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# Molecular biology of oxygen free radicals

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The knowledge regarding oxygen free radicals (termed also as active oxygen species) showed an explosion-like increase during the last decade. Unfortunately, however, the interpretation of their physiological roles is by far not clear. Therefore, it seems to be necessary to outline the general trends and most important aspects of their molecular biology even in the frame of such a Workshop dedicated to a specialized problem like phagocytic cell disorders.

## The concept of free radicals

Free radicals are defined as chemical agents having an odd number of electrons, i.e., an unpaired electron on their external electron shell [24]. The most general characteristics of the free radicals is that apart from some exceptional cases, the unpaired electron usually cannot be sufficiently stable, i.e., the free radicals react rather quickly with the adjacent molecules, and consequently, they never reach considerably high concentrations in any chemical or biochemical system. This situation has always represented a serious difficulty for the progress of free radical science. It should be stressed that in the living systems a wide variety of different types of free radicals occur, however, the greatest importance can be attributed to those which derive from the molecular oxygen.

Although the role of free radicals in chemical synthetic processes, polymerization reactions, etc. has been recognized long decades ago, most of the biochemists have been neglecting the significance of their function in the living systems for many years. The discovery of superoxide dismutase (SOD) [13, 14] represented an important step in the free radical research, nevertheless, this approach to biology has not been generally accepted. It is obvious that the most important parameter on the basis of which one could unequivocally judge the role of free radicals would be the turnover rate of these radicals. Unfortunately, turnover measurements on the free radicals still represent a serious methodological problem. Therefore, the importance of free radicals in biological systems is based largely on circumstantial or indirect evidence

even today. It seems to be amazing that in spite of the fact that the implication of free radical reactions in biological processes such as aging was proposed already 32 yers ago [9], the progress in this field was extremely slow, and even today it can only be concluded from data actually available that oxygen free radicals do occur in biological systems [8, 19—21] and are involved in numerous biological phenomena such as cellular aging, mutagenesis, inflammation and other pathologies [1, 2, 10, 12, 15, 16, 22, 23, 26, 27, 31, 32].

## Some important data about oxygen free radicals

It is generally accepted that the molecular oxygen has to undergo a tetravalent reduction for being activated as a biological electron acceptor in the terminal oxidation. To best of our knowledge, tetravalent reduction is performed in one single step only by a few enzymes like the cytochrome oxidase, lactase, ascorbic acid oxidase, whereas numerous other enzymes (cytochrome P-450, xanthine oxidase, aldehyde oxidases, etc) catalyze only monovalent reduction of the $O_2$ molecule. This latter process leads to the formation of superoxide anion radicals ($O_2^-$.), which are dismutated very actively by the SOD [13, 14]. The product of this reaction is hydrogen peroxide which is much less harmful per se for the cells than the superoxide radicals, nevertheless, it is also eliminated. Two main systems perform this job: 1) the catalases (wherever present) which are sensitive only for relatively high hydrogen peroxide concentrations, and (2) the glutathione peroxidase (GPO) reacting also with the low concentrations of $H_2O_2$. However, GPO requires a substrate (glutathione) for this reaction which may not always be available in the cells [7]. As a consequence, it can be assumed that some freely diffusing $H_2O_2$ is always present in living systems and may participate in various reactions. One of the effect of the presence of $H_2O_2$ can be direct lipid-peroxidation [3, 28], however, probably more important is the reaction of $H_2O_2$ with transition metals like $Fe^{2+}$ (Fenton-reaction) [4]. This type of reaction generates OH· free radicals which are extremely reactive [33]. They will pick up an electron from any of the neighboring molecules within about 2 molecular collisions, i.e., they will form covalent cross-links in or between the organic molecules. Due to the extremely high reactivity of the OH· radicals, practically no enzymatic or any other protection is possible against them except that which has been provided by the evolution of the life: the continuous replacement of the damaged components by resynthesis and degradation of the waste products.

It has been shown that even in vivo a sufficient amount of $Fe^{2+}$ is available [5] for a Fenton-reaction, and that the OH· radicals deriving from this reaction are able to attack the amino acids and proteins very efficiently even under mild chemical conditions

[6, 40, 41]. Furthermore, rapid aging of young rats could be achieved by increasing the $Fe^{2+}$ content of the brain by injecting iron solutions into the cerebrospinal fluid (CSF) [18].

