Irena Kruk

Environmental Toxicology and Chemistry of Oxygen Species

The Handbook of Environmental Chemistry

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Volume 2 Reactions and Processes Part I

O. Hutzinger
Editor-in-Chief

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Environmental Toxicology and Chemistry of Oxygen Species

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Environmental chemistry is a rather young and interdisciplinary field of science. Its aim is a complete description of the environment and of transformations occuring on a local or global scale. Environmental chemistry also gives an account of the impact of man's activities on the natural environment by describing observed changes.

"The Handbook of Environmental Chemistry" provides the compilation of today's knowledge. Contributions are written by leading experts with practical experience in their fields. The Handbook will grow with the increase in our scientific understandig and should provide a valuable source not only for scientists, but also for environmental managers and decision makers.

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Preface

Environmental Chemistry is a relatively young science. Interest in this subject, however, is growing very rapidly and, although no agreement has been reached as yet about the exact content and limits of this interdisciplinary discipline, there appears to be increasing interest in seeing environmental topics which are based on chemistry embodied in this subject. One of the first objectives of Environmental Chemistry must be the study of the environment and of natural chemical processes which occur in the environment. A major purpose of this series on Environmental Chemistry, therefore, is to present a reasonably uniform view of various aspects of the chemistry of the environment and chemical reactions occurring in the environment.

The industrial activities of man have given a new dimension to Environmental Chemistry. We have now synthesized and described over five million chemical compounds and chemical industry produces about hundred and fifty million tons of synthetic chemicals annually. We ship billions of tons of oil per year and through mining operations and other geophysical modifications, large quantities of inorganic and organic materials are released from their natural deposits. Cities and metropolitan areas of up to 15 million inhabitants produce large quantities of waste in relatively small and confined areas. Much of the chemical products and waste products of modern society are released into the environment either during production, storage, transport, use or ultimate disposal. These released materials participate in natural cycles and reactions and frequently lead to interference and disturbance of natural systems.

Environmental Chemistry is concerned with reactions in the environment. It is about distribution and equilibria between environmental compartments. It is about reactions, pathways, thermodynamics and kinetics. An important purpose of this Handbook, is to aid understanding of the basic distribution and chemical reaction processes which occur in the environment.

Laws regulating toxic substances in various contries are designed to assess and control risk of chemicals to man and his environment. Science can contribute in two areas to this assessment; firstly in the area of toxicology and secondly in the area of chemical exposure. The available concentration ("environmental exposure concentration") depends on the fate of chemical compounds in the environment and thus their distribution and reaction behaviour in the environment. One very important contribution of Environmental Chemistry to the above mentioned toxie substances laws is to develop laboratory test methods, or mathematical correlations and models that predict the environ-

mental fate of new chemical compounds. The third purpose of this Handbook is to help in the basic understanding and development of such test methods and models.

The last explicit purpose of the Handbook is to present, in concise form, the most important properties relating to environmental chemistry and hazard assessment for the most important series of chemical compounds.

At the moment three volumes of the Handbook are planned. Volume 1 deals with the natural environment and the biogeochemical cycles therein, including some background information such as energetics and ecology. Volume 2 is concerned with reactions and processes in the environment and deals with physical factors such as transport and adsorption, and chemical, photochemical and biochemical reactions in the environment, as well as some aspects of pharmacokinetics and metabolism within organisms. Volume 3 deals with anthropogenic compounds, their chemical backgrounds, production methods and information about their use, their environmental behaviour, analytical methodology and some important aspects of their toxic effects. The material for volume 1, 2 and 3 was each more than could easily be fitted into a single volume, and for this reason, as well as for the purpose of rapid publication of available manuscripts, all three volumes were divided in the parts A and B. Part A of all three volumes is now being published and the second part of each of these volumes should appear about six months thereafter. Publisher and editor hope to keep materials of the volumes one to three up to date and to extend coverage in the subject areas by publishing further parts in the future. Plans also exist for volumes dealing with different subject matter such as analysis, chemical technology and toxicology, and readers are encouraged to offer suggestions and advice as to future editions of "The Handbook of Environmental Chemistry".

Most chapters in the Handbook are written to a fairly advanced level and should be of interest to the graduate student and practising scientist. I also hope that the subject matter treated will be of interest to people outside chemistry and to scientists in industry as well as government and regulatory bodies. It would be very satisfying for me to see the books used as a basis for developing graduate courses in Environmental Chemistry.

Due to the breadth of the subject matter, it was not easy to edit this Handbook. Specialists had to be found in quite different areas of science who were willing to contribute a chapter within the prescribed schedule. It is with great satisfaction that I thank all 52 authors from 8 contries for their understanding and for devoting their time to this effort. Special thanks are due to Dr. F. Boschke of Springer for his advice and discussions throughout all stages of preparation of the Handbook. Mrs. A. Heinrich of Springer has significantly contributed to the technical development of the book through her conscientious and efficient work. Finally I like to thank my family, students and colleagues for being so patient with me during several critical phases of preparation for the Handbook, and to some colleagues and the secretaries for technical help.

I consider it a privilege to see my chosen subject grow. My interest in Environmental Chemistry dates back to my early college days in Vienna. I received significant impulses during my postdoctoral period at the University of California and my interest slowly developed during my time with the National Research

Council of Canada, before I could devote my full time of Environmental Chemistry, here in Amsterdam. I hope this Handbook may help deepen the interest of other scientists in this subject.

Amsterdam, May 1980 *O. Hutzinger*

Seventeen years have now passed since the appearance of the first volumes of the Handbook. Although the basic concept has remained the same some changes and adjustments were necessary.

Some years ago publishers and editor agreed to expand the Handbook by two new open-ended volume series: Air Pollution and Water Pollution. These broad topics could not be fitted easily into the headings of the first three volumes. All five volumes series are integrated through the choice of topics and by a system of cross referencing.

The outline of the Handbook is thus as follows:

1. The Natural Environment and the Biochemical Cycles,
2. Reactions and Processes,
3. Anthropogenic Compounds,
4. Air Pollution,
5. Water Pollution.

Rapid developments in Environmental Chemistry and the increasing breadth of the subject matter covered made it necessary to establish volume-editors. Each subject is not supervised by specialists in their respective fields.

A recent development is the 'Super Index', a subject index covering chapters of all published volumes, which will soon be available via the Springer Homepage http://www.springer.de or http://www.springer-ny.com or http://Link.springer.de.

With books in press and in preparation we have now published well over 30 volumes. Authors, volume-editors and editor-in-chief are rewarded by the broad acceptance of the 'Handbook' in the scientific community.

May 1997 *Otto Hutzinger*

Contents

Introduction

All respiring organisms need oxygen for the generation of energy, and thus their lives are supported by molecular oxygen (O_2). If the concentration of oxygen is greater than that normally occurring in the atmosphere, the living organisms are exposed to oxygen toxicity. Molecular oxygen is not extremely reactive, although it contains unpaired electrons, i.e. it possesses a radical nature. The electronic structure of molecular oxygen means that it can form more reactive species by univalent reduction. Such reactive oxygen species arising play an important physiological function and can also cause damage to cell constituents.

"Life" in an oxygen environment involves a composite balance between endogenous generation of toxic oxygen species and the ability of organisms to protect themselves against them. Toxicity due to the oxygen species occurs in unbalanced conditions, i.e. inordinate generation of the oxygen species or defense mechanisms insufficient to neutralize their production.

The term "toxic oxygen species" is restricted in this book to a number of oxygen free radicals[1]: superoxide anion radical ($O_2^{\cdot-}$), hydroxyl radical ($HO^{\cdot}$), peroxy radical ($RO_2^{\cdot}$) alkoxy radical ($RO^{\cdot}$) hydrogen peroxide (H_2O_2) as well as the oxygen molecule in the electronically excited state called singlet oxygen ($^1O_2$).

However, suggestions that toxic oxygen species are important intermediates in chemistry and biology have been made for at least half a century, and interest in their generation in a cell has increased enormously in the last three decades. The reasons for this are as follows:

- the oxygen species were identified spectroscopically and were shown to survive long enough to be reactive chemically;
- they were shown to be responsible for a wide variety of damage effects to cell constituents like nucleic acids, amino acids, proteins, lipids, carbohydrates;
- many endogeneous processes generating highly reactive oxygen species have been recognized (e.g. respiration, phagocytosis, autoxidation of catecholamines, carboxylation or hydroxylation reactions);
- the oxygen species are formed by exogenous sources like ionizing radiation, cigarette smoke, cytostatic drug therapy, redox reactions of herbicides and pesticides;

[1] A free radical is any species actually existing, that possesses one or more unpaired electrones occupying outer orbitals.

- the participation of oxygen radicals in several human diseases has been postulated;
- effective defense mechanisms preventing oxygen species damage to cell constituents are recognized and research is in progress for new compounds playing the role of oxygen species scavengers;
- sufficient information on the presence of $^1O_2$ in high concentrations in polluted environments is available.

This book describes the chemical structure of toxic oxygen species, their sources and formation, their chemical reactivity with some biological compounds, methods of identification, biochemical, clinical and environment aspects of their formation, and finally cell protection against such oxygen species cellular toxicity. This information provides the basis for understanding the oxygen species role in environmental pollution and health hazards.

Chemical Aspects

1 Properties of Activated Oxygen Species

1.1
A Brief Survey of the Quantum Notation of Atoms and Molecules

1.1.1
The Hydrogen Atom and the Schrödinger Equation

The electronic structure of atoms and molecules, their interactions, spectra and the nature of the chemical bond are explained in a modern way by quantum mechanics. The behaviour of the electron in the atom is described by Schrödinger's equation, formulated in 1926 (Fig. 1).

Let us consider the simplest atom which is the hydrogen atom. For this atom, the Schrödinger equation may be written as follows:

$$-\frac{\hbar^2}{2m}\nabla^2\Psi + V\Psi = E\Psi$$

where $\hbar = h/2\pi$, and h is Planck's constant;
m is the mass of the electron;

∇^2 is the Laplace operator $\left(\nabla^2 \equiv \frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2} + \frac{\partial^2}{\partial z^2}\right)$;

V is the potential energy;
E is the total energy.

The equation must be solved for Ψ, which assumes many values and is called the wave function. The solutions of Schrödinger's equation involve the existence of various levels of energy and states described by the wave functions.

The wave function Ψ depends on the space coordinates of the electron, $\Psi = \Psi(x,y,z)$.

The exact position of the electron in an element of the volume ($dV = dx\,dy\,dz$) containing point (x_o, y_o, z_o) is related to the square of the absolute value of the wave function describing the stationary state

$$dP = |\Psi(x_o, y_o, z_o)|^2 dV$$

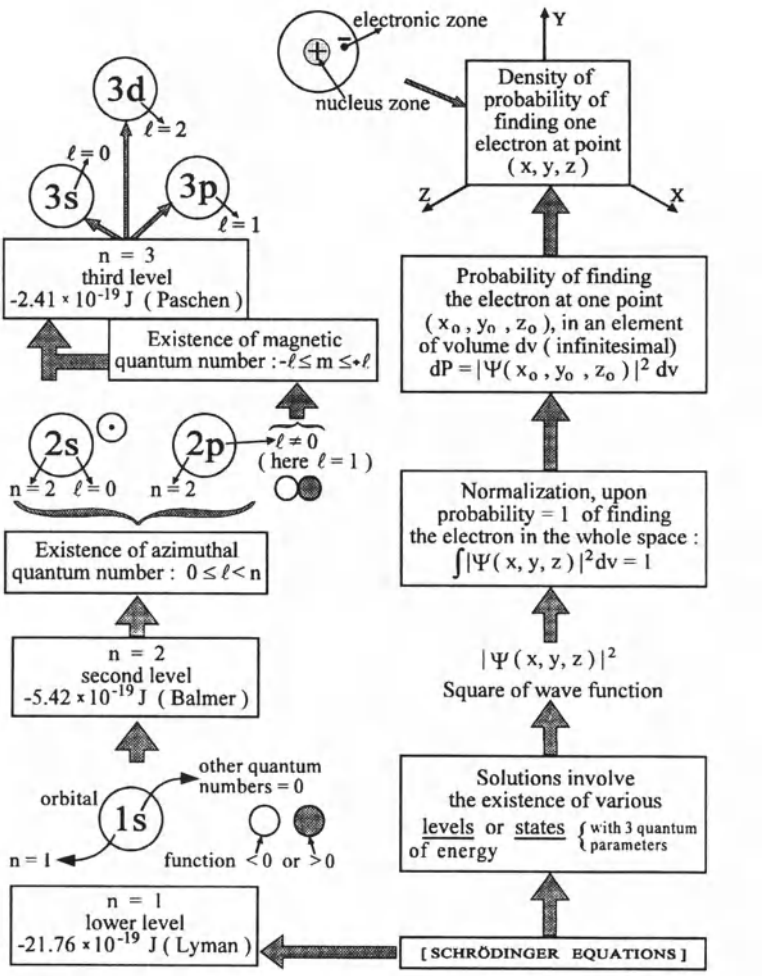


Fig. 1. Illustration of atomic orbitals

According to this interpretation, the normalization condition is fulfilled, i.e.

$$\int |\Psi(x, y, z)|^2 dV = 1$$

The $|\Psi|^2$ value in some small element of a space has exactly the sense of probability density of finding the electron in this space. In this sense, the electron may be considered as a diffuse cloud, contrary to the Bohr theory, where the electron in the hydrogen atom is treated as a negatively charged particule moving along defined circular orbits.

The Ψ functions describing only one electron, i.e. one-electron wave functions, are called orbitals.

The solutions of the Schrödinger equation involve the existence of three characteristic quantum numbers of an atomic orbital: the principal quantum number n, the azimuthal quantum number ℓ, and the magnetic quantum number m.

The n number may assume the values 1, 2, 3, 4 .... This number determines the energy of the electron in the atom and the radius of the orbitals. The next quantum number, ℓ determines the orbital momentum of an electron in its motion around the nucleus. The azimuthal quantum number may assume the values 0, 1, 2, 3, ..., n-1 for a given value of n. The ℓ number also defines the shape and symmetry of the orbital.

The magnetic quantum number m defines the orientation of the angular momentum of an electron about a specified direction (e.g. z–axis, external magnetic or electric fields). The components of the angular momentum of an electron may take only the following values:

$$m_z = m\hbar$$

For a given value of number ℓ, the m number may take on the $2\ell + 1$ values:

$$0, \pm 1, \pm 2, \pm 3, \ldots, \pm \ell.$$

Orbitals having different values of number ℓ are conventionally marked as follows:

$\ell = 0,$	1,	2,	3,	4, ...
orbitals s	p	d	f	g ...

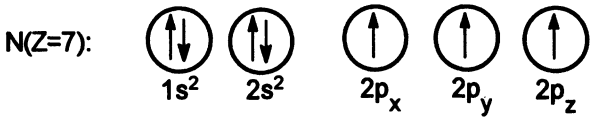
All orbitals with $\ell = 0$ are called "s - orbitals", and they have spherical symmetry (Fig. 2). If $\ell = 1$, the orbital is called a "p-orbital". There exist three kinds of p orbitals: p_x, p_y, p_z, for a given n, since for $\ell = 1$ there are three different values of the magnetic quantum number m $(-1, 0, +1)$. The p orbitals resemble "dumbbells" in shape and they have a cylindrical symmetry with respect to a given axis. They differ only in orientation.

From the above discussion thus far we can conclude that for a given value of n, there can be one s orbital, three p orbitals, five d orbitals and seven f orbitals. For example, if n = 3, the ℓ values associated with this n are:

$\ell = 0$	$\ell = 1$	$\ell = 2$
$(m = 0)$	$(m = -1, 0, +1)$	$(m = -2, -1, 0, +1, +2)$
one s orbital	three p orbitals	five d orbitals

The full description of the electron state in the atom requires an additional quantum number called the spin quantum number, s. The spin quantum number determines the orientation of the electron spin, e.g. in an external magnetic field. There are two possible orientations of the electron spin $(s = +1/2)$ and $(s = -1/2)$. The orientation may be graphically marked by arrows $\uparrow$ $(s = +1/2)$ and $\downarrow$ $(s = -1/2)$. According to the Pauli exclusion principle each orbital can be occupied by at most two electrons under the condition that these spins are not parallel $(\uparrow\downarrow)$.

Let us consider the ground-state electronic configuration of the nitrogen atom:



The 1 s and 2 s orbitals are already filled with the electron pairs, and they will not take part in bond formation. The remaining orbitals only contain one electron. Such description is in accordance with the Hund's rule, which says that for a ground state, electrons prefer to occupy separate orbitals (the number of electrons in the atom having parallel spins is maximum). All three orbitals $2p_x$, $2p_y$, $2p_z$ have the same values of energy.

For a given n, the proper values of energy E, obtained from the Schrödinger equation solution for the hydrogen atom, are described by the same relationship as that from the Bohr theory, i. e.:

$$E = -\frac{2\pi^2 me^4}{h^2 n^2}$$

where m is the electron mass;

e is the magnitude of the electron charge;
n is the principal quantum number.

If n = 1, the electron occupies the 1s orbital, i.e. the electron possesses the lowest energy. This state is called the ground state. For n > 1 the states are energetically higher, and are called excited states. Transition of the electron from the higher state characterized by n_2 to the lower state characterized by n_1 is accompanied by light emission:

$$h\nu = E_2 - E_1 = \frac{2\pi^2 me^4}{h^2}\left[\left(\frac{1}{n_1}\right)^2 - \left(\frac{1}{n_2}\right)^2\right]$$

where $n_2 > n_1$;

ν is the light frequency.

Transitions of the electron from higher orbitals characterized by $n_2 = 2, 3, 4, 5 \ldots$ to the orbital having $n_1 = 1$ form the spectral series called the Lyman series. Transitions of the electron from higher orbitals to $n_1 = 2$ gives the Balmer series; to $n_1 = 3$ the Paschen series; to $n_1 = 4$ the Brackett series; and $n_1 = 5$ the Phund series. For any given series n_2 may take all integral values greater than the n_1 value.

1.1.2 Molecular Orbitals

Atoms form molecules, and they are held together by chemical bonds. Let us consider the simplest kind of molecule, i.e. the hydrogen molecule, H_2, which consists of two nuclei and two electrons. In this molecule two atomic orbitals of two isolated H-atoms approach one another and a big cloud, called a molecular orbital, is formed (Fig. 2).

In a molecule the electron shells are calculated using the molecular orbital method in the form of the linear combination of atomic orbitals. In this method

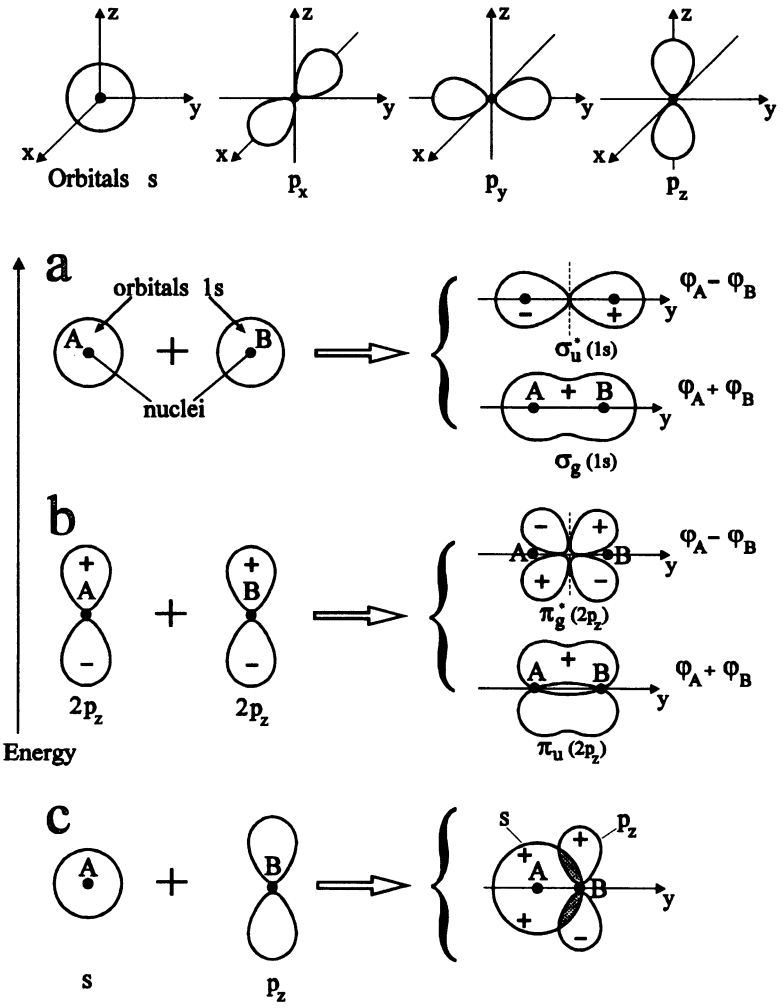


Fig. 2. Formation of σ and π-bonds; φ_A and φ_B are the 1s orbitals of both hydrogen atoms A and B, respectively

molecular orbitals are considered to be linear combinations of atomic orbitals of atoms forming a molecule as follows:

$$\Psi(\tau) = \sum_{i=1}^{n} C_i \cdot \varphi_i(\tau)$$

where τ denotes the total number of space coordinates of the electron, φ_i ($i = 1, 2, 3, \ldots, n$) are atomic orbitals, and C_i are numerical coefficients.

The molecular orbital, like the atomic orbital, is a one-electron function, i.e. it depends only on the space coordinates of the i-th electron. The electron wave function of a molecule can be written as a number of molecular orbitals of particular electrons:

$$\Psi_e = \Psi_1 \cdot \Psi_2 \ldots \Psi_n$$

and its energy is equal to the energy sum corresponding to these orbitals.

For the hydrogen molecule, the molecular orbital describing each one of both electrons of H_2 in the ground state is the linear combination of the 1s orbitals of both hydrogen atoms (denoted as A and B). Marking these orbitals by φ_A and φ_B we describe the molecular orbitals of electrons 1 and 2 as follows:

$$\Psi(1) = C_A \varphi_A(1) + C_B \varphi_B(1)$$

$$\Psi(2) = C_A \varphi_A(2) + C_B \varphi_B(2)$$

Finding the final form of the molecular orbitals from the Schrödinger equation for the H_2 molecule is a difficult example, so we will try to approach this question for H_2^+. The ion consists of two nuclei and one electron. The molecular orbital of the H_2^+ ion is

$$\Psi = C_A \varphi_A + C_B \varphi_B$$

where φ_A and φ_B are the 1s orbitals of both hydrogen atoms, A and B, respectively.

The solution of Schrödinger's equation gives two values for the H_2^+ energy which correspond to both molecular orbitals Ψ_g and Ψ_u:

$$\Psi_g = C_g(\varphi_A + \varphi_B)$$

$$\Psi_u = C_u(\varphi_A - \varphi_B)$$

The Ψ_g orbital is energetically more stable than the atomic orbitals of separate H atoms. On the other hand, the Ψ_u orbital is less stable. The Ψ_g orbital is called the bonding orbital, the Ψ_u the antibonding orbital. The relative energies of these orbitals are shown in Fig. 2.

The values of C_g and C_u can easily be obtained from the normalization conditions of the above-mentioned wave functions, and they are

$$C_g = \frac{1}{\sqrt{2}(1 + S_{AB})}$$

$$C_u = \frac{1}{\sqrt{2}(1 - S_{AB})}$$

The values of the S_{AB} integrals determine the so-called overlapping of the atomic orbitals φ_A and φ_B, which is a measure of coincidence.

Thus, from each pair of atomic orbitals we can obtain two molecular orbitals: bonding and antibonding. The bonding and antibonding properties of molecular orbitals result from electron density in the regions between the atomic nuclei given by Ψ_g^2 and Ψ_u^2. Bonding orbitals have an incrased electron density as compared to antibonding ones.

Figure 2a illustrates the overlap of two 1s orbitals with sigma (σ) molecular orbitals formation: bonding (σ), and antibonding (σ^*). Overlap of two $2p_y$ orbitals leads to the formation of bonding $\sigma(2p)$ and antibonding $\sigma^*(2p)$ orbitals. Sigma orbitals are orbitals with an axial symmetry. The σ orbital has a plane symmetry, and the rotation about a symmetry axis does not change the sign of the wave function, $\Psi(\tau)$.

Figure 2b shows pi(π) molecular orbital formation from isolated $2p_z$ orbitals. Pi orbitals are antisymmetrical with respect to the plane of the nuclear configuration. The π-orbital has one and only one nodal plane containing the internuclear axis, the rotation about which through 180° changes the sign of the wave function. The formation of molecular orbitals $\sigma, \sigma^*, \pi, \pi^*$ is common in molecules considerably larger than H_2 and other diatomic molecules.

It is worth noting that the bond formed by a molecular orbital is stronger if overlapping of atomic orbitals forming the bond is greater. For example, the overlap of 2s and $2p_z$ orbitals (Fig. 2c) is ineffective due to the same absolute values of the overlap integrals and opposite signs, giving $S_{AB} = 0$. Similarly, the p_x and p_z orbitals of the A and B atoms do not overlap.

Let us consider the electronic configuration of the nitrogen molecule, N_2. From the electronic configuration of the ground state of the nitrogen atom we notice that the 1s and 2s orbitals are filled with paired electrons and they will not be involved in formation of molecular orbitals. If we assume that the two $2p_y$ orbitals are pointed at one another then their overlap gives rise to a σ bond accommodated by two $2p_y$ electrons, which may be denoted as $\sigma(2p_y)$. Overlap of the $2p_x$ and $2p_y$ orbitals, $\pi(2p_x, 2p_y)$, forms two π bonds each of which will hold two electrons. The molecular-orbital calculation for the nitrogen molecule leads to the following structure of the nitrogen molecule:

$$N_2: KK(\sigma_g(2s)^2(\sigma_u^*2s)^2(\pi_u 2p)^4(\sigma_g 2p)^2$$

where KK indicates the (1s) electrons of the nitrogen atoms $(1s\sigma)^2(1s\sigma^*)^2$.

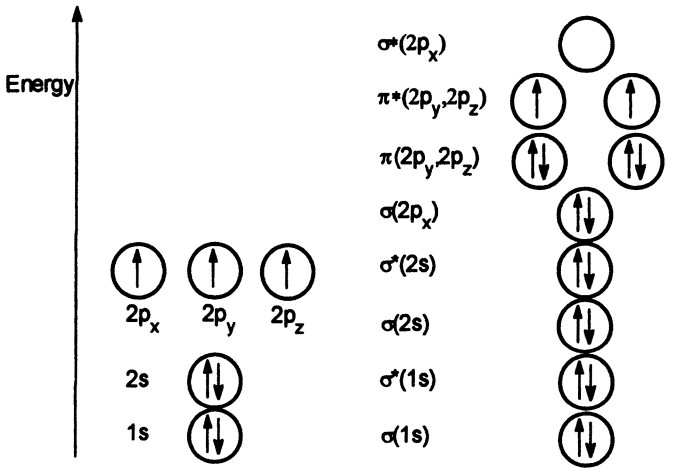
The $\sigma_g(2p)$ orbital has higher energy than the twice-degenerated orbital $\pi_u(2p)$ (i.e. containing four electrons). Thus, the N_2 molecule possesses one σ bond and two π bonds ($N \equiv N$).

The dimensional structure of molecules indicates that their bonds have exactly determined directions in space. This is attainable by hybridization of the atomic orbitals. The hybridized orbitals are linear combinations of orbitals due to the same atom and have similar values of energy. Hybridized orbitals have

directional character and therefore they are suitable for being overlapped by the atomic orbitals of other atoms showing similar symmetry. Details can be obtained from quantum chemistry literature.

1.2 Oxygen

The oxygen atom possesses eight electrons and forms the diatomic oxygen molecule which has a biradical nature, becuase two last electrons are located in different molecular orbitals. These two unpaired electrons have parallel spin and occupy the highest antibonding $\pi^*(2p_y)$ and $\pi^*(2p_z)$ orbitals (circles designate atomic orbitals in the case of the oxygen atom and molecular orbitals for the oxygen molecule):



The electronic structure of the ground state of the oxygen molecule can be written as follows [1]:

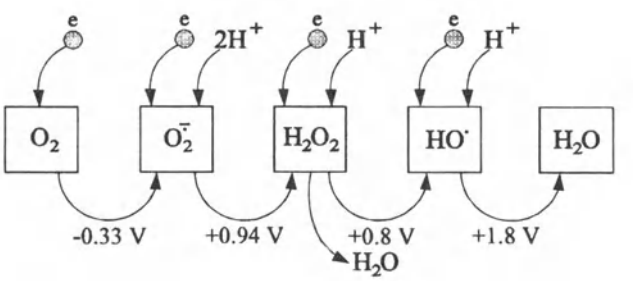
$$O_2[KK]\,(2s\sigma)^2(2s\sigma^*)^2(2p_x\sigma)^2(2p_y\pi)^2(2p_z\pi)^2(2p_y\pi^*)^1(2p_z\pi^*)^1$$

where KK means the (1s) electrons of the oxygen atoms $(1s\sigma)^2(1s\sigma^*)^2$, and fully occupied K shells, whereas stars refer to antibonding orbitals.

The state is known as the triplet sigma state ($^3\Sigma_g^-$). This means that O_2 exists as a triplet molecule and can act like a biradical since the highest occupied orbitals according to Hund's rule of maximum multiplicity are a pair of doubly

degenerated antibonding orbitals with two electrons having parallel spins. The parallel spins ($s = +1/2$ or $s = -1/2$) give rise to three possible states corresponding to the components of spin angular momentum $(+1, 0, -1)$ in the presence of a strong magnetic field. The oxygen molecule therefore shows paramagnetic properties in the ground state. The Σ notation indicates a state having a molecular electron cloud of rotational symmetry[2].

The triplet multiplicity distinguishes O_2 from the nonradical biological molecules of singlet multiplicity. The oxygen molecule in its ground state is rather a weak oxidant because the parallel electron spin arrangement prevents the direct addition of the electron pair to a molecule. In order to form a bond, a spin inversion should occur which is slow in comparison to the lifetime of collisional complexes. Simultaneous two-electron reactions with organic molecules which are in the singlet state require a spin conversion. As a consequence most of the reactions involving the oxygen molecule in the ground state are one-electron reactions. Therefore, if energetically possible, the one-electron reduction of the O_2 molecule predominates over the two-electron reduction [2]. The protective mechanisms are responsible for survival of life and control of oxygen concentration, and they cause its complete reduction to H_2O [3]. The reduction of the oxygen molecule involves the successive steps illustrated by Scheme 1.



Scheme 1. Reductive detoxication of oxygen molecule

As can be seen, the initial step is a reduction of O_2 by the univalent pathway resulting in the formation of O_2^-, and the next step is a reduction of O_2^- to H_2O_2. The addition of three electrons to each O_2 molecule results in the formation of the HO radical and the final step of the O_2 molecule reduction involves generation of the H_2O molecule. There are several reports dealing with the values of the reduction potentials of the oxygen molecule. The scheme con-

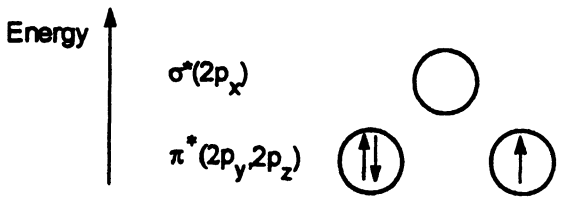
[2] The Σ state corresponding to the symmetric function is denoted by minus (–), and to the nonsymmetric function by plus (+). The states denoted by g are even (gerade), whereas those denoted by u are odd (ungerade).

tains the values for which there is the greatest consensus in the literature data [4].

Electronic structures of the oxygen radicals and the singlet states of the oxygen molecule differ only by the configuration of (π*2p) orbitals.

1.3 Superoxide Radical

We saw in Scheme 1 that the superoxide anion radical is the product of one-electron reduction of O_2, the additional electron entering one of the π antibonding orbitals of the molecular oxygen in the ground state. The electronic configuration of the highest occupied orbitals of O_2^- may be presented as follows:



In aqueous solution the superoxide anion radical (O_2^-) exists in the equilibrum state with the hydroperoxy radical (HOO·):

$$HO_2^{\cdot} \rightleftharpoons H^+ + O_2^- \qquad K^3_{diss} = 1.6 \cdot 10^{-5}\ mol \cdot \ell^{-1}$$

The O_2^- and its conjugate acid, HOO·, absorb in the UV region in aqueous solution at wavelengths λ_{max} = 225 nm (ε = 1440 ± 80 $\ell \cdot mol^{-1} \cdot cm^{-1}$) and λ_{max} = 245 nm (ε = 2350 ± 120 $\ell \cdot mol^{-1} \cdot cm^{-1}$), respectively [5]. The hydroperoxy radical is a weak acid whose pK[4] is 4.69 ± 0.08 [6]. The stability of the O_2^- species strongly depends on the solvent. For example in nonaqueous, aprotic[5] solvent O_2^-

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[3] K_{diss} is the dissociation constant, i.e. the equilibrium constant governed by the law of mass action. The equilibrium constant for dilute solutions is equal to the ratio of the product of concentrations of the final reaction products to the product of the concentrations of the reactants. Each concentration must be raised to the power equal to the number of moles of given substances in the reaction equation. This is true for reversible reactions in a dynamic equilibrium set at constant temperature. For O_2^- dissociation, the equilibrium constant may be expressed as follows:

$$K_{diss} = \frac{[H^+][O_2^-]}{[HO_2^-]}$$

where brackets denote the concentration in moles per litre (mol · ℓ^{-1}). If numbers of moles of products and reactants are the same, the dissociation constant is a dimensionless quantity.

[4] $pK = -\lg K_{diss}$.

[5] Aprotic substances are those which are unable to yield or to accept a proton.

has a lifetime of the order of minutes, whereas in highly alkaline aqueous solution it is no more than seconds.

In aqueous solution, both in acid and neutral pH ranges, O_2^- and $HOO^\cdot$ species undergo the dismutation reaction

$$HOO^\cdot + HOO^\cdot \rightarrow H_2O_2 + O_2 \quad (1)$$

$$HOO^\cdot + O_2^- \rightarrow HOO^- + O_2 \quad (2)$$

The rate constants[6] of the disproportionation reaction are rather high $(0.2-1.04) \cdot 10^6\ \ell \cdot mol^{-1} \cdot s^{-1}$ in the pH range (0.0–7.7) [7]. On the other hand, the rate constant of O_2^- recombination

$$O_2^- + O_2^- \rightarrow O_2^{2-} + O_2 \quad (3)$$

is small, $k \ll 100\ \ell \cdot mol^{-1} \cdot s^{-1}$ [8].

---

[6] The rate of the first-order reaction $A \xrightarrow{k} B$ can be expressed as the derivative of the concentration of the substance that has reacted in time t

$v - d[A]/dt = k[A]$.

The rate of disappearance of the substance A is proportional to its concentration, where k is the proportionality coefficient called the first-order rate constant of reaction. The rate constant can be calculated by integration of the equation

$-d[A]/[A] = k\,dt$

between the limits $[A_1]$ at time t_1, and $[A_2]$ at time t_2:

$$k = \frac{1}{(t_1 - t_2)} \ln \frac{[A_1]}{[A_2]} - \qquad [s^{-1}]$$

Thus, the first-order rate constant is expresed in seconds raised to the power minus one (s–1). The rate of a bimolecular reaction, e.g. A + B → products, is proportional to the square of the reagent concentration or the product of concentration of two reagents to the power one:

$$v - \frac{d[A]}{dt} = k[A][B]$$

and for [A] = [B] the latter part of the equation can be written

$$- \frac{d[A]}{dt} = k[A]^2$$

of after transformation

$$- \frac{d[A]}{[A]^2} = k\,dt.$$

Integration of this equation between the limits $[A_1]$ at $t = t_1$ and $[A_2]$ at $t = t_2$ yields an expression

$$k = \frac{1}{(t_2 - t_1)} \left(\frac{1}{[A_1]} - \frac{1}{[A_1]} \right) \quad [\ell \cdot mol^{-1} \cdot s^{-1}]$$

which is expressed in litres per mole and per secound $[\ell \cdot mol^{-1} \cdot s^{-1}]$

As can be seen from Scheme 1, O_2^- can be produced either by the univalent reduction of the O_2 molecule or by the univalent oxidation of hydrogen peroxide.

The O_2^- sources which have been reported may be divided into two main groups 1. physico-chemical sources and 2. biological sources.

The variety of the reactions and biological systems able to generate O_2^- species, and the rapid expansion of new methods for their discovery may mean that this review is incomplete.

1.3.1 Physico-Chemical Processes for Superoxide Radical Generation

Several physico-chemical methods have been shown to produce O_2^-.

(a) Cathodic reduction of molecular oxygen in aprotic solvents [9, 10] or in water [11, 12] in a reversible one-electron way:

$$O_2 + e^- \rightleftharpoons O_2^- \tag{4}$$

(b) Dissolving KO_2 in aprotic solvent using crown ethers [13].

(c) Synthesis of tetramethylammonium superoxide [14, 15].

(d) Irradiation of air-saturated aqueous solutions by γ-rays, X-rays or electron beams from accelerators [16]:

$$H_2O \xrightarrow{\gamma, X} e_{aq}^-, HO^{\cdot}, H^{\cdot}, H_2, H_2O_2, H^+ \text{ and } HO^- \tag{5}$$

Solvated electrons (e_{aq}^-) and H-atoms react with oxygen molecules to form O_2^- (Eq. 4) as follows:

$$O_2 + H^{\cdot} \rightarrow HO_2^{\cdot} \xrightarrow{HO^-} O_2^- + H_2O \tag{6}$$

The HO radical produced in the reaction at Eq.(5), in the presence of formate ion or H_2O_2, is converted into $HO^{\cdot}{}_2$ and O_2^- by the following reactions:

$$HO^{\cdot} + HCOO^- \rightarrow CO_2^- + H_2O \tag{7}$$

$$CO_2^- + O_2 \rightarrow O_2^- + CO_2 \tag{8}$$

$$HO^{\cdot} + H_2O_2 \rightarrow HO_2^{\cdot} + H_2O \tag{9}$$

(e) Electrolysis of O_2 in alkaline solutions containing quinoline or triphenylphosphine oxide [17, 18].

(f) Ultrasonification of water [19, 20].

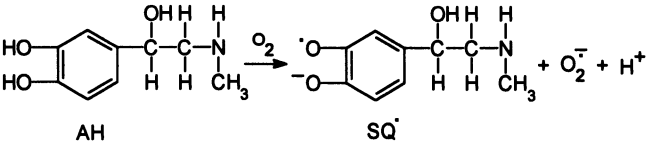
(g) Oxidation of H_2O_2 in the presence of cations or anions such as Co^{2+}, Cu^+, Ce^{4+}, IO_4^- [21], e.g.

$$Ce^{4+} + H_2O_2 \rightarrow Ce^{3+} + H^+ + HO_2^{\cdot} \tag{10}$$

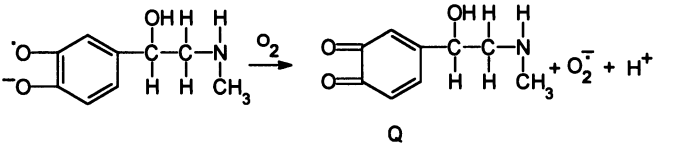
(h) Formation of metal (Me) – O_2 molecular complexes [22, 23]. The change in the redox behaviour of the transition metal ions[7] is due to their interaction with the O_2 molecule. Especially important in biochemistry is the fact that complexes of Fe and also of Cu, Mn, Mo or V, according to their order of abundance in nature, form oxy-complexes and release the O_2^- radicals:

$$Me^{n+} + O_2 \rightarrow MeO_2 \leftarrow Me^{(n+1)+} + O_2^- \tag{11}$$

(i) Reduction of oxygen molecules during autoxidation of some organic compounds like thiols [24], polyphenols [25–27], antibiotics [28–30], pesticides [31, 32] and semiquinones [25]. As an example, a theoretical reaction sequence forming O_2^- during autoxidation of biogenic catecholamines may be written [25, 27]:



AH SQ· (12)



Q (13)

where AH denotes the catechoalmine molecule, SQ· is the semiquinone free radical, and Q is the quinone of AH.

During autoxidation of thiols, the thiol anion undergoes oxidation in the presence of a transition ion to yield thiyl radical which can react with the O_2 molecule to give O_2^- [24]:

$$HS\text{–}R\text{–}S^- + Me^{n+} \rightarrow HS\text{–}R\text{–}S^{\cdot} + Me^{(n-1)+} \tag{14}$$

$$HS\text{–}R\ RM\ S^{\cdot} + O_2 \rightarrow \underset{\diagdown\ \diagup}{S\text{–}S} + H^+ + O_2^- \qquad R \tag{15}$$

(j) Deactivation of the triplet state of some dyes and pigments [33, 34]. All dye and pigment molecules are excited to singlet states by the absorption of light and then transformed to triplet states. Several compounds are sensitizers (S) when irradiated with visible light and thus they can produce O_2^- via the electron transfer process:

$$^1S_0 \xrightarrow{h\nu} {}^1S_1 \xrightarrow{ISC} {}^3S_1 \tag{16}$$

$$^3S_1 + O_2 \rightarrow S^{+\cdot} + O_2^- \tag{17}$$

---

[7] $Me^{n+} + e^- \rightarrow Me^{(n-1)+}$ (reduction: gain of electron)
$Me^{n+} - e^- \rightarrow Me^{(n+1)+}$ (oxidation: loss of electron)
n denotes valency of metal.

The reaction involves the electronic excitation of the sensitizer to the singlet state ($^1S_1$) which can undergo intersystem crossing (ISC) to form the metastable triplet state ($^3S_1$) of the sensitizer. The excited triplet state interacts with an O_2 molecule by electron transfer to form an O_2^- radical. The deactivation of the long-lived triplet states is essentially important for sensitized photoxidation of many organic stubstances and the above photo-redox reaction involving radical species is classified as a type I photosensitized reaction [33].

It is worth mentioning that ultraviolet radiation of melanins, dark-coloured pigments of high molecular weight formed by the enzymatic oxidation of phenols, mainly tyrosine, dopa, tyramine, etc, wide spread in the animal and plant Kingdom, also generates O_2^- in this way [35].

Apart from the above-mentioned physical factors initiating generation of O_2^- radicals, a new factor – temperature – is suggested. It has been shown [36] that the concentration of O_2^- species increases with increase in temperature. The observed increase may be explained by an increase of the oxygen concentration in a cell as a result of the restraining respiratory chain and oxygen utilization via hyperthermia [37].

1.3.2 Biological Sources of Superoxide Radical

The superoxide anion radical is formed in all aerobic organisms. O_2^- is not a main product of the biological reduction of molecular oxygen because rules of quantum mechanics lead to a spin restriction that favours its divalent reduction [38]. In living systems the redox reaction proceeds either under enzymatic control or the radicals are formed radomly in the presence of the potential electron donors. A number of enzyme systems are known to catalyze the transfer of electrons in a one-electron way.

Although the majority of the biological sources of O_2^- remain otherwise unknown, the essential sources producing substantial amounts of O_2^- radicals may be specified.

(a) Enzymes including xanthine oxidase [39], aldehyde oxidase [40], and dihydroorotic dehydrogenase [41] have been shown to produce the O_2^- radical. A number of flavoprotein hydroxylases, oxidases and dehydrogenases show the ability for O_2^- production [42] as well as galactose oxidase [43], indoleamine dioxygenase [44] and other enzymes [45, 46], which act via formation of this radical as an intermediate. It is interesting to mention that flavoprotein oxidase can catalyze direct reduction of O_2 even in the absence of cytochrome or cytochrome oxidase. It is possibly due to the presence of riboflavin, i.e. the prosthetic group of flavoproteins which are stable in their semiquinone form or at one-electron reduced forms. The O_2^- generation can occur by the reaction at Eq. (13).

(b) Mitochondrial and microsomal electron chains. Subcellular organelles such as mitochondria [47, 48] and chloroplasts generate O_2^- [49, 50]. Intramitochondrial O_2^- concentration is about 8 pmol $\cdot \ell^{-1}$ [51]. About 75% of all the O_2^- production in a cell originates just from the mitochondrial membrane.

(c) Transport of molecular oxygen by hemoglobin (Hb) catalyzed by protein also leads to O_2^- formation during oxidation of hemoglobin to methemoglobin [52]

$$Hb\text{–}Fe^{2+} \rightleftharpoons Hb\text{–}Fe^{3+}\text{–}O_2^- \tag{18}$$

(d) Continous generation of O_2^- radicals by members of the respiratory chain in the reduced form such as ubiquinone, cytochrome b and the iron-sulfur centres of the succinate dehydrogenase-cytochrome b system [53]. Especially large amounts of O_2^- are liberated during the respiratory burst accompanying phagocytosis by human polymorphonuclear leucocytes or granulocytes [54]. During phagocytosis oxygen consumption by polymorphonuclear leucocytes increases about 80 times, and the consumed oxygen is converted into the oxygen species. For example, human neutrophils stimulated by a chemotactic factor can release at least 1.35 nmol of O_2^- per million cells per min at 300 K [55].

(e) Autoxidation and oxidation of biogenic catecholamines (dopamine, noradrenaline and adrenaline) by ceruloplasmin and/or in the presence of trace amounts of transition metal ions [56–59], melanins [60] as well as by a wide variety of normal and malignant cell types stimulated by cytokine-growth factor or tumour cells [61].

(f) Synthesis of prostaglandins and tromboxans [62]. The O_2^- generation involves a variety of radical redox reactions yielding other oxidants such as H_2O_2, $HO^\cdot$, $^1O_2$, $RO^\cdot$, $ROO^\cdot$.

1.4 Hydrogen Peroxide

Hydrogen peroxide may be formed as a result of two types of reactions:

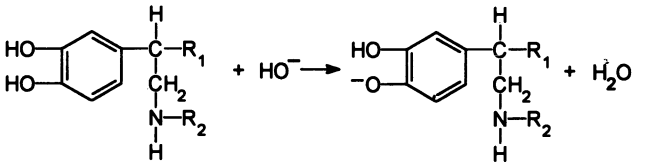
(a) Univalent reduction of molecular oxygen to O_2^- relates to the catalytic and non-catalytic dismutation of O_2^-. For this reason H_2O_2 is always present in all systems producing O_2^-, e.g. according to the reaction at Eq. (1) [63]. However this reaction is rather slow ($k = 4.5 \cdot 10^5\ \ell \cdot mol^{-1} \cdot s^{-1}$) [64] to have important biological meaning, but is strongly catalyzed by transition metal ions [65], especially iron ion:

$$Me^{(n+1)+} + O_2^- \xrightarrow{2\,H^+} Me^{n+} + H_2O_2 \tag{19}$$

Trace amounts of iron are present in most biological systems. It has been found that the iron concentration in liver cytosol may reach even 130 mmol $\cdot\ \ell^{-1}$ with average values of 55 mmol $\cdot\ \ell^{-1}$ [66]. It is well documented that H_2O_2 is continuously generated in vivo during phagocytosis and by endothelial cells [67–69]. Oxidation of thiols such as cysteine or glutathione with O_2^- can also yield H_2O_2 [70]:

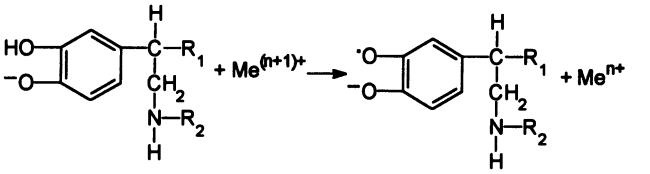
$$\begin{array}{l} HS\text{–}CH_2\text{–}CH\text{–}COOH \\ \qquad\quad | \\ \qquad\quad NH_2 \end{array} + HO_2^- \longrightarrow \begin{array}{l} {}^\cdot S\text{–}CH_2\text{–}CH\text{–}COOH \\ \qquad\quad\ \ | \\ \qquad\quad\ \ NH_2 \end{array} + H_2O_2 \tag{20}$$

Likewise, oxidation, both spontaneous as well as enzyme and metal ion catalyzed, of catecholamines, melanins and other biologically important polyphenols is accompanied by H_2O_2 generation. The reactions predicted by theory in which H_2O_2 can be generated during oxidation of biogenic catecholamines (dopamine, noradrenaline, adrenaline) are as follows [25]:



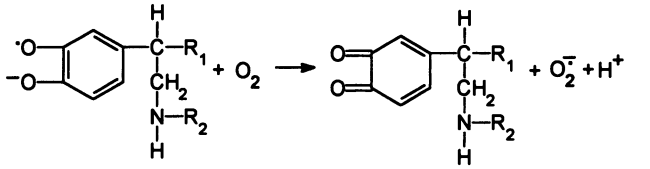
(21)

AH AH^-



(22)

SQ·



(23)

Q

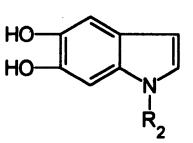
$R_1 = H$	$R_2 = H$	Dopamine
$R_1 = OH$	$R_2 = H$	Noradrenaline
$R_1 = OH$	$R_2 = CH_3$	Adrenaline

$$AH^- + O_2^- + 2H^+ \rightarrow SQ^\cdot + H_2O_2 \quad (24)$$

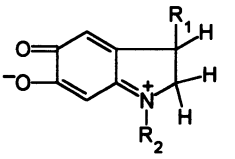
$$Q + O_2^- + H^+ \rightarrow SQ^\cdot + H_2O_2 \quad (25)$$

where AH is the catecholamine molecule, $SQ^\cdot$ denotes the semiquinone free radical of AH and Q is the quinone of catecholamine.

Catecholamines undergo ring closure forming 5,6-dihydroxyindole compounds as well as the corresponding semiquinone radical and quinones of these compounds. Thus, the similar reactions generating both O_2^- and H_2O_2 molecules can be repeated for the above-mentioned intermediates [58, 59]:



5,6-Dihydroxyindole



Quinone

(b) Divalent reduction of O_2, which is carried out by numerous oxidases like xanthine oxidase, uricase, and D-amino acid oxidase [48, 63]. The enzymes generating H_2O_2 by divalent reduction are usually localized within peroxisomes.

It is widely documented that H_2O_2 generation occurs in microsome [71], tissue homogenates [72], illuminated chloroplasts [73], and mitochondria isolated from different sources [48]. It was demonstrated that H_2O_2 could be generated at concentrations ranging from 10^{-9} to 10^{-7} mol $\cdot \ell^{-1}$ in perfused rat liver [74]. Submitochondrial particles containing either NADH[8] or succinate are also known to be efficient sources of H_2O_2 [48]. The rate of H_2O_2 generation in mitochondria depends on metabolic states [75], which may suggest that H_2O_2 is produced by components of the respiratory chain such as ubiquinone, cytochrome b and the transition metal-containing centre of the succinate dehydrogenase cytochrome b complex [48].

It is worthwhile to note that under pathophysiological conditions such as radiotoxemia, carcinogenesis, and strong mental stress, the yield of endogenous H_2O_2 production is increased. Among all oxygen species resulting from reduction of molecular oxygen, H_2O_2 in the most stable and readily determined.

1.5 Hydroxyl Radical

Superoxide radical and H_2O_2 are relatively non-toxic per se but they are precursors of the most powerful oxidant occurring in biological systems, i. e. the HO radical. The hydroxyl radical has not been detected directly in vivo. Its detection is generally inferred from its strong oxidative properties since it reacts with many cell components with rate constants of the order of 10^9–10^{11} $\ell \cdot \text{mol}^{-1} \cdot \text{s}^{-1}$, and from the presence of $HO^{\cdot}$-scavengers in cell components.

However some controversy arises concerning what are the sources of HO radicals in biological systems, and there exist several reactions by which $HO^{\cdot}$ may be producted in a cell, which are generally accepted. A number of the physicochemical reactions involved in environment can be specified.

(a) Photolysis of hydrogen perocide [21]

$$H_2O_2 \xrightarrow{h\nu} HO^{\cdot} + HO^{\cdot} \tag{26}$$

(b) Irradiation of oxygenated aqueous solution by γ-rays, X-rays or electron beams from accelerators (reaction at Eq. (5)) [6].

(c) One-electron transfer to ozone [76, 77].

$$O_3 + e \rightarrow O_3^- \text{ Pd } O_2 + O^- \tag{27}$$

$$O^- + H_2O \rightleftharpoons HO^{\cdot} + HO^- \tag{28}$$

$$O_3^- + H_2O \rightleftharpoons HO_3 + HO^- \tag{29}$$

$$HO_3 \rightarrow HO^{\cdot} + O_2 \tag{30}$$

[8] Nicotinamide adenine dinucleotide, reduced form.

These reactions may play a crucial role in ozone toxicity [78].

(d) The Haber-Weiss and Fenton reactions [79–82]:

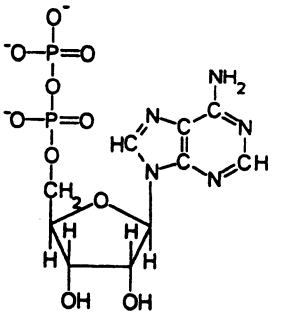
$$O_2^{\cdot-} + H_2O_2 \longrightarrow HO^{\cdot} + HO^- + {}^1O_2 \tag{31}$$

The reaction of $O_2^{\cdot-}$ with H_2O_2 is slow: $k = 3.0 \pm 0.6\ \ell \cdot mol^{-1} \cdot s^{-1}$ [83] and has only historical meaning under the name of the Haber-Weiss reaction. This reaction is accelerated by the presence of many transition metal ions of varing redox potential (Fe^{2+}, Cu^+, Cr^{2+}, Co^{2+}, etc.) or their complexes, as H_2O_2 reacts rapidly with them:

$$H_2O_2 + Me^{n+} \longrightarrow HO^{\cdot} + HO^- + Me^{(n+1)+} \tag{32}$$

The latter reaction when Fe^{2+} was used is known as the classic Fenton reaction, and the mixture of Fe^{2+} salts and H_2O_2 is called Fenton reagent. The $Me^{(n+1)+}$ ions can return to the Me^{n+} form in the presence of $O_2^{\cdot-}$ or H_2O_2, according to the reactions at Eqs. (19) and (10), respectively. Additionally, the $O_2^{\cdot-}$ radical may form a complex with some transition metal compounds, to become more stable and longer-lived than free $O_2^{\cdot-}$. Such a complex may also react with H_2O_2 to yield $HO^{\cdot}$.

The Haber-Weiss reaction modified by Fenton is believed to be the most important source of $HO^{\cdot}$ in biological systems. The conditions for such a type of reaction to occur exist in a cell because various forms of complexed iron are present in it, for example in heme or ADP[9] [84, 85]:



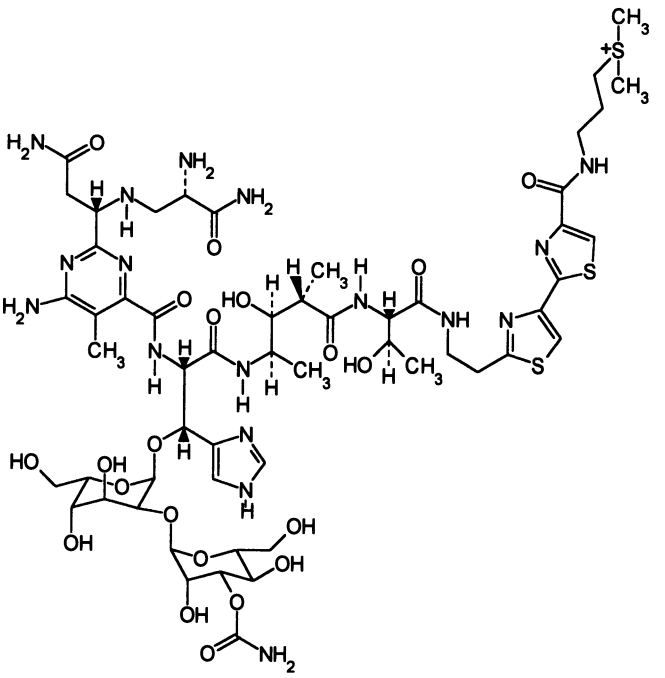
ADP

$$ADP\text{–}Fe^{3+} + O_2^{\cdot-} \rightarrow ADP\text{–}Fe^{2+} + {}^1O_2 \tag{33}$$

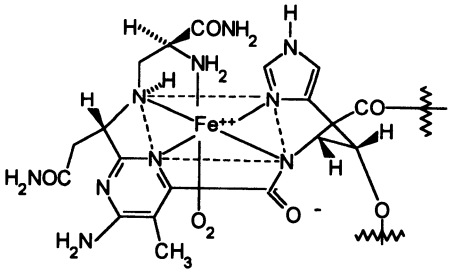
$$ADP\text{–}Fe^{2+} + H_2O_2 \rightarrow ADP\text{–}Fe^{3+} + HO^{\cdot} + HO^- \tag{34}$$

Cytotoxic action of bleomycin (BLM), an antibiotic showing antitumour activity, is also assumed to lead to the $HO^{\cdot}$ formation in a similar way [86]:

[9] Adenosine 5′-diphosphate.



BLM



(BLM)Fe^{2+}

$$BLM + Fe^{3+} \rightleftharpoons (BLM)\,Fe^{2+} \tag{35}$$

$$(BLM)\,Fe^{2+} + O_2 \longrightarrow (BLM)\,Fe^{3+} + O_2^- \tag{36}$$

$$(BLM)\,Fe^{2+} + O_2^- \xrightarrow{2H^+} (BLM)\,Fe^{3+} + H_2O_2 \tag{37}$$

$$(BLM)\,Fe^{2+} + H_2O_2 \longrightarrow (BLM)\,Fe^{3+} + HO^{\cdot} + HO^- \tag{38}$$

Similarly, Cu^{2+} ions readily form complexes with amino groups of proteins and other biomolecules and they undergo reductions to Cu^{+} ions which are able to generate $HO^{\cdot}$ by a Fenton-type reaction in the presence of H_2O_2 [87].

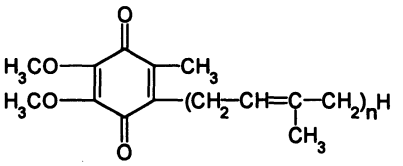
(e) Reactions of organic radicals, e.g. of semiquinone, thiyl and even simple ones such as those of ethanol or propanol, with H_2O_2 [21, 24, 25, 27]:

$$\dot{C}H_2OH + H_2O_2 \longrightarrow CH_2O + H_2O + HO^{\cdot} \quad (39)$$

Mitochondrial ubisemiquinone ($UQH^{\cdot}$) is an important catalyst in HO radical production [88]:

$$UQH^{\cdot} + H_2O_2 \longrightarrow UQ + HO^{\cdot} + HO^{-} \quad (40)$$

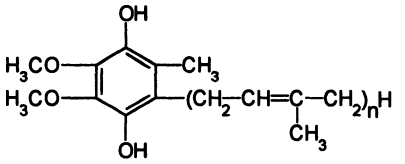
The same compound is responsible for the generation of $O_2^{\cdot-}$ by mitochondria. Ubihydroquinone (koenzyme Q) CoQ_6 occurs in certain microorganisms, and CoQ_{10} is present in mitochondria of the majority of mammals.



n = 6 CoQ_6

n = 10 CoQ_{10}

Coenzyme Q – oxidized form



n = 6 CoQ_6

n = 10 CoQ_{10}

Coenzyme Q – reduced form

Similarly, the thiyl radical generated during autoxidation of thiols (reaction at Eq. (14)) can react with H_2O_2 to give $HO^{\cdot}$ as follows [24]:

$$HS\text{–}R\text{–}S^{\cdot} + H_2O_2 \longrightarrow \underset{\backslash\,/}{S\text{–}S}\;(R) + HO^{-} + HO^{\cdot} \quad (41)$$

(f) Decomposition of peroxynitrite in the presence of protons [89]. Peroxynitrite is formed during the interaction of the nitric oxide radical ($NO^{\cdot}$) with $O_2^{\cdot-}$ [90]:

$$NO^{\cdot} + O_2^{\cdot-} \rightarrow ONOO^{-} \quad (42)$$

$$ONOO^{-} + H^{+} \rightarrow NO_2^{\cdot} + HO^{\cdot} \quad (43)$$

Both NO and $O_2^{\cdot-}$ radicals are generated by vascular endothelial cells [91].

(g) Finally, the generation of HO radicals and also $O_2^{\cdot-}$ has been observed from photoexcited powdery semiconductors such as TiO_2, WO_3, CdS and Fe_2O_3 in aqueous hydrogen peroxide solution [92].

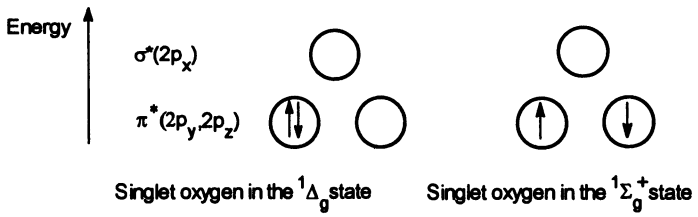
1.6
Singlet Oxygen

Superoxide anion radicals, hydrogen peroxide and hydroxyl radicals are formed in normal biochemical processes as well as on exposure of biological systems to light or irradiation. These oxygen species are known to be precursors of an energetically richer form of molecular oxygen called singlet oxygen.

Singlet oxygen was discovered in 1924, but an explosive development of study of its physico-chemical and expecially biological properties has been recorded since 1963, and more than one hundred papers are published annually [93].

1.6.1
Spectroscopic Properties

Absorption of a suitable portion of energy by the oxygen molecule, e.g. by exposure, irradiation or by its mode of formation, may change the electronic structure such that new states are formed. Molecular oxygen has two low-lying singlet states, lower, $^1\Delta_g$, and higher, $^1\Sigma_g^+$. Electronic configurations of these states differ from the ground state configuration only by structure of the antibonding π-orbitals, and may be depicted as follows:



The $^1\Delta_g$ state is not a radical, because the electrons in the highest occupied orbitals occupy the same $\pi^*(2p_y)$ orbital and their spins must be antiparallel, according to the Pauli principle. It has the following configuration of molecular orbitals [94]:

$$O_2\,[KK\,(2s\sigma)^2(2s\sigma^*)^2(2p_x\sigma)^2(2p_y\pi)^2(2p_z\pi)^2(2p_y\pi^*)^2\,].$$

In the second excited state, $^1\Sigma_g^+$, the electronic configuration of the oxygen molecule is identical to that of the ground state, but in this state the last two electrons have antiparallel spins. Since the transition from the $^1\Delta_g$ state to the $^3\Sigma_g^-$ state is spin-forbidden, $^1\Delta_g$ is a relatively long-lived species. On the contrary, the $^1\Sigma_g^+$ state is sufficiently short-lived due to a spin-allowed transition to the $^1\Delta_g$ state. Radiative lifetimes of $O_2(^1\Delta_g)$ and $O_2(^1\Sigma_g^+)$ in the gas phase, estimated by integrated absorption measurements, are 45 min and 7–12 s, respectively [95]. The $^1\Delta_g$ state of the O_2 molecule is much more stable as ascertained by the determined lifetime and will be mainly referred to in this book.

Collisions of singlet oxygen with other molecules shorten the lifetimes drastically. For example, for O_2-O_2 molecular pair singlet excited states (also cal-

led $^1O_2$-dimoles or $^1O_2$-dimers) such as 2 $O_2(^1\Delta_g, {}^1\Delta_g)$, 2 $O_2(^1\Delta_g, {}^1\Sigma_g^+)$ and 2 $O_2(^1\Sigma_g^+, {}^1\Sigma_g^+)$, formed during collision of the single molecules of oxygen in excited state $^1\Delta_g$, $^1\Sigma^+_g$, the following radiative lifetimes have been calculated: 1.5, 1.7 and 0.3 s, respectively [95].

In solution the lifetimes are decreased drastically and vary from 10^{-6} to 10^{-3} s for the $^1\Delta_g$ state and from 10^{-11} to 10^{-9} s for the $^1\Sigma_g^+$ state [96, 97]. They are strongly dependent upon the type of solvent. The shortest lifetime of $^1\Delta_g$, about 2 ms, is observed in water, the longest in Freon-11 (about 1000 ms) [98]. The lifetime is usually increased by a factor of 10 in deuterated solvents [99]. The solvent effects on the lifetime of $^1O_2$ are discussed in some detail by Kearns [98] and are beyond the scope of this book as they are not concerned with biological conditions.

The $^1\Delta_g$ and $^1\Sigma_g^+$ states have energies of 95 and 158 kJ · mol^{-1} above the triplet ground state, respectively [100]. A greater number of papers have appeared which describe a light emission accompanying radiative deactivation of electronically excited oxygen as well as spectroscopic comparison with its absorption bands observed in the gaseous state at high presure [95–97, 101–104].

Although the $^1\Sigma_g^+ \leftrightarrow {}^3\Sigma_g^-$ transitions in the single oxygen molecule, observed at 762.1 nm, are highly electric dipole forbidden on the basis of both spin and symmetry, and the $^1\Delta_g \leftrightarrow {}^3\Sigma_g^-$ transitions at 1268.7 nm, in addition, orbitally forbidden, these transitions are observed in the absorption and emission spectra due to the extraordinary metastability of the $^1\Delta_g$ and $^1\Sigma_g^+$ states. The forbidden transitions in the single molecules became spin-allowed for the stimultaneous transitions in the collision pairs. The emission from single molecule oxygen excited states and from O_2–O_2 molecular pair excited states is observed with gaseous oxygen as well as in solution. In the latter case, the emission comes from metastable singlet oxygen trapped in gas bubbles. The transitions are shown in Fig. 3.

The emission observed at 1070 nm corresponds to the vibrational levels $(1 \rightarrow 0)$ of the $O_2[^1\Delta_g \rightarrow {}^3\Sigma_g^-]$ transition. The remaining transitions presented in Fig. 3 correspond to the simultaneous transition of two excited oxygen molecules to the ground state in a two molecule-one photon process. For example, the $2[^1\Delta_g] \rightarrow 2[^3\Sigma_g^-]$ transitions with the vibrational quantum numbers of (1, 0), (0, 0) and (0,1) are observed at 580, 640 and 700 nm, respectively [104]. The emission at 381 nm is the (0, 0) transition in the $2[^1\Sigma_g^+] \rightarrow [^3\Sigma_g^-]$ system.

For exhaustive detail related to spectral properties of $^1O_2$, the reader is referred to the excellent review of Khan and Kasha [105].

1.6.2 Generation of Singlet Oxygen

There exists several sources generating $^1O_2$. These sources fall into five groups:

- chemical
- physical
- photosensitized reactions

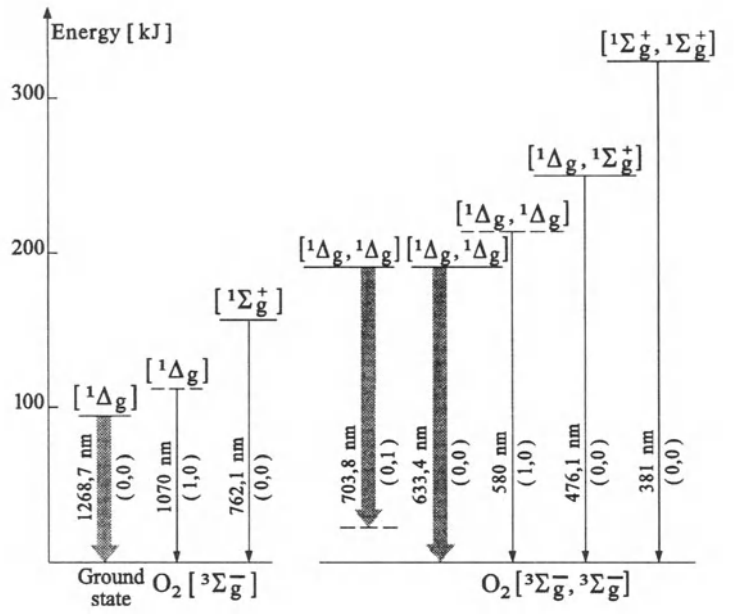


Fig. 3. Emission transitions of molecular oxygen observed for single molecule states and simultaneous transition O_2–O_2 molecular pair states with vibronic components

- biological
- pharmaceutical origin

1.6.2.1 *Chemical Sources*

(1) The reaction hypochloride ion (OCl^-) with hydrogen peroxide in aqueous solution generates both $^1\Delta_g$ and $^1\Sigma_g^+$ states of $^1O_2$ [105]:

$$OCl^- + H_2O_2 \rightarrow HOCl + HO_2^- \quad (44)$$

$$HO^-_2 + HOCl \rightarrow HOOCl + HO^- \quad (45)$$

$$HOOCl + HO^- \rightarrow H_2O + ClOO^- \quad (46)$$

$$ClOO^- \rightarrow {}^1O_2 + Cl^- \quad (47)$$

A similar mechanism of $^1O_2$ generation is proposed for the decomposition of H_2O_2 by bromine or chlorine in alkaline solution [106, 107]:

$$H_2O_2 + Br_2 \rightarrow H^+ + Br^- + HOOBr \quad (48)$$

$$HOOBr + HO^- \rightarrow H_2O_2 + BrOO^- \quad (49)$$

The bromoperoxy anion undergoes decomposition to $^1O_2$ and bromate anion according to the reaction at Eq. (47). It is worth noting that the emission is intensive and can even be observed visually in a darkened room.

(2) Hydrogen peroxide disproportionation catalyzed by molybdate ions (MoO_4^{2-}) in moderately alkaline solution [108]. The MoO_4^{2-} reacts catalytically with H_2O_2 to form $^1O_2$, and MoO_6^{2-} anion is formed as an intermediate:

$$MoO_4^{2-} + 2H_2O_2 \rightarrow MoO_6^{2-} + 2H_2O \quad (50)$$

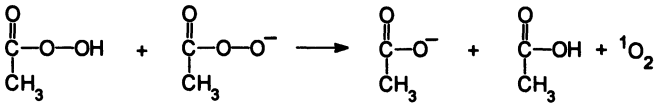
$$MoO_6^{2-} \rightarrow MoO_4^{2-} + {}^1O_2 \quad (51)$$

The $^1O_2$ formation has also been reported during base catalyzed disproportionation [109]:

$$H_2O_2 + HO^- \rightleftharpoons HOO^- + H_2O \quad (52)$$

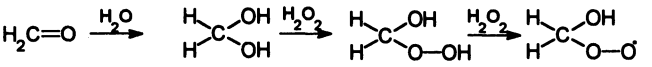
$$H_2O_2 + HOO^- \rightarrow H_2O + HO^- + {}^1O_2 \quad (53)$$

(3) Decomposition of organic peracids in alkaline solution, e.g. peracetic acid [107]



(54)

(4) The Trautz-Schörigin reaction: the oxidation of formaldehyde by H_2O_2 in the presence of pyrogallol, known as the Trautz-Schörigin reaction [110, 111], is a very efficient source of $^1O_2$. This species may also be generated during peroxidation of formaldehyde in the presence of several biologically important polyphenols and quinones, and the following reactions sequence is proposed [111–114]:



(55)

$$CH_2O + H_2O_2 \rightarrow CH_2(OH)OO^- \quad (56)$$

$$CH_2(OH)OO^- + SQ^{\cdot} \rightarrow CH_2(OH)OO^{\cdot} \quad (57)$$

$$2CH_2(OH)OO^{\cdot} \rightarrow CH_2(OH)OOCH_2OH + {}^1O_2 \quad (58)$$

The yield of the $^1O_2$ generation was strongly enhanced in the presence of semiquinones ($SQ^{\cdot}$) compared to that found during peroxidation of CH_2O alone [113, 114].

(5) Autoxidation or peroxidation of a number of biologically important phenols and polyphenols [58, 115–120] such as gallic acid, pyrocatechol, dopa, biogenic catecholamines, and tetracyclines.

