Free Radicals in Biology

8

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8.1 Introduction

As discussed above (see Part II), the appearance of any photon in a system is a consequence of the relaxation of some electronically excited state (EES):

$$P^* \rightarrow P + hv$$

Thus, the phenomenon of luminescence automatically requires constant appearance of electronic excitation within the system, and conditions for its further radiative relaxation (Scheme 8.1).

If the system is illuminated by external light, such electron-excited states (EES) may appear due to its absorption. This way is active in photosynthetic systems, in which there are many "adjustments" for inactivating EES to prevent uncontrolled dangerous processes with their participation (photochemical reactions).

Aside from this specific case, the main sources of electron excitation in biological systems are free-radical processes, proceeding by chain, including branched-chain mechanisms. Thus, spontaneous photon emission of biological systems, or biological autoluminescence (BAL), is almost exclusively chemiluminescence (see Chap. 5, Sect. 5.2).

Moreover, we can safely make the following statements:

1. In the absence of special external influences, all known cases of BAL relate specifically to chemiluminescence.
2. Spontaneous generation of EES in all known biological systems (and BAL at their relaxation) occurs as a result of free-radical processes, or at least with intermediate free-radical products. (A separate but very important case, photoinduced luminescence of photosystems also occurs through free-radical stages, although the initial energy comes from external illumination).

Thus, the phenomenon of biological autoluminescence is inextricably linked with free-radical processes, and the study of its mechanisms is almost entirely the study of free radicals.

Accordingly, this part of this book, devoted to molecular mechanisms of BAL, is actually a gradual step-by-step proof of the above statements. The next Chaps. 9, 10, 11, 12, 13, and 14, show detailed investigation of the origin of free radicals in different model and biological systems, as well as important, dangerous, and vital processes with their participation. For a better organization and a clear understanding of this huge and complicated material, this chapter is devoted to a general overview of the role of free radicals in biology, their classification and methods of experimental and theoretical work with them.

8.2 One-Electron Oxygen Reduction as a Source of Free Radicals

As is well known, "ordinary" molecules have two electrons in their upper filled electron orbital. It is the interaction of these paired electrons magnetic moments that ensure stability of chemical compounds. Contrary to that, free radicals are molecules containing one or more unpaired (single) electrons at the upper orbitals, which make them chemically active particles. The radical "seeks" to reclaim the missing electron, taking it from other molecules with which it collides, or to donate the unpaired electron to other species, with higher electron affinity.

Unlike most other molecules, oxygen in its ground state is triplet, $^3O_2$, that is, it contains two unpaired electrons, forming a bi-radical. Thus, it is prone to accept two additional electrons, usually one by one, forming a number of intermediate species, that line up in a chain of one-electron oxygen reduction (Scheme 8.2).

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In the first step, the oxygen molecule ($^3O_2$, Scheme 8.2a) accepts an electron, forming a mono-radical $O_2^{-\bullet}$ (Scheme 8.2b), which is also an anion, since the particle as a whole is negatively charged. The exact name of this molecular particle is radical dioxide, or dioxyl, but the well-established name is superoxide anion-radical. Superoxide is involved in a variety of processes, both as an oxidizing agent and as a reducing agent (see Sect. 8.3.2). In addition, in neutral or acidic media, it quickly adds a proton, transforming into a neutral hydroperoxide radical $HOO^{\bullet}$ (Scheme 8.2c). The latter forms hydroperoxide anion HOO^- upon further one-electron reduction (Scheme 8.2d). HOO^-, in its turn, attaches a proton, forming a neutral hydrogen peroxide molecule (Scheme 8.2e).

The scheme of the above reactions can be represented as follows (Scheme 8.3):

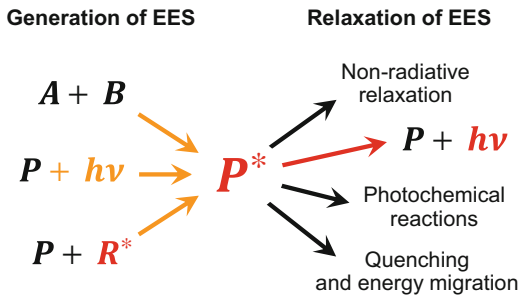
The H_2O_2 (HOOH) molecule also enters into further reactions, forming a whole spectrum of possible products, many of which are also radicals – including the most reactive radical $OH^{\bullet}$.

In the presence of oxygen, this chain of reactions is one of the main sources of free radicals both in biological and in model systems.

8.3 Reactive Oxygen Species

All the radicals shown above (Scheme 8.2 and 8.3), as well as the hydrogen peroxide molecule and a number of other compounds, are usually classified as the family of reactive oxygen species (ROS).

Although the term itself is extremely widespread in the scientific literature, there is no clear generally accepted definition of it, and many authors put it in different meanings. Here we present the widest possible set of radicals and molecules attributed to ROS (Table 8.1). Some of these compounds would be more correctly attributed to active forms of other substances, which is what a number of authors are doing (see column 3).



Scheme 8.1 Generation and relaxation of electronically excited states (EES)

8.3.1 Singlet Oxygen

As already discussed above, oxygen molecule in the ground, unexcited state is *triplet* ($^3O_2$). Under the action of radiation in the near infrared region, the triplet oxygen passes into an excited, *singlet* state ($^1O_2$) (see reviews (Krasnovsky 2007, 2015) for the history of its discovery, and its main properties). The energy difference between the ground state and singlet oxygen is 0.98 eV per molecule, which corresponds to a transition in the near IR range ($\lambda \cong 1270$ nm).

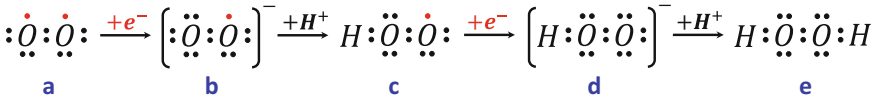
A direct transition between the singlet and triplet states is very unlikely due to spin exclusion. Therefore, direct $^3O_2 \rightarrow {}^1O_2$ excitation under the action of IR light practically does not occur, as well as spontaneous relaxation $^1O_2 \rightarrow {}^3O_2$ (the average lifetime of $^1O_2$ in the gas phase is estimated from minutes to dozens of minutes (Wang et al. 2020; Krasnovsky 2015)). In solutions, the molecules $^1O_2$ willingly enter into reactions with other molecules, and the $^1O_2$ lifetime is reduced to microseconds or less, primarily due to quenching by water molecules.

Singlet oxygen is chemically very active, which is used in photodynamic therapy of cancerous tumors (Krasnovsky 1998). The patient is given photodynamic sensitizers (hematoporphyrin, phthalocyanines), then the tumor site is irradiated with laser light, and the tumor tissue is selectively destroyed. This is a very effective method, especially in the postoperative period after surgical removal of the main tumor (Gunaydin et al. 2021). Upon irradiation, the sensitizer molecule (AH) transforms into an excited triplet state (^{3}AH), reacts with molecular oxygen (which is in the ground, triplet state $^3O_2$), forming the extremely reactive singlet oxygen $^1O_2$:

$$AH + h\nu \rightarrow {}^3AH$$

$$^3AH + {}^3O_2 \rightarrow AH + {}^1O_2$$

Scheme 8.2 One-electron oxygen reduction



Scheme 8.3 One-electron oxygen reduction

$$O_2 + e^- \rightarrow O_2^{-\bullet}$$
$$O_2^{-\bullet} + H^+ \rightarrow HOO^{\bullet}$$
$$HOO^{\bullet} + e^- \rightarrow HOO^-$$
$$HOO^- + H^+ \rightarrow HOOH$$

Table 8.1 Radicals and molecules classified as reactive oxygen species. Alternative options for classification are presented in the last column: RCS – reactive chlorine species, RNS – reactive nitrogen species, RLS – reactive lipid species

Formula	Name	Alternative classification
$^1O_2$	Singlet oxygen	ROS
$O_2^{-\bullet}$	Superoxide anion-radical	ROS
H_2O_2	Hydrogen peroxide	ROS
$OH^{\bullet}$	Hydroxyl radical	ROS
O_3	Ozone	ROS
ClO^-	Hypochlorite	RCS
$NO^{\bullet}$	Nitrogen monoxide (nitric oxide)	RNS
$ONOO^-$	Peroxynitrite	RNS
$L^{\bullet}$	Alkyl (lipoalkyl)	RLS
$LO^{\bullet}$	Alkoxyl (lipoxyl)	RLS
$LOO^{\bullet}$	Dioxil (peroxyl, lipoperoxil)	RLS
LOOH	Hydroperoxide (lipohydroperoxide)	RLS

The $^1O_2$ molecule is further involved in the processes of damage to cancer cells.

