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Peculiarities of the free radicals biogenesis – implications in pathobiochemistry

Z. Gârban [1,2], Mirela Ahmadi-Vincu [2,3], R. Ujhelyi [2,4], F. Muselin [2,5]

[1]*Former - Banat`s University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" Timişoara, Faculty of Food Products Technology,Department of Biochemistry-Molecular biology, Calea Aradului No. 119, RO-300645 Timişoara;*
[2] *Working Group for Xenobiochemistry, Romanian Academy-Branch Timişoara, Bd. M.Viteazu No.24, RO-300 223 Timişoara;*
[3] *Banat`s University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" Timişoara, Faculty of Veterinary Medicine, Department of Biochemistry, Calea Aradului No. 119, RO-300 645 Timişoara;*
[4] *S.C. CaliVita International, Medical Department, Timişoara*
[5] *Banat`s University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" Timişoara, Faculty of Veterinary Medicine, Department of Toxicology, Calea Aradului No. 119, RO-300 645 Timişoara*

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Abstract

Free radicals biogenesis is of interest both for the metabolism of nutrients in biochemistry and for the biotransformation of chemical xenobiotics in xenobiochemistry. The perfection of the physico-chemical methods of investigation allowed the evaluation of the harmful effects induced by the free radicals having as target to find more effective investigations of some important biomarkers in the biochemistry and pathobiochemistry. A brief presentation of these issues in the form of a review is followed in this paper. In a context regarding the harmful effects of free radicals it is mentioned the pro-oxidant and anti-oxidant effects. There are also mentioned the delayed consequences of the of free oxygen radicals action in the appearance of degenerative diseases, ischemic diseases a.o. consequent to oxidative stress, knowing the protective activity of some food antioxidants.

Keywords: free radicals, oxidative stress, various diseases, pathobiochemistry

1. Introduction

Regarding free radicals - in terms of the development of knowledge - is known that the progress in their study has encountered numerous difficulties due to their reactivity and the low duration of their chemical activity. Evolutive, it was initially stated that radicals cannot exist as free radicals due to increased reactivity [1]. Subsequently, Gomberg [2] isolated a stable free radical, i.e. triphenyl methane $(C_6H_5)_3CH$. In the period that followed, free radicals of aliphatic hydrocarbons were obtained.

In biochemistry, the role of free radicals was revealed when they were detected in the enzymatic oxidation-reduction reactions [3]. Marking of free radicals is done by writing the specific sign - a point on the right - at conventionally used for: radicals (–R) (e.g.: $–CH_3$, $–C_2H_5$, $–O–C_6H_5$); functional groups (e.g. –OH); simple molecules (e.g. O_2) or even atoms (e.g. –H).

Corresponding author: *e-mail:* zeno.garban@yahoo.com

In all cases, however, is given up to emphasize the valence, the free radicals (FR) are noted directly. So, in the cases mentioned above they will be noted:

$$R^{\cdot},\quad CH_3^{\cdot},\quad C_2H_5^{\cdot},\quad OH^{\cdot},\quad O_2^{\cdot},\quad H^{\cdot}$$

Significant advances in free radical studies have been made with the introduction of the concept of „oxidative stress" in researches on redox reactions occurring in biology and medicine [4,5]. But as an updated definition, the oxidative stress represents an imbalance between pro-oxidants and anti-oxidants, leading to a disruption of redox signaling and control having as result the molecular injury [6, 7].

For oxidative stress there are known various specific forms, among which can be mentioned: nutritional oxidative stress; dietary oxidative stress; postprandial oxidative stress; physiological oxidative stress; photo oxidative stress, i.e. ultraviolet (UV-A, UV-B, infrared-A); radiation-induced oxidative stress; nitrosative stress; reductive stress [8, 9].

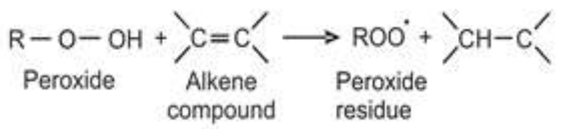
1. Interactions specific to free radical biogenesis

Studies on free radicals indicate that their production can be achieved in the course of various specific reactions: a) homolytic; b) photolytic; c) enzymatic [5, 10, 11].

