ Glucose + H_3PO_4

(*e*) $CH_3COCOOH + 2H^+ \longrightarrow CH_3CHOHCOOH$

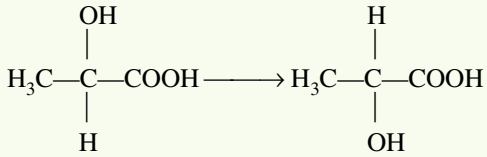
(*f*) $CH_3COCOOH \longrightarrow CH_3CHO + CO_2$

(*g*) 3-phosphoglycerate $\longrightarrow$ 2-phosphoglycerate

(*h*) Tryptophan $\longrightarrow$ Tryptamine + CO_2

(*i*) L-lysine $\longrightarrow$ D-lysine

(*j*) Acetycholine $\longrightarrow$ Acetic acid + choline

(*k*)


(*l*) Glutaric acid + oxaloacetate $\longrightarrow$ α-ketoglutarate + aspartic acid