8.3.2 Superoxide Anion-Radical

Superoxide anion-radical (radical dioxide or dioxyl) is the first product in the chain of one-electron oxygen reduction (Scheme 8.2 and 8.3).

In the cell and in model systems, it is formed due to the reaction of oxygen with transition metals:

$$O_2 + Me^{n+} \rightarrow O_2^{-\bullet} + Me^{(n+1)+},$$

in the mitochondrial respiratory chain and during oxidation of NADPH with molecular oxygen using NADPH oxidase (see more in Chap. 14).

Superoxide has the properties of either an oxidizing agent or a reducing agent, depending on the partner. If there are no other partners, two superoxide molecules readily react with each other, entering into a dismutation reaction, where one radical $O_2^{-\bullet}$ acts as an oxidizing agent, and the other one as a reducing agent:

$$O_2^{-\bullet} + O_2^{-\bullet} + 2H^+ \rightarrow H_2O_2 + O_2$$

The spontaneous course of such a reaction is impeded by the electrostatic repulsion of two anions, and in proton-less media (nonpolar solvents, lipid layers of membranes or simply strongly alkaline media) superoxide radicals can exist for minutes or even hours.

In the cell, the superoxide dismutation reaction is mainly enzymatic, due to catalysis by the enzyme superoxide dismutase (SOD, see Chap. 14):

$$2O_2^{-\bullet} \xrightarrow{SOD} H_2O_2 + O_2$$

In neutral and acidic media, superoxide anion quickly and spontaneously (without enzymes) attaches a proton, turning into a neutral hydroperoxide radical:

$$O_2^{-\bullet} + H^+ \rightarrow HOO^{\bullet}$$

$HOO^{\bullet}$ quickly attaches an electron to form the hydrogen peroxide anion:

$$HOO^{\bullet} + e^- \rightarrow HOO^-$$

By attaching a proton, this anion turns into a neutral molecule H_2O_2:

$$HOO^- + H^+ \rightarrow HOOH$$

Besides other reactions, superoxide readily reacts with nitric oxide, constantly produced in the cell, with a rate constant of ~7×10^9 $M^{-1}s^{-1}$ (Huie 1993), so that nearly every collision between these species results in an irreversible reaction:

$$O_2^{-\bullet} + NO^{\bullet} \rightarrow ONOO^-$$

The formed peroxynitrite is a strong oxidizing and nitrating agent (Szabó et al. 2007), and its conjugated peroxynitrous acid homolyzes forming NO_2 and $OH^{\bullet}$ (Beckman et al. 1990):

$$ONOO^- + H^+ \leftrightarrows ONOOH \rightarrow ONO^{\bullet} + OH^{\bullet}$$

8.3.3 Hydrogen Peroxide and Hydroxyl Radicals

Hydrogen peroxide plays a huge role in the cell, including regulatory pathways.

Spontaneously, in the absence of enzymes, H_2O_2 can either undergo thermal decomposition:

$$H_2O_2 \xrightarrow{\text{spontaneous}} 2OH^{\bullet},$$

which is highly unlikely under normal conditions, or one-electron reduction on transition metal ions in the *Fenton reaction* (Halliwell and Gutteridge 2015):

$$H_2O_2 + e^- \xrightarrow{Me^{n+} \rightarrow Me^{(n+1)+}} HO^- + HO^{\bullet}$$

In particular, in the most common situation with iron ions:

$$H_2O_2 + Fe^{2+} \rightarrow OH^- + OH^{\bullet} + Fe^{3+}$$

In both of these reactions, an extremely chemically aggressive hydroxyl radical $OH^{\bullet}$, the most reactive of all ROS, is formed.

Hydroxyl radicals cause destruction in all vital cellular components. Penetrating into biological membranes, they initiate reactions of lipid chain oxidation, leading to membrane dysfunction, primarily as a barrier for ions and water-soluble molecules. In proteins, $OH^{\bullet}$ oxidize thiol groups and histidine residues, thereby inactivating most of the enzymes. Acting on nucleic acids, $OH^{\bullet}$ cause mutations. Many known mutagens act by forming hydroxyl radicals during their metabolism (Korkina et al. 1992; Moody and Hassan 1982; Reha-Krantz 2013). Thus, the Fenton reaction plays an important role in the development of a wide range of pathological processes.

In the cell, the most important reaction with the participation of H_2O_2 is glutathione oxidation, catalyzed by glutathione peroxidase:

$$2GSH + H_2O_2 \xrightarrow{\text{glutation peroxidase}} GSSG + H_2O,$$

Glutathione plays the role of the main reservoir of sulfhydryl groups in the cell, and a decrease in its level leads to the oxidation of protein thiols and a change in activity of a number of enzymes. In addition, this reaction is accompanied by a decrease in redox potential.

Thus, the H_2O_2 molecule (and, accordingly, its precursor, $O_2^{-\bullet}$) plays the role of a regulator of a number of important processes in the cell, that is, behaves as a second messenger (see more in Chap. 14).

At the same time, being a potential source of the $OH^{\bullet}$ radical, hydrogen peroxide can trigger many destructive processes.

8.3.4 Other Reactive Oxygen Species

Many authors also consider hypochlorite a reactive oxygen species. It is formed by the interaction of hydrogen peroxide with chloride ions under the action of myeloperoxidase:

$$H_2O_2 + Cl^- \xrightarrow{\text{mieloperoxidase}} H_2O + ClO^-$$

In the presence of transition metals, ClO^- enters into the reaction, which was studied by A.N. Osipov and colleagues using the spin trap method (Osipov et al. 1993):

$$ClO^- + H^+ + Fe^{2+} \xrightarrow{\text{spontaneous}} Cl^- + OH^{\bullet} + Fe^{3+},$$

generating the $OH^{\bullet}$ radical.

Considering the wide prevalence of Cl^- ions both in the cell and in the extracellular fluid, this path of $OH^{\bullet}$ generation turns out to be no less and even more significant than the classical Fenton reaction.

Strictly speaking, hypochlorite could be attributed to active forms of chlorine, as well as peroxynitrite – to active forms of nitrogen.

Lipid radicals include three types of radicals – alkyl, alkoxyl and dioxyl radicals – which appear during lipid chain oxidation (see Chap. 11). Many authors also classify them as reactive oxygen species, although, in fact, these are more like reactive lipid species. Lipid hydroperoxides (LOOH) can also be classified as reactive lipid species, since they are easily involved in Fenton-like reactions, as well as subjected to the peroxidase action, thus forming lipid radicals and branching the lipid peroxidation chains. All these connections will be thoroughly covered in Chaps. 10 and 11.

8.4 Classification of Radicals

A convenient classification of free radicals occurring in the human body, and in biological systems in general, was proposed by Yu.A. Vladmirov (Vladimirov et al. 2017) (Fig. 8.1).

First of all, radicals formed in the cell and in the body as a whole can be divided into *natural*, spontaneously formed in the system, and *alien*, generated under certain external influences.

