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Mini-review

Free radicals, reactive oxygen species, oxidative stress and its classification

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ABSTRACT

Reactive oxygen species (ROS) initially considered as only damaging agents in living organisms further were found to play positive roles also. This paper describes ROS homeostasis, principles of their investigation and technical approaches to investigate ROS-related processes. Especial attention is paid to complications related to experimental documentation of these processes, their diversity, spatiotemporal distribution, relationships with physiological state of the organisms. Imbalance between ROS generation and elimination in favor of the first with certain consequences for cell physiology has been called "oxidative stress". Although almost 30 years passed since the first definition of oxidative stress was introduced by Helmut Sies, to date we have no accepted classification of oxidative stress. In order to fill up this gape here classification of oxidative stress based on its intensity is proposed. Due to that oxidative stress may be classified as basal oxidative stress (BOS), low intensity oxidative stress (LOS), intermediate intensity oxidative stress (IOS), and high intensity oxidative stress (HOS). Another classification of potential interest may differentiate three categories such as mild oxidative stress (MOS), temperate oxidative stress (TOS), and finally severe (strong) oxidative stress (SOS). Perspective directions of investigations in the field include development of sophisticated classification of oxidative stresses, accurate identification of cellular ROS targets and their arranged responses to ROS influence, real *in situ* functions and operation of so-called "antioxidants", intracellular spatiotemporal distribution and effects of ROS, deciphering of molecular mechanisms responsible for cellular response to ROS attacks, and ROS involvement in realization of normal cellular functions in cellular homeostasis.

Contents

Abbreviations: BOS, basal oxidative stress; ETC, electron transport chain; HOS, high intensity oxidative stress; 8-OHG, 8-hydroxyguanine; G6PDH, glucose-6-phosphate dehydrogenase; GPx, glutathione-dependent peroxidase; GR, glutathione reductase; GSH, glutathione reduced; GSNO, S-nitrosoglutathione; GSSG, glutathione oxidized; IDH, $NADP^+$-isocitrate dehydrogenase; IOS, intermediate intensity oxidative stress; LOOH, lipid peroxides; LOS, low intensity oxidative stress; MDA, malonic dialdehyde; NADP-ME, NADP-malic enzyme; NOE, no observable effect point; ·NO, nitric oxide radical; 6PGDH, 6-phosphogluconate dehydrogenase; 8-oxodG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; 8-oxoGua, 8-oxo-7,8-dihydroguanine; PPP, pentose phosphate pathway; RNS, reactive nitrogen species; ROS, reactive oxygen species; RS, reactive species; $O_2^{\cdot-}$, superoxide anion radical; SOD, superoxide dismutase; TBA, thiobarbituric acid; TBARS, thiobarbituric acid reactive substances; TRR, thioredoxin glutathione reductase.

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1. Introduction

Free radicals were first described by Moses Gomberg more than a century ago [1]. For a long time they were not considered to present in biological systems due to high reactivity and consequently short living time. More than 30 years later, Leonor Michaelis [2] proposed that all oxidation reactions involving organic molecules would be mediated by free radicals. Although that statement generally was wrong, it stimulated interest to the role of free radicals in biological processes. In 1950th, free radicals were found in biological systems [3] and immediately supposed to be involved in diverse pathological processes [4] and aging [5,6]. Since that time our knowledge on involvement of free radicals in living processes extended enormously. Clearly, long time and actually up to now they have been mainly suggested to play deleterious roles considering mainly as damaging species. This point of view was strengthened by discovery of McCord and Fridovich [7] who described the first protective enzyme against free radicals called superoxide dismutase. In 1970–1990th understanding of free radicals as only deleterious species for biological systems was substantially challenged by several important discoveries. The first, free radicals were found to be responsible for combating of infection agents by immune system [8–11]. The second, in 1980th vascular endothelial cells were found to produce nitric oxide from L-arginine, and this accounted for the biological activities attributed to endothelium-derived relaxing factor (EDRF) [12–15]. That discovery opened the second avenue for free radical research, their signaling function, initially for nitric oxide and further for other reactive species [16–19]. Finally, the level of free radicals was found to be regulated by hormones like insulin [20], and they were suggested to be regulators of core metabolic pathways [21]. Therefore, it is absolutely clear now that free radicals are active participants in diverse processes and they cannot be considered anymore as only damaging agents, but real players in many normal functions of living organisms.

The field of free radicals or more common reactive species (RS) research in biological systems is one of the most dynamic, but also is among most complicated due to many reasons [22]. Main of them are: (i) low stability and high reactivity resulting in low steady-state concentrations; (ii) high diversity of reactions they can participate in; (iii) complicated spatiotemporal distribution in cell space and extracellularly; (iv) dependence on physiological state of the organism, and (v) absence of technical tools for reliable evaluation of their absolute and even relative levels. Because of presence of many complications in free radical studies and extensive involvement of new researchers from different fields in this area and due to obvious universality and importance of RS-related processes, this paper aims to clarify and highlight some basic terms used, processes they are involved in, describe the concept of oxidative stress and give general classifications of this sort of the stress.