An important feature of the short-lived radical species such as OH· radicals must be emphasized here. It is easy to show (and well known in polymer chemistry) that the cross-linking effect of the OH· radicals depends very strongly on the density of the actual chemical system. If the system is highly diluted (as in the cytosol), intermolecular cross-linking will not occur, since the dissolved molecules are too far from each other, i.e., one radical will react with only one molecule. If however, the density of the system is larger (as in the membranes), the probability of the formation of intermolecular cross-links will considerably increase. Therefore the same rate of radical formation may be much more efficient in polymerizing the molecular components in systems of higher density. In other words, it can be expected that OH· radical attack destroys the membrane components to a higher extent than the cytosolic components. This expectation agrees with the fact that some membrane proteins of hepatocytes display tenfold shorter half-life than do the average proteins in the cytoplasm [30]. Therefore, the possibility that the cell membrane damage represents one of the most important rate-limiting factors during the function of all cellular systems cannot be ignored.

## Free radical induced membrane damage

The best known mechanism of membrane damage induced by free radicals is lipid peroxidation. This pathway was proposed as the origin of the age pigment (lipofuscin) accumulation. Details of this process are treated in various reviews [3, 25, 29]. An essential point of lipid peroxidation is that polyunsaturated fatty acids are decomposed by direct or indirect peroxidation processes resulting in malondialdehyde (MDA) as an end-product. MDA is extremely reactive with primary amino groups of proteins and gives rise to the so-called Schiff-bases showing a characteristic fluorescence.

Lipid peroxidation increases the rigidity of the lipid layer. Althoug hit is known that lipid peroxidation decreases the activities of some membrane bound enzymes and the age-dependent decrease of the activities of a great number of hormone-receptor complexes has been ascribed to such phenomena, it seems to be of even more general importance that lipid peroxidation alters the overall physicochemical properties of the cell membrane. Since the physicochemical state of the membrane lipids can directly determine basic functional parameters like permeability of the cell membrane for the monovalent ions (and probably also for water) [see for ref: 38, 39], it can be concluded that it is worthwhile to analyze the possible functional consequences of the radical-induced physicochemical changes.

The other possibility for free-radical induced membrane damage is that oxyradicals (mainly OH·) are able to attack directly the membrane proteins and result in a cross-linking of them [17, 40]. It should be stressed that this phenomenon must be of greater importance than generally assumed, since the OH· radicals are formed in the aqueous phase, and because of their high reactivity, they will perform most probably the reactions in the same phase, while only a part of them can reach the relatively deep, hydrophobic regions of the cell membrane.

Therefore, there are good reasons for assuming that the functioning of the living cells is accompanied all the time by a continuous formation of lipid-lipid, lipid-protein or protein--protein cross-links; however, these phenomena occur with higher probability in the membranes than in the cytosol. In addition to this free-radical induced damage, the cell plasma membrane is exposed to another damaging factor: the residual heat formation during each discharge of the resting transmembrane potential [see for details: 35]. Membrane structure and function are continuously altered by these phenomena. The damaged components need to be replaced by newly synthesized ones, and must be degraded by the lysosomal system, since otherwise the cells could not maintain their homeostasis. In the young and adult ages a strict equilibrium is maintained between the decomposition and replacement of the damaged membrane components; therefore, no accumulation of the waste-products (lipofuscin) occurs. This does not mean that damage is not taking place to the membranes in the younger ages. As a matter of fact, because of the higher metabolic rate in the young tissues, an even higher amount of lipofuscin-like products comes into being in the same time period than in the old systems. However, the rate of elimination of these products is able to keep up with the requirement of th cells [37, 39]. This assumption has recently been verified when it was shown that brain cells filled up with lipofuscin-like pigments even in young rats, if some of the lysosomal enzymes (thiol-proteases) were inhibited by leupeptin for a couple of days [11], in other words, if the equilibrium between formation and elimination rates of lipofuscin was disturbed.

## The membrane hypothesis of aging

Even if the biological role of oxygen free radicals is accepted, there is a paradoxical situation which needs explanation: the aggressive chemical nature of the oxygen free radicals remains unchanged during the whole human life span. Young individuals consume more oxygen per unit of mass and time (i.e., there is an even more intense radical formation in the younger ages) as compared to older ones. Yet it is a fact that young cells and organisms are able to grow and differentiate, while older ones progressively deteriorate in their structure

and functional performance. This discrepancy must be explained by biological structure itself. Theoretical evidence is available [37] showing that any explanation of general validity for this discrepancy should consider only the really common properties of all living systems.

Considering the basic properties of the cell membrane, a working hypothesis was outlined first in 1978 called the membrane hypothesis of aging (MHA) [34]. This concept proposes a cell physiological mechanism which is able to explain the contradictory effects and consequences of the free radicals on biological systems at young and old ages. Numerous experimental models have been tested from the point of view of MHA and none of them resulted in any inherent contradiction. Details can be found in our original as well as some recent review papers summarizing [34 – 42] the available evidence demonstrating that the free-radical induced membrane damage is a key event in both maturation and aging of cells.

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