1.1. Homolytic reactions

Are more common in case of peroxidic compounds and metallic ions.

a) peroxidic compounds - in the presence of alkene compounds:



b) metallic ions – transfer of electrons (e.g.: of Fe^{2+} or Fe^{3+}) in the presence of hydrogen peroxide (H_2O_2) and adenosine diphosphate (ADP)

$$Fe^{2+} + H_2O_2 \xrightarrow[-e^-]{} Fe^{3+} + OH^{\cdot} +$$

$$Fe^{3+} + H_2O_2 \xrightarrow{+e^-} Fe^{2+} + HO_2^{\cdot} +$$

Such processes can be encountered *in vivo* when the peroxides interact with hemoglobin. For example, it is known that hydrogen peroxide (H_2O_2) is formed during metabolic processes in mitochondria and cytoplasma.

Hydrogen peroxide (H_2O_2) in the presence of transition metal ions Fe^{2+}/Cu^{+} (being in cell) can be transformed. In these conditions the so called „Fenton reaction" is occurring:

$$H_2O_2 + Fe^{3+}/Cu^{+} \longrightarrow OH^{\cdot} + OH^{\cdot} + Fe^{2+}/Cu^{2+}$$

hydroxyl radicals are generated and the ionic states of the involved metals in the reaction is changed.

There is also a reaction in which the same transition metals participate as catalysts of the hydrogen peroxide transformation (H_2O_2) known as „Haber-Weiss reaction" :

$$H_2O_2 + O_2^{\cdot} \xrightarrow{Fe/Cu} HO^{\cdot} + HO^{\cdot} + O_2$$

resulting finally hydroxyl radicals, too.

1.2. Photolytic reactions

Are generated by the energy (E) provided by the ionizing radiation. This effect can also be retarded (after cessation of direct exposure). Such an effect – as a result of which free radical is formed – also occurs in the radiolysis of water:

$$H_2O \xrightarrow{E} H_3O^{+} + H^{\cdot} + OH^{\cdot} + e^{-}_{aq} + H_2O_2 + H_2$$

Due the radiolytic effect in the water the radicals, the aquated electrons ($e^{-}aq$), ions (H_3O^{+}) and molecules (H_2O_2, H_2) are formed.

Considering the high water content of living organisms (about 70-80%), it is easy to see the effect of free radicals biogenesis in biological systems. The issue – from biomedical point of view – concerns radiobiology, radioprotection and radio-pathology (e.g. mutagenic, teratogenic, carcinogenic effects).

1.3. Enzymatic reactions

These enzymatic reactions are partially involved in the free radical production. Most of the radical biogenesis reactions are redox. In the case of enzymatic reactions it should be mentioned that the production of free radicals occurs not only in current metabolic reactions but also in the biotransformation of xenobiotics that enter the organism through various routes: digestive (with food and water), respiratory (with inspired air) and transcuta-neous (handling of chemical substances).

Detection of free radicals by *in vitro* and/or *in vivo* investigations requires the use of accuracy and high resolution methods. In chemical and biochemical analytical practice for such research UV-VIS spectroscopy and electron spin resonance (ESR) are used.

Spectrometric method has been used in the study of kinetic reactions of flavin-enzymes that produce free radicals. The ESR method has proven effective in detecting free chlorophyll radicals during photosynthesis. ESR was also applied to the study of FR in cancerous tissues. But, due to the high amount of water in living organisms the ESR method has some difficulties.

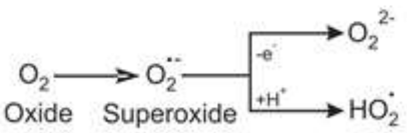
2. Free radicals in biological systems

In biological systems - implicitly in the human organism - free radical biogenesis is a natural and / or an accidental process [12-15]. Details of these processes are not the object of this review. Reactive oxygen species together with nitric oxide are mainly factors involved directly in self-defense system.