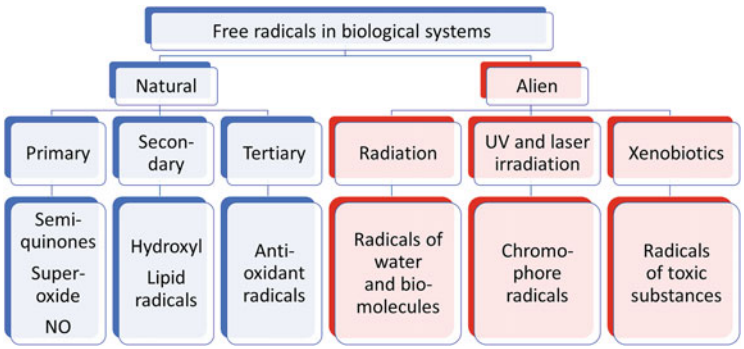


Fig. 8.1 Classification of free radicals in biological systems according to Yu.A. Vladimirov

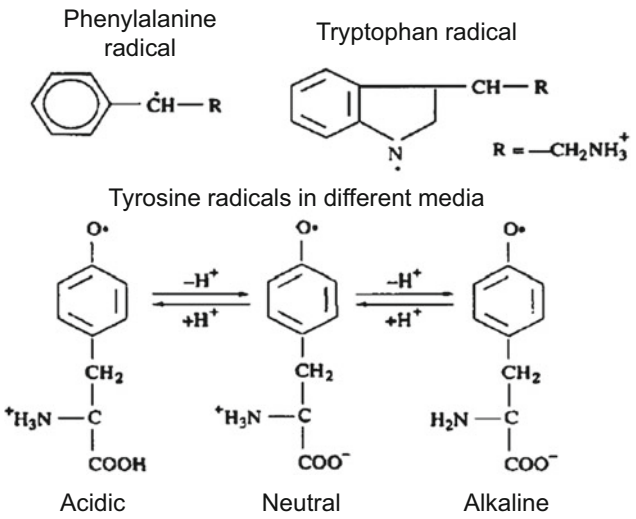


Fig. 8.2 Free radicals formed by the action of UV irradiation on amino acids. (Adapted from Vladimirov et al. (1966), with permission from the authors)

8.4.1 Alien Radicals

Alien radicals are those formed under the influence of adverse environmental factors: radiation or UV exposure, chemicals, etc. For instance, hard radiation produces water radicals and organic radicals; UV irradiation produces chromophore radicals, etc. The primary action of UV is photoionization, that is, "knocking out" an electron from a molecule. Thus, it ionizes aromatic amino acid residues in proteins, leading to formation of tyrosine, tryptophan, and phenylalanine radicals, through an intermediate state of radical-cation:

$$AH + h\nu \rightarrow e^- + AH^{\bullet +} \rightarrow e^- + A^{\bullet} + H^+$$

Formulas of aromatic amino acid radicals are shown in Fig. 8.2.

The formed radicals can react with adjacent amino acids, which leads to protein damage and to reactions of protein and/or lipid chain oxidation in the membrane structures of the cell.

When various poisons and toxins are metabolized in the body, they also form free radicals that destroy cells and tissues. For example, microsomal oxidation in the liver involves free-radical stages of the cytochrome P450 active center, which easily generate superoxide (see Chap. 14), but normally remain localized in the active center, causing no harm to the cell. Yet, in the presence of hepatotropic toxins, for example, carbon tetrachloride CCl_4, radicals can escape from the active center of P450 and initiate chain oxidation of lipids, leading to the liver tissue destruction.

8.4.2 Natural Radicals

Natural radicals can be classified into primary, secondary, and tertiary.

8.4.2.1 Primary Radicals

Primary radicals are those specially formed in the body as a result of enzymatic reactions. They are necessary for normal functioning of living cells and the body as a whole (see Chap. 14). These radicals include the following:

1. Respiratory chain electron carrier radicals: ubisemiquinone ($Q^{\bullet}$) and flavin radicals such as $FAD^{\bullet}$.
2. Superoxide $O_2^{-\bullet}$, formed by the enzyme NADPH oxidase in cell membranes, primarily of phagocytes, in the mitochondrial respiratory chain and on transition metal ions. $O_2^{-\bullet}$ is a source of other reactive oxygen species: hydrogen hydroperoxide H_2O_2, hydroxyl radical $OH^{\bullet}$, singlet oxygen $^1O_2$, hypochlorite ClO^-.
3. Nitrogen monoxide $NO^{\bullet}$, which is synthesized by a group of enzymes called β-synthases. $NO^{\bullet}$ is a natural factor in vascular relaxation and regulation of blood pressure. In particular, nitroglycerin, an important drug used in coronary artery disease, acts by stimulating $NO^{\bullet}$ formation.

Primary radicals are rapidly converted into molecular products, due to their high reactivity, and specific enzyme systems responsible for their further transformation (see Chap. 14). The formed molecular products are usually also chemically very active.

8.4.2.2 Secondary Radicals

Secondary radicals are formed when peroxide molecules and some other active compounds are broken down in one-electron reduction or oxidation processes. Hydroxyl and lipid radicals seem to play the most important role in biological systems.

Hydroxyl radicals are formed in the *Fenton reaction* (Halliwell and Gutteridge 2015):

$$H_2O_2 + Fe^{2+} \xrightarrow{\text{spontaneous}} OH^- + OH^\bullet + Fe^{3+}$$

and in the *Osipov reaction* (Osipov et al. 1993):

$$ClO^- + H^+ + Fe^{2+} \xrightarrow{\text{spontaneous}} Cl^- + OH^\bullet + Fe^{3+}$$

Lipid radicals ($LO^\bullet$, $L^\bullet$, and $LOO^\bullet$) are formed when lipid molecules (LH) are oxidized by other, already existing radicals:

$$X^\bullet + LH \rightarrow XH + L^\bullet$$

(where $X^\bullet$ is a primary radical, or in general any radical present in the system), as well as at the interaction of transition metal ions (e.g., Fe^{2+}) with lipid hydroperoxides (LOOH):

$$LOOH + Fe^{2+} \rightarrow LO^\bullet + OH^- + Fe^{3+}$$

These reactions trigger a chain process of lipid peroxidation, which no longer requires primary radicals or metal ions and develops independently as long as there are lipids and molecular oxygen (see Chaps. 10 and 11):

$$LO^\bullet + LH \rightarrow LOH + L^\bullet$$

$$L^\bullet + O_2 \rightarrow LOO^\bullet$$

$$LOO^\bullet + LH \rightarrow LOOH + L^\bullet$$

forming three types of radicals replacing each other in this chain: alkyl $L^\bullet$, alkoxyl $LO^\bullet$, and dioxyl $LOO^\bullet$.

Lipid peroxides LOOH formed during this process, also react with Fe^{2+} and other metals, generating new peroxidation cycles $L^\bullet \leftrightarrow LOO^\bullet$.

Thus, an uncontrolled process of peroxidation can potentially lead to oxidation of all lipids in the membrane, a change in its viscosity and surface charge, and, as a result, to electrical breakdowns of the membranes and their destruction.

In nature, these processes are limited by recombination of free radicals, for example.,

$$LOO^\bullet + LOO^\bullet \rightarrow \text{molecular products} + h\nu$$

and reactions with the so-called antioxidants – molecules that are readily oxidized by free radicals, turning them into molecules and forming relatively inert radicals of their own:

$$LOO^\bullet + InH \rightarrow LOOH + In^\bullet$$

These processes will be discussed in more detail in Chaps. 10 and 11.

Other secondary radicals of utmost importance in the cell are *amino acid* and *protein radicals* formed during their oxidation by primary radicals (see above and also Chap. 12).

Secondary radicals have high chemical activity and, as a rule, harm the surrounding molecules, cells, and the body as a whole, participating in the chain processes of peroxidation.

8.4.2.3 Tertiary Radicals

Tertiary radicals are antioxidant radicals ("radical traps" or inhibitors of free-radical processes) – $In^\bullet$. They have a complex spatial structure, shielding the active site from the surrounding molecules, and therefore are much less reactive than secondary radicals. Thus, they "isolate" the unpaired electron and quench further development of oxidation chains.

The $In^\bullet$ radicals themselves can be either beneficial or harmful, depending on the circumstances. In particular, α-tocopherol radicals can turn out to be prooxidants, reacting with lipid hydroperoxides (Izmailov and Vladimirov 2003).

8.5 Methods of Research on Free Radicals

Although free radicals themselves are not the direct subject of this monography, and the methods of their study – all the more, some of them will be immediately necessary for us in the subsequent sections. It was these methods that made the processes of light generation in biological systems available for discovery and research. Thus, without their understanding and application, some of the subsequent sections will be completely incomprehensible.