Singlet oxygen generated during oxidation of the compounds mentioned above is believed to be a product of the interaction between the reduced oxygen

intermediates such as O_2^-, $HO^\cdot$ and H_2O_2, (see reactions at Eqs. (1), (3), (31), and (33)). Additionally, it has been shown [105] that the oxidation of O_2^- produces singlet oxygen:

$$O_2^- \rightarrow {}^1O_2 + e^- \tag{59}$$

as well as the reduction of HO radical by O_2^- [121]:

$$O_2^- + HO^\cdot \rightarrow HO^- + {}^1O_2 \tag{60}$$

(6) The electron transfer from O_2^- to radical cations ($X^{+\cdot}$) [122]:

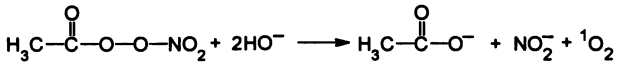
$$X^{+\cdot} + O_2^- \rightarrow X + {}^1O_2 \tag{61}$$

(7) The decomposition of the oxygen complexes of the transition metals, for example potassium peroxochromate (K_3CrO_8) in aqueous solution [123]:

$$4CrO_8^{3-} + 2H_2O \rightarrow 4CrO_4^{2-} + 4HO^- + 7O_2 \tag{62}$$

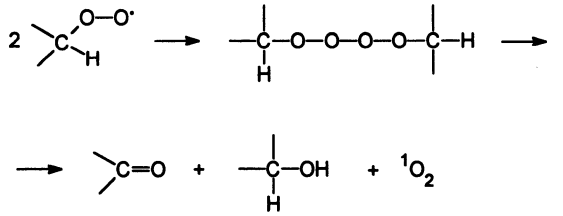
The decomposition of this ion in basic solution also liberates O_2^-.

(8) The base-catalyzed decomposition of peroxyacetyl nitrate, a compound generated by sunlight in polluted atmospheres containing hydrocarbons and nitrogen oxides [124]:



(63)

(9) The dismutation of two secondary butylperoxy radicals (so called Russel termination mechanism) [125–127]:



(64)

and the self reaction of hydroperoxy radicals [128]:

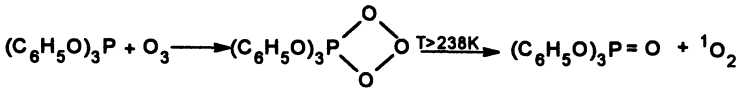
$$2HOO^\cdot \rightarrow H_2O_2 + {}^1O_2 \tag{65}$$

(10) Photolysis of ozone by ultra-violet light [129]

$$O_3 \xrightarrow[\lambda < 260\ \text{nm}]{h\nu} {}^1O_2 + O({}^1D)\ ^{10} \tag{66}$$

[10] Electronically excited oxygen atom with an energy of about 2 eV above the ground state, $O(^3P)$.

(11) Spontaneous decomposition of the adducts formed between phosphite esters and ozone [130]. In the reaction of triphenyl phosphite with ozone at 203 K in a methylene chloride solution the adduct is formed, which is stable at low temperatures. When the adduct is allowed to warm it decomposes to phosphate and $^1O_2$ is evolved:



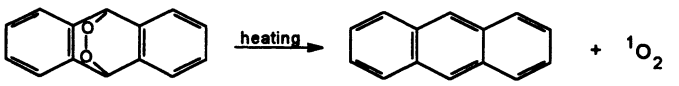
(67)

Ethers and alcohols also react with O_3 to yield $^1O_2$ [131].

(12) Oxidation of nitric oxide by ozone [132]

$$NO + O_3 \rightarrow NO_2 + {}^1O_2 \tag{68}$$

(13) Decomposition of endoperoxides [133]; aromatic hydrocarbons such as anthracene, rubrene and tetracene, cyclohexandiene derivatives, furans, and sulfides form endoperoxides during photosensitized autoxidation. These peroxides undergo decomposition on heating to release $^1O_2$ and the parent substrate:



(69)

1.6.2.2 *Physical Methods*

(1) Electric discharge [134, 135]; in this method molecular oxygen of high purity (99.999%) is passed through a discharge zone (the quartz tube), especially constructed in order to obtain maximum absorption by oxygen, with linear flow at a pressure 1–10 torr. Microwaves are generated by magnetron, e.g. type 12 T 202 Bosch (Germany) operating at 2450 MHz with a power up to 200 W. The atomic oxygen also generated during the electric discharge is removed with mercury vapour passed through the discharge zone by formation of a mercuric oxide ring.

(2) Laser irradiation [135, 136]; the next method of the direct generation of singlet oxygen requires high oxygen pressures, up to 140 atm, since the absorption intensity is proportional to the square of the oxygen concentration. Therefore the reaction vessel must be a high pressure cell which withstands such high pressure. The 1.065 μm beam from the Nd-YAG laser (multimode C.W. at a power of 4–6 W) can be used, for example, in order to obtain the $^1\Delta_g$ state.

1.6.2.3
Type II Photosensitized Reactions

Sensitizers which may react by the Type II mechanism are organic dyes. The process of $^1O_2$ production via photosensitized reactions, a basic method for generating $^1O_2$ in a biological system, has been shown to occur according to the following equation [137, 138]:

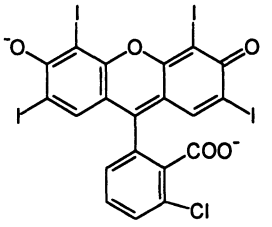
$$^1S_o + h\nu \rightarrow {}^1S_1 \xrightarrow{ISC} {}^3S_1 \xrightarrow{O_2} {}^1S_o + {}^1O_2 \qquad (70)$$

$$k \approx 10^9\ \ell \cdot M^{-1} \cdot s^{-1}$$

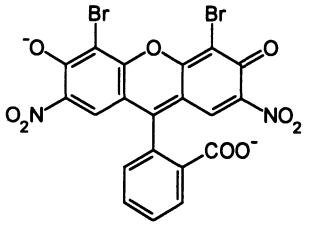
where ^1S_o is the dye molecule in the ground state, $^1S_1$ and $^3S_1$ are the dye molecule in electronically excited singlet and triplet states, respectively, and ISC denotes intersystem crossing.

The photosensitized reaction proceeds by the energy transfer from the triplet state of the sensitizer to the oxygen molecule producing $^1O_2$. The most effective sensitizers are, therefore, those which give a long-lived triplet state. Dyes such as rose bengal, eosin, methylene blue and pigments (flavins, porphyrins, chlorophylls) and aromatic hydrocarbons (anthracenes) are very effective sensitizers.

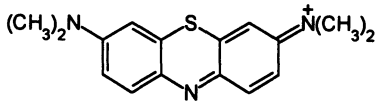
Rose bengal, eosin and methylene blue are sensitizers very often used in laboratories for $^1O_2$ generation.



Rose Bengal

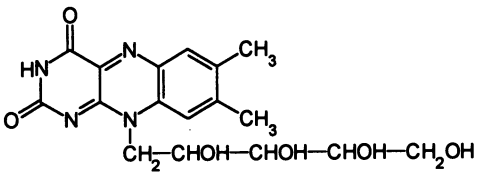


Eosin B

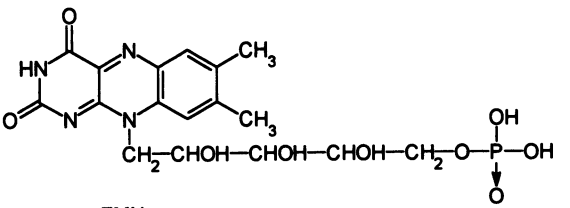


Methylene Blue

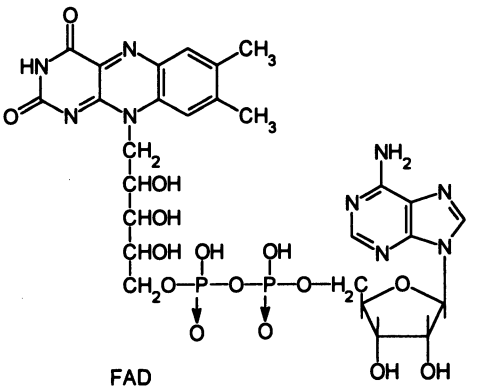
Flavins such as riboflavin (vitamin B_2), present in lean meat, liver, kidney, cereal germ and milk is a prosthetic group in a large number of systems carrying out cellular oxidation. Riboflavin is the basic structural unit of coenzyme FMN[11] and FAD[12].



Riboflavin (vitamin B_2)



FMN

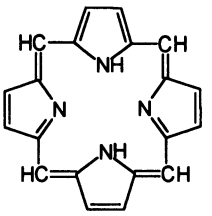


FAD

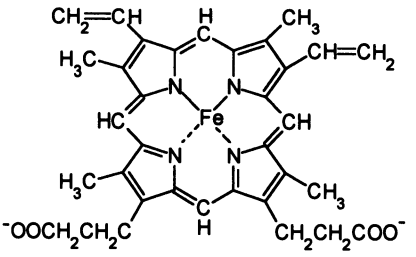
Porphyrins are porphins with hydrocarbon substituents in the pyrrole and pyrrolenine rings. Porphin is the main constituent part of blood heme and chlorophylls a and b. Heme is the prosthetic group known as ferroprotoporphyrin IX bound to a protein globin through a coordinate bond. Heme contain ferrous ion (Fe^{2+}) in the centre of the molecule with four nitrogen ligands pro-

[11] Flavin mononucleotide.
[12] Flavin adenine dinucleotide.

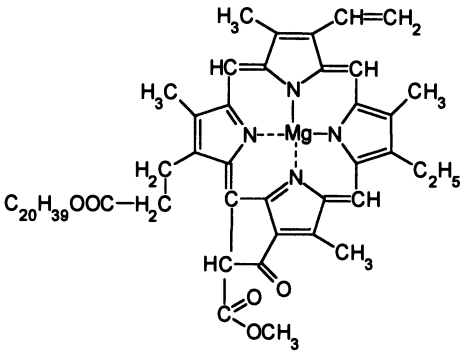
vided by the protoporphyrin and another from a histidine in the protein and the sixth coordination position may be occupied, e.g. by O_2. In this case Fe^{2+} is oxidized to the ferric ion (Fe^{3+}) and methemoglobin is formed.



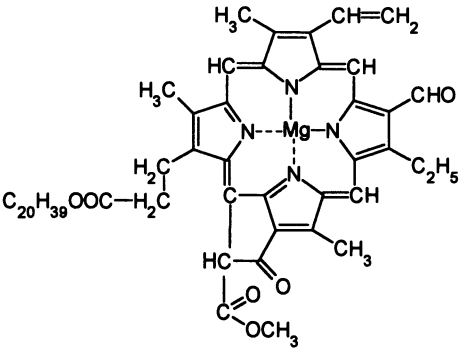
Porphin



Heme

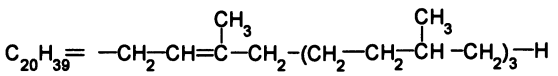


Chlorophyll a



Chlorophyll b

where



It has been shown that several endogeneous as well as exogeneous compounds can sensitize the $^1O_2$ formation both in the gas and liquid phase [139, 140]. The rate of the $^1O_2$ formation depends mainly on the oxygen concentration and on the type of sensitizer.

Some olefins and aromatic compounds from automobile exhausts can also act as sensitizers [130, 131].

1.6.2.4 *Biological Systems*

The $^1O_2$ formation in biological systems has been described in several excellent reviews [141–143]. Special attention has been paid to the formation of $^1O_2$ with the participation of a number of enzyme systems like myeloperoxidase, microsomal lipid oxidase, chloroperoxidase, xanthine oxidase, lactoperoxidase, lipoxidase, prostaglandin oxygenase, cyclooxygenase, and lipooxygenase.

The enzyme system consisting of a peroxidase (myeloperoxidase, chloroperoxidase, lactoperoxidase), H_2O_2 and a halide ion such as bromide, chloride or iodide (X^-) has been reported to produce $^1O_2$ by the following mechanism [144–149]:

$$H_2O_2 + H^+ + X^- \xrightarrow{\text{peroxidase}} H_2O + HOX \quad (71)$$

$$H_2O_2 + HOX \rightarrow H_2O + H^+ + X^- + {}^1O_2 \quad (72)$$

The halide ion plays the role of a cofactor and is oxidized by H_2O_2 and peroxidase, producing $^1O_2$. This method of $^1O_2$ generation is similar to that of the chemical reaction of the OCl^- plus H_2O_2 interaction, the reactions at Eqs. (45)–(47).

Singlet oxygen generation by xanthine oxidase when incubated with one of its substrates has been reported in several review [150–153]. Most authors postulate the $^1O_2$ generation by a metal catalyzed Haber-Weiss reaction (Eqs. (31) and (32), since the xanthine oxidase system generates O_2^- and H_2O_2, and/or via the dismutation of O_2^- formed through an electron transfer.

Very compelling evidence has shown that $^1O_2$ is produced by the microsomal lipid oxidase in the presence of NADPH[13] through the self-reaction of lipid peroxy radicals according to the Russel mechanism reaction at Eq. (64) [154–157]. However, several investigators have obtained divergent results dealing with the identification of $^1O_2$, but these discrepancies may be mainly attributed to technical differences in experiments.

The Russel mechanism has also been postulated to be responsible for the $^1O_2$ generation when prostaglandin-endoperoxide synthethase acts on arachidonic acid [158, 159].

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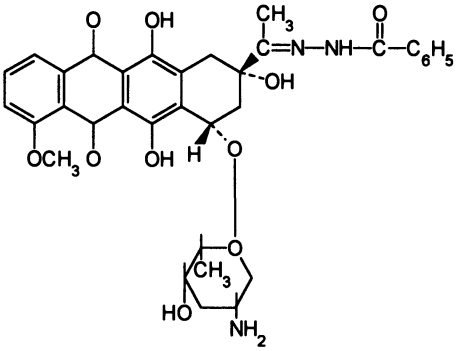
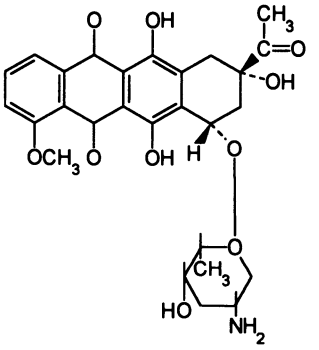
[13] Nicotinamide adenine dinucleotide phosphate, reduced form.

1.6.2.5
Pharmaceutical Substances

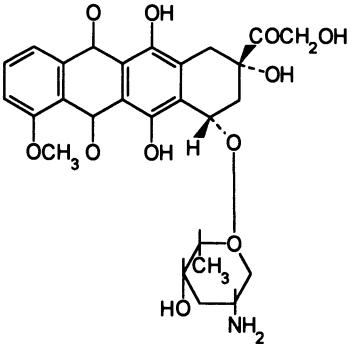
Drugs used in the treatment of cancer or various infectious disease, e.g. bleomycin, rubidazone, daunorubicin, adriamycin, armorubicin (the structura analog of adriamycin) or mitomycin C can be responsible for $^1O_2$ generation [160–164].



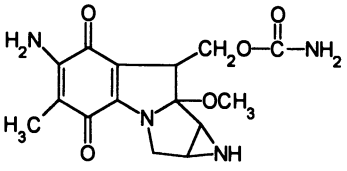
Rubidazone



Daunorubicin



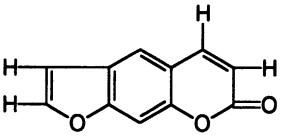
Adriamycin



Mitomycin C

For example, the mechanism of DNA [14] degradation by bleomycins, a group of glycopeptide antibiotics showing antineoplastic activity, has been found to be related to its ability to generate reactive oxygen species as well as $^1O_2$, according to the reactions at Eqs. (35–(38), (60), (65).

It is worth noting that psoralen and derivatives, compounds used in therapy of certain skin diseases, are good sensitisers.



Psoralen

Although there exist many more reactions generating toxic oxygen species and $^1O_2$, their discussion is limited by space in this book. This chapter has attempted to select from available literature the particularly significant reactions from the environmental and health point of view, at the present state of knowledge in this field.

[14] Deoxyribonucleic acid.

2 Role of Transition Metal Ions in Generation of Oxygen Species and Their Interconversion

Of the oxygen free radicals which could potentially be involved in the generation of singlet oxygen, $HO^{\cdot}$, $O_2^{\cdot-}$ and $ROO^{\cdot}$ (i.e the three which are involved in excess in biological systems) seem to be the most likely candidates. Many of the oxygen species such as $O_2^{\cdot-}$, $HO^{\cdot}$, O, O_3 and $^1O_2$ are always present in a cell since they are involved by exposure to sunlight, the earth's natural background of high energy radiation and by a normal physiological mechanism. The autoxidation of industrially important chemicals and the oxidation of several compounds in the atmosphere are also efficient sources of these species.

The oxygen species easily undergo interconversion into each other under physiological conditions (Fig. 4). For a description and discussion of the known interconversion reactions of $^1O_2$ and other oxygen species, the reader is referred to compilations by Singh [165] and Bielski et al. [5]. The compilations also contain free energies and rate constants for oxygen species conversion in aqueous solution.

Most transition metals play an important role in the generation and decomposition of active oxygen species due to their ability to participate in oxidation-reduction reactions. Their variable valency allows them to take part in radical reactions, and they can act as donors and acceptors of electrons. Especially important for living organisms are, according to their order of abundance in nature, iron, copper, manganese, molybdenum and vanadium ions. Activation or

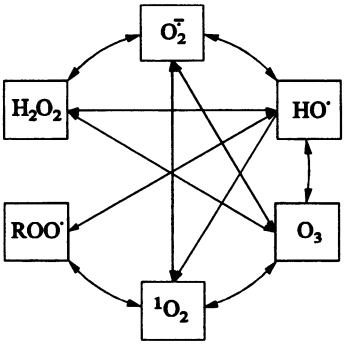


Fig. 4. Interconversion of oxygen species

deactivation of the oxygen molecule by the metal ions may also result from the ability of the ions to form reversible complexes or to change the conformation of a biomolecule. Formation of the oxy complex, which is a diamagnetic molecule, removes the restrictions for the oxygen reactions with molecules in the singlet state. It causes an increase in the oxidation properties of the oxygen complexes compared to the oxygen molecule alone. The variation in the redox behaviour of the metal ion and its spin state results from the type of ligands and its field geometry.

The variation of the metal ion redox properties by its coordination sphere is responsible for the broad spectrum of the transition metal ions interaction with the oxygen molecule. All of the transition metal properties are important in a normal physiological mechanism as well as in pathological conditions associated with the deregulation of the oxygen molecule activation.

In humans 70% of the iron is present in hemoglobin and about 25% as storage iron (myoglobin, various enzymes and transferrin). In aqueous solution, iron occurs in two oxidation states – divalent as ferrous (Fe^{2+}) and trivalent as ferric (Fe^{3+}). The physiologically stable form of iron is trivalent, and biological systems are able to transport iron in its stable triplet state. Activation of the oxygen molecule by iron ions is a normal physiological reaction, as iron is involved in the transport of O_2 by hemoglobin (reaction at Eq. (18)). Hemoglobin formulates with the oxygen molecule a superoxo-Fe^{3+} complex [22, 166, 167].

Transferrin, the transport glycoprotein, can bind at most two moles of iron per mole at physiological pH [168]. The next protein, similar to transferrin, released by phagocytic cells, lactoferrin, is present in several body fluids and in milk. It also binds 2 moles of iron per mole of protein [169].

Iron transferred within a cell is stored as ferritin and hemosiderin. Ferritin consists of a protein shell surrounding an iron core which can contain as many as 4500 atoms of iron per molecule of protein. Ferritin is present in all mammalian cells, with the greatest concentration in the liver. Iron enters ferritin as Fe^{2+} and is converted into the oxyhydroxide form of Fe^{3+} in the presence of the protein as an oxidant. Iron can be released from ferritin as the divalent ferrous ion (Fe^{2+}). Several models have been proposed for the formation of ferritin and iron release from it [170, 171].

In lysosomes, ferritin can be transformed into an insoluble compound known as hemosiderin.

Iron is also a component of the terminal oxidase of the mitochondrial electron-transfer pathway, cytochrome oxidase and several other enzymes such as microsomal cytochrome P450, both heme and non-heme containing dioxygenases, hydroxylases of phenylalanine, tyrosine and tryptophan, and xanthine oxidase [172].

Enzymes which are involved in defence mechanism against $O_2^{\cdot-}$ and H_2O_2 like SOD[1], catalase and peroxidase also contain iron atoms.

[1] Superoxide dismutase.

Apart from the above-mentioned complexes of iron ions with high-molecular-weight compounds, iron also chelates with low-molecular-weight chelating substances such as ADP, ATP[2], organic acids, membrane lipids or DNA[3] [173].

The stability of the oxy-complexes $[MeO_2]^{n+}$ strongly depends on the pH. In the presence of proton donors they undergo decomposition to yield H_2O_2 (Fig. 5). Furthermore it is generally accepted that H_2O_2 yields HO radicals when it reacts with Me^{n+} ions through the Haber-Weiss reaction. According to this mechanism the oxy-complex of the metal being bound to a cell compound would produce HO radicals almost at the point where it was bound [174–176].

The figure explains the increase of the toxicity of H_2O_2 by metal ions through the formation of either HO· or a metal peroxocomplex or a higher valency state of the metal ion ($Me^{(n+2)+} = O$) which is also a highly oxidizing species [22]. Both species may have great reactivities towards most biological molecules. They can initiate lipid peroxidation and alcohol oxidation, which will, in turn, lead to the formation of the organic oxy radicals such as peroxy radicals ($RO_2^{\cdot}$), alkoxy radicals (RO·), hydroperoxides, and peroxides (ROOR). Recently, there has been growing awareness of the participation of oxy radicals in metabolism, pathology, stress physiology, ageing, carcinogenesis and in neuro and brain biochemistry [169].

The enhancement of the toxicity of $O_2^{\cdot-}$ caused by transition metal ions is observed in many biological processes, such as eschemia [177–179], the action of drugs like bleomycin, adriamycin [96, 180, 181], and toxins [169], phagocytosis [54, 168], and other human diseases. It has been observed that bleomycin damages DNA only in the presence of iron salts, oxygen and reducing agents [182].

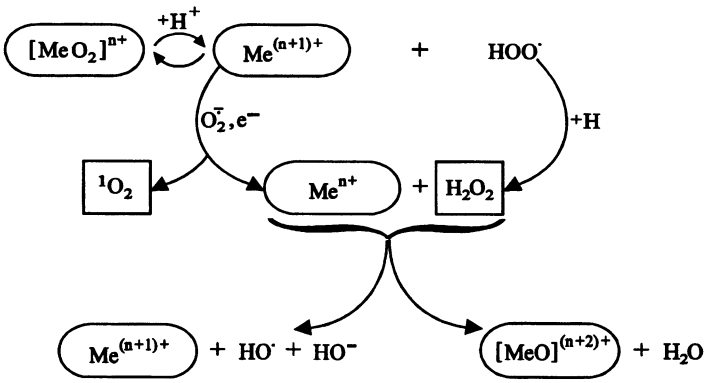


Fig. 5. Role of the transition metal in activation of molecular oxygen

[2] Adenosine triphosphate.
[3] Deoxyribonucleic acid.

Iron and copper complexes are the best known compounds for reactions with $O_2^{\cdot-}$. It is interesting to note that many iron, copper and manganese compounds catalyze the dismutation of $O_2^{\cdot-}$ with high efficiency.

There are several conflicting reports dealing with the activity of transferrin – and lactotransferrin bound – iron in generating HO radicals under physiological conditions [183–189]. Some authors found the activity as good [183–185], and others very poor, even equal to zero [186–188]. But the authors are in agreement with the activity of these complexes in iron-overload conditions [189]. Ferritin and hemosiderin are also able to stimulate $HO^{\cdot}$ formation [190, 191], although hemosiderin is less active in this respect.

The mechanism of the metal-catalyzed dismutation of $O_2^{\cdot-}$ and enormous importance of the metal ion as a component of many enzymes in the protection of a cell against oxygen toxic species will be discussed in Chap. 8.

It is worthwhile to mention that oxidation with participation of Fe^{3+} ions is selective and can be very fast ($k \approx 10^8\ \ell \cdot mol^{-1} \cdot s^{-1}$), although ligands decrease the rate constant to a value of $< 10^4\ \ell \cdot mol^{-1} \cdot s^{-1}$ [80, 192]. Radical reduction by Fe^{2+} is also very fast.

Copper ion is a weaker oxidizing agent. Oxidation by Cu^{2+} is slower than the ones observed with Fe^{3+} [192]. Copper is absorbed from the diet as complexes with amino acids and peptides [193, 194]. The main protein of human plasma containing copper is a ceruloplasmin. It contains six or seven copper ions atoms per molecule. Copper linked to amino acids or small peptides is able to interact with $O_2^{\cdot-}$ and H_2O_2 and to produce $HO^{\cdot}$ and $^1O_2$ according to Fig. 5. In contrast, copper incorporated into ceruloplasmin is only released at low pH in the presence of a reducing agent, and therefore binding copper plays an important role as an extracellular antioxidant [195, 196].

Chelating agents can play a double role in some in vivo systems. Some of them can degrade certain cell constituents by generating the toxic oxygen species. On the other hand the chelating agents protect a cell against oxidative damage by pulling the metal ions away from its sensitive sides.

For this reason the metal chelates are widely used in pharmacology [169]. For example desferrioxamine, the most effective chelating agent, is used in therapy for patients suffering from thalasaemia or to prevent iron-overload occurring, e.g. during repeated blood transfusion. An iron-overload also occurs during increased dietary absorption, or transfusion when increased accumulation of iron is not compensated by an increase in its excretion. In iron-balance the adult human being absorbs and excretes about 1 mg of iron per day. Disturbances in the iron metabolism will lead to either iron-overload or to its deficiency.

3 Reactions of Oxygen Free Radicals and Singlet Oxygen

3.1 General Consideration

Oxidation is a general process but the oxygen molecule in the triplet state reacts with low rate constant. This process is strongly accelerated because oxygen in most oxidation reactions takes part as "activated species", i.e. as $O_2^{\cdot-}$, $HO^{\cdot}$, $RO^{\cdot}$, $^1O_2$, H_2O_2. There is no doubt that these oxygen species are involved in the development of various pathological states in situations when the balance between prooxidant factors and antioxidants gets out of a control.

In order to consider a number of important complications caused by these species in biological systems, in the autoxidation of industrially important compounds and compounds occurring in the atmosphere, elucidation of basic mechanisms of chemical reactions, especially those of oxidation and reduction, created by these radicals and other oxygen species will be examined.

A free radical is a chemical species possessing one or more unpaired electrons occupying an outer orbital. Upon acceptance of an electron a free radical is converted into an anion, while on giving an electron up a free radical becomes a cation. Molecular oxygen ($^3\Sigma_g^-$) is a species containing two unpaired electrons in π^* antibonding orbitals. The definition of a free radical also embraces the hydrogen atom and most transition metal ions. Before considering the chemical reactivity of $O_2^{\cdot-}$, the formation of free radicals and their reactions are briefly summarized.

Any free-radical chain reaction involves three steps: initiation, propagation, and termination.

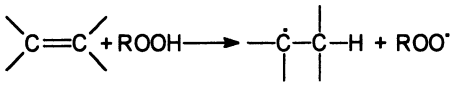
3.1.1 Initiation

Free radicals are formed in several types of processes [197].

(a) The homolysis[1] of molecules containing weak bonds (C–C, O–O, C–N=N, C–Me^n).

[1] During homolysis two electrons constituting a bond are separated (cleavage of the bond) and two free radicals are generated.

Several factors may influence the homolysis of bonds, e.g. the presence of other molecules (alcohols, acids, etc.). For example, olefins accelerate the decomposition of hydroperoxides by either a hydrogen atom transfer or an attack on an oxygen atom:



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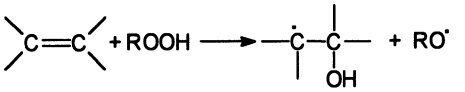


(74)

The homolysis of bonds may be exerted by the following factors:

- radiolysis (homolysis effected by ionizing radiation)
- photolysis (homolysis caused by light)
- pyrolysis (thermal homolysis).

(b) Reactions with the participation of redox systems involving one-electron transfer by a molecule with paired electrons to an oxidizing agent (e.g. to the cation of a transition metal):

$$ROOH + Co^{3+} \rightarrow ROO^{\cdot} + Co^{2+} + H^{+}, \tag{75}$$

or from transition metal ion to organic molecule:

$$ROOH + Fe^{2+} \rightarrow RO^{\cdot} + HO^{-} + Fe^{3+} \tag{76}$$

(c) Interactions with compounds known as pollutants, i.e. O_3, NO_2, $^1O_2$.

(d) Enzymic processes playing an important role in the production of radicals in vivo. Possible mechanisms for the initiation of oxygen radical reactions in vitro and in vivo have been discussed in Chap. 5.

3.1.2 Propagation

Free radicals may enter into propagation reactions of five main types [197, 198].

3.1.2.1 *Atom or Group Transfers*

In these reactions most frequently the free radical ($X^{\cdot}$) abstracts either a hydrogen from aliphatic compounds (RH), or a halogen atom from the molecule to give secondary radicals. The following examples illustrate common processes:

$$X^{\cdot} + RH \rightarrow R^{\cdot} + XH \tag{77}$$

$$X^{\cdot} + CCl_4 \rightarrow CCl_3^{\cdot} + XCl \tag{78}$$

Hydrogen atoms being univalent are very easily attacked by free radicals and they are abstracted most often because of their abundance in all biomolecules.

Of considerable biological importance also are reactions involving sulfur atoms:

$$X^{\cdot} + RSSR \rightarrow RS^{\cdot} + XSR \quad (79)$$

$$X^{\cdot} + RHS \rightarrow RS^{\cdot} + XH \quad (80)$$

The abstraction of hydrogen atoms occurs as the first step of the propagation process during autoxidation reactions. Autoxidation may be defined as chained processes of oxidation by molecular oxygen. They start as a result of the formation of free radicals due to the action of a so-called initiator. The role of the initiator can be played by foreign radicals, singlet oxygen, ions of variable valency and even oxygen molecules at higher temperature. For example, the autoxidation of saturated hydrocarbons (RH) proceeds as follows:

$$\text{initiator } (X^{\cdot}, {}^1O_2, {}^3O_2, Me^n) \rightarrow R^{\cdot}, HOO^{\cdot}$$

$$R^{\cdot} + O_2 \rightarrow ROO^{\cdot} \quad (81)$$

$$RH + ROO^{\cdot} \rightarrow ROOH + R^{\cdot} \quad \text{etc.} \quad (82)$$

$$ROO^{\cdot} + R^{\cdot} \rightarrow ROOR \quad \text{(chain termination)} \quad (83)$$

$$HOO^{\cdot} \rightarrow HO^{\cdot} + O^{\cdot} \quad (84)$$

$$HOO^{\cdot} \rightarrow H^+ + O_2^{\cdot-} \quad \text{(chain branching)} \quad (85)$$

The formation of the peroxy radical ($ROO^{\cdot}$) may occur with participation of both ${}^3O_2$ and ${}^1O_2$. Considering the kinetics of the autoxidation process, we must remember that the total reactivity of a substrate depends on the rate constants of the initiation, propagation and termination processes.

However, oxidation is a general process, and while oxygen in the triplet state is a "poor" oxidant, it is involved in most oxidation reactions in the form of "activated species", e.g. $O_2^{\cdot-}$, $HO^{\cdot}$, ${}^1O_2$, H_2O_2, $RO^{\cdot}$. All of these are oxidants relative to H_2O. There is no doubt that these species are involved in the development of various pathological states in situations where the balance between prooxidant and antioxidant factors gets out of a control.

3.1.2.2
Radical Addition to Double Bonds

The radical addition occurs according to the scheme

$$X^{\cdot} + CH_2 = CH_2 \rightarrow X - CH_2 - CH_2^{\cdot} \quad (86)$$

The addition reactions to aromatic rings play an important role in many biological systems, e.g. the addition of the ketyl radical of isopropanol to DNA bases [199] or in the disproportionation reactions of bilirubin [200].

Basically all free radicals are expected to add to aromatic rings.

3.1.2.3 *Electron Transfer Reactions*

Organic free radicals and both their cationic and anionic forms create pairs of thermodynamically reversible oxidation-reduction systems:

$$R^{\cdot} \rightleftharpoons R^{+} + e^{-}, \qquad R^{\cdot} + e^{-} \rightleftharpoons R^{-}$$

This type of reaction plays a crucial role in biomolecule damage caused by ionizing radiation. For example, the NAD[2] free radical ($NAD^{\cdot}$) can be generated by reduction of (NAD^{+})[3]. Reaction of $NAD^{\cdot}$ with O_2 occurs through one-electron transfer leading to formation of O_2^{-}, and the rate is very fast ($k = 2 \cdot 10^9\ \ell \cdot mol^{-1} \cdot s^{-1}$) [201]:

$$NAD^{\cdot} + O_2 \rightarrow NAD^{+} + O_2^{-} \tag{87}$$

Reduction of NAD^{+} by hydrated electrons or CO_2 regenerates $NAD^{\cdot}$ by intramolecular electron transfer:

$$NAD^{+} + e^{-}(H_2O)_n \rightarrow NAD^{\cdot} + nH_2O \tag{88}$$

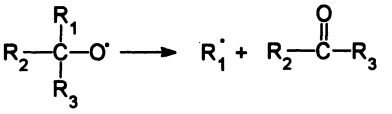
Similarly, internal electron transfer occurs in cytochrome c (cyt c), a heme protein, [202]

$$Fe^{2+} - \text{cyt c} \xrightarrow{-e} [Fe^{2+}\text{cyt c}]^{+\cdot} \rightarrow Fe^{3+} - \text{cyt c} \tag{89}$$

During oxidation ferrocytochrome c forms ferrocytochrome c – cation radical with the odd electron placed in the porphyrin ring as a intermediate, with subsequent occurrence of a rapid internal electron transfer.

3.1.2.4 *β-Scission*

This process is the reverse of radical addition. A stable radical is then eliminated as a result of the unpairing of electrons of the bond in the β-position to the odd electron [197]



(90)

The radical with an odd electron on the oxygen atom is converted into a radical with an odd electron on carbon.

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[2] Nicotinamide adenine dinucleotide.

[3] The oxidized form of nicotinamide adenine dinucleotide.

3.1.2.5
Rearrangements

The oxidation of saturated hydrocarbons at high temperature is a classic example of this type of reaction. The mechanism reaction proceeds by transformation of the hydrocarbon peroxides followed by their decomposition to olefines.

3.1.3
Termination

The chain termination step can be presented in a simplified way as

$$2\,R^{\cdot} \rightarrow R\text{–}R$$

The process involves conversion of the free radicals into stable molecules or the formation of only slightly active radicals which are incapable of propagating the chain reactions. According to the products obtained two types of the radical chain termination are known.

3.1.3.1
Recombination (the Union of two Radicals)

$$C_2H_5^{\cdot} + C_2H_5^{\cdot} \rightarrow C_4H_{10} \qquad (91)$$

3.1.3.2
Disproportionation

This process leads to disproportionation – the formation of two products, one saturated and one unsaturated

$$C_2H_5^{\cdot} + C_2H_5^{\cdot} \rightarrow CH_2 = CH_2 + CH_3 - CH_3 \qquad (92)$$

The latter two reactions represent homotermination processes where terminating radicals share the same origin, whereas, for example, the reaction at Eq. (2) is an example of a cross-termination reaction.

3.2
Reactions of the Superoxide Radical

The superoxide radical, also known as the superoxide anion, is a free radical with negative charge. $O_2^{\cdot-}$ is the anion of an acid $HO_2^{\cdot}$ in an equilibrium depending on pH.

$$H^+ + O_2^{\cdot-} \rightleftharpoons HO_2^{\cdot} \qquad (pK = 4.69) \qquad (93)$$

Since the pK of $HO_2^{\cdot}$ is 4.69, the $O_2^{\cdot-}$ radical is the main form present at physiological pH values. In aqueous and aprotic solvents $HO_2^{\cdot}$ reacts similarly to the

RO_2 radical and its reactivity will be discussed latter, whereas the $O_2^{\cdot-}$ species can be involved in three types of reactions – as an oxidant, as a reducing agent, and as a nucleophile.

The lifetime of the $O_2^{\cdot-}$ radical is in the millisecond range and, in the absence of other compounds able to react with the radical, it reacts with itself giving H_2O_2 and singlet oxygen (reaction at Eq. (65)).

3.2.1 Superoxide Radical as an Oxidant

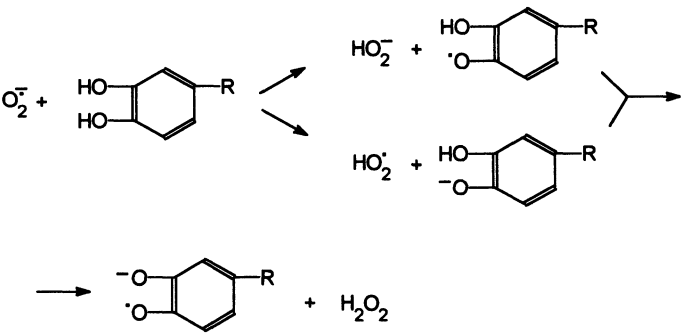
Superoxide radical as a weak base reacts with water at a high rate ($k = 2.5 \cdot 10^8$ $\ell \cdot mol^{-1} \cdot s^{-1}$) [203]

$$2\,O_2^{\cdot-} + H_2O \rightleftharpoons O_2 + HO_2^- + HO^- \qquad (94)$$

The species can promote proton transfer from much weaker acids than water, e.g. weakly acidic organic compounds (HB), especially when these compounds are solvents or are involved at high concentration [204, 205]

$$2\,O_2^{\cdot-} + HB \rightarrow O_2 + HO_2^- + B^- \qquad (95)$$

One of the best known reactions characterizing $O_2^{\cdot-}$ as an oxidant is the oxidation of catecholamines and other diphenols to semiquinone radicals [206–208] (reaction at Eq. (96)). The first step is the proton transfer from phenol to $O_2^{\cdot-}$ followed by HO_2^- formation or hydrogen atom transfer with $HO_2^{\cdot}$ generation. These reactions constitute a reaction chain in which H_2O_2 is produced.



(96)

The superoxide radical oxidizes alcohols, aldehydes and unsaturated ketones [207, 208]. In a general form, this reaction may be formulated as

$$RCHO + O_2^{\cdot-} \rightarrow O_2 + HO^- + RCO_2^{\cdot} \qquad (97)$$

Effective basicity of $O_2^{\cdot-}$ is especially important for biological systems since the species reacts with some biological compounds, e.g. with ascorbic acid ($k = 1.6 \cdot 10^4$ $\ell \cdot mol^{-1} \cdot s^{-1}$) [209], ascorbate radical anion ($k = 5 \cdot 10^9$ $\ell \cdot mol^{-1} \cdot s^{-1}$), bilirubin and biliverdin ($k = 2.3 \cdot 10^4$ and $7 \cdot 10^3$ $\ell \cdot mol^{-1} \cdot s^{-}$ respectively) [210],

α-tocopherol ($k = 1.7 \cdot 10^4\ \ell \cdot mol^{-1} \cdot s^{-1}$), compounds containing the thiol group (-SH), e.g. cysteine [211], ceruloplasmin ($k = 1.8 \cdot 10^6\ \ell \cdot mol^{-1} \cdot s^{-1}$) [5]. The reaction of $O_2^{\cdot-}$ with thiols may be illustrated as

$$O_2^{\cdot-} + RSH \xrightarrow{H^+} RS^{\cdot} + H_2O_2 \tag{98}$$

It has been recognized that $O_2^{\cdot-}$ also reacts with some enzymes such as dehydrogenases, e.g. the lactate dehydrogenase-NADH complex ($k = 1 \cdot 10^5\ \ell \cdot mol^{-1} \cdot s^{-1}$) [204]:

$$\text{(lactate dehydrogenase)-NADH} + O_2^{\cdot-} \rightarrow$$
$$\rightarrow \quad \text{(lactate dehydrogenase)-NAD}^{\cdot} + H_2O_2, \tag{99}$$

or superoxide dismutase (SOD) ($k = 2.4 \cdot 10^9\ \ell \cdot mol^{-1} \cdot s^{-1}$) [212]. In the presence of these enzymes the dismutation of $O_2^{\cdot-}$ to H_2O_2 is accelerated. Also, $O_2^{\cdot-}$ shows a high reactivity towards oxidases ($k \approx 10^6 - 10^7\ \ell \cdot mol^{-1} \cdot s^{-1}$) [5]. It is worthwhile to mention the relatively high reactivity of $O_2^{\cdot-}$ towards collagen, a main protein of connective tissue ($k = 9 \cdot 10^6\ \ell \cdot mol^{-1} \cdot s^{-1}$, pH 7).

The superoxide radical reacts with hydroperoxides by a process involving reduction and cleavage of the O–O bond

$$O_2^{\cdot-} + HOOH \rightarrow HO^- + HO^{\cdot} + O_2 \tag{100}$$

An analogous reaction has been postulated for tetrabutyl hydroperoxide in acetonitrile [213]. The rate of the latter reaction is too low to be important. All these reactions are strongly catalyzed by metal ions with variable valencies [5].

In the case of linoleic acid hydroperoxide (LOOH) the reaction at Eq. (100) occurs with the same mechanism and the $LO^{\cdot}$ is formed at $k = 7 \cdot 10^3\ \ell \cdot mol^{-1} \cdot s^{-1}$ [214]. For diacetyl peroxide and ethyl hydroperoxide the rates are higher and they reach values of $2 \cdot 10^7$ and $3 \cdot 10^4\ \ell \cdot mol^{-1} \cdot s^{-1}$, respectively [214].

Finally, $O_2^{\cdot-}$ is the weak oxidizing species, although in the presence of proton donors it dismutates rapidly to $HO_2^{\cdot}$ and O_2 (reaction at Eq. (94)).

The oxidizing properties of $O_2^{\cdot-}$ result from the formation of the proton induced dismutation of this species to $^1O_2$, $HO_2^{\cdot}$, HO_2^-, $HO^{\cdot}$ and H_2O_2. Thus, protons or metal cations are necessary to catalyze the oxidation process in aprotic solvents. In contrast, in aqueous solutions $O_2^{\cdot-}$ easly oxidizes transition metal ions such as Mn^{2+} and Cu^+ through the following scheme ($k = 1.3 \cdot 10^7\ \ell \cdot mol^{-1} \cdot s^{-1}$, pH = 7.3, $k = 8.1 \cdot 10^9\ \ell \cdot mol^{-1} \cdot s^{-1}$, pH = 7, respectively) [5, 215]:

$$Me^{(n+1)+} + O_2^{\cdot-} \rightarrow [Me^{(n+2)+}(O_2^{2-})] \tag{101}$$

$$[Me^{(n+2)+}(O_2^{2-})] + O_2^{\cdot-} \xrightarrow{H_2O} Me^{(n+1)+} + O_2 + H_2O_2 \tag{102}$$

3.2.2 Superoxide Radical as a Nucleophile

The superoxide radical shows a high nucleophilicity towards typical S_N2 substrates comparable to the best known nucleophiles such as thiocyanate or

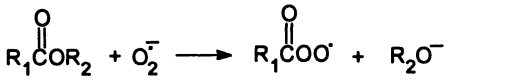
thiophenoxide [203]. Both electrochemical data and a study using radical traps have shown that such substrates are alkyl halides (RX) and tosylates [203, 216, 217]. The nucleophilic attack of O_2^- on the alkyl halides in benzene, pyridine or dimethylformamidine occurs with the formation of dialkyl peroxide and elimination of products even in excited states, such as $^1O_2$ (reaction at Eq. (64)), by the following reactions [216]:

$$RX + O_2^- \rightarrow RO_2^{\cdot} + X^- \quad (103)$$

$$RO_2^{\cdot} + O_2^- \rightarrow RO_2^- + {}^1O_2 \quad (104)$$

$$RO_2^- + RX \rightarrow ROOR + X^- \quad (105)$$

Nucleophility of O_2^- towards carbonyl systems have also been observed [203, 204]. The superoxide radical reacts with esters yielding carboxylic acids and alcohols [218, 219], as well as with acyl halides, yielding diacylperoxides [220]. The sequence of reactions for ester hydrolysis with the participation of O_2^- is proposed by Sawyer and Gibian [203] as follows: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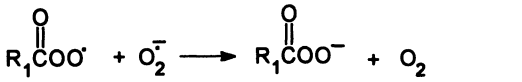
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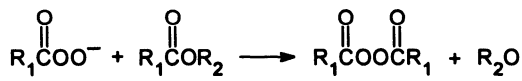
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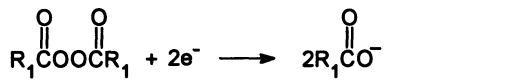
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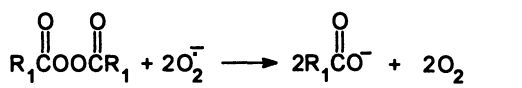
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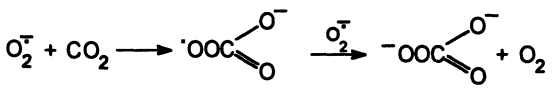
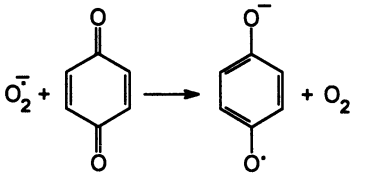
The oxidation of ascorbic acid, mentioned above as a radical process, may also occur as a result of the nucleophilic attack of O_2^- on the lactone and subsequent oxidation.

Another example of the O_2^- reaction as a nucleophile via the S_N2 mechanism is its reaction with CO_2 in aprotic solution [221]:


(111)

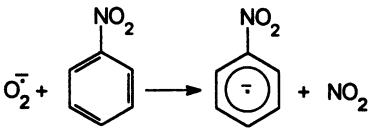
3.2.3
Superoxide Radical as a Reducing Agent

The superoxide anion radical is able to act as a one-electron reducing agent in aprotic solution. The species reacts rapidly with para-benzoquinone ($k \approx 9 \cdot 10^8\ \ell \cdot mol^{-1} \cdot s^{-1}$) to form the semiquinone [222]:



(112)

and with nitrobenzenes to form the anion radical [204]



(113)

The superoxide radical can reduce sulfur dioxide to dithionite in dimethylformamide [203]

$$O_2^{\cdot-} + SO_2 \rightarrow O_2 + SO_2^{\cdot-} \quad (114)$$

$$2\,SO_2^{\cdot-} \rightarrow S_2O_4^{2-} \quad (115)$$

Cations such as Fe^{3+}, Mn^{3+} and Cu^{2+} efficiently catalyze the dismutation of $O_2^{\cdot-}$ to $^1O_2$ and H_2O_2. The first step of the catalysis is the reduction of the metal ion (reaction at Eq. (19)). Analogous reactions occur with chelated iron [223] and manganese, as, for example

$$(Mn^{3+}) + O_2^{\cdot-} \rightarrow (Mn^{2+}) + O_2, \quad (116)$$

and with many metalloenzymes containing copper, zinc, manganese, iron [224–227] such as superoxide dismutases (SOD):

$$SOD\text{–}Cu^{2+} + O_2^{\cdot-} \rightarrow SOD\text{–}Cu^{+} + O_2 \quad (117)$$

$$SOD\text{–}Cu^{+} + O_2^{\cdot-} \xrightarrow{2H^+} SOD\text{–}Cu^{2+} + H_2O_2 \quad (118)$$

The next characteristic reaction for $O_2^{\cdot-}$ in aqueous solution is an electron transfer to organic halides, e.g. bromouridine (BrU) [228]:

$$O_2^{\cdot-} + BrU \rightarrow O_2 + Br^- + U^{\cdot} \quad (119)$$

Another interesting reaction of $O_2^{\cdot-}$ as a reducing agent is its ability to react rapidly ($k = 1.60 \cdot 10^9\ \ell \cdot mol^{-1} \cdot s^{-1}$) with singlet oxygen [229]:

$$O_2^{\cdot-} + {}^1O_2 \rightarrow {}^3O_2 + O_2^{\cdot-} \quad (120)$$

Such a high rate of reaction means that the O_2^- radical is an effective quencher of $^1O_2$. Similarly, the superoxide radical reacts with the hydroxyl radical:

$$O_2^- + HO^\cdot \rightarrow O_2 + HO^- \quad (121)$$

The second-order rate constant for the reaction of O_2^- with $HO^\cdot$ is also high ($k = 1 \cdot 10^{10}\ \ell \cdot mol^{-1} \cdot s^{-1}$) [5].

The superoxide radical as the anion of an acid $HO_2^\cdot$ acts as an electron-pair donor to protons, ions of alkali metals and alkaline earth ions in aprotic media [230, 231]. In a general form the formation of the coordination complexes with metals may occur as follows:

$$Zn^{2+} + 2\,O_2^- \rightarrow Zn(:O_2^\cdot)_2 \quad (122)$$

$$Zn(:O_2^\cdot)_2 \rightarrow O_2 + ZnO_2 \quad (123)$$

The complexes are transiently stable and decompose to yield O_2 and the metal peroxide. Formation of the analogous complexes have been observed in the case of several metal ions, i.e. Ca^{2+}, Cd^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Y^{3+}, Cr^{3+} and Li^+ [232]. The latter lithium ion forms a stable $Li(:O_2^\cdot)$ complex with O_2^-.

3.3 Reactions of Organic Oxygen Radicals

There is no doubt that organic oxy radicals, besides their participation in food chemistry, degradation of polymers, etc., can be involved in neuro- and brain chemistry in the development of various pathological states, stress physiology, radiation biology or in drug metabolism [169].

The oxy radicals can be divided into three major types:

- peroxy radicals ($ROO^\cdot$)
- alkoxy radicals ($RO^\cdot$)
- aryloxy radicals ($ArO^\cdot$)

where R denotes an alkyl radical, and Ar an aromatic ring.

The organic free radicals ($R^\cdot$) formed by abstraction of a hydrogen atom from the parent compounds during, for instance, homolysis or redox reaction, can react with an oxygen molecule to form the peroxy radical (reaction at Eq. (81)). As is easy to see, oxygen prevents the regeneration of the original compound. Formation of the organic radicals by abstraction of a hydrogen atom from alkyl compounds are much more favourable, due to their lower bond energies ($\sim 403.7\ kJ \cdot mol^{-1}$) in a comparison to abstraction reactions from an aromatic ring ($\sim 469.3\ kJ \cdot mol^{-1}$) [233].

There are several types of aryloxy radicals, e.g. phenoxy derived from the hydroxy and polyhydroxy derivatives of the benzene molecule and substituted benzenes, naphthoxy derived from the hydroxy derivatives of naphthalene, etc. Organic oxygen radicals undergo most types of free radical reaction [234, 235]. This aspect of free radical chemistry is beyond the scope of this book. In this chapter a brief discussion of the most characteristic reactions of the RO_2 and RO

radicals will be given. Peroxy radicals undergo such reactions as HO radicals or as O_2^- depending on the pH value, whereas RO· reacts similarly to the HO radicals, which will be discussed below.

Let us consider the three general types of reactions caused by RO_2 radicals.

3.3.1 Electron Transfer

Peroxy radicals show high acceptance of an electron when they react with O_2^- or metal cations undergoing a reduction [80, 235]

$$RO_2^{\cdot} + Me^{2+} \rightarrow RO_2^- + Me^{3+} \quad (124)$$

The reaction at Eq. (104) also illustrates this process.

3.3.2 Recombination of Peroxy Radicals

Peroxy radicals are able to undergo decomposition on different pathways depending on whether they are primary, secondary or tertiary [80]:

$$2\,ROO^{\cdot} \rightarrow 2\,RO^{\cdot} + O_2 \quad (125)$$

$$2\,ROO^{\cdot} \rightarrow R\text{–}O\text{–}O\text{–}R + O_2 \quad (126)$$

The reaction at Eq. (126) proceeds by a tetroxide, ROOOOR, which decomposes to give two alkoxy radicals, or ROOR and oxygen molecules. The balance between the existence of the alkoxy radicals as a potential toxic species whose diffusion causes further oxidation, and their termination to ROOR, depends on the medium viscosity.

If the peroxide radical possesses an α-hydrogen atom, another reaction of the decomposition becomes possible:

$$2R_2CH\text{–}O\text{–}O^{\cdot} \rightarrow R_2C{=}O^* + R_2CHOH + O_2 \quad (127)$$

or

$$2R_2CH\text{–}O\text{–}O^{\cdot} \rightarrow R_2C{=}O + R_2CHOH + {}^1O_2 \quad (128)$$

The latter reactions are very important in the oxidation of unsaturated fatty acids in mammalian membranes containing phospholipids. The recombination of peroxy radicals, known under the name of the Russel reaction, releases about 400 kJ · mol^{-1} of energy which is distributed between the products and excited carbonyl compounds in the triplet or singlet states and/or singlet oxygen is formed [236, 237]. The reactions are very important from the point of view of singlet oxygen generation.

3.3.3
Hydrogen Abstraction

Peroxy radicals abstract hydrogen atoms from such molecules as aldehydes, phenols, thiophenols, sulfhydryl compounds, aromatic amines or benzylic and allylic hydrocarbons, giving rise to a new free radical, e.g.

$$RO_2^{\cdot} + CH_2O \rightarrow ROOH + CHO^{\cdot} \quad (129)$$

$$RO_2^{\cdot} + RSH \rightarrow ROOH + RS^{\cdot} \quad (130)$$

The reaction at Eq. (129) is especially important in both the autoxidation and the lipid peroxidation. In the autoxidation process the hydrogen abstraction occurs as a step of the sequence of the propagation phase.

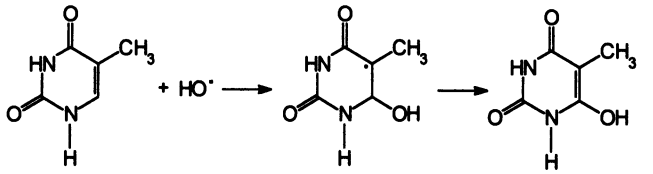
3.4
Reactions of the Hydroxyl Radical

The hydroxyl radical is the most reactive unstable oxidizing species among the oxygen species and therefore reacts with a large variety of organic compounds as well as with several inorganic ions ([238] and references therein). The high reactivity (rate constants usually exceed $10^9\ \ell \cdot mol^{-1} \cdot s^{-1}$) means that $HO^{\cdot}$ may react with the first encountered molecule.

Three basic types of reaction of HO radicals may be distinguished, depending on the substance present in solution: addition, hydrogen abstraction and electron transfer.

3.4.1
Addition to Double Bonds

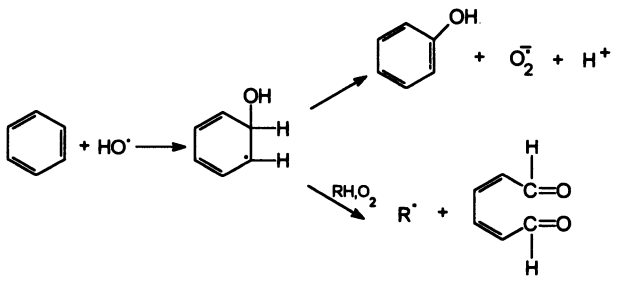
In these reactions $HO^{\cdot}$ adds to a double bond giving a secondary radical. The reaction with pyrimidine bases, for example with thymine, occurs as follows [239]:



(131)

and leads to destruction of the thymine molecule, since the thymine radical undergoes further reactions in which a stable product with changed structure is generated.

The addition of HO radical to the benzene molecule can transform benzene to phenol [240]. If the oxygen molecule and organic molecule (RH) are present in the reaction medium the radical product converts into dialdehyde:



(132)

The benzene toxicity can result from dialdehyde formation which is a compound known as a mutagenic agent. Furthermore, the organic free radicals formed in the reaction can also react with O_2 to form a peroxy radical.

3.4.2 Hydrogen Abstraction

Reactions with hydrogen-containing molecules are an important special case of reactions giving rise to a new free radical [241]. Such reactions very often constitute a main step in chain reactions, i.e. in oxidation reactions effected by oxygen. Rate constants for both types of HO· reaction, e.g. hydrogen addition as well as hydrogen abstraction, are of the same order of magnitude and exceed $10^9\ \ell \cdot mol^{-1} \cdot s^{-1}$ in the case of many biomolecules ([198] and references therein, [242]). Hydroxyl radicals react with a majority of enzymes with rate constant values about $10^{11}\ \ell \cdot mol^{-1} \cdot s^{-1}$. The abstraction of hydrogen atoms by HO· from alcohols may lead to the generation of other radicals having their unpaired electrons located on carbon atoms situated at different distances from the HO group depending on the chain length. The following reactions are typical examples:

$$CH_3OH + HO^{\cdot} \rightarrow {}^{\cdot}CH_2OH + H_2O \quad (133)$$

$$CH_3CH_2CH_2OH + HO^{\cdot} \rightarrow {}^{\cdot}CH_2CH_2CH_2OH + H_2O \quad (134)$$

In the latter reaction, an unpaired electron may be located on the other carbon atoms and also on the oxygen atom. This radical is resistant to oxidation, in contrast to the radical formation in the reaction at Eq. (133), which is readily oxidized [241]:

$$\dot{C}H_2OH \xrightarrow{O_2} O_2CH_2OH \xrightarrow{OH^-} CH_2O + O_2^- \quad (135)$$

The high reactivity and low selectivity of HO· means that the radical formed in a cell may react with the first encountered biomolecules like enzymes, nucleic acids and bases, amino acids, proteins, carbohydrates and lipids [242]:

$$DNA + HO^{\cdot} \longrightarrow DNA^{\cdot} + H_2O \xrightarrow{O_2} \text{degradation products} \quad (136)$$

Of particular importance to biological systems is the fact that $HO^{\cdot}$ reacts with sulfhydryl compounds, SH-containing molecules, with a rate constant near $10^{10}\ \ell \cdot mol^{-1} \cdot s^{-1}$ [243]:

$$R\text{–}SH + HO^{\cdot} \rightarrow R\text{–}S^{\cdot} + H_2O_2 \tag{137}$$

3.4.3 Electron Transfer

Hydroxyl radicals react rapidly with easily-oxidizable inorganic and organic ions such as CO_3^{2-}, Cl^-, I^- and CNS^-, and an electron transfer occurs from the ion to $HO^{\cdot}$ [238]:

$$I^- + HO^{\cdot} \rightarrow I^{\cdot} + HO^- \tag{138}$$

$$CO_3^{2-} + HO^{\cdot} \rightarrow CO_3^{\cdot-} + HO^- \tag{139}$$

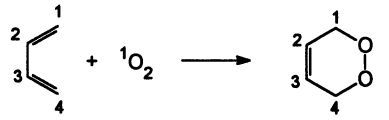
As the HO radical is a strong oxidizing agent, the intermediates formed by reaction of $HO^{\cdot}$ with various of various biological substrates have been widely studied ([84] and references therein).

3.5 Reactions of Singlet Oxygen

The high reactivity of $^1O_2$ to both organic and inorganic compounds has been pointed out by several authors [241, 244–259], and five main types of mechanism of its interaction are to be considered.

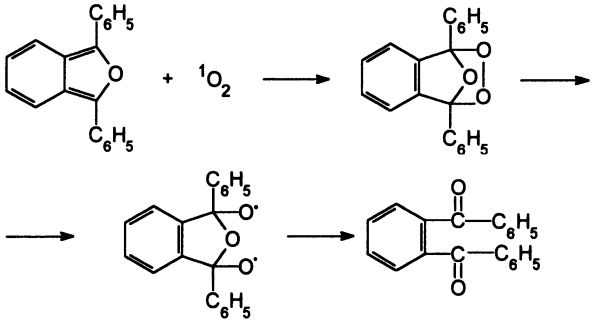
3.5.1 1,4-Addition to Dienes and Conjugated cis-Dienes

The 1,4-addition of $^1O_2$ to conjugated double bonds occurs giving their endoperoxides. This type of reaction is similar to the well known Diels-Alder reaction:



(140)

An example is the oxidation of 1.3-diphenylisobenzofuran (known as a quencher of $^1O_2$) to dibenzoyl benzene [244]:



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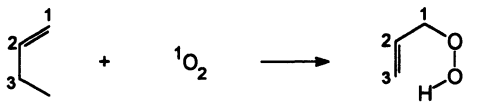
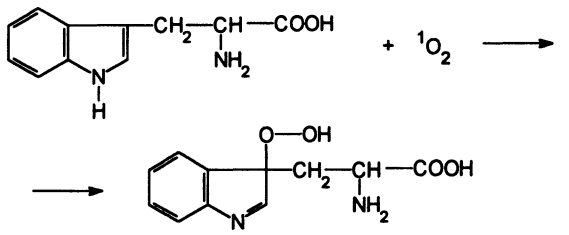
3.5.2
"Ene" Reactions with Olefins having Two or More Allylic Substituents

During this reaction the double bond is shifted to a neighbouring position and allylic hydroperoxides are formed:



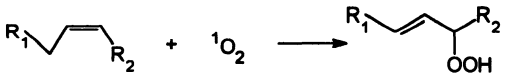
(142)

Of particular importance to biological systems is the chemical trapping of $^1O_2$ by tryptophan or histidine [241]:



(143)

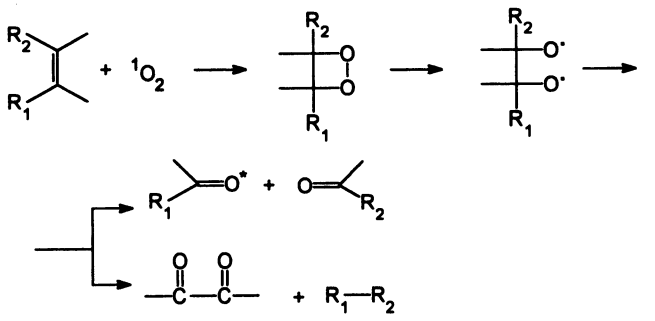
as well as the formation of lipid peroxides from unsaturated fatty acids [244]:



(144)

3.5.3
1,2-Cycloaddition Reactions

Cycloaddition of the electrophilic $^1O_2$ to π-electron rich or sterically hindered olefins leads to 1,2-dioxetanes. Dioxetanes are stable at low temperatures, but at room temperature they usually undergo decomposition forming two carbonyl groups. Due to the efforts of numerous research teams, it is now well established that dioxetane decomposition proceeds along two pathways. In one of these, breaking of C–C and O–O bonds takes place with the production of electronically excited carbonyl groups in a fraction of the resulting carbonyl-containing compounds [245, 246]. These reactions are accompanied by chemiluminescence [247]. The other leads to C–C and C-heteroatom cleveage [248, 249], such as

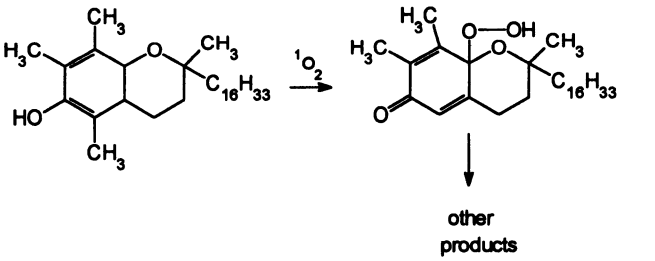


(145)

where R_1 and R_2 denote, for example, H and OH, SH, NH_2, respectively.

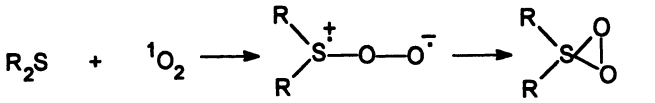
3.5.4 Oxidation Reactions

Owing to its strong electrophilic affinity, $^1O_2$ reacts with substituted phenols, sulfides and amines. Phenols, an important class of both biological and synthetic antioxidants, undergo oxidation to hydroperoxides of the corresponding quinones [250]. One of the most popular biological protective antioxidants is α-tocopherol (vitamin E), inhibitor of lipid peroxidation [251, 252]:



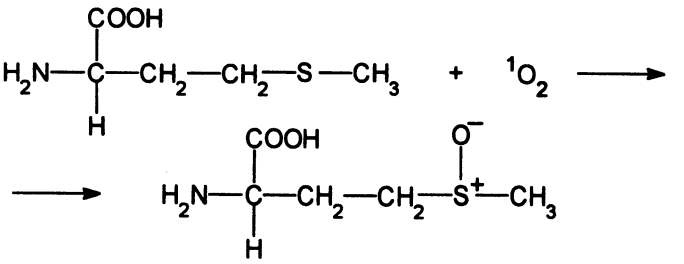
(146)

This singlet oxygen mechanism of oxidation of sulfides and disulfides is well known. Sulfides are oxidized to sulfoxides, disulfides give thiolsulfinates [253]. The overall chemical change in dialkyl sulfides is as follows:



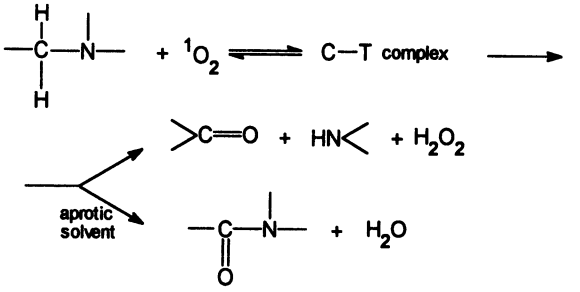
(147)

An example is the oxidation of methionine, a natural amino acid containing a sulfide linkage:



(148)

Amines with low ionization potentials undergo oxidation with $^1O_2$ through the formation of a charge-transfer (C-T) intermediate [254, 255]:



(149)

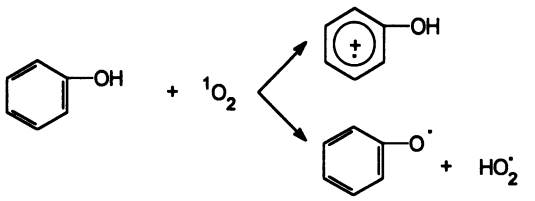
According to this mechanism the quenching of $^1O_2$ by biogenic catecholamines was observed when high concentrations of the amines were used ($\geq 10^{-2}$ mol · ℓ^{-1}) [256].

3.5.5 Electron Transfer

It should be noted that most of the singlet oxygen reactions may proceed by electron transfer from the electron rich compound (C) to $^1O_2$, giving a radical cation and superoxide ion or a charge-transfer complex [255]:

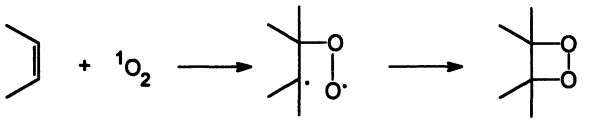
$$C + {}^1O_2 \rightarrow (C^+O_2^-) \rightarrow CO_2 \tag{150}$$

For example, phenols react with $^1O_2$ with electron transfer rather than hydrogen abstraction [257]:



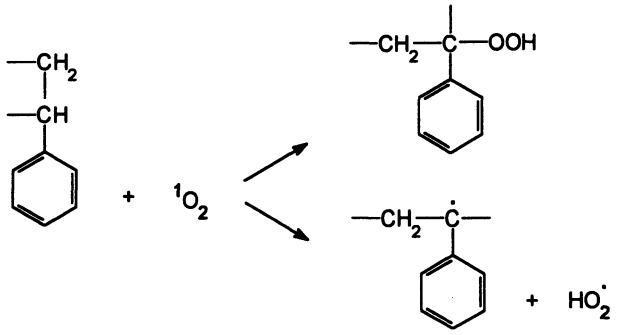
(151)

A biradical mechanism has been postulated for the formation and decomposition (reaction at Eq. (145)) of dioxetanes [258]:



(152)

Rånby and Rabek have postulaed that the initial stage of the photo-oxidative degradation of polystyrene involving $^1O_2$ may also occur by the radical mechanism [259]:



(153)

The intermediary of the biradical mechanism has also been postulated in the $^1O_2$ oxidation of 1.3-diphenylisobenzofuran (reaction at Eq. (141)).

4 Detection of Oxygen Species and Singlet Oxygen

4.1 Introduction

Many oxygen free radicals, as electrophilic agents, show a high reactivity when reacting with themselves or with most organic compounds at almost diffusion-controlled rates: they therefore never reach a high concentration, especially in biological systems. The radicals generally absorb in the UV region and their observation is very complicated. The above-mentioned properties explain why the detection and identification are usually difficult tasks. In the last two decades this problem has become the subject of extensive studies [260–376].

Two basic methods for the detection of oxygen species are conveniently used:
first physical methods (including conductimetry, ultra-violet and visible spectrophotometry as well as electron spin resonance spectroscopy (ESR)), and then chemical methods (application of scavengers and product analysis).

(1) Physical methods without modification are not applicable to detect the oxygen species in a cell, because they would require concentration of the species which are unachievable in living systems. Thus, the direct detection of the species remains almost imposible in biological media. The sensitivity of free radical detection in biological systems in these techniques can be improved, for example, by lyophilization (microwave absorption is decreased by the presence of water), a way of slowing down the rate of radical disappearance by rapidly freezing a sample, or using a compound which forms a stable long-lived free radical as a result of covalently bonding with an unstable oxygen species. This compound is called a spin trap and the method spin trapping. As mentioned in Chap. 1, both oxygen radicals ($O_2^{\cdot-}$, $HO^{\cdot}$) as well as $^1O_2$ are short-lived and therefore spin trapping is suitable for these oxygen species detection. For detecting the oxygen species in aqueous solutions, nitrones ($R = N^+ - O^-$) or nitroso ($R - N = O$) compounds are the spin traps most often used. Oxygen species form with the trap adduct (a nitroxide-free radical) which is "long-lived" and has an ESR spectrum characteristic of the oxygen species.

(2) Chemical methods are used for identification of oxygen species in both chemical and biological systems. Oxygen species, being very reactive, are involved in oxidation and reduction reactions with a number of compounds,

and they are removed from a medium by specific substances called scavengers. Analysis of products formed allows for individual determination of particular oxygen species.

Both types of detection methods, physical and chemical, are discussed below for particular oxygen species, i.e. the $O_2^{\cdot-}$, HO radicals and $^1O_2$.

4.2 Detection of the Superoxide Anion Radical

Both $O_2^{\cdot-}$ and its conjugate acid, $HO_2^{\cdot}$, show distinct absorption spectra in the low UV region. The superoxide anion has its absorbance maximum at wavelength 245 nm ($\varepsilon = 2350\ \ell \cdot mol^{-1} \cdot cm^{-1}$, pH 10.5). For $HO_2^{\cdot}$ these values are 225 nm and $\varepsilon = 1400\ \ell \cdot mol^{-1} \cdot cm^{-1}$, (pH 1.5) [5]. The reduction potential of the pair $O_2/O_2^{\cdot-}$ at pH 7 is – 0.33 V, and –0.46 V for the pair $O_2/HO_2^{\cdot}$ [260].

Methods used for identification of superoxide radical include

- oxidation reactions,
- reduction reactions,
- inhibition by specific scavengers (enzymes), and
- Electron Spin Resonane spectroscopy and spin trapping.

Not all the methods mentioned are specific for $O_2^{\cdot-}$ determination.

4.2.1 Oxidation Reactions

The superoxide radical reacts with many organic compounds to give products that can be detected directly or after separation by gas-liquid chromatography or high performance liquid chromatography, using, for example, spectrophotometric methods [1], fluorescence and chemiluminescence detection, or a polarographic method [2].