However, some data are mentioned regarding: a) the formation of free oxygen radicals (with maximum biological distribution); b) sources of free radicals; c) pathochemical implications and antioxidant protection.

2.1. Free radicals formation

Molecular oxygen can – under certain conditions – generate free radicals of the type: superoxide or hydroxyl and peroxide ion. Their biogenesis can be represented as follows:



and from peroxide the reaction can proceed in the manner:

$$O_2^{2-} \xrightarrow{+H^+} HO_2^- \xrightarrow{+H^+} H_2O_2 \xrightarrow[-e]{+H^+} OH^\bullet + H_2O$$

The resulting compounds may participate in the superoxide dismutase reaction that can evolve in two ways:

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

$$HO_2^\bullet + O_2^{\bullet -} + H^+ \longrightarrow H_2O_2 +$$

These reactions involve the enzyme called „superoxide dismutase" (SOD). The admission of this enzyme to biological systems in 1968 was conditioned by the *in vivo* production of the rapidly decomposing radical. The SOD enzyme is presented as a metal-enzymatic complex containing Mn, Fe (for prokaryotes), Fe or Cu-Zn (eukaryotes). This enzyme interferes with constant reactions having a rate 10^8-10^9 $M^{-1}s^{-1}$ (e.g.: SOD from mito-chondria $6.0 \cdot 10^8$; SOD from the $2.3 \cdot 10^9$ in the cytoplasma of red blood cells).

Besides the free radicals of oxygen in pathological conditions, other types of free radicals can also form under the action of radiation.

2.2. Sources of free radicals

Free radicals can be formed during oxidation-reduction reaction specific for the biochemical transformations concerning the metabolization of nutrients and/or biotransformation of chemical xenobiotics acceding into the organism [11, 16]. The development of these processes is conditioned by the topochemical peculiarities of the cell [14, 17].

To the forming of free radicals can contribute not only the cellular organelles with special attributes (e.g.: mithocondria, peroxisomes) in redox processes but also the cellular cytoplasm by the metabolic processes that integrate carbohydrates, lipids and proteins. Some peculiar aspects are presented below.

a) Mitochondria - as cytoplasmic organelle - compete for FR formation. Thus, O_2 is formed during the breathing in the mitochondria. The process involves the participation of O_2 brought by hemoglobin, a number of redox enzymes (nicotinamide adenine nucleotide: NAD^+, cytochrom-oxidase etc.) and metalloproteins present in mitochondria.

Afterwards the superoxide is dismuted - by the SOD of the mitochondrial matrix - according to the reaction:

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

Finally, the decomposition of H_2O_2 is made by the action of glutathione-peroxidase:

$$2\,G-SH + H_2O_2 \xrightarrow{\text{G-SH peroxidase}} G-S-S-G + 2\,H_2O$$

b) Peroxisomes - cytoplasmic organelles specific to animal cells (mainly present in hepatocytes) compete to form H_2O_2 in large quantities as a result of some oxidation reactions such as:

$$\text{Amino acids} \xrightarrow[-H_2O_2]{\text{Oxidase}} \alpha\text{-ceto acids} + H_2O_2$$

$$\text{Hydroxy acids} \xrightarrow[-H_2O_2]{\text{Oxidase}} \alpha\text{-ceto acids} + H_2O_2$$

$$\text{Uric acid} \xrightarrow[-H_2O_2]{\text{Uric-oxidase}} \text{Allantoin} + CO_2 + H_2O_2$$

The decomposition of H_2O_2 is mainly done under the action of catalase:

$$H_2O_2 + H_2O_2 \xrightarrow{\text{Catalase}} 2\,H_2O$$

Also, the decomposition of H_2O_2 may occur in the presence of oxidizing derivatives (such as phenols, alcohols) which pass into oxidized forms (quinones, aldehydes) the decomposition occurs under the action of peroxidase:

$$H_2O_2 + \text{Phenols} \xrightarrow{\text{Peroxidase}} H_2O + \text{Quinone}$$

c) Cellular cytoplasm – may be the place of free radicals formation, too during various metabolic processes, e.g.: glycolysis as the degradation process of carbohydrate macromolecules; lipolysis in the decomposition phase of fatty acids; reconversion of carbohydrates into lipids (fact explained by the presence in large number of liposomes in adipose tissue); biodegradation of nucleic acids - DNA and RNA [18, 19].