Therefore, here we devote a special section to the methods of free-radical research, with accents dictated by their necessity for the subsequent presentation.

These methods can be divided into three main categories: biophysical, biochemical methods and methods of kinetics and mathematical modeling.

8.5.1 Biophysical Methods

Biophysical methods can also be called direct methods, since they directly detect the presence of unpaired electrons in the system (the electron-spin resonance method), or the process of their recombination (chemiluminescence).

8.5.1.1 Electron-Spin Resonance

As is known, the electron-spin resonance (ESR) or electron-paramagnetic resonance (EPR) method is a classical approach for detecting particles with nonzero spin, including free radicals. Description and explanation of the method can be found elsewhere (Wertz 1986; Hammes and Hammes-Schiffer 2015; Weil and Bolton 2006), while here we emphasize its limitations in relation to the study of BAL generation.

As most of free radicals are very reactive, their stationary concentration is usually extremely low, even at a high rate of their appearance in the system. It is usually considered to be in average an order of magnitude less than the sensitivity threshold of the ESR method, which is estimated as $\sim 10^{-10} - 10^{-8}$ M (Mason and Fann, https://www.niehs.nih.gov/research/resources/epresr/sensitivity/index.cfm; Vladimirov 1967). Thus, classical ESR allows detecting radicals in biological systems only at their artificial initiation (i.e., under special model conditions), or in very specific situations, such as respiratory burst (Allen et al. 1972; Babior et al. 1973) (however, the new technologies arriving these years offer very promising prospects and might change the whole field in the nearest future (Probst et al. 2017)).

A successful modification of ESR for such issues is the spin trapping method (Janzen and Blackburn 1968; Saprin and Piette 1977; Haywood 2013). It consists in adding into the system special compounds, which react with the radicals to be detected, forming a new radical with a longer lifetime, known as a spin adduct. The added compound is called the spin trap.

Thus, adding a spin trap into the system greatly increases the concentration of unpaired electrons, making them detectable by ESR, but does not interfere with the processes leading to the generation of the studied radicals. Besides, there are spin traps that mainly react with a specific type of radicals, which makes it possible to detect them separately with rather high specificity (see more in Khramtsov (2018); Dikalov et al. (2018) and Samouilov et al. (2004)).

8.5.1.2 Chemiluminescence

As will be shown below, chemiluminescence (CL) necessarily occurs during recombination of certain types of free radicals. Accordingly, it clearly indicates the presence of these radicals in the system. On the one hand, this limits its applicability only to "luminescent radicals" (say, $ROO^{\bullet}$, but not $R^{\bullet}$ – see Chap. 10); on the other hand, it makes CL a specific detection method, applicable for analysis of concrete processes and their mechanisms.

Other advantages of CL as a method for observing radicals are its noninvasiveness and kineticity. As a rule, CL detection does not require any processing of the system and is possible in dynamics (i.e., allows its real-time observation). That is why the discovery of chemiluminescence (Vladimirov and Litvin 1959; Tarusov et al. 1961a, b; Tarusov et al. 1962; Vasil'ev 1965a, b) and its further use greatly simplified kinetic experiments, since in some cases, instead of sampling and analyzing the probes, it is enough to observe its luminescence kinetics.

8.5.1.2.1 Sensitivity

The sensitivity of CL, as a method to report free radicals in the system, can be roughly calculated as follows.

Let us consider lipid peroxidation – the process, responsible for the greatest proportion of biological autoluminescence (see Chaps. 10 and 11). As will be shown in Chap. 10, the main chemiluminescent reaction in this process is recombination of peroxide radicals:

$$ROO^{\bullet} + ROO^{\bullet} \xrightarrow{k_6} \text{molecular products} + h\nu$$

whose rate equals:

$$v = k_6[LOO^{\bullet}]^2 \tag{8.1}$$

and the CL intensity is:

$$I_{h\nu} = N_A V_{cuv} \cdot \varphi_{cl} \cdot k_6[LOO^{\bullet}]^2 \tag{8.2}$$

where $I_{h\nu}$ is CL intensity (photons/s), N_A – Avogadro number, V_{cuv} – cuvette volume (l), φ_{cl} – quantum yield of chemiluminescence, k_6 – reaction rate constant ($l \cdot mol^{-1} \cdot s^{-1}$), $[LOO^{\bullet}]$ – radical concentration (M).

The reaction rate constant k_6 and the quantum yield of CL φ_{cl} are obviously the two main parameters determining the CL intensity. Both of them can be estimated from extraneous data (Vasil'ev 1965a, b; Shlyapintokh et al. 1966).

For example, k_6 for a number of organic compounds at 15 °C is estimated as: 1.9×10^7 $l \cdot mol^{-1} \cdot s^{-1}$ (ethylbenzene), 5×10^5 $l \cdot mol^{-1} \cdot s^{-1}$ (ethyl linoleate), 2.8×10^4 $l \cdot mol^{-1} \cdot s^{-1}$ (cumene) (Shlyapintokh et al. 1966). φ_{cl}, which is defined as:

$$\varphi_{cl} = \frac{\text{number of emitted photons}}{\text{number of product molecules}} \tag{8.3}$$

can be represented as:

$$\varphi_{cl} = \varphi_{ex} \cdot \varphi_{lum} \tag{8.4}$$

where:

$$\begin{aligned} \varphi_{ex} &= \frac{\text{number of excited product molecules}}{\text{total number of product molecules}} \\ &- \text{quantum yield of excitation} \\ \varphi_{fl} &= \frac{\text{number of emitted photons}}{\text{number of excited product molecules}} \\ &- \text{quantum yield of luminescence} \end{aligned} \tag{8.5}$$

Both φ_{ex} and φ_{fl} can be estimated from other sources (Vasil'ev 1965a, b): $\varphi_{ex} = 10^{-5}$~10^{-4}, $\varphi_{fl} = 10^{-4}$~10^{-3}. Whence $\varphi_{cl} = 10^{-9}$~10^{-7} photons per formed product molecule, that is, per 2 radicals $ROO^{\bullet}$, disappearing in this reaction (Vasil'ev 1965a, b).

Altogether, from the approximated numbers above, one generated quantum of CL will correspond to $10^{-12} - 10^{-10}$ M, i.e. $\mathbf{10^{11} - 10^{13}}$ reacted radicals:

$$[LOO^{\bullet}] \cong \sqrt{\frac{I_{h\nu}}{6 \times 10^{23} \cdot V_{cuv} \cdot \left(10^{-9} \sim 10^{-7}\right) \cdot \left(10^{5} \sim 10^{7}\right)}} \tag{8.6}$$

Now if the photodetecting system collects ~10% of the total light emitted by the object (this depends on the optical system of the device and can be more), and the reaction cuvette is 10 ml, the minimal detectable concentration of radicals will be estimated as 10^{-9}~10^{-11} M. This makes the CL method 1–2 orders of magnitude more sensitive than the classical ESR.

Another important difference between the methods is the information they give in general. While ESR detects the free-radical concentration as is, CL shows the rate of radical disproportionation. Importantly, the higher the radical reactivity (k_6 in Eq. 8.1), the lower their stationary detectable concentration is, and consequently, the less efficient is ESR and the more efficient the CL method.

8.5.1.3 Activated Chemiluminescence

In addition to analyzing intrinsic chemiluminescence (including its kinetics and spectral composition), a very informative method is the use of selective activators – molecules that "readily" react with certain free radicals, or accept the product excitation energy due to its physical transfer, and have a high quantum yield of luminescence. Their use appeared convenient if the system intrinsic CL is below the setup sensitivity threshold, if the researcher needs to reduce the volume of experimental material, or if it is necessary to reveal specific radicals involved in the process.

Widespread selective activators of CL are luminol (Allen and Loose 1976; Allred et al. 1980), lucigenin (Allen 1982) and coumarin derivatives (see more in Gomes et al. 2005 and Vladimirov and Proskurnina 2009). Other dyes such as eosin, fluorescein, and acridine orange are not selective, but these dyes can also be used in a number of cases. Thus, the measurement of chemiluminescence in combination with activators is a highly sensitive and selective technique for studying free-radical processes, similarly to spin trapping in ESR.