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[1] Spectrophotometry is useful for monitoring of compounds absorbing any wavelength of 400–750 nm (visible light) or 200–400 nm (ultraviolet region). Appropriate wavelengths are selected from the spectrum by passing light through a prism or a diffraction grating. If a sample absorbs light then the reaction can be monitored by the changes in optical density (absorbance) at the wavelength absorbed. The absorbane is determined by the formula

$$A = \log (I/I_0)$$

where I_0 is the intensity of the incident light and I is the intensity of the light transmitted through the sample. The absorbance depends on the concentration of the light-absorbing substance according to the Beer-Lambert law:

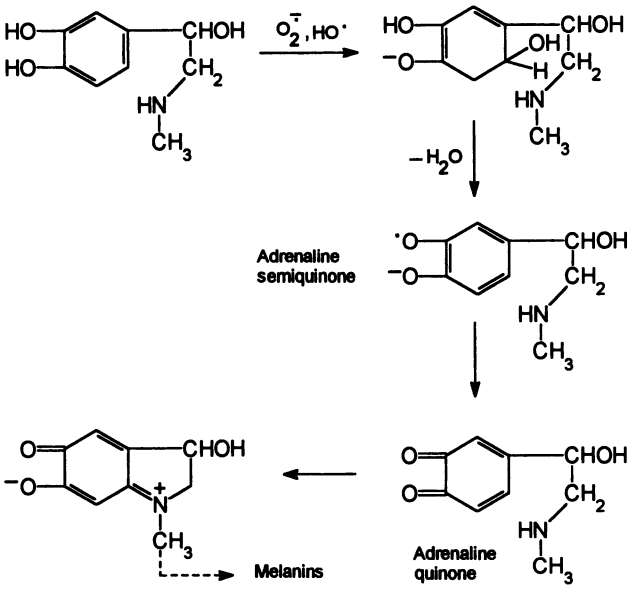
$$A = \varepsilon c \ell$$

where ε is the molar extinction coefficient,
c is the molar concentration of a substance, and
ℓ is the length of the light-path through the sample.

Spectrophotometry can be used satisfactorily only at low absorbance to avoid interactions between absorbing molecules.

A number of compounds have been shown to be oxidized by $O_2^{\cdot-}$ in solution, but we shall cite only the most typical reactions:

- adrenaline and other substituted phenols,
- 1,2-dihydroxy benzene-3,5-disulfonic acid (commercial name tiron),
- hydroxylamine,
- para-phenylenediamine,
- sulfite,
- NADH



(154)

(a) Aqueous solutions of adrenaline saturated with air or molecular oxygen undergo oxidation to adrenochrome [261]. The process may be initiated by both HO and $O_2^{\cdot-}$ radicals and a common intermediate, adrenaline semiquinone, is formed.

In both cases identical intermediates and products are obtained apart from the initial formation of the HO·-adrenaline adduct. Formation of adrenochrome

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[2] Polarography (or voltametry) is based on the application of an increasing voltage between two electrodes immersed in the test solution, whose composition determines the current flowing at each instant. If a fixed voltage is applied between the electrodes, then the current intensity depends on the electrical conductance of the solution. The electrical conductance of the solution is the sum of the contribution of all ions present, and is the reciprocal of the electrical resistance between the electrodes. The course of the reaction may be followed by measuring the change of electrical conductance with time. This method is termed conductiometry. If an uncharged molecule is converted into ions during the reaction we can obtain (at low concentration of diluted compound) a linear response between current flow and sample concentration.

can easily be detected spectrophotometrically by following the increase in absorbance at 310 nm ($\varepsilon = 2170\ \ell \cdot mol^{-1} \cdot cm^{-1}$) or at 485 nm ($\varepsilon = 4470\ \ell \cdot mol^{-1} \cdot cm^{-1}$), at pH 7.6 [206]. It is worthy mentioning that $HO^{\cdot}$ reacts with adrenaline faster than $O_2^{\cdot-}$ [261]. Moreover, adrenaline generates $O_2^{\cdot-}$, H_2O_2 and $^1O_2$ [119] during autoxidation, and these active products can react with adrenochrome causing its further transformation.

The simultaneous interaction of $O_2^{\cdot-}$ and $HO^{\cdot}$ with adrenaline and generation of other oxygen species leads to complexities in measuring $O_2^{\cdot-}$ even in simple systems, and should be considered during interpretation of results obtained. Furthermore, in cells, the quinoidal products of the adrenaline oxidation can be reduced by reducing agents, such as ascorbate, to semiquinone free radicals. These radicals may also enter into reactions with $O_2^{\cdot-}$ and $HO^{\cdot}$. The structural analogues of adrenaline, noradrenaline and dopamine are also oxidized by $O_2^{\cdot-}$ to the corresponding quinones [262]. Thus, these compounds may be used for the detection of the superoxide radical. In addition, specificity of the method is often provided by using other effective $O_2^{\cdot-}$ scavengers, for instance SOD, which inhibits oxidation of adrenaline.

(b) Oxidation of Tiron by $O_2^{\cdot-}$ occurs with a rate constant of about $10^8\ \ell \cdot mol^{-1} \cdot s^{-1}$ [263] giving a stable semiquinone form easily detectable by ESR. The application of Tiron to the $O_2^{\cdot-}$ detection is comparable to the method using adrenaline. The oxidation of Tiron by $HO^{\cdot}$ must also be taken into consideration since the HO radical reacts with Tiron in a similar fast manner as in the case of adrenaline [264].

(c) Oxidation of hydroxylamine by $O_2^{\cdot-}$ occurs in a wide range of pH values (1.1–10.5) and may be followed by pulse radiolysis, stopped flow and spectrophotometric methods. The generation of nitrite radicals ($NO_2^{\cdot}$) is detected in the form of diazo compounds in the presence of sulfanilic acid and 2-naphthylamine [265]. The reaction of hydroxylamine with $O_2^{\cdot-}$ or $HO_2^{\cdot}$ in the presence of formate and chelating agent (EDTA)[3] can easily be measured by decay of radical absorption and bleaching of hydroxylamine at 250–270 nm [266]. Hydroxylamine also reacts with HO radicals with a rate constant about 10^5 times higher than that for $O_2^{\cdot-}$ radical [261].

(d) Oxidation of *p*-phenylenediamine by $O_2^{\cdot-}$ is monitored as an increase in absorption at 490 nm.

(e) Oxidation of sulfite to sulfate can be detected by the decrease in the absorbance at 235 nm [267], as well as by a polarographic method [268].

(f) Oxidation of NADH[4] catalyzed by lactate dehydrogenase [269] may be observed by the disappearance of the absorbance at 340 nm and an increase at 260 nm in air-saturated solution containing EDTA and formate in the pH range of 4.5–9.0. The reaction shows a high sensitivity and specificity for $O_2^{\cdot-}$ detection.

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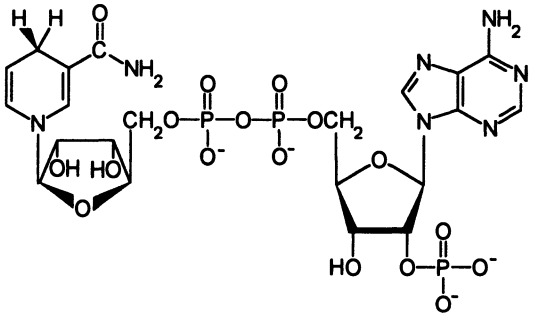
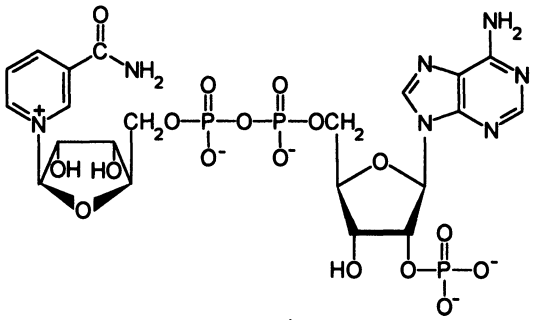
[3] Ethylendiaminetetraacetic acid.

[4] The reduced form of nicotinamide adenine dinucleotide.



NADH

The oxidized form of coenzymes have only a single absorbance maximum at 260 nm, due to the adenine and nicotinamide rings, the reduced form has two maxima, one at 260 nm and the second, also strong, at 340 nm. Additionally the reduced form exhibits fluorescence with a maximum at 450 nm, whereas the oxidized form is non-fluorescent.



NAD^+

4.2.2 Reduction Reactions

Reduction reactions include mainly:

- cytochrome c,
- tetranitromethane,
- nitroblue tetrazolium salts.

(a) Ferricytochrome c is reduced by O_2^- to ferrocytochrome c with a rate constant of $1.1 \cdot 10^6\ \ell \cdot mol^{-1} \cdot s^{-1}$ at neutral pH [270]. (For details dealing with cytochromes see Chap. 7):

$$Fe^{3+} - \text{cytochrome c} + O_2^- \rightarrow Fe^{2+} - \text{cytochrome c} + O_2 \qquad (155)$$

The rate of the reaction decreases with increasing pH.

The reduction of cytochrome c can easily be followed spectrophotometrically at 550 nm, the absorbance maximum of ferrocytochrome c in the visible region.

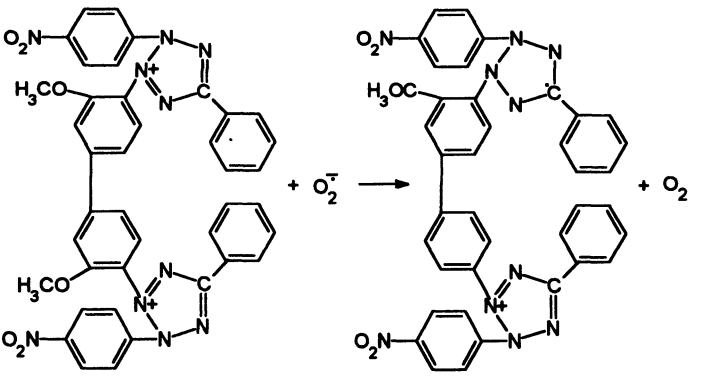
However ferricytochrome c also reacts with other reductants such as e_{aq}, $HO^{\cdot}$ or CO_2^{-}, and the yield of ferrocytochrome c formation is very high (82–104%) with respect to the amount of O_2^{-} generated [270]. The specificity of the O_2^{-} determination and usefulness of the reaction for the detection of the radical in biological systems containing proteins can be increased when acetylated cytochrome c is substituted for cytochrome c [271]. It should be mentioned that the measurement of O_2^{-} using cytochrome c cannot be carried out in the presence of other strongly reducing substances, e.g. cytochrome P-450 reductase.

(b) Tetranitromethane undergoes conversion to the nitro anion form:

$$O_2N\text{–}C(NO_2)_2\text{–}NO_2 + O_2^{-} \longrightarrow C(NO_2)_3^{-} + NO_2 + O_2 \qquad (156)$$

The rate constant for the reaction with O_2^{-} at pH 5.6–6.2 is the highest among those obtained for organic compounds ($2 \cdot 10^9\ \ell \cdot mol^{-1} \cdot s^{-1}$) [272]. The reaction can be followed spectrophotometrically at 350 nm. The weak point of the method is that the gas is toxic and reacts with cell components, generating O_2^{-} radical.

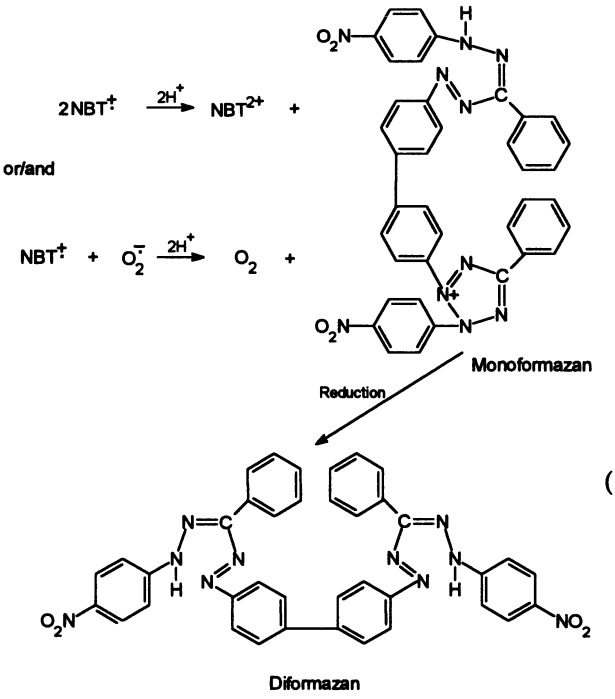
(c) *p*-Nitro-blue tetrazolium salts are reduced by O_2^{-} to form a slowly precipitating product, insoluble in water, called formazan. The most popular, and used in histology, is nitroblue tetrazolium chloride $(NBT)Cl_2$ of which cation (NBT^{2+}) is reduced by O_2^{-} to form diformazan (reaction at Eq. (157))



NBT^{2+} Tetrazolinyl radical (NBT^{+}) (157)

The reaction mechanism is complex and the second-order rate constant for this reaction has been quoted as $5.88 \cdot 10^4\ \ell \cdot mol^{-1} \cdot s^{-1}$ at the pH range 7–11 [273].

The diformazan formation can be measured spectrophotometrically at 560 nm. However, the method is very simple to use, but is unspecific because the hydroxyl radicals can also form diformazan [228].



(157 continued)

4.2.3
Inhibition by Specific Scavengers

The next most popular methods for O_2^- detection are reactions involving radical scavengers such as the enzymes SOD and peroxidases.

4.2.3.1
Superoxide Dismutase

SOD catalyzes the dismutation of O_2^- with formation of H_2O_2 (see reactions at Eqs. (117) and (118) [224–227]. The rate constants for the interaction of superoxide dismutases with O_2^- are $1.9 \cdot 10^8 - 5.3 \cdot 10^9\ \ell \cdot mol^{-1} \cdot s^{-1}$ depending on the pH value and the kind of metal present in the enzyme molecule [274]. The decay of O_2^- radical can be measured spectrophotometrically by following the decrease in absorbance at 250 nm. For detection of O_2^- in complicated biological systems, SOD is used very often as the competitive scavenger, together with *p*-nitro-blue tetrazolium as detecting agents.

4.2.3.2
Peroxidases

Peroxidases show high sensitivity and specificity in the reaction with O_2^-, giving the so-called compound III of peroxidase as a product, which can be detected spectrophotometrically. The α-band of the complex shows a maximal absor-

bance at 598 and 630 nm for lacto-peroxidase and myelo-peroxidase, respectively [276–277].

4.2.4 Chemiluminescent Probes

Chemiluminescence is defined as the generation of electromagnetic radiation (ultraviolet, visible and infrared) by chemical reactions. The process often referred to as "cold light", involves the generation of chemically excited species and their decay to a ground state with light emission:

$$\text{Substrates} + \text{Oxidant} \rightarrow P_1^* + P_2 + \ldots \quad \text{(chemiexcitation)}$$

$$P_1^* \rightarrow P + h\nu \quad \text{(luminescence)}$$

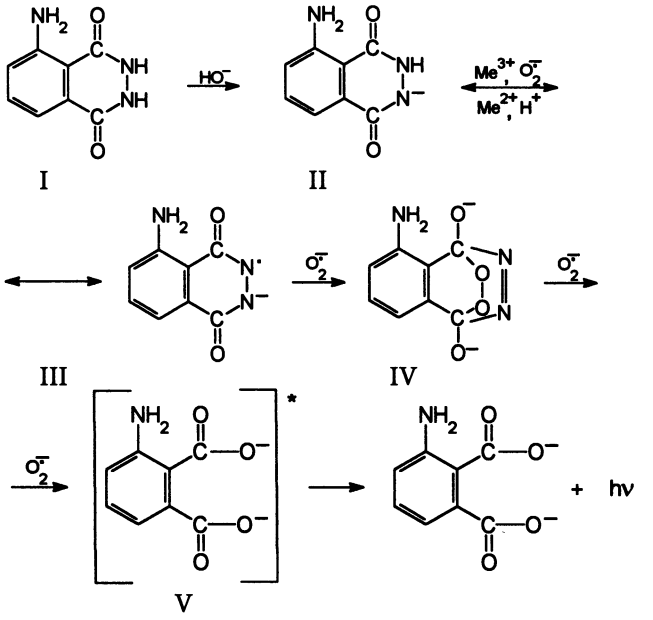
where P_1^* and P_1 denote products formed in the electronically excited state and the ground state, respectively.

The chemiluminescence yield of the above reactions is considered to be the product of the efficiency for generation of P_1^* and the efficiency of its radiative decay to the ground state. The yield is equal to the ratio of the number of photons emitted during a reaction divided by the number of molecules which reacted. To be chemiluminescent the reaction must fulfil three essential features: it must be exothermic; at least one product must be able to transfer itself into the electronically exited state; and the excited product must lose its energy by light emission or transfer it to a fluorophore. Some details dealing with chemiluminescence are discussed below. The most popular chemiluminescent probes are luminol and lucigenin.

4.2.4.1 *Luminol (5-amino-2,3-dihydro-1,4-phthalazine-dione)*

There are several experimental data showing that $O_2^{\cdot-}$ can participate in chemiluminescence reactions of luminol as an oxidant ([278, 279 and references therein]). The chemical basis for the chemiluminescent analysis of $O_2^{\cdot-}$ is existence of redox reactions involving $O_2^{\cdot-}$ and luminol, in which energy is released as visible light. The following reaction scheme has been reported for the formation of the light emitter [278]:
Luminol (I) at alkaline pH undergoes deprotonation yielding monoanion (II). The luminol monoanion forms a luminol radical (III) by one-electron transfer. The radical reacts with $O_2^{\cdot-}$, and an endoperoxide (IV) is formed which then decomposes, giving an electronically excited 3-aminophthalate dianion (V).

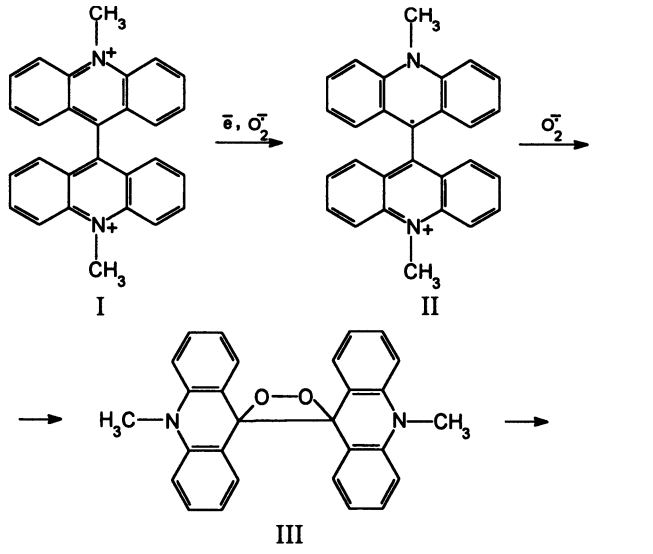
The latter compound emits light when it returns to its ground state. Oxidation of luminol is characterized by a high quantum yield of chemiluminescence achieving a value of 0.012 [280], and by blue (λ_{max} ~425 nm) emission in aqueous solution. However, the chemiluminescence of luminol has been postulated as one of the first simple assays for $O_2^{\cdot-}$ determination, but the method always requires a check for the absence of HO radical, since this species undergoes a univalent reduction by the luminol monoanion (II) yielding the luminol radical (III).



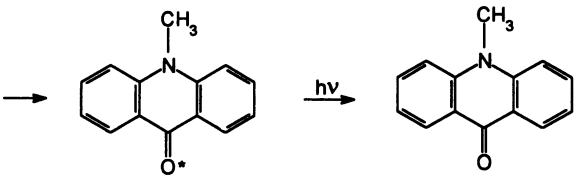
(158)

4.2.4.2
Lucigenin (10,10′-dimethyl-9,9′-biacridinium dinitrate)

Chemiluminescence arising during oxidation of lucigenin has been the most extensively studied and is well recognized. It has been established that the emission is observed during oxidation of lucigenin with $O_2^{\cdot-}$, H_2O_2 or $^1O_2$, and formation of the dioxetane intermediate is a common step of the chemiluminescence ([281 and references therein]):



(159)



IV

(159 continued)

The scheme involves one-electron reduction of lucigenin (I) to its radical (II) which reacts quickly with O_2^- to give the dioxetane (III). Decomposition of the dioxetane ring gives two molecules of *N*-methylacridone (IV); one of those has an electronically excited carbonyl group, which then emits light during its return to the ground state. The quantum yield, ϕ, of chemiluminescence from lucigenin is also high like that of luminol (0.01–0.02) [281], and the respective colour is blue-green ($\lambda_{max} = 440$ nm). Since the dioxetane formation from lucigenin has also been observed during oxidation of its alkaline solution by H_2O_2 as well as by oxidation of diene compounds formed from lucigenin by two-electron reduction, the determination of O_2^- always requires a test for the absence of H_2O_2 and $^1O_2$.

4.2.5
Electron Spin Resonance Spectroscopy and Spin Trapping

Electon spin resonance is a technique that detects the presence of unpaired electrons in a sample. The method can be applied to two important classes of samples, i.e. free radicals and some transition metal ions, since both contain unpaired electrons. Sensitivity of the method is high as it allows one to detect radicals at concentration 10^{-10} mol · ℓ^{-1}. An unpaired electron possesses a magnetic moment resulting from its spin (s = 1/2). In the presence of an external strong magnetic field, $\vec{B}$, the magnetic moment can orientate itself either parallel or antiparallel to field $\vec{B}$. These two orientations of the spin define two energy states, which differ in energy of the electron. If a sample containing a free radical is subjected to the electromagnetic radiation of the appropriate frequency ν, perpendicular to the external field, an energy is absorbed by the electron in the lower energy state and its transition to a higher state is induced (Fig. 6). The absorbed energy is equal to the energy gap between these two energy states, and is given by the equation

$$h\nu = g\beta B$$

where h is the Planck constant; g denotes the effective value of the g-factor equal to 2.0023 for a free electron and for some free radicals; B is the value of the magnetic field at the resonance and β is the Bohr magneton ($\beta = 0.92731 \cdot 10^{-23}$ J · T). The above equation constitutes the basic condition in ESR spectroscopy, and the absorbed energy usually comes from the microwave region of the electromagnetic radiation spectrum, corresponding to a frequency of about 9 GHz (X-band).

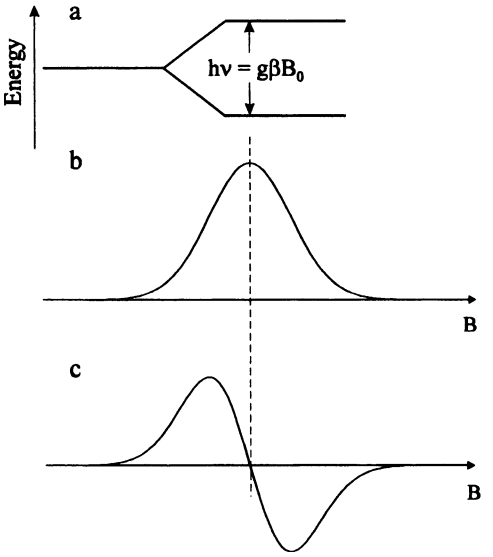


Fig. 6. **a** Energy-level splitting of an electron in an applied magnetic field. **b** Absorption curve. **c** Its first derivative.

The ESR signals are usually presented as the first derivative of the absorption signal. The absorption spectrum presented in the shape of a single "peak" is due to a free electron if the free radical contains atomic nuclei having a magnetic moment equal to zero.

A number of atomic nuclei such as hydrogen and nitrogen possess their own spin (I) (nuclear spins H = 1/2, N = 1), and the spin generates a local magnetic field at the electron. Moreover, in a external magnetic field, the nuclear spin can have $(2 \cdot I + 1)$ orientations which differ in energy. The electron spins interact with the nuclear spins, these interactions lead to the hyperfine structure of a spectrum, and thus the spectrum is split into $(2 \cdot I + 1)$ lines. The magnitude of the splitting between lines is called the hyperfine splitting constant (A). If a number of equivalent nuclei in the radical molecule is n, the ESR spectrum consists of $2nI + 1$ lines. For example, the ESR spectrum of the 2,2,6,6-tetramethyl-piperidine-N-oxyl (TEMPO) consists of a triplet line, as for N the nuclear spin I = 1 (Fig. 7).

The basic ESR spectrum can be characterized by four main parameters: signal intensity, linewidth (ΔB), g-value, and multiplet structure, characterized by hyperfine splitting constant A.

The g-values can easily be obtained from the equation

$$g = \frac{h\nu}{\beta B_0} = 0.0714475 \frac{\nu(\mathrm{GHz})}{B(\mathrm{T})}$$

where B_0 is the applied or external magnetic field at resonance and T denotes tesla [$1T = 10^4$ Gauss (Gs)].

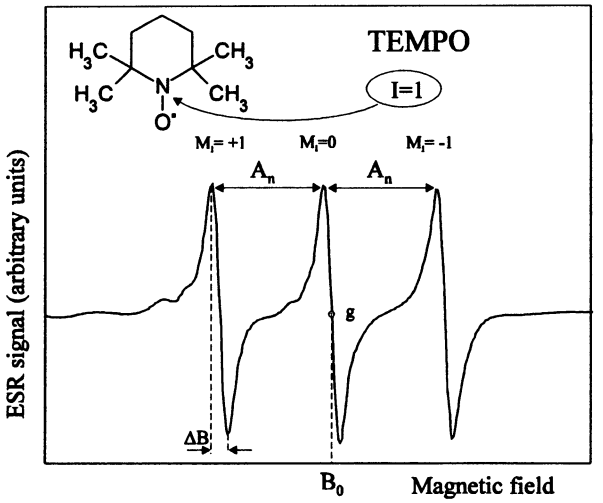
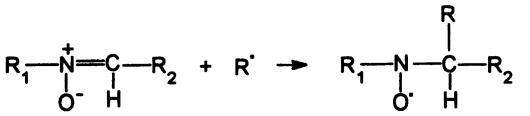


Fig. 7. The ESR spectrum of the TEMPO spin trap

The ESR signal intensity is proportional to $(\Delta B)^2$. The g and A values are usually anisotropic, i. e. they often depend on the direction of the molecular axes relative to the applied magnetic field.

However, a number of free radicals can be detected directly using the ESR method, but there exist several factors, such as the high reactivity, too low concentration of free radicals, or the spectral line broadening (e. g. HO radicals react with various compounds at rates controlled by diffusion) making it impossible to register. These difficulties can be removed by using the spin-trapping technique.

The basic reaction for the spin-trapping technique is the addition reaction of a "short-lived" radical ($R^{\cdot}$) to nitrone as a trap as follows [282–286]:



(160)

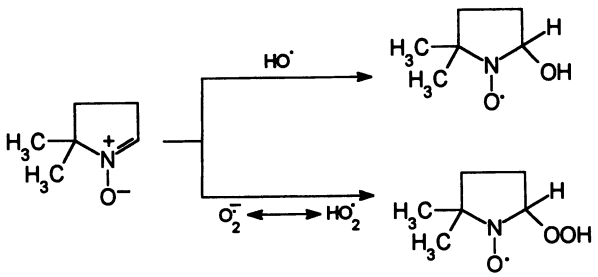
where $R^{\cdot}$ may be $O_2^{\cdot-}$, $HO^{\cdot}$, etc.

The trapped radical ($R^{\cdot}$) is bonded to the α-carbon of the trap and a "long-lived" nitroxide radical is generated. The radical can easily be detected by electron spin resonance spectroscopy. Both the β-proton and the nitrogen hyperfine splitting of the nitroxide radical are sensitive to the type of $R^{\cdot}$. In most cases, traps used for identification of $O_2^{\cdot-}$ are also suitable for detection of HO radicals. To identify the trapped radical, in principle two parameters of the ESR spectrum of the nitroxide radical are needed: g-factors and hyperfine splittings.

A few nitrone spin traps have been used for $O_2^{\cdot-}$ detection as follows [282–295]:

4.2.5.1
5,5-Dimethyl-1-pyrroline-N-oxide (DMPO)

DMPO is able to trap $O_2^{\cdot-}$ and $HO^{\cdot}$ species and to form spin adducts with these radicals [284]:

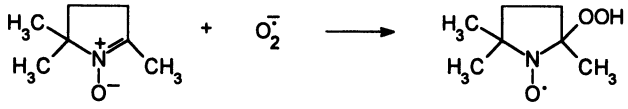


(161)

Both spectra are distinguishable by hyperfine splitting constants. The DMPO–OH adduct shows an ESR spectrum consisting of a quartet with intensity ratio 1:2:2:1. The spectrum is rather stable and shows hyperfine splittings: A_N = 14.9 Gs, A_H = 14.9 Gs, and g-factor = 2.0060 [284] with additional small hyperfine splitting observed under high resolution. In contrast, the DMPO–OOH adduct is highly unstable and is converted into the DMPO–OH adduct. The ESR DMPO–OOH adduct spectrum exhibits hyperfine splittings A_N=14.3 Gs; A_H^{β} = 11.7 Gs and A_H^{γ} = 1.25 Gs, g = 2.0061 [283]. These data deal with $O_2^{\cdot-}$ generated by photolysis of H_2O_2. Neither g-factor nor hyperfine splittings varied with a change in the pH value. The half-lifetimes of the DMPO–OH and DMPO–OOH adducts were reported to be about 2.5 h and 1.5 min (pH 5) or 27 s (pH 9), respectively [286]. The rate constants for HO, HO_2, and $O_2^{\cdot-}$ radicals trapping were found to be $3.4 \cdot 10^9$, $6.6 \cdot 10^3$ and 10 $\ell \cdot mol^{-1} \cdot s^{-1}$, respectively.

4.2.5.2
2,5,5-Trimethyl-1-pyrroline-N-oxide (TMPO)

TMPO is a spin trap structurally similar to DMPO, but without β-hydrogen. The superoxide radical reacts with TMPO to form the nitroxide radical [286]:



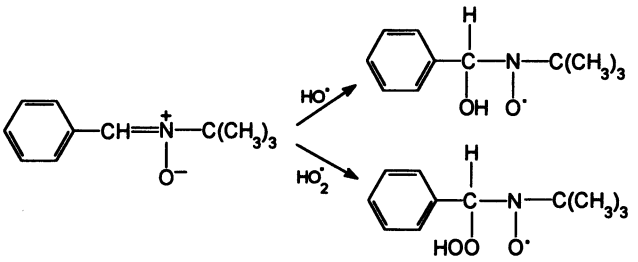
(162)

Unlike DMPO, since the TMPO trap does not contain β-hydrogen, it is less susceptible to hydrogen abstraction, and therefore the DMPO–OOH adduct is stable. Unfortunately, the identification of the trapped species is limited, since one hyperfine split, i.e. A_N = 15.6 Gs is obtained [286].

TMPO is much better at detecting the HO radical as the trap reacts with this radical with a rate constant of $3.8 \cdot 10^9$ $\ell \cdot mol^{-1} \cdot s^{-1}$ [284]. The ESR signal is characterized by A_N = 15.7 Gs. The hyperfine splittings for TMPO–OH and TMPO–OOH adducts are weakly distinguishable.

4.2.5.3
α-Phenyl-N-tert-butyl nitrone (PBN)

PBN can act as a trap for $O_2^{\cdot-}$ and $HO^{\cdot}$ and forms very stable spin adducts.

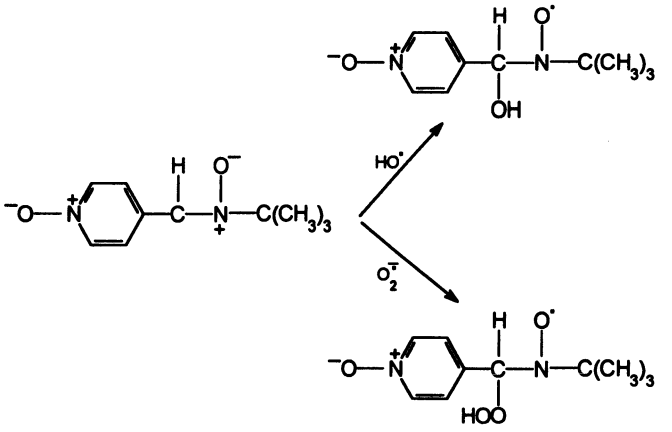


(163)

The ESR spectrum consists of a triplet of doublets with a g-factor of 2.0057 [287]. For $HO_2^{\cdot}$ and $HO^{\cdot}$ species adducts the spectra show hyperfine splittings A_N = 14.8 Gs, A_H^{β} = 2.75 Gs and A_N = 15.3 Gs, A_H^{β} = 2.75 Gs, respectively [285]. The use of PBN is convenient for determination of both oxygen radicals generated in aqueous solution.

4.2.5.4
α-(4-Pyridyl-N-oxide)-N-tert-butyl nitrone (4-POBN)

4-POBN is known as a poor spin trap for $O_2^{\cdot-}$ and a very efficient one for HO radicals. It reacts with $HO^{\cdot}$ with a rate constant $1.9 \cdot 10^9\ \ell \cdot mol^{-1} \cdot s^{-1}$ [286] at physiological pH ranges. Both ESR spectra formed by reaction of $O_2^{\cdot-}$ as well as $HO^{\cdot}$ with 4-POBN consist of a triplet of doublets assigned to the 4-POBN-OOH and 4-POBN-OH adducts [286–288, 290].



(164)

The spectra differ with splitting constants and therefore can easily be distinguished. The 4-POBN-OOH nitroxide gives a spectrum with A_N = 14.16 Gs and A_H^{β} = 1.75 Gs, whereas the spectrum ascribed to the 4-POBN-OH adduct shows,

apart from the hyperfine splitting from nitroxide nitrogen and β-type hydrogen, additional splitting from γ-type hydrogen: A_N = 14.97 Gs, A_H^β = 1.68 Gs, and A_H^γ = 0.34 Gs [286, 290].

The review of spin traps in this book is limited to those which are most widely used. There exist several others and some of them will be discussed below. Although the spin trapping method is a very useful one, considerable experimental caution is required because nitrones are very reactive compounds, and they can easily be oxidized and reduced to hydroxylamines giving other radical products without the oxygen radical trapping. This problem is especially significant when somebody wants to detect oxygen radical generation by biological systems, where the nitrones can be reduced by enzymes and other biomolecules.

Therefore, additional evidence is needed during an application of spin traps. A simple method for verifying that the oxygen radicals have been trapped is to perform a study of the effects of oxygen free radical inhibitors on the ESR spectra. For example, if the generation of the spin trap-OOH adduct is due to the trapping of O_2^-, the addition of SOD should prevent the adduct formation and a decrease in the ESR signal amplitude would then be observed.

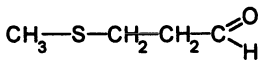
It becomes evident, from the above review of O_2^- detection methods, that reaction of O_2^- with acetylated cytochrome c and the oxidation of NADH in the presence of lactate dehydrogenase seem to be the most specific methods for O_2^- radical detection.

4.3 Detection of the Hydroxyl Radical

Besides the above-mentioned chemiluminescent and ESR spin trapping methods used for $HO^\cdot$ detection, the other main methods for detecting these radicals are as follows:

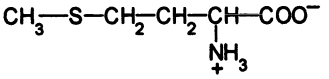
(1) Detection of ethylene ($H_2C=CH_2$) formation during reaction of hydroxyl radical with:

(a) methional (β-methyl-mercapto-propanal) [261]:

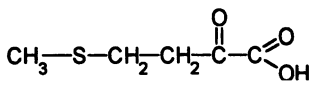


The rate constant of the reaction is high ($8.2 \cdot 10^9\ \ell \cdot mol^{-1} \cdot s^{-1}$), and the reaction is specific, since it occurs without participation of O_2^-.

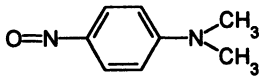
(b) methionine in the presence of pyridoxal phosphate [296]:



(c) keto-mercapto-butanoic acid [297]:



(2) *p*-Nitroso-dimethyl aniline (NDA) bleaching:



The bleaching of the strong absorption of NDA at 440 nm ($\varepsilon = 3.4 \cdot 10^4\ \ell \cdot mol^{-1} \cdot cm^{-1}$) [298] can easily be followed spectrophotometrically. The method is sensitive and specific as the interaction of $HO^{\cdot}$ with NDA occurs with high efficiency, $k = 1.2 \cdot 10^{10}\ \ell \cdot mol^{-1} \cdot s^{-1}$ [299], and other oxygen species such as $^1O_2$ and $O_2^{\cdot-}$ do not bleach NDA. However, it has been reported that the bleaching of NDA was also observed in the presence of other reactive intermediates formed during oxidation of 6-HO-dopamine and dihydroxyfumaric acid [261].

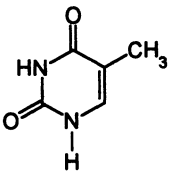
(3) Oxidation of 2,2'azino-di-(3-ethylbenzenthiazoline-6-sulfonate) (ABTS) to a long-lived radical-cation [85], as follows:

$$ABTS + HO^{\cdot} \rightarrow ABTS^{\dot{+}} + HO^- \qquad (165)$$

The rate constant of the above reaction is estimated to be $k = 1.2 \cdot 10^{10}\ \ell \cdot mol^{-1} \cdot s^{-1}$. The absorption band of the radical-cation can easily be measured at 415 nm.

(4) Oxidation of thymine or thymidine [300, 301], for example:

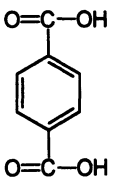
thymine + $HO^{\cdot}$ → N-formylpyruvylurea,



Thymine

detected by high pressure liquid chromatography.

Terephthalic acid (THA) ($C_6H_4(COOH)_2$)

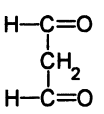


THA

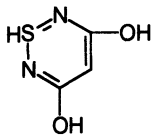
is also reported to be a very sensitive detector of HO radicals.

THA is a non-fluorescent compound which, after the reaction with $HO^{\cdot}$, gives a product showing fluorescence with a maximum at 426 nm ($\lambda_{exc} = 312$ nm).

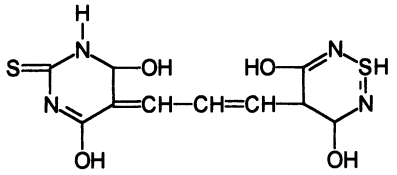
(5) Degradation of the sugar deoxyribose into a thiobarbituric acid reactive compound malonaldehyde. This compound forms with thiobarbituric acid the coloured (2:1) thiobarbituric acid-malonaldehyde adduct which in acid solution absorbs at 532 nm. The resulting adduct can be detected spectrophotometrically or spectrofluorometrically as it emits at 553 nm [169].



Malonaldehyde

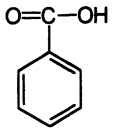


Thiobarbituric acid



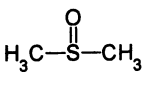
(2:1) Thiobarbituric acid – malonaldehyde adduct

(6) Formation of $^{14}CO_2$ from benzoic acid [302]

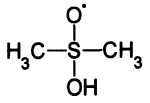


labelled with ^{14}C in the carboxyl group.

(7) Dimethylsulfoxide (DMSO) method; in this method $HO^{\cdot}$ adds to DMSO giving a radical adduct which decomposes into a few products, among them being methane gas which may easily be detected by gas-liquid chromatography.



DMSO



Adduct

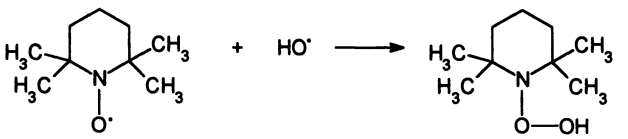
(8) Scavenging of hydroxyl radical, highly electrophilic species, and one of the strongest oxidizing agents. The radical reacts with several inorganic and organic compounds, the so-called $HO^{\cdot}$ scavengers, with nearly diffusion-controlled rate (10^8–10^{10} $\ell \cdot mol^{-1} \cdot s^{-1}$). Among them are alcohols (methanol, ethanol, propanol, isopropanol, *i*-butanol, *t*-butanol, glycerol, mannitol), formate, urea, sodium benzoate, benzoquinone, hydroquinone, ascorbate, amino acids (cysteine,

histidine, methionine, tryptophan), glutathione red, HCO_3^-, N_3^- ions or phenols [295, 302–304]. The addition of one of these scavengers should remove the radical from the system. The rate constants for the reactions of HO and O_2^- radicals with the biologically important compounds, mentioned above, are given in [5, 302, 303].

Recently, a hydroxyl radical optic-fiber sensor has been reported [304] which shows good stability and linearity during detection of HO· with nitrophenol. Nitrocatechol exhibits a strong absorption with λ_{max} = 510 nm. As nitrophenol is immobilized in XAD-7 methacrylate beads, reflectance spectroscopy must be used to detect the decrease in the band intensity.

(9) Infrared absorption spectrum detection. Hydroxyl radical trapped in solid argon at 12 K shows the characteristic infrared absorption line at 3548.2 cm^{-1} [305].

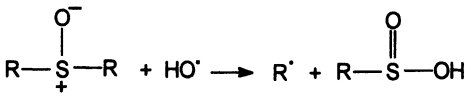
(10) Electron Spin Resonance method. The identification of HO· by ESR described in this chapter should be completed with the other nitrone spin traps such as 2,2,6,6-tetramethylpiperidine-N-oxyl (TEMPO). The method is based on the fact that a decrease in the concentration of stable-N-oxyl radicals after the reaction with HO· is observed [306]. The interaction of HO radical with TEMPO occurs with high yield ($k = 4 \cdot 10^9\ \ell \cdot mol^{-1} \cdot s^{-1}$) [307], as follows:



(166)

A typical ESR spectrum of this trap has been shown in Fig. 7. The ESR spectrum consists of a triplet line with a g-value of 2.0068 and hyperfine splitting constants A_N = 1.6 ± 0.02 mT. The decrease in the signal amplitude was observed, e.g. for autoxidation of tetracycline antibiotic in the presence of Co^{2+} as the hydroxyl radicals source [308].

The next group of spin traps which allows one to detect secondary radical products resulting from the reaction of HO· with methyl ethyl, methyl *n*-propyl sulfoxides or sulfones [309, 310], are nitroso-*tert*-butane (NtB) known as scavengers for alkoxy, acyl, alkyl or aryl radicals [311, 312]. For example, reaction of HO· with aliphatic sulfoxides gives alkyl radical [309]:



(167)

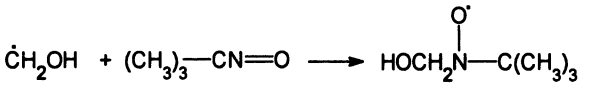
which, trapped by NtB, gives a strong ESR signal due to the spin adduct. The spin trapping technique has been succesfully used to demonstrate HO· generation in several studies, e.g. anthracycline antibiotics [293].

Similarly, secondary radical resulting from the reaction of HO radical with alcohols (methanol or ethanol) as follows [313]:

$$HO^{\cdot} + CH_3OH \rightarrow H_2O + \dot{C}H_2OH \quad (168)$$

$$HO^{\cdot} + CH_3CH_2OH \rightarrow H_2O + CH_3\dot{C}HOH \quad (169)$$

can be trapped, for example, by NtB or DMPO spin traps [314]:



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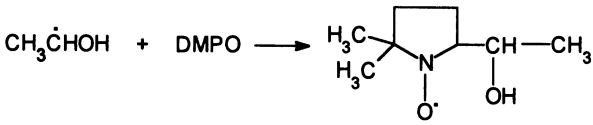


(171)

4.4 Detection of Singlet Oxygen

Several techniques have been used to identify the generation of $^1O_2$ in chemical and biological systems. They can be divided into four man groups:

1. quenching of $^1O_2$ by specific compounds called scavengers;
2. analysis of products of chemical reactions with $^1O_2$ participation;
3. solvent and deuterium isotope effect on lifetime of $^1O_2$;
4. spectroscopic evidence.

4.4.1 Quenching of Singlet Oxygen

Two kinds of quenching may be distinguished – physical and chemical. During physical quenching $^1O_2$ undergoes deactivation to the ground state ($^3\Sigma_g^-$) without chemically reacting with a quencher:

$$^1O_2 + Q \xrightarrow{k_q} {}^3O_2 + Q \quad (172)$$

The chemical quenching involves reaction of $^1O_2$ with a quencher leading to the formation of a new product:

$$^1O_2 + Q \xrightarrow{k_r} QO_2 \quad (173)$$

Both processes very often occur simultaneously, and the quenching is their sum: $k_Q = k_q + k_r$. To be practically effective, a quencher should deactivate $^1O_2$ at a rate constant of at least 10^7–10^8 $\ell \cdot mol^{-1} \cdot s^{-1}$ order of magnitude [250].

Methodology and techniques used for measuring of the rate constant of the total quenching as well as k_r and k_q are outside the scope of this book (see, for example, [315]).

The energy transfer and charge transfer mechanisms have been established during physical quenching of $^1O_2$. The energy transfer mechanism involves deactivation of $^1O_2$ to the ground state ($^3\Sigma_g^-$) and excitation of a quencher to the triplet state [306]:

$$O_2(^1\Delta_g) + {}^1Q \rightarrow O_2(\Sigma_g^-) + {}^3Q \tag{174}$$

The maximal rate of the process has been reported to be about $2 \cdot 10^{10}\ \ell \cdot mol^{-1} \cdot s^{-1}$ [315].

The next mechanism responsible for $^1O_2$ quenching, e.g. the charge transfer process, involves interaction between an electrophilic singlet oxygen molecule with electron donors, A:

$$A + {}^1O_2 \rightleftharpoons [A^+O_2^-]^1 \rightleftharpoons [A^+ O_2^-]^3 \rightleftharpoons A + {}^3O_2 \tag{175}$$

The rate constants for the charge transfer quenching are lower than that for energy transfer ($k_q \leq 10^9\ \ell \cdot mol^{-1} \cdot s^{-1}$) [315]. Several types of compounds known as $^1O_2$ quenchers, such as amines, phenols, thiols and other electron-rich substances, quench $^1O_2$ by the charge transfer mechanism.

One of the earliest recognized classes of compounds showing $^1O_2$ quenching abilities are carotenoid pigments. These compounds quench $^1O_2$ physically [316]. The quenching abilities of carotenoids result from the length of the conjugated polyene chain. Carotenes containing 11 or more conjugated C=C bonds quench $^1O_2$ nearly at a diffusion-controlled rate. The most popular $^1O_2$ quencher, β-carotene, destroys singlet oxygen with a high efficiency. Thus, one molecule of β-carotene quenches about 250 molecules of $^1O_2$. Several values of quenching rate constants have been reported for β-carotene starting from $1.4 \cdot 10^9$ to $3 \cdot 10^{10}$ $\ell \cdot mol^{-1} \cdot s^{-1}$ depending on the kind of a solvent [317, 318]. This quencher is very often used as a test for involvement of $^1O_2$ in chemical and biological systems. A list of carotenoids known to quench $^1O_2$, with their rate constants and chemical formulae, is given in [250, 319, 320] and their chemical formulae are presented in Chap. 7.

The next important group of $^1O_2$ quenchers, less effective than carotenoids, involves compounds containing a nitrogen atom, such as both aliphatic and aromatic amines [321–323], nitroxides [324], nitrones [325], nitroso compounds [326], azomethine dyes [327], amino acids and proteins [328–330], bilirubin and biliverdin [331–333]. It is worth mentioning that chlorophyll a as well as bacteriochlorophylls c and d (pigments of the chlorosome antenn as green photosynthetic bacteria) are strong physical quenchers of $^1O_2$ with rate constants $8.0 \cdot 10^7$–$11.2 \cdot 10^9\ \ell \cdot mol^{-1} \cdot s^{-1}$ [334, 335].

Phenols, a group of compounds widespread in the plant and animal kingdoms, scavenge $^1O_2$ by a combination of chemical reactions and physical quenching with the rate constants achieving $6 \cdot 10^8\ \ell \cdot mol^{-1} \cdot s^{-1}$ ([315, 324 and references therein]). An interesting observation made by Foote et al. [336] during a study of the rate constant of $^1O_2$ quenching by 2,4,6-trisubstituted

phenols, showed that the logarithm of the total quenching rate constants is a linear function of the phenol half-wave oxidation potential. Similar observations were made for certain phenol methyl ethers and amines [337].

Sulfides and disulfides have also been found to quench $^1O_2$ and again both quenching and oxidation reaction occur during persulfoxide formation as a common intermediate [338, 339]:

$$R_2S + {}^1O_2 \rightarrow [R_2S^+ - OO^-] \tag{176}$$

Metal complexes are the next group of compounds recognized as $^1O_2$ quenchers. They are widespread in nature and some chelates are extremely reactive. For example, it has been reported that SOD, a natural metal chelate, exhibits a high quenching rate towards $^1O_2$ ($k_r = 1.6 \cdot 10^9\ \ell \cdot mol^{-1} \cdot s^{-1}$ [340]). However, the experiments carried by other authors [315] indicated a scavenging of the precursor of $^1O_2$, i.e. O^-_2 radical rather than $^1O_2$. The detailed mechanism of $^1O_2$ quenching by metal complexes is not well known. Among the synthetic metal complexes, quenching ability increases in the order Zn, Pd, Pt, Cu, Co(II), Ni(II) [315]. Some of the Ni(II) chelates exhibit $^1O_2$ quenching ability comparable with β-carotene [341].

Singlet oxygen quenching is also observed by inorganic anions such as N_3^-, Br^-, I^-, $S_2O_3^{2-}$, $NCSe^-$ or O_2^-. Inorganic anions quench $^1O_2$ at rates which vary from about $1.2 \cdot 10^6$–$7 \cdot 10^9\ \ell \cdot mol^{-1} \cdot s^{-1}$ [342–344]. The superoxide radical anion shows extraordinary high $^1O_2$ quenching efficiency ($k_q = 7 \cdot 10^9\ \ell \cdot mol^{-1} \cdot s^{-1}$) by an electron transfer, as follows [343]:

$$^1O_2 + O_2^- \rightarrow O_2^- + {}^3O_2 + \text{heat} \tag{177}$$

Both physical and chemical quenching ability towards $^1O_2$ is shown by NADH and its analogues [345] undergoing one-electron oxidation:

$$^1O_2 + NADH \rightarrow O_2^- + NAD^{\cdot} + H^+ \tag{178}$$

Besides the substances described above, a large number of other compounds showing $^1O_2$ quenching ability have been reported. The details of the interaction between $^1O_2$ and its quenchers as well as the quenching rates are outside the scope of this review. They are compiled in [315, 324, 339, 345–347]. However, a few quenchers among those most often used by investigators have been examined in some detail, and their rate constants are given in Table 1.

Table 1. The total rate constants for $^1O_2$ quenching

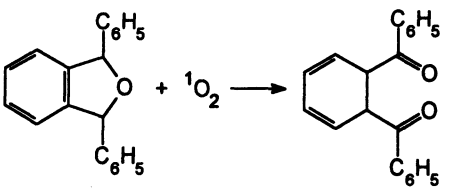
Compound	k_Q [$\ell \cdot mol^{-1} \cdot s^{-1}$]	Solvent	Reference
Carotens			
β-Carotene	$1.3 \cdot 10^{10}$	C_6H_6	[346]
Crocin	$1.8 \cdot 10^9$	H_2O	[320]
Amines			
1,4-Diazabicyclo[2.2.2]octane	$7.3 \cdot 10^6$	CH_3OH	[337]
(DABCO)	$1.5 \cdot 10^7$	CH_3OH	[315]

Table 1 (continued)

Compound	k_Q [$\ell \cdot mol^{-1} \cdot s^{-1}$]	Solvent	Reference
Diethylamine	$1.88 \cdot 10^6$	CH_3OH	[337]
Diphenylamine	$6.1 \cdot 10^6$	CH_3OH	[337]
Phenols			
α-Tocopherol (vitamin E)	$6.2 \cdot 10^8$	CH_3OH	[336]
Hydroquinone	$7.0 \cdot 10^7$	C_6H_5-CH_3OH (4:1)	[337]
Amino acids and proteins			
Tryptophan	$3.6 \cdot 10^7$	H_2O-CH_3OH (1:1)	[328]
Methionine	$2.5 \cdot 10^7$	H_2O-CH_3OH (1:1)	[328]
Histidine	$3.2 \cdot 10^7$	H_2O-CH_3OH (1:1)	[328]
Superoxide Dismutase (SOD)	$8.2 \cdot 10^8$	D_2O	[340]
Sulfides			
Cysteine	$8.9 \cdot 10^6$	D_2O	[339]
Glutathione	$2.9 \cdot 10^6$	D_2O	[339]
Bilirubin	$2.5 \cdot 10^8$	$CHCl_3$	[331]
Biliverdin	$8.0 \cdot 10^8$	freon 113	[333]
Chlorophyll a	$(1.5–6) \cdot 10^{10}$	D_2O	[333]
	$< 10^9$	C_6H_6	[334]
	$8 \cdot 10^7$	acetone	[335]
NADH	$7.5 \cdot 10^7$	CH_3CN-CH_3OH(1:4)	[345]
L-Ascorbic acid (vitamin C)	$8.3 \cdot 10^6$	H_2O	[348]
	$2.5 \cdot 10^6$	D_2O	[348]
	$5.8 \cdot 10^8$	H_2O	[349]
	$6.28 \cdot 10^8$	D_2O	[349]
5,5-Dimethylcyclohexane-dione-1.3	$3.0 \cdot 10^7$	H_2O	[349]
Nickelocene	$2.8 \cdot 10^9$	chloroform	[350]

4.4.2 Analysis of Products

During reaction with $^1O_2$ some of the reviewed scavengers yield specific products which can easily be analyzed by spectrophotometric or chromatographic techniques. For example, 1,3-diphenylisobenzofuran (DPBF) is widely used as $^1O_2$ scavenger as it reacts rapidly with $^1O_2$, giving *o*-dibenzylbenzone:

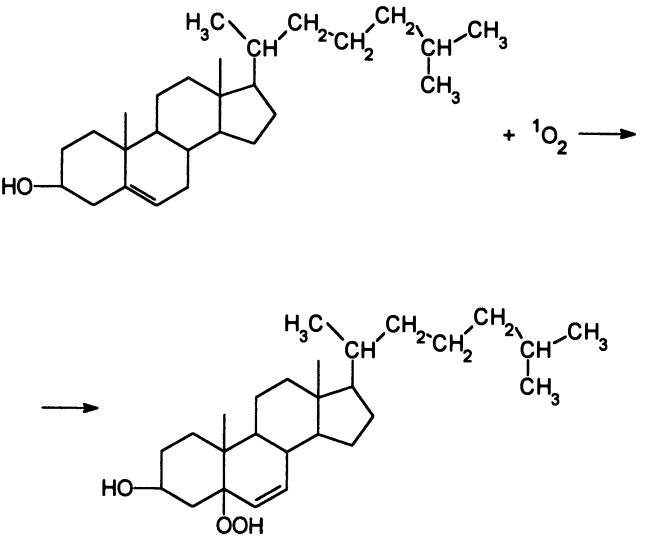


(179)

Its usage is based on the decrease of absorbance [157, 351] as well as fluorescence [352] due to removing DPBF from the reaction system after the reaction with $^1O_2$. The rate of disappearance of DPBF can be followed by monitoring the

absorbance at 415 nm and fluorescence intensity at 458 nm when excitation is 405 nm.

Cholesterol, like other olefins with alkyl substitutents, undergoes "ene" reaction with $^1O_2$, forming the allylic hydroperoxide 3-β-hydroxy-5α-cholest-6-ene-5-hydroperoxide [353]:



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When cholesterol is oxidized on a radical pathway several other hydroperoxides are produced [303]. The next compound, tryptophan, reacts with $^1O_2$ and forms several products including kynurenine, *N*-formyl kynurenine and 2-carboxy-3a-hydroxy-1,2,3,3a,8,8a-hexahydropyrrolo-(2,3b)-indole [241].

There is also evidence that β-carotene, the *cis* isomer, inhibits $^1O_2$ converting itself into the *trans* isomer [354].

4.4.3 Solvent and Deuterium Isotope Effect

As was mentioned in Chap. 1, the lifetime of $^1O_2$ in solution is very sensitive both to the kind of the solvent and to the solvent deuteration [96, 98]. Both $^1O_2$ properties have been used to demonstrate $^1O_2$ generation [98 and references therein]. The effect of both factors on the lifetime of $^1O_2$ for chosen solvents are given in Table 2. The details dealing with solvent and solvent deuterium effects on the $^1O_2$ lifetime, methods of measurement, and values of lifetime, have been compiled, e.g. in [98, 355].

The solvent deuteration $^1O_2$ lifetime dependence, also known as the heavy-water effect, is a very simple and popular method for evaluating the participation of $^1O_2$ in several chemical and biological processes.

Table 2. Solvent influence on the lifetime of singlet oxygen

Solvent	τ[μs]	References	Solvent, Medium	τ[μs]	References
H_2O	2	[96]	D_2O	67	[356]
CH_3OH	7	[96]	D_2O	20	[98]
C_2H_5OH	12	[98]	H_2O/CH_3OH (1:1)	3.5	[98]
C_6H_6	24	[96]	D_2O/CH_3OD (1:1)	35	[98]
$CHCl_3$	60	[96]	$CDCl_3$	300	[98]
CCl_4	700	[96]	polymer matrice	10–50 ms	[357]

4.4.4 Spectroscopic Evidence

Spectroscopic evidence can be classified according to the apparatus used as:

- spectrophotometric determination;
- ESR spin trapping;
- chemiluminescence.

4.4.4.1 *Spectrophotometric Determination*

The spectrophotometric method developed by Kraljić and Mohsni [358] is based on bleaching of *p*-nitrosodimethylaniline (RNO) caused by the intermediate product formed during interaction of $^1O_2$ with imidazole (A) or histidine being a transannular peroxide (AO_2):

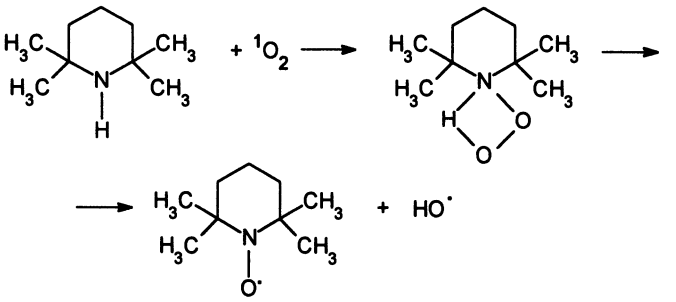
$$^1O_2 + A \rightarrow AO_2 \quad (181)$$

$$AO_2 + RNO \rightarrow -RNO + \text{products} \quad (182)$$

The bleaching of RNO can simply be followed by monitoring the decrease in optical density of RNO at 440 nm. The method is sensitive and selective for detection of $^1O_2$ in aqueous systems, and has been used to identify the generation of $^1O_2$ during autoxidation of catecholamines [118] and some tetracycline antibiotics [92, 359] as well as during photoxidation of some dyes [358].

4.4.4.2 *ESR Spin Trapping*

The ESR spin trapping method for the detection of $^1O_2$ was originally set up by Lion and Van de Vorst [360] and has been successfully applied to study its generation in several chemical systems, initiated by the light as well as in the dark [361–363]. This method involves the formation of stable nitroxide radical of 2,2,6,6-tetramethylpiperidine-*N*-oxyl (TEMPO) from the sterically hindered amine: 2,2,6,6-tetramethylpiperidine (TEMP):



(183)

Figure 8a shows as an example the formation of TEMPO detected during autoxidation of methacycline. The spectrum consists of a triplet line and can be analyzed in terms of three parameters: g = 2.0064, hyperfine splitting A_N = 16.0 Gs, and linewidth $\Delta B = 0.38°$ Gs [120]. The splitting is typical for the hyperfine interaction between the unpaired electron and the nitrogen nucleus (I = 1). All parameters obtained are the same, within error limits, as those reported for TEMPO [360].

When $^1O_2$ quencher was added to the reaction mixture, a large decrease in the ESR signal amplitude was observed (Fig. 8b). Figure 8c presents a very weak ESR signal characteristic for the nitroxide radicals induced by autoxidation of TEMP in the absence of methacycline.

It was reported that TEMP is a very specific $^1O_2$ spin trap since neither O_2^- nor HO· and H_2O_2 were able to generate TEMPO [364].

In order to detect $^1O_2$ in neutral solution using the heterocyclic amine it should be remembered that the amine must show a pK value below 8 as does the 2, 2, 6, 6-tetramethyl-4-piperidone (pK = 7.6) trap [363].

The N-substituted 2,2,4,4-tetramethylpiperidines can also be used for the detection of other oxygen species. For example, a chloroamine (Cl substituted for H atom) was reported to exhibit specific spin-trap property for O_2^- detection [364].

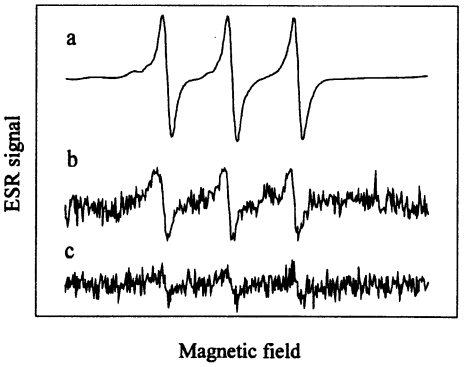
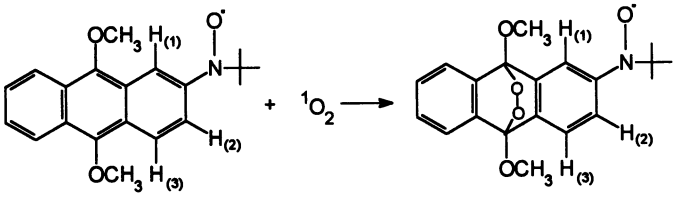


Fig. 8. **a** ESR spectrum of the 0.25 mol · ℓ^{-1} TEMP + 0.5 mmol · ℓ^{-1} $CoCl_2$ + 1 mmol · ℓ^{-1} methacycline system. **b** The same as **a** but in the presence of $^1O_2$ scavenger (1 mmol · ℓ^{-1} sodium azide). **c** The same as **a** but in the absence of methacycline. Temperature 295 K

It is important to remember that sterically hindered piperidine derivatives can be used for detection of the oxygen species in aqueous solution, where their concentration is quite low owing to short lifetime of $^1O_2$ or very fast dismutation of $O_2^{\cdot-}$ (reaction at Eq. (94)).

The next nitroxide ESR group of traps for the $^1O_2$ detection are anthracenyl *tert*-butyl nitroxides (I) [365] which, after reaction with $^1O_2$, give corresponding endoperoxides (II), and characteristic changes in their ESR spectrum can easily be observed. For example, compound I showing a six-line ESR spectrum upon reaction with $^1O_2$ converts into endoperoxide nitroxide (II):



I II (184)

of which the ESR spectrum consists of three groups of lines. The groups are due to N-splitting, and each group shows nearly equivalent coupling to H(1) and H(3) and smaller coupling to H(4) [365].

The ESR spin-trapping technique used for detection of oxygen species, however indirect, provides sensitive and very popular spectroscopic evidence. New compounds showing trapping properties are steadily being synthetized and new applications are currently being reported [366–369].

4.4.4.3 *Chemiluminescence*

Chemiluminescent emission occurs in liquid, gas and solid phases and finds application in clinical, toxicological, chemical and environmental analysis [370–376]. The emission can be characterized by several parameters, among which the chemiluminescence emission intensity (I) and spectral distribution $I_\lambda = f(\lambda)$ show great impact on the application of this method. Chemiluminescence kinetics, $I = f(t)$, give information about the rate of formation and deactivation of excited products, whereas the chemiluminescence spectrum reflects the energy released during the reaction and the kind of emitters.

For the detection of $^1O_2$, the measurement of the light emission from the single and paired excited oxygen molecules is of significance. As has been presented in Chap. 1, singlet oxygen gives characteristic emission spectra during radiative deactivation to the ground state. If the quantum yield of the emission is high, conventional equipment can be used to obtain the spectra. The apparatus consists, in its simplest form, of a sample cuvette, a reagent addition device, monochromator and light measuring device (photomultiplier, silicon photodiodes). When the quantum yield is low a set of cut-off filters consisting of coloured glass, according to the method of Vasiliev [377], and a single-photon counting technique are usually used to record the spectra. A typical spectrum recorded using this method is shown in Fig. 9.

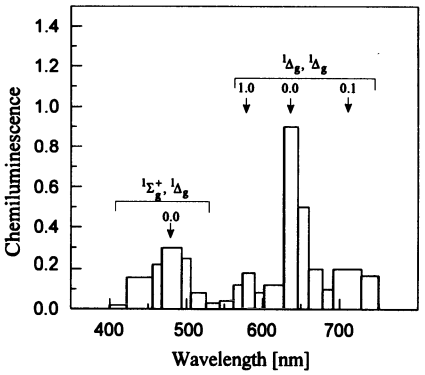


Fig. 9. Spectral distribution of chemiluminescence measured during the decomposition of 0.3 mol · ℓ^{-1} hydrogen peroxide in phosphate buffer pH 11, temperature 310 K

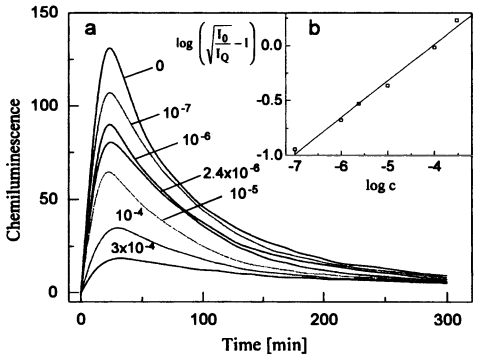


Fig. 10. **a** The influence of $^1O_2$ quencher (bilirubin) on the chemiluminescence from pyrogallol (numbers indicate the bilirubin concentration in mol · ℓ^{-1}). **b** The Stern-Volmer plot for several concentrations of the quencher. Concentration of substrates after mixing: 0.34 mmol · ℓ^{-1} pyrogallol, 2 mmol · ℓ^{-1}). KOH, 295 K

The most specific and direct method for measurement of $^1O_2$ is the observation of the fundamental luminescence at 1268 nm ("monomol emission") corresponding to the 0.0 band of the $[^1\Delta_g]_{v=0} \rightarrow [^3\Sigma_g^-]_{v=0}$ transition [376, 378–393].

Also, the $^1\Delta_g$ emission, resulting from the $[^1\Delta_g]_{v=0} \rightarrow [^3\Sigma_g^-]_{v=1}$ transition at 1588 nm can be observed in solution [378–394].

These emissions in the infra-red region can be directly measured using a germanium diode as the photodetector or indirectly after the energy transfer from $^1O_2$ to a thin polymer film. The blue shifted emission is then measured [395].

In the last few years new high-sensitivity spectrometer systems for time- and spectral-resolved near infrared detection of $^1O_2$ in photobiological and photochemical systems have been developed [396–400].

Another criterion used for examination of $^1O_2$ generation is finding the quenching constants of $^1O_2$ by specific quenchers. The Stern-Volmer plot [401] for the ratio of chemiluminescence intensity from $O_2[^1\Delta_g]$, when the quencher is added to the reaction system (I_Q) and without the quencher (I_0), is used to find the quenching constant k_Q:

$$\frac{I_0}{I_Q} = 1 + k_Q\tau[Q]$$

where [Q] is the concentration of the quencher and τ is $O_2[^1\Delta_g]$ lifetime in a given solvent. The plot of (I_0/I_Q) vs quencher concentration of the $O_2[^1\Delta_g] \rightarrow O_2[^3\Sigma_g^-]$ emission gives a straight line in a wide range of quencher concentrations. While the chemiluminescence results from the radiative deactivation of the pairs of the oxygen molecule $O_2[^1\Delta_g, {}^1\Delta_g] \rightarrow 2\,O_2[^3\Sigma_g^-]$, the emission intensity at 634 nm is proportional to the square of the $O_2[^1\Delta_g]$ concentration and therefore the ratio (I_0/I_Q) must be replaced by $\sqrt{I_0/I_Q}$ (Fig. 10).

Environmental Aspects

5 Biological Damages Caused by Reactive Oxygen Species

Within the past three decades accumulating evidence has pointed out the intermediancy of oxygen species in the chemical modification of natural and xenobiotic compounds present in cells and tissues. A great number of international conferences as well as published papers, and books, all devoted to the reactive oxygen species and their role in the environment, indicate the importance and immediate interest of the problem. This chapter is not an exhaustive review, but presents the chemical mechanisms which have been shown to operate during damage to compounds of biological and industrial interest as well as in living systems. The possible medical implications of these species, chemical reactivity are also discussed.

The intention of the author was to gather together the experimental data which implicate the active oxygen radicals and singlet molecular oxygen in the oxidative degradation of cell constituents such as

- proteins (especially amino acid residues)
- lipids (mainly polyunsaturated)
- carbohydrates (e.g. hyaluronic acid, collagen)
- nucleic acids (e.g. DNA)

and assess their pathogenic properties.

Discussing the types of reactions in which the reactive oxygen species are involved, we are aware of the extreme reactivity and eventually the destructive power which they can show under unbalanced conditions. An imbalance results from excessive generation of activated oxygen species or when protective mechanisms are exceeded or put out of order. Normal metabolism involves the ordered production of reactive oxygen species playing an important physiological function since free radical reactions are a basic part of the homeostasis in cellular processes. Moreover, the radical biochemistry plays a key role in the origin of aerobic forms of life.

Reactive oxygen species, besides playing an important physiological function, can also cause extensive damage. There is no doubt that oxygen species, especially $HO^{\cdot}$ and $^1O_2$, are involved in a majority of human diseases, although very high reactivity of the species makes it impossible to measure directly their cytotoxic action in vivo. The enormous volume of literature on the participation of both oxygen free radicals and $^1O_2$ in pathological states consider these species as very dangerous agents. Many toxic effects may result from the species reaction towards enzymes, membranes, nucleic acids or polysaccharides (Fig. 11). The

damage may be a result of the direct action of the species with biomolecules, or from the action of toxic products of reactions initiated by these species. Figure 11 also covers endogenous and exogenous processes and factors which can increase the rate of radical production in vivo under unbalanced conditions. They include three basic processes: radiation, photolysis and oxidation-reduction reactions.

The last process is accelerated by an increase in the amount of metal ions (Fe, Cu, Zn, etc.) so-called "iron-overload", discussed in Chap. 2 as well as oxidation of the cell reductants such as ascorbate, thiols, nucleotides or monosaccharides, accumulation of very reactive quinones, for example, products of the catecholamines oxidation or xenobiotics reduction by enzymes.

It has been reported that the oxygen species generation occurs mainly in the intracellular site, for example, in lysosomes and the endoplasmic reticulum

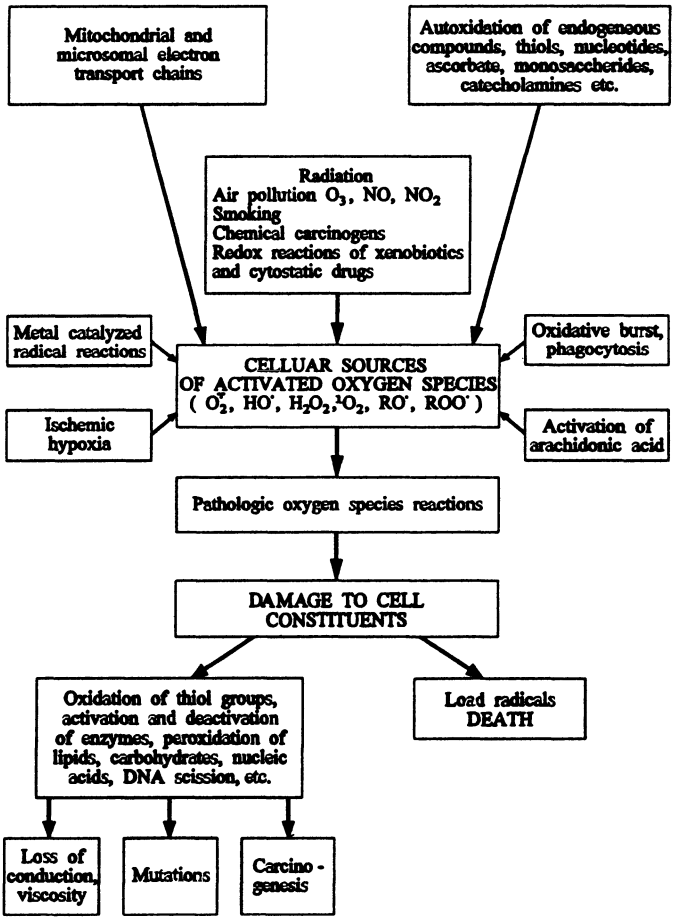


Fig. 11. Schematic representation of cellular sources of oxygen species and these species damage to cell constituents

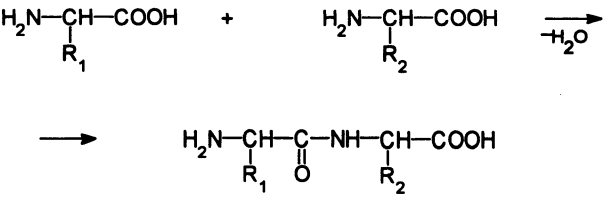
[402]. The degradation of extracellular space components including, for instance, hyaluronic acid and collagen can also be possible, since it has been reported that a large group of the compounds generating O_2^- and $HO^\cdot$ in various model systems degrade these compounds [403–405].