2.3.Pathobiochemical implications and antioxidant protection

Free radicals are involved in triggering pathological conditions. These include acute inflammation, some cardiovascular diseases (especially in the evolution of atherogenic processes), hepatic insufficiency, respiratory failure, and so on. [5].

Antioxidant systems protects the body from free radicals, with removing the reactive species of oxygen in aerobic life. The issue of Oxidative Factors - Antioxidant Systems is a major direction of interdisciplinary studies in biochemistry, molecular biology, pathobiochemistry and, last but not least, nutrition and pharmacology.

In antioxidant systems during the formation of free radicals synergic action occurs on different levels in relation to enzymatic antioxidant [15].

Accepting the existence of a process of radical biogenesis regarding the oxygenated radicals, the concept of „oxidative stress" was imposed. This concept is justified by the injury produced by FR at cellular level by the action of ($O_2^{\cdot}$). The triggered biochemical processes lead to detectable disturbances at the level of molecules which are involved in the pathogenesis and acceleration of degradative morphofunctional processes that hurry senescence.

Under these circumstances, research in biochemistry, molecular biology, nutrition and pharmacology has focused on detecting chemical compounds (nutritional principles and drugs) with a distinct antioxidant role.

It is worth noting in this context the beneficial role of food nutrients. Among these are vitaminic micronutrients (vitamins A, C, E) and minerals (food rich in Cu, Zn, Se), but also some lipids (e.g. triacylglycerols) containing unsaturated fatty acids.

In medical practice, „oxidative stress" has been counteracted by the use of specific chemotherapies, e.g.: phenothiazines and barbiturates - recommended for central nervous system disorders; allopurinol (xanthine oxidase inhibitor) – recommended for rheumatic diseases; vitamins A, C, E administered as food supplements.

3.Biomarkers in the evaluation of oxidative stress

In pathobiochemistry it is important to develop the use of biological markers (biomarkers) that can allow the evaluation of oxidative stress. This method of investigation, currently available only in specialized laboratories, allows knowledge of the role of pro-oxidants and anti-oxidants as well as their endogenous and exogenous sources.

The World Health Organization [20] has defined a biomarker as any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease. The most important biomarkers are those which are closely correlated with the pathobiochemical and pathophysiological process of the disease.

The role of oxidative stress in pathobiochemistry and pathophysiology of various diseases are known and so the quantification of the reactive oxygen species (ROS) is very useful. Excessive ROS in vivo can modify the molecules of DNA, lipids, proteins and carbohydrates [21, 22].

Below there are exemplified some biomarkers generated in the organism: elevated malondialdehyde and isoprostanes detected in serum and urine samples - reflect the lipid peroxidation related to a cardiovascular risk factor; decreased blood levels of the antioxidant vitamins (A, C and E) and decrease of serum bilirubin can be found in Crohn`s disease (inflammation of the intestinal wall); elevated protein carbonyl levels in neurodegenerative diseases, obesity, diabetes mellitus, age-related macular degeneration, human anemia, sickle cell disease, newborn bronchopulmonary dysplasia, hepatocellular carcinoma a.o.

Information on oxidative stress are of great importance because it is involved in many diseases [18, 21, 22]. Among the most common diseases are the: inflammatory diseases (arthritis, vasculitis, respiratory disease syndrome); ischemic diseases (heart disease, stroke, intestinal ischemia), acquired immunodeficiency syndrome, digestive disease (gastric ulcer, Crohn`s disease); neurological disorders (Alzheimer`s disease, Parkinson`s disease) a.o.