8.5.2 Biochemical Methods

Biochemical methods of free-radical research are mostly indirect, which is their obvious disadvantage comparing to ESR and CL. Yet, they can be quite useful in some situations (see below), and most importantly, it is these methods that allowed detailed studies of CL mechanisms, bringing it to the present state of a research method.

Biochemical methods are divided into biomarker and inhibitory. Biomarker analysis consists in detecting specific substances, generated during the process under study, which can be used to assess its efficiency and/or direction. Inhibitory analysis consists in introducing some highly specific substance, which suppresses a definite (known beforehand) stage of the process, detecting and analyzing its effect.

8.5.2.1 Biomarkers

The biomarker approach is widely used in clinical free-radical research. These are chemicals that appear in the system as a result of radical attack on biologically important compounds: lipids, proteins, nucleic acids (see reviews (Kulikov and Grishina 2015; Proskurnina and Vladimirov 2015)). These processes are usually pathological, if it relates to medicine, and the biomarkers are mostly stable enough for chemical or spectroscopic detection. This determines their wide clinical application.

For instance, during oxidation of fatty acids, along with hydroperoxides, other compounds are formed: alcohols, ketones, aldehydes, epoxides, polymers, etc. (Badings 1960; Ellis 1950; Ellis et al. 1961; Evans 1961; Fenell and Skellon 1954; Karnojitzky and Vial 1966; Knight et al. 1951; King 1956; Perkins 1960) (see Fig. 8.3). All these substances can be quantitatively detected by chromatographic methods and mass-spectrometry.

One of the most important marker reactions, which has been used in lipid peroxidation research for more than 50 years, is malonic dialdehyde (MDA), formed after a repeated radical attack on polyunsaturated fatty acids (PUFA) (Evans 1961; Mas-Bargues et al. 2021; Aguilar Diaz De Leon and Borges 2020) (see Fig. 8.4a).

Reacting with thiobarbituric acid (TBA), it gives a specific colored and fluorescent substance MDA-TBA_2 (Suslova 1971) (see Fig. 8.4b; see more in Mas-Bargues et al. (2021) and Aguilar Diaz De Leon and Borges (2020)). In PUFA oxidation it is formed in close relation with the free-radical processes and thus remains widely used as a marker for them. However, today we know that both MDA and TBA are rather nonspecific reactants, and in fact the colored reaction with TBA reflects a general presence of MDA-like species, commonly called TBARS (TBA-reactive substances), which cannot be used as the sole indicator of lipid peroxidation in complex biological samples (Mas-Bargues et al. 2021).

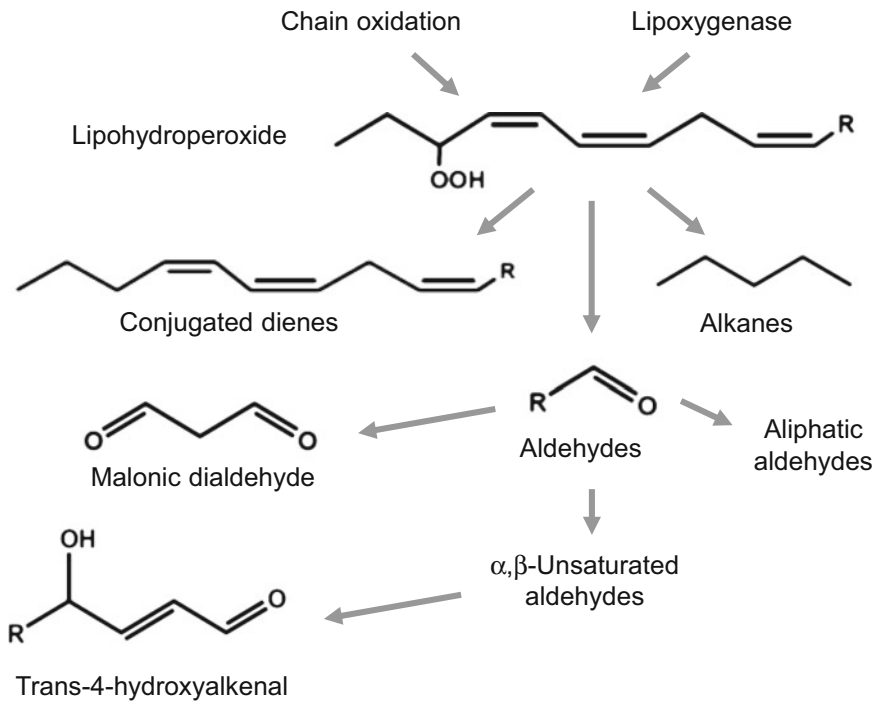


Fig. 8.3 Biomarkers of lipid peroxidation. (Reprinted with permission from Vladimirov et al. (2017). Copyright 2017, Vladimirov et al.)

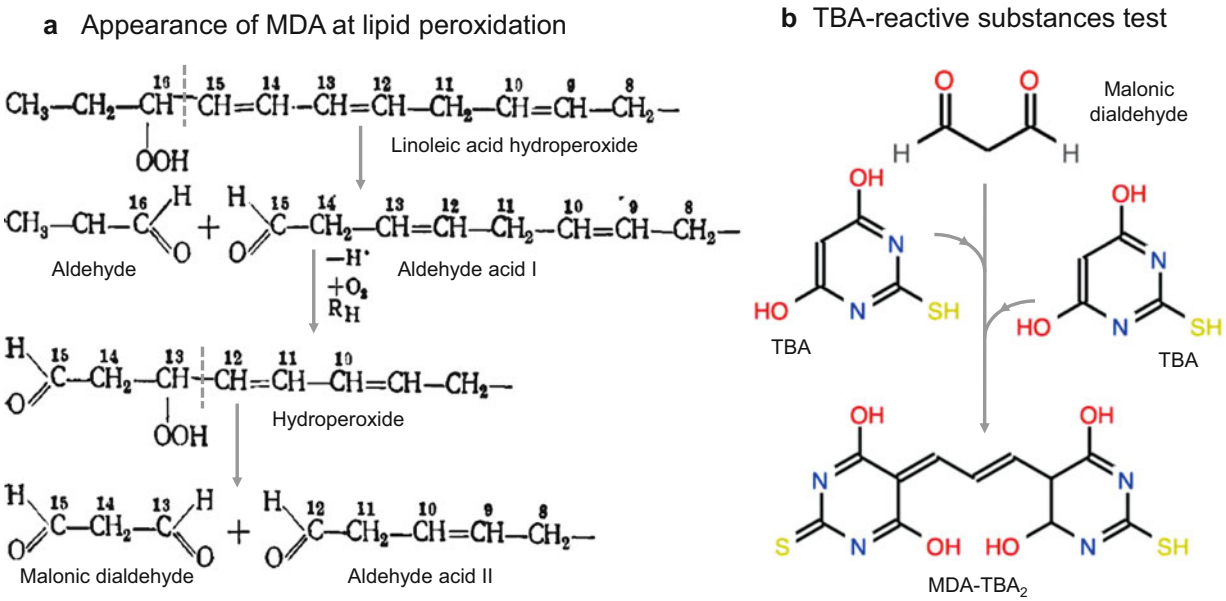


Fig. 8.4 Formation of malonic dialdehyde during the oxidation of linoleic acid (**a**) and a general scheme of its reaction with thiobarbituric acid (**b**). (**a** – reprinted from Vladimirov and Archakov (1972), with permission from the authors)

Yet, though lacking enough specificity (and sensitivity either), this reaction is still widely used for assessing the general rate of lipid and hydrocarbon peroxidation, which is quite convenient in combination with other methods (Forman et al. 2015).

In proteins, an important marker of free-radical oxidation is hydroxyphenylalanine, oxidized phenylalanine.