5.1 Reactivity of Hydroxyl Radicals with some Biologically Important Compounds

Lets us consider the above-mentioned four main groups of cell constituents susceptible to damage caused by oxygen radicals.

5.1.1 Proteins

It is generally stated that proteins are polypeptides of molecular weights more than a few thousands, although there is no sharp distinction between peptides and proteins. Linear peptides have one α-NH_2 and α-COOH group which are not involved in peptide bond formation, so-called terminal amino or carboxyl. Formation of peptide bonds, i.e. amide links between the α-carboxyl of one amino acid and the α-amino of another is accompanied by the elimination of water.



(185)

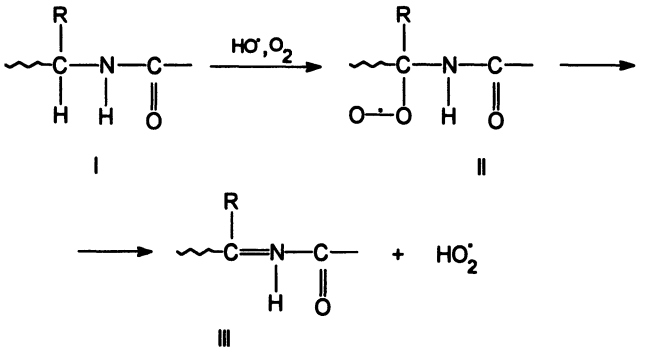
Polypeptides are built up according to the same amide link formation of several amino acids.

Proteins are present in cells and outside them in very high concentrations and they must be critical targets for both oxygen free radicals and $^1O_2$. Proteins may be damaged by reactions of amino acid residues with oxygen species and/or by interaction of aldehydes with –SH and $-NH_2$ groups of the protein molecule [406, 407]. The damage to amino acids residues may lead to changes, for example, in enzyme activity. Aldehydes are formed during lipid peroxidation of fatty acids and they are considered to be "toxic second messengers" for the primary generated free radicals [408]. Dean and Pollak [409] have shown in vitro that $HO^\cdot$ generated during steady-state gamma radiolysis degrades proteins under aerobic conditions. A similar effect was reported when proteins were exposed to the H_2O_2 + Cu/histidinyl system generating HO radicals [410].

Degradation of proteins results in the formation of a large number of products depending on the kind of a protein, its concentration, the number of

amino groups contained in the protein molecule and the rate constant of the radical reaction with the amino acid residues [406, 411].

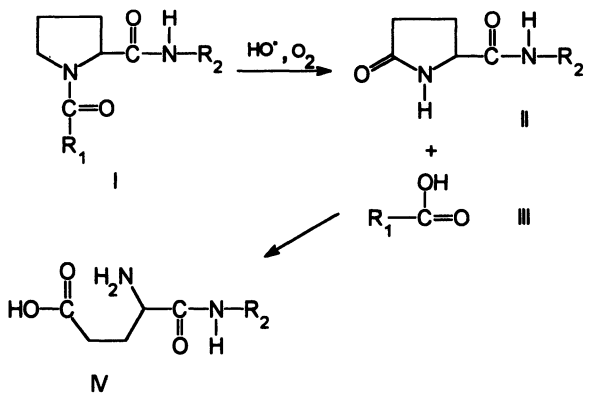
Several hypothetical reaction schemes have been proposed for protein fragmentation. For example, the scheme proposed by Garrison [412] for the model polypeptides (I) involves H-atom abstraction at the α-carbon position. The next stages are peroxy radical formation (II) and its elimination followed by the iminopeptide (III) formation:



(186)

The latter compound decomposes easily under mild hydrolysis, giving a few compounds such as dicarbonyl derivative acids or ammonia.

The next mechanism worth mentioning for protein fragmentation, given by Wolff et al. [406], postulates protein cleavage of proline residues:



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The reaction scheme involves hydroxylation of proline following the formation of 2-pyrrolidone intermediate as a result of hydrogen atom abstraction by HO radical at the gamma carbon. Under physiological conditions the latter products undergo spontaneous hydrolysis at the peptide-bond giving product II and acid III. Product II undergoes degradation during a mild hydrolysis giving new N-terminal glutamate residues (IV).

Special attention has been paid to oxygen active species reactions with -SH containing molecules and complicated biological polymers, for example enzymes

(see [212, 269, 303, 413–415]). Many compounds react with HO radicals at high rates, but for interactions with O_2^- these rate constants are usually lower. The rate constants for reactions of the O_2^- and HO radicals with several compounds of biologically interest are listed in Tables 3 and 4.

The mechanism of oxygen free radicals reactions with compounds containing the -SH groups was discussed in Chap. 3. Enzyme molecules as large molecules contain several amino acid residues: thus oxygen free radicals can interact with all possible residues, destroying them.

In order to determine damage of an enzyme, measurements of its catalytic activity before and after reaction with a mediator of cytotoxicity were carried out [243]. If the reaction is catalyzed by an enzyme:

$$\text{enzyme} + \text{substrate} \xleftrightarrow{K_w} \text{enzyme-substrate complex} \xrightarrow{k_{cat}} \xrightarrow{k_{cat}} \text{enzyme} + \text{products}$$

its Michaelian rate is given by the equation

$$v = k_{cat}[E]_o[S]/(K_w + [S]) \tag{188}$$

where [S] and $[E]_o$ denote the concentration of substrate and the initial (i.e. total) concentration of an enzyme, respectively. When the amino acids residue of the enzyme is damaged or its conformation changed, for instance by oxygen radicals, the value of k_{cat} becomes nearly equal to zero for the enzyme considered.

Table 3. Rate constants for reaction of O_2^- with some biologically important compounds

Substrate	$k\,[\ell \cdot mol^{-1} \cdot s^{-1}]$	pH	References
Cysteine	$> 5.0 \cdot 10^4$	7.0	[416]
Histidine	1.0	7.0	[303]
Methionine	0.33	7.0	[303]
Tryptophan	24	7.0	[303]
Papain	$\leq 6.0 \cdot 10^5$	6.0	[417]
Riboflavin semiquinone	$7.1 \cdot 10^8$	7.0	[418]
NADH-Lactate dehydrogenase complex	$1.0 \cdot 10^5$	7.5–9	[269]
Peroxidase Compound I	$1.6 \cdot 10^6$	7.2–8.8	[413]
Adenosine triphosphate-iron (II)	$1.1 \cdot 10^6$	7.0	[419]
Ascorbate radical anion	$< 2.3 \cdot 10^8$	7.4	[420]
Ascorbic acid	$2.7 \cdot 10^5$	7.4	[232]
Flavin adenine dinucleotide semiquinone	$2.2 \cdot 10^8$	7.0	[418]
Gluthatione red	$6.7 \cdot 10^5$	3.6, 8.7	[421]
Methemoglobin (enzyme from bovine blood)	$1.4 \cdot 10^9$	7.0	[422]
Adrenaline	$5.4 \cdot 10^4$	8.8	[423]
Cytochrome c	$5.8 \cdot 10^5$	7.3	[424]
Ceruloplasmin	$1.8 \cdot 10^6$	7.8	[425]
Superoxide dismutase	$2.4 \cdot 10^9$	7.4	[212]

Table 4. Rate constants for reaction of $HO^{\cdot}$ with some biologically important compounds

Substrate	$k[\ell \cdot mol^{-1} \cdot s^{-1}]$	pH	Reference
Ascorbate	$1.0 \cdot 10^{10}$	7	[303]
Ethanol	$1.0 \cdot 10^{9}$	7	[302]
Cysteine	$8.0 \cdot 10^{9}$	7	[426]
Histidine	$5.0 \cdot 10^{9}$	7	[303]
Tryptophan	$1.9 \cdot 10^{10}$	7	[303]
Methionine	$8.5 \cdot 10^{9}$	7	[303]
Glutathione red	$8.0 \cdot 10^{9}$	7	[427]
Mannitol	$2.1 \cdot 10^{9}$	7	[428]
Imidazole	$8.7 \cdot 10^{9}$	6.8	[429]
Alcohol dehydrogenase	$2.2 \cdot 10^{11}$	7	[414]
Lactate dehydrogenase	$2.1 \cdot 10^{11}$	7	[212]
Papain	$4.7 \cdot 10^{10}$	6.4	[415]
Superoxide dismutase	$5.3 \cdot 10^{10}$	7	[303]
Guanidine derivative	$(1.7-9.0) \cdot 10^{9}$	7.4	[430]
H_2-receptor antagonists like cimetidine	$1.48 \cdot 10^{10}$	7.4	[430]
Green tea polyphenols	$1.7 \cdot 10^{10}$	7	[431]
Quercitin	$1.5 \cdot 10^{10}$	7	[431]
Caffeic acid	$7.4 \cdot 10^{9}$	7	[431]

In this case the initial amount of the active enzyme molecules can be calculated from the ratio of v_d/v, where v_d denotes the rate of catalysis for the same quantity of enzyme solution after exposure to radicals.

The decrease in the enzyme concentration (δ) is then given by the equation

$$\delta = \frac{[E]_o(v - v_d)}{v} \tag{189}$$

The loss of a number of enzyme molecules per energy of 100 eV is called G(-activity) [243]. The G-value for enzymes containing n active residues showing independent activity in the enzyme molecule must be expressed as "molecular units of active site" lost per energy of 100 eV, and is given by the expression

$$\frac{n \cdot [E]_o (v - v_d)}{v} \tag{190}$$

This value shows how dangerous the radical reaction is that occurred to the enzyme and allows one to compare sensitivities of the enzyme for its interaction with various radicals. An important parameter characterizing a degree of damage caused by active species is inactivation probability, f(i), of the compound per a single radical of a given active species, i. If one radical species is presented in a medium, f(i) is equal to G(-activity)/G_i, whereas for several radical species values of f(i) must be calculated from the equation

$$G(\text{-activity}) = \sum_i (G_i \cdot f(i)) \tag{191}$$

Table 5. The inactivation probabilities f(i) for some enzymes during their interaction with O_2^- and HO radicals

Enzyme	f(HO·)	$f(O_2^-)$	Reference
Papain	0.20[1]	0.33	[417]
	0.06	–	[414]
Trypsin	0.05	0.03	[427]
Lactate dehydrogenase from beef heart	0.18	0.02	[212]
Alcohol dehydrogenase from yeast	0.12	0.12	[432]

[1] This value was measured in aerated solution, while the other were obtained in oxygen free solutions. Values of pH were in the range of 5.8 to 7.5

where G_i are values obtained during exposure to particular species reacting. For example for $G_{HO\cdot}$; $G_{O_2^-}$, we obtain $f_1(HO\cdot)$, $f_2(O_2^-)$, respectively.

Table 5 shows, as an example, the inactivation probabilities exerted by O_2^- and HO· radicals for chosen enzymes. As can be seen from the table only a small proportion of the reacting radicals damages the biologically active residues or exerts conformational changes, having influence on the catalytic activity of the enzyme.

Moreover, the presence of molecular oxygen in solution can significantly increase the inactivation coefficient as is observed in the case of papain. This increase may result from the participation of oxygen as a radical trap in the radical chain reactions initiated by the damaging radical:

$$\begin{aligned} &\text{enzyme} - H + HO\cdot \rightarrow \text{enzyme}\cdot + H_2O_2 \\ &\text{enzyme}\cdot + O_2 \rightarrow \text{enzyme} - O_2^{\cdot} \rightarrow \text{loss of enzyme activity} \end{aligned} \qquad (192)$$

The similar increase in the inactivation probability has been observed in the reaction of glyceraldehyde-3-phosphate dehydrogenase with HO· in aerated solution [433]. The loss of activity of both enzymes caused by HO· correlates with loss of –SH groups [417, 433].

The role of O_2^- in inactivation of trypsin and lactate dehydrogenase is very small, practically insignificant, in contrast with alcohol dehydrogenase. The large value of $f(O_2^-)$ observed in the case of papain is explained by observations that –SH groups are easily accessible for binding acyl groups of the enzyme [417].

5.1.2 Polyunsaturated Fatty Acid Peroxidation

The most frequently reported toxic effect of oxygen species, and at the same time well documented, deals with the oxidative breakdown of polyunsaturated fatty acids. This process is commonly known as "lipid peroxidation".

The term "lipid" deals with a very heterogenous group of biomolecules that are insoluble in water, but which dissolve in non-polar solvents. The most important lipids in humans are fats, oils, steroids (cholesterol, steroid hormones), fat soluble

vitamins (A, D, E, K) and fatty acids derivatives (prostaglandins, prostacyclins, thromboxanes). Fatty acids occurring in higher animals have usually chain lengths of 16, 18 or 20 carbon atoms. Saturated fatty acids are rather unreactive compounds, unlike unsaturated fatty acids, especially those having conjugated bonds, e.g. linoleic acid, vitamin A.

However, lipid peroxidation is a chain reaction which may be initiated by several oxygen species: $I^{\cdot} = HO^{\cdot}, O_2$ (diradical), $^1O_2$, $RO^{\cdot}$, $RO_2^{\cdot}$, ferryl ion (FeO^{2+}), perferryl compound (FeO_2^{+}) or atomic oxygen [434]. It is widely belived that the most important initiator with respect to the production of lipid peroxidative tissue damage is the hydroxyl radical [435]. The detailed nature of an initiator has not been established yet. Three main steps may again be distinguished during the lipid peroxidation: initiation, propagation and termination [179, 436, 437].

Let us consider the peroxidation of the part of a fatty acid molecule with two unsaturated bonds (Fig. 12), for example, linoleic acid with a chemical formula:

$$CH_3(CH_2)_4-CH=CH-CH_2-CH=CH-(CH_2)_7-\overset{\overset{O}{\|}}{C}-OH \qquad (193)$$

Identical mechanisms may be written for fatty acids containing one or three unsaturated bonds as well as for the cholesterol molecule.

The scheme involves hydrogen atom abstraction from a fatty acid (LH) by initiating species ($I^{\cdot}$) following by the formation of lipid radical, ($L^{\cdot}$), (an alkyl radical), with an electron situated on the carbon atom adding onto carbon atoms connected with double bonds. The electron may be shifted to other carbon atoms followed by the double bonds moving closer together with arising isomeric forms, (b). This step is followed by the oxygen molecule adding onto the free radical and the formation of a lipid peroxy radical ($LOO^{\cdot}$), (c). The lipid peroxy radical is unstable and undergoes reaction abstraction with a hydrogen atom from neighbouring molecules or a variety of compounds including other lipid molecules, nucleic acids, antioxidants, denoted as RH, to form lipid hydroperoxide (LOOH) and an alkyl radical ($R^{\cdot}$), (d). The diene conjugated hydroperoxides show the characteristic absorption at 232–235 nm. The hydroperoxide generates a number of new free radicals which are the products of its spontaneous or metal catalyzed decomposition, such as alkoxy radical ($LO^{\cdot}$) and hydroxy radical, ($HO^{\cdot}$), (e). Both radicals, $HO^{\cdot}$ and $LO^{\cdot}$, may terminate by abstraction of hydrogen atoms from neighbouring molecules, RH, forming LOH, H_2O and the next radicals from the RH molecules, (f). The newly formed radicals propagate the chain reaction whereby more and more unsaturated fatty acids oxidize into hydroperoxides and other products.

Also, the other carbon atoms occurring in the fragment of the lipid peroxide, $LO^{\cdot}$ connected with carbon atoms with double bonds can be attacked by free radicals, forming unstable products of the bis-hydroperoxide type, decomposition of which leads to the formation of an aldehyde and another alkyl radical, (g). All these radicals can cause polymerization at the dienic bonds and at isolated-C=C-bonds. Apart from the polymerization, the cross-linking of the alkyl radicals also occurs by mechanisms shown in reactions (h) and (i).

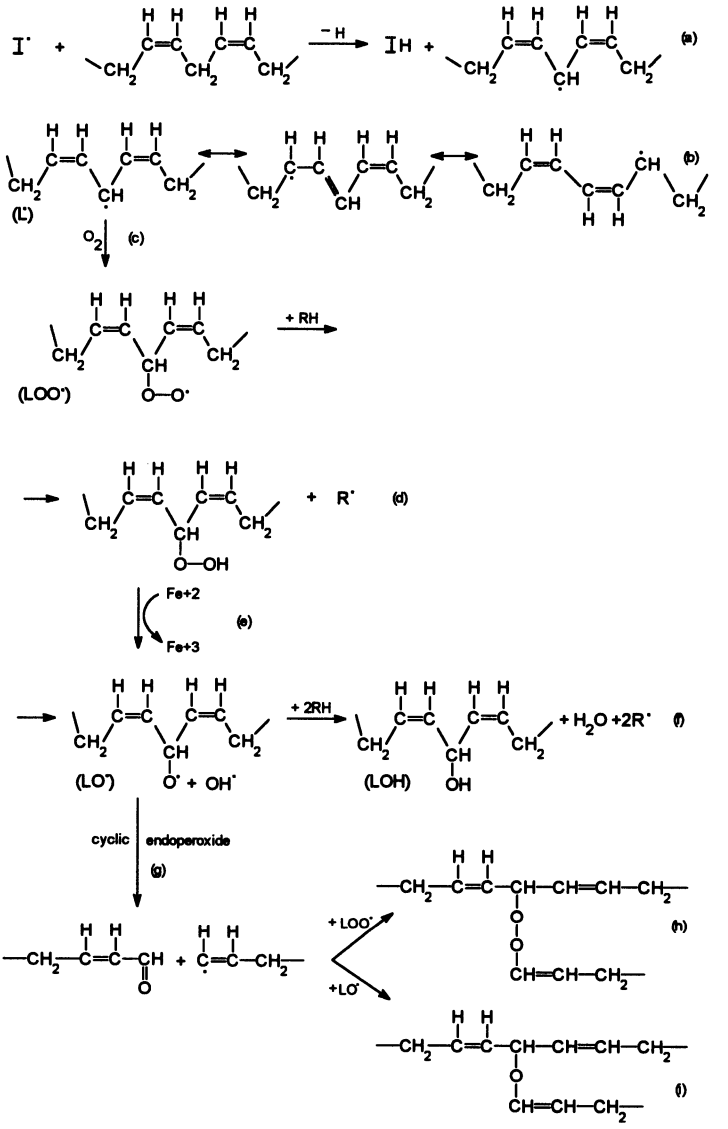
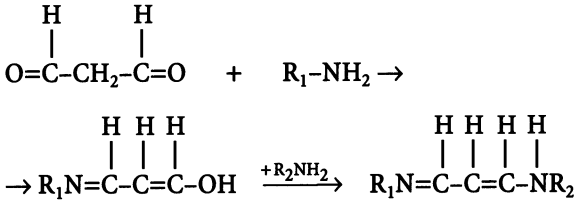


Fig. 12. Schematic sequence of lipid peroxidation

As is known, compounds containing a carbonyl group, e.g. aldehydes, react with the side-chain amino groups of proteins, free amino acids or nucleic acids either by intramolecular bridging or intermolecular cross-linking forming new compounds called Schiff bases:

$$R_1\text{-}\overset{H}{\underset{|}{C}}\text{=O} + R_2\text{-NH}_2 \rightarrow R_1\text{-}\overset{H}{\underset{|}{C}}\text{=N-}R_2 \qquad (194)$$

For example, malonaldehyde, known to be a very reactive and toxic compound, can react with two molecules containing -NH_2 groups forming aminoiminopropene Schiff base [438, 439]:



(195)

The latter compound shows a characteristic fluorescence with a maximum at 430 nm, when λ_{exc} = 360 nm [440]. This spectroscopic property gives another technique for monitoring the lipid peroxidation products generated even in vivo. For example, products of lipid oxidation have been found in plasma as well as in lung tissue extracts after intraveneous injection of cobra venon factor causing lung injury resulting from damage to their vascular endothelial cells [441].

Moreover, products of the lipid peroxidation showing fluorescent properties may be usuful as very sensitive markers for peroxidative damage to lipids induced by the active oxygen species, e.g. during ischemic injury of myocardium and bowel or acute respiratory distress syndrome [442, 443].

The next property of aldehydes worth mentioning is their ability to react with -SH groups on protein molecules, resulting in damage to proteins.

Lipids are naturally occurring substance in the tissues of animals. They include mainly fats and waxes, i.e. esters of glycerol and phospholipids. Almost all mammalian membranes contain phospholipids, many of which are unsaturated fatty acids.

Single phospholipids are all derivatives of glycerol, and they contain two acyl groups and a phosphodiester group on the third hydroxyl.

The probability of interaction of proteins with peroxidizing lipids has long been recognized. Biochemical changes in proteins exposed to lipid peroxidation have been observed to be similar to those induced by ionizing radiation, i.e. loss of enzyme activity, destruction of amino acids, polymerization, crosslinking or scission.

Apart from the above-mentioned malonaldehyde, all kinds of aldehydes can be formed during lipid peroxidation. Among them, 4-hydroxy-2,3-*trans*-nonenal, considered as "a toxicological second messenger" [407], is able to migrate rather far from the place of its creation, even to other cells.

Not all oxidative toxicity exerted by oxygen radicals can be ascribed to the fragmentation of lipids. The next very important consequence is the generation of a new propagatory agent, $^1O_2$ which might contribute to the chain reaction, causing more initiation, by the breakdown of the lipid hydroperoxides [379]:

$$LOOH + O_2^{\cdot-} \rightarrow LO^{\cdot} + {}^1O_2 + HO^- \quad (196)$$

$$2LOOH \rightarrow LHOH + LO^{\cdot} + {}^1O_2 \quad (197)$$

It has been reported that decomposition of hydroperoxides, alkoxy and peroxy radicals is strongly catalyzed by iron ions [436]. In muscle tissue the iron-containing compounds are mainly heme pigments, ferritin and transferrin. They act as catalysts in the presence of H_2O_2 (see for example reactions at Eqs. (34) and (38), or when the iron ions are released [444]:

$$LOOH + Me^{n+} \rightarrow LO^{\cdot} + Me^{(n+1)+} + HO^{-} \quad (198)$$

$$LOOH + Me^{(n+1)+} \rightarrow LOO^{\cdot} + Me^{n+} + H^{+} \quad (199)$$

The proof of involvement of this strong oxidant includes observation of the infrared emission (1268° nm) which is due to singlet oxygen and the quenching effect exerted by $^1O_2$-scavengers. Moreover, this catalytic effect of Fe-containing biologically important compounds has also been reported for cytochromes and methemoglobin. The decomposition of hydroperoxide lipids is also accelerated by copper ions ([426 and papers cited therein]). A compilation of biologically important iron complexes exerting effect on decomposition of lipid peroxides has been given by Halliwell and Gutteridge [437]. This paper also includes the effect of Fe-ions on fragmentation of some fatty acids and lipids in microsomal fractions and on compounds containing a carbonyl group. For example, the catalytic effect of iron chelators on the lipid peroxidation of raw turkey meat has been well documented [445].

It should be mentioned that a magnetic field exerts an effect on lipid peroxidation. The observations carried out during non-enzymatic peroxidation of lipids in the liposomes model system have shown that lipid peroxidation is accelerated even by a weak steady magnetic field [446]. This property of magnetic fields is important since magnetic fields are used therapeutically, for example in magnetic resonance imaging high-frequency hypothermia of tumours or magnetocontrolled transport of drugs [447].

However, unsaturated acids with the divinylmethane bond system

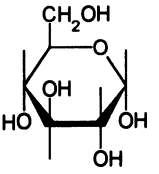
$$-CH=CHCH_2-CH=CH-$$

for which the mechanism of oxidative degradation has been discussed above, are not synthesized by the human organism, but they must be supplied to the organism with food, usually as vitamins. They are constituents of cell wall lipids and are responsible for a semipermeability of the walls (e.g. regulation of the Ca^{2+} influx).

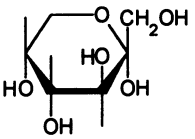
5.1.3 Carbohydrate Degradation

Carbohydrates are the simple sugars (monosaccharides) widely distributed in living nature, e.g. grape sugar-glucose, fruit sugar-fructose, mannose, ribose.

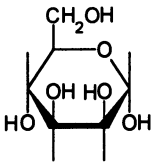
Plants contain polysaccharides – high-molecular-weight polymers built up by repeated condensations of simple sugars with releasing H_2O. For example, a disaccharide molecule contains two monosaccharide residues united by a glycosidic linkage (with elimination of a water molecule).



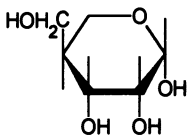
Glucose



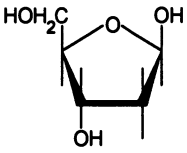
Fructose



Mannose

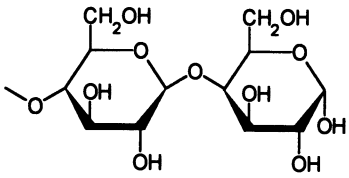


Ribose



Deoxyribose

The properties of polysaccharides depend on the nature of the sugar unit, the type of linkage and the length and degree of branching of the chain. Polysaccharides containing a few monosaccharide units are called oligosaccharides. Usually they are the anhydrite forms of two to four molecules of the basic structural units. Polysaccharides may be divided into two categories: straight-chain polysaccharides (e.g. cellulose) and branch-chain polymers (e.g. starch). Cellulose, glycogen are examples of homopolysaccharides containing the same carbohydrate units.



Glycogen

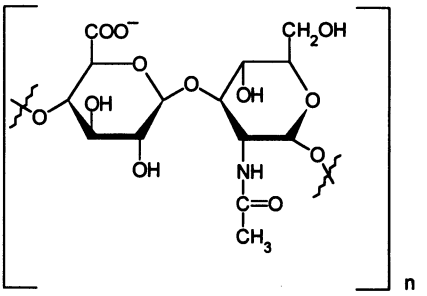
Starch and glycogen play important roles in biological processes. Starch is the principal food reserve carbohydrate in plants, whereas glycogen is the animal starch present in liver tissue (it may contain up to 20% by weight of glycogen). Cellulose is the chief constituent of the cell walls of all plants.

Of interest are heteropolysaccharides (they contain two or more carbohydrate units), sugars which contain amino sugars such as glucosamine, galactosamine. They are present in blood, nervous tissue and connective tissue.

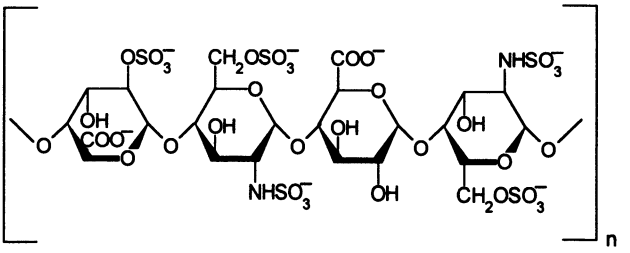
A very important group of sugars is glycosaminoglycans, known as the mucopolysaccharides. They are linear polymers of repeated disaccharides, usually hexosamines. Hyaluronic acid and heparin are the most important among the glycosoaminoglycans. Hyaluronic acid contain 400–25000 repeating units and is the basis of joint gel.

Heparin is a powerful inhibitor of blood clotting in the human body and also participates in concentrating the signal substances regulating cell growth.

Oxidative destruction of polysaccharides may result in their depolymerization, and in hyaluronic acid and collagen degradation [448]. Hyaluronic acid, a glycosoaminoglycan is an integral structural component of the intercellular milieu as well as of vitreous and synovial fluids. The degradation of this acid has important implications in the function of the interstitial space, and in the alteration of the chemical properties of synovial and vitreous fluids [448 and references therein].



Units of hyaluronic acid



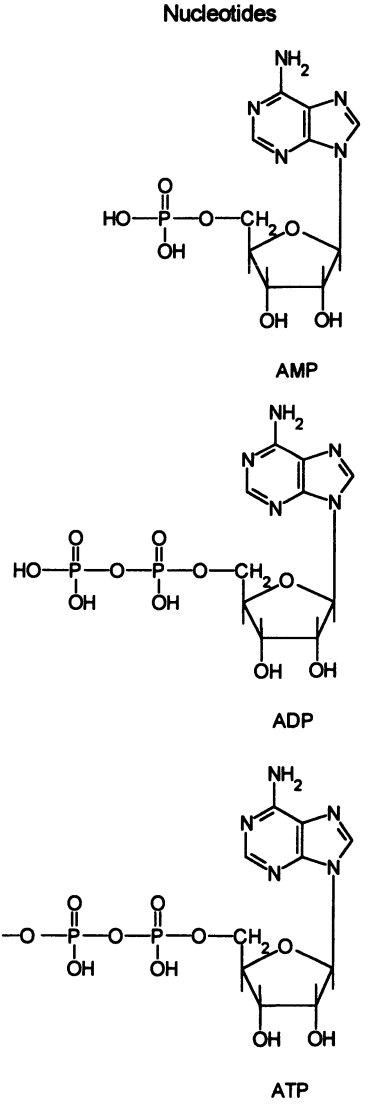
Units of heparin

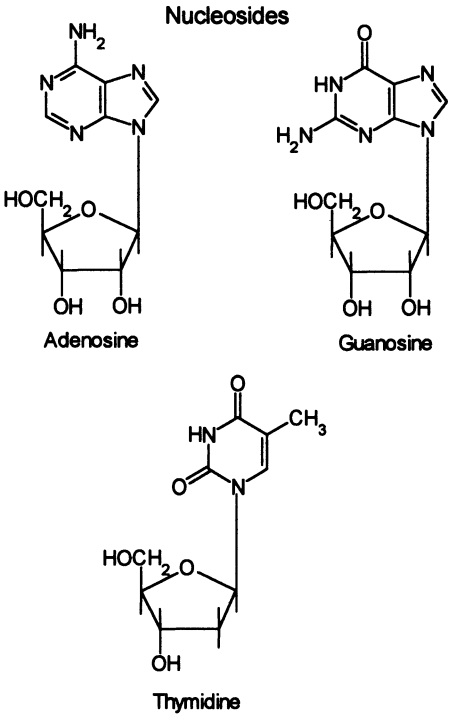
It has been suggested that the hydroxyl radical is a main radical species responsible for hyaluronic acid degradation. They cause breaking the glycosidic (acetal) linkages between monomers, resulting in decrease of solution viscosity.

5.1.4
Nucleic Acid Damage

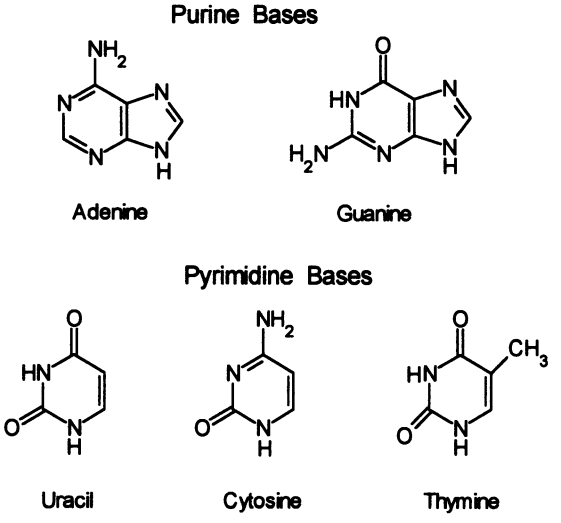
Nucleic acids are high molecular weight polymers (polynucleotides) of smaller units called nucleotides. Nucleic acids occur in the nucleus of each cell and in the plasma, being rather weakly bound to protein. Nucleotides are the phosphoric esters (phosphates) of nucleosides.

Nucleotides as the structural units of nucleic acid are joined through the ester linkage of the phosphoric acid residue to the hydroxyl group of the sugar molecule of the next nucleotide. Examples are adenosine monophosphate (AMP), adenosine diphosphate (ADP) and adenosine triphosphate (ATP).



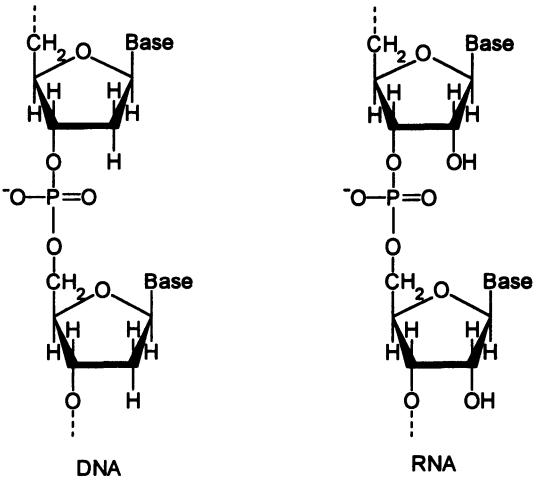


Successively, the nucleosides are *N*-glycosides pyrimidine and purine heterocyclic bases with pentoses, ribose and deoxyribose. The C–N bond between the sugar and the base involves the carbon atom (C-1) on the part of the sugar. Two purine bases – adenine and guanine and three pyrimidine bases – uracil, thymine and cytosine occur mainly in nucleosides.



The nucleic acids of the cell nucleus contain deoxyribonucleic acid (DNA), whereas the nucleic acids of the cell plasma contain ribonucleic acid (RNA). Both nucleic acids are polymers containing one mole of sugar and one phosphoric acid residue per one mole of the heterocyclic base. RNA contains phosphoric acid, D-ribose, adenine, guanine, cytosine and uracil; DNA contains phosphoric acid, D-2-deoxyribose and mixture of two purine bases – adenine and guanine and two pyrimidine bases – thymine and cytosine.

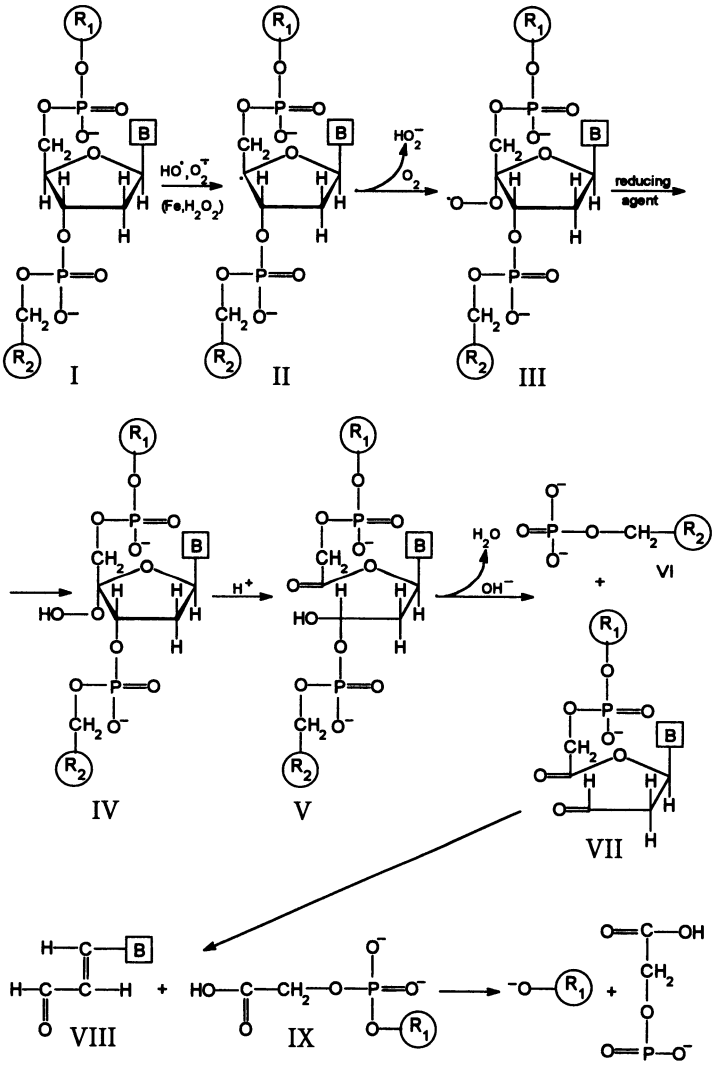
Information on the cell damage caused by oxygen active species has been compiled by the observation of their direct and indirect effects and the study of oxidative stresses that have been exerted by the relevant species. Among the cell constituents, DNA, although deeply embedded inside the cells, is susceptible to the oxygen radicals attack as well as to $^1O_2$. Oxygen radicals can attack the DNA components (sugar, phosphate backbone or bases), causing several modifications. The base loss or its damage, sugar fragmentation and a strand break with terminal fragments of sugar residue have been well documented [449–452]. The main role in DNA cleavage has been described for the HO radical [453].



The cytotoxicity of several antineoplastic antibiotics in clinical use against cancer cells, such as bleomycin, adriamycin or neocarcinostatin have been found to be related to their ability to induce intracellular DNA strand breaks (see for example [454] and recent reviews [455, 456]). Due to the effort of numerous research groups, it is now well established that DNA degradation, for example that catalyzed by bleomycin, proceeds along two pathways; in one of these, an oxygen-dependent, partially reduced oxygen species generated by bleomycin is responsible for the damage [457]. The studies carried out in vitro have shown that degradation of DNA by bleomycin occurs in the presence of Fe^{2+} ions and molecular oxygen and is enhanced in the presence of H_2O_2 and reducing agents such as thiols or ascorbate [452, 457–459].

The mechanism of DNA degradation by drugs has been the subject of extensive studies, (see for example [45, 460, 461]). Giloni et al. [460] proposed a hypo-

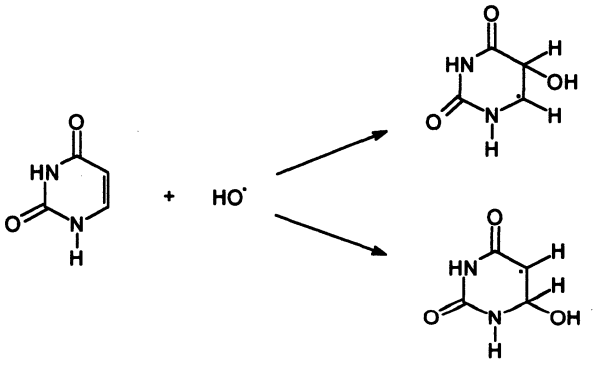
thetical mechanism of DNA cleavage by the bleomycin + Fe(II) + O_2 system (Scheme 2). It seems likely that the mechanism presented in the scheme may be obligatory in the case of other compounds generating active oxygen species ($HO^{\cdot}$, $O_2^{\cdot-}$, $^1O_2$). The generation of the above species during bleomycin treatment by the Fe(II) + O_2 or Fe(III) + H_2O_2 system has been well documented [120, 462–467]. The mechanism shown in Scheme 2 suggests abstraction of a hydrogen atom from the C(4′) position of deoxyribose moiety by an oxygen radical followed by the formation of radical II. The detailed mechanism of radical II formation is given in [468]. The newly formed radical reacts with molecular oxygen to give the peroxy radical, III.



Scheme 2. Hypothetical mechanism of DNA cleavage by bleomycin – Fe(II) – O_2; B means the heterocyclic base

The next stages are the formation of the hydroperoxy compound IV (in the presence of reducing agent) and its decomposition by cleavage of the C(3′)–C(4′) bond to hemiaminacetal, V, with elimination of water. The base-catalyzed hydrolysis of the phosphodiester bonds of compound V gives free phosphate ester, VI, and compound VII. The latter compound is further decomposed to afford the base-propenal, VIII, and the phosphate ester of glycolic acid, IX. After alkali treatment a free base release is possible [462].

All main bases, both pyrimidine and purine, may be damaged by oxygen species. Recent studies by Catterall et al. [468] dealing with the reaction of HO· with main pyrimidine nucleobases (uracil, thymine and cytosine), nucleosides and nucleotides, polynucleotides and RNA[1], carried out using the spin-trapping technique, have confirmed that HO· radicals attack the C(5)–C(6) double bond in the pyrimidine ring. The attack occurs at the rate constant about $5 \cdot 10^9\ \ell \cdot mol^{-1} \cdot s^{-1}$ mainly at the C(5) and to a lesser extent at the C(6) position [429]. For example, uracil reacts with HO· radical to form two adducts:



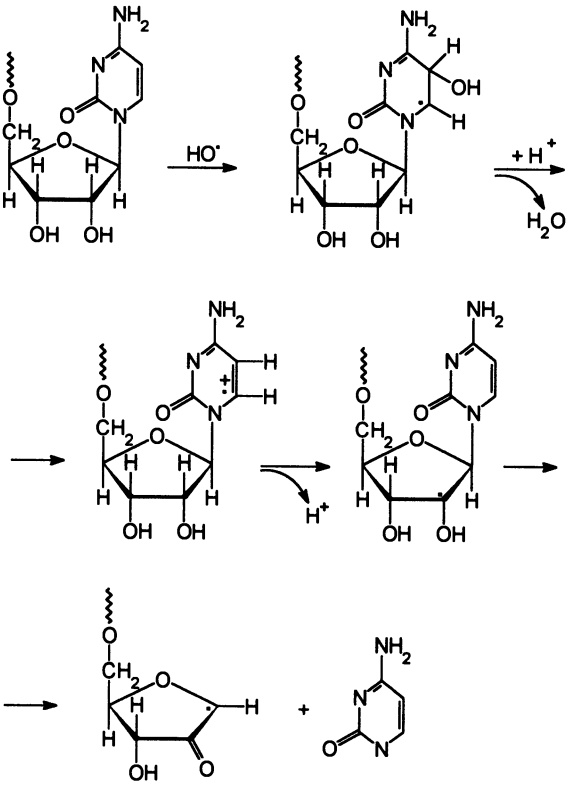
(200)

Such initial damage to the nucleobases can be transferred to neighbouring sugar molecule [468] following release of the base (Scheme 3). Thymine and cytosine as well as their derivatives show similar behaviour like uracil when reacting with HO· [469 and references cited therein]. It has been reported [452] that the thymine damage during DNA treatment with H_2O_2 results in formation of four of its derivatives: thymine glycol, methyltartronylurea, urea and 5-hydroxymethyluracil.

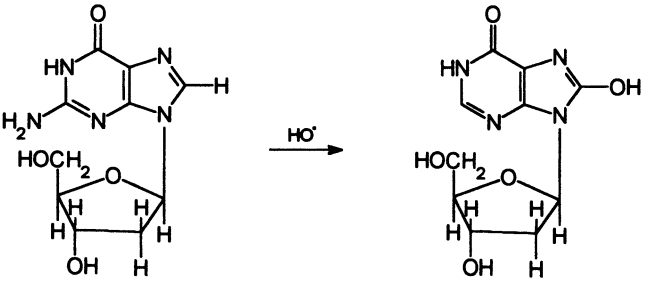
The reactions of HO· with purine bases adenine, guanine and their derivatives have been intensively studied during the past decades but the exact mechanism of these complicated processes is not yet well known (see [469, 470]).

It has been proved that guanine in the DNA molecule undergoes hydroxylation when treated with oxygen radicals under physiological conditions in vitro [471]. The hydroxylation of deoxyguanosine by the hydroxyl radical gives 8-hydroxydeoxyguanosine (reaction at Eq. (201)).

[1] Ribonucleic acids.



Scheme 3. Release of the base from cytidine

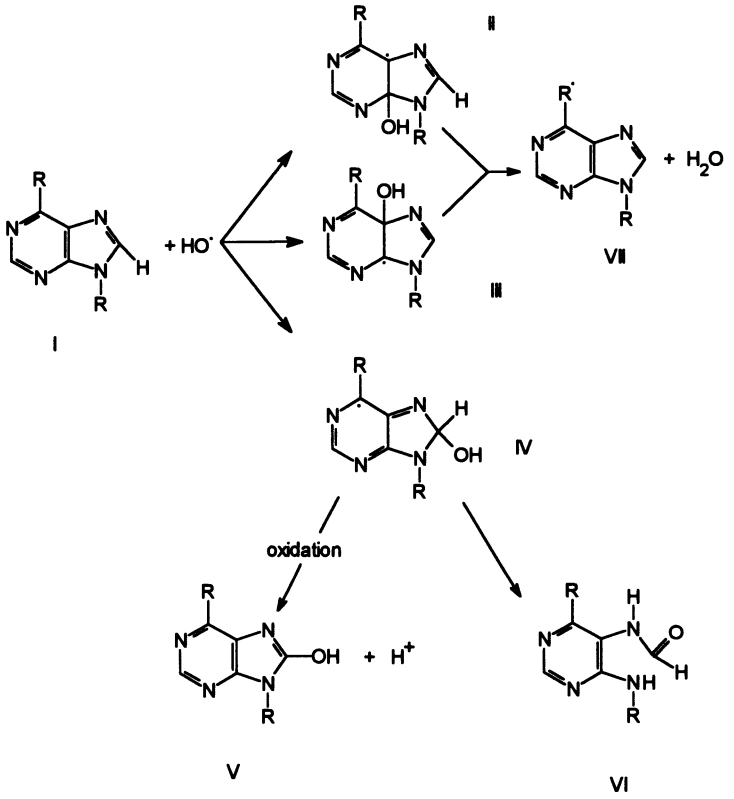


(201)

The 8-hydroxy-2′-deoxyguanosine formation is an indicator of in vivo DNA damage in mammals [472, 473] and may be regarded as evidence of the involvement of the HO radical in DNA degradation.

For 6-substituted purine bases and purine-9-riboxides, three intermediates of their reactions with HO· have been distinguished: C(4), C(5) and C(8) sites adducts [474, 475], radicals II, III and IV, respectively (Scheme 4).

The purine-8-OH radical shows strongly reducing properties and can undergo oxidation, giving product V, or spontaneous imidazole ring-opening reaction



Scheme 4. Degradation of purine derivatives by hydroxyl radicals

following reduction and protonation with a formamidopyrimidine derivative compound of type VI being formed. The purine-4-OH and purine-5-OH radicals undergo a dehydration process to form radical products easily removed by antioxidants. Thus the purine-8-OH radical is considered to be more toxic than these two remaining radicals.

The toxicity of $HO^{\cdot}$ towards DNA has been used in an experimental approach providing information on how protein binds to the DNA molecule to make footprints of protein-DNA complexes [476].

However the major damage to biological systems, for example by radiation, is started by $HO^{\cdot}$ as a product of the radiolysis of water present in living tissues. Other oxidizing species ($^1O_2$, $ROO^{\cdot}$, H_2O_2, O, O_3, cosmic rays) can also initiate reactions leading to similar damage.

5.2 Photodynamic Effect

The photodynamic effect may be defined as the damaging effect of the combination of sensitizing compounds (exogenenous or endogenous), light, and

oxygen exerted on all classes of organisms. A great variety of compounds showing sensitizing properties have been studied [477–480].

Photosensitized oxidation usually involves light absorption by the sensitizer molecule in the ground state ($^1S_0$) and its electronic excitation to the singlet state ($^1S_1$). The excited singlet state is short-lived (~ 10^{-8} s) and the sensitizer molecule undergoes an internal conversion process to give the long-lived (~ 10^{-1} to 10^{-4} s) triplet state ($^3S_1$). Two major classes of photo-oxidation reactions sensitized by organic compound exist. They are classified as Type I and Type II mechanisms [478, 481].

In the Type I mechanism, sensitizer in the excited state reacts with the substrate RH directly, by hydrogen abstraction or electron transfer:

$$^3S_1 + RH \rightarrow \dot{S}H + R^{\cdot} \tag{202}$$

The substrate radical ($R^{\cdot}$) produced undergoes a radical-chain autoxidation to give oxidized substrate – hydroperoxide (ROOH), peroxide (ROOR) or a radical dimer (R–R) [482]. The Type II mechanism (Fig. 13) involves $^1O_2$ formation in $^1\Delta_g$ and $^1\Sigma_g^-$ states as the result of energy transfer from the excited triplet or singlet level of the sensitizer to the molecular oxygen:

$$^3S_1 + O_2[^3\Sigma_g^-] \rightarrow {}^1S_o + {}^1O_2[^1\Delta_g \text{ or } {}^1\Sigma_g^+] \tag{203}$$

$$^1S_1 + O_2[^3\Sigma_g^-] \rightarrow {}^1S_o + {}^1O_2[^1\Delta_g \text{ or } {}^1\Sigma_g^+] \tag{204}$$

This reaction is very fast for almost all sensitizers ($k \sim 1-3 \cdot 10^9\ \ell \cdot mol^{-1} \cdot s^{-1}$) [479]. The $^1\Sigma_g^+$ state is much shorter-lived than the $^1\Delta_g$ state and is belived to undergo internal conversion to the $^1\Delta_g$ state. The electron transfer from the excited sensitizer ($^3S_1$) to the oxygen molecule can also occur, generating the O_2^- ion and an oxidized form of the sensitizer, but with less efficiency ($k \approx 10^7\ \ell \cdot mol^{-1} \cdot s^{-1}$):

$$^3S_1 + O_2[^3\Sigma_g^-] \rightarrow S^+ + O_2^- \tag{205}$$

The reactive oxygen states ($^1\Delta_g$, $^1\Sigma_g^+$) either show a decay, regenerating oxygen in the ground state ($^3\Sigma_g^-$), or react with acceptor (A) giving peroxide (AO_2). As reported in Chap. 3, three main kinds of substrates (A) are subject to this process: compounds containing an isolated double bond with a β-hydrogen atom; cyclic conjugated hexadienes; polycyclic aromatic hydrocarbons with condensed rings, e.g. anthracene or heterocyclic compounds such as furans. The quantum yield of the AO_2 peroxide formation strongly depends on the kind of sensitizer and the medium of $^1O_2$ formation with regard to its lifetime.

Photosensitized oxidation occurring by the Type II mechanism is enhanced in D_2O and other solvents with prolonged $^1O_2$-lifetime and inhibited by addition of $^1O_2$-quenchers, allowing one to distinguish the photoreaction types.

The competition between Type I and Type II reactions depends on the natue of the sensitizer and oxidizable substrate, pH, concentration of molecular oxygen and state of the medium in which the photoxidation is carried out (liquid or solid) [483].

A compound possesses the property of being an effective sensitizer if it forms a long-lived triplet state with a high quantum yield. Many pigments, such as

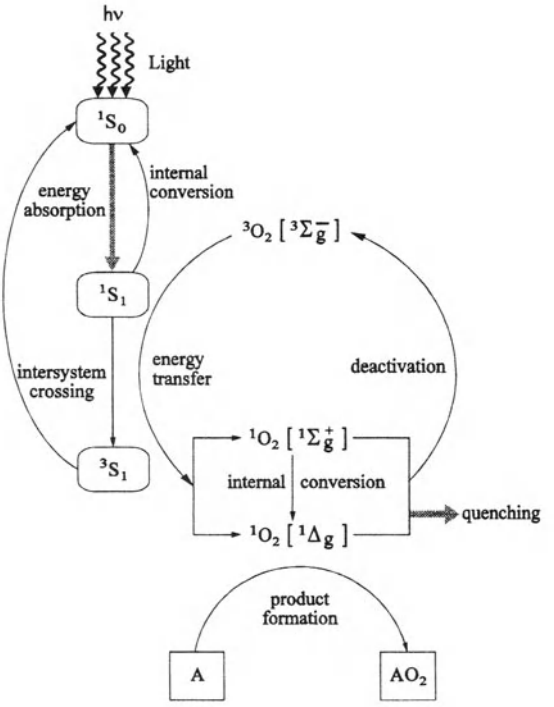


Fig. 13. Schematic illustration of the photodynamic effect

xanthene dyes (eosine, fluorescein, rose bengal); thiazine dyes (thionine, methylene blue); the chromophores of the photosystem II reaction centres e.g. P680, chlorophyll, flavins, hematoporphyrin; the polycyclic aromatic hydrocarbons with condensed rings, e.g. anthracenes; benzophenone; rubrene, heterocyclic compounds such as furans or some drugs e.g. tetracyclines and even nucleic bases, show photosenitizing properties (see for example [477, 484–487]).

For a substance to be a sensitizer, the energy difference between the excited triplet and singlet ground states must reach at least 94.3 kJ · mol^{-1}, i.e. at least the energy difference between the O_2 ($^3\Sigma_g^-$) and O_2 ($^1\Delta_g$) states. Figure 14 shows values of triplet energy of some sensitizers and oxygen [477]. As can be seen from Fig. 14, the triplet energies of sensitizers vary over a wide range of values (142–287 kJ · mol^{-1}). During absorption of light, the aromatic hydrocarbons and the dyes give (π, π^*) triplets, whereas the compounds containing the carbonyl group usually generate (n, π^*) triplets.

Singlet oxygen in the $^1\Delta_g$ state having the excitation energy of 94.3 kJ · mol^{-1} may be generated by all sensitizers for which the triplet energy is higher than this value, whereas the $^1\Sigma_g^+$ state may be produced only by the sensitizers having triplet energy above 157.1 kJ · mol^{-1}. All sensitizers shown in Fig. 14 fulfil this condition, except the methylene blue which can produce only $^1\Delta_g$ state.

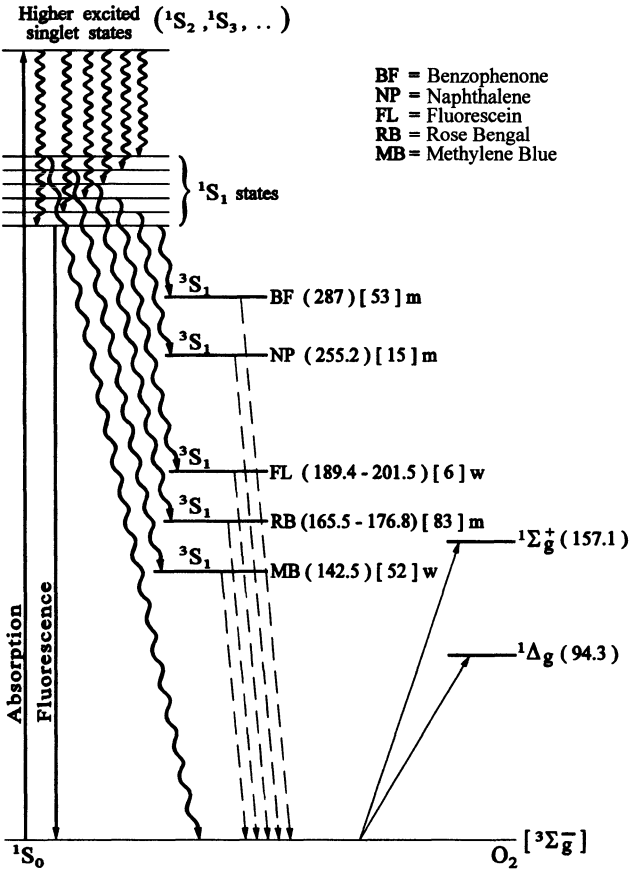
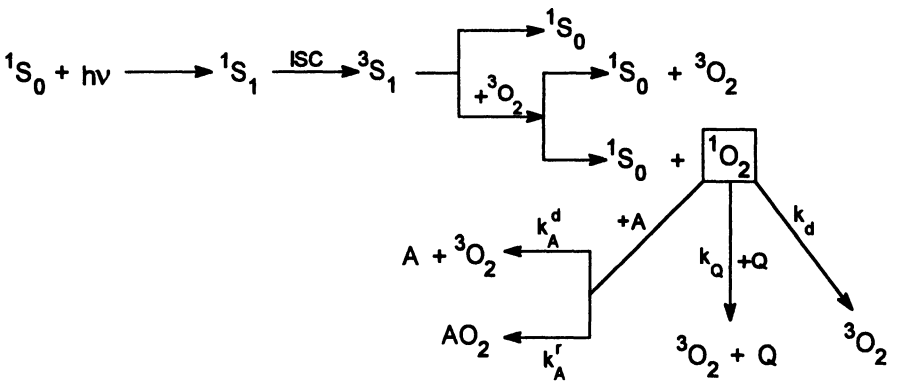


Fig. 14. Diagram of energy transfer from the excited singlet ($^1S_1$) and triplet ($^3S_1$) states of a sensitizer to molecular oxygen observed in methanol (m) or water (w). *Numbers in round brackets* mean the excitation energy of a sensitizer molecule in kJ · mol^{-1}

5.2.1 Kinetic Characterization

Determination of the quantum yield of $^1O_2$ formation (ϕ^1O_2) is very important in consideration of its strong oxidative properties as an oxidant in vitro and in vivo. Since the generation of $^1O_2$ during photosensitized oxidation of the substrate is a rather complicated process [488], the simplest general scheme will be analyzed. In order to define ϕ^1O_2 let us consider oxidation of compound A (acceptor) in the presence of sensitizer (S) in aerated solution. Because the quantum yield of the peroxide AO_2 formation increases with its concentration and is independent of absorbed light intensity, the scheme which leads to the photostationary expression for the overall quantum yield of AO_2 formation is as follows (Scheme 5) [488]:



Scheme 5. Schematic presentation of the photostationary expression for determination of the quantum yield of the substrate (A) oxidation

It is seen from Scheme 5 that after excitation by light absorption, the dye is converted to the triplet state by intersystem crossing (ISC). In this state the dye reacts with oxygen $^3O_2$ forming $^1O_2$ or undergoes collisional deactivation to the ground state ^1S_o. This reaction, like the reactions of $^1O_2$ with substrate A, leads to different processes in competition, depending upon the conditions, such as formation of AO_2 or $^1O_2$ deactivation, spontaneous or caused by presence of the $^1O_2$ quencher (Q).

Scheme 5 does not cover all possible processes, e.g. fluorescence or phosphorescence ($^1S_1 \rightarrow {}^1S_o$, $^3S_1 \rightarrow {}^1S_o$ transitions, respectively) or the reaction of the excited dye with substrates other than A.

The quantum yield of substrate oxidation ϕ is given by

$$\phi = \phi\,{}^1O_2 \frac{k_A^r[A]}{k_d + k_A^r[A]} = \phi\,{}^1O_2 \frac{[A]}{\frac{k_d}{k_A^r} + [A]} = \phi\,{}^1O_2 \frac{[A]}{\beta + [A]} \qquad (206)$$

where ϕ^1O_2 is the quantum yield of $^1O_2$ formation;

k_d is the rate of $^1O_2$ decay ($k_d = \tau^{-1}_{^1O_2}$ for the appropiate solvent);
k_A^r is the rate constant for reaction of substrate A with $^1O_2$ [488].

The constant β, defined as $k_d/k_A^r = 1/k_A^r \cdot \tau$, is the relative reactivity index of the acceptor (A). Its value represents the concentration of the acceptor at which half the reactive species generate product AO_2. If the concentration of the acceptor is high, then

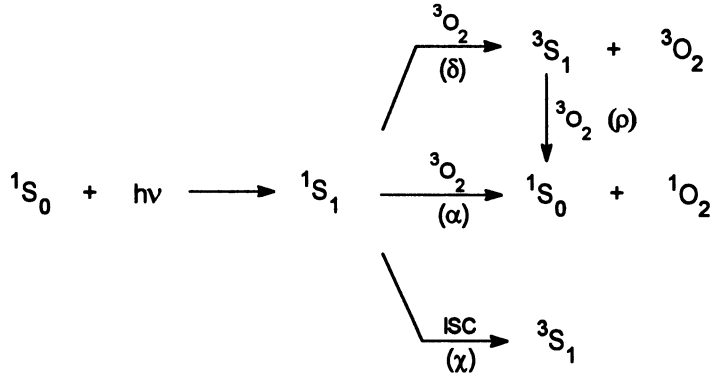
$$k_A^r \cdot [A] \gg k_d$$

and the limiting quantum yield of the acceptor oxidation is the quantum yield of the triplet state of the sensitizer formation $\phi\,{}^3S_1$

$$\phi' = \phi\,{}^3S_1 \frac{k_A^r[A]}{k_d + k_A^r[A]}, \frac{1}{\phi'} = \frac{1}{\phi\,{}^3S_1}\left(1 + \frac{\beta}{[A]}\right) \tag{207}$$

A plot of $1/\phi$ or $1/\phi'$ vs $1/[A]$ should be a straight line and may be used for the β value determination. If the β value is independently determined, the latter equation may be used for $\phi\,{}^3S_1$ determination. Similarly, knowledge of the β value leads to determination of the quantum yield of $^1O_2$ formation.

Let us analyse factors having an influence on the quantum yield of $^1O_2$ formation. If we assume that the triplet state of the sensitizer is completely quenched, and that the quenching of the $^1S_1$ state by oxygen is also significant, the following kinetic scheme in which oxygen quenching of the dye singlet and triplet states may be given (Scheme 6) [488]:



Scheme 6. Quenching of the singlet and triplet states of a dye by molecular oxygen

The overall quantum yield of $^1O_2$ formation is described by

$$\phi\,{}^1O_2 = \alpha\, p(O_2) + \gamma \cdot \rho \tag{208}$$

where

- α is the fraction of the quenching which leads to $^1O_2$ ($^1\Delta_g$) formation;
- $p(O_2)$ is the quenching probability of the $^1S_1$ state by oxygen in the ground state. For sensitizers showing fluorescence this value is easily found, by the detection of fluorescence decrease since the emission is strongly quenched by $^3O_2$;
- ρ is the probability of $^1O_2$ generation during interaction of $^3S_1$ with oxygen molecule;
- γ is the overall quantum yield of the $^3S_1$ formation.

The γ parameter is the sum of the spontaneous intersystem crossing yield (χ) and yield of the oxygen induced ($^1S_1 \rightarrow {}^3S_1$) transition ($\xi$):

$$\gamma = \chi + \xi$$

where

$$\chi = \phi_{ISC}[1 - p(O_2)]$$
$$\xi = \delta\, p(O_2)$$

and ϕ_{ISC} is the quantum yield of the intersystem crossing process;
δ means the fraction of the quenching which leads to the $^3S_1$ generation.

Thus $\gamma = \phi_{ISC}[1 - p(O_2)] + \delta\, p(O_2)$.

Substituting the latter expression for "γ" in Eq. (208) we obtain

$$\phi\, {}^1O_2 = [\alpha + \rho(\delta - \phi_{ISC})]p(O_2) + \rho\, \phi_{ISC} \tag{209}$$

When the excited singlet state of sensitizer ($^1S_1$) is effectively quenched by oxygen, i.e. $p(O_2) = 1$, ϕ^1O_2 may be approximately extrapolated to unity. Then $\alpha + \delta = 1$ and $\rho = 1$. This means that $^1O_2$ is generated by interaction of oxygen in the state $^3\Sigma_g^-$ and the sensitizer molecule in the $^3S_1$ state.

The ϕ^1O_2 value may be obtained from Eq. (206). If deactivation of $^1O_2$ by substrate A is negligible (k_A^r and k_A^d small), extrapolation of Φ (Eq. (206)) to the initial concentration of the acceptor (A) gives values of ϕ^1O_2.

If deactivation of $^1O_2$ by acceptor (A) is taken into account [488], then Eq. (206) becomes

$$\phi = \phi^1O_2 \frac{k_A^r[A]}{k_d + (k_A^r + k_A^d)[A]} = \phi^1O_2 \frac{[A]}{\frac{k_d}{k_A^r} + \frac{k_A^r + k_A^d}{k_A^r}[A]} \tag{210}$$

In this case the reactivity index $\beta = \dfrac{k_d}{k_A^r + k_A^d} = \dfrac{k_d}{k_A}$, where $k_A = k_A^r + k_A^d$.

The rate constand k_d may also be easily determined from Eq. (206) using an effective $^1O_2$ quencher, having the quenching rate (k_Q) equal to the diffusion controlled rate constant ($\sim 10^{10}\ \ell \cdot mol^{-1} \cdot s^{-1}$) [489]. As can be seen from Scheme 5, in the presence of quencher Q, Eq. (206) takes the following form:

$$\phi = \phi\, {}^1O_2 \frac{k_A^r[A]}{k_d + k_Q[Q] + k_A^r[A]} \tag{211}$$

or

$$\frac{1}{\phi} = \frac{1}{\phi\, {}^1O_2}\left(1 + \left[\frac{k_Q[Q]}{k_A^r} + \frac{k_d}{k_A^r}\right]\frac{1}{[A]}\right). \tag{212}$$

The plot of $1/\phi$ vs $1/[A]$ at given concentration of the quencher [Q] gives a straight line. Since the inclination of the line is the linear function of the quencher concentration, and the value of k_Q is assumed to be known, the value of k_d may be obtained.

The rate constants k_d and k_A^r are often determined by monitoring the decrease of 1,3-diphenylisobenzofuran (DPBF), a compound known as a good $^1O_2$ quencher, by detecting the decrease of its absorption band with maximum at 415 nm or fluorescence intensity at 458 nm (λ_{exc} = 405 nm) due to $^1O_2$. The technique has been proposed by Young et al. [490] and is also useful for determining the β-value and rate constant k_A of $^1O_2$ quenching by another compound competing with the oxidation of DPBF by $^1O_2$.

We have applied this technique to the determination of β values of catecholamines (CAT) during rose bengal sensitized photooxidation of DPBF [256].

The photooxidation of DPBF by a $^1O_2$ mechanism in the presence of CAT occurs according to Scheme 7.

The kinetic equation of this process may be written

$$-\frac{d[DPBF]}{dt} = K\left(\frac{k_Q[DPBF]}{k_Q[DPBF] + k_A[CAT] + k_d}\right) \tag{213}$$

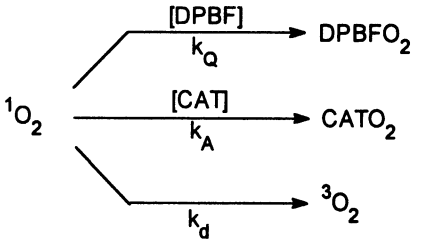
where K is the rate of formation of $^1O_2$, and is dependant upon light intensity, dye concentration, etc.; k_d is the rate of decay of $^1O_2$ to the ground state; and k_A, k_Q are the rate constants for reactions of CAT and DBPF with $^1O_2$, respectively. At very low concentration of DPBF (about 10^{-6} mol · ℓ^{-1}), using a fluorescence technique, a first-order disappearance of DPBF is observed:

$$-\frac{d[DPBF]}{dt} = K\left(\frac{k_Q[DPBF]}{k_A[CAT] + k_d}\right) \tag{214}$$

where the slope of the first order plot

$$\frac{d[DPBF]}{dt} \text{ vs } [DPBF] \tag{215}$$

$$S = K\frac{k_Q}{k_A[CAT] + k_d} \tag{216}$$



Scheme 7. Photoxidation of 1,3-diphenylisobenzofuran (DPBF) in the presence of catecholamines (CAT)

and

$$S_o = K\frac{k_Q}{k_d} \tag{217}$$

in the absence of CAT.

The ratio of the slope without and with the catecholamine is given by:

$$\frac{S_o}{S} = 1 + \frac{k_A}{k_d}[CAT] = \frac{I_o}{I} \tag{218}$$

where I_o, I are the fluorescence intensity of DBPF in the absence and presence of CAT, respectively. A plot of I_o/I against [CAT] should yield a straight line with a slope $\frac{k_A}{k_d} = \frac{1}{\beta}$.

Table 6 presents rate constant (k_A) and reactivity indexes (β) for catecholamines and other chosen compounds. The data indicate that for effective $^1O_2$ quenchers we obtain very small β-values, such as for β-carotene, which means that this compound reacts with $^1O_2$ with almost a diffusional controlled rate constant.

The values of quantum yield of singlet oxygen formation have been determined for several sensitizers including those biologically important (see for instance [477, 486, 487]). For example, the quantum yields of $^1O_2$ formation determined during photosensitized oxidation of 2,5-dimethylfuran in methanol solution at 293 K using rose bengal, benzaldehyde and fluorenone as sensitizers, have been reported to be 83, 64 and 7 % respectively [477]. Rose bengal, methylene blue and hematoporphyrins are very good sensitizers, whereas the dyes having a high triplet energy such as benzophenone or flavins show rather high ability to undergo the Type I processes.

Later studies [486] have shown that the triplet excited states of adenine and the pyridine bases (uracil, thymine and cytosine) also produce $^1O_2$ via the Type II photodynamic mechanism. The quantum yield of singlet oxygen formation found for these bases are 3, 13, 1 and 2 %, respectively.

All DNA bases, nucleotides and dinucleosides have high-energetic triplets, usually higher than 300 kJ · mol^{-1} [487]. This means that they should be capable

Table 6. Rate constants k_A and reactivity indexes β for chosen compounds in methanol

Acceptor	$(k_A^r + k_A^d)$ [$\ell \cdot mol^{-1} \cdot s^{-1}$]	β [$mol \cdot \ell^{-1}$]	Reference
Sodium azide	$2.2 \cdot 10^8$	$4.1 \cdot 10^{-5}$	[489]
1,3-Diphenylisobenzofuran	$1.4 \cdot 10^9$	$6.7 \cdot 10^{-6}$	[489]
β-Carotene	$1.5 \cdot 10^{10}$	$6.1 \cdot 10^{-7}$	[490]
Catecholamines	$(1.0-2.3) \cdot 10^6$	$(4.0-9.2) \cdot 10^{-2}$	[256]
Histidine	$5.0 \cdot 10^7$	$3.0 \cdot 10^{-3}$	[317]
Methionine	$3.0 \cdot 10^7$	$5.0 \cdot 10^{-5}$	[317]

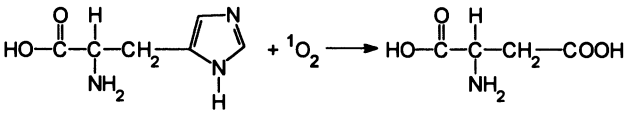
of producing $^1O_2$ during photosensitization, but the observed effect is rather poor. The yields of $^1O_2$ generation observed for the guanine base and guanine-containing residues (e.g. DNA, thymidyl (3′→5′)-2′-deoxyguanosine) were almost undetectable. These observations may result from a high susceptibility of the guanine base and its derivatives on oxidation by $^1O_2$, in contrast to other bases being at least two orders of magnitude less susceptible [491, 492]. Although the Type II photosensitized oxidation occurs mainly in solution, it can also occur in a gas phase using naphthalene as the sensitizer, in a gas-solid phase when both sensitizer and substrate are adsorbed, e.g. on silica gel [493], and in a solid-liquid phase when the sensitizer is on polymers, e.g. polystyrene [494] or an ion-exchange resin [495].

5.2.2
Biochemical Implications of the Photodynamic Effect

Although much progress in research work in this field has already been made, the complexity of photosensitized reactions and the number of intermediates make the identification of these intermediates very difficult and only the end degradation products have been isolated and characterized. In view of the potentiality for photodynamic damage by photooxidation of certain amino acids, peptides, phenols, nucleotides, indoles and lipids, these compounds have been of great interest to photochemists and photobiologists, and will be discussed in turn.