2. Free radicals and reactive oxygen species

In the most cases, the terms "free radicals" and "reactive oxygen species (ROS)" are used interchangeably. If in many cases that is correct, in some ones it is wrong. Therefore, to clarify the issue I will provide below the information how to discriminate between these two terms in order to provide understanding based on modern knowledge in the field. Probably, analysis of ROS generation, interconversion and elimination is the easiest way to do that. These processes are schematically presented in Fig. 1. In living organisms under aerobic conditions more than 90% of oxygen consumed is reduced directly to water by cytochrome oxidase in electron-transport chain (ETC) via four-electron mechanisms without ROS release [23,24]. In eukaryotes, the system is represented by ETC, located in

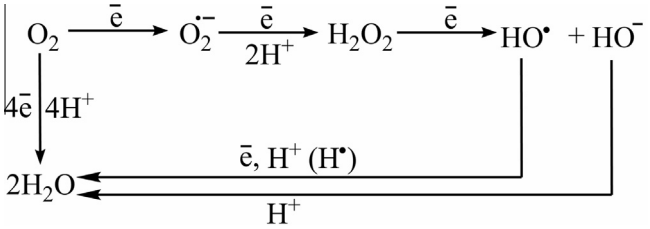

Fig. 1. Reduction of molecular oxygen via four- and one-electron schemes. Description in the text.

internal mitochondrial membrane, whereas in prokaryotes ETC components are located in plasmatic membrane. Operation of ETC is coupled with oxidative phosphorylation to produce energy in ATP form. Much less than 10% of oxygen consumed is reduced via one-electron successive pathways resulting in conversion of molecular oxygen to superoxide anion radical ($O_2^{\bullet-}$) followed by one-electron reduction with concomitant accepting of two protons to yield hydrogen peroxide (H_2O_2) (Fig. 1). This compound is not a free radical, but is chemically more active than molecular oxygen due to which is included in the ROS group. Hydrogen peroxide molecule accepting one more electron is split up to hydroxyl radical ($HO^\bullet$) and hydroxyl anion (OH^-). Finally, $HO^\bullet$ interacts with one more electron and proton resulting in formation of water molecule. In biological systems, this reaction is mainly realized through abstraction of hydrogen atom from different compounds such as proteins and lipids resulting frequently in initiation of chain processes. Summarizing described above, $O_2^{\bullet-}$, H_2O_2, and $HO^\bullet$ collectively are called reactive oxygen species, but only $O_2^{\bullet-}$ and $HO^\bullet$ are free radicals, whereas H_2O_2 is not. Reactive oxygen species include not only mentioned above $O_2^{\bullet-}$, H_2O_2, and $HO^\bullet$, but also diverse peroxides, like lipid peroxides, and peroxides of proteins, and nucleic acids. Moreover, their homeostasis is closely related to many other reactive species (RS), such as reactive carbonyl species (glyoxal, methylgyoxal) [25]. There are also many other RS of nitrogen (nitric oxide, peroxynitrite, and related compounds) [26–28], carbon [27,29,30], sulfur [31], halogens [32], etc. However, in this short review I will focus only on ROS.

3. Generation, conversion, and elimination of ROS in living systems

After ROS discovery in living organisms, the interest of researchers was focused on identification of mechanisms of their generation. To date, many of them have been disclosed. It has been established that in eukaryotic cells over 90% of ROS are produced by mitochondria [24]. Probably of that amount dependently on specific situation most ROS are generated via escaping of electrons from mitochondrial ETC mainly from coenzyme Q to molecular oxygen. The electrons escaping ETC interact with molecular oxygen to give $O_2^{\bullet-}$. Further it spontaneously or enzymatically is converted to H_2O_2 and $HO^\bullet$. The amount of electrons escaping ETC varies broadly and depends on physiological state of organisms. Total amount of ROS produced by mitochondria seems is poorly controlled by the cell at this stage and to maintain low ROS steady-state level cells possess finely regulated antioxidant systems. In addition, minor amounts of ROS are produced by ETC located in/at endoplasmic reticulum (ER), plasmatic, and nuclear membranes as well as some oxidases. Production of ROS in ER is mainly connected with operation of hydroxylation system represented by cytochrome P450 family enzymes. At oxidation of substrates, some portion of electrons escape ETC and similarly to mitochondria, joins O_2 giving rise to $O_2^{\bullet-}$ and products of its transformation. The products formed during hydroxylation reaction also may