Phenylalanine is also oxidized enzymatically in the cell, without participation of radicals, but this leads to formation of para-hydroxyphenylalanine, that is, a natural amino acid tyrosine. If proteins containing phenylalanine are attacked by a hydroxyl radical, then various isoforms of hydroxyphenylalanine are formed, including ortho-hydroxyphenylalanine (sometimes called ortho-tyrosine), which is not normally present in the human body (Fig. 8.5).

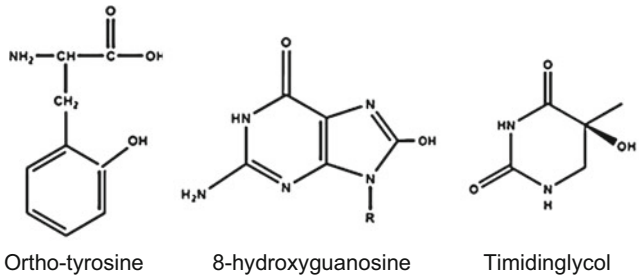


Fig. 8.5 Biomarkers of oxidative damage to proteins (o-tyrosine) and nucleic acids (8-hydroxyguanosine and thymidine glycol). (Reprinted with permission from Vladimirov et al. (2017). Copyright 2017, Vladimirov et al.)

A radical attack on nucleic acids leads to the formation of unusual derivatives of nitrogenous bases, for example, thymine or guanidine. Figure 8.5 shows the structural formulas of 8-hydroxyguanosine and thymidine glycol, substances that are absent in the blood under normal conditions.

8.5.2.1.1 Diene Conjugation

Diene conjugation is one of the most important markers for free-radical oxidation of polyunsaturated fatty acids (PUFA), an important component of biological membranes and blood lipoproteins. As known, PUFA are the least stable component of biological membranes and become the first targets for free radicals at their appearance. Therefore, detecting diene conjugation can be a sensitive test for the entire process of lipid peroxidation, which is of great importance both in normal cell metabolism and especially in the development of pathological conditions.

Besides, diene conjugation is a very accurate test specifically for free-radical processes (in contrast to other biomarker methods, which usually do not give complete confidence in the free-radical origin of the marker product). All this gives the diene conjugation method a special value and determines its wide use in free-radical research.

As is well known, the peculiarity of the PUFA chemical structure is double bonds, separated in the chain by the so-called methylene bridges (–CH_2–, see Fig. 8.6a). The carbon atoms in such bridges appear the most vulnerable targets for radical attack, as they have the weakest bonds with hydrogen atoms (Fig. 8.6a, see Chap. 10). A proton and an electron from this carbon atom are transferred to the radical (usually $OH^{\bullet}$), and the second, unpaired electron remains on the carbon. This electron is indistinguishable from the other four electrons that previously formed the neighboring double bonds, that is, a π-electron system of five identical electrons is formed (Fig. 8.6b-c). Such a compound is called alkyl radical ($R^{\bullet}$), and in case of lipids, it is denoted by $L^{\bullet}$. When molecular oxygen appears in the environment, it attacks $R^{\bullet}$ in lateral positions of the π-electron system, as it is thermodynamically advantageous to form a

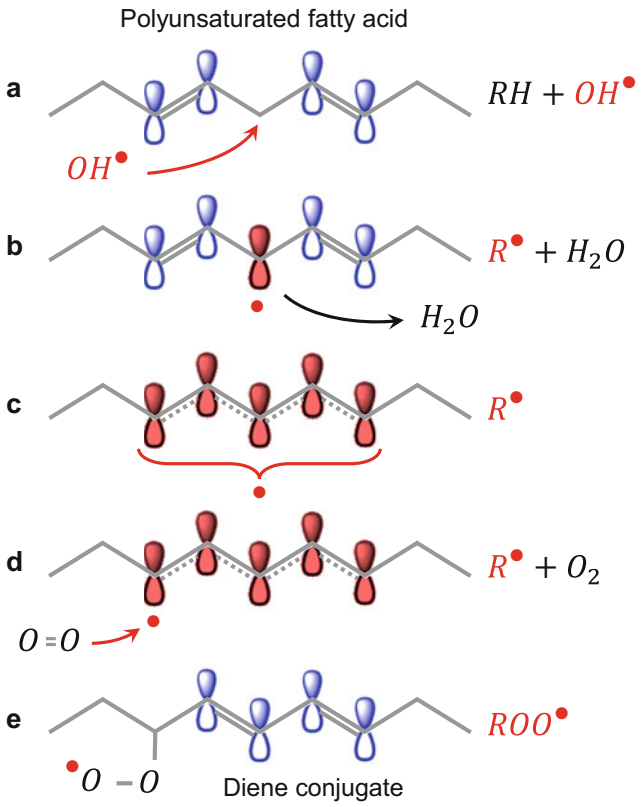


Fig. 8.6 Diene conjugation reaction scheme. (**a**) The site of the polyunsaturated fatty acid is attacked by a hydroxyl radical. (**b**) An alkyl radical of PUFA. (**c**) The unpaired electron is delocalized among the 5 carbon atoms with free π-orbitals. (**d**) A system of 5 carbon atoms containing a delocalized π electron is attacked by an oxygen molecule. (**e**) The lipoperoxyl radical, formed during the previous action, contains two conjugated double bonds (diene conjugate). (Adapted with permission from Vladimirov et al. (2017). Copyright 2017, Vladimirov et al.)

system of conjugated double bonds (Fig. 8.6d-e). As a result, a new radical $ROO^{\bullet}$ appears, with the unpaired electron belonging to the oxygen atom (Fig. 8.6d, e). This can be proved by the ESR method, since the signal of radicals in which an unpaired electron belongs to carbon ($R^{\bullet}$) differs from the signal of radicals in which a single electron is "centered" on oxygen.

As a result of the above process, the remaining four π-electrons form a conjugated system (conjugated diene), which can be easily detected by spectrophotometry: conjugated dienes absorb at 233 nm, while unconjugated double bonds absorb only in very short wavelength UV (less than 200 nm) (O'Brien 1969; Freeman and O'Brien 1967; Recknagel and Ghoshal 1966a, b). As an example, Fig. 8.7 shows absorption spectra of the peroxidation products generated by irradiating a phospholipid suspension with UV. As once can see, 1–2 minutes of irradiation is enough to form diene conjugates with the absorption maximum at ~235 nm (Fig. 8.7, curves b, c). Further irradiation causes further photochemical transformation and appearance of

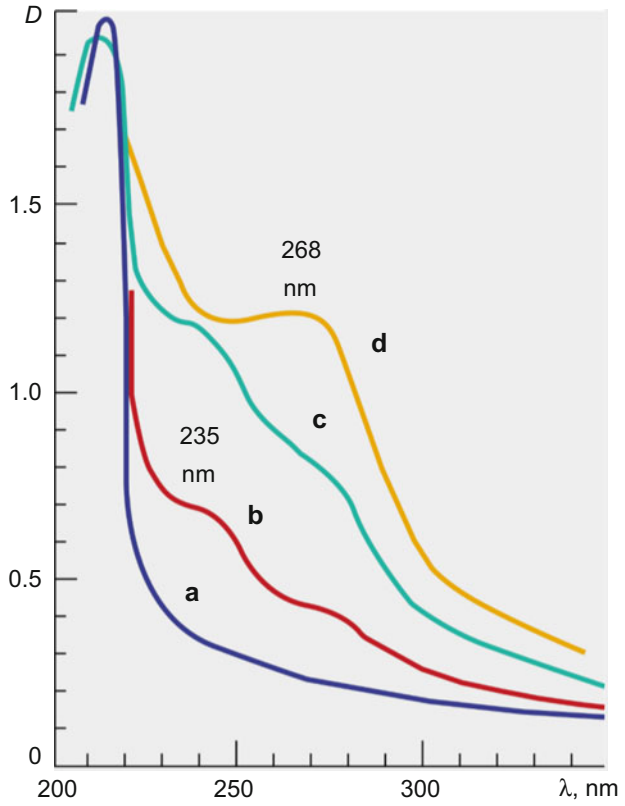


Fig. 8.7 Absorption spectra of freshly prepared phospholipid solutions in n-heptane, irradiated with a mercury quartz lamp for different time: a – without irradiation; b – 1 minute irradiation; c – 2 minutes irradiation; d – 22 minutes irradiation. (Redrawn with permission from Potapenko et al. (1972). Copyright 1972, the authors)

carbonyl compounds, absorbing at 260–280 nm (Fig. 8.7, curves c, d).