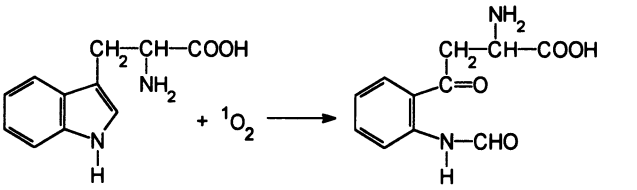
5.2.2.1
Amino Acids and Their Derivatives

Amino acids such as methionine, histidine, cysteine, tryptophan and their derivatives are susceptible to the $^1O_2$ attack, and therefore they are affected during the sensitized photooxidation (see for example [496]). For example, methionine undergoes oxidation to the sulfoxide (reaction at Eq. (148)), whereas cysteine is oxidized to cysteic acid [497]. During the photooxidation of histidine, $^1O_2$ mechanism cleavage of the imidazole ring is observed [479]:



(219)

whereas the photooxidation of tryptophan occurs by cleavage of the enamine double bond and mixtures of different products, among which *N*-formyl-kynurenine, has been reported [498]:



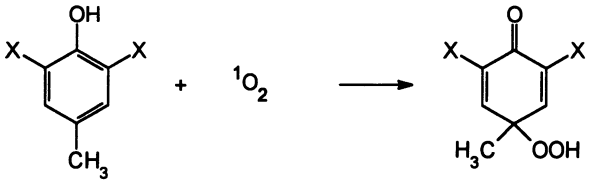
(220)

N-formylkynurenine

In the case of tyrosine, the attack of $^1O_2$ has been reported to occur at the phenolic ring site [499]. Amino acids present in peptides and proteins undergo a similar reaction as those given above.

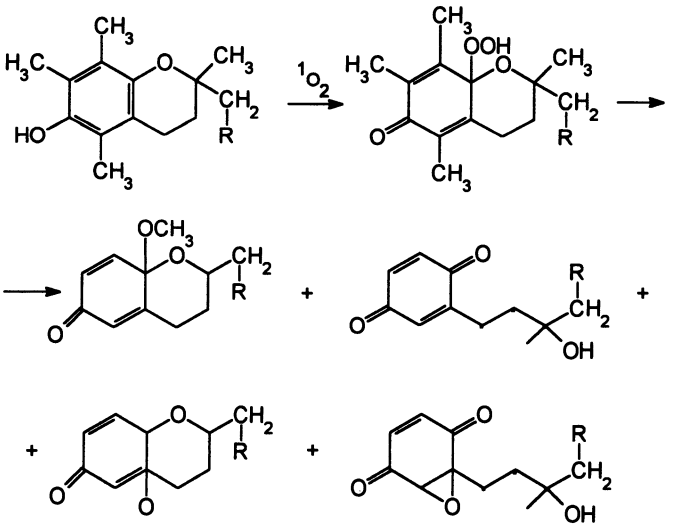
5.2.2.2 *Phenols*

Phenols are also susceptible to photooxidation by singlet oxygen. For example, photooxidation of phenols sensitized by methylene blue in CH_2Cl_2 leads to production of hydroperoxide [250]:



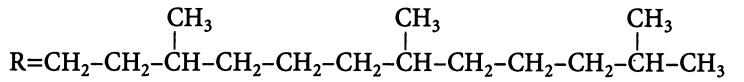
(221)

Similarly tocopherols, among which α-tocopherol (vitamin E), a biological protective phenolic compound in hydrophilic phase, is oxidized by $^1O_2$ with very high rate ($\sim 10^8\ \ell \cdot mol^{-1} \cdot s^{-1}$) according to the following mechanism [500]:



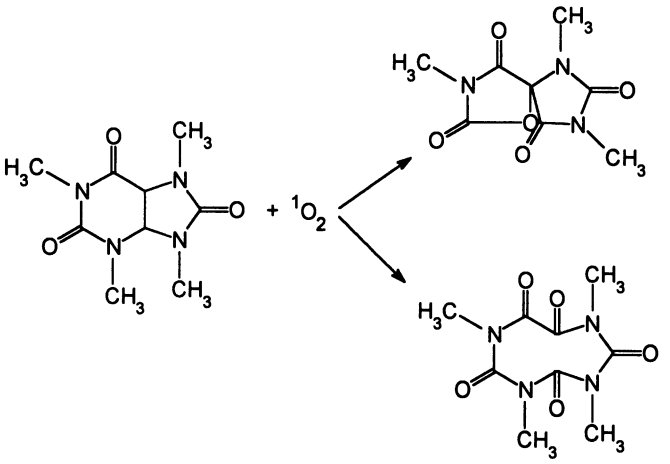
(222)

where



5.2.2.3
Nucleic Acid Components

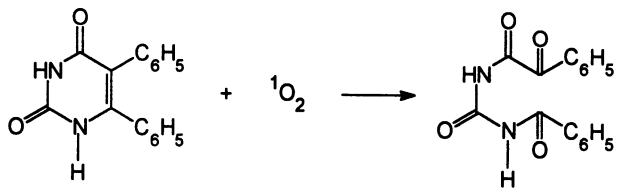
It has been reported [491, 501, 502] that $^1O_2$ can cause the oxidation of major cell components such as DNA or RNA. The study carried out on model systems has shown that heterocyclic dienes and enamine double bonds are targets for $^1O_2$ attack (see [503]). For example tetramethyluric acid (ketonic form) reacts with $^1O_2$ as follows [504]:



(223)

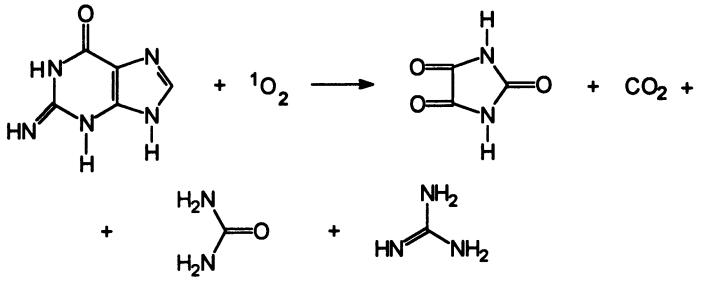
In vitro study performed by Cadet et al. [505]on 3′,5′-di-*O*-acetyl-2′-deoxy-guanosine oxidized by $^1O_2$ showed formation of the degradation products, e.g. deoxy-ribosyl-cyanuric acid and deoxyribosyl-4,8-dihydro-4-hydroxy-8-oxo-guanine from this purine derivative.

Another example of interaction of $^1O_2$ with nucleic acid components was reported by Foote [480]. It has been observed in this study that photooxidation of uracil derivatives gives carbonyl compounds via the enamine double bond cleavage:



(224)

Similarly, the photosensitized oxidation of guanine and guanosine occurs with a ring cleavage leading to the generation of several compounds such as guanidine, carbon dioxide, derivatives of urea and parabanic acid (reaction at Eq. (225)) [506, 507]:

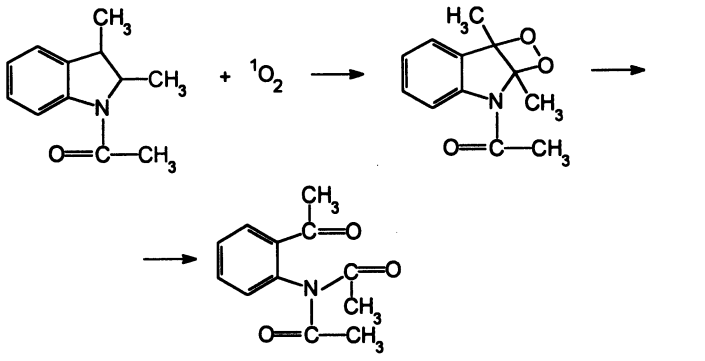


(225)

If we compare the ability of the HO radical and $^1O_2$ to oxidize nucleic acid components, we will find experimental data showing that HO· reacts with both pyrimidine and purine components, whereas $^1O_2$ shows the most activity towards guanine and related purines. Several reports dealing with photodynamic oxidation of nucleotides and nucleic acids have proved that the guanine residue in nucleic acid is the most readily and selectively damaged [506]. Compounds identified during oxidation of guanosine and its derivatives as well as other nucleic acid bases by $^1O_2$ generated by other methods than the Type II reaction were found to be different from those discussed above (see for example [508]).

5.2.2.4 *Indoles*

Dye-sensitized photooxidation of the next biologically important compounds such as electron-rich *N*-alkylated indole derivatives has been shown to give end products containing carbonyl and amide groups by way of dioxetane stage formation and 2,3-bond cleavage (reaction at Eq. (226)) [509, 510], whereas 2,3-disubstituted indoles give mainly ene reaction products, i.e. 3-hydroperoxyindolenines (reaction at Eq. (227)) [511, 512]:



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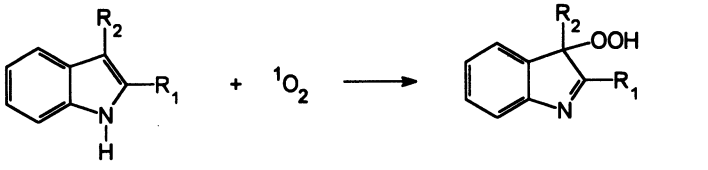


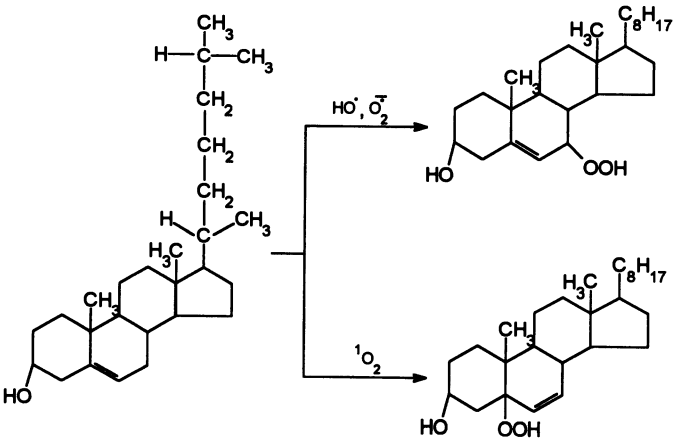
(227)

5.2.2.5 *Lipids*

Fatty acids and lipids are photosensitized by singlet oxygen to allylic hydroperoxides with the double bond shifted to the allylic position ([480, 513 and references therein]):

$$R_1\text{-CH=CH-CH}_2\text{-}R_2 + {}^1O_2 \rightarrow R_1\text{-}\underset{\displaystyle \underset{\displaystyle OOH}{|}}{CH}\text{-CH=CH-}R_2 \qquad (228)$$

For example, cholesterol is photooxidized via the singlet oxygen way to 3β-hydroxy-cholest-6-ene-5α-hydroperoxide, whereas this sterol oxidized by oxygen free radicals gives the 7-hydroperoxides, (reaction at Eq. (229)) [353].



(229)

Hydroperoxides generated during oxidation by $^1O_2$, in turn, can decompose to give several types of secondary free radicals. These lipid radicals can participate in the propagation reactions of lipid peroxidation (see Fig. 12), and neighbouring cell constituents can be further targets.

Coming to the end of this review of the chemical basis of damaging reactions observed during the photodynamic effect, it is worth mentioning that there are several important factors controlling the Type II photosensitized oxidation such as ionization or half-wave oxidation potentials of the acceptor, hindering effect of the interaction between singlet oxygen and the acceptor, solvent dependence of the singlet oxygen lifetime, temperature effect determining the stability of the peroxide type intermediates, kind of sensitizer conditioning the Type II oxidation, and the singlet oxygen generation in the $^1\Delta_g$ state having relative longer lifetime than the $^1\Sigma_g^+$ state, or presence of $^1O_2$ quenchers (antioxidants). Thus, biological and medical consequences of the photodynamic effect are conditioned by these factors as well as the protective level of the biological systems against the $^1O_2$ action.

5.2.3
Biological and Medical Aspects of the Photodynamic Effect

Problems connected with photodynamic action have aroused considerable interest during the last few years. However research on this problem has been going on since 1903, and Kautsky (1939) [514] was the first researcher who proposed the mechanism of dye-sensitized photooxidation reaction in the presence of molecular oxygen as an energy transfer from the sensitizer to molecular oxygen following $^1O_2$ generation and its reaction with an oxygen acceptor.

The discovery of the red chemiluminescence bands at 638 and 700 nm of $^1O_2$ by Khan and Kasha in 1963 [105], the solvent effect on $^1O_2$ lifetime, especially its prolongation in D_2O (deuterium effect), and development of a novel method for direct measurement of the lifetime in solution by Merkel et al. in 1971 [99, 317] as well as observation of quenching effect exerted by certain specific scavengers, reported in a number of papers (1968–1974) [316–318, 322], turned the attention of photobiologists onto $^1O_2$ as the responsible agent for the dye-sensitized inactivation of aqueous solutions of enzymes [328, 514]. There is considerable evidence of the importance of the role of $^1O_2$ in the photooxidation of biomolecules. Photodynamic action shown by $^1O_2$ in vivo is very complex on the whole nevertheless several different techniques were used for demonstrating the intermediacy of this species in this process [480, 515]. In 1974 Ito and Kobayashi first demonstrated the participation of $^1O_2$ in vivo systems such as acridine orange sensitized photooxidation of yeast cells using a combination of two criteria for $^1O_2$ participation: $^1O_2$-quencher (NaN_3) and D_2O as a solvent [516].

The photodamaging effects have been observed in all classes of organisms starting from multicellular plants and ending in humans. This effect can operate in membrane damage, carcinogenesis, mutagenesis, disturbance in metabolism or reproduction and in numerous other biological effects. All the above-mentioned medical and biological disorders have a source in $^1O_2$ reactions with molecules of biological interest such as amino acids, proteins and peptides, nucleosides, nucleotides, lipids and other cell constituents.

The model studies carried out by several scientific groups (see for example [517 and references cited therein]) on simple systems that mimic biological membranes such as micelles, reverse micelles, liposomes and vesicles as well as biological membranes, organelles and cells have shown that the $^1O_2$ damaging activity strongly depends on the locus of its production. The damage is nearly site-specific with regard to $^1O_2$ lifetime, e.g. about 1.03 μs in human plasma [518], and the presence of reactive compounds showing $^1O_2$ quenching property. A longer lifetime allows $^1O_2$ to diffuse far from the place of its generation. Kanofsky has reported that $^1O_2$ may diffuse in the membrane core for a distance of about 0.16 micrometers [518]. This value is about 2.5 times higher than the mean diffusion distance for $^1O_2$ in aqueous solution. This unique feature of $^1O_2$ (generation and reaction sites can be different) differentiates $^1O_2$ behaviour from the oxygen radical reactions.

The toxicity of $^1O_2$ to the cell in photosensitized reactions depends on the localization of the sensitizers, on whether the sensitizer is free or bound to the cell constituents, as well as on the value of the excited triplet state energy and the

value of the environmental pH [516]. For example, acridine dyes are bound to the mitochondria as well as to yeast cells [519], eosines are distributed more in cytoplasm and protoporphyrin in liposome [516].

The distribution of sensitizer in a cell may be an important determinant of the photodynamic biological activities. An excellent overview dealing with photomodification of biological membranes and the role of $^1O_2$ in this process in in vitro research is given in [520 and several references cited therein]. A survey of in vivo studies is reported by Ito [516].

The above-mentioned ability of the nucleic bases for photosensitized generation of $^1O_2$ might have significant biological consequences. Singlet oxygen as a relatively long half-life species (10–50 μs) can travel for a long distance and cause strand breaks of DNA chain with the damage being located at neighbouring sugar sites. This oxidant reacts with DNA causing single-strand breaks and biological consequences similar to those caused by HO radicals [521]. It is generaly accepted that $^1O_2$ is generated under various pathophysiological conditions in mammalian tissues. Studies carried out in recent years [522, 523] have shown that $^1O_2$ damaging DNA bases causes misreading of the DNA template and results in mutagenicity and carcinogenicity. The mutagenicity exerted by $^1O_2$ has been reported, for example, in bacteria and mammalian shuttle vectors [524, 525].

Singlet oxygen is known as a genotoxic agent. This species exerts influence on the genetic information and on DNA replication [524]. A review of the genotoxic properties of $^1O_2$ has recently been published [501].

In vitro study has shown that *E. coli* DNA polymerase I is stopped when copying single-strand DNA possessing guanine oxidized by $^1O_2$ [526]. If such a blockade in replication occurs in vivo, the lesions in DNA will lead to death of a cell. The toxicity of $^1O_2$ in *E. coli* in terms of growth and survival is well documented in Nakano et al. [527]. Moreover, it is suspected that DNA damage is a main step in gene induction [528, 529], and recently it has been suggested that $^1O_2$ generated interacellularly is able to induce human immunodeficiency virus type 1 (HIV-1) reactivation from latently injected lymphocytes and monocytes [530]. The mutagenic consequences of DNA damages has been well documented by Decuyper-Debergh et al. [524].

Ito [516] has classified the inactivation of the cell into three modes: (1) the sensitizer is localized outside the cell and $^1O_2$ attacks the cell from outside; (2) the sensitizer occurs in the cytoplasm and $^1O_2$ reacts with the cell constituents from inside; (3) the sensitizer is bound to DNA and $^1O_2$ also attacks from inside.

Singlet oxygen generated during the photodynamic effect is capable of injuring and even killing cells [142]. The possible involvement of $^1O_2$ in tissue damage in different disease conditions has attracted great attention in recent years and we will discuss only a small fraction of this important field of research. The evidence obtained has established that singlet oxygen generated intracellularly, i. e. in the cytoplasmic region, reacts mainly with enzymes, DNA, tRNA and other subcellular structures causing a mutagenic effect, whereas produced outside a cell it attacks the cell surface and thus the cellular membrane lipids. Ito and Kobayashi [531] have collected good evidence for the induced membrane damage of yeast cells by $^1O_2$ generated outside the cell during the photodynamic effect.

Of particular interest to all biologist and biochemist research groups has been the localization of oxygen species generation intracellularly and the site of damage caused in biological systems. Up till now, investigation of the ability of localization of these reactive species generated in vivo has met with limited success. As described in Chap. 4, the ESR spin trapping technique, one of the most frequently applied methods for detecting both oxygen free radicals and $^1O_2$, does not allow for the simultaneous observation of their generation and reaction sites with cell constituents. Recently the research has been focused primarily on the use of fluorescence microscopy techniques in combination with linked nitroxide traps for fluorophores. The method has been successfully used to differentiate the various types of biological damage caused by singlet oxygen and oxygen radicals [532] in cells. For example, this method allows one to detect oxygen radical generation by stimulated human neutrophils.

A few diseases have been postulated to involve the photodynamic effect. For example, porphyrias, which are defects in the blood porphyrin metabolism, have several different syndromes [533]. One of the disease, erythropoietic protoporphyria, is mediated by generation of $^1O_2$ during the photosensitization of porphyrins occurring in the skin, and patients suffer with edema and erythremia. Because blood from these patients contains increased concentration of protoporphyrin in the red cells [534] acting as sensitizers in the photodynamic effect, activation of the lipid peroxidation by $^1O_2$ is observed which leads to increase in red cell membrane permeability [535]. There are many examples of red blood cells being damaged during photooxidation in the presence of endogeneous or exogeneous sensitizers. Denaturation of hemoglobin and damage of the erythrocyte membrane have been proposed as a reason for oxidative hemolytic diseases. Hemolysis of erythrocytes has been observed for a number of photosensitizers [536, 537].

Lamola et al. [538] observed hydroperoxide cholesterol formation during protoporphyrin sensitization in normal and ghost red blood cells in patients suffering from erythropoietic protoporphyria. These patients are subject to swelling, erythema and lesion on exposure to light. Their red blood cells contain a large amount of free protoporphyrin which is a very good sensitizer for the Type II reaction.

The increased permeability of lysosomes and cell membranes towards liquids and inorganic ions as a reason of accumulation of fluid in a cell during edema has been postulated by Allison et al. [539].

From the medical point of view the next important consequence of the photodynamic effect is an induction of collagenase expression in human skin fibroblasts. Collagenase, synthesized under unbalanced conditions, cleaves the α-chain of interestitial collagenases I and III causing damage to the connective tissue in human fibroblasts under in vitro and in vivo conditions [540]. The next report worth mentioning [541] deals with induction of human heme oxygenase gene, the rate-limiting enzyme in the heme degradation pathway, via the Type II mechanism. Similarly, Keyse et al. [542] have reported transcriptional activation of the human heme oxygenase gene in cultured skin fibroblasts induced by oxidative stress.

Nye et al. [543] using the ESR spectroscopy have reported that epithelial cells, a cell type known to undergo oxidant stress, are photodynamically inactivated

in the presence of methylene blue, causing changes in respiratory epithelial cell morphology and function.

Photosensitized inactivation of lysozyme and dysfunction of ATP-ases occurs probably via tryptophan destruction [544], whereas a loss of lipoamide and alcohol dehydrogenase activity is related to histidine oxidation [545].

Another potential consequence of photosensitized generation of singlet oxygen involves carcinogenesis. The evidence is convincing that $^1O_2$, as a powerful oxidant able to induce a cellular pro-oxidant state, can act as a promotor of carcinogenesis [546].

It has been observed that many carcinogenic polycyclic hydrocarbons are good sensitizers showing photodynamic properties [547], for example 3,4-benzopyrene [548]. It has been shown that illumination of mice after injection of sensitizers produced skin tumours, although single treatment of mice with either sensitizer or illumination does not give the tumour formation. One of the hypothetical theories of carcinogenicity involving $^1O_2$ postulates generation of this species by the polynuclear hydrocarbon bound to the cell constituents during exposure to light [548]. Singlet oxygen reacts with acceptors giving hydroperoxides. In vivo, such hydroperoxides or products of their decomposition, e.g. oxides, epoxides or hydroxylated compounds, are electrophilic carcinogenic species capable of reacting with nucleophils within the cell to stimulate metabolic activation leading to tumour growth [549].

According to the above-mentioned mechanism the DNA strand breakage induced by $^1O_2$ can elicit secondary metabolic reactions, particularly polyadenosine diphosphate ribose (ADP)-ribosylation of chromosonal proteins [550]. Because concentration of $^1O_2$ generated during the dye-sensitized photooxidation can reach a relatively high level, this oxidant may result not only in changing chromatin conformation but also in excessive poly ADP-ribosylation, which causes depletion of ATP, NAD, inhibition of DNA synthesis or even cell death [551–553].

There also exists good evidence that some drugs are phototoxic because of their ability to act as photosensitizers. One example showing this property is tetracycline which can cause a number of phototoxic effects in patients when they are exposed to sunlight. Clinical symptoms are edema, papules, erythema or photo-oncholysis [484]. Another member of the tetracycline antibiotics group, doxocycline, damages neutrophils [554].

Finally, there are several reports considering singlet oxygen as a causative factor leading to plant senescence [555].

5.3 Cytotoxicity of Oxygen Free Radicals and Their Possible Role in Human Diseases

5.3.1 Oxygen Radical Induced Cytotoxicity

As has been shown in Chap. 1 there are two possibilities for reactive oxygen free radical generation: enzymatic and random (in the presence of electron donors).

The enzymatic generation of oxygen free radicals includes a number of biological redox reactions, both enzymatic and spontaneous, which generate $O_2^{\cdot-}$. Several oxidative enzymes have been shown to catalyze the univalent reduction of O_2 to $O_2^{\cdot-}$, e.g. xanthine oxidase, flavin oxidase or aldehyde oxidase. The superoxide anion radical dismutates spontaneously or catalytically in the presence of a family of enzymes called SODs, forming H_2O_2. The spontaneous dismutation is a slow process, of second order with respect to $O_2^{\cdot-}$ concentration, while the enzymatic process is rapid, of first order in this radical concentration. Superoxide anion radical can, in turn, react with H_2O_2 to produce $HO^{\cdot}$ (reaction at Eq. (31)).

The random generation of oxygen species includes one-electron reduction of molecular oxygen by a low spin transition metal (Cu, Fe, Co, Mn, Cr, Ti, Ni), irradiation of H_2O which constitutes the main tissue component, the redox reactions of quinones, semiquinones or the transfer of electron from compounds containing a lone pair of electrons (–N̈–, –S̈–, –P̈–, –S̈e–) to the oxygen molecule.

However, not all cellular sources of $O_2^{\cdot-}$ are identified, although several important have been well recognized (see [39–52, 556]. It has been shown that whole cells and subcellular organelles are involved in oxygen free radical production (see Chap. 1).

Oxygen free radicals have been postulated to be involved in development of various pathological states. Interest in oxygen free radicals as potential toxic intermediates dates to the early 1940s and an enormous amount of literature has been published on this subject. It is impossible to discuss the wide range of pathological states and diseases in which they have been reported to be involved, and we will therefore concentrate our discussion only on those which are well documented and are considered to be connected with the presence of physical and chemical pollutants in the environment. Oxygen free radicals can be toxic and very dangerous when the balance between prooxidation conditions and antioxidants counteraction gets out of the cells, control. Besides being toxic, they play an important role in the physiological processes for the maintenance of homeostasis in tissues.

Del Maestro [557] has proposed two categories of disease states – increased generation of free radicals and decreased generation of free radicals. Both processes may occur intracellularly and extracellularly. In the case of increased generation both intracellular and extracellular generation of free radicals may occur simultaneously. Several disease states have been reported to result from intracellular injury of organelles such as mitochondria, lysosomes or peroxisomes exerted by oxygen free radicals generated under pathological conditions. The main reason for the disease state is an alteration of protective and control mechanisms acting in a cell that can lead to uncontrolled radical reaction of oxidation.

Chemicals, drugs, irradiation, toxins, deficiency of antioxidants, e.g. vitamins, and ageing are considered to operate as sources of increased intracellular generation of free radicals. Human beings are exposed to a great number of physical and chemical pollutants present in the environment (see Fig. 11). Long-lasting or chronic exposure to these pollutants may lead to damage of cell constituents or even cell death. Ultraviolet radiation, therapeutic radiation and

chemical carcinogens present in air pollution (O_3, NO, NO_2) or smoking generate an increased radical flux in the intracellular and extracellular space of a cell.

The increased extracellular production of oxygen free radicals occurs during acute inflammatory (burns, infections) and chronic inflammatory (rheumatoid arthritis, connective tissue disorders, vasculitis) conditions. Oxygen free radicals are then released mainly in the extracellular space, for example by polymorphonuclear leucocytes, monocytes and macrophages.

It is widely believed that, among oxygen free radicals and general oxygen species, the most important free radical species with respect to the cell damage is the HO radical. Hydroxyl radical is a short-lived species, with half-life times in the range of nanoseconds, but this radical reacts rapidly with almost every molecule met in a cell. The $O_2^{\cdot-}$ radical and H_2O_2 are rather low reactivity species and are not expected to be highly toxic, but they can be converted to $HO^{\cdot}$ through the Fenton reaction catalyzed by endogenous stores of transition metal ions. In the healthy cell the catalytic activity of metal ions is well controlled because the metal ions are compartmentalized by incorporation into macromolecular structures such as cytochromes, ferritin and hemoglobin, or are bound to enzymes.

Hydrogen peroxide is a stable enough compound and can diffuse for a long distance to a vulnerable part of the cell, generating HO radicals by the reaction with ferrous ion often bound to the phosphates of the DNA backbone and to protein associated with chromatin. This hypothesis for H_2O_2 toxicity has received widespread acceptance and has been supported indirectly by a number of model studies [437, 558, 559]. In healthy cells of different types, iron is stored as bound to ferritin. Cells also contain insoluble iron chelates, e.g. hemosiderin and also low molecular weight iron chelates or a number of cytosolic iron-containing enzymes. Each iron form can potentially by involved in the Fenton reaction.

Hydroxyl radicals generated by activated phagocytes near endothelial surfaces may cause changes in intravascular granulocyte behaviour. Besides possible bacterial killing activity they can oxidize lipids of the intracellular organelles, and also cause alteration of other macromolecules, e.g. lipids, DNA, RNA according to reactions described in Chap. 5 and therefore increase microvascular permeability. The increased generation of HO radicals, e.g. during the splitting of water molecules, may result in alteration of components of the extracellular space and plasmalemmal peroxidation [558].

The importance of metal ions in the generation of oxygen species has been discussed in Chaps. 1 and 2 (see reactions at Eqs. (10), (11), (18), (19), (33), (34), (37), (38)). The metal ions, especially iron, play an important role in lipid peroxidation as shown in Chap. 5, causing fission of an O–O bond transferring lipid peroxide into alkoxy and peroxy radicals (reactions at Eqs. (198), (199)). Peroxy and alkoxy radicals, like $HO^{\cdot}$, may cause DNA damage. Finally, a number of toxic products is generated during the reaction of metal complexes with lipid peroxides (aldehydes, epoxides). Peroxidation of the cellular membrane bound lipids has been considered as important in the development of ischemic brain damage [560]. Lastly, the direct demonstration of the occurrence and time course of increased HO radicals production in the injured brain has been demonstrated by Hall et al. [561].

5.3.2 The Role of Oxygen Radicals in Human Diseases

Decreased generation of oxygen radicals in a cell also leads to disease states. For example, people suffering from psychiatric diseases (schizophrenia, paranoid psychosis and other mental aberrations) have increased intracellular levels of SOD (an enzyme which eliminates O_2^-, see reactions at Eqs. (117), (118)). Michelson [562] suggested that some psychiatric diseases may result from increased elimination of O_2^- by SOD giving, as an example, children suffering from mongolism. It was found that these children had 50% higher intracellular concentration of SOD [563]. Similarly, the decreased ability of O_2 reduction to O_2^- by inflammatory cells causes increased susceptibility of humans to some kinds of infections. Babior [564] has reported the occurrence of this defect in chronic granulomatous diseases and myeloperoxidase deficiency.

Halliwel and Gutteridge [565] have suggested that oxygen free radicals may be involved in at least 100 sorts of disease and that enhanced production of the radicals occurs in most human diseases [566]. A big role in oxygen radical production during disease states has been postulated for catalytic activity of metal ions. In healthy cells this activity is well controlled by incorporation into macromolecular structures, but during diseases a structural disorder or the action of chemicals or viruses causes a metal ion to be released and become involved in abnormal redox reactions. If the redox reactions release strongly electrophilic oxygen species such as HO radicals or $^1O_2$ near targets, namely DNA, RNA and proteins, then damaging oxidation may occur. But there is little direct evidence supporting oxygen radical etiology of diseases because of the short life of HO radicals, which strongly hinders their detection in vivo. However, a few examples of successful detection of HO radicals using the ESR spin-trapping method has recently been reported [567, 568]. Additionally, the ESR spin-trapping method combined with microdialysis has been satisfactorily used for the HO radical detection in the processes of brain ischemia and reperfusion damage [569].

The presence of $HO^\cdot$ in the mitochondrial fraction during reperfusion after ischaemia has been measured using salicylic acid as a chemical trap. Salicylic acid reacts with $HO^\cdot$ forming hydroxylated benzoic acid [570].

Recently, Liu [571] has reported the double microdialysis fibre technique for both generation and detection of HO radicals in vivo as a method of overcoming difficulties in studying damage to cells caused by HO radicals. In vivo HO radical mediation of the toxicity of paraquat and copper using the ESR technique has also been reported [572].

Let us now consider several of the most common human diseases in which there is experimental support for oxygen free radical involvement, such as

- carcinogenesis
- anticancer action
- atherosclerosis
- immunopathologies
- oxidative stress diseases
- ageing and related diseases
- emotional distress

5.3.2.1 *Carcinogenesis*

This mechanism may be defined as the multi-stage process of normal cell transformation to the malignant states dependent on two factors – inherited and environmental [565]. The hypothesis that oxygen free radicals are involved in carcinogenesis finds support in the fact that in living systems redox reactions proceed under enzymatic control, otherwise radicals are generated randomly in the presence of electron donors. Many carcinogenic factors (ionizing radiation, ultraviolet waves, drugs and other chemicals, metal ions) stimulate the generation of free radicals. For example, the involvement of free radicals in melanomas may result from their involvement with melanins because these biopolymers take up oxygen forming O_2^- and H_2O_2 [573, 574]. Two possible pathways of cytotoxicity are possible: (1) conversion of a potent carcinogen to free radical form in vivo or (2) its indirect participation in radical formation. Many carcinogenic compounds are therefore free radicals. Moreover, it has been experimentally found that many scavengers of oxygen free radicals inhibit various stages of carcinogenesis, whereas studies performed on animals have shown that polyunsaturated fatty acids increase the carcinogenic properties of some chemicals (see for example [565, 573]). The free radical theory of carcinogenesis and other human diseases is based on evidence of the increased formation of oxidants in the course of some diseases and on greater than average evidence that antioxidants are beneficial in some diseases.

Although the role of free radicals in some types of cancers such as bronchial cancer, leukaemia, cervical cancer or melanoma is now well established as a result of almost 50 years of research, up till now it remains difficult to fit gathered data into a unified scheme of carcinogenesis.

It is generally accepted that oxygen free radicals may play an important role in the two major stages of carcinogenesis, initiation and promotion, since carcinogenic substances are known to act as electron donors and/or electron acceptors. Therefore they may adversely affect the pathway or the kinetic equilibrium of the electron transport as well as change the concentrations of normally occurring free radicals of the cell, or introduce some new ones. The first example probably occurs with DNA damage (modification of purine and pyrimidine bases) mediated by oxygen free radical and a carcinogen [575]:

$$\begin{matrix}\text{DNA} \\ \text{carcinogen}\end{matrix} + [\,O_2,\ Me^{n+}/Me^{(n+1)+},\ O_2^-,\ H_2O_2,\ RO^{\cdot},\ RO_2^{\cdot}\,] \longrightarrow \begin{matrix}\nearrow\ \text{DNA}^{\cdot} \rightarrow \text{strand breaks} \\ \searrow\ \text{carcinogen}^{\cdot}\end{matrix} \qquad (230)$$

$$\text{DNA}^{\cdot} + \text{carcinogen}^{\cdot} \rightarrow \text{DNA} - \text{carcinogen adduct} \qquad (231)$$

The carcinogen molecule may be covalently bound to the DNA molecule with the participation of oxygen free radicals. The critical step for tumour initiation

is considered to be the formation of the DNA-carcinogen adduct in the endoplasmic reticulum, and its distribution within the chromatin.

Hydrogen peroxide, shown in reaction at Eq. (230) as an inductor of DNA damage and therefore as a carcinogen initiator, is able to carry out these functions by site-specific $HO^{\cdot}$ formation. It has been reported that cancer may result from the excess of H_2O_2 in peroxisomes when this oxidant cannot be fully decomposed by catalase and may reach a nucleus [565]. The role of metal ions in DNA radical formation and following its damage by $O_2^{\cdot-}$ and probably, in turn, the $HO^{\cdot}$ formation, has been observed by Shires [576] in isolated rat liver nuclei.

Ionizing radiation and ultraviolet light are potent carcinogens which generate oxygen free radicals in tissues. Hydroxylated derivatives of many polycyclic hydrocarbons and aromatic amines are recognized to be very active carcinogens in vivo. It is also worth mentioning that about one-third of all cancers can be related to the presence of carcinogens in tobacco products. The role of carcinogenic factors in damage to the unsaturated fatty acids of membrane phospholipids and to membrane cholesterol has been well documented [577, 578]. Coon reported that endoplasmic reticulum plays very important role in activating carcinogens by normally controlled free radical reactions such as formation of hydroperoxides and oxygen species [579].

Many promotors react with membranes causing inflammation, enhance mitotic activity and accelerate tumour production. Membranes, besides their architectural role in delimiting the cell and its organelles, exert an effect on the structure and activity of the regulatory enzymes, fitting and maintaining them in phospholipid bilayers as well as controlling transport electrons, ions and other nutrients. Cell regulation occurs with participation of DNA, nucleotides and prostaglandins [580]. These messengers are made from phospholipid-dependent enzymes [577] and influence whether DNA is going to be replicated, or whether the cell is going to divide. If DNA is damaged by a carcinogen, the cell is divided several times, and therefore the controlled synthesis of macromolecules and membrane proteins is very important in the carcinogenesis process. Dempoulos et al. have reported [577] that over 30 phospholipid-dependent enzymes and enzymatic systems are present in plasma membranes, endoplasmic reticulum and mitochondria. If free radical reactions damage phospholipid fatty acids, the functions of membrane enzymes will be changed, and alterations may occur in cell division regulation.

Phorbol-12-myristate-13-acetate, a compound known as a strong promoter of carcinogenesis, stimulates generation of $O_2^{\cdot-}$ by polymorphonuclear leucocytes [581]. Zucker et al. [582] have reported powerful platelet aggregation abilities of this ester in the oxygen free radical path. Platelets are involved in hemostasis, preventing blood loss from injured vessels. When endothelial cells are damaged, platelets adhere to collagen and membranes, and they also adhere together. The aggregation and adhesion of platelets depend on releasing platelet activation factors, e.g. thromboxanes.

Figure 15 presents a possible way of cellular injury and increased granulocyte rolling by $O_2^{\cdot-}$. Phorbol ester, by stimulation of the $O_2^{\cdot-}$ generation on platelet

surface, may form HO radicals via the Haber-Weiss reaction. The HO radicals can enhance the membrane phospholipase A_2 activity following arachidonic acid release, because the membrane phospholipids are a main source of arachidonic acid [583].

Prostaglandins (PGE_2 and PGF_{1a}) and thromboxanes (TAA_2 and THB_2) are the main products of controlled arachidonic acid metabolism and also other polyunsaturated fatty acids containing 20 carbon atoms in their molecules [584, 585]. Prostaglandins have a wide range of function, e.g. stimulation of steroidogenesis in the ovary, inhibition of gastric secretion, lowering of blood pressure or generation of inflammatory responses. Thromboxanes and prostaglandins are metabolically related compounds. Platelets contain a high level of arachidonic acid and the enzyme-tromboxane A_2 synthetase. Released arachidonic acid is oxidized to the endoperoxide intermediate PGG_2 (9,11-*endo*-peroxy-15-hydroperoxyprostaglandin) with participation of the enzyme cyclooxygenase (an enzyme present in almost all mammalian tissues), oxygen and Fe(III) heme. This means that the arachidonic acid oxidation occurs with the participation of HO radicals generated from lipid peroxides or H_2O_2. On the other hand, an excess of lipid peroxides or H_2O_2 can inactivate cyclooxygenase. Thus supply of arachidonic acid as well as the balance between hydroperoxide generation and their removal by antioxidants control the rate of prostaglandin synthesis. We must remember that the prostaglandins are biologically active lipids being involved in the regulation of numerous physiological processes and playing an important role in many diseases that involve inflammation and tissue damage [565]. The antioxidants, both endogeneous and exogenous, e.g. drugs, chemicals, can exert an effect on prostaglandins synthesis. PGG_2 is further transformed into PGH_2 (9,11-*endo*-peroxy-15-hydroxyprostaglandin) by a peroxidase which is part of the same protein as cyclo-oxygenase. Both PGG_2 and PGH_2, as unstable intermediates, are rapidly converted into other products such as PGE_2, PGF_{1a}, thromboxanes A_2 and B_2, prostacyclines PGI_2 or HHT (12-hydroxy-5,8,10-heptadecatrienoic acid) depending on the kind of enzymes present in the tissue. For example, platelets are rich in the enzyme thromboxane A_2 synthetase, and predominantly generate thromboxane A_2 and B_2, HHT and malonaldehyde (MDA). Thromboxane A_2 shows both vasoconstrictor and powerful platelet aggregation properties, whereas thromboxane B_2 is the stable and inactive form. In contrast, the endothelial cells synthesize prostacyclin PGI_2 which dilates blood vessels and acts as a powerful inhibitor of platelet aggregation. The ratio of thromboxane to prostacyclin is considered to be important in controlling blood flow and platelet aggregation.

Figure 15 covers only selected products released in the "arachidonate cascade" formation, which occurs with participation of HO radicals, and whose products may be potentially important in the disease states. For details, see [448, 565 and references therein].

As is easy to see, the oxygen radicals may be involved in the release of arachidonic acid, in the generation of PGG_2, in the transformation of PGG_2 to PGH_2 and, finally, they may stop the production of PGI_2 and cause micro-occlusions by the formation of lipid peroxides.

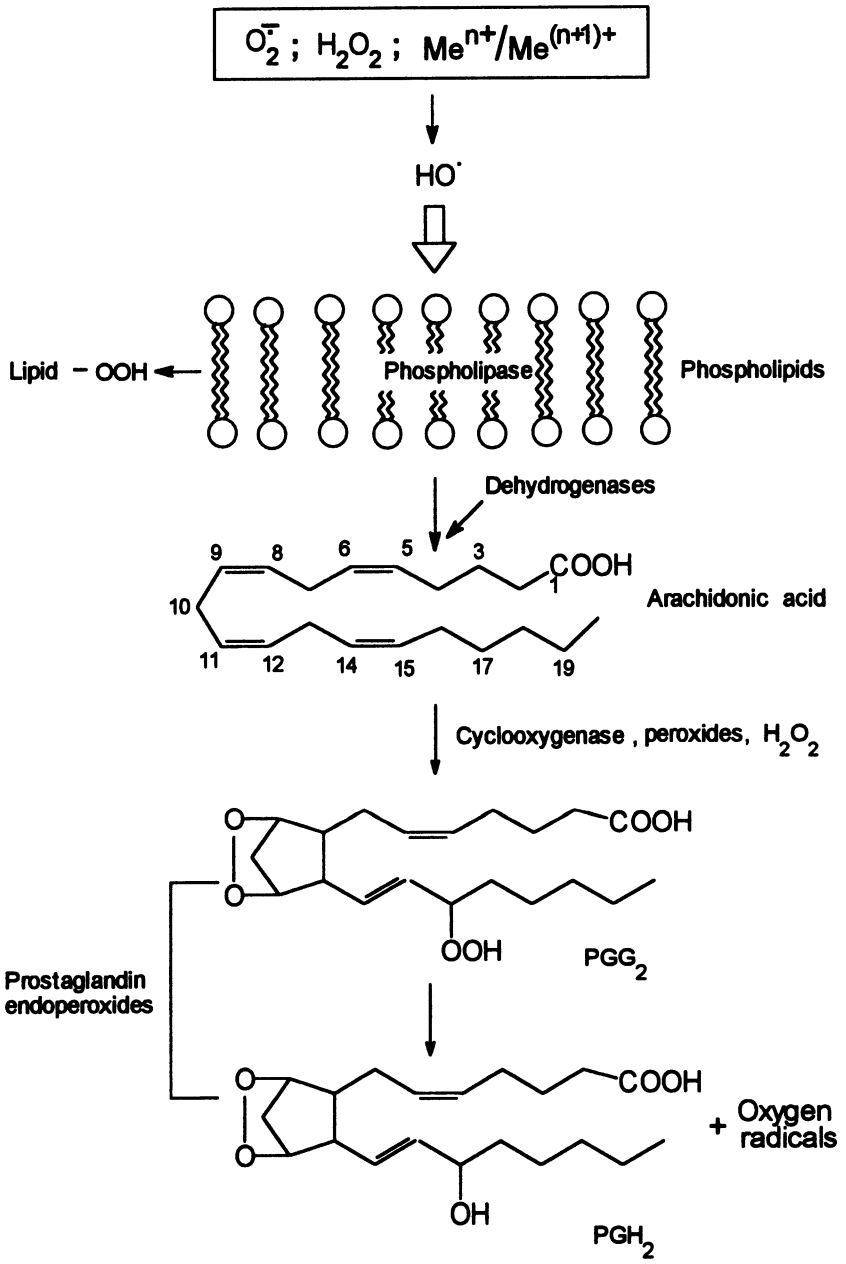


Fig. 15. Schematic illustration of arachidonic acid transformation

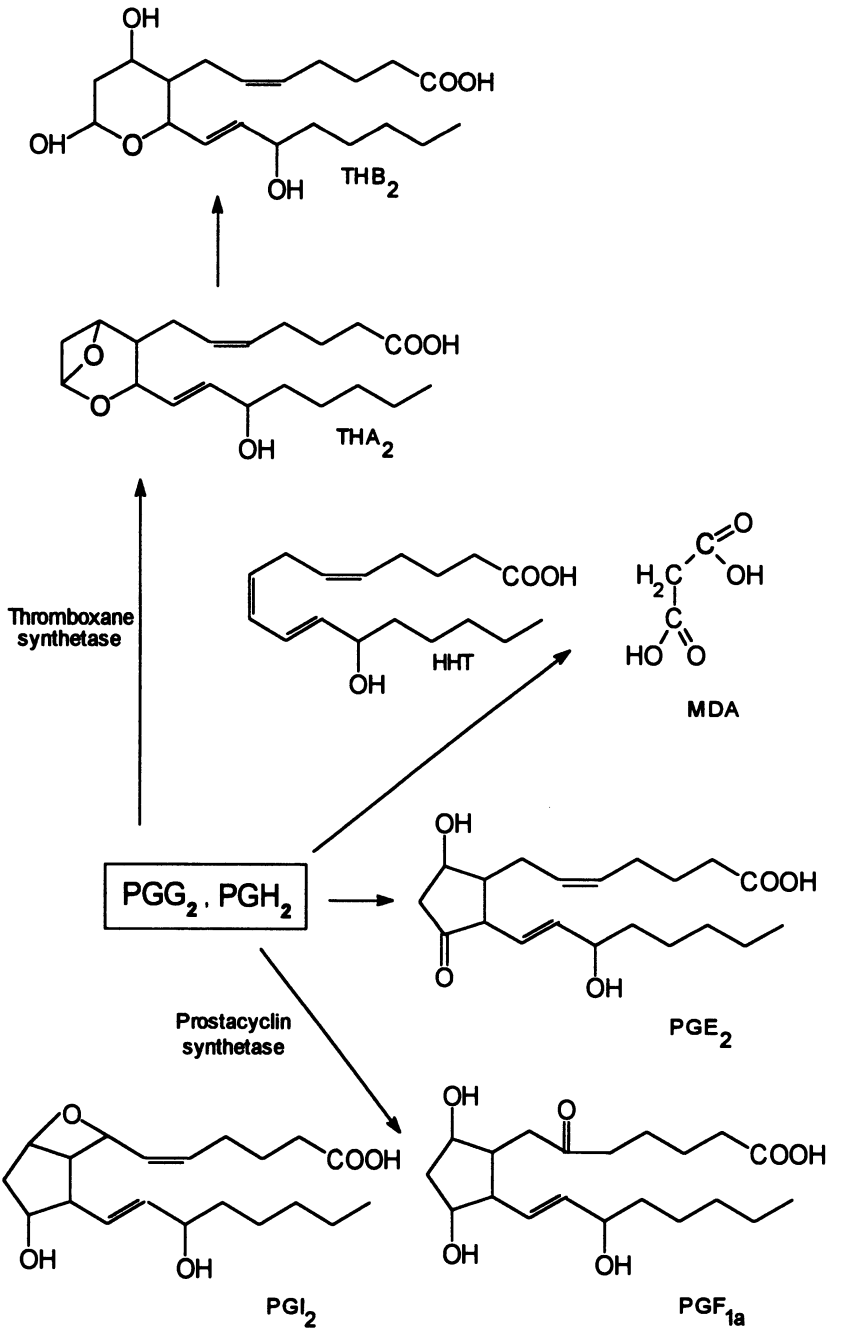


Fig. 15 (continued)

Finally, independently of the carcinogenesis pathway, the activated carcinogens or oxygen free radicals are able to react with target nucleophiles and to generate biochemical changes leading to tumour induction [586].

5.3.2.2 *Anticancer Action*

Free radical reactions are involved not only in the promotion and progression of cancer but also in the mechanism of action of anticancer drugs. A number of chemotherapeutic agents are phenols or quinones. These agents may undergo cyclic reduction and autoxidation generating oxygen radicals, H_2O_2 and singlet oxygen. Anthracycline antibiotics (daunomycin, adriamycin) are examples of anticancer drugs containing quinone structures [587, 588]. The quinone form of the drug can be reduced by enzymes to the semiquinone, which can combine directly with cellular constituents or form oxygen radicals. Because many cells are dividing during carcinogenesis, drugs used in therapy should counteract cell proliferation, for example by blocking synthesis of DNA or RNA. The above-mentioned products of the redox reaction of chemotherapeutic agents do have these properties.

5.3.2.3 *Atherosclerosis*

Atherosclerosis is a disease of the arteries resulting in a thickening of the inner part of the vessels. Ischaemic damage to the brain and heart are consequences of atherosclerosis. The ischaemic damage is a major cause of death. Although the detailed mechanism of the origin of atherosclerosis is not well known, there is a commonly accepted theory that the disease starts with damage to the vascular endothelium by $O_2^{\cdot-}$ and H_2O_2 produced, e.g. by monocytes and macrophages or by oxidative stress [588–593]. The authors reported that phagocytes cause the development of atherosclerosis because their activation may injure endothelial cells by generation not only of $O_2^{\cdot-}$ and H_2O_2 but also $NO^{\cdot}$ and hydrolytic enzymes, to such huge concentrations that protection and repair systems cannot work adequately. Human blood contains several complexes of proteins connected with lipids, so-called, lipoproteins. The increased concentration of lipids in blood may produce large quantities of lipid peroxides which may be involved in endothelial injury. Similarly, sterols such as cholesterol may also be involved in atherosclerosis because this agent undergoes oxidation to products known to be toxic to arterial smooth muscle cells [565, 594, 595]. This theory has found confirmation during therapy with probucol, a compound with two phenolic rings which shows powerful chain-breaking antioxidant properties and is used for lowering the level of blood cholesterol.

Atherosclerosis may lead to the blockade of an essential artery and to damage of the brain and heart, as was mentioned above, resulting in complete oxygen deprivation of these tissues, in other words to ischaemia. Restriction in blood flow leads to lowering of oxygen concentration below normal, i. e. hypoxic injury.

The brain is very sensitive to hypoxic injury, and is unable to survive ischaemia for longer than a few minutes. If the ischaemia or hypoxia state are prolonged, the tissue may be saved by reoxygenation (reperfusion) with blood by introducing molecular oxygen. This process, although saving the hypoxic or ischaemic tissue, may also cause additional damage to the tissue with participation of oxygen radicals [560]. An indicator of such type of damage in the initial phase of ischaemia is the enhanced capillary permeability which results in edema formation, whereas the more advanced phase exhibits a microscopic or gross tissue destruction [596, 597]. It has been reported that HO radicals generated during postischemic myocardial dysfunction cause heart dysfunction and stunning in the canine heart [598, 599]. In vitro studies on isolated adult rat cardiac myocytes subjected to anoxia/reoxygenation have shown $HO^{\cdot}$ generation [600].

There is also a widespread belief that oxygen radicals are responsible for secondary neuronal damage in central nervous system injury and a wide variety of neurodegenerative diseases [601, 602]. Traumatic injury to the brain or spinal cord very often leads to tissue degeneration involving lipid peroxidation [603].

However, although direct measurement of oxygen free radical generation in vivo is not technically feasible, indirect evidence that these radicals are mediators of damage during myocardial ischaemia and reperfusion is strong. It would seem, too, that oxygen radicals might play an important role in Parkinson's disease. Some evidence consistent with this suggestion has been provided by Adams and Odunze [604]. They have shown that increased lipid peroxidation and both iron and SOD levels, as well as decreased concentrations of glutathione peroxidase, occur in the brains of patients with Parkinson's disease.

Tissue damage caused by ischaemia/reperfusion, disease or toxins occurring with participation of oxygen free radicals is accompanied by increased formation of prostaglandins, leukotrienes, interleukins, interferons and so-called tumour necrosis factors [605]. These compounds have been reported to be involved in a wide range of human diseases, such as Keshan disease (occurring when diet is chronically deficient in selenium) or in neurological disorders observed in people with defective intestinal fat absorption during chronic dietary deficiency of vitamin E [606].

5.3.2.4 *Immunopathologies*

Increased radical generation may also cause immunological disorders. Some evidence for the importance of oxygen free radicals in autoimmune disease has been provided by Alam et al. [607]. They reported creation of new antigenic materials as a result of HO radical attack on DNA and RNA. Halliwell and Gutteridge [565] reported that oxygen free radicals generated during phagocytosis may involve a damaging response causing abnormal activation of phagocytes, so-called chronic inflammation and autoimmune disease. Cells are fitted out with mechanisms preventing the formation of agents acting

against their own components (antibodies). Any disturbance in performing this duty enables for formation of autoantibodies that can bind to the normal cell components and provoke attack by phagocytic cells.

There is also abundant evidence that oxygen species ($O_2^{\cdot-}$, $HO^{\cdot}$, H_2O_2) contribute to the tissue injury associated with inflammatory disorders such as rheumatoid arthritis [608, 609]. The disease is accompanied by disturbance in the body's iron metabolism. Gutteridge [610] has reported that a large decrease in iron concentration in plasma of patients suffering from rheumatoid arthritis is observed. The decrease in plasma iron results from a decrease in hemoglobin concentration and is accompanied by the increased depositon of protein containing Fe ions in the synovial membranes, where iron is mainly present as ferritin. Superoxide anion radical released in the phagocytosis process is able to liberate Fe from ferritin. Similarly hemoglobin may be degraded by H_2O_2 and release iron ions.

The iron ions may be involved in the Fenton reaction generating $HO^{\cdot}$, which degradates hyaluronic acid. This free radical theory of rheumatoid arthritis finds confirmation in some reports showing that drugs used in anti-inflammatory therapy are very efficient inhibitors of HO radicals, which results from their structure. It has been shown [565] that plasma of rheumatoid patients treated with aspirin (acetylsalicylic acid) contained higher concentrations of 2,3-dihydroxybenzoate than control groups, consuming aspirin. This derivative is formed during interaction of the HO radical with the aromatic ring of aspirin.

5.3.2.5
Oxidative Stress Diseases

Of particular importance is damage exerted in endothelial cells subjected to oxidative stress. Oxidative stress results from imbalance between reactive oxygen species generation and antioxidant defences caused, for example, by lowering of vitamin concentrations (ascorbate, vitamin E), reduced glutathione or when generation of oxygen species or the amounts of macromolecules susceptible to oxidative damage are increased. The oxidative stress state causes perturbation of the cell metabolism, DNA and protein damage, NAD^+ depletion and excessive intracellular Ca^{2+} release [589, 590].

There exists evidence that oxidative stress plays a role in tissue damage associated with diabetes [611, 612] and ageing [613–619]. The increased oxidation has been considered to be a result of an increase in redox catalysts, e.g. Cu^{2+} ions and/or compounds having oxidant properties or generating free-radicals, for example, monosaccharides (glucose). Some evidence consistent with this suggestion has been reported by Nath et al. [614]. They have observed an increase of Cu^{2+} concentration associated with ageing in lens and in idiopathic cataract. (Cataract, i.e. lens opacity, occurs in humans frequently after the sixth decade, and results from cross-link and aggregation crystallins containing mainly lens proteins formed by free radical reactions.) Both Cu^{2+} ions and autoxidation of monosaccharides are able to generate H_2O_2 and $HO^{\cdot}$ [615], thereby causing protein fragmentation and conformational changes. In vitro

studies carried out on protein exposure to glucose in a glycosylation model of diabetes mellitus and ageing have shown that the enhancement of fragmentation of protein, observed in the presence of Cu^{2+}, is dependent on $HO^{\cdot}$ generation [616].

5.3.2.6
Ageing and Related Diseases

The free radical theory of ageing has been widely considered by several scientific groups. They postulate that ageing results from random damage to tissues, caused by free radicals generated in the process of normal aerobic metabolism. The antioxidant ability of many tissues are not able to protect the cell against increased production of oxygen radicals. Moreover, Halliwell and Gutteridge [565] suggest that protective mechanisms acting in a cell can only cope with 99.9% of free radicals generated during the normal metabolism. The remaining amount of free radicals (0.1%) involve very slow progressive damage during all human life. The most convincing evidence of the increased formation of free radicals during ageing is enhanced accumulation of intracellular pigment known as lipofuscin showing green-yellow fluorescence. Lipofuscin is considered by a number of scientific groups to be adducts formed by the condensation of lipid aldehydes (generated during peroxidation) with primary amines, proteins or other compounds having amino groups. They accumulate in tissue, notably in brain, as this organ has a high content of fatty acids very susceptible to peroxidation, and certain parts of the human brain contain a large amount of non-heme iron [620]. Accumulation of this pigment in neurons during ageing may have drastic consequences.

It has also been suggested that oxygen species might contribute to the process of delimitation in multiple sclerosis [621]. Increased production of $O_2^{\cdot-}$ in the blood of patients suffering from multiple sclerosis has been reported [622].

Other evidence of the importance of oxygen free radical reactions in some diseases has been provided by Bolli et al. [623]. They have reported that the increased generation of the radicals occurs in angina and myocardial stunning.

Myocardial necrosis and contractile failure following the administration of large doses of catecholamines are considered to be mediated by short-lived oxygen metabolites generated during autoxidation of catecholamines.

5.3.2.7
Emotional Distress

Emotional painful stress releases high concentrations of catecholamines. Catecholamines are able to produce $O_2^{\cdot-}$ radicals which, in turn, form other unstable products of oxygen metabolism including $HO^{\cdot}$ and $^1O_2$ [56–59, 116–118]. These species may promote peroxidation of membrane phospholipids following permeability changes in the membrane and intracellular calcium overload. This leads to depletion of high-energy phosphates as a result of acti-

vation of calcium dependent ATP-ases and impairment of mitochondrial energy release, and finally to cardiomyopathy [623].

It is worth mentioning here that lipid peroxidation caused by oxygen radicals also plays an important role in the pathogenesis of gastrointestinal diseases induced by ischaemia-reperfusion and various kinds of stress [619].

Several diseases and clinical conditions other than the above-mentioned, such as hemolytic disease, malaria, and porphyria involving the participation of oxygen radicals have also been suggested. For details see [565 and references therein].

6 Possible Useful Roles of Reactive Oxygen Species

We have already seen postulated in Chap. 5 that a great amount of damage to cell constituents involves reactive oxygen species. In the present chapter we will mention and discuss some major and well known examples showing useful roles.

6.1 Oxygen Free Radicals

It is well known that reactive oxygen species play an essential role in the control of cell functions. Although our knowledge about the useful role of oxygen radicals is limited, we can find in the literature a number of data suggesting the involvement of free radical reactions in normal physiological processes for maintenance of homeostasis in tissues.

The controlled generation of oxygen species during cellular metabolism may be summarized as follows.

1. Electron transport respiration and active site of cytochrome P-450 [624, 625].
2. The synthesis of cellular messengers such as prostaglandins [626, 627], thyroxine [628, 629] or leukotrienes [630].
3. The synthesis of deoxyribonucleotides [631], prothrombin and the blood coagulation factors VII and IX with participation of vitamin K_1 (phylloquinone) [632, 633].
4. Participation in post-translational protein turnover [634].
5. Defensive systems of cells against invading microorganisms [635, 636].
6. Activity of some enzymes, e.g. peroxidases, dioxygenases, hydroxylases, pyruvate metabolizing enzymes [637, 638].
7. Participation in development and differentiation [639, 640].
8. Membrane potential generation [641, 642].
9. Metabolism of xenobiotics and toxins [643].
10. Metabolism of ethanol [565].
11. The wound response of plant tissues [644].
12. The synthesis of lignin [645] and melanin [646].
13. The control of vascular tone [647, 648].
14. Modulation of secondary messengers, e.g. cyclic GMP [649, 650].

15. The activation of human platelets [651].
16. The oxidative degradation of humic acids [652].

The main role in regulation of the level of oxygen free radicals involved in normal physiological processes is due to antioxidant enzymes.

6.2 Singlet Oxygen

Generation of $^1O_2$ in the chemical way as well as during photosensitization reactions is sometimes useful in medicine and industry. The best established uses are reported below.

6.2.1 Phagocytosis

Oxidative killing of invading microorganisms, for example bacteria by cells of the blood, is covered by phagocytosis. Although the exact mechanism of the microorganisms killing is not known in detail, several scientific groups have reported a crucial role for oxygen species such as $O_2^{\cdot-}$, H_2O_2, $HO^{\cdot}$ and $^1O_2$ in this process.

There are two different cell types able to protect organisms against foreign bodies: first polymorphonuclear leucocytes, i.e. cells possessing a multilobed nucleus and relatively short life span (less than one day), and then the mononuclear leucocytes which have a single nucleus and are longer living. A mononucleotide in the blood stream finally becomes a tissue macrophage. Phagocytic cells play several functions such as recognition of the invading organism, killing and removing of foreign bodies.

During phagocytosis, polymorphonuclear leucocytes, mononuclear leucocytes and macrophages show a sudden increase in the oxidative metabolism which is called the respiratory burst. The leucocytes can leave the blood circulation and accumulate in large numbers at a site of infection, whereas macrophages are gathered in the tissues or suspended in tissue fluids. All these cell types can recognise the ingested microorganisms [653].

Several stages may be distinguished during the phagocytosis (Fig. 16). The first is recognition of bacteria by the phagocytic cell. Some foreign cells are bound to the surface of polymorphonuclear leucocytes, while others have to be coated with serum proteins of the microbicidal system (opsonins). This process is called opsonization. The mechanism of the recognition of the foreign particles has been discussed in several papers [654–666]. Such microorganisms ingested by phagocytic cells are encircled by part of an inverted plasma membrane, and so-called pseudopodia are formed. At this time the increase of the lysomal enzymes activity is observed. The consumption of oxygen is enhanced 10- to 15-fold within a few seconds of contact with invading particicles [667] and a cycle of the chemical reaction is started. For example, an increase in hexose-monophosphate, HMP, shunt activity, which metabolizes glucose to glucose-6-phosphate and further to 6-phosphogluconate using NADPH or NADH as a sub-

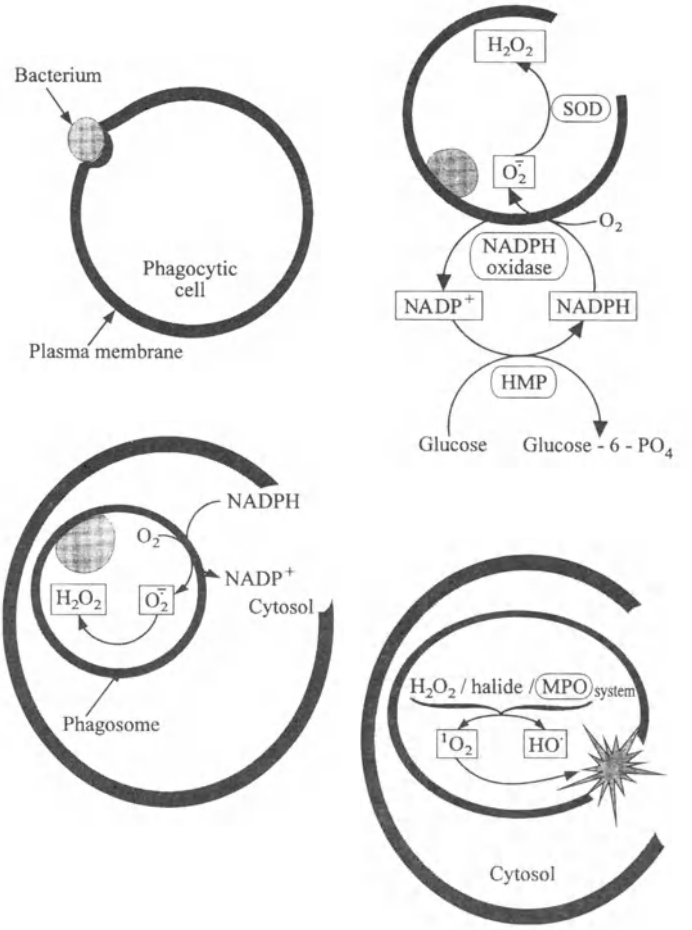


Fig. 16. Intracellular killing of microorganisms

strate, is observed. In the case of NADPH the reaction occurs by a transfer of electrons from NADPH to O_2. The first product from the toxic oxygen species group are large amounts of O_2^- generated by NADPH oxidase [557,668]. Both the spontaneous and the SOD-catalyzed recombination of O_2^- can lead to H_2O_2 production (see reaction at Eq. (118)). It has been found that concentration of H_2O_2 in the extracellular medium may reach the value of 0.6 mmol $\cdot \ell^{-1}$ or higher during the phagocytosis [667].

Further, during the phagocytosis, the pseudopodia completely encloses the bacteria inside the plasma membrane, forming the phagocytic vacuole, which flows in cytosol and is called a phagosome.

The cycle of the chemical reactions with participation of the above-mentioned enzymes is continued. The next stage of the phagocytosis is a connection of phagosome with granules containing lysosomal enzymes and proteins and their deliverance into the phagosome. This process is accompanied by genera-

tion of new oxygen species such as $HO^{\cdot}$ and $^1O_2$ having very strong bactericidal capabilities. The studies performed with polymorphonuclear leucocytes isolated from human and animal blood have shown that these toxic oxygen species are the most potent agents for microbe killing [669].

It has been established that the main reactions responsible for the $^1O_2$ generation during phagocytosis are as follows.

- The reaction of H_2O_2 with hypochloride ion (ClO^-) catalyzed by a peroxidase, one of the principal lysosomal enzymes located in polymorphonuclear leucocytes called myeloperoxidase (MPO). (Fig. 16) [102, 144, 157, 670–672].
- The interaction between $O_2^{\cdot-}$ and H_2O_2 (the Haber-Weiss) reactions at Eqs. (31) and (32)).
- The direct oxidation of $O_2^{\cdot-}$ to $^1O_2$ as shown by reaction at Eq. (59).

The physiological concentration of Cl^- ions in serum and in polymorphonuclear leucocytes was reported by Krinsky [157, 673] to be about 100 mmol $\cdot \ell^{-1}$. The reactions given in Fig. 16 besides the $^1O_2$ generation may also cause halogenation, proteolysis and decarboxylation of bacterial walls [673]. These reactions generate lipid peroxides, toxic aldehydes and chloroamines, which can participate in the microorganism killing.

6.2.2
Photodynamic Therapy

6.2.2.1
Tumours and Viruses

The photodynamic effect, besides its damaging interaction with biomolecules, finds applications in the photodynamic therapy of tumours. A number of dyes act as photosensitizers generating $^1O_2$ via the Type II mechanism and oxidation of a substrate is performed. Identification of $^1O_2$ as the cytotoxic agent in photoinactivation of tumours is well documented [674–684]. The increasing interest in the application of the photodynamic effect in the photodynamic therapy of tumours is currently the subject of intense research on new sensitizers. The potential candidate for biological photosensitizer must fulfil the following six criteria:

- specific accumulation in tumour tissues;
- a lack of cytotoxicity;
- to be a hydrophobic compound;
- to have expanded system of π-electrons enabling it to show a strong absorption in the long wavelengths region of the spectrum;
- the absorption band of the sensitizer should not overlap absorption bands of other chromophores occurring in tissues;
- to have a high value of the intersystem crossing ($^1S_1 \rightarrow {}^3S_1$) yield.

These criteria are fulfilled, for example, by compounds containing the tetrapyrrole unit, such as naphthaloprophyrins, phthalocyanines, naphthalocyanines, bacteriochlorins, purpurins [685, 686]. Porphyrins and their analogues as well

as metallo-derivatives are very often used in photodynamic therapy [683, 687]. Porphyrins are efficient sensitizers for the photodynamic generation of $^1O_2$ and they are compounds occurring in skin or tumours. The use of hematoporphyrin derivatives as effective agents in tumour phototherapy relies on their increased concentration in tumour cells over that in normal cell [688]. The maximal quantum yield of $^1O_2$ generation for some metallic derivatives of hematoporphyrin has been found to be 50% [689]. Antrapyrazoles and phthalocyanines are more reactive as photosensitizer compounds in comparison with the hematoporphyrin derivative in $^1O_2$ production [686]. They are used in treatment of several kinds of tumours. Furocumarin derivatives (psoralens) are used for treatment of psoriasis and other types of skin diseases [677].

Porphycene and its derivatives, absorbing in the 320–400 nm region, also show chemical and photophysical properties superior to the those of the hematoporphyrin derivatives [676]. Phototherapy is a valuable treatment for the local control of different kinds of human tumours as well as helpful in their surgery [681]. It should be mentioned that phototherapy can show significant side effects such as cutaneous photosensitivity lasting even one month, and patients must avoid exposure to sunlight [690].

The Type II mechanism of photosensitized oxidation also finds application in inactivating viruses in blood and red cells [691] (see Chap. 5). Hematoporphyrin derivatives, such as aluminium phthalocyanine derivatives, because of the red-shift of their absorption spectra relative to the absorption maximum of hemoglobin that eliminates the site effect, i.e. phototoxicity, are very good sensitizers in this therapy [692]. In recent years activation of hematoporphyrin and its derivatives by ultrasonics has been found to occur and has been reported to be useful in the treatment of cancer in the $^1O_2$ generation way [693 and references cited therein].

Hypericin dye has also been found to be very efficient in the photodynamic therapy of cancer [694].

6.2.2.2 *Newborn Jaundice*

Newborn infants, particularly those born prematurely, are subject to jaundice which is associated with the lack of glucuronyl transferase, which converts the liposoluble bilirubin into the water soluble conjugate with glucuronic acid. The lack of the enzyme means that excess bilirubin can be stored in the skin and brain. The treatment of neonatal jaundice consists of application of the Type II reaction in which bilirubin plays two roles simultaneously – sensitizer and $^1O_2$ acceptor. The irradiation of the infant with light of wavelength $\lambda \approx 450$ nm corresponding to the bilirubin maximal absorption causes an electronic excitation of the bilirubin molecule. Singlet oxygen formed in an energy transfer process from the excited triplet state of bilirubin to molecular oxygen acts as a strong oxidant at a high rate constant of about $10^9\ \ell \cdot mol^{-1} \cdot s^{-1}$ causing destruction of bilirubin to degradative products soluble in water [694].

The phototherapy of jaundice does not involve additional photodynamic damage because the rate constant value of the interaction of bilirubin with $^1O_2$ is about 1000 times greater than that of cholesterol [695].

6.2.3 Industrial Application

The Type II mechanism of photooxidation can be used for removing low amounts of oxygen from closed atmospheres. Low level oxygen atmospheres are used in order to decrease deterioration of packaged foods, oxidation of essences and chemicals, in chemical reactors and vessels or for anaerobic microbiology [696].

For example, the method proposed for removing oxygen from transparent packages proposes generation of $^1O_2$ in a polymeric film such as ethyl cellulose or cellulose acetate containing a photosensitizer and acceptor of $^1O_2$. Both compounds are dissolved and immobilized in the film. Illumination of the polymeric film generates $^1O_2$ according to reactions presented in Fig. 13, which reacts with the acceptor, thereby being consumed.

Also, singlet oxygen generated in the dark during the reaction of *N*-chloro-4-hydroxy-2,2,6,6-tetramethylpiperidine with sodium perborate can be used as an oxidative bleaching agent [697]. This strong oxidant shows the capability for destruction of many organic pollutants and has been used in advanced oxidation processes for wastewater treatment (see Chap. 8).

7 Cell and Tissue Mechanisms of Protection Against Oxygen Free Radicals and Singlet Oxygen Damage

Organisms are equipped with effective defense systems preventing damage to cell constituents by reactive oxygen species. Cells and tissues are protected by a multiplicity of antioxidants mechanisms.

The question of how the cell protects itself against oxygen species damage has attracted a great deal of attention which has generated numerous reports. It is beyond the scope of this chapter to give a full review, and we shall therefore restrict ourselves to the main enzymatic systems and endogeneous antioxidants. Protectors vary from complex enzyme systems to low molecular weight oxygen free radicals and $^1O_2$ scavengers [698–701].

7.1 Protection by Enzyme Systems

Enzymes are the most efficient catalyst known in chemistry. Almost all physiological reactions are catalysed by enzymes, which accelerate the rate of reactions by factors of 10^6–10^{12}. Enzymes lead to rapid attainment of equilibrium under natural conditions. Enzymes are mainly proteins, exhibiting high specificity, i.e. selectivity for a single substrate and the ability to be controlled. Two classes of substances, coenzymes and prosthetic groups, participate in reactions catalyzed by enzymes. They are non-protein compounds synthesized from vitamins. Coenzymes do not remain permanently bound to the enzyme and they are reduced, acylated or phosphorylated in a cell. They exist in high concentrations in cells. At this time coenzymes become substrates for intracellular enzymes, which reverse these processes. For example, coenzymes nicotinamide nucleotides (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) exhibit a redox function undergoing reduction in the two-electron ways.