Generally, free radical biogenesis involves proteins through numerous enzymes that compete with metabolic processes. It can be argued that the issue of this field - presenting nutritional and therapeutic interest is an objective of preventive medicine.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

References

1. Ostwald, W., *Elektrochemie. Ihre Geschichte und Lehre*, Verlag von Veit und Comp., Leipzig, 1896
2. Gomberg, M., An instance of trivalent carbon: triphenylmethyl, *J. Am. Chem. Soc*., 1900, 22(11), 757-771
3. Michaelis, L., Free radicals as intermediate steps of oxidation-reduction, *Cold Spring Harbor Symposia on Quatitative Biology*, 1939, 7, 33-49.
4. Sies, H. (Ed.), *Oxidative stress: Oxidants and antioxidants,* Academic Press, London, 1991
5. Halliwell, B.; Gutteridge, J.M.C., *Free Radicals in Biology and Medicine*, 4th ed., Oxford University Press, Oxford, 2007.
6. Voet, D.; Voet, G. Judith, *Biochemistry*, 4th edition, J. Wiley and Sons Inc., New York, 2011.
7. Sies, H., Oxidative stress: a concept in redox biology and medicine, *Redox Biology*, 2015, 4, 180-183.
8. Fink G. (Ed.), *Encyclopedia of stress*, 2nd edition, Elsevier, Amsterdam, 2007
9. Jones, D.P., Radical-free biology of oxidative stress, *American Journal of Physiology-Cell Physiology*, 2008, 295(4), C849–C868.
10. Moslen M.T. - Protection against free radical-mediated tissue injury, pp.203-215, in *Free Radical Mechanisms of Tissue Injury* (Moslen M.T., Smith C.V., Eds.), CRC Press, Boca Raton, FL., 1992.
11. Gârban, Z., *Biochimie: Tratat comprehensiv, Vol.I*, ediţia 5-a, Editura Academiei Române, 2015
12. Nagata C., Kodama M., Kimura T., Aida M. – Metabolically generated free radicals from many types of chemical carcinogens and binding of radicals with nucleic acid bases, pp. 43-54, in „*Carcinogenesis: Fundamental mechanisms and environmental effects*" (Pullman B., Ts'o P.O.P., Gelboin H., Eds.), D.Reidel Pub. Comp., Dordrecht, 1980.
13. Sahini V. Em., Radicalii liberi în ţesuturile biologice, *St. cerc. Biochim.*, 1989, 32(1), 7-10.
14. Olinescu R. – *Radicalii liberi în fiziopatologia umană*, Editura Tehnică, Bucureşti, 1994.
15. Dejica, D., *Stresul oxidativ în bolile interne*, Casa Cărţii de Ştiinţă, Cluj-Napoca, 2000.
16. Aruoma, O.I., Nutrition and health aspects of free radicals and antioxidants, *Food Chem. Toxicol*., 1994, 32, 671-683.
17. Armstrong D., Sohal R.S., Cutler R.G., Slater T.F. (Eds.) – *Free Radicals in Molecular Biology, Aging and Disease*, Raven Press, New York, 1984.
18. Lobo, V., Patil, A., Phatak, A., Chandra, N., Free radicals, antioxidants and functional foods: Impact on human health, *Pharmacognosy Review*, 2010, 4(8), 118-126.
19. Gârban Z., Herman V., Muselin F., Ahmadi-Vincu Mirela, Ujhelyi R., Avacovici Adina - Integrative and transdisciplinary aspects in pathobiochemistry. *Scientifical Papers Veterinary Medicine Timişoara,* 2016, 49(1), 88-104
20. *** WHO, *Biomarkers in Risk assessment: Validity and Validation*. Geneva: WHO, 2001.
21. Ho, E., Galougahi, K.K., Liu, C.-C., Bhindi, R., Figtree, G.A., Biological markers of oxidative stress: Applications to cardiovascular research and practice, *Redox Biology*, 2013,1, 483–491
22. Frijhoff, J., Winyard, G.P., Zarkovic, N. et al., Clinical Relevance of Biomarkers of Oxidative Stress, *Antioxidants and Redox Signaling*, 2015, 23 (14), 1144-1170