Altogether, since the appearance of conjugated dienes can occur *only* as a result of radical formation in the system (as above), their presence in the peroxidation products directly indicates the free-radical mechanism of PUFA peroxidation, which makes this method especially valuable for free-radical biology and medicine.

8.5.2.2 Inhibitory Analysis

The principle of inhibitory analysis is quite simple: a substance that intercepts radicals is introduced into the system, and the resulting changes are observed. For different radicals, one can find one or another interceptor-inhibitor. For instance, for superoxide anion-radical, it is the enzyme superoxide dismutase; for hydrogen peroxide, it is catalase; α-tocopherol or carotenoids can serve as traps for lipid radicals. Mannitol, benzoic acid, or ethanol are used to trap the hydroxyl radical.

Thus, the role of superoxide in phagocyte bacterial killing can be investigated by adding superoxide dismutase. As this experiment results in decreasing the number of killed bacteria, an important role of superoxide in bacterial destruction is suggested.

For more information and practical recommendations see reviews (Emanuel 1976; Kostyuk and Potapovich 2009; Shinohara and Ishiguro 1989).

Table 8.2 Simplified diagram of peroxidation processes

$[\text{initiation}] \xrightarrow{\omega_i} X^{\bullet}$
$X^{\bullet} + RH \xrightarrow{k_1} XH + R^{\bullet}$
$R^{\bullet} + O_2 \xrightarrow{k_2} ROO^{\bullet}$
$ROO^{\bullet} + RH \xrightarrow{k_3} ROOH + R^{\bullet}$
$ROO^{\bullet} + ROO^{\bullet} \xrightarrow{k_6} \text{molecular products} + h\nu$
$ROO^{\bullet} + InH \xrightarrow{k_7} ROOH + In^{\bullet}$

Above the arrows are the rate constants of the processes

8.5.3 Kinetic and Simulation Methods

In the study of chain reactions, kinetic methods play a role that is perhaps even more important than in the study of any other chemical processes. The reason is that, unlike simple chemical transformations, chain processes cannot be divided into separate reactions and investigated with the common research techniques: isolating products at each stage, measuring stoichiometry, etc. In a chain reaction, initiation, chain development and termination occur simultaneously, and the researcher can judge about these basic elementary reactions only indirectly by the final macroscopic result: decrease in the initial compound, oxygen consumption, or accumulation of the final products. This is why to establish the mechanism of the process, one needs to study its kinetics, applying math simulation: construct a system of differential equations for the whole supposed set of elementary reactions, solve it under different conditions, and compare theoretical results to the experimental data. This allows one to draw conclusions about the mechanisms, and change, if necessary, the equations, and therefore the proposed scheme of the process.

Let us consider the simplified classic scheme of peroxidation processes (Table 8.2, see more in Chap. 10) and see what we can learn about it using this approach.

Here $X^{\bullet}$ are the primary radicals appearing in the system due to some initiation process with the rate ω_i, RH are organic molecules, subjected to peroxidation (usually lipids or hydrocarbons), $R^{\bullet}$ – their radicals, $ROO^{\bullet}$ – peroxy radicals, ROOH – hydroperoxides, InH and $In^{\bullet}$ – inhibitor and its radical. The above scheme is greatly simplified for convenience (a more complete scheme and its proof are given in Chap. 10); the rate equations are as follows (Table 8.3):

To study the behavior of such a reaction system, a number of techniques are used, the main of which are:

Table 8.3 Rate equations for a simplified peroxidation scheme (Table 8.2)

1	$[\dot{X^{\bullet}}] = \omega_i - k_1[X^{\bullet}][RH]$
2	$[\dot{RH}] = -k_1[X^{\bullet}][RH] - k_3[ROO^{\bullet}][RH]$
3	$[\dot{R^{\bullet}}] = k_1[X^{\bullet}][RH] - k_2[R^{\bullet}][O_2] + k_3[ROO^{\bullet}][RH]$
4	$[\dot{ROO^{\bullet}}] = k_2[R^{\bullet}][O_2] - k_3[ROO^{\bullet}][RH] - k_6[ROO^{\bullet}]^2 - k_7[ROO^{\bullet}][InH]$
5	$[\dot{ROOH}] = k_3[ROO^{\bullet}][RH] + k_7[ROO^{\bullet}][InH]$
6	$[\dot{InH}] = -k_7[ROO^{\bullet}][InH]$

where, for any substance x, $[x]$ is its concentration, $[\dot{x}] \equiv \frac{d[x]}{dt}$ is the rate of its change

8.5.3.1 Give Priority to One of the Parallel Processes

If one of the components can be formed or consumed by several competing paths, the researcher can create special conditions under which one of these reactions will proceed incomparably faster than the others, and the rest can be neglected.

For example, in the initiation reaction $X^{\bullet} + RH \xrightarrow{k_1} XH + R^{\bullet}$ various radicals can act as initiator $X^{\bullet}$:

- $OH^{\bullet}$, formed in the Fenton and Osipov reactions, during ROOH thermal decomposition and in a number of other processes.
- $HOO^{\bullet}$, generated at one-electron oxygen reduction.
- $RO^{\bullet}$, formed during ROOH decomposition by transition metals: $ROOH + Fe^{2+} \rightarrow RO^{\bullet} + OH^{-} + Fe^{3+}$, etc.

Each of these reactions has its own rate constant, and taking all of them into account makes even the first stage of initiation incredibly difficult. If the research task does not require simultaneous consideration of these processes, the system can be simplified to one or two main ways of initiation.

For instance, in Karpukhin et al. (1963) and Shlyapintokh et al. (1966), peroxidation in fatty acid or phospholipid suspensions was initiated by ionizing or UV irradiation of constant intensity. Because there were practically no other ways of initiation in the system, it can be assumed that:

$$\omega_i = \text{const} \sim I_{\text{illumination}}$$

In Emanuel et al. (1965); Emanuel and L'askovkaya (1961); Lemon et al. (1951); and Kern and Willersin (1955), also in suspensions, peroxidation was initiated by Fe^{2+}. Then the main reactions of initiation were (Table 8.4):

Thus, the initiation rate could be assumed as:

$$\omega_i = \mu k_0[O_2]\left[Fe^{2+}\right] + \nu k_p[ROOH]\left[Fe^{2+}\right]$$

Table 8.4 Basic initiation reactions upon adding Fe^{2+} to model systems

$O_2 + Fe^{2+} \xrightarrow{k_0} Fe^{3+} + O_2^{-\bullet}$
$ROOH + Fe^{2+} \xrightarrow{k_p} Fe^{3+} + RO^{\bullet} + OH^{-}$

where the constants μ and ν reflect the contribution of the resulting radicals $O_2^{-\bullet}$ and $RO^{\bullet}$ to further processes.

In the absence of oxygen, we get:

$$\omega_i = \nu k_p[ROOH]\left[Fe^{2+}\right]$$

In the presence of oxygen, but in the absence of ROOH, at the initial stages (when ROOH did not have time to accumulate due to peroxidation), we get:

$$\omega_i = \mu k_0[O_2]\left[Fe^{2+}\right]$$

8.5.3.2 "Playing on Characteristic Times": Consider "Slow Variables" as Parameters

All reactions have their own rates and, accordingly, characteristic times of change of variables. If we are interested in the processes occurring during a time $\sim\tau_0$, then all variables with characteristic times $\tau \gg \tau_0$ can be considered parameters.

So, for example, in reactions (Table 8.4), with an excess of added Fe^{2+}, at the initial stages one can consider $[Fe^{2+}] \cong$ const.

Then, in the absence of oxygen $\omega_i \sim [ROOH]$.

In the presence of oxygen, but in the absence of ROOH, $\omega_i \sim [O_2]$.

Later, however, the Fe^{2+}waste and the ROOH accumulation become significant, and the equation for ω_i becomes more complicated again.