A prosthetic group is a non-protein compound of an enzyme which remains associated with enzymes for a reaction cycle. It is sometimes covalently linked to proteins or less tightly bound and easily removable from the enzymes.

The enzymatic cellular defence line is presented schematically in Fig. 17.

The defence mechanism includes the following main enzymes.

- Cytochrome oxidase system,
- Superoxide dismutases,
- Catalases,

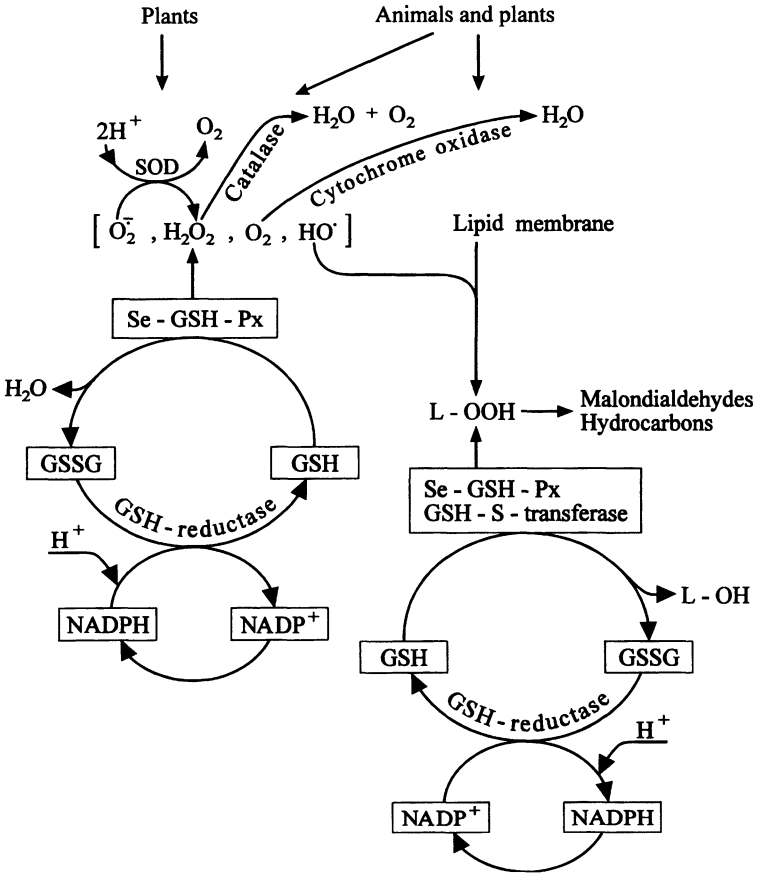


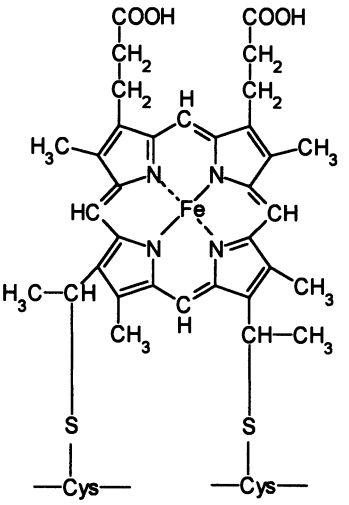
Fig. 17. Simplified scheme of the cellular enzymatic defence mechanisms. Superoxide dismutase (SOD) catalyses dismutation of O_2^-; catalase reduces H_2O_2 to water; cytochrome oxidase reduces molecular oxygen to water; selenium-dependent glutathione peroxidase (Se-GSH-Px) reduces H_2O_2 and organic peroxides using glutathione (GSH) as a hydrogen donor; glutathione reductase regenerates GSH from its oxidized form (GSSG) in the presence of nicotinamide adenine dinucleotide phosphate (NADPH)

- Peroxidases,
- Ceruloplasmin, transferrin.

7.1.1 Cytochrome Oxidase System

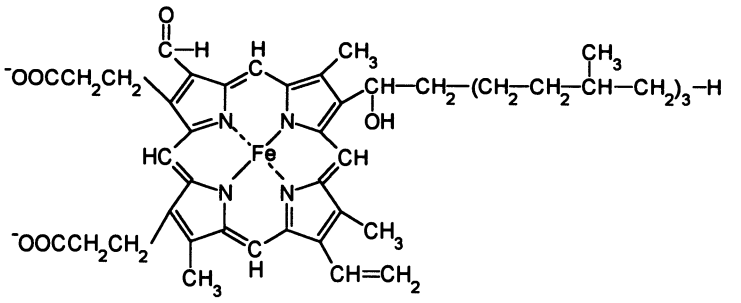
Cytochromes are respiratory pigments occurring in all aerobic cells. They are a group of proteins containing iron and transferring electrons. Mammalian cytochromes are of three types: cytochromes a, b and c. They differ in the structure of their prosthetic groups, although they are always a type of heme. Four of the six ligands to the iron ion come from protoporphyrin and two from protein. The last ligands are usually histidine. During the catalysis the heme of cytochromes

undergoes reversible oxidation ($Fe^{3+} \overset{e^-}{\rightleftharpoons} Fe^{2+}$), but the redox potential is different in different cytochromes. In cytochromes a and b the prosthetic group is heme which is bound non-covalently to protein, whereas cytochromes c have the same prosthetic group but linked by thioether bonds to cysteine side-chains in the protein.



Cytochrome c

It is stated that fifth and sixth coordination positions of Fe ion in cytochrome c are occupied by histidine and methionine residues. The prosthetic group of a-type cytochromes (a, a_3) has one formyl group -CHO on C-8 and long hydrophobic 15-carbons farnesyl side chain on C-2. This group is known as heme A.



Heme A

Cytochromes a are called cytochrome oxidase. All cytochromes occur in the mitochondrial electron transport chain, and b-types of cytochromes also occur in microsomal and mitochondrial monooxygenase systems and in leukocytes. Oxidized forms of cytochromes a and a_3 can take electrons from the reduced

forms of cytochromes c thus being transferred back to the reduced form with ferrous ion. The ion is next oxidized by molecular oxygen to the Fe^{3+} state.

Copper atoms can also act as electron carriers in proteins and form the prosthetic group of a number oxidases.

Cytochrome oxidase system prevents the release of O_2^-, H_2O_2 and $HO^{\cdot}$ radicals into a cell by tetravalent reduction of molecular oxygen to water. The majority of oxygen reduction by aerobic organisms is carried out by cytochrome oxidase [702].

7.1.2 Superoxide Dismutases

The excess of superoxide anion radicals in the cell is removed by superoxide dismutases (SODs) (E.C.1.15.1.1, molecular weigh about 30000). SODs are present in both the cytoplasm and the mitochondria of most aerobic cells keeping the O_2^- concentration at low levels. SODs catalyze the dismutation of O_2^- into O_2 and H_2O_2 according to reactions at Eqs. (117) and (118) [703]. Numerous reports have been published since 1969, when McCord and Fridovich discovered SOD [704], showing that the catalytic property of SODs results from the presence of metal ions at each active site. In eukaryotic cells of plants, animals and yeast three forms of SODs are present: the Cu Zn SOD in cytoplasm, a Mn-dependent one in the mitochondria matrix, and an extracellular SOD [705]. The SODs contain two protein subunits each of which contains one Cu^{2+} and one Zn^{2+} ion. The Zn^{2+} ion does not show the catalytic property but it stabilizes the enzyme.

Manganese containing SODs (Mn SODs) catalyse the same reaction as do the CuZn SODs, but they are not so resistant to heating or chemicals acting as CuZn SODs. The Mn SODs extracted from higher organism contain four protein subunits each of which contains 0.5 – 1.0 Mn ion, while the bacterial enzymes possess only two subunits. The third type of SOD enzymes isolated from bacteria and a few higher plants such as tomato, mustard or water lily, contain Fe ion at their active sites. Fe SODs have not been reported to occur in animal tissues. The Fe-containing SODs hold two protein subunits and one or two Fe^{3+} ions per molecule of the enzyme. During the cycle the Fe^{3+} ions are reduced by O_2^- to the Fe^{2+} state, as are the above-mentioned SODs containing Cu^{2+} ion [565]. The catalytic activity of Mn SODs and Fe SODs decrease at pH > 7. The Fe SODs and Mn SODs contain similar sequences of amino acids in their molecules, while differing from CuZn SODs. It is worthwhile mentioning that concentrations of SODs in human liver is especially high in comparison to concentration in other human tissues.

Similarly, animal liver also contains the highest concentration of SODs compared to other organs. Hemoglobin also contains relatively high concentration of SODs (about 260000 molecules of the enzyme per red blood cell, i.e. $\approx 460\ \mu g \cdot g^{-1}$) [706].

Superoxide dismutases scavenger O_2^- radicals with great efficiency. The rate constant of dismutation of O_2^- in the presence of the enzyme is about 10^4-fold greater than that of the spontaneous dismutation [565].

Numerous studies in vitro and in vivo have supported the protective role of the SODs against tissues damage. For example, Nilson et al. [294] have reported

that treatment of rabbits with SOD before ischaemia and reperfusion decreased radical formation by 85% compared to the untreated control group.

7.1.3 Catalases

Catalases occur in most aerobic cells. All major animal tissues and organs contain catalase. High amounts of the enzyme are contained in erythrocytes and liver (100–1500 catalase activity units per milligram of protein), whereas in skeletal muscle, brain or in heart the enzyme concentration is much lower (30–50 enzyme activity units per milligram of protein).

The enzyme consists of four protein subunits containing a heme (Fe^{3+}) as the prosthetic group bound to its active site [565], and usually a molecule of NADPH which stabilizes the enzyme. Catalase and SODs may act synergistically when they are present at the same time in a cell [707] because catalase catalyzes the decomposition of H_2O_2 produced in a cell, for example, during SODs catalyzed dismutation of $O_2^{\cdot-}$ radicals [708, 709]:

$$E - Fe^{3+} - OH^- + H_2O_2 \rightarrow E - Fe^{3+} - OOH^- + H_2O \quad (232)$$

$$E - Fe^{3+} - OOH^- + H_2O_2 \rightarrow E - Fe^{3+} - OH^- + O_2 + H_2O \quad (233)$$

Catalase may also act in peroxidase-type reaction when concentration of H_2O_2 is low and electron donors are present in a medium, e.g. as alcohols or formate [710–714] (for this type of reaction see below).

Another type of catalase also important in protection against H_2O_2, is the so-called "pseudocatalase". The enzyme consists of six protein subunits each of which contains one Mn^{3+} ion and has a relative molecular mass of 172000 [565]. Pseudocatalase occurs in several microorganisms, e.g. streptococci. The following reactions have been postulated to be responsible for H_2O_2 decomposition by this enzymes [565]:

$$E - Mn^{3+} + H_2O_2 \xrightarrow{2H^+} E - Mn^{5+} + 2H_2O \quad (234)$$

$$E - Mn^{5+} + H_2O_2 \longrightarrow E - Mn^{3+} + 2H^+ + O_2 \quad (235)$$

7.1.4 Peroxidases

The most important antiperoxidative bioprotector occurring in most organisms is glutathione peroxidase (GSH-Px), (GSH:H_2O_2 oxidoreductase, E.G. 1.11.1.9.), discovered in 1957 by Mills [715], an enzyme of molecular weight about 80000.

Two major GSH-Px activities have been recognized: a selenium-dependent GSH-Px which removes H_2O_2 and organic hydroperoxides, and a selenium-independent GSH-Px which utilizes organic peroxides, decreasing the propagation stage of the lipid peroxidation [716, 717]. The enzyme converts lipid hydroperoxides to the less toxic lipid alcohols (Fig. 17). This is a widespread

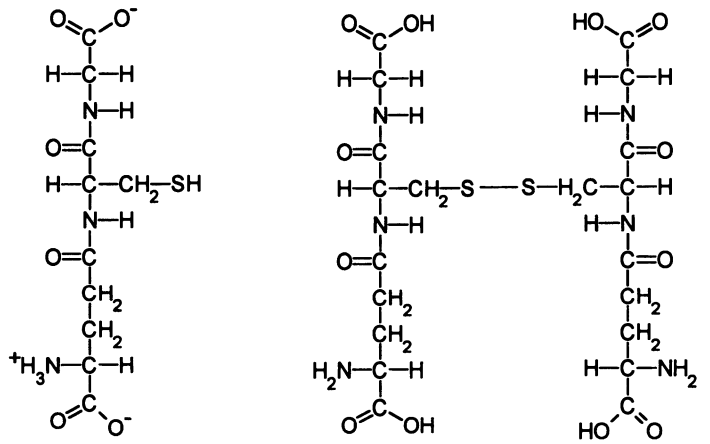
enzyme present in human and animal tissues, in plants, and in some bacteria at concentrations ranging from 1 $\mu mol \cdot g^{-1}$ to 10 $mmol \cdot g^{-1}$.

Glutathione peroxidase consists of four protein subunits, each of which contains one atom of selenium at its active site, probably incorporated into molecules of cysteine, replacing their sulfur atoms. The enzyme uses the low-molecular-thiol containing tripeptide (glutamic acid-cysteine-glycine), glutathione (GSH), as a hydrogen donor:

$$2GSH + H_2O_2 \rightarrow G\text{–}S\text{–}S\text{–}G + 2H_2O \qquad (236)$$

Glutathione undergoes oxidation to unreactive forms GSSG. In this case two molecules of GSH are connected with participation of oxidized-SH groups of cysteine, and a disulfite bridge (–S–S–) is formed:

Glutathione



reduced form oxidized form

Because the ratios of GSH/GSSG in normal cells in kept rather high (10:1), it means that glutathione must be regenerated in tissues. Probably glutathione is regenerated by NADPH in the reaction catalyzed by glutathione reductase (E.C. 1.6.4.2):

$$GSSG + NADPH + H^+ \rightarrow 2GSH + NADP^+ \qquad (237)$$

Tissues are supplied in NADPH from the oxidative pentose phosphate pathway [565]. The first reaction of this pathway is an enzymatic dehydrogenation of glucose-6-phosphate by glucose-6-phosphate dehydrogenase (E.C.1.1.1.49.) with generation of 6-phosphogluconate:

$$\text{glucose-6-phosphate} + NADP^+ \rightleftharpoons \text{6-phosphogluconate} + NADPH + H^+ \qquad (238)$$

The latter product undergoes oxidative decarboxylation and the process is controlled by the supply of $NADP^+$ to glucose-6-phosphate dehydrogenase:

$$\begin{aligned} &\text{6-phosphogluconate} + NADP^+ \rightarrow \\ &\quad \rightarrow CO_2 + NADPH + H^+ + \text{D-ribose-5-phosphate} \end{aligned} \tag{239}$$

Both glutathione peroxidase and catalase contain hematin (Fe^{3+} protoporphyrin IX) as prosthetic group, and they commonly occur in animal tissues where they catalyze similar reactions.

If H_2O_2 is produced in cells in physiological conditions this oxidant is probably removed by GSH-Px, but under pathological conditions the excess H_2O_2 is decomposed by catalase, according to reactions at Eqs. (232) and (233).

For most peroxidases the decomposition of H_2O_2 can be described by the following reactions [717]:

$$E - Fe^{3+} - OOH^- + DH_2 \rightarrow E - Fe^{3+} - O^- + DH^\cdot + H_2O \tag{240}$$

$$E - Fe^{3+} - O^- + DH_2 \rightarrow E - Fe^{3+} - OH^- + DH^\cdot \tag{241}$$

$$2DH^\cdot \rightarrow DH_2 + D \tag{242}$$

where the $E - Fe^{3+} - OOH^-$ substrate is the product of the reaction at Eq. (232), and DH_2 is the substrate.

As it was mentioned above, liver contains high concentrations of both enzymes; catalase occurs mainly in the peroxisomes, whereas GSH-Px is found in the cytosol and in the matrix of mitochondria. Depending on the place of H_2O_2 generation, this oxidant will be removed by the enzyme occurring at the higher concentration. The role of GSH-Px in protecting the liver against peroxidation has been substantiated by Sies and Summer [718].

It is worthwhile mentioning a little about the element selenium which shows intermediate properties between a metal and a non-metal. This "bio-element" has been shown to protect against damage caused by heavy metals such as Hg, Cd or Ag [719], has been used against cancer and infection [720–723], and yet an excess of selenium is toxic.

It has been reported that the indispensability of selenium in organisms results from the function of GSH-Px as an antiperoxidative bioprotector in the organism [724, 725]. A diet poor in selenium and inhibition of GSH-peroxidase and glucose-6-phosphate dehydrogenase cause tremendous susceptibility of erythrocytes to damage as reported by Bus and Gibson [716]. It is commonly accepted that glutathione peroxidase, glutathione reductase and glucose 6-phosphate dehydrogenase form an integrated antioxidant system in a cell.

Other GSH-dependent peroxidases have also been recognized, such as a GSH-dependent vitamin E free radical reductase [726]. This enzyme converts oxidized vitamin E to the non-oxidized form.

The next heme-containing enzyme is cytochrome-c peroxidase (Cyt-c-Px). The enzyme removes excess H_2O_2 by the rapid formation of an enzyme-H_2O_2

complex, which undergoes a decomposition regenerating the enzyme by cytochrome-c(Fe^{2+}) [565]:

$$\text{cyt c-Px-}H_2O_2\text{ complex} + 2\text{cyt c }(Fe^{2+}) \rightarrow \rightarrow \text{cyt c-Px} + 2\text{cyt c }(Fe^{3+}) + 2HO^- \quad (243)$$

The enzyme occurs in yeast mitochondria and in some bacteria.

Several bacteria also contain a peroxidase which oxidizes NADH into NAD^+ using H_2O_2 (so-called NADH peroxidase). Other examples of peroxidases are horseradish peroxidases occurring in roots of the horseradish plant, lactoperoxidase found in milk, and myeloperoxidase occurring in phagocytic cells (see Chap. 6). Both enzymes can catalyze oxidation of glutathione by H_2O_2.

The next group of known peroxidases, chloroperoxidase and bromoperoxidase, also decompose H_2O_2 like the peroxidases described above, and they also catalyze an introduction of halogen atoms and halide ions into substrates in the presence of H_2O_2 [565]:

$$S\text{–}H + X^- + H_2O_2 + H^+ \rightarrow S\text{–}X + 2H_2O \quad (244)$$

where X^- denotes the halide ion, and S–H is the substrate.

It is worthwhile mentioning ascorbate peroxidase, contained in chlorplasts of higher plants, which also catalyses H_2O_2 decomposition to water:

$$\text{ascorbate} + H_2O_2 \rightarrow \text{dehydroascorbate} + 2H_2O \quad (245)$$

7.1.5 Ceruloplasmin, Transferrin

Human plasma shows powerful antioxidant properties [727, 728] as it contains numerous high and low molecular weight compounds showing redox activity and able to react with organic and inorganic oxygen radicals [729]. Among high molecular weight plasma antioxidants, ceruloplasmin is a copper-containing oxidase of the α_2-globulin fraction of mammalian blood plasma (ferro-O_2-oxidoreductase, E.C.1.16.3.1). Ceruloplasmin is synthesized by the liver.

Three important functions of ceruloplasmin are recognized: transport of copper out of the liver, transformation of iron ion from the Fe^{2+} state into the biologically inactive Fe^{3+} state with concomitant reduction of O_2 to H_2O, and oxidation of biogenic amines in the central nervous system [730]. Ceruloplasmin also catalyzes the dismutation of O_2^- [731]:

$$4O_2^- + 4H^+ + O_2 \rightarrow 4O_2 + 2H_2O \quad (246)$$

The next important enzyme occurring in plasma is transferrin. The enzyme is an iron-transporting antioxidant, which is saturated with iron only in the disease state. Transferrin behaves as an iron chelator since its antioxidant property is proportional to the unsaturated fraction of the enzyme. This enzyme is a natural iron chelator which prevents free radical generation with metal ion participation [729]. For example, iron released during hemoglobin degradation is removed and attached to transferrin for transport to storage sites of the cell.

7.2 Protection by Low Molecular Weight Compounds

Protective mechanisms, besides the above – mentioned enzymatic ones, can be divided into hydrophobic ones (working in those areas of a cell in which thermodynamic factors are responsible for the separation of hydrophobic non-polar groups away from water) and hydrophilic ones, which control generation of oxygen species in the water or ionic regions of a cell. This raises the question of what properties the compounds involved in the protection against oxidative damage should have. In order to answer this question, we should consider mechanisms in which these compounds will be involved. Compounds present in cells, inhibiting or slowing down oxidation of biosubstrates, are called "antioxidants". They act in different ways and they may be or may not be consumed during oxidation processes [565]. The main mechanisms of antioxidant action are:

- removing excess molecular oxygen from a cell
- scavenging HO radicals of very common initiators for the oxidation of biological important compounds to lipid peroxides
- decomposition of lipid peroxides to unreactive products such as alcohols
- chelating metal ions, which protect against generation of oxygen toxic species, e.g. HO, RO_2, RO radicals
- scavenging intermediate radical products, e.g. $ROO^{\cdot}$, $RO^{\cdot}$ in order to break the chain reactions of the oxidation process
- scavenging singlet oxygen

Many compounds working as antioxidants show multiple protection mechanisms. Compounds which repair damage to biomolecules also play the role of antioxidants. Compounds which may exhibit controlling ability in hydrophobic regions are mainly carotenoids and tocopherols.

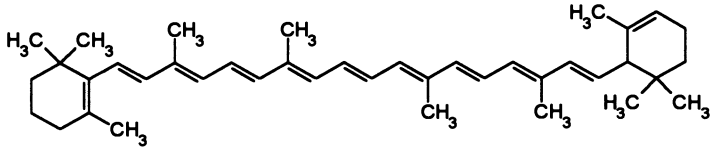
7.2.1 Carotenoids

Carotenoids are the most extensive group of compounds involved in the protection of living organisms against photo-sensitized oxygenation.

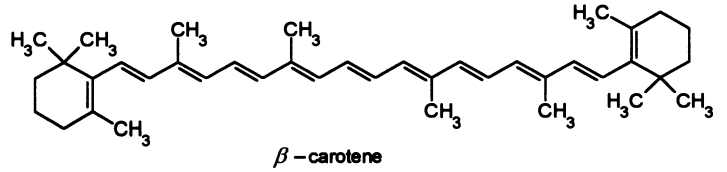
Fifty carotenoids are recognized, but the most important are α-carotene, β-carotene and γ-carotene. Carotenoid pigments are very effective quenchers of $^1O_2$ [316, 346, 731, 732]. They are very often used as diagnostic tools for the generation of singlet oxygen in chemical and biological systems, and knowledge of their rate constants for quenching of $^1O_2$ is very important.

Carotenoids are so widely distributed in nature that their yearly production has been estimated at about 10^8 tons [733]. For example, α-carotene occurs in carrots, tomatoes and the green leaves of many plants. This carotene is rapidly converted in the organism into vitamin A_1 and they are therefore called provitamins. Also, β-, and γ-carotenes, after oxidation in the organism, form vitamin A_1. The β-carotene is converted in the organism into two molecules of vitamin

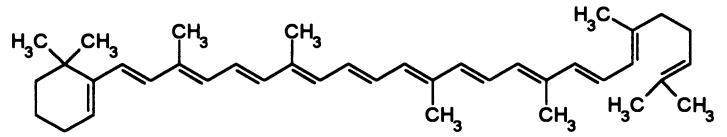
Carotenoids



α – carotene



β – carotene



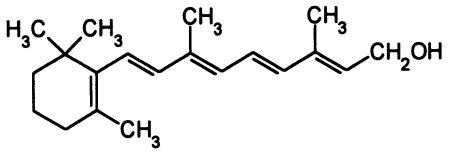
γ – carotene

A_1. The conversion involves oxidative cleavage in the centre of the β-carotene chain.

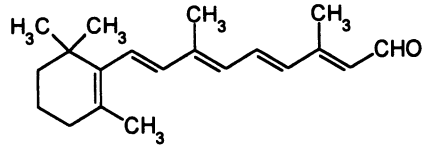
Vitamin A is a polyunsaturated alcohol also occurring in milk, butter, eggs and liver. More than 95% of vitamin A in plasma occurs as retinol. Retinol is transported to the tissues bound to a special kind of protein, and is reversibly converted to its aldehyde (retinal) by alcohol dehydrogenase.

Deficiency of vitamin A leads to the degeneration of mucus-secreting cells, cessation of growth, reproductive disorders, failure of bone remodelling or night blindness. In humans, vitamin A deficiency exerts the most obvious effects on eyes, causing irreversible corneal opacity.

Vitamin A



retinol



retinal

Carotenoids protect photosynthetic organisms from damage caused by their own excited states of chlorophylls, because these dyes are the most effective sensitizers. Naturally occurring carotenoids also protect non-photosynthetic organisms against other sensitizers. The most popular carotenoids showing extraordinary high quenching efficiency towards $^1O_2$ are the dyes containing nine or more conjugated double bonds such as β-carotene, lycopene, fucoxanthin, lutein, violaxanthin or neoxanthin.

Three mechanisms whereby carotenoids protect cell against photodynamic effect have been postulated [250 and references therein] (Fig. 18).

In two mechanisms, the energy transfer from the excited triplet state of a sensitizer ($^3S_1$), occurring in living organisms, e.g. chlorophyll, or from excited oxygen ($^1O_2$) to the carotene molecule, either protects the formation of $^1O_2$ (the first case) or causes its deactivation (the second case). These energy transfer reactions are diffusion controlled and should compete with other mechanisms. In both cases the electronically excited carotenoid is deactivated to its ground state and the excess of energy is released into the cellular environment.

In the third mechanism, carotenes are considered to be preferred substrates for $^1O_2$, being oxidized to a carotene-O_2 adduct, in spite of the higher rate constant of the carotene and $^1O_2$ reaction [346], in comparison to the reaction of $^1O_2$ with other biomolecules ($k \approx 1.5 \cdot 10^{10}\ \ell \cdot mol^{-1} \cdot s^{-1}$). These three mechanisms are responsible for the decrease of cell damage during both types of photodynamic reactions (Type I and Type II, see Chap. 5).

β-Carotene contains 11 conjugated double bonds and is located in lipid globules inside the chloroplast interthylakoid space. This compound is an integral component of photosynthetic reaction centres in all green plants and algae, and plays the role of a light-harvesting pigment and of a protector of photosynthetic apparatus against photodamage. It has been reported [734] that

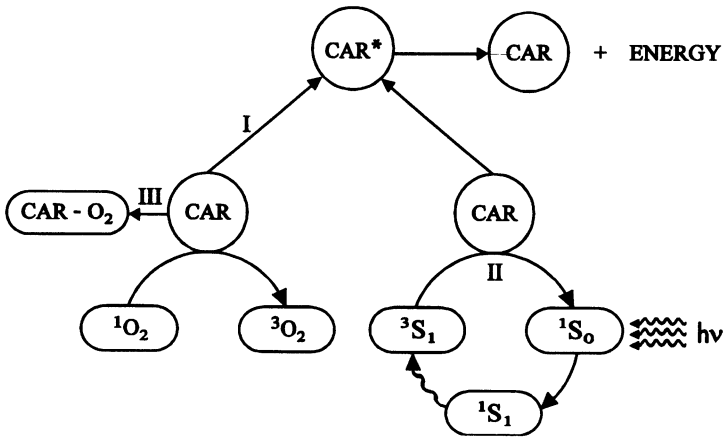
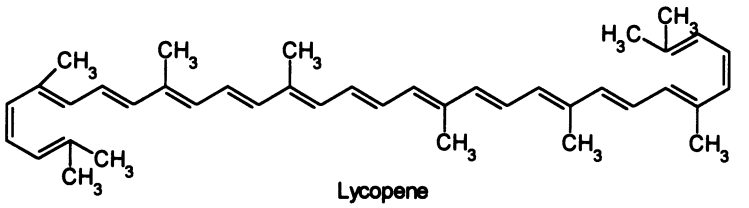


Fig. 18. Mechanisms of cell protection by carotenoid (CAR) pigments during photodynamic effect. $^1S_0$ is the ground state of a sensitizer, $^1S_1$ and $^3S_1$ are the excited singlet and triplet states of a sensitizer, respectively. *The asterisk* denotes the electronic excited state of CAR

β-carotene and canthaxanthin inhibit neoplastic transformation induced in certain cells (10T1/2) by chemical and physical factors, as well as β-carotene bound to the human lymphoid cells quenching $^1O_2$ [735]. Carotenoids have recently been reported to enhance gap junctional communication and to inhibit lipid peroxidation [736–738]. The β-carotene protects cells either by preventing the formation of $^1O_2$ or its deactivation [739, 740]. The most efficient biological carotenoid at $^1O_2$ quenching is lycopene [741]. Lycopene, the red dye, is an aliphatic hydrocarbon occurring in tomato and other plants. Its structure includes 11 conjugated π-bonds.



Lycopene

Epstein has reported that β-carotene can act as an anticancer agent, as he has observed the slower development of skin tumours in mice treated with β-carotene [742].

7.2.2
Tocopherols and Tocotrienols

Tocopherols are a group of fat-soluble compounds called vitamin E, first isolated from the oil of wheat-germ. At present eight compounds are recognized as exhibiting the effect of vitamin E, among which is α-tocopherol, the most active form of this vitamin group. Four compounds have a long aliphatic skeleton consisting of 16 carbon atoms (tocopherols α, β, γ and δ), and another four compounds (tocotrienols α, β, γ and δ) have three double bonds in their skeletons.

Tocopherols contain an aromatic ring system with the hydroxyl group and an isoprenoid side chain. α-Tocopherol differs from the other tocopherols by the presence of one more methyl group in the benzene ring and is localized in cellular membranes, being a major lipid-soluble antioxidant present at much higher concentrations than other antioxidants in plasma lipoproteins. This compound is readily oxidized and reduced, and prevents unsaturated lipids, particularly membrane lipids, from peroxidation by providing hydrogen atoms [743, 744]:

$$LOO^{\cdot} + Toc\text{–}OH \rightarrow LOOH + Toc\text{–}O^{\cdot} \quad (247)$$

where $LOO^{\cdot}$ denotes lipid peroxide radical, and Toc–OH is tocopherol.

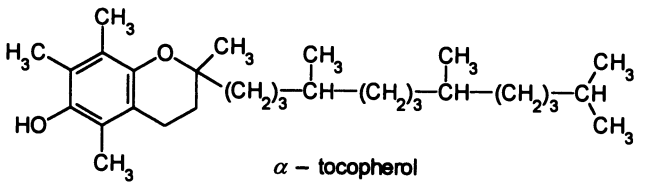
The Toc – $O^{\cdot}$ radical is relatively unreactive and can scavenge the second $LOO^{\cdot}$ radical:

$$Toc\text{–}O^{\cdot} + LOO^{\cdot} \rightarrow \text{non-radical products} \quad (248)$$

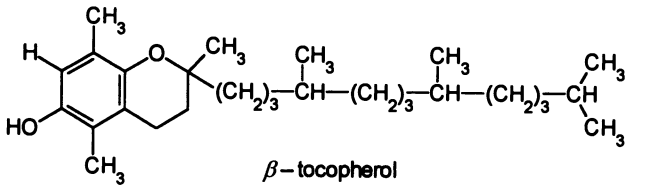
or the radical may be converted to the reductant form by ascorbic acid (vitamin C) [743], quinones, e.g. ubiquinone [744], vitamin K [745], or by a membrane-bound glutathione-dependent vitamin E free radical reductase (see [407 and references therein]):

$$\text{Toc-O}^{\cdot} + \text{vit C} \rightarrow \text{Toc-OH} + \text{vit C}^{\cdot} \tag{249}$$

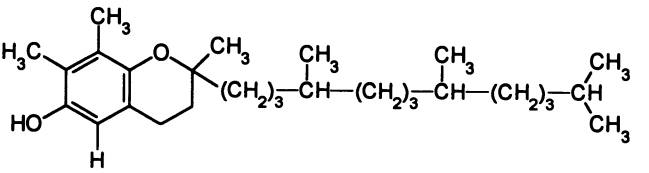
Tocopherols



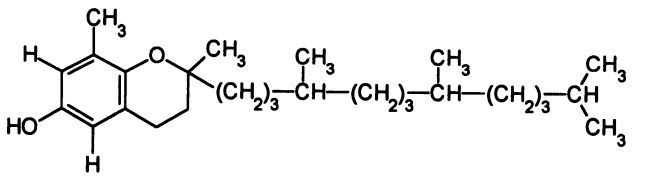
α – tocopherol



β – tocopherol



γ – tocopherol



δ – tocopherol

Krasnovsky and Kagan [746] have observed that α-tocopherol present in the photoreceptor membrane plays the role of protector of the retina against the damage caused by $^1O_2$ because this antioxidant is known as a very efficient

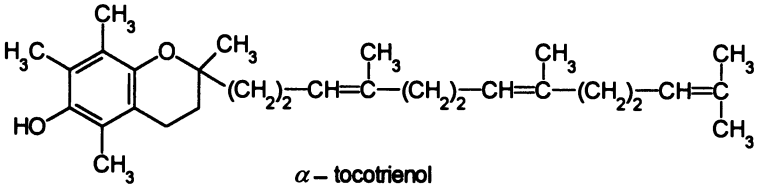
$^1O_2$-scavenger. The known rate constants, k_q, for quenching of $^1O_2$ by tocopherols range from 10^7 to 10^8 $\ell \cdot mol^{-1} \cdot s^{-1}$ and decrease in the following order:

$$\alpha\text{-tocopherol} > \gamma\text{-tocopherol} > \delta\text{-tocopherol} > \beta\text{-tocopherol.}$$

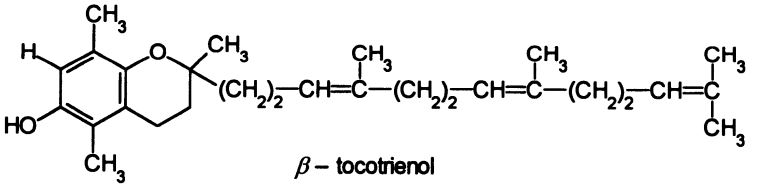
This property is confirmed by several observations that preincubation of the red blood cells of patients suffering from protoporphyria with α-tocopherol inhibits the oxidative damage and hemolysis erythrocytes caused by irradiation of the cells [250].

The human diet usually provides an adequate daily intake of vitamin E from plants, thus vitamin E deficiency is rare but can occur in premature infants or during oxidative stress induced, e. g. by dynamic or endurance exercise training. Prolonged vitamin E deficiency in humans causes decrease of lifetime of erythrocytes, muscular dystrophy, and multiple sclerosis.

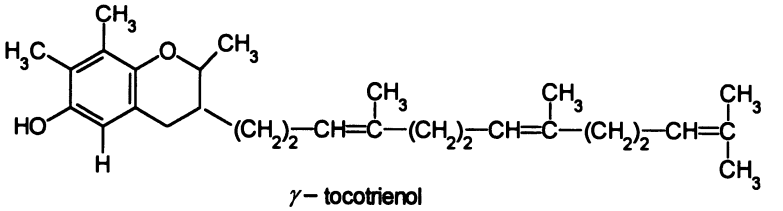
Tocotrienols



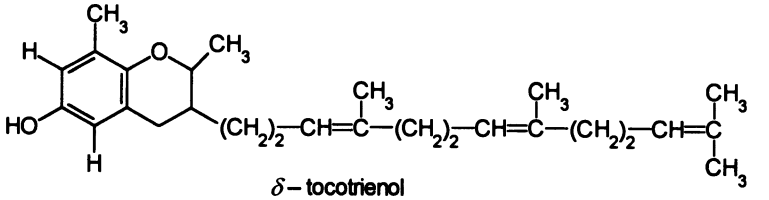
α – tocotrienol



β – tocotrienol



γ – tocotrienol



δ – tocotrienol

However, the prooxidant effect of vitamin E on radical initiated oxidation of low density lipoprotein, e.g. cholesterol-bearing protein, has also been recently observed [747].

7.2.3 Thiols

The next important group of compounds showing antioxidant properties are thiols. Thiols are low molecular weight R-SH compounds, both reactive powerful scavengers of $HO^{\cdot}$ radicals [338, 339, 748–750] and $^1O_2$ quenchers [338, 339, 751, 752] at the physiological pH. For HO radicals, most R-SH compounds have rate constant about $10^9\ \ell \cdot mol^{-1} \cdot s^{-1}$ (see Table 4), and they are a major factor of defense, because they can compete with biologically important molecules, e.g. enzymes, proteins or DNA:

$$R\text{–}SH + HO^{\cdot} \rightarrow R\text{–}S^{\cdot} + H_2O \tag{250}$$

or

$$R\text{–}SR + HO^{\cdot} \rightarrow R\text{–}SR^{+\cdot} + HO^{-} \tag{251}$$

and therefore the concentration of oxidized biological important substrates fall in a cell. The thiol-containing compounds may repair the enzymes damaged in the reaction at Eq. (192), as follows:

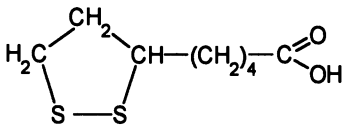
$$\text{enzyme}^{\cdot} + R\text{–}SH \rightarrow \text{enzyme} - H + R\text{–}S^{\cdot} \tag{252}$$

The protection by thiols against the $O_2^{\cdot-}$ generation is also possible:

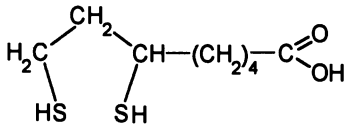
$$R\text{–}SH + O_2^{\cdot-} \xrightarrow{H^+} R\text{–}S^{\cdot} + H_2O_2 \tag{253}$$

For details see [243 and references therein].

Several reports have appeared in connection with the sulfur-containing compounds protection of biomolecules against damage caused by $^1O_2$ or oxygen radicals, for example protection of deoxyribose against damage by HO radical [749], protection against skin photosensitivity [748], prevention of DNA by lipoic and dihydrolipoic acids [753]. Lipoic acid (thiocitic acid) is one of the central coenzymes of three dehydro-genase complexes in mitochondria. The acid plays the role of a hydrogen acceptor in a number of oxidation-reduction processes, turning to dihydrolipoic acid.



Lipoic acid



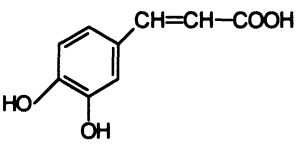
Dihydrolipoic acid

This antioxidant is used in therapy of diabetes mellitus, ischaemia reperfusion injury, radiation damage, AIDS and other causes where scavengers of free radicals are needed [752, 754, 755].

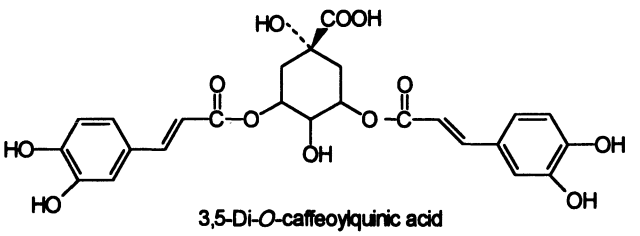
Glutathione and several thiols and related compounds nontoxic to humans have been used against phototoxic site effects during photoradiation therapy [750]. Wefers et al. [751] reported a methionine protective effect against plasmid DNA damage caused by $^1O_2$. It is worthwhile mentioning a family of low molecular weight proteins called metallothioneins, which are very rich in sulfur, as they contain about 23–33% cysteine [565]. These proteins occur in the cytosol of eukaryotic cells, mainly in liver, kidney and gut. They play a crucial role in storage of heavy metal ions (Cd^{2+}, Hg^{2+}) and in regulation of Cu^+ and Zn^{2+} ions metabolism.

7.2.4
Plant Polyphenols

Plant tissues are rich in a wide variety of polyphenolic compounds. As polyphenols play an essential role in plant growth and reproduction, during the last 20 years the rate of discovery of new polyphenolic compounds structures has doubled. Several classes of polyphenolic compounds can be found in living plants: polymers, lignins, flavonoids, phenolic acids, stilbenes, coumarins, catechins, tanins etc. Phenolics occur in plants in different conjugated form, e.g. with anthocyanins, organic bases, sugars, sulfates. For example, caffeic acid (3,4-dihydroxycinnamic acid), of which derivatives, e.g. 3,5-di-*O*-caffeoylquinic acid, strongly inhibit the peroxidation of linoleic acid, and form an astonishing number of combinations:

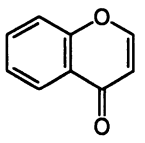


Caffeic acid

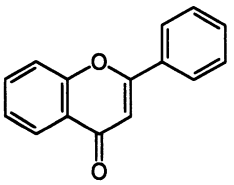


3,5-Di-*O*-caffeoylquinic acid

A large number of papers deal with flavonoids (polyphenolic pigments widely present in plants), e.g. with kaempferol, quercetin and myricetin, apigenin, luteolin, tricetin. Flavonoids are derivatives of 2-phenyl-2,3-benzo-γ-pyrene (flavone)



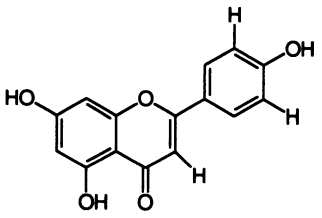
2,3 - Benzo - γ - pyrene



Flavone

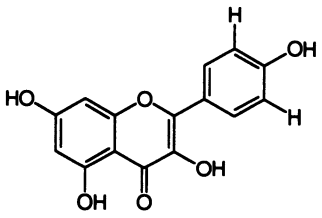
FLAVONOIDS

Flavones

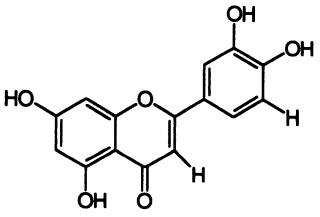


Apigenin

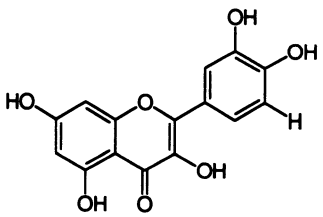
Flavonols



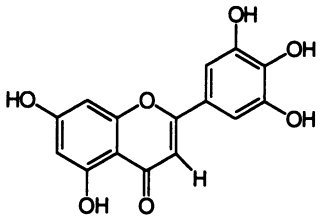
Kaempferol



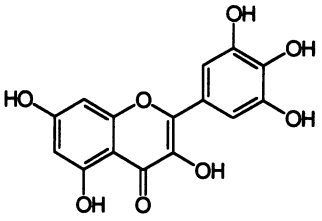
Luteolin



Quercetin



Tricetin

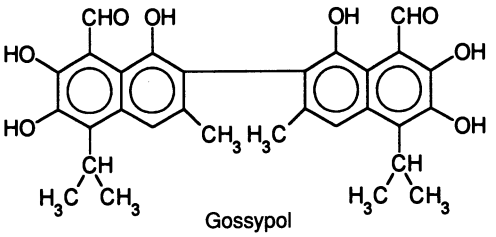


Myricetin

They are used in medical therapy, e.g. as anti-inflammatory and antiallergic drugs. Their protective activity has been identified as $^1O_2$ quenching [756, 757] and oxygen radical scavenging [758–760].

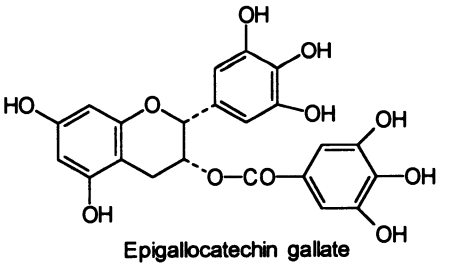
Flavonoids also show the ability to inhibit enzymes involved in lipid peroxidation such as lipo-oxygenase or cyclo-oxygenase. This attribute results from their antioxidant activity [761, 762]. However, prooxidant properties were also observed for the same compounds [763].

Polyphenols present in green tea also show high HO radical scavenging abilities [764]. Phenolic compounds like quercitin, gossypol and myricetin have also been found to quench lipid peroxidation in rat liver microsomes under iron mediation [763].



Gossypol

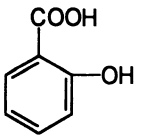
The next polyphenolic compound (–)-epigallocatechin, present in green tea and responsible for its taste, has been found to inhibit some types of cancer promotion in animals, acting as a free radical scavenger.



Epigallocatechin gallate

The inhibitory effect of high molecular weight polyphenols having orthotrihydroxyphenyl structures is stronger than that of the simpler polyphenols.

Similarly, the very popular drug aspirin, used as an anti-inflammatory agent, in vivo deacetyled to salicylic acid, acts as antioxidant by preventing peroxidative degradation of the rat lens lipids. The sodium salt of aspirin (sodium salicylate) is strongly antiseptic and is used as a drug for treatment of rheumatioid arthritis.



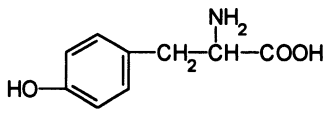
Salicylic acid

Lastly, it has been reported [765] that L-deprenyl (selegiline), an inhibitor of type B monoamine oxidase, decreased the HO radical formation and migral injury induced by administration of 1-methyl-4-phenyl-pyridinium ion. This reagent was used as a possible neuroprotective agent for patients with Parkinson's disease in its early stages. Its action is described as suppressing the liberation of dopamine from neurons, followed by amine oxidation both enzymatically and via the oxygen way, and formation of H_2O_2 which, in turn, generates HO radicals [766]. L-Deprenyl acts like hydroxyl radical scavengers and suppresses formation of melanin from dopamine, which is also accompanied by $HO^{\cdot}$ generation.

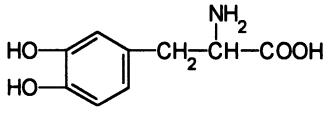
7.2.5 Catechols

Catechols (*o*-dihydroxybenzenes) are strong reducting agents easily undergoing conversion into *o*-benzoquinones in the semiquinone way. They are good antioxidants and this property is due to their radical scavenging activity. For example, dihydroxy-phenylalanine (abbreviated to DOPA), a melanin precursor, has been shown to be selectively toxic to human pigmented melanoma cells in vivo. The amino acid L-DOPA and its analogues are oxidized to the corresponding orthoquinones within melanoma cells with participation of tyrosinase as a catalyst. The quinone, a very reactive species, reacts with nucleophiles (e.g. -SH groups) of a specific enzyme inactivating it, or generating oxygen free radicals or $^1O_2$ [116–119, 767]. Catechols are also able to quench leukemia cells [767]. Biogenic catecholamines – dopamine, noradrenaline and adrenaline – also show the above-mentioned property. They act as neurotransmitters in the central and peripheral nervous system. Noradrenaline and adrenaline are hormones synthesized in the brain, in the nerve terminals and in the adrenal modulla from tyrosine.

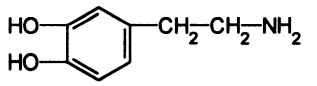
Catecholamines are synthetized from tyrosine. Tyrosine is hydroxylated by a monooxygenase to DOPA, which is decarboxylated to dopamine. Dopamine is hydroxylated to noradrenaline by dopamine monooxygenase. Finally, adrenaline is formed by methylation of noradrenaline. Catecholamines are released into the blood-stream from the adrenal modulla.



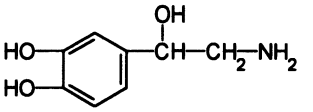
Tyrosine



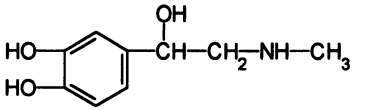
DOPA



Dopamine



Noradrenaline



Adrenaline

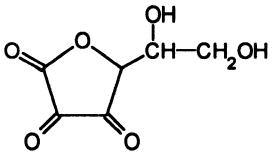
Many quinones, like phenolic compounds, are powerful antioxidants and have been used in industry to prevent autoxidation of unsaturated fats [565, 768–770]. Although polyphenols and quinones are able to scavenger HO radicals, they also deactivate $^1O_2$ quite efficiently (Table 1).

7.2.6 Melanins

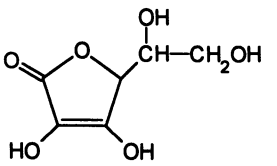
Melanins are natural high molecular weight pigments containing polyphenol-semiquinone units. The polymers absorb a wide range of electromagnetic radiation (UV, visible and infra-red) and participate in redox reactions. They are present in human skin, dark hair, feathers, bacteria and in the seeds of some plants. Melanins are localized within intracellular granules called melanosomes, and possess photoprotective properties. Having free radical centres in their structure they can act as scavengers of free radicals generated, for example, during UV-mediated photolysis of H_2O [771].

7.2.7 Vitamin C

The next very important antioxidant occurring in the hydrophobic phase of a cell is ascorbic acid (vitamin C).



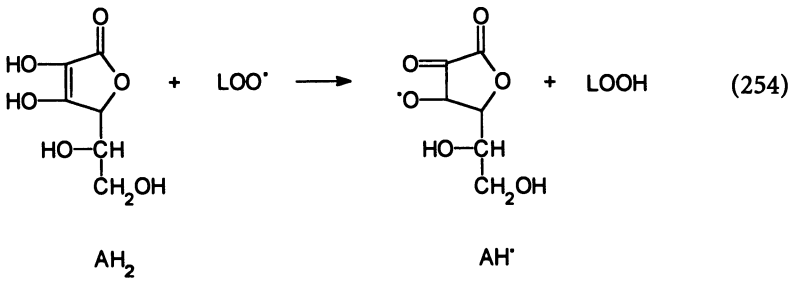
Ascorbic acid – oxidized form



Ascorbic acid – reduced form

Vitamin C plays an important role in the regulation of biological oxidation-reduction processes. It is found in citrus fruits, strawberries, rosehips, blackcurrants, potatoes and green vegetables. A deficiency of vitamin C increases the sensitivity of the organism to bacterial infection and toxins. The disease resulting from vitamin C deficiency occurs in those who subsist for a long period on a diet deficient in fresh vegetables and fruits, and is characterized by weakness, loss of teeth, peripheral hemorrhages, and is called scurvy. All the symptoms of vitamin C deficiency result from the lack of collagen in scar and connective tissues, because procollagen is not secreted by fibroblasts in the absence of the vitamin.

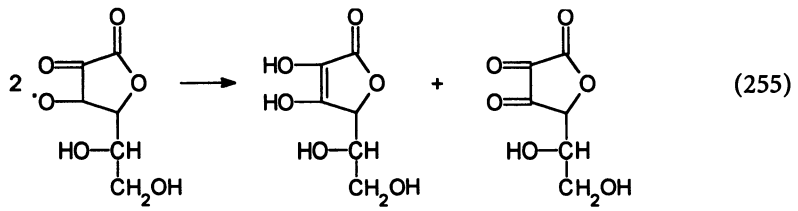
Vitamin C (AH_2), as a scavenger of radicals, undergoes oxidation in the presence of lipid peroxy radicals, giving rise to monodehydroascorbic acid radical ($AH^{\cdot}$).



(254)

AH_2 $AH^{\cdot}$

Dismutation of these radicals regenerates ascorbic acid and produces dehydroascorbic acid (A) [772].



(255)

A

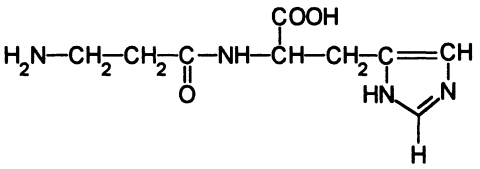
Ascorbic acid in the presence of metal ions can generate $O_2^{\cdot-}$, H_2O_2 and HO radicals, and thus may also act as the promoter of autoxidation reactions [773]. Additionally, ascorbic acid is partially metabolized to oxalate, which can form urinary stones.

7.2.8
Alcohols

Alcohols, known as efficient scavengers of HO radicals (see Table 4), constitute an important group of antioxidants. For example, mannitol and glycerol have been reported to protect DNA from breakage caused by HO radicals produced by UV and gamma radiation [774].

7.2.9
Peptides and Proteins

Peptides and albumin are also good antioxidants. For example carnosine, the histidine containing dipeptide (β-alanyl-L-histidine) and its N_1-, and N_2- methylated derivatives, such as anserine and ofidine, are known to reduce lipid oxidation [775].



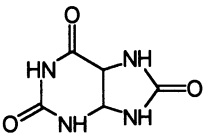
Carnosine

They occur in large amounts in skeletal muscles of vertebrates (about 2% of the net weight tissue). There exist several reports dealing with pronounced antioxidative properties of dipeptides. Thus, carnosine has been stated to inhibit lipid oxidation catalyzed by iron, hemoglobin, lipo-oxidase and $^1O_2$ by 35–96%, and is recommended as an excellent natural antioxidant in processed foods [776]. Complexes of carnosine with Zn^{2+} and Cu^{2+} ions scavenge O_2^- radicals similar to SODs [777]. Moreover, as reported by Pavlov et al. [778], carnosine also interacts with HO radicals at a high rate, forming a stable product.

Plasma consists of about 80 proteins, which are classificated as albumin and globulin depending on their solubility in water or dilute salt solution. Albumin is synthesized in liver and is the smallest but the major protein of plasma. In a normal healthy person the concentration of albumin in plasma is estimated to be 3.2–5.1/100 ml. This protein plays an important role in the maintenance of colloid osmotic pressure. Albumin is also a good antioxidant, binding copper ions [407].

7.2.10
Uric Acid

Uric acid, one of the key compounds in the synthesis of purine relatives, is a powerful scavenger of HO and RO_2 radicals, $^1O_2$, O_3 and hypochlorous acid. This antioxidant also binds iron and copper ions [407]. Uric acid is excreted by man and other primates. In birds and reptiles the acid removes excess of nitrogen like urea in other mammals.



Uric acid

Uric acid is present in human blood plasma at relatively high concentrations estimated as decimal parts of mmol · ℓ^{-1}.

Cells possess well organized antioxidant and repair systems in order to protect themselves against the damage caused by oxygen species. Antioxidant activities are well established for the majority of the above-mentioned antioxidants. Organisms contain many other compounds able to protect organs against cancer, inflamation, and oxidative stress [779]. For details concerning repair systems working in organisms, see for example, [565].

Several compounds have been synthetized in order to increase foodstuffs storage life. Among them are butylated hydroxyanisole (anisole = methoxybenzene), butylated hydroxytoluene, propyl gallate, etc. For example, butylated hydroxyanisole added to fat, e.g. butter, prolongs its storage life even to a few years [565].

It should be remembered that antioxidants may also exert very dangerous side effects: they may not be selective and cut off physiological reactions generating oxygen free radicals, such as mitochondrial electron transport or synthesis of prostaglandins. Many antioxidants contain metal ions because they occur as chelates, and they may affect synthesis and activity metal-dependent enzymes.

8 Role of Oxygen Species in Air and Water Environments

It is generally accepted that oxygen reactive species play an important role in polluted environments since they are implicated in the mechanism of the toxic action of many pollutants in which free radicals and singlet oxygen are late stages in the process of cell injury.

In order to discuss the role of oxygen species in environment disorders it is necessary to consider the various kinds and sources of pollutants as well as the basic chemical and photochemical reactions taking place in both air and water pollution.

8.1 Air Environment

8.1.1 General Considerations

Earth's atmosphere consists of a mixture of gases. The atmosphere extends to an altitude of between 800 and 1000 km above the surface of the Earth. The lower layer of the atmosphere (10–15 km), the most important for life and geochemical processes, is called the troposphere. The troposphere is normally a mixture of the following gases: nitrogen (~ 78%), oxygen (20.94%), argon (~ 1%), carbon dioxide (~ 0.032%), noble gases (neon, helium, krypton, xenon) occurring at concentration of $1.8 \cdot 10^{-3}\%$–$8 \cdot 10^{6}\%$), nitrogen dioxide ($1 \cdot 10^{-6}\%$), nitric oxide ($\sim 2 \cdot 10^{-5}\%$), nitrogen monoxide ($5 \cdot 10^{-5}\%$), methane ($\sim 1 \cdot 10^{-4}\%$), ozone ($2 \cdot 10^{-6}\%$), ammonia ($1 \cdot 10^{-6}\%$), sulfur dioxide ($2 \cdot 10^{-8}\%$) [780]. Also, changing amounts of steam, hydrocarbons, dust of Earth and cosmic origin, microorganisms and small amounts of radiation from radon-222, thorium-232, radium-226, carbon-14 and potassium-40 are present in the troposphere.

Water vapour is continually being exchanged with the Earth by processes of evaporation and precipitation. In the troposphere water vapour forms clouds, rain, snow. Also, the combustion of fuels and the cooling towers of electrical power stations produce water vapour in the troposphere. Unfortunately, people are exposed daily to a great number of potential toxins. Five major natural pollutants may be distinguished with respect for their abundance in the air (over 90% all recognized pollutants in the air): carbon monoxide, sulfur oxides, hydrocarbons, nitrogen oxides and particulates (including lead). Also other gases, e.g. hydrochloric acid, hydrofluoric acid or dust emitted during an erup-

tion of volcanoes may be found in the atmosphere. The origin of the pollutants may be specified as natural and man-made [780].

8.1.2 List and Origin of Pollutants

Three main sources of pollutants may be distinguished: combustion of fuels (obtaining energy for power and heating); emissions from transport vehicles using fuels; and waste gases, dust and heat from chemical manufacturers, cement and brick factories, iron and steel factories or power plants.

The burning of fuels is the first major source of pollutants released into the atmosphere. Fuels contain mainly carbon and when they undergo complete combustion the main gaseous product is CO_2. In the case of incomplete combustion, CO is one of the combustion products. For example, incomplete combustion of coal releases the following products: smoke, tarry droplets of non-oxidized hydrocarbons and carbon oxide. When a fuel contains sulfur, SO_2 is also produced during combustion.

Road transport is the second major source of pollutants. They result mainly from the rather low efficiency of fuel conversion to energy by engines. An incomplete combustion releases CO (the major product), nitrogen oxides (NO_x), sulfur oxides (SO_x), unburt hydrocarbons, aldehydes, and lead. The third main source of air pollutants is industry. Industry produces smoke, SO_2, toxic gases, heavy metals, complex organic compounds, dust and grit.

Transportation, fuel combustion, industrial processes, the combustion of most biological materials (agricultural burning, forest fires, coal waste) or solid waste storage are examples of anthropogenic sources. The basis source of pollutants is transportation emitting the highest concentration of carbon monoxide. It may be worth remembering that pollutants resulting from the mans actions may introduce tremendous amounts of different pollutants into the environmental atmosphere. For example, it has been stated that emission of the air pollutants caused by one person constitutes 2–3 kg per day in some of the most industrial countries of the world, such as USA [780]. OECD reports that world-wide pollutants released into the atmosphere in 1990 as a result of human activity was estimated to be as follows: 99 million tonnes of sulfur oxides (SO_x), 68 million tonnes of nitrogen oxides (NO_x), 57 million tonnes of suspended particulate matter and 177 million tonnes of carbon monoxide (CO) [781]. The global emission rates of NO_x increased by almost 30%, and emission of SO_x by about 18% during the period 1970–1986 [782].

Total national emissions of SO_2 and NO_x during 1985 for several european countries are shown in Figs. 19 and 20 [783].

Annual emission of SO_x and NO_x in 1980 and 1991 only from energy consumption is shown in Figs. 21 and 22 [784].

The data summarized in Figs. 19–22 show that the emission of pollutants by highly industrialized countries is of minor importance in environmental pollution. For this reason many industrialized countries have made considerable progress in decreasing emission of the major air pollutants. Examples of this activity are illustrated in Fig. 23–26 [784–786].

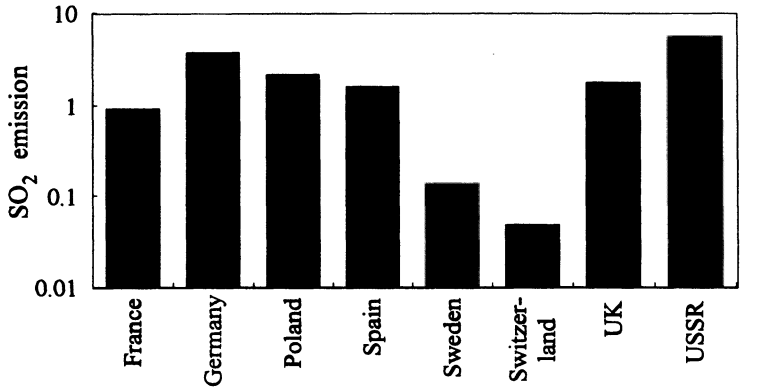


Fig. 19. Total national emissions of SO_2 as equivalent of sulfur in megatonnes during 1985

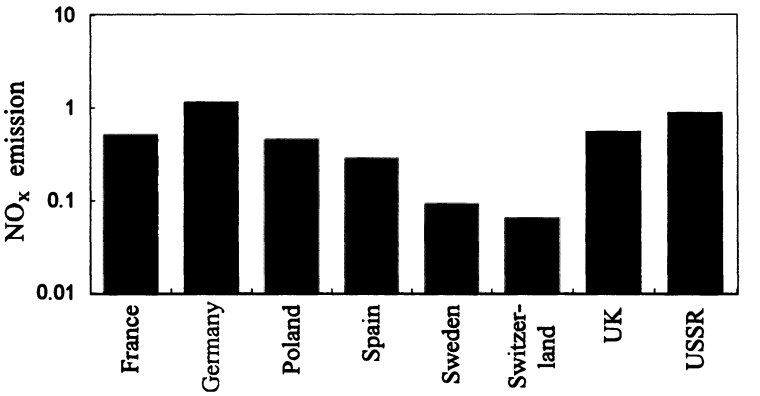


Fig. 20. Total national emissions of NO_x as equivalent of nitrogen in megatonnes during 1985

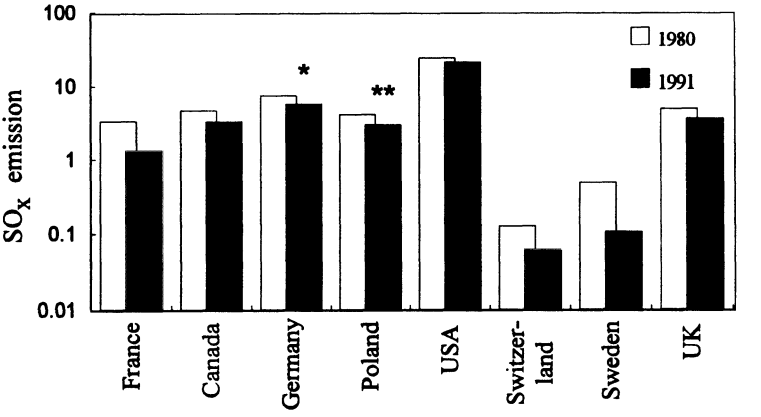


Fig. 21. The annual emissions of SO_x in megatonnes of sulfur from energy consumption (* means for 1990, ** only SO_2)

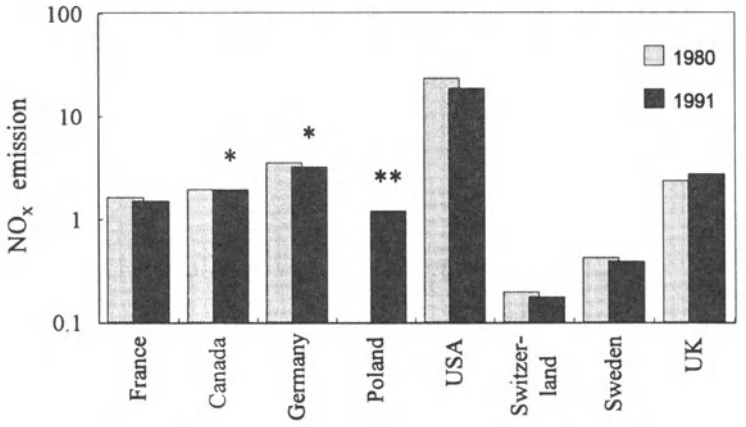


Fig. 22. The annual emissions of NO_x in megatonnes of nitrogen (* means for 1990, ** only NO_2)

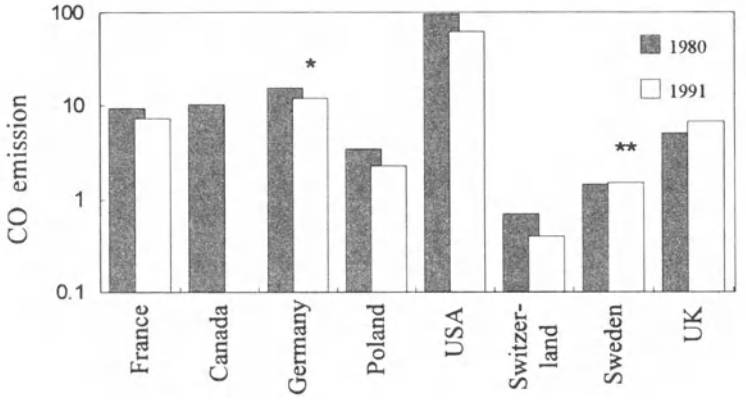


Fig. 23. The annual emissions of CO in megatonnes of carbon (* means for 1990, ** for 1988)

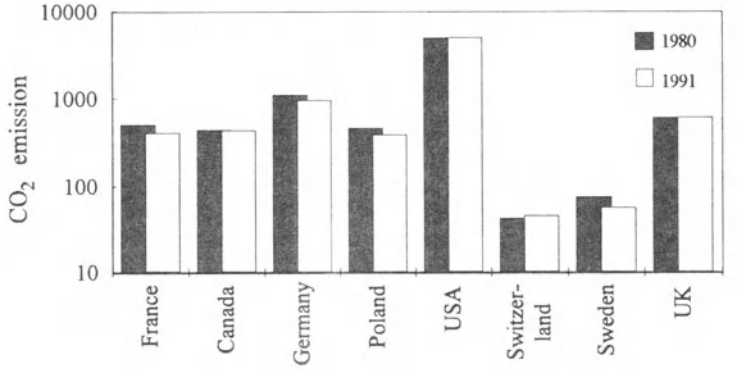


Fig. 24. The annual emissions of CO_2 in megatonnes of carbon

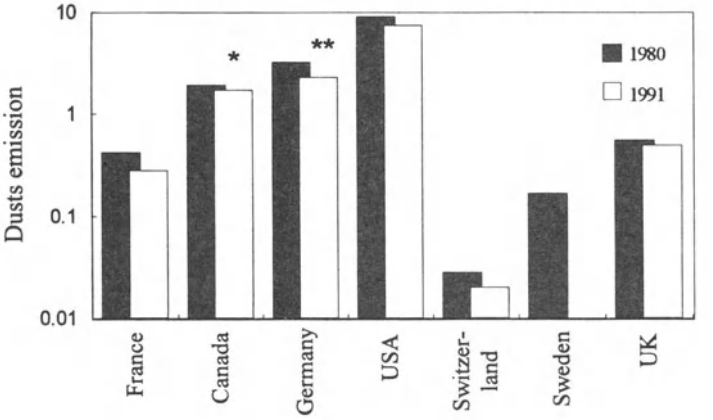


Fig. 25. The annual emissions of dusts in megatonnes (* means for 1985, ** for 1990)

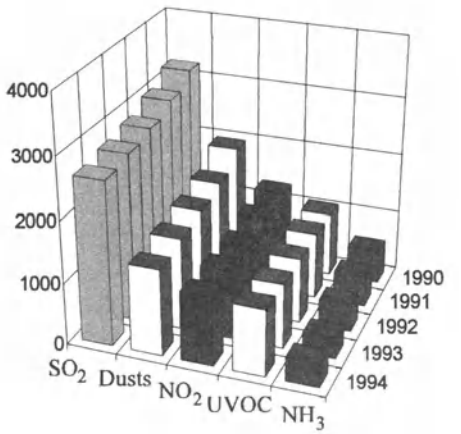


Fig. 26. Total emissions from Poland of SO_2 as sulfur, dusts, NO_2 as nitrogen, unsaturated volatile organic compounds (UVOC) and NH_3 in megatonnes per year

Chemical substances released by the above-mentioned sources directly into the atmosphere are called primary pollutants. They undergo chemical changes in the presence of molecular oxygen, H_2O and UV solar energy and secondary products are formed (e.g. sulfur acids arises from SO_2 in the presence of steam). The majority of trace species emitted into the atmosphere is in reduced forms (e.g. hydrogen sulfide) and when they return from the atmosphere to the Earth's surface they are oxidized during photochemical reactions (e.g. sulfuric acid).

These two types of processes, photochemical reactions and chemical oxidation, are very important in the pollution process of the atmosphere and in smog formation (smog formation will be detailed later). Photochemical reactions are concerned basically with the formation of electronically excited states, and their

behaviour follows several processes including light emission or nonradiative decay, conversion to other excited states, energy transfer (which can result in the formation of other molecule in the electronically excited state), and chemical reactions. For the past three decades photochemistry has made great progress in connection with photobiology as well as with atmospheric and space sciences. The photosensitized reactions no doubt play an important role in many of the reactions leading to the material transfer type.

Both photochemical reactions and chemical oxidations are often accompanied by the formation of the reactive oxygen species, i.e. oxygen radicals and singlet oxygen.

8.1.3
The Role of Excited Molecules in Pollutant Formation

Two main processes may be distinguished during the photochemical reactions: the primary process and the secondary process, and two kinds of reactions occurring in the atmosphere can be involved: homogeneous, i.e. occurring in the gas phase, and heterogeneous occurring within or on aerosols.

The primary process is the absorption of photon by a molecule (A) with formation of the electronically excited molecule, A*:

$$A \xrightarrow{h\nu} A^* \quad \text{absorption}$$

The electronically excited molecule, A*, may undergo several conversions:

- ionization

$$A^* \rightarrow A^+ + e^-$$

- dissociation

$$A^* \rightarrow A_1 + A_2 + \ldots$$

- reaction with a neighbouring molecule, for example molecule B

$$A^* + B \rightarrow D + \ldots$$

- the radiative processes, presented in Fig. 27

$$^1A^* \rightarrow {}^1A + h\nu \text{ (fluorescence)}$$

$$^3A^* \rightarrow {}^1A + h\nu \text{ (phosphorescence)}$$

- collision deactivation

$$A^* + S \rightarrow A + S$$

In this process the electronically excited molecule, A* loses its excitation energy during collision with a molecule of a different kind. Also, sensitized reactions are possible:

$$A^* + S \rightarrow A + S^*$$

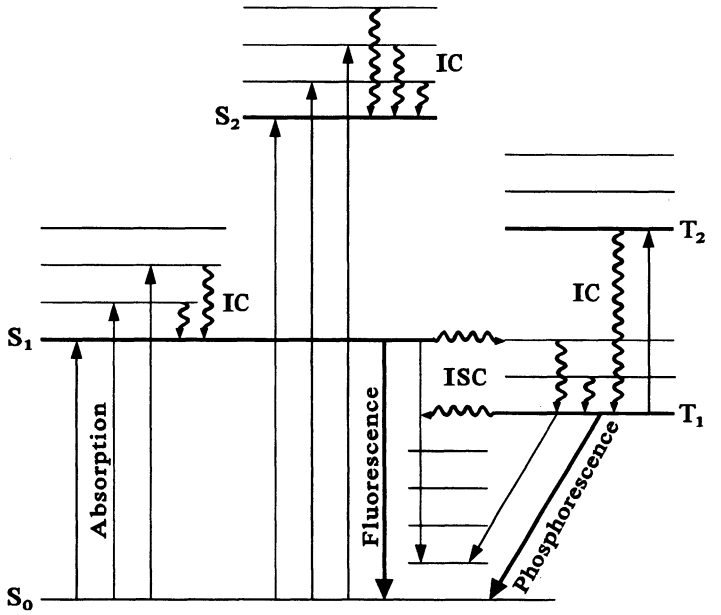


Fig. 27. Transition between the excited states in a molecule according to Jabłoński. S_1, S_2 denote first, second excited singlet states, and T_1, T_2 denote first, second excited triplet states. *Thick horizontal lines* marked S_0, S_1, S_2 are the lowest vibrational levels for each electronic state. The radiationless transitions between the states of the same multiplicity like S_1, S_2 or T_1, T_2 are called the internal conversion (IC), whereas the radiationless transition between the states of different multiplicity ($S_1 \rightarrow T_1$) is called the intersystem crossing (ISC). The emission due to the transition $S_1 \rightarrow S_0$ is called fluorescence, whereas the emission accompanied with the transition between $T_1 \rightarrow S_0$ is called phosphorescence. Lifetimes for the fluorescence transitions are typically around 10^{-8} s, and for the phosphorescence transitions they range from milliseconds to minutes. *Straight arrows* denote the radiative transitions, whereas *wavy arrows* mean the radiationless transition

in which molecule S undergoes electronic excitation as a result of the energy transfer from molecule A* (see also Chap. 5). During the two latter processes, molecule A* generally falls down to the ground state.

A large number of primary products may arise during the photochemical processes, e.g. atomic oxygen (O), nitrogen oxides (NO_x), atomic hydrogen (H), aldehydes (RCHO), nitrosyl (HNO) and the radicals hydroxyl ($HO^{\cdot}$), alkoxy ($RO^{\cdot}$), alkyl ($R^{\cdot}$), acyl ($RCO^{\cdot}$) and formate ($HCO_2^{\cdot}$).

The secondary processes include chemical reactions in the troposphere and lower stratosphere of different molecules arising during the primary processes. These reactions utilize solar energy and are called photochemical pollution reactions. For details see [482]. Rate constants of the photolysis products formation depend on radiation intensity, exposure time, year season, the atmosphere stability, pollutants concentration, presence of compounds absorbing the sunlight and their concentration.

8.1.4
Pollutants Production by Chemical Oxidation

The next very important process in pollutants formation is chemical oxidation. For example, oxidation of sulfur (S) by atomic oxygen, molecular oxygen, ozone or RO· radicals results in formation of sulfur dioxide (SO_2), sulfur trioxide (SO_3); oxidation of organic compounds, such as hydrocarbons yields aldehydes, ketones and carboxylic acids, compounds known as pollutants. Combustion sources can produce very high concentrations of carbon monoxide (CO). It is interesting to note that the high concentration of molecular oxygen in the atmosphere thermodynamically favours oxidation of the gases emitted from the Earth, although O_2 does not react easily with most reduced gases under normal conditions. The reason for this is the relatively strong O=O bond (bond energy ~ 501 kJ · mol^{-1}). By contrast free radicals, as species having an unpaired electron in their outer shell and thus showing an affinity for adding a second electron, are very strong oxidizers. Of free radicals arising in the atmosphere, the hydroxyl radical is the strongest in this respect.

8.1.4.1
Carbon Oxides

Combustion, the man activity for obtaining heat energy, i.e. rapid oxidation of carbon-containing fuels (coal, fuel oil), natural gases (methane, ethane) or bottled gases (propane, butane) leads to emission of carbon oxides (CO, CO_2), may be presented shortly as

$$2C + O_2 \rightarrow 2CO \quad (256)$$

$$2CO + O_2 \rightarrow 2CO_2 \quad (257)$$

The main product of carbon burning is CO as the reaction at Eq. (256) is ten times faster than that at Eq. (257). Similarly, burning of hydrocarbons gives CO_2 as the end product:

$$C_nH_m + O_2 \rightarrow CO_2 + H_2O + \text{energy} \quad (258)$$

It has been established that transportation releases about 90% of all CO into the atmosphere in large cities, and about 80% of CO arises from methane (CH_4) released during decay of organic matter. It is worthwhile to emphasize the importance of hydroxyl radicals involved in this process [780]. Burning of gaseous fuels (methane, ethane) as well as liquid hydrocarbons, e.g. gasoline for automobiles (benzene, hexane, octane, isooctane) emits great amounts of pollutants. The major sources of methane released into the atmosphere are the anaerobic fermentation of organic material in swamps, tropical rain forests, and paddies. Model calculations indicate that oxidation of methane generates about 20–50% of the CO released into the atmosphere [787]. Many gases emitted in the air, even at low concentrations, can exert multiple functions, i.e. act as acids, toxins or oxidants. Among environmental sources of CO emission, forest fires are the most important.

Cigarette smoke can also contribute significantly to CO concentration [788].

Carbon monoxide is very dangerous to both human and animal life, as CO passes through the lungs directly into the blood, nearly irreversibly binding with hemoglobin. Hemoglobin then loses its ability as a carrier of oxygen, leading to anoxemia. The heart and brain, as the most sensitive to oxygen deprivation, may undergo damage, the extent of which depends on the concentration of CO in the air and the exposure time. At low concentration of CO, i.e. ppm (parts per million) man may suffer only a headache. (The natural background is 0.1 – 0.2 ppm [788].)

During the past decade, understanding of associations between pollution level and pathogenesis of human diseases has grown significantly. The link between air pollution and human morbidity and even mortality have been reviewed and discussed in [788 – 790].

The limit of CO concentration for chronically exposed human beings is 5 – 30 ppm [780]. Because automobiles release large amounts of CO, the reduction of CO and other toxic compounds in combustion gases is very important in environmental prevention. There exists several reports dealing with identification of not only CO but also organic compounds in diesel exhaust particles, e.g. aliphatic hydrocarbons, heterocyclic compounds and their toxic pulmonary, carcinogenic and mutagenic effects on rats exposed chronically by inhalation [791, 792 and papers cited therein]. The first very significant action of the automobile constructors has been the adaptation of a catalytic control devices reducing carbon and nitrogen oxides, hydrocarbons, benzene, etc.

Moreover, the recently patented Vehicle Airpollution Control System makes it possible that cars will become driving "smog exhausters", able to remove even 90% toxic substances from the air. The action of the "smog exhauster" is proposed as follows. An electric blower in the engine chambers blows the ground ambient polluted air through filters. Four steps of filtration have been proposed. The first step converts O_3 into O_2 and removes soot molecules, and the next stages reduce nitrogen oxides, SO_2, CO, H_2S, hydrocarbons and benzene using filters with active carbon. The motor of the blower may be driven by solar batteries, enabling "cleaning" of about $8.6 \cdot 10^{10}$ dm^3 of ground air i.e. the volume polluted by one million cars daily (during 8 h) [data from Auto-Świat, No. 10, 1995].

Carbon monoxide is easily oxidized to non-toxic carbon dioxide by hydroxyl radicals yielding atomic hydrogen [793]:

$$CO + HO^{\cdot} \rightarrow CO_2 + H \tag{259}$$

The hydrogen atom is very reactive and reacts with molecular oxygen in a three-body process to form the hydroperoxy radical, $HO_2^{\cdot}$ [794]:

$$H + O_2 + M \rightarrow HO_2^{\cdot} + M \tag{260}$$

where M represents air molecules as a third collision reactant.

A very important source of CO_2 in the atmosphere is the respiration process of man, animals and combustion. Carbon dioxide is removed from the atmosphere during photosynthesis as the substrate of this process, and thus is necessary for the life of green plants.

It should be noted here that the concentration of CO_2 in the atmosphere is continuously increasing, on average by 2.5 ppm per year, causing an increase in the Earth's temperature (the so-called greenhouse effect), because the CO_2 molecules absorb heat, and prevent its escape from the troposphere (the lower 10–15 km of the Earth's atmosphere) into the higher atmosphere. Therefore the temperature of the Earth's atmosphere is increasing.

8.1.4.2 *Sulfur Compounds*

Sulfur compounds, especially in the oxide forms (SO_2, SO_3) are very important contributors to air pollution in the highly industrialized areas. The SO_2 is the index pollutant for sulfur oxides. This compound is also generated from natural sources such as volcanoes, sulfur springs, and decaying organic matter, and the natural background is about 0.0002 ppm. The man-made sources of SO_2 are combustion of fossil fuels, wood pulp processing, petroleum refining coke ovens.

On the world scale, about one third of SO_2 is released by industrial sources, whereas the other two-thirds comes from natural sources, e.g. volcanoes. The sulfur oxides arise mainly durning burning fuels (coal, oil) containing sulfur

$$S + O_2 \rightleftharpoons SO_2 \tag{261}$$

$$2SO_2 + O_2 \rightleftharpoons 2SO_3 \tag{262}$$

and during metallurgy processes involving sulfur ores, e.g.

$$2ZnS + 3\,O_2 \rightarrow 2ZnO + 2SO_2 \tag{263}$$

Additionally, hydrogen sulfide (H_2S) and mercaptans may contribute to air pollution.

In atmospheric air the concentration of SO_2 is low because of the presence of water vapour. Sulfur dioxide dissolves in H_2O giving sulfurous acid (H_2SO_3)

$$SO_2 + H_2O \rightarrow H_2SO_3 \tag{264}$$

Sulfur trioxide (SO_3) forms sulfuric acid (H_2SO_4) upon reaction with water

$$SO_3 + H_2O \rightarrow H_2SO_4 \tag{265}$$

These acids play a significant role in the acidity of rain.

Sulfur compounds can exert toxic effects on plants, man and different materials and their damaging effect depends on concentration and exposition time. The damaging effect observed on plants mainly affects leaves, which show a splotchy bleaching pattern, sometimes the spots becoming a reddish-brown colour, disturbing in this manner the photosynthesis process, which leads to decreasing growth and cropping. It has been reported [795] that damage to pine trees at a distance of 48 km from a metallurgy centre reaches 60–100% at a concentration of sulfur oxides of about 0.5 ppm and an exposure time of 7 h.

Sulfur oxides act as irritants and they increase respiratory symptoms at a concentration ≥ 5 ppm, but lower concentrations may increase the respiratory symptoms in older people suffering from different chronic lung diseases. Human health troubles mainly concern inhalation of acid aerosol (H_2SO_4 and NH_4HSO_4) which causes bronchospasm in asthmatics and chronic bronchitis [796–798]. These are induced by droplets depositing on the surface of the airways of the lungs [788].

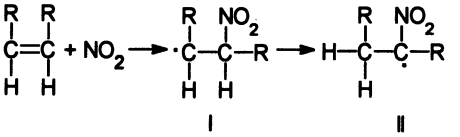
Other sulfur compounds, e.g. ammonium sulfate $(NH_4)_2SO_4$, hydrogen sulfide (H_2S) (released, e.g. during the anaerobic processes of biological substance deposition, by volcanoes or other hot sources), organic sulfides, mercaptans, carbon disulfide (CS_2) often formed in the polluted atmosphere may also cause health problems. For example hydrogen sulfide is oxidized in the presence of oxygen yielding SO_2:

$$2H_2S + 3O_2 \rightarrow 2SO_2 + 2H_2O \tag{266}$$

8.1.4.3 *Nitrogen Compounds*

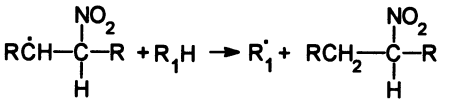
Several nitrogen compounds such as nitrogen oxides, i.e. nitric oxide (NO), nitrogen dioxide (NO_2), nitrous oxide (N_2O), dinitrogen trioxide (N_2O_3), dinitrogen pentoxide (N_2O_5) and nitric acid (HNO_3) are present in the atmosphere. Most involved in air pollution are NO and NO_2, often denoted in the literature as NO_x and HNO_3. These compounds are coupled chemically by a series of reactions acting in the cycle among each other. A main source of NO and NO_2 are biological processes, particularly some organisms such as bacteria. Among these two nitrogen oxides, NO is emitted into the air in considerably greater amounts. Nitrogen is essential for life, but plants assimilate nitrogen in the form of nitrates, ammonia, further amino acids and finally urea. A great role in this respect is played by some organisms, as mentioned above, by combustion of atmospheric nitrogen, oxidation processes, action of solar energy and industrial conversion. Biological conversion is the most important in the transformation of nitrogen to its organic compounds and reduction back to nitrogen by denitrifying bacteria. The concentration of nitrogen oxides from natural sources is independent of population or industry presence, whereas man-made nitrogen oxides are concentrated mainly in urban areas, where they are released into the atmosphere during burning of coal, oils and natural gas, or as pollutants arising from motor vehicle exhausts. It has been estimated that average values of NO and NO_2 lifetimes in the atmosphere are 4 and 3 days respectively [795, 799]. These oxides convert themselves into HNO_2 or HNO_3, being removed from the air by rain or dust. It is well known that a high concentration of NO_2 is toxic because this compound can attach to hemoglobin and cause oxygen deprivation, similar to CO_2. Also, NO plays an important biological role in pathological, physiological and pharmacological processes [796–802]. For example, the presence of NO_x in the air at concentrations about 3.5 ppm for 21 h causes partial necrosis of cotton tissues, whereas concentrations of 10 ppm of NO cause decrease of the photosynthesis rate by 60–70% [795]. Furthermore, nitrogen oxides cause oxidative

stress and hydroperoxides (ROOH) are produced [803]. The following reaction is an example of the hydroperoxide formation



(267)

Radical (I) arising during the NO_2 attack on the ethylene group of the unsaturated fatty acid is able to abstract a hydrogen atom from the other molecule of unsaturated fatty acid, and a free radical chain reaction occurs



(268)

$$R_1^{\cdot} + O_2 \rightarrow R_1O_2^{\cdot} \quad (269)$$

$$R_1O_2^{\cdot} + R_1H \rightarrow R_1OOH + R_1^{\cdot} \text{ etc.} \quad (270)$$

Nitrogen dioxide has powerful oxidizing properties and is known as a poisonous gas. The high reactivity of nitrogen oxides (NO, NO_2), similar to that of free radicals, results from the fact that they contain odd numbers of electrons. Nitrogen oxides can convert organic peroxides into $RO^{\cdot}$ and form hydroxyl radicals [804]:

$$RO_2^{\cdot} + NO \rightarrow RO^{\cdot} + NO_2 \quad (271)$$

$$RO^{\cdot} + H_2O_2 \rightarrow RHO + HO_2^{\cdot} \quad (272)$$

$$HO_2^{\cdot} + NO \rightarrow HO^{\cdot} + NO_2 \quad (273)$$

Nitrogen oxide is the active metabolite released from nitrovasodilator drugs able to activate soluble guanylate cyclase and to increase intracellular cGMP, thus causing vasodilation [804] and they can cause eye and nasal irritation, lung damage and heart stress.

Both nitrogen oxides are present in cigarette smoke. It is worthwhile saying a little about cigarette smoke as a main pollutant of "house air".

Cigarette smoke contains a variety of oxidants and autoxidizable components such as phenols and hydrogen peroxide which are involved in active oxygen species formation [805–808]. It has been found that cigarette smoke contains about 10^{15} radicals per puff, such as $HO^{\cdot}$, $ROO^{\cdot}$ and carbon-centred radicals. The smoke also contains high concentrations of NO, hydrocarbons having one or two bonds, and traces of metal ions (Fe, Cu, Cd) which may be involved in reactions with hydrogen peroxide to give hydroxyl radicals. The reactions of H_2O_2 with NO and NO_2 may also be a source of hydroxyl radicals in cigarette smoke:

$$NO + H_2O_2 \rightarrow HNO_2 + HO^{\cdot} \quad (274)$$

$$NO_2 + H_2O_2 \rightarrow HNO_3 + HO^{\cdot} \quad (275)$$

Cigarette tar also contains very stable semiquinones and over 3000 aromatic compounds [565]. Cigarette smoking, as well as passive smoking by nonsmokers exposed to smoke, predisposes one to lung cancer, heart disease and atherosclerosis [809–812]. Two possible pathways for alterations in lung function by cigarette smoke have been proposed [813]. The first is damage of α-1-proteinase inhibitor (a protein present in blood plasma) by methionine oxidation. The second mechanism of proteinase inactivation is activation of leucocytes followed by generation of hydrogen peroxide, hypochlorous acid and oxygen radicals. Recent studies have shown alteration of lung morphology and increased levels of vitamin A in guinea pig lungs exposed to both mainstream and sidestream cigarette smoke [814].

It is interesting to mention another nitrogen-containing compound, ammonia (NH_3). The main sources of NH_3 are direct volatilization from soils, decomposition of urea, and release during industrial processes. Ammonia cycles through the atmosphere acting as a base in solution, and thus it can neutralize acid compounds, e.g. HNO_3 and H_2SO_4, in rain water, although its rate of removal from the atmosphere is higher than its rate of emission into it [787].

8.1.4.4 *Hydrocarbons*

Hydrocarbons represent a very important class of atmospheric pollutants. Forests, vegetation, bacterial decomposition of organic matter (gives methane gas), incomplete combustion of coal, oil, wood, and production of gasoline release about 85% hydrocarbons into the air. Some hydrocarbons are carcinogenic agents, e.g. benzo-α-pyrene. The toxicity of the aromatic hydrocarbons is observed at concentrations > 25 ppm. Ethylene (C_2H_4) is toxic for plants even at concentrations <1 ppm, as are acetylene (C_2H_2) and propylene (C_3H_6). They limit plant growth and cause necrosis of leaves and flowers.

It may be of interest to mention a large variety of other atmospheric trace gases such as chlorine and its compounds: hydrogen chloride (HCl), chlorinated hydrocarbons (chloroform, $CHCl_3$; carbon tetrachloride, CCl_4; freons $CFCl_3$ and CF_2Cl_2 used in refrigerator systems and as filler of aerosol spray), methyl chloroform (CH_3CCl_3) used as a cleaning and decreasing agent, pesticides, herbicides, bromine, etc. In particular freons are very dangerous for the environment because they destroy the protective ozone layer. Pesticides and herbicides have a high vapour pressure and a large quantity of these compounds escapes into the air. Bromine comes mainly from automobiles because ethyl bromide (C_2H_5Br) is added to petrol.

8.1.4.5 *Particulates*

The next very important group of air pollutants are particulates. Particulates consist of fine solids or liquid droplets suspended in air. Their size determines their behaviour and time of residence in the atmosphere. The smaller sized particulates are smoke and gas-born solids, mist (liquid droplets) and aerosols

(solid or liquid). The larger sized particulates are grit (solid phase), fly ash, dust (solid phase) and soot [790].

The highest concentration of particulate matter in the atmosphere usually occurs over urban and industrial areas. A large concentration of particulates may also be present in homes and public buildings from tobacco smoke.

Particulates, both solid and liquid, come from natural sources, e.g. dry soil, volcanic eruption, from industrial processes entailing grinding or spraying, dust, ashes produced during combustion, and from automobile. For example, coal mine dust, a mixture which contains different minerals, trace metals (mainly iron) and organic substances, causes various pulmonary diseases in coal miners [815–817]. Recent studies have shown that these compounds are involved in the increased production of carbon-centred and hydroxyl radicals which may be involved in pneumoconiosis. For details see [818 and references cited therein]. Among the particulates, very important pollutants are dusts as they contain a large variety of trace metals. Chemical reactions needing a catalyst may occur on its surface. Dusts may contain lead, cadmium, nickel, mercury, copper, iron, manganese, vanadium, chromium, beryllium, arsenic. They come from burning of gasoline, fuels, industrial processes, e.g. galvanization, electroplating, rubber tires and plastic containers.

Particulates are potentially harmful to human health, and their toxic effects depend on particle properties. For example, smoke and fumes can increase the atmospheric turbility and reduce the intensity of the solar radiation on the Earth [790]. Particulates alter immune defences, cause pulmonary problems and respiratory irritation [788].

It is well recognized that cadmium, for example, causes mitochondrial dysfunction, lipid peroxidation and testicular cancer [819–821]; nickel compounds cause lung cancer and respiratory syndromes and lead is also very dangerous because it accumulates in the body and can cause headaches, anemia and brain damage, particularly to the newborn, by regional lipid peroxidation [822]; mercury produces renal injury, affects redox enzyme activity and lipid peroxidation, influences interacellular calcium levels in rat T lymphocytes and is a neurotoxic agent [823–825]; vanadium causes a decrease in the vitamin C level in plasma and increases malondialdehyde concentration in the blood, generating hydroxyl radicals during its one-electron reduction by flavoenzymes/NADPH [826]. The above-mentioned metals deplete glutathione and SH-groups of protein and are known to produce superoxide anion radicals, hydroxyl radicals and hydrogen peroxide. For details see the review paper of Stohs and Bagchi [827].

Metals such as iron, copper, chromium or vanadium are able to undergo redox reactions, thus generating oxygen free radicals (reaction at Eq. (32)). They involve neurotoxicity, hematotoxicity and nephrotoxicity. Lead is also considered as a major pollutant. Four main sources of lead may be distinguished: automobile emissions, industrial emissions, crustal weathering and leaded paint [788]. Lead emited into the atmosphere can deposit on leafy vegetables and fruits. The response to lead exposure is complex, leading to serious health problems such as kidney disease and neurological impairment. Primarily, lead affects children.

During the last decade there has been growing danger caused by progress in jet propulsion techniques, leading to a problem with beryllium and its compounds, which are components of rocket fuel.

Radiactivity also appears to have a significant role in environmental pollution. It comes from both natural and man-made sources. Background radiation comes from nuclides emanating from three sources: cosmic rays, rocks and soil, and food and drinking water. The man-made sources of radiation are X-rays used in medicine, fall-out during nuclear weapons testing or nuclear reactors (e.g. Chernobyl accident in 1986) and use of radioisotopes in industry and nuclear reactors [790].

Finally, it is worth saying a little about asbestos, i.e. mineral fibres containing silicon oxides, magnesium, aluminium, iron, sodium, calcium and water. Few mineral silicates which crystallize into fibrous forms can be considered as asbestos minerals. They are defined as cotton-like fibrous minerals having unusual tensile strength and being heat resistant. Asbestos can be divided into serpentine and amphiobole minerals. Asbestos fibres are hazardous to human health and plants. They have small size and low density and are therefore easily transported by wind and water. Asbestos is also released by automobiles. Workers having long-lasting contact with asbestos are liable to suffer from cancer. The response to exposure in humans leads to medical problems such as pleural thickening and calcification, pulmonary fibrosis or asbestosis, and lung and gastrointestinal cancer [828]. Active oxygen species are considered to be secondary causes of asbestos, silica and other inorganic minerals toxicity [829–831].

At present our attention focuses on reactions generating the oxygen species mainly ozone, hydroxyl radical and singlet oxygen, their reactions with pollutants and the role they play in smog formation, plant senescence processes and polymer degradation.

8.1.5 Ozone

Ozone is one of the most toxic gases produced in photochemical smog. The gas is an important shield against solar radiation in the upper atmosphere (stratosphere) absorbing solar radiation below 290 nm. Due to the ozone, the solar radiation reaching the troposphere is not energetic enough to cause photolysis of the main gases such as molecular oxygen, nitrogen, carbon dioxide or water vapour, but is able to cause the photolysis of trace gases, e.g. ozone.

Ozone is present in unpolluted air at concentrations ranging from 0.01 to 0.02 ppm, whereas in very polluted air the gas reaches concentrations up to 0.2 ppm [832, 833].

A main source of ozone is in situ photochemical generation in the stratosphere by reaction of ground state atomic oxygen, $O(^3P)$, with molecular oxygen in the presence of air molecules (M) [834, 835].

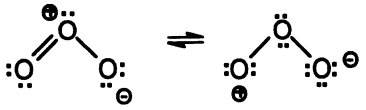
$$O(^3P) + O_2 + M \rightarrow O_3 + M \qquad (M = N_2 \text{ or } O_2) \tag{276}$$

The atomic oxygen arises in the air during photochemical dissociation of molecular oxygen and/or nitrogen dioxide

$$O_2 \xrightarrow{h\nu} 2O \tag{277}$$

$$NO_2 \xrightarrow{h\nu} NO + O \tag{278}$$

Ozone is transported from the lower stratosphere downward to the troposphere, and its concentrations are usually maximal in the late morning or afternoon and decrease in the evening. This is due to ozone loss during reactions with NO and Earth's surface. Ozone can show both electrophilic and nucleophilic properties. This results from its electronic arrangement into a dipolar molecule with both a positive and a negative centre:



Ozone is strongly biologically active because forms free radical products [836]. Its reaction with unsaturated fatty acids forms radicals, thereby decreasing the time required for the acids' autoxidation. Mechanisms of fatty acid oxidation were discussed in Chap. 5 and are given elsewhere; see for example [832]. Ozone is also known as a potent toxic agent to plants and microbes.

Fluorinated hydrocarbons (CF_2Cl_2, $CFCl_3$), used in aerosol sprays deplete the layer of ozone in the upper atmosphere by photodissociation to chlorine atoms ($Cl^{\cdot}$), which react with ozone removing it from the air:

$$Cl^{\cdot} + O_3 \rightarrow ClO + {}^1O_2 \tag{279}$$

$$ClO + O \rightarrow Cl + {}^1O_2 \tag{280}$$

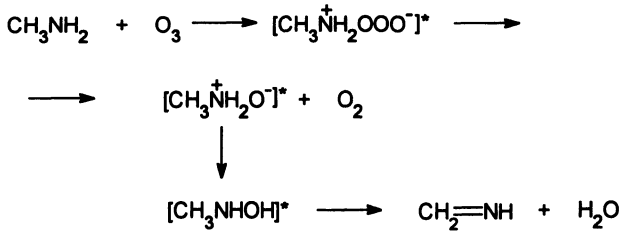
These reactions release energies of 161 and 263 kJ · mol^{-1}, respectively, which are sufficient for singlet oxygen formation [837].

In atmospheric air, ozone plays important functions in the transformation of other pollutants, mainly nitrogen oxides and olefines, transforming them into both nonradical and radical products [838, 839]:

$$NO_2 + O_3 \rightarrow NO_3 + {}^1O_2 \tag{281}$$

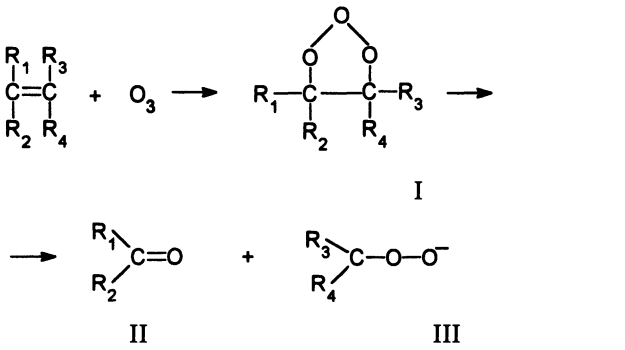
$$2NO_2 + O_3 \rightarrow N_2O_5 + O_2 \tag{282}$$

and also the reaction at Eq. (68). Ozone reacts with a variety of different organic compounds containing a nitrogen atom, including nitrites, hydrazines, nitriles, diazo compounds and amines. The reaction mechanisms are not presently known in detail, and are being extensively studied [835]. The experimental data obtained up till now show, e.g. that the initial reaction of ozone with amines and hydrazines proceeds via addition at the nitrogen atom, with formation of the ozone-amine or hydrazine adduct:



(283)

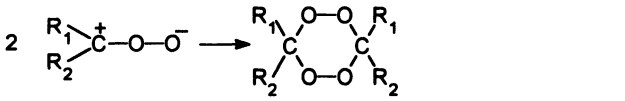
In the reactions with olefines, ozone as an electrophilic agent can add across double and triple carbon-carbon bonds to form in the initial stage the nonradical intermediate – ozonide. Reactions of ozone with olefines are well recognized and it has been established that they often produce several complex products. For example, ozone reacting with ethylene, propylene, butane and pentene forms aldehydes and carbon oxides, whereas with heptane and hexane it forms ozonides [803, 834, 835, 840, 841]:



(284)

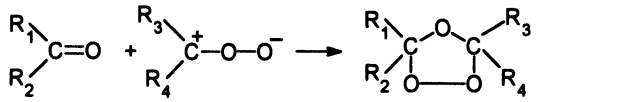
Adduct (I) undergoes decomposition to aldehyde or ketone (II) and zwitter ion (III). The zwitter ion can undergo transformation via several pathways.

(a) Dimerization with alkyldiene superoxide formation



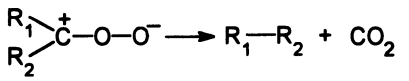
(285)

(b) Reaction with own aldehyde or ketone, forming ozonide



(286)

(c) Decomposition with formation of several different products depending on the kind of substituents R_1 and R_2, e.g.



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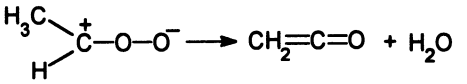

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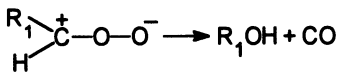
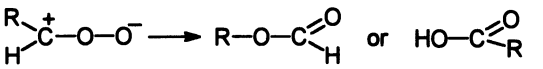


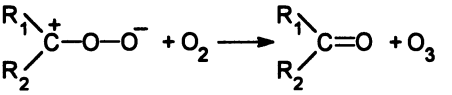
(289)

(d) Rearrangement, e.g.



(290)

(e) Reactions with molecular oxygen, nitrogen oxide or olefines



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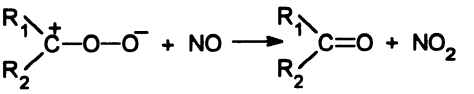

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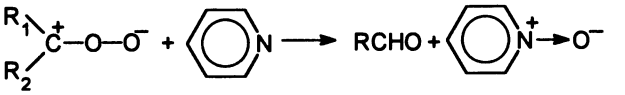

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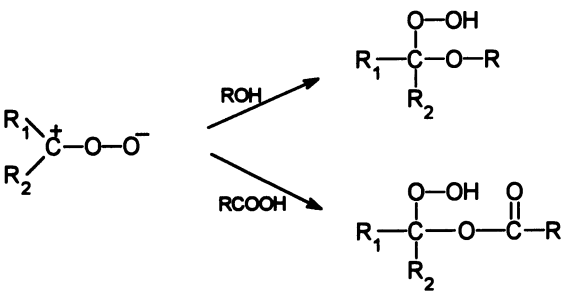
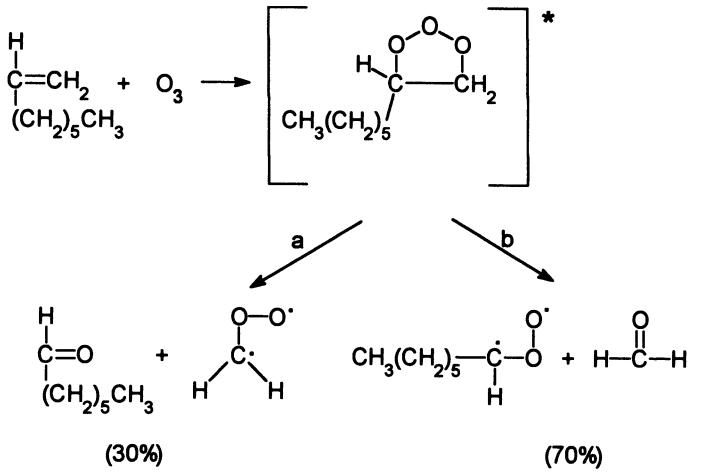


(294)

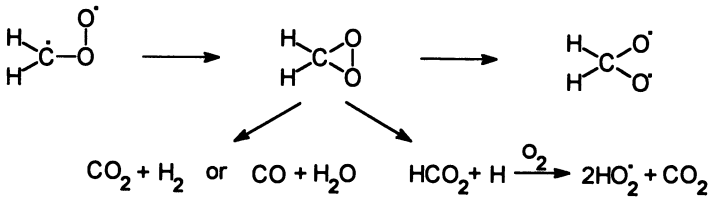
Another reaction significant with respect to radical production is the reaction of ozone with 1-octane followed by formation of electronically excited adduct.

The adduct can decompose in two ways with different efficiency, yielding two pairs of products such as carbonyls and biradicals [839]:



(295)

The energy-rich biradicals can undergo subsequent bimolecular reactions or undergo unimolecular rearrangement and fragmentation. For example, the simple biradical $\dot{C}H_2O\dot{O}$ forms different toxic products such as carbon monoxide, carbon dioxide, hydroperoxy radical:



(296)

Similarly, the higher biradicals, e.g. $R_1R_2\dot{C}OO\cdot$ are involved in more complex reactions [835].

The $R\dot{C}HOO\cdot$ biradicals have been reported to react with several pollutants such as carbon monoxide, nitrogen oxides and sulfur dixoide giving toxic products as follows [840]:

$$R\dot{C}HOO\cdot + NO \longrightarrow RCHO + NO_2 \quad (297)$$

$$R\dot{C}HOO\cdot + NO_2 \longrightarrow RCHO + NO_3 \quad (298)$$

$$R\dot{C}HOO\cdot + SO_2 \xrightarrow{H_2O} RCHO + H_2SO_4 \quad (299)$$

$$R\dot{C}HOO\cdot + H_2O \longrightarrow RCOOH + H_2O \quad (300)$$

Niki et al. [842] have hypothesized that initial reactions ozone with more complex alkenes, e.g. polyalkenes, cyclic alkenes and haloalkenes are analogous.

Although the gas-phase reactions ozone with olefines as well as with various other groups of organic compounds and their role in the pollution of Earth's

atmosphere are not well recognized, a great number of rate constants of ozone reactions with organic compounds have been found. For review see [835].

From the biological point of view it may be worth mentioning reactions of ozone with other compounds containing nitrogen in their molecule, such as amines, hydrazines or diazenes as reactions being the source of hydroxyl radicals [843–845].

$$R_2NH + O_3 \rightarrow R_2N^{\cdot} + HO^{\cdot} + O_2 \tag{301}$$

$$NH_2NH_2 + O_3 \rightarrow NH_2\dot{N}H + HO^{\cdot} + O_2 \tag{302}$$

$$RH{=}NH + O_3 \longrightarrow RN{-}N^{\cdot} + HO^{\cdot} + O_2 \tag{303}$$

$$\downarrow$$

$$R^{\cdot} + N_2$$

Oxidation of thiols is a further example of the reaction between ozone and biologically important compounds. For example, cysteine is oxidized by ozone to cysteic acid [803].

$$\begin{aligned} &HOOC{-}\underset{\displaystyle NH_2}{\underset{|}{CH}}{-}CH_2{-}SH \xrightarrow{O_3} HOOC{-}\underset{\displaystyle NH_2}{\underset{|}{CH}}{-}CH_2{-}S{-}S{-}CH_2{-}\underset{\displaystyle NH_2}{\underset{|}{CH}}{-}COOH + \\ &+ HOOC{-}\underset{\displaystyle NH_2}{\underset{|}{CH}}{-}CH_2{-}SO_3H \end{aligned} \tag{304}$$

Also, other biologically important compounds containing thiol groups such as glutathione or enzymes are oxidized to the corresponding disulfides.

The next important type of ozone interaction with organic compounds are reactions which have been reported to be accompanied by free radical formation:

$$RH + O_3 \rightarrow R^{\cdot} + O_2 + HO^{\cdot} \tag{305}$$

Ozone also reacts rapidly with organic hydroperoxides [846, 847]:

$$ROOH + O_3 \rightarrow ROO^{\cdot} + HO^{\cdot} + O_2 \tag{306}$$

Photolysis of ozone to atomic oxygen also leads to hydroxyl radical generation according to the reaction at Eq. (66) [794, 848–851].

The reaction at Eq. (66) is immediately followed by reactions:

$$O(^1D) + M \rightarrow O(^3P) + M \qquad (M = N_2 \text{ or } O_2) \tag{307}$$

$$O(^1D) + H_2O \rightarrow 2HO^{\cdot} \tag{308}$$

This very short review of the reactivity of ozone with some pollutants shows that this compound may be toxic for all living organisms with respect to pro-

ducts generated in the polluted air. It is well known that a great number of people suffer from diseases caused by ozone [803]. A number of alterations in tissues caused by ozone may be specified: hemoglobin is oxidized to the ferriform; extracts of lungs of animals exposed to ozone action show the presence of conjugated dienes; ozonolysis of unsaturated fatty acids leads to the accumulation of fatty acid ozonides; the thiol content of red blood cells of animals is significantly decreased after their exposure to ozone; the activity of acid phosphatase, lysozyme and other enzymes was observed to be decreased. In vitro studies showed that ozonides of fatty acids are very toxic as their injection involves acute lung edema as well as increases in the permeability of the microvasculature. Evidence gathered by Lippmann [788] shows the consequences of ozone exposure on humans in relation to ambient O_3 levels and exposure time.

Studies with pinto bean leaves also showed that their exposition to 0.35 ppm ozone for 20–30 min caused decrease of RNA content in chloroplast ribosomes [803].

8.1.6
Hydroxyl and Hydroperoxide Free Radicals

Hydroxyl radicals control the atmospheric concentration of many trace gases, and their interaction with the gases are very important because these reactions are often primary and rate limiting steps in the chain of oxidation reactions.

Hydroxyl radicals may be generated in the atmosphere by several reactions and it is difficult to determine the priority of any of them. The most important reactions may be summarized as follows.

(a) Photolysis of ozone (reactions at Eqs. (66) and (308))

(b) Photolysis of hydrogen peroxide (reaction at Eq. (26))

(c) Photolysis of the nitrous and nitric acids

$$HNO_2 \xrightarrow{h\nu} HO^{\cdot} + NO \tag{309}$$

$$HNO_3 \xrightarrow{h\nu} HO^{\cdot} + NO_2 \tag{310}$$

Also, other compounds arising during atmospheric oxidation such as CH_2O, CH_3O_2H, carbonyls and alkylhydroperoxides may generate the hydroperoxy and hydroxyl radicals during photolysis of these compounds by ultraviolet radiation [794].

(d) Oxidation of methane to the methoxy radical ($CH_3O^{\cdot}$) [852]

(e) Reaction of hydroperoxide radical with nitrogen oxide, ozone or atomic oxygen [794, 836, 853]

$$HO_2^{\cdot} + O_3 \rightarrow 2O_2 + HO^{\cdot} \tag{311}$$

$$HO_2^{\cdot} + O \rightarrow {}^1O_2 + HO^{\cdot} \tag{312}$$

(see also reaction at Eq. (273))

(f) Reaction of ozone with hydrogen atom

$$H + O_3 \rightarrow O_2 + HO^{\cdot} \tag{313}$$

or with some organic compounds as well as their hydroperoxides (reactions at Eqs. (305) and (306)).

(g) Reaction of nitrogen dioxide with hydrogen atom [843]

$$H + NO_2 \rightarrow HO^{\cdot} + NO \tag{314}$$

(h) Decomposition of alkyllperoxy radical ($CH_3O_2^{\cdot}$)

$$RCH_2OO^{\cdot} \rightarrow RCHO + HO^{\cdot} \tag{315}$$

(i) Reactions of ozone with other compounds containing nitrogen (see reactions at Eqs. (301)–(303)).

The HO and HO_2 radicals are interconverted very rapidly, thus they are in photochemical equilibrum and are often considered as a sum of $HO^{\cdot}$ and $HO_2^{\cdot}$ and designated by HO_x. The HO radical does not react with the main constituents of air but it is a primary oxidant in the troposphere and is mainly responsible for oxidation of hydrocarbons, CO, SO_2, NO_2, CH_4 or H_2S.

The balance between hydroxyl radical generation and its disappearance depends mainly on the yield of HO_x radicals production, transformation to $HO_2^{\cdot}$, reconstruction of $HO^{\cdot}$ from $HO_2^{\cdot}$, the radical-radical type interaction removing HO_x radicals from the air and their chemical reactions with atmospheric trace gases. Under midday conditions, stationary state concentration of HO radicals has been estimated to range from about $1 \cdot 10^7$ radicals $\cdot$ cm^{-3} to a few $\times 10^6$ radicals $\cdot$ cm^{-3} [854].

Several techniques for HO radicals detection have been discovered which utilize both their physical and chemical properties. For example, direct physical measurement can be carried out using Laser Induced Fluorescence and Long-Path Absorption Spectroscopy [855–858]. In both techniques, lasers are usually used as light sources. In the case of absorption spectroscopy, the beam of light from the laser is sent a known distance and is reflected back to a detector and concentration of the HO radicals is calculated from Beer's law (For details see [858]).

The fluorescence technique needs the excitation wavelength of 282 nm and fluorescence results from nonresonant transition near 308 nm [856, 857].

Chemical methods use the chemical reactivity of hydroxyl radicals such as H-atom abstraction from C–H and O–H bonds, $HO^{\cdot}$ addition to unsaturated bonds C=C, C≡C and aromatic rings as well as their interaction with compounds containing –SH, –S–, $-NH_2$, > NH and > N-groups; these were discussed in Chap. 4. For example, the application of radiochemical technology to $HO^{\cdot}$ concentration measurement in the troposphere relies on use of ^{14}C labelled species and monitoring ^{14}CO oxidation [858–860]:

$$^{14}CO + HO^{\cdot} \rightarrow {}^{14}CO_2 + H \tag{316}$$

The radioactive $^{14}CO_2$ produced is then cryogenically enriched and measured by using a scintillation counter. The second most popular method for $HO^{\cdot}$ concentration detection is observation of decay of hydrocarbons in the urban atmosphere [861] or detection of methyl chloroform in the air [862].

Another very popular method of $HO^{\cdot}$ detection employs an ESR spin trapping technique and its precision is estimated to be ± 25 % [863]. In the last few years great progress has been made, discovering suitable methods and computer programmes for $HO^{\cdot}$ detection in the troposphere. For details see [864 and references cited therein].

The HO_x radicals react with the large variety of trace gases emitted into the atmosphere. The most significant reaction in which the hydroxyl radical participates is the oxidative transformation of most organic substances emitted into the atmosphere [865]. They are responsible for the formation of pollutants participating in urban photogenerated smog or acid rains. A knowledge of the reactions of hydroxyl radicals with organic compounds and other traces gases present in the atmosphere is needed in order to prepare risk assessment and foreseeing generation of new pollutants. Now over 500 organic compounds are emitted into the Earth's atmosphere and those man-made that are involved in reactions with hydroxyl radicals have been identified and rate constants have been determined [793, 794]. In cleaner regions of the atmosphere, the main reaction in which the $HO^{\cdot}$ radical participates is with carbon monoxide (reaction at Eq. (259)) and with methane [866]

$$HO^{\cdot} + CH_4 \rightarrow H_2O + CH_3^{\cdot} \tag{317}$$

The methyl radical ($CH_3^{\cdot}$) reacts immediately with molecular oxygen to produce the methylperoxy free radical ($CH_3O_2^{\cdot}$)

$$CH_3^{\cdot} + O_2 + M \rightarrow CH_3O_2^{\cdot} + M \qquad (M = N_2 \text{ or } O_2) \tag{318}$$

The latter reaction may be followed by oxidation of nitric oxide to nitrogen dioxide

$$CH_2O_2^{\cdot} + NO \rightarrow CH_3O^{\cdot} + NO_2 \tag{319}$$

The methoxy radical ($CH_3O^{\cdot}$) reacts rapidly with molecular oxygen giving formaldehyde (CH_2O), well known as a strongly toxic compound

$$CH_3O^{\cdot} + O_2 \rightarrow HCHO + HO_2^{\cdot} \tag{320}$$

A similar reaction scheme has been proposed for non-methane hydroxarbons [867]. The hydroperoxy radicals resulting from the latter reaction are converted back to hydroxy radicals mainly in reactions with nitrogen oxide as well as with ozone (reactions at Eq. (273) and (311)).

The atmospheric cycling of hydroxyl radicals is presented schematically in Fig. 28.

As one can see from the figure, many hydroxyl radicals may be lost during their reaction with NO_2. By this means, about 50 % of HO_x compounds may be removed from the atmosphere [868]:

$$HO^{\cdot} + NO_2 + M \rightarrow HNO_3 + M \tag{321}$$

Another significant product of the hydroxyl radical reaction with nitrogen dioxide is hydrogen oxoperoxonitrate, (ONOOH) [869]

$$HO^{\cdot} + NO_2 \rightleftharpoons ONOOH \tag{322}$$

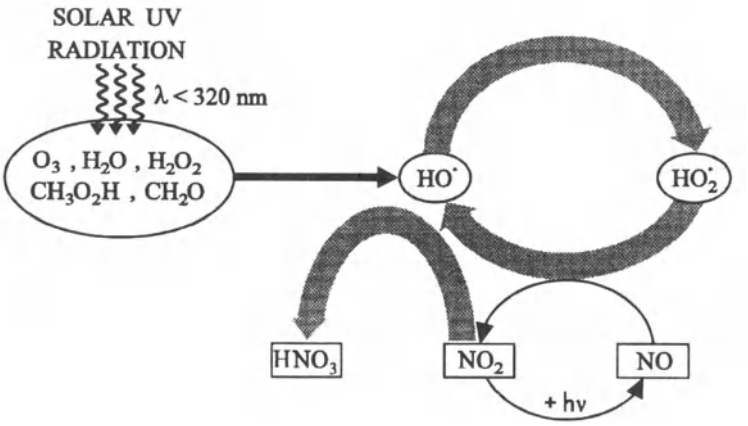


Fig. 28. Atmospheric cycling of the hydroxyl radical

a strong oxidant which may be formed in vivo from nitrogen monoxide and superoxide anion radical near activated macrophases [870, 871]; see reaction at Eq. (42).

The cytotoxic properties of both hydrogen oxoperoxonitrate as well as its anionic form, oxoperoxonitrate ($ONOO^-$) ($pK_a = 6{,}8$) [872] to the cell are high. They initiate lipid peroxidation [873], react at a high rate with sulfhydryls [874], and cause inactivation of α-1-proteinase inhibitor by peroxynitrate [875].

The next most important reaction of the hydroxyl radical is reaction with sulfur dioxide which starts a process forming sulfuric acid [849]:

$$SO_2 + HO^{\cdot} + M \rightarrow HSO_3 + M \quad (323)$$

$$HSO_3 + O_2 \rightarrow HO^{\cdot} + SO_3 \quad (324)$$

The latter product, sulfur trioxide, dissolves easily in water, giving sulfuric acid (see reaction (265)).

The above reactions remove about 30% of SO_2, 90% of CO, CH_4, alkanes, isoprene and isopropene from the atmosphere as well as 50% of terpenes [875].

A small amount of hydroxyl radicals is removed from the air by the following reactions [876–878]:

$$HO^{\cdot} + H_2O_2 \rightarrow H_2O + HO_2^{\cdot} \quad (325)$$

$$HO^{\cdot} + HO_2^{\cdot} \rightarrow H_2O + O_2 \quad (326)$$

$$HO^{\cdot} + CH_3OOH \rightarrow CH_3OO^{\cdot} + H_2O \quad (327)$$

$$HO^{\cdot} + CH_3OO^{\cdot} \rightarrow CH_3OOH + O_2 \quad (328)$$

$$NO + HO^{\cdot} + M \rightarrow HONO + M \quad (M = N_2 \text{ or } O_2) \quad (329)$$

In polluted atmosphere the hydroxyl radical forms with nonmethane hydrocarbons and nitrogen oxides a very toxic pollutant, peroxyacetyl nitrate, $CH_3COO_2NO_2$ [879]:

$$CH_3CHO + HO^{\cdot} \rightarrow CH_3CO^{\cdot} + H_2O \quad (330)$$

$$CH_3CO^{\cdot} + O_2 \rightarrow CH_3COO_2^{\cdot} \quad (331)$$

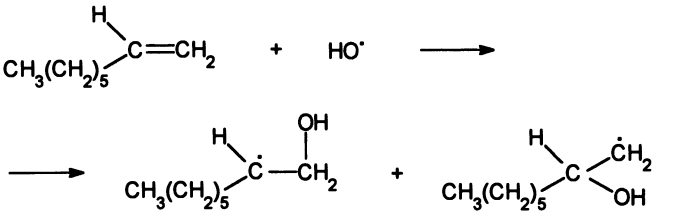
$$CH_3CHOO^{\cdot}{}_2 + NO \rightarrow CH_3COO_2NO_2^{\cdot} \quad (332)$$

Peroxyacetyl nitrate acts as a reservoir for nitrogen and peroxy radicals and is strongly mutagenic for plants and animals. This compound in reaction with hydroperoxy radicals forms peracetic acid

$$CH_3COO_2^{\cdot} + HO_2^{\cdot} \rightarrow CH_3COOOH + O_2 \quad (333)$$

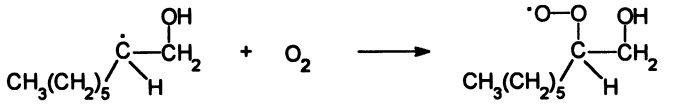
Peracetic acid and peroxyacetyl nitrate are gas-phase plant toxics, they have a long lifetime in the upper troposphere, and may occur in the environment in precipitation at nmol · ℓ^{-1} to µmol · ℓ^{-1} concentrations.

Lastly, let us consider in more detail the interaction of the hydroxyl radical with 1-octane, a compound known to participate in photochemical smog formation occurring in the air at concentrations of 40–48 ppb (parts per billion). It has been reported that 1-octane undergoes photooxidation in a similar way as 1-alkenes, e.g. 1-hexene [880, 881]. The reaction is started by addition of the hydroxyl radical to one side of the unsaturated C=C bond with formation of radical and nonradical products [839]:



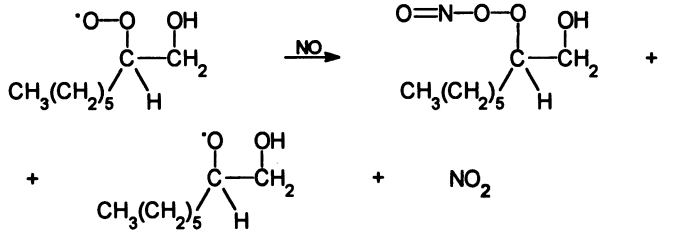
(334)

The arised radical species is proposed to react with molecular oxygen giving rise to alkylperoxy radical:



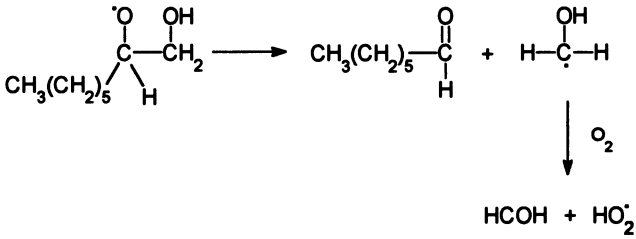
(335)

The alkylperoxy radical may react with nitrogen oxide present in the polluted atmosphere leading to nitrate and hydroxy alkoxy radical formation with partial oxidation of NO to NO_2.



(336)

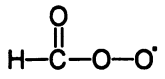
The hydroxy alkoxy radical formation is followed by its decomposition to heptanal and lastly formaldehyde and hydroperoxy radical may be generated.



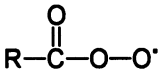
(337)

The decomposition reaction dominates for a number of smaller, up to 4 hydroxy, alkoxy radicals [881]. The hydroxy alkoxy radical may isomerize and may react with molecular oxygen, and thus several different radicals and products may be formed.

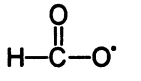
In the polluted air, besides the above-mentioned oxygen radicals such as O_2^-, HO_2^-, HO^-, RO^-, RO_2^-, formyl (HCO^-) and acyl (RCO^-), other radicals may also arise under the influence of sunlight radiation, e.g. peroxyformyl, peroxyacyl, formate and acidic:



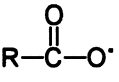
peroxyformyl



peroxyacyl



formate



acidic

The most important reaction generating radicals is photolysis of aldehydes which results in alkylic and formyl radical production. It is worth noting that photolysis of nitrous and nitirc acids produces hydroxyl radical and atomic oxygen, whereas photolysis of alkyl nitrite gives alkoxy radicals [882].

It is interesting to underline that HO radicals play an important role in controlling climate, because methane (a greenhouse gas) is removed from the atmosphere mainly during its reaction with HO^- (reaction at Eq. (317)). Moreover, this reaction initiates a series of subsequent reactions leading to the production of carbon monoxide. It is calculated that about 20–50% of CO present in the atmosphere is generated by methane oxidation. Hydroxyl radicals also participate in CO generation from other hydrocarbons, e.g. isoprenes and terpenes [787], although the main loss of CO results from its reaction with HO^-.

There exist many more reactions which generate free radicals, and a variety of other toxic products may be generated in the polluted atmosphere [883–886] but their discussion is prohibited by space limitation.

8.1.7 Singlet Oxygen

It has become well recognized during the past three decades that singlet oxygen plays an important role in both the natural and polluted tropospheres [876, 877, 887–891]. This reactive species may play a significant role in the pathology of all living organisms from plants to man (Chap. 5).

Several sources of singlet oxygen in the polluted atmosphere have been reported [890–903].

(a) Direct absorption of solar radiation by molecular oxygen

$$O_2(^3\Sigma_g^-) \xrightarrow{h\nu} {}^1O_2(^1\Delta_g, {}^1\Sigma_g^+) \tag{338}$$

This way of singlet oxygen generation is of rather low efficiency because of suppression by the electric dipole selection rules. The collision of the molecules releases this suppression [900] (see also Chap. 1).

(b) Collisionally induced absorption of solar energy

$$O_2(^3\Sigma_g^-) + M \overset{h\nu}{\rightleftharpoons} O_2(^1\Delta_g, {}^1\Sigma_g^+) + M \qquad (M = O_2, N_2) \tag{339}$$

(c) Energy transfer from organic molecules excited to the triplet state (donor) to oxygen molecules; see reactions at Eqs. (203) and (204). It may be worth remembering that the energy transfer occurs not only from dyes but also from organic molecules, e.g. aromatic hydrocarbons in gasoline [891] such as benzaldehyde, benzene and naphthalene in the gas phase excited by solar energy. The transfer energy has been observed for gas-liquid, gas-solid systems as well as homogeneous gaseous systems [904].

(d) Photolysis of ozone in the Hartley spectral region (320–200 nm); see reaction at Eq. (66).

In the polluted urban atmosphere and in the upper atmosphere the photolysis of ozone is an especially important source of singlet oxygen. Atomic oxygen arising in the reaction at Eq. (66) may react with molecular oxygen giving rise to another molecule of singlet oxygen [889]:

$$O(^1D) + O_2 \rightarrow O(^3P) + O_2(^1\Sigma_g^+) \tag{340}$$

(e) The oxygen atoms recombination

$$O + O + M \rightarrow {}^1O_2 + M \tag{341}$$

The latter reaction may be a dominant source of $^1O_2(^1\Delta_g)$ in the Earth's atmosphere during the night.

(f) Reactions of ozone with nitrogen oxides (see reactions at Eqs. (68) and (282). The reaction at Eq. (68) is known in the literature as generation of singlet oxygen in the atmosphere via "chemiexcitation" because it is accompanied by direct generation of $^1O_2$ or nitrogen dioxide in the electronically excited state (NO_2^*). The excited molecule of NO_2 may transfer its energy to molecular oxygen yielding the latter molecule in the excited $^1\Delta_g$ or $^1\Sigma_g^+$ states.

Also, reaction of hydroxyl radicals with atomic oxygen is highly exothermic and generates $^1O_2$:

$$HO^{\cdot} + O \rightarrow H + {}^1O_2 \tag{342}$$

(g) Reactions of ozone with a variety of other atmospheric trace compounds such as sulfides, sulfoxides, amines and fluorinated hydrocarbons giving rise to chlorine atoms during photodissociation produce singlet oxygen (e.g. reaction at Eq. (279)) [903, 904].

Lastly it is worth mentioning a reaction which may be an important source of $^1O_2$ in the polluted air, i.e. the reaction of hydrogen peroxide with nitrogen oxide [905]. Singlet oxygen generated by the above-mentioned multiplicity of reactions occurring in the atmosphere loses its excitation energy by the following main processes [891, 906].

(a) Radiative decay (simultaneous emission)

$$^1O_2 \rightarrow O_2(^3\Sigma_g^-) + \text{light emission} \tag{343}$$

(for details see Chap. 1).

(b) Collisionally induced emission

$$^1O_2 + M \rightarrow O_2(^3\Sigma_g^-) + M + \text{light emission} \tag{344}$$

(c) Collisional quenching

$$^1O_2 + M \rightarrow O_2(^3\Sigma_g^-) + M \tag{345}$$

or

$$^1O_2(^1\Sigma_g^+) + M \rightarrow {}^1O_2(^1\Delta_g) + M \tag{346}$$

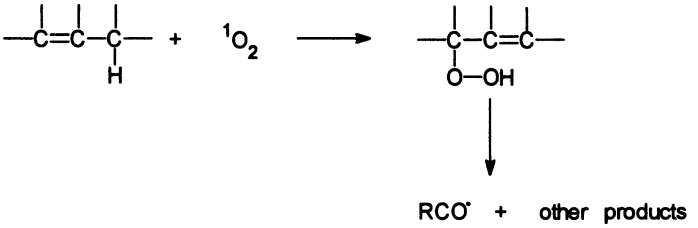
(d) Chemical reactions. Singlet oxygen, as a very strong oxidant, reacts with a broad variety of organic and inorganic compounds with high efficiency (see Chap. 3).

It should be noted here that most singlet oxygen formed even in polluted air is quenched by molecular oxygen which occurs in excess: therefore this species might exert influence on the balance between atomic oxygen and ozone in the higher atmosphere. It has been found that the mean concentration of $^1O_2$ in the atmosphere is about 10^8 molecules $\cdot$ cm^{-3} [876], and may be sometimes 10^2 times higher than the concentration of hydroxyl radicals. Methods of singlet oxygen detection are reported in Chap. 4. Recently, a new method for $^1O_2$ detection in the atmosphere has been developed by Ogawa et al. [907]. In this method, α-terpinene reacts with $^1O_2$ giving ascaridole, which in turn is determined by gas chromatography. Using this method the maximal concentration of $^1O_2$ in the polluted troposphere of suburban Kyoto was measured to be about $5 \cdot 10^{11}$ molecules $\cdot$ cm^{-3}.

It has also been reported that singlet oxygen is a major contributor to the airglow in the stratosphere and the mesosphere. The measurements of the day and night glow from $^1O_2$ in the middle atmosphere has been discussed in details by Wayne [889].

The major types of reactions of $^1O_2$ with organic and inorganic compounds are discussed in Chap. 3. The most significant role of $^1O_2$ in both the natural and polluted lower atmospheres is generation of organic free radicals participating in a conversion of NO to NO_2.

It is worth noting here that the generation of unstable hydroperoxides during $^1O_2$ addition to olefines produces the acyl radicals ($RCO^{\cdot}$) needed for the radical oxidation of NO to NO_2 as follows [908, 909]:



(347)

$$RCO^{\cdot} + {}^3O_2 \rightarrow RCO_3^{\cdot} \quad (348)$$

$$RCO_3^{\cdot} + NO \rightarrow RCO_2 + NO_2 \quad (349)$$

Faust and Allen [910] have reported that singlet oxygen might play an important role in photochemical reactions occurring in clouds and fogs. This suggestion found confirmation in paper by Lelieveld and Crutzen [911] which showed that reactions with participation of singlet oxygen can exert an effect on the cycling of ozone in the troposphere.

Singlet oxygen is an important species not only with respect to the pathology of several diseases, discussed in Chap. 5, but it may also play a significant role in smog-producing reactions.

As one can see from Chap. 5, amino acids and pyrimidine bases undergo oxidation with singlet oxygen and their oxidation leads to considerable biological damage. Both plants and animals contain the porphyrin structure in chlorophyll and hemoglobin molecules, so the damaging effect of singlet oxygen is not surprising considering its high reactivity as an electrophilic agent [912, 913].

It is also worth mentioning that not only living systems but also natural and synthetic compounds, e.g. polymers, can be affected by singlet oxygen [914]. It has been reported that the reactivity of singlet oxygen may be larger in droplets of clouds and fogs than in the gas phase [910].

8.1.8
Hydrogen Peroxide and Formaldehyde

Hydrogen peroxide is produced by cells generating $O_2^{\cdot-}$ as well as by several enzymes, e.g. urate oxidase, glycollate oxidase or D-amino-acid oxidase. This oxidant arises during photosynthesis and in the breathing process. If the steady state of H_2O_2 concentration estimated to be 10^{-7}–10^{-9} mol · ℓ^{-1}, e.g. in the rat liver, is exceeded then this compound can cause ATP depletion, inactivation of some enzymes by damage to their thiol groups [565]. Hydrogen peroxide is also

known as a precursor of the HO radical (Chap. 1). The main source of hydrogen peroxide in the gas phase is dismutation of hydroperoxy radicals arising during ozone photolysis (reaction at Eq. (1)). The next toxic agent, formaldehyde, is both emitted into the atmosphere as primary pollutant and produced as an oxidation product of hydrocarbons (reaction at Eq. (320)). The aldehyde plays a very important role in smog formation. Photolysis of CH_2O or its reaction with hydroxyl radicals (Fig. 28) generates HO_2 radicals. The HO_2 radical oxidizes NO into NO_2. Note that the HO radical is also responsible for the formation of NO_3 as well as generation of nitric acid (reaction at Eq. (321)). Formaldehyde in a free form is a central metabolite for assimilatory and dissimilatory sequences. Formaldehyde can be produced, for example, during oxidation of methanol by methanol oxidase, or by methanol dehydrogenase, from nitroamines in the presence of demethylase enzyme system or in the presence of aromatic hydrocarbons. This compound can also arise as a result of different stress effects, infections, radiation, or chemical reagents. Formaldehyde can take part in spontaneous methylation and formylation reactions with proteins and nucleic acids. Epidemiological studies have suggested the possibility of the human risk from formaldehyde exposure as well as nasal cancer in rats. It is evident that there is a regulated metabolism of formaldehyde in a cell, and its cytotoxicity depends on this compound generation and consumption. The water-soluble gases such as hydrogen peroxide and formaldehyde are important trace species in the chemistry of the troposphere. Both gases have natural backgrounds in the atmosphere mainly as a result of the chemical degradation of hydrocarbons. Hydrogen peroxide and formaldehyde have been found to occur in the troposphere in both gas and aqueous phases. Their concentrations in the gas phase are estimated to the about 40 $\mu mol \cdot \ell^{-1}$ and they strongly depend on the season, with maximum during summer [915].

Results obtained from aircraft on the vertical distribution of hydrogen peroxide show that concentration of hydrogen peroxide increases with altitude in the lower troposphere and reaches the maximal value close to the surface of clouds, where it is about ten times higher than in the boundary layer. Hydrogen peroxide and formaldehyde are present in cloud, fog, rain water and in other precipitations because of their high solubility in water [916]. Hydrogen peroxide is an important oxidant of sulfur dioxide in cloud and rain water [917]. This oxide is very important in the formation of acidity in cloud water, e.g. by reactions at Eqs. (274) and (275). The increased natural emission of isoprene from forests by pollution of air is also an important source of H_2O_2 in the atmosphere [918]. For example, it has been found that nitrogen oxides increase the natural emission of isoprene from deciduous trees. The isoprene photooxidation increases the formaldehyde concentration oxidation of which leads to formation of hydroperoxide radicals and further to hydrogen peroxide generation.

The mechanisms of reactions discussed in this chapter and the role of the oxygen species constitute only some of the important chemical processes occurring in the natural and polluted troposphere. These reactions are very complex, making their examination experimentally difficult. Therefore the elucidation of the reaction mechanisms has been sought.

Natural and man-made emissions of pollutants lead to their regional and global distribution and they can act as acids, oxidants and precursors to toxics.

It should be stressed here that the total dangerous effect of pollutants on humans and plants is far greater than the sum of each pollutant's effect separately. This is a result of the multiplicity of chemical and photochemical reactions occurring in the atmosphere and will be detailed below.

8.2 Biochemical and Economic Aspects of Troposphere Pollution

8.2.1 Smog

In both popular and scientific literature we find the therm "smog" which reflects the worldwide danger of air pollution due to chemical and industrial pollution.

Under certain conditions, mainly if air is not able to circulate, the concentration of pollutants may become relatively high. For example, concentrations of nitrogen oxide, sulfur dioxide and aldehydes may reach values of 5–30 pphm (1 pphm = $2.445 \cdot 10^9$ mol $\cdot$ dm^{-3}); ketones, nitrous acid, the acyl and alkyl nitrates about 10 pphm; peroxides and nitric acid about 1 pphm, whereas the rate of atomic oxygen generation and nitrogen oxide is estimated to be 100–500 pphm $\cdot$ h^{-1}, alkyl, alkoxy and acyl radicals – even 10 pphm $\cdot$ h^{-1} and hydroxyl radicals less than 1 pphm $\cdot$ h^{-1} [795].

Reactions between the pollutants generate a dense, yellow-brown fog, containing ozone and strongly toxic superoxides such as peroxyacyl nitrate, sulfur oxides (SO_2, SO_3), carbon oxides (CO, CO_2), nitric oxides (NO_x), hydrocarbons, formaldehyde, other aldehydes, ketones, paraffins, olefins, aromatics and sulfuric acids. Two kinds of smog are discerned: (a) photochemical, arising with participation of the solar energy, and (b) chemical, arising in the absence of sunlight, i.e. during dark reactions [919].

(a) Photochemical smog which is called the Los Angeles type, since it was observed first in Los Angeles, arises in the tropical climate, mainly from pollutants emitted from automobile exhausts. Some key reactions in photochemical smog formation involve NO to NO_2 conversion, and consequently the occurrence of ozone at high levels. Therefore ozone is a major indicator of photochemical smog presence. Photochemical smog consists of a mixture of primary and secondary pollutants of which ozone and polyaromatic hydrocarbons are considered to be of significance. A key process in the formation of photochemical smog is the photolysis of nitric dioxide into nitric oxide and atomic oxygen, (see reaction at Eq. (278)) with simultaneous formation of ozone (reaction at Eq. (276)) [889, 909]. The next important reaction in smog formation is that of hydrocarbons with atomic oxygen giving raise to C_nH_mO radical, followed by its reaction with molecular oxygen, as follows:

$$C_nH_m + O \rightarrow C_nH_mO^{\cdot} \tag{350}$$

$$C_nH_mO^{\cdot} + O_2 \rightarrow C_nH_mO_3^{\cdot} \tag{351}$$

The latter free radical (acyl peroxy radical) may be involved in reaction with NO_2 to yield peroxyacyl nitrate

$$C_nH_mO_3^{\cdot} + NO_2 \rightarrow C_nH_mO_3NO_2 \quad (352)$$

as well as with hydrocarbons giving aldehydes and ketones as the and products. The peroxy radicals are efficient catalysts of H_2SO_4 production from SO_2 (reactions at Eqs. (264) and (265)). The acyl peroxy radical also reacts rapidly with molecular oxygen, giving ozone,

$$C_nH_mO_3^{\cdot} + O_2 \rightarrow C_nH_mO_2^{\cdot} + O_3 \quad (353)$$

and furthermore the $C_nH_mO_2$ radical may be involved in oxidation of nitric oxide to nitrogen dioxide, similarly to the reaction at Eq. (319).

Ozone and peroxyacyl nitrate are main components of smog. They are very dangerous to humans causing alteration of lung function or throat symptoms.

The presence of hydrocarbons in the atmosphere, especially that polluted by industry, increases the rate of oxidation of SO_2 to SO_3, and a great part is played in this catalysis by radicals such as alkyl, alkyl peroxy and acyl peroxy. Also, nitrated polycyclic aromatic hydrocarbons (nitroarenes), known for their mutagenic activity, are emitted by automobiles and represent important constituents of urban air.

During photochemical reactions, considerable amounts of inorganic radicals such as HO and HO_2 are also generated, and are involved in oxidation of SO_2 to SO_3 (as in reaction at Eq. (316)). The influence of HO_2 radicals on photochemical smog formation is well know, and the reaction at Eq. (273) is reported to play a crucial role [920, 921].

Another oxygen species, namely singlet oxygen, also plays an important role in photochemical smog formation. Reactions generating this strong oxidant have been discussed above, among which the most important in smog formation are the reactions of ozone with nitrogen oxide (reaction at eq. (281)) and of ozone with atomic oxygen [837]:

$$O_3 + O \rightarrow {}^1O_2 + O \quad (354)$$

Singlet oxygen, similar to ozone, plays a key role in the atmospheric cycling of nitrogen oxides (Fig. 29).

The possible role of singlet oxygen in photochemical smog formation in the lower atmosphere was first reported by Leighton in 1961 [921]. Smith and Wayne [887] have developed the hypothesis of singlet oxygen participation in photochemical smog formation in respect of its relatively long lifetime at atmospheric pressure, i.e. 0.05 s, and its high and efficient reactivity with olefines in the gas phase. The mechanisms of photochemical smog formation are discussed in detail by Demerjian et al. [922].

It has been reported that smog caused plant foliage damage in both Los Angeles and Tokyo [883]. Photochemical smog does not cause short-term lethality on a large scale, but it decreases immunity of living organisms against disease, and attacks respiratory tracts, causing eye and throat irritation. In large urban areas, e.g. London metropolitan area, photochemical smog is present in the summer over an area of about 48 square km [883].

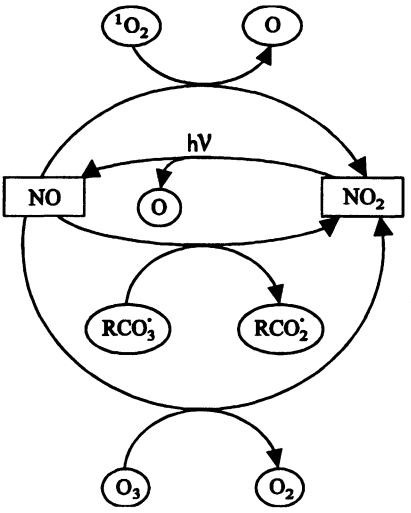


Fig. 29. Participation of ozone and singlet oxygen in the atmospheric cycling of nitrogen oxides

(b) The second kind of smog – chemical smog – arises without participation of solar energy during burning of coal and other fuels. This smog contains mainly oxides of sulfur and carbon, toxins and smokes. The smog is often called London smog or sulfuric smog. Absorption of environmental pollutants, mainly chemicals, may occur at all external surfaces of the body including the mouth, the gastrointestinal tract, the lungs, and the skin. Chemical compounds are also introduced into the body by simple diffusion. For this reason chemical smog blisters living organisms, causing large-scale morbidity and even death. For example, air pollution and smog formation in London was the cause of death of four thousand people in 1952/53, and of more than one thousand in 1956 [883, 923].

8.2.2 Acid Rain

The term "acid rain" was introduced in the 19th century by the England scientist Angus Smith, in order to describe precipitations near Manchester. This phenomenon may be described as rainfall mainly acidified by sulfuric and nitric acids [786, 924–926]. Sulfur dioxide and nitrogen oxides are carried out on winds, combine with water to give the above-mentioned acids, and fall to Earth in rain, snow, fog or in a dry state. They enter the soil and reach the roots of trees, as well as pollute lakes and streams. Acid rain occurs in many places in North America, Canada [926] and Europe (Germany, Poland, Austria) [923]. It is well established that sulfur oxides are responsible for about 70%, and nitric oxides for 30% of the acidification of precipitation. The formation of acid rain may be quickly explained as follows. Natural trace gases and man-made pollutants are mainly

emitted into the Earth's atmosphere in reduced or only partially oxidized form. There they usually undergo oxidative transformation to acids, becoming water-soluble pollutants. The pollutants may fall directly down onto the Earth's surface or can be washed down by precipitation [924, 925].

For example, sulfur dioxide of which emission to the atmosphere is estimated to be about 300 million tons per year [867] is very soluble in water, giving sulfurous acid (reaction at Eq. (264)), or forms sulfuric trioxide and further sulfuric acid (reaction at Eq. (265)). The mechanism of the sulfuric acid formation from SO_2 is as follows: gaseous SO_2 diffuses to the surface of a water droplet, dissolves, and hydrolyzes forming HSO_3^- and SO_3^{2-} ions. These ions are oxidized to SO_4^{2-} during reaction with O_3 and H_2O_2 and finally dissolve in the droplets.