$[LH] \cong$ const can often be considered as well.

8.5.3.3 "Playing on Characteristic Times": Tikhonov's Theorem for "Fast Variables"

Let us, again, consider processes occurring at characteristic times $\sim\tau_0$. Variables with $\tau \ll \tau_0$ are called "fast": they manage to change much faster than the "experimenter sees" and "instantly" reach a certain level determined by the balance of their formation and consumption and then remain at it. In physical chemistry this statement is known as the Bodenstein–Semenov principle; in mathematics, in a strict form, it follows from the Tikhonov theorem (Tikhonov 1952). This "quasi-stationary" state is determined by the parameters and the current value of the "slow" variables and, therefore, can gradually change.

Thus, the system dynamics is divided into three phases:

- The initial (very short) period – reaching the quasi-stationary state: fast variables (x_j) change from initial

Table 8.5 Quasi-stationary solutions of the associated system in Table 8.3

1`	$\omega_i - k_1[X^\bullet][RH] = 0$
3`	$k_1[X^\bullet][RH] - k_2[R^\bullet][O_2] + k_3[ROO^\bullet][RH] = 0$
4`	$k_2[R^\bullet][O_2] - k_3[ROO^\bullet][RH] - k_6[ROO^\bullet]^2 - k_7[ROO^\bullet][InH] = 0$

concentrations to a quasi-stationary state with slow variables remaining practically constant.

- The main period – the quasi-stationary state: slow variables change according to their equations; fast variables "instantly" reach their quasi-stationary state for each value of the slow variables.
- The final (short) period is a significant change in slow variables, at which the conditions for the Tikhonov theorem are violated.

8.5.3.4 Practical Application

In practice, such "fast" variables are often concentrations of free radicals, which generally react much faster than other molecules. Thus, the concentrations of $X^\bullet$, $R^\bullet$, and $ROO^\bullet$, at a constant initiation rate, "instantly" (for most tasks immeasurably fast) reach such a quasi-stationary level, remaining almost constant until the entire RH pool is consumed, or new initiators appear. In Table 8.3, this allows replacing Eqs. 1, 3, and 4 with the following (Table 8.5):

Then from 1`:

$$[X^\bullet] = \frac{\omega_i}{k_1[RH]} \tag{8.7}$$

(i.e., at a constant initiation rate, while the concentration of RH can be considered constant, $[X^\bullet] = \text{const}$ also).

From 1` and 3`, we get:

$$\omega_i = k_2[R^\bullet][O_2] - k_3[ROO^\bullet][RH] \tag{8.8}$$

Then, the rate of the substrate consumption (reaction 2 in Table 8.3) will look like:

$$[\dot{RH}] = -k_1[X^\bullet][RH] - k_3[ROO^\bullet][RH] = -k_2[R^\bullet][O_2] \tag{8.9}$$

and the substrate consumption rate (i.e., the rate of the entire peroxidation process) will be completely determined by the reaction

$$R^\bullet + O_2 \xrightarrow{k_2} ROO^\bullet$$

In a particular case, if there are no inhibitors in the system (which corresponds to model systems with hydrocarbon suspensions), $[InH] = 0$, and Eq. 4` in Table 8.5 will take the form:

$$k_2[R^\bullet][O_2] - k_3[LOO^\bullet][RH] - k_6[ROO^\bullet]^2 = 0$$

That is, $k_6[ROO^\bullet]^2 = \omega_i$, and therefore:

$$[ROO^\bullet] = \sqrt{\omega_i/k_6}$$
$$[R^\bullet] = \frac{1}{k_2[O_2]}\left(\omega_i + \frac{k_3[RH]}{\sqrt{k_6}}\sqrt{\omega_i}\right) \tag{8.10}$$

Thus, the peroxidation rate will be:

$$\omega = -[\dot{RH}] = \omega_i + \frac{k_3[RH]}{\sqrt{k_6}}\sqrt{\omega_i} \tag{8.11}$$

If the initiation rate is small and [RH] is large, then the predominant contribution to RH consumption will be made by the second term, and $[\dot{RH}] \sim \sqrt{\omega_i}$. It is this ratio shown in Emanuel et al. (1965), which was used as a proof of the very scheme of peroxidation processes.

Otherwise, if there are inhibitors in the system, then, as a rule, the rate of $ROO^\bullet$ disappearance is determined mainly by the reaction with InH, and not by recombination (as $[ROO^\bullet]$ is very small in any case). Thus, the term $k_6[ROO^\bullet]^2$ can be neglected, and eq. 4` in Table 8.5 will take the form:

$$k_2[R^\bullet][O_2] - k_3[ROO^\bullet][RH] - k_7[ROO^\bullet][InH] = 0$$

That is, $k_7[ROO^\bullet][InH] = \omega_i$, and therefore:

$$[ROO^\bullet] = \frac{\omega_i}{k_7[InH]}$$
$$[R^\bullet] = \frac{\omega_i}{k_2[O_2]}\left(\frac{k_3[RH]}{k_7[InH]} + 1\right) \tag{8.12}$$

and the rate of peroxidation:

$$\omega = -[\dot{RH}] = \omega_i\left(\frac{k_3[RH]}{k_7[InH]} + 1\right) \tag{8.13}$$

Thus, while in the absence of inhibitors, the peroxidation rate has two components: $\sim\omega_i$ and $\sim\sqrt{\omega_i}$, addition of inhibitors to the system makes the second component insignificant, replacing it with a component also proportional to ω_i.

8.5.3.5 Summary

In all cases, the original system is simplified by dividing it into relatively autonomous subsystems and a separate analysis of each of them. Using these methods, it is possible to investigate individual stages of the process: to check the correctness of specific kinetic equations and to obtain experimental values for the rate constants. Different variants of peroxidation process, depending on the concentrations of various components and rate constants, were analyzed in more detail in Vladimirov and Archakov (1972) and the literature cited there, and are also given further in Chaps. 10 and 11.

8.6 Summary and Announcement for the Next Chapters

As is well known, free radicals are the most reactive substances in the living cell, and thus the cause of damage and destruction. Their leading role in almost all elderly diseases and body aging is generally recognized and is the subject of numerous studies (Wei et al. 1989; Harman 1981; Beckman and Ames 1998; Valko et al. 2007; Smallwood et al. 2018; Tavassolifar et al. 2020). This set the generalized terms such as oxidative stress or reactive oxygen species.

At the same time, free radicals appeared important participants in regulation of living cells, triggering a lot of important processes (Iles and Forman 2002; Suzuki et al. 2011; Yang et al. 2012; Zhang et al. 2016). The general belief that their action is nonspecific and always destructive, was supplemented with new data: the action of various radicals can be unexpectedly specific, and most of the effects seem adapted by living nature for the benefit of the organism. Thus, superoxide radical, the main radical formed in living cells, serves as a source of hydrogen peroxide, which, in turn, is the main molecule that carries out redox signaling based on thiol-disulfide transitions in proteins (Finkel 2011; Fomenko et al. 2007; Weerapana et al. 2010). Lipid radicals participating in fatty acids oxidation turned out to be intermediate products in the biosynthesis of the most important lipid mediators (Xiao et al. 2011; Yu et al. 2009). Controlled cell death turned out to be triggered by radicals formed in the depths of lipoperoxidases, primarily cytochrome c in the complex with anionic lipids (Liu et al. 1996; Yang et al. 1997). These radicals generate other lipid radicals, whose action leads to the main events of apoptosis: a cascade of enzymatic reactions in the cytoplasm and the attraction of phagocytes to destroy the cell.

Research of free radicals, initially seeming impossibly difficult due to their high reactivity, appeared feasible mainly due to chemiluminescence and kinetic methods, which uncovered many sources of free radicals in the living cell. The initial idea that the main catalysts for their appearance are transition metals, including iron, turned out too simplified and in many cases simply incorrect. The main sources of free radicals in real cells appeared reactions involving a wide range of enzymes, mainly metalloproteins, including heme proteins.

All this flurry of new data, as well as the classically accepted free-radical processes, will be discussed in the following chapters.

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