Similarly, nitrogen oxides form acids with water as follows

$$NO + NO_2 + H_2O \rightarrow 2HNO_2 \tag{355}$$

$$NO_2 + NO_3 \rightarrow N_2O_5 \tag{356}$$

$$N_2O_5 + H_2O \rightarrow 2HNO_3 \tag{357}$$

Acids are also formed by these oxides during their reactions with H_2O_2 (reactions at Eqs. (274) and (275)) or with both organic and inorganic radicals, e.g. reactions at Eqs. (298) and (321).

Carbon dioxide also dissolves in water, forming the weak carbonic acid

$$CO_2 + H_2O \rightarrow H_2CO_3 \tag{358}$$

and forms an equilibrium with bicarbonate and carbonate ions; thus normally the pH of rain should be about 5.6. Large amounts of SO_2, NO_x and other pollutants are present in the atmosphere and they cause a decrease of rainfall pH to 3.5–4.5 [786].

Precipitation also contains a number of organic and inorganic gases and aerosol species soluble in water. The formation of compounds responsible for the acidification of precipitation is strongly coupled with photochemical formation of oxidants, which is thus connected to smog chemistry. For example, formaldehyde, hydrogen peroxide, sulfate and nitrate have been identified in precipitation samples collected from the air [927]. It is worth mentioning that the acidity of rivers and lakes also arises from precipitation.

Acid rains have received a great deal of attention from scientists because of their damaging effects on crops, forest ecosystems, rivers and animals as well as on building materials, metals, cotton, wool, linen, nylon, paper goods or skin. Building materials are especially susceptible to corrosion. The damaging effect of acid precipitation results from the formation of calcium sulfate ($CaSO_4$) which has twice as large a volume as calcium carbonate:

$$CaCO_3 + H_2SO_4 \rightarrow CaSO_4 + H_2CO_3 \tag{359}$$

Stone erosion is increased by CO_2 because carbonic acid forms soluble calcium and magnesium hydrogen carbonates on contact with the stone.

Nash and Gries [928], using lichens as indicators air pollution, especially SO_2, have shown several changes in photosynthesis and respiration, nitrogen fixation and pigment degradation.

8.2.3 Plant Senescence-Like Processes

During the last two decades a strong interest has arisen in both the beneficial and deleterious effects of free radicals such as $O_2^{\cdot-}$, $HO^{\cdot}$, $RO^{\cdot}$, $ROO^{\cdot}$, singlet oxygen or H_2O_2 on maturation and senescence of various plant organs – leaves, flowers and fruits [929–934]. As it was discussed in Chap. 6, oxygen free radicals play an important role in the normal functioning of a cell, e.g. $O_2^{\cdot-}$ participates in the respiratory process, and phytopathological resistance from diseases may be a result of free radical destruction of pathogens [935].

Plant senescence has been considered as a multifactorial process depending on several factors, e.g. irradiation, temperature, oxygen concentration, virus infection, presence of metals and levels of atmosphere pollution (acid rain, concentration of ozone, nitrogen oxides, sulfur oxides or carbon oxides, automobile fuel exhaust gas components, tobacco smoke, some herbicides) [936–938]. These external factors have biochemical consequences and important implications for oxygen species production and plant ageing.

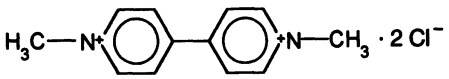
It is worth mentioning here that plants contain a number of photosensitizers such as chlorophylls, riboflavin, griseofulvins, anthraquinones, hypercins, psoralens, porphyrins, polyacetylenes, and cinnamic acid derivatives able to give rise to singlet oxygen in the presence of light and molecular oxygen [930, 939].

The balance between oxygen species generation and their removal by free radical scavengers as well as enzymatic protective mechanisms is necessary for plant growth. Plants have, like man, developed defence mechanisms against the damaging effects of oxygen toxic species. For example, catalase and SOD, the enzymes able to remove H_2O_2 and $O_2^{\cdot-}$ from a cell, are an integral part of Photosystem I where the generation of $O_2^{\cdot-}$ together with $HO^{\cdot}$ and $^1O_2$ in chloroplasts occurs [940], while SOD is present in the stroma of chloroplasts. The ability of chloroplasts to decompose the excess of H_2O_2 is due to the activity of the following enzymes: ascorbate peroxidase, dehydroascorbate reductase and glutathione reductase because the chloroplasts do not contain catalase. Plants also produce large concentrations of vitamins C and E as well as a group of purine bases – phytohormones which play a protective role against oxygen species [931].

However, the ripening and senescence of leaves, flowers and fruit is a complex process which has been found to be correlated with increases in lipids peroxidation and membrane permeability [941, 942]. The increased generation of oxygen species or damage to the protective mechanisms in a cell (e.g. by pollution of the atmosphere) increases the rate of lipids peroxidation and amounts of toxic products of their oxidation; see Chap. 5.

It has been reported that the product of lipid peroxidation which accumulates with ageing of pear and banana fruits is spectrally identical with lipofuscin from mammals [943].

Lastly, a great deal of attention has been focussed on oxygen radical toxicity to plants of some herbicides. Herbicides belong to one of the three main groups of pesticides, i.e. substances used to control fungi, insects and other animals attacking food production. The two other groups of herbicides are insecticides (e.g. nicotine, DDT, organochlorines, chlorinated hydrocarbons) and fungicides (e.g. captan, organic mercury compounds or phenolic substances). The most important herbicides are paraquat and diquat. These organic substances are used to destroy or suppress the growth of plants. The damaging effect of paraquat is accelerated by light, oxygen and transition metal ions [944–946]. For example, the killing of green plants by bipyridyl herbicides, e.g. paraquat (methyl viologen, PD^{2+}, 1,1′-dimethyl-4,4′-bipyridylium dichloride) or diquat (1,1′-ethylene-2,2′-dipyridylium ion) has been related to O_2^- and H_2O_2 generation:



(PD^{2+}) paraquat dichloride

$$PD^{2+} + e \rightarrow PD^{\cdot+} \quad (360)$$

$$PD^{\cdot+} + O_2 \rightarrow PD^{2+} + O_2^- \quad (361)$$

Paraquat ion, PD^{2+}, undergoes one electron cyclic reduction and oxidation. The reduction of PD^{2+}, e.g. by the cell enzymes gives the stable monocation radical ($PQ^{\cdot+}$), a powerful reducing agent which reacts at high rate with oxygen, generating O_2^-. Superoxide anion radical can rapidly dismutate to hydrogen peroxide.

The monocation radical can also reduce redox active metal ions [946]:

$$PD^{\cdot+} + Me^{n+} - \text{complex} \rightarrow PD^{2+} + Me^{(n-1)+} - \text{complex} \quad (362)$$

of which the reduced form generates hydroxyl radicals and singlet oxygen in reaction with H_2O_2 via a Fenton-type reaction (reaction at Eq. (31)).

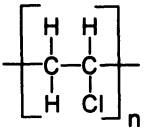
Paraquat is highly toxic to all living organisms including animals and man. Its poisoning causes respiratory failure, probably by damage to epithelial cells in the lung. For details dealing with biological damage see [947].

8.2.4 Degradation of Polymers

8.2.4.1 *Classification of Involved Compounds*

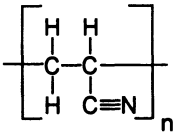
Polymers are used on a large scale as objects of daily use or materials in industry. Several examples of their applications may be specified [780]. Hence, vinyl plastics are used for electrical installation, films, tubing containers. Among them:

polyvinyl chloride (PVC)



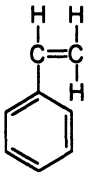
finds use in phonograph records, sheet plastic wrap production;

polyacrylonitrile



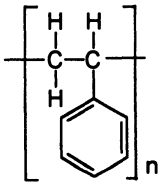
is used for fibre, orlon, acrilans production;

styrene (vinylbenzene)



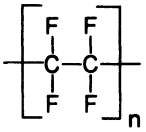
is industrially important in the production of plastics and synthetic rubbers. The basic application of styrene is its polymerization to polystyrene.

Polystyrene



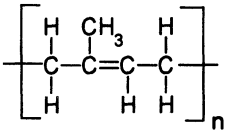
shows thermoplastic and light transparent properties. The polymer finds application for electrical installation and moulded objects.

Polytetrafluoro-ethylene

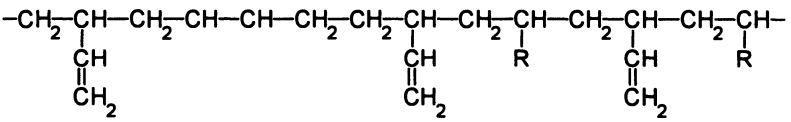


shows chemical resistance and finds application in moulded objects, electrical installation and teflon production.

Natural rubber



is a polymer of molecules of the composition $(C_5H_8)_n$ obtained from certain trees. It consists of isoprene units, with a polymerization degree ranging between n = 5000 and n = 8000, and molecular weight of 100000–150000. Synthetic rubbers are commercial synthetics widely used in the automobile industry, for example for manufacturing gasoline pipes, for their good resistance to hydrocarbons, or for the production of tyres. They are produced by polymerization of 1,3-butadiene (CH_2=CH–CH=CH_2) with unsaturated compounds such as acrylonitrile (butadiene-acrylonitrile rubber) or styrene (styrene-butadiene rubber):



where R denotes C≡N or C_6H_5, respectively. Similarly, if isobutylene is copolymerized with butadiene, butyl rubber is obtained.

Plastics compete with traditional materials, e.g. replace metal elements used in the automobile construction. Increasing application of plastics for industrial purposes has led to studying of the role of atmospheric pollutants in their ageing.

8.2.4.2 Degradation

The importance of oxygen free radicals, singlet oxygen, ozone and other constituents of the polluted atmosphere in the oxidative degradation of both natural and synthetic polymers has been pointed out by several authors [914, 948–958]. Reactions of oxygen species with polymers are very important industrially with respect to deterioration of the product quality.

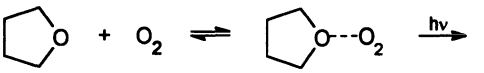
Two kinds of processes responsible for polymer degradation may be distinguished: autoxidation (oxidation of polymers in air, usually occurring under mild chemical conditions), and oxidation which occurs with participation of light energy (photo-oxidation), heating or external oxidants [948]. Usually both types of oxidation reactions occur simultaneously. They may result in formation of carbonyl and hydroxyl groups, chain scission, crosslinking reactions, and thus in modification of the polymer structure. These processes cause changes of physical and chemical properties of the polymer involving the deterioration of product quality and substantial financial losses. Oxygen, temperature and light are the three most accelerating agents in the degradation of polymers. Photo-oxidation of polymers is a particular problem of considerable technological importance from an industrial viewpoint as the accelerated oxidation of polymers occurs in the presence of light, one of the most frequently met oxidative factor.

Purely synthetic polymers contain single bonds C–C, C–H, C–O, C–N, C–Cl, C–F and are not subject to photodegradation by visible light because they do not

absorb light of longer wavelengths than 190 nm [953]. Photodegradation reactions may be acceleratd by impurities of internal and external origin [951]. The main internal impurities are due to the presence of chromophoric groups which are built into a polymer chain during the polymerization process carried out, e.g. in the presence of air.

External impurities come from the manufacturing, contact with metals, or solvents used in the polymerization process and from the polluted environment. During the processing of polymers thermal oxidation generates carbonyl groups which easily undergo excitation during the exposition of polymers to light. Moreover, there exist contacts with parts of the machinery involving metallic impurities able to undergo redox reactions in the polymer structure, and thus able to generate oxygen reactive species such as O_2^-, H_2O_2, $^1O_2$ and $HO^\cdot$. It has been reported that some salts and metal oxides, e.g. TiO_2 [959] and ZnO [949], accelerate the photooxidation rate of nylons, whereas CuO and Cu_2O exhibit a sensitizing effect in oxidation of natural rubber [960]. Several polynuclear aromatic compounds such as quinones, polycyclic aromatic hydrocarbons, benzophenones and peroxides are absorbed from the atmosphere and may be introduced in polymer oxidation. In particular, alkyl and aryl peroxides are important in both thermal and photodegradation of polymers, because they give rise to alkoxy radicals. Also organic impurities, e.g. residues of solvents such as ketones, aromatic hydrocarbons, benzene, chloroform, tetrahydrofuran and carbon tetrachloride, are responsible for polymer degradation [951]. It has been reported that polyolefines exposed to the air may absorb aromatics released during combustion [961], and generate singlet oxygen.

Many solvents undergo photooxidative degradation forming radicals and new compounds which may accelerate the degradation of polymers. Some of them form charge transfer complexes (CTC) with molecular oxygen, which are a source of oxygen radicals. For example, tetrahydrofuran forms, O_2^-, HO_2 and HO radicals [951], as follows:



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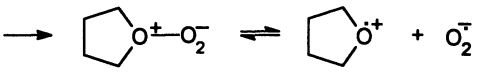

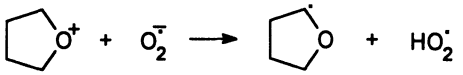

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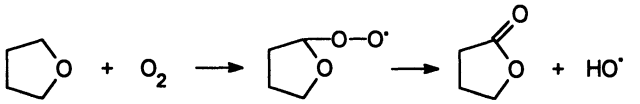


(365)

Polymers of commercial usage also contain compounds which, e.g. added in the processing of polymers into plastic or rubber materials, play the role of thermostabilizers and antioxidants, but which may also be a source of impurities, being involved in the photosensitizing reactions.

Role solvents, metal compounds, organic impurities and internal chromophoric groups in accelerated degradation of polymers is detailed by Rånby and Rabek [951].

Two main mechanisms of initiation of oxidative degradation of polymers may be distinguished: initiation by free radicals and with participation of singlet oxygen [953].

Initiation by Free Radicals. The free radical process involves radical formation by the photo-dissociation of impurities (AB) present in a polymer matrix when the excitation energy reaches the energy of bond disruption:

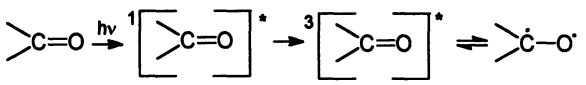
$$AB \xrightarrow{h\nu} A^{\cdot} + B^{\cdot} \tag{366}$$

The radicals formed may abstract hydrogen atom from the polymer chain giving alkyl polymer free radical ($R^{\cdot}$)

$$RH + A^{\cdot} \text{ or } B^{\cdot} \rightarrow R^{\cdot} + AH \text{ or } BH \tag{367}$$

This reaction is known as the initiation step of the chain branching.

The next mechanism of free radical formation during the degradative oxidation of polymers containing impurities with chromophoric groups, e.g. carbonyl group in their structure, is formation of a biradical



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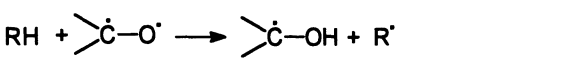
The oxygen atom in a carbonyl group excited to the triplet state is electron deficient and behaves as an electrophilic alkoxy radical being involved in the hydrogen atom abstraction



(369)

If molecular oxygen is present the alkyl radical ($R^{\cdot}$) reacts very rapidly with oxygen giving polymer peroxy radical, $ROO^{\cdot}$, according to reaction at Eq. (81), able to abstract hydrogen atoms from the same or other polymer molecules (reaction at Eq. (82)) and hydroperoxide, ROOH is formed. Hydroperoxide groups, present on the polymer, may decompose themselves thermally or photochemically. The energy of the RO-OH bond dissociation is about 176 kJ $\cdot$ mol^{-1}, thus the energy of wavelengths above 300 nm is sufficient for cleavage of this bond to form polymer oxy radical, $RO^{\cdot}$ and hydroxyl radical, $HO^{\cdot}$. The oxy radicals are very reactive and they can abstract hydrogen atoms from the

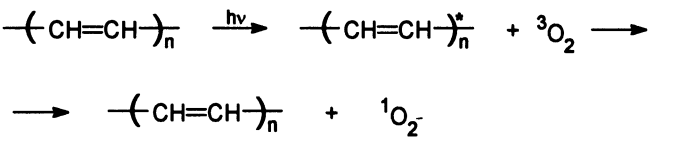
neighbouring molecules, and hydroxyl groups in photooxidized polymer are formed as well as a new polymer alkyl radical ($R^{\cdot}$). The hydroxyl groups are assumed to be generated along the polymer chain. The oxidation of polymer occurs by a similar mechanism as that proposed for peroxidation of polyunsaturated fatty acids, presented in Fig. 12. For details dealing with possible reactions occurring during free radical oxidation, e.g. of 1,2-polybutadiene, see work by Lucki et al. [952].

Free radicals may also be generated with participation of traces of impurities present in a polymer containing chromophoric groups. The groups can undergo excitation to the long-lived triplet excited state during absorption of light following energy transfer from this state to polymer [962]:

$$^3S_1 + RH \rightarrow {}^1S_1 + (RH)^* \tag{370}$$

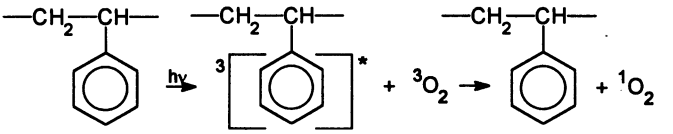
$$(RH)^* \rightarrow R^{\cdot} + H^{\cdot} \tag{371}$$

Initiation by Singlet Oxygen. Singlet oxygen, a very important agent in the oxidative degradation of polymers, can be generated thermally or photochemically. During exposure of polymer to ultraviolet radiation singlet oxygen may be formed directly in the energy transfer between photoexcited polyene-units and molecular oxygen [948]:



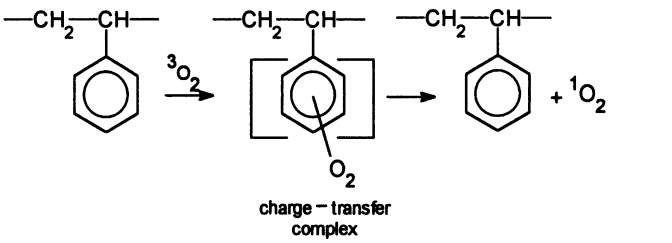
(372)

or from excited triplet state of benzene rings, e.g. in polystyrene:



(373)

The next important way of singlet oxygen formation is the energy transfer from charge-transfer complex to molecular oxygen [953]:

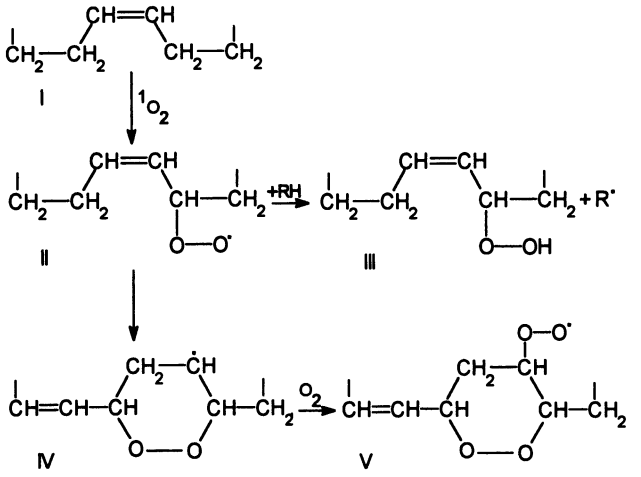


(374)

Moreover, industrial polymers contain a high number of carbonyl groups, and therefore singlet oxygen can be generated in polymers by intramolecular energy transfer from the excited triplet of the carbonyl group to molecular oxygen with high efficiency. Large amounts of pollutants released into the atmosphere from automobile exhaust can also concentrate in polyolefin materials exposed to this atmosphere [963] and act as photosensitizers.

Molecular oxygen and singlet oxygen play an important role in acceleration of polymer degradation. These species generated in a polymer or on its surface are involved immediately in polymer oxidation. The role of singlet oxygen in the degradation of polymers has been demonstrated by many workers in the last 30 years. In 1968 Trozzolo and Winslow [964] showed that reactions of $^1O_2$ with polyethylene resulted in the formation of hydroperoxide groups. More extensive evidence for the participation of $^1O_2$ in degradative oxidation of polymers was obtained by Kaplan and Kelleher in 1970 [965] and Rabek and Rånby in 1976 [966] and was presented during the EUCHEM Conference on Singlet Oxygen Reactions with Polymers held at Södergan on Lidingö, Stockholm, Sweden on September 2–4, 1976. The attention of scientists has focussed on the mechanism of singlet oxygen generation, its reactions with synthetic and natural polymers, and the problems of stabilization of polymers against oxidation by singlet oxygen.

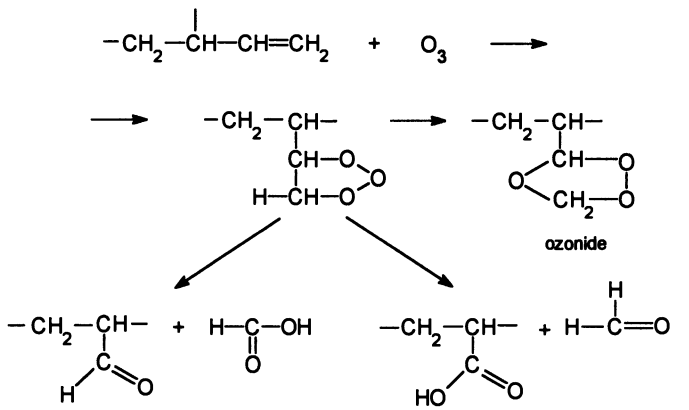
Studies are being continued on photochemical reactions in the polluted atmosphere and new spectroscopic methods for measurement of the role of singlet oxygen in oxidation of polymers are being applied. For example, recent studies by Billingham and Then [957] have shown that an early stage of polymer oxidation may be measured by using the chemiluminescent method. It has been reported that α- and β-unsaturated carbonyls located in the main chain of polymers are the luminescent centres formed during oxidation of polyolefins, rubbers or aliphatic amides [967]. Many commercially important polymers contain C=C bonds and $^1O_2$ can attack double bonds, and so may react with olefines, both in liquid and gaseous phases, giving allylic hydroperoxides [962, 968]. For example, photooxidation of polydiene polymer, compound I, gives a polymer hydroperoxide, compound III:



(375)

or a cyclic peroxide-hydroperoxide structure (IV) by cycloaddition of $^1O_2$ across a conjugated double bond. The cyclic hydroperoxides may also show a free radical structure and form new peroxy radicals (V) in the presence of molecular oxygen. Decomposition of cyclic hydroperoxides often results in formation of α- and/or β-unsaturated aldehydes and other low-weight products.

Initiation by Atomic Oxygen and Ozone. There are experimental data showing that commercial polymers are oxidized by atomic oxygen and ozone in air containing these pollutants. Comparative studies of 1,2-polybutadiene degradation by oxygen, atomic oxygen, singlet oxygen and ozone carried by Lucki et al. [952] in solution and/or in solid state showed some differences in the kind of products formed, but all these oxidants caused crosslinking reactions. For example, reaction of ozone with 1,2-polybutadiene gives ozonides and high-weight products of the polymer degradation, which contain aldehyde, carboxylic and carbonyl groups along the backbone chain [952]:



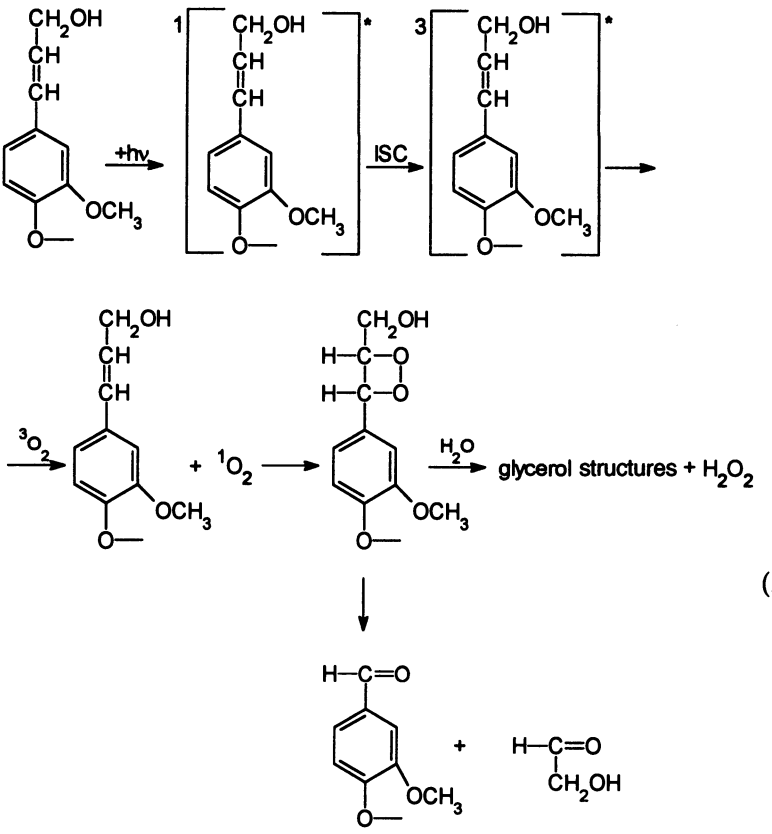
(376)

The Case of Lignin Degradation. It is worth looking at the photodegradation of natural polymers, e.g. lignin. Lignin is a natural polymeric structural material of woody plants and is associated with cellulose and hemicellulose. They account for about 30% of the dry weight of wood. Lignins contain three types of chromophoric structures: aromatic α-carbonyl groups, ring-conjugated double bonds and biphenyl structures [969], all of them able to undergo electronic excitation. This process is followed by energy transfer to molecular oxygen giving rise to singlet oxygen. Native lignins and lignins modified by various pulping processes (wood, high yield pulps and paper) contain functional groups reactive towards $HO^{\cdot}$, $^1O_2$ and O_3, e.g. phenylpropane units, cinnamyl alcohol or cinnamaldehyde structures [969]. Studies using lignin model compounds have shown that the yellowing process results from sunlight induced oxidation of lignin units to phenoxy radicals and quinones [913, 970].

Both excited carbonyl-containing compounds and $^1O_2$ can abstract hydrogen atoms from lignin units and form phenoxy radicals and quinone structures.

Singlet oxygen may also add across the C=C bond to form a dioxetane structure, which during cleavage gives new carbonyl-containing compounds.

For example, Gellerstedt et al. [970] have proposed the following mechanism for lignin photooxidation of cinnamoyl units (alcohols of the end group of the lignin) as illustrated by the case of coniferyl alcohol methyl ether:



(377)

where ISC denotes intersystem crossing.

The above examples of degradative oxidation of both synthetic and natural polymers show that the reactions have an extremely complex nature and they involve a wide variety of free radicals and oxygen species as intermediates.

The damaging influence of light on biopolymers of living organisms observed in all classes of organisms in the presence of oxygen, i.e. the photodynamic effect and its physiological consequences are discussed in Chap. 5.

Polymeric materials used on a large scale for commercial purpose must be resistant to photodegradation. For example, polymeric materials for the packing industry should show a mean photostability of 1–4 years, whereas polymers used for the building and machine industries need 10–40 years [948]. Therefore the main effort of studies in the field of polymers is directed mostly towards the increase of plastic photo- and thermo-stability. Some polymers are poorly photostable, e.g. polyolefines, poly (vinyl chloride) films (PVC), polystyrene,

nylons, rubbers, cellulose, and they must be prevented from oxidation by addition of photostabilizers.

Protection Against Photodamage. Photostabilizers are compounds which should be good ultraviolet absorbers, chromophoric group quenchers, free radical and singlet oxygen scavengers and be able to decompose hydroperoxides [948].

During the last decade there has been increasing interest in the properties of polymers and protection against oxidative degradation with respect to the wide range of their practical applications. Because, for a number of years, new techniques have been available for preparing singlet oxygen and oxygen free radicals, researchers have been able to state the role of these oxygen species in polymer degradation in model studies [971–973]. It has also been possible to find a number of singlet oxygen effective quenchers which are useful in the protection of polymers against light aging. For example, it has been reported that the polyolefins, polyethylene and polypropylene are stabilized against photodegradation by metal complexes, compounds known as effective singlet oxygen quenchers [971, 974]. Moreover, carotenoids isolated from plants are very efficient quenchers of singlet oxygen, and were reported to have a high protective effect on synthetic polymers such as polystyrene [975] and *cis*-1,4-polybutadiene [976]. Similarly, oxidation of 1-polybutadiene by singlet oxygen has been reported to be inhibited by hindered amines (known as $^1O_2$-quenchers) [977]. For a review, see work by Pospišil [978]. It is worth mentioning that oxidation of some polymers with $^1O_2$ has been used as a method for obtaining elastomers [979], and for the oxidation of butyl rubber for transforming this polymer into functional derivatives [980]. Furthermore steady-state and time-resolved phosphorescence (emission at 1270 nm) from $^1O_2$ has been reported to be useful for the characterization of many properties of solid organic polymers [981–983].

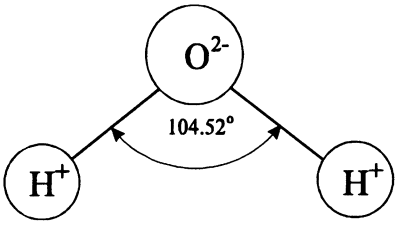
8.3 Aquatic Environment

8.3.1 Usual Components Present in Natural Water

Chemistry of the water environment has been well recognized and is discussed in a tremendous number of papers and books, and only a few of them will be cited here. This chapter attempts to select particular examples of chemical polutants and to synthesize from available literature a coherent view on the role of highly reactive oxygen species, especially singlet oxygen in chemistry of the water environment.

The water molecule consits of two atoms of hydrogen combined with one atom of oxygen. The molecule is a dipole, the O–H bonds forming an angle of 104.52°.

The opposite charges on hydrogen and oxygen atoms cause the molecules to form additional weak bonds, so-called "hydrogen bonds". It is the reason why water has several very important physical and chemical properties. In nature water is a main component of plant and animal organisms (about 78% of body weight).



Water is essential for the existence of life on Earth. There exists the worldwide danger of water-shortage due to rapid growth of human population, and to increasing needs for industry, agriculture and domestic purposes. For example, a man drinks annually about five times his weight of water, i. e. on average about 25 cubic meters during his lifetime. However 70% of the Earth's surface is covered by H_2O, but 98% of that water is salty and unfit for drinking or agriculture. For example, the Colorado River is 28 times as salty as its headwaters, making it toxic for aquatic life [984].

Water occurring in nature, i. e. natural water, is a universal solvent for both polar and ionic substances. Therefore, water is a mixture of H_2O and different substances and compounds of organic and inorganic origin. These additives may be divided into natural and foreign constituents. Among the natural additives are sand, loams, clay, parts of soil, humus substances, SiO_2, cations (e. g. Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, Mn^{2+}), anions (CO_3^{2-}, NO_2^-, NO_3^-, Cl^-, HO^-, PO_4^{3-}) and gases. Gases originate from the air (O_2, N_2, CO_2, SO_2, SO_3, noble gases), from chemical processes occurring in the atmosphere (e. g. CO_2, H_2S, N_2, CH_4, SO_2, SO_3), from decomposition of organic substances under both aerobic and anaerobic conditions (e. g. CH_4, H_2S, H_2, CO_2, N_2), and from radioactive sources, e. g. tritium, helium or radon [795].

A simple universal classification of water quality for clear and polluted is difficult, and it requires consideration of water quality criteria and standards. The term water quality means different things to different people, depending on place and uses. For example, drinking water must not give rise to ill health and must be attractive to the drinker. Thus drinking water must be free of colour, taste, odour, and excessive amounts of mineral and organic matter.

Water acceptability may be characterized by physical and chemical parameters [985, 986].

The main physical criteria include the following.

- Turbidity, i. e. cloudiness caused by sediment suspended in water. Turbidity should be not more than one unit.
- Colour (not more than five colour units[1] is preferred).
  Colour influences biological activity of water organisms by attenuation of the solar radiation in the water.

[1] A unit of colour equivalent to the colour produced by 1.0 mg · dm^{-3} of platinum in the form of chloroplatinate ion by the platinum-cobalt method.

- Temperature controls all forms of life in water and the solubility of inorganic materials.
  The most desirable range is from 4 to 10 °C. Temperatures above 27 °C are unsuitable, and above 32 °C are unfit for public use.
- Taste and odour indicate undesirable impurities caused by dead or decaying organic matter, living organisms, industrial wastes (especially phenolic compounds), dissolved gases (CH_4, H_2S, etc.), and dissolved minerals (e.g. chlorides, metalic salts, sulfates). A good objective for odour is three as the threshold odour number.
  Transparency, sedimentation rate, density, diffusion rate, particulate matter, and mixing are also physical parameters used for examination of water [986].Chemical standards for water acceptability may be summarized, as follows.

8.3.1.1
Hydrogen Ion Concentration, pH

The pH of natural waters ranges from 5.5 to 8.6. For drinking water, the pH should be 6.5–8.5. Pure natural water is weakly dissociated and has a pH of 7.

8.3.1.2
Salt Content

Salt content depends on the amounts of acids and bases present in water. Alkalinity and acidity of water are expressed in terms of $CaCO_3$. Alkalinity refers to Na_2CO_3, $CaCO_3$, $MgCO_3$ and hydroxide content, e.g. $Fe(OH)_3$. Good waters contain less than 80 mg · dm^{-3} of $CaCO_3$ [985]. The H_2CO_3 concentration determines the pH of the water by its protolysis and exchange with CO_2 from the atmosphere and the biosphere [987]. The CO_2–carbonate system is the most important in the hydrosphere, as it controls the pH. The CO_2 concentration in surface waters is dependent on its atmospheric concentration. From the reaction at Eq. (358) it can be seen that in solution CO_2 forms carbonic acid which dissociates in two steps:

$$H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \tag{378}$$

$$HCO_3^- \rightleftharpoons H^+ + CO_3^{2-} \tag{379}$$

These reactions are pH dependent, and the maximal concentration of CO_2 occurs at pH < 4, whereas the maximal concentrations of HCO_3^- and CO_3^{2-} ions are at pH 8 and above pH 11.5, respectively [986].

The chemical equilibrium state for the CO_2–HCO_3^- system occurs at pH 6, and for the HCO_3^- and CO_3^{2-} at pH about 10. In old ground water the water reaches the equilibrium state with respect to the $CaCO_3$ concentration.

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[2] The pH is defined as the negative logarithm of the molar hydrogen ion, H^+, concentration: $pH = -\log [H^+]$.

8.3.1.3
Dissolved Oxygen

Oxygen solubility in natural water depends mainly on temperature, pressure, salinity and thickness of the surface film. The concentration of oxygen in surface water is mainly a function of the oxygen exchange between water and atmosphere. In deeper water the oxygen content depends on its consumption by the biological and chemical processes.

8.3.1.4
Nitrate and Nitrites

Excess concentrations of nitrate (NO_3^-) and nitrite (NO_2^-) ions are toxic to aquatic organisms and to humans.

8.3.1.5
Phosphates

This group of compounds have little effect in unpolluted waters.

8.3.1.6
Metals

Trace concentrations of metals (e.g. Cu, Fe, Ni, Zn, Mn) are required for normal organism growth and metabolism. These metals can be toxic if their level is high.

8.3.1.7
Organic Substances

In unpolluted waters the total concentration of organic substances is low, i.e. ≤ 2 ppm.

8.3.1.8
Radioactivity Level

Very low levels of radioactivity come from geological sources (radioactive soil and rock) and may be enhanced by radioactive wastes. Concentration of radionuclides in water is usually determined in $Bq \cdot m^{-3}$ or $pCi \cdot dm^{-1}$.[3]

There is a danger of water shortage caused by increasing chemical pollution, industrial consumption and mismanagement as well as replacement of natural resources of energy such as coal, gas, wood or oil by water (e.g. hydro power plant). Therefore we should be aware of water quality and quantity protection.

8.3.2
Categories of Water Pollutants

Substances present in the hydrosphere become pollutants when their concentration is high enough to affect the water environment. As water is a universal

[3] 1 pCi = 10^{-12} Ci (curie). Curie is equal to $3.7 \cdot 10^{10}$ disintegrations per second = $3.7 \cdot 10^{10}$ Bq (becquerel).

solvent a great number of various substances occur in water systems. Chemical reactions between pollutants can create new pollutants.

Natural waters contain pollutants in the form of suspensions (insoluble materials, mainly mineral tailings), dissolved impurities (acids, alkalis, heavy metals, insecticides, cyanides, and other toxics) and floating materials (oils, greases, foam and other solids). Several sources of water pollution may be distinguished: industrial processes, domestic wastes, agricultural and urban activities, energy production, transportation and mine drainage [780]. Solid and liquid waste products containing pollutants are the main source of water pollution. They may be divided into sewage, industrial and agricultural origin. Sewage come from domestic and commercial premises, land drains, some industrial plants, and agricultural activity [883].

Four basic kinds of water pollutants have been recognized [795]:

- chemical (oils, detergents, phenols, dyes, hydrocarbons, carbohydrates, carboxylic acids, sugars, nitrates, phosphates, acids, alkalis, heavy metals, organo-pesticides, sulfides, cyanides, cyanates, minerals, or volatile metal compounds, e.g. Pb from leaded gasoline, Hg, radioactive isotopes);
- physical (wood, leaves, metal pieces, rubber, plastics, paper);
- biological (bacteria, viruses, parasites and other organisms) – microorganisms present in drinking water may be major health hazards, and several diseases can be transmited by water, e.g. fever, cholera, gastroenteritis or typhoid;
- thermal (hot water from nuclear fuel cycle) – heat enhances the effect of chemical pollutants.

Impurities (the mineral constituents) and pollutants are introduced into water during water circulation in nature. The total content of impurities and pollutants depends on the surroundings with which the water is in contact.

In respect to stability, two types of water pollutants may be distinguished [780]:

- degradable, i.e. those that can be broken into low-weight molecules as a result of natural, chemical, physical and biological processes;
- nondegradable, e.g. plastics, aluminium products, or chlorinated hydrocarbons applied as pesticides.

The pollutant concentration in the water may be broken down into two categories: those tolerated and those constituting grounds for rejection [986]. As both the raw water quality and standards as well as pollutant limits depend on the end use (municipal, industrial, agricultural, recreation, fish and animals), they are different for different uses [884, 986, 988].

In much of the research carried out on the generation of secondary pollutants, especially oxygen toxic species in natural water systems, the major emphasis has been put on molecular oxygen. Oxygen, as a very important particular component of water, comes from the air and is released during the photosynthetic process of aquatic plants. Photosynthesis is a very complicated process, but with simplification may be represented by the following reaction (upon water photolysis):

$$CO_2 + H_2O \xrightarrow[\text{chloroplast}]{\text{solar energy}} \frac{1}{n}(CH_2O)_n + O_2 \qquad (380)$$

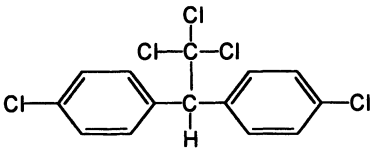
All living green plants and plants having chloroplasts assimilate carbon dioxide and, in the presence of solar energy, form carbohydrates and release molecular oxygen. This process occurs with a high yield during strong insolation of the water surface. Oxygen is consumed by aquatic organisms during respiration, it oxidizes inorganic substances present in water, and is used for biochemical decomposition of organic substances. This give rise to the environmental tests of Chemical and Biological Oxygen Demand (COD, BOD). Oxygen is present in surface waters, shallow underground water and drop waters. Its content is crucial for water quality because the presence of oxygen is necessary for the existence of the majority of aquatic organisms participating in the process of self-purification of water. The oxygen content decreases when pollution of water by organic substances increases or when hot industrial wastes are introduced. If the concentration of oxygen dissolved in water falls to 40% of the saturation level, disorder in biocenosis and death of fishes are observed.

Organic substances occurring in natural waters originate from a large number of sources. Waters contain organic compounds of both natural and synthetic origin. The natural organic compounds include amino acids, proteins, carboxylic acids, carbohydrates, phenols, humic acids and vitamins. The synthetic organic compounds are, for example, pesticides, herbicides, chlorinated hydrocarbons (e.g. DDT), polychlorinated biphenyls and organophosphorus compounds. The synthetic compounds are introduced into the water system indirectly by runoff from agricultural areas, from the atmosphere as precipitation, and as waste materials. Oil contains organic fractions and although organic substances occur in water at relatively low concentrations in comparison to inorganic substances, they may be very toxic.

Water contains about 20% volatile organic compounds and 80% is referred to as the nonvolatile fractions [989, 990]. Many of the compounds present in drinking and river water are alkanes, phthalates and polychlorinated terphenyls. The possible toxic effects on health result from long term exposure to organic substances in drinking water, mainly volatile pesticides (fumigants and solvents). Fumigants are pesticides used to treat insects in soils. They include cyanide compounds, methylbromide, dibromochloropropane, and ethylene dibromide [988]. Among the nonvolatile organic compounds are polychlorinated terphenyls, detergents, pharmaceuticals and epoxy resin constituents. A list of organic substances which were identified in rivers and drinking water, their concentrations and the highest permissible amounts are given, for example, in [925, 985, 986, 988, 989, 991, 992].

Some organic compounds can be strongly toxic to organisms and often cause biological implications. The most important toxins are pesticides, cyanides, halogenated hydrocarbons, polynuclear aromatic hydrocarbons and polychlorinated biphenyls.

Pesticides can enter water systems as leachates from agricultural areas. The most toxic pesticides are the organochlorine compounds. These compounds were commonly used in agriculture in the past. The best known chlorinated insecticide, DDT, 1,1,1-trichloro-2,2-bis (*p*-chlorophenyl)ethane has a half-life of 2.5–5 years. It shows high stability and persistence in the environment (even up to 25 years) [883]:



DDT

Persistent pesticides can concentrate in food chains, and have been found in animals and the human body. Maximum permissible level for all pesticides in drinking water may not exceed 0.5 mg · m^{-3} [988]. The chlorinated hydrocarbon insectides have been replaced by organophosphorus insecticides with short lifetime. However, although they do not persist in the environment, they are highly toxic to humans, causing neurotoxicity. The health effects exerted by other halogenated hydrocarbons are presented in Fig. 30 [988].

Polychlorinated biphenyls are by-products of the plastics, rubber and paper industries. They exert similar physiological effects to DDT, and in 1969 they were responsible for the death of thousands of birds in the Irish Sea [883].

Polynuclear aromatic hydrocarbons have been established as carcinogenic, and a limit of 0.2 mg · m^{-3} is established by the World Health Organization (WHO) for their presence in drinking water [883].

Cyanides (CN^-) are very toxic compounds to all living organisms, and the safety limit is recommended to be 50 mg · m^{-3} for drinking water [883].

The next very important organic compounds found in water are humic acids and fulvic acids. These organic acids are highly polymerized, and usually insoluble in water. Humic acids have molecular masses from 5000 to 100000, and

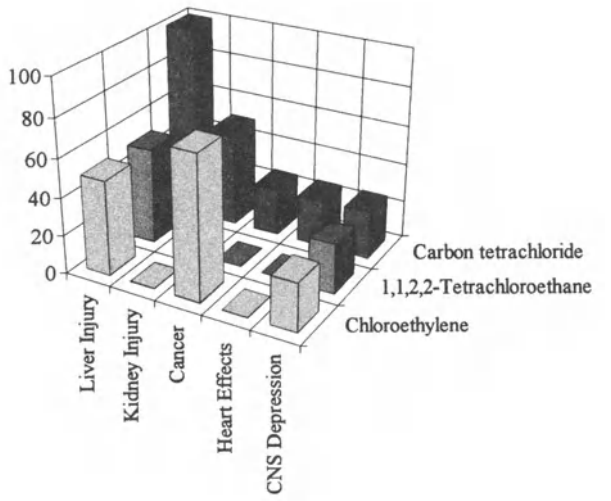
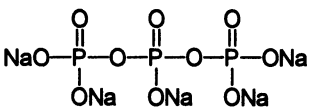


Fig. 30. Health effects of some halogenated hydrocarbons (CNS means the central nervous system)

fulvic acids from 300 to 2000. The final produts of humic matter degradation are yellow organic acids having molecular masses less then 300. The main functional groups of fulvic and humic acids are alcoholic and phenolic hydroxyls and carbohydrates. These acids influence the chemical composition of the water entering streams and lakes and, as a result, soil-water interactions. The humic substances form complexes with metallic ions, they are good sensitizers for singlet oxygen, and influence water pH. On the other hand, humic and fulvic acids reduce metal toxicity by complexation with, e.g. aluminium, which is toxic to aquatic life, especially fish [786].

The risks associated with the disposal of cytotoxic drugs have been discussed in detail by Richardson and Bowron [993]. Cytotoxic drugs used in a cancer therapy and immunosuppressive agents are the most toxic drugs used by man. Because they are often used by people at home, their disposal into the aquatic environment is very difficult to control. Trace amounts of cytotoxic drugs have been determined in river and drinking water samples. For example, bleomycins, a group of glycopeptide-derived antibiotics having antitumour activity, has been found to occur at concentrations of $< 5-17$ mg $\cdot$ dm^{-3}, i.e. about one millionth of the normally prescribed daily dose for adult patients (20–30 mg $\cdot$ m^{-2}), assuming an average 2 ℓ of water drunk per day [994]. It is worth mentioning phenolic compounds, for which the upper limit in drinking water is 0.002 ppm.

The next important group of water pollutants are nitrients – nitrogen and phosphorus. They are essential for maintaining growth and metabolism of all organisms. Nitrogen and phosphorus are usually present in polluted waters as nitrates and phosphates. These nutrients occur initially in the inorganic form and are converted to the organic form during metabolism. These nutrients are assimilated by organisms in the form of the nitrate ion, NO_3^- and the phosphate ion, HPO_4^{2-}. Small amounts of these ions are normally present in unpolluted waters, as needed for biological life. Wastes from chemical fertilizer plants and the use of fertilizers for obtaining higher crop yields are sources of water pollution by NO_2^-, NO_3^- and HPO_4^{2-} ions. Other sources of phosphates are detergents and complex phosphates introduced into water distribution systems for the prevention of corrosion. Many detergents contain condensed phosphates, usually tripolyphosphates, where the hydrogen ions are replaced by sodium ions, Na^+, giving sodium tripolyphosphate:



Sodium tripolyphosphate

The WHO European limit for NO_3^-/NO_2^- is 45 mg $\cdot$ dm^{-3}, and for phosphates < 0.2 mg $\cdot$ dm^{-3} [985, 992].

Another form of inorganic pollutants are metal ions. Many heavy metals like Co^{2+}, Zn^{2+}, Cd^{2+}, Ni^{2+}, CrO_4^{2-}, Hg^{2+}, Pd^{2+}, Cu^{2+}, Fe^{2+}/Fe^{3+} and Tl^+ have been found to pollute water at toxic concentrations [795, 995–998].

Metals occurring in waters may be classified into highly toxic (mercury, cobalt, thallium, nickel and chromate) and less toxic (arsenate, lead, zinc, silver). For example, copper has been found to occur in concentrations ranging from 300 to 2000 ppm in sewage sludge, whereas this metal occurs in unpolluted water at concentrations around 0.003 ppm [780], and the toxic effect of the metal is observed at concentration of 0.1 ppm.

The next very toxic metal mercury, Hg, occurs in surface waters in the form of inorganic compounds, Hg^{+}, Hg_2^{2+}, $HgCl_3^{-}$, $HgCl_4^{-}$, HgS_2^{-2}, and organic derivatives of the R–Hg–X type, where R denotes organic substituent, and X halide. Mercury in water originates mainly from industrial wastes and precipitates. Mercury is used in industry, e.g. during production of polyvinyl chloride, neon light tubes, thermometers, pharmaceuticals, batteries, as well as in agriculture. Mercury occurs in coal and petroleum products, and is released into the air during their burning. Rainfall contains mercury as a result of air pollution by burning liquid fuel and coal. The most toxic mercury compounds are the alkyl derivatives, e.g. methyl mercury cation (CH_3Hg^{+}) and dimethyl mercury ($(CH_3)_2Hg$). For example, CH_3Hg^{+} is not metabolized in the body and binds with thiol groups of amino acids and proteins, accumulating in organisms and damaging the brain cells. Certain fish die if concentrations of organo-mercurials reach values of $4 \cdot 10^{-6} - 2 \cdot 10^{-5}$ g · m^{-3}. Mercury also accumulates in the human body and its half-life (inorganic salts and organo-mercurials) is about two months. Permissible concentrations of mercury in drinking water are ≤ 0.001 g · m^{-3} [795].

Special mention should also be paid to cadmium, Cd, because it has been reported that a great number of rivers contain mercury and cadmium at concentrations 100 times higher than permissible. The increased concentration of these metals causes the death of fish. The main sources of cadmium in water are electroplating plants, zincgalvanizing iron using cadmium [985], lead and zinc mining and fossil fuel burning [988]. Cadmium is water soluble and is distributed to the kidney and liver. The cadmium concentration in drinking water should not exceed 0.01 mg · dm^{-3} [992].

It is also worth saying a little about lead, Pb. Lead in drinking water comes from several sources including industrial wastes and precipitates (Fig. 31).

Especially great concentrations of lead in the air are released by automobiles, because tetraethyl lead, $Pb(C_2H_5)_4$ is used for ethylization of gasoline. With CO_2 dissolved in water in the presence of oxygen lead can form acid lead carbonate, $Pb(HCO_3)_2$. In natural waters lead can occur in the form of soluble and insoluble compounds. Solubility of lead decreases with increase in pH values, being the highest at pH < 7. Lead shows toxic properties, it accumulates in liver, kidney, bone, and the central nervous system. Lead is very easy absorbed from the intestinal tract (100 times better than aluminium) and is stored within the hair shaft [999]. Acute lead poisoning is rare, but chronic lead poisoning is important. This toxic metal can interfere with the synthesis of heme, can damage red blood cells and affects blood pressure. Lead is also classified as a probable human carcinogen [988]. A lead concentration of 0.3 g · m^{-3} involves lead-poisoning (permissible concentration of lead stands at about 0.1 g · m^{-3}) [883].

Beside the above-mentioned metals, a number of organometallic compounds used for commercial purposes interact with the aqueous environment. For

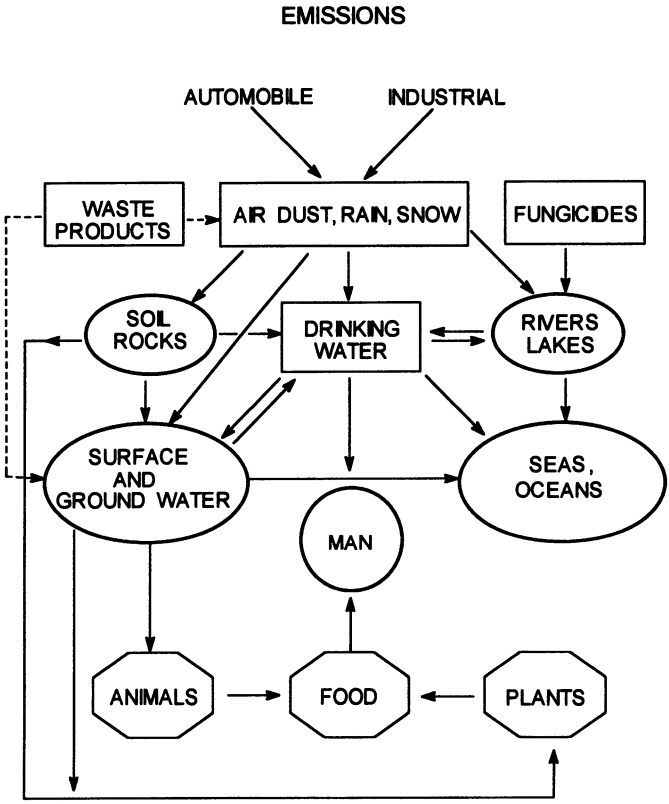


Fig. 31. Pathways of pollutants from the water environment to man

example, organomercury or organotin compounds used as pesticides, gasoline additives (methyl- and ethyl-leads), polymers (organosilicons), catalysts, additives to polymers or organo-arsenic compounds applied in medicine and agriculture are used to the extent of several thousands tonnes annually. The abovementioned arsenic comes from a geological source (soil, bedrock) and is present in wastes from pesticide manufacture and smelting operations. However, although is an essential dietary element in small concentrations, higher concentrations cause damage to the central nervous system and brain. Exposure to chronic arsenic poisoning produces different deleterious health effects including lung and skin cancers. The permissible concentration of arsenic in drinking water is 0.05 mg · dm^{-3} [988].

The occurrence and pathways of organometallic compounds in the environment have been very well discussed in a monography of Craig [1000].

There are many other metals present in drinking water. Many of these metals are essential elements in the diet, e.g. iron, copper, zinc, cobalt, manganese, molybdenum and chromium. These biometals are not considered as a major health hazard, although in certain individuals a sensitivity is observed to some

of the metals, e.g. copper and iron. It has been reported that high levels of iron in the body cause an increase in the carcinogenic process [1001]. This process may be a result of abnormal oxygen radicals production, e.g. the hydroxy radical and/or singlet oxygen. According to the modified iron-catalyzed Haber-Weiss reactions discussed in Chap. 1, the most important in this respect are intracellular reactions that generate Fe^{2+} and H_2O_2. The iron-induced carcinogenesis is interpreted as a result of oxygen species induced damage to DNA [995, 996, 1002–1004].

There is increasing evidence that iron present in asbestos is responsible for an increase in the risk of cancer in humans caused by these minerals. This problem has been discussed by Hardy and Aust [998]. In this paper a model for chemical reactions occurring between asbestos fibres and an aqueous environment, responsible for active oxygen species generation is discussed. A strong correlation between the ability of asbestos fibres to produce active oxygen species, especially $HO^{\cdot}$, and the mortality rate from mesothelioma in humans is shown.

Asbestos fibres, because of their small size and low density, can be readily transported by wind and water, and thus enter a variety of environmental pathways. The pathways by which asbestos are most frequently introduced into the aquatic system may be summarized as follows: precipitation acts as a collector introducing fibres from air into the hydrological cycle by rain and snow. Stream and ground-water, by contact with asbestos-bearing bedrock and surface materials, are the major sources of asbestos fibres in the water supply. Most water supplies contain asbestos fibres in the order of $10^5 - 10^6$ fibres $\cdot$ dm^{-3}. Water supplies with high fibre levels are located in areas geologically rich in asbestos. Other substantial sources of asbestos fibres are cement pipes and other materials used to transport and store drinking water [829].

The next kind of water pollutant is radioactive waste. The increase in production of nuclear energy in the world has been followed by disposition into the environment of more amounts of radioactive waste. Radioactive waste contains radionuclides with long half-lives and they are stored on land or disposed of at sea. Naturally occurring isotopes stem from the decay of uranium-238, uranium-235, and thorium-232. In drinking water the series of isotopes stemming from uranium-238 are of the greatest importance (uranium-237, radium-226 and radon-222). Nucleotides discharged into water are a health hazard and act as carcinogens. Samples of water should be examined several times per year to control the radiation level. For example, the average radioactivity limit for drinking water containing radium-226 and 228 is 5 pCi $\cdot$ dm^{-3} for a period of one year [988].

8.3.3
Generation of Reactive Oxygen Species

Ionizing radiation (cosmic rays, X-rays, γ-rays) and ultra-violet radiation decompose the water molecule, forming several oxygen products (Fig. 32) known for their strong oxidant properties. The species can interact between themselves and with neighbouring molecules to start a chain of radical reactions. In the last 20 years there has been a growing interest in the occurrence of oxidants in natural surface waters because of their involvement in redox reactions of

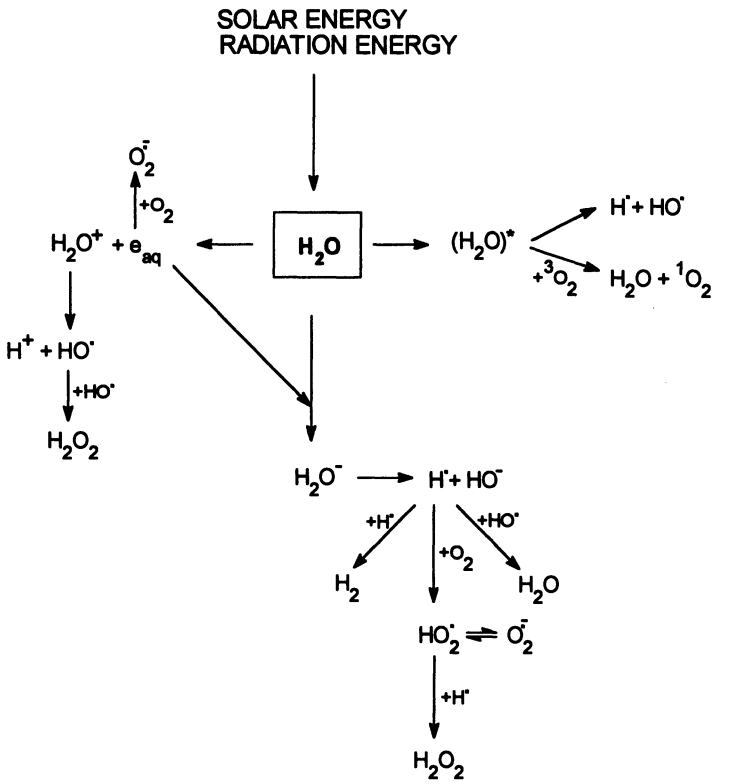


Fig. 32. Generation of oxygen species during photolysis of water. *Star* denotes vibrationally excited molecule of water, e_{aq} denotes hydrated electron

organic and inorganic compounds. It has been found that in quasi-steady state in sunlight, sea water concentration of O_2^- reaches 10–100 nmol · ℓ^{-1} [1005, 1006].

Miciński et al. [1007] have reported that photochemical production of this oxygen species in the eastern Caribbean occurs with rates ranging from 0.1 to 6 nmol · min^{-1} · dm^{-3} during full sun irradiation in spring. The much higher concentration of O_2^- has been measured in polluted surface waters.

Photolysis of phenolic compounds has been postulated to be a source of the anion radical [1008–1011]. Phenols, nitrophenols and oxalates are pollutants very often met in waters as they are used in large amounts as fungicides and bactericides. Photolysis of phenolic compounds releases electrons in their hydrated form (e_{aq})

$$ArOH \xrightarrow{h\nu} ArO^\cdot + H^+ + e^-_{aq} \qquad (381)$$

and they can reduce oxygen according to the reaction at Eq. (4).

Superoxide anion radical is a quantitatively dominant species among other oxygen species arising in natural waters. These radicals' recombination leads to

the formation of hydrogen peroxide. Hydrogen peroxide has been reported to be found in samples of rain and cloud waters [1012–1015]. Concentration of H_2O_2 in these waters has been reported to be about 100–150 $\mu mol \cdot \ell^{-1}$ during summer [905].

Analysis of cloud water and fog water samples have shown that hydroxyl radicals are also formed by sunlight in these waters. Rates of hydroxyl radical generation in the water samples have been estimated to be 0.3–30 $\mu mol \cdot h^{-1} \cdot dm^{-3}$ under midday insolation [910]. In addition, hydroxyl radicals may be generated in surface waters, for example, by decomposition of ozone [1016]:

$$O_3 + H_2O \rightarrow 2HO^{\cdot} + O_2 \tag{382}$$

and in reaction of ozone with the hydroperoxide radical (reaction at Eq. (311)), or as a product of the following reactions [992]:

$$O_3 + e_{aq} \rightarrow O_3^{\cdot -} \tag{383}$$

$$O_3^{\cdot -} + H^+ \rightarrow HO^{\cdot} + O_2 \tag{384}$$

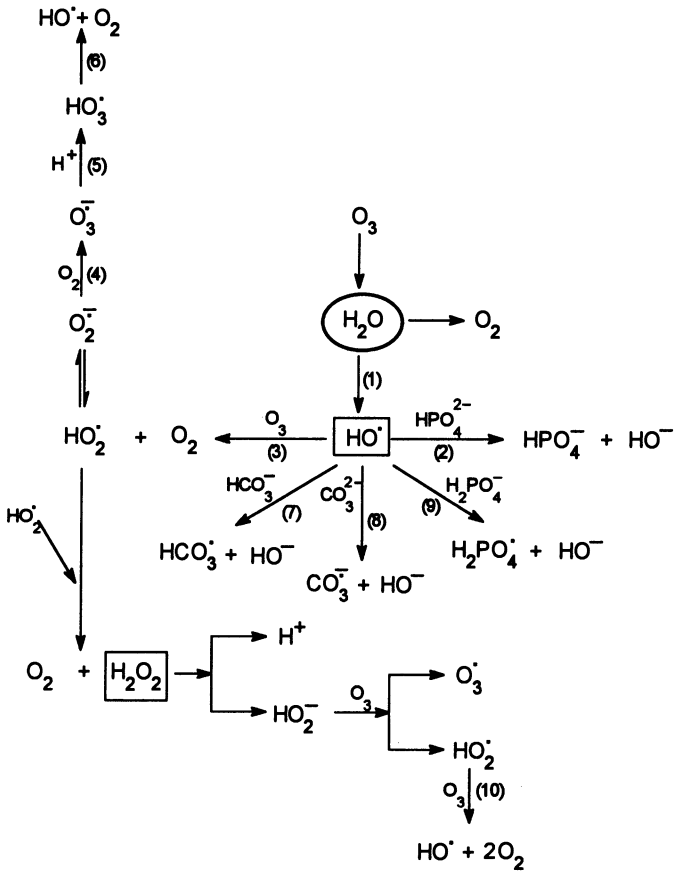
The decomposition of ozone in water accompanied by $HO^{\cdot}$ generation is presented in Fig. 33. As shown in Fig. 33, the hydroxyl radical is a product of a series of chemical reaction sequences that require an oxygen presence. The chemistry of the hydroxyl radical in water controls the rate at which many water pollutants are oxidized to the another kinds of radicals or are removed from the water [992]. Processes that occur with high rate constants are of primary importance in controlling the concentration of $HO^{\cdot}$ in water; those that have a negligible effect on the hydroxyl radical levels occur with much lower rate constants.

The next strong oxidant, singlet oxygen, is formed in natural waters by energy transfer from vibrationally excited molecules of water (see Fig. 32) [1017–1021]. This process occurs constantly in lakes, rivers, oceans and water vapour in the atmosphere. Several research groups have observed the formation of $^1O_2$ in waters by detection of energy absorption at 762 nm, corresponding to the transition in the oxygen single molecule [1022]:

$$O_2(^3\Sigma_g^-) + h\nu \rightarrow O_2(^1\Sigma_g^+) \tag{385}$$

Absorption in the regions of 1066 and 1266 nm, which is due to singlet oxygen, was observed, although heavily masked by strong absorption of water in this spectral region.

Singlet oxygen may be generated during photolysis of water as a product of interaction of transient oxygen species arised by several mechanisms, as discussed in Chap. 1. Natural and polluted waters contain organic compounds which can act as sensitizers and generate singlet oxygen in the energy transfer way. The primary sensitizer responsible for $^1O_2$ generation in natural waters is dissolved organic matter. For example, some natural humic substances are good sensitizers for $^1O_2$ production in surface waters [1009, 1018]. Secondary sensitizers also represent a wide group of water pollutants. The chromophoric impurities present in waters such as dyes, oils, pigments (chlorophyll, myoglobin, porphyrin)



$k_1 = 1.1 \cdot 10^4\ \ell \cdot mol^{-1} \cdot s^{-1}$
$k_2 < 10^7\ \ell \cdot mol^{-1} \cdot s^{-1}$
$k_3 = 3 \cdot 10^9\ \ell \cdot mol^{-1} \cdot s^{-1}$
$k_4 = 1.6 \cdot 10^9\ \ell \cdot mol^{-1} \cdot s^{-1}$
$k_5 = 5 \cdot 10^{10}\ \ell \cdot mol^{-1} \cdot s^{-1}$
$k_6 = 1.4 \cdot 10^5\ s^{-1}$
$k_7 = 1.5 \cdot 10^7\ \ell \cdot mol^{-1} \cdot s^{-1}$
$k_8 = 4.2 \cdot 10^8\ \ell \cdot mol^{-1} \cdot s^{-1}$
$k_9 < 10^5\ \ell \cdot mol^{-1} \cdot s^{-1}$
$k_{10} = 1.6 \cdot 10^9\ \ell \cdot mol^{-1} \cdot s^{-1}$

Fig. 33. Decomposition of ozone in water

and phenols can generate singlet oxygen after their electronic excitation by visible and near-UV light via energy transfer from the excited triplet state of chromophoric impurities to molecular oxygen. Recent studies carried by Tratnyek and Hoigně [1019] on waste-water samples used in the textile industry show that water contains dyes, brighteners, wetting agents and salts, and is usually pipelined into local waste-water without treatment. These water pollutants have been shown to act as good sensitizers for generation of singlet oxygen [1021]. Significant singlet oxygen generation by commerical textile dyes and military smoke constituents was also reported [1020]. The steady-state concentrations of $^1O_2$ photochemically generated by sunlight action near the water surface during

summer are found to be about three times higher than winter values. The highest values of $^1O_2$ formed in water from coloured rivers of North Florida containing high concentrations of organic substances were reported to be 8–22 pmol · ℓ^{-1} [1017].

8.3.4 Role of Reactive Oxygen Species in Drinking Water Treatment

Considering the properties of potentially hazardous pollutants present in water systems it is obvious that they can exert heavy toxic effects on the ecosystem. This may be summarized as follows: suspended particle solids cause turbidity in cooling water as well as raising its temperature; floating materials (fats, greases and waste oil) entering the water system form a thin film on the water surface which prevents the exchange of molecular oxygen with the atmosphere; chemical oxidation of both inorganic and organic compounds reduces concentration of oxygen dissolved in water; toxic substances, bacteria and viruses, and radioactive substances cause physiological changes in living organisms. This means that the environment is degraded, human health is affected, yet people themselves are responsible for these unwanted effects, as most pollution is caused by hazardous waste disposal and transportation. For this reason reduction of pollutants discharged into the environment to extremely low levels is required. Very important activities in waste minimization are destructive waste treatment technologies, e.g. advanced oxidation processes, that can completely oxidize organic compounds to CO_2, H_2O and salts [992, 1023]. Among advanced physical and chemical water treatments such as disinfection, flocculation, chlorination, coagulation, sedimentation, filtration, refining, UV irradiation and ozonization, irradiation with UV, ozonization and chlorination generate various active oxygen species which react with bacteria, humic acids and chemical pollutants [988, 1023, 1024].

In this respect oxygen active species ($HO^{\cdot}$, $O_2^{\cdot-}$, $HO_2^{\cdot}$, $^1O_2$, H_2O_2) and ozone are very good oxidizers.

Many studies have shown that singlet oxygen and oxygen radicals are widespread in the aquatic environment and may be important oxidants acting in this environment. They may play the double role of both oxidant and antioxidant. As oxidant, oxygen species may exert harmful effects on living water organisms, e.g. photodynamic effect [1025]. Oxygen species may also be very important antioxidants transferring pollutants present in lakes and rivers into nontoxic products by their oxidative degradation, usually in the free radical or singlet oxygen way [1026–1032]. For example, photodegradation of methyl and ethyl mercury in sea water and their dealkylation have been reported by several authors [1026, 1033–1036].

The oxygen active species ($HO^{\cdot}$, $O_2^{\cdot-}$, $HO_2^{\cdot}$, $^1O_2$) used in advanced oxidation processes as water treatment systems for reduction of pollutant levels are very strong oxidants. For example, hydroxyl radical has a very high oxidation potential (2.8 V) compared to molecular oxygen (1.9 V) or ozone (2.07 V) [1023].

Additionally, HO radicals and $^1O_2$ show very high values of second order rate constants for a variety of organic compounds [1037, 1038] (see also Tables 1

and 4). Oxygen radicals and singlet oxygen in advanced oxidation proceses are generated during the combination of the primary oxidants H_2O_2, ozone or irradiation with UV or transition metal ions (mainly iron ions).

Ozone shows bactericidal effects and is widely used in water treatment. The decomposition of ozone in water was presented in Fig. 33. The reaction involves multi-step chain reactions leading to $HO^{\cdot}$ generation and its reactions with water pollutants [992, 1039–1041]. It is estimated that about 50% of the ozone concentration in water is transformed into hydroxyl radicals [1041]. Ozone reacts directly with inorganic compounds such as chlorite ion, nitrite ion, ammonia, sulfites and sulfates, oxidized metal ions and hydrazines, but for most organic compounds, even unsaturated ones indirect ozonization is needed, which involves generation of $HO^{\cdot}$. In this case the combination of ozone with H_2O_2 is successfully used.

Hydrogen peroxide alone is also a strong oxidant used in wastewater treatment for destruction of inorganic pollutants, e.g. sulfur compounds. The combination of H_2O_2 with UV irradiation involves the photolysis of H_2O_2 into two HO radicals, similar to the reaction of H_2O_2 with ozone [1023].

Hydroxyl radicals and singlet oxygen are also generated in the combination of H_2O_2 with iron salts, according to the Fenton's reaction (reactions at Eqs. (31) and (32)).

The usefulness of advanced oxidation processes and their high efficiency means that kinetic models based on generation of highly reactive oxygen intermediates, especially hydroxyl radicals and $^1O_2$, for the study of destruction of hazardous organic compounds in air and drinking water, industrial wastes or ultrapure water are under investigation [1042–1060].

In the past decade a marked interest has been shown in water examination and control. Several highly sensitive and specific methods of analytical chemistry as well as spectroscopic methods have been applied to this; see, for example [1061–1063]. It is worth mentioning here a little about the use of biosensors for environmental monitoring, which complement analytical methods. They are intended for the detection of biologically available metals in samples taken from the environment [1064]. Microorganisms present in water respond to adverse environmental conditions, e.g. elevated temperature, presence of chemicals, solvents, metals or reactive oxygen species, by increased expression of stress proteins. Many marine phytoplankton species produce bioluminescence, i.e. emit a visible blue light resulting from their biochemical processes. The presence of some toxic agents inhibits the intensity of light emitted by bioluminescent bacteria, and this phenomenon may also be used as a sensitive test for determination of the presence of toxic compounds in water [1065, 1066].

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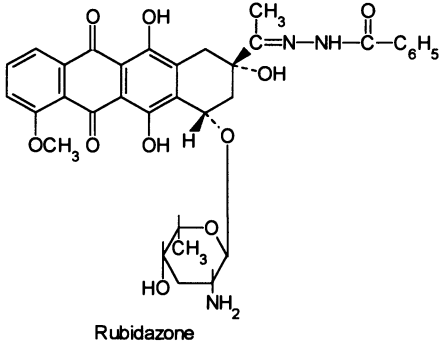
Subject Index

Errata

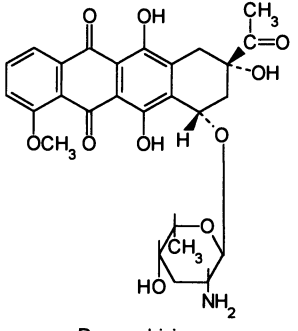
The values of C_g and C_u on pages 10 and 11 should be:

$$C_g = \frac{1}{\sqrt{2\,(1 + S_{AB})}} \qquad C_u = \frac{1}{\sqrt{2\,(1 - S_{AB})}}$$

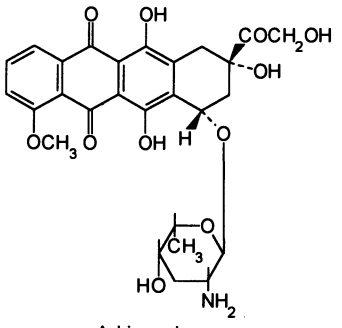
The structural formulas of Rubidazone, Daunorubicin and Adriamycin on page 35 are incorrect. Their correct structures are as follows:



Rubidazone



Daunorubicin



Adriamycin

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