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# Reactive Oxygen Species and Cellular Defense System

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## Abstract

Reactive oxygen species (ROS) is a collective term used for oxygen-derived free radicals (superoxide, hydroxyl radical, nitric oxide) and non-radical oxygen derivatives of high reactivity (singlet oxygen, hydrogen peroxide, peroxyxynitrite, hypochlorite). ROS can be either harmful or beneficial to the body. An imbalance between formation and removal of free radicals can lead to a pathological condition called as oxidative stress. However, the human body employs molecules known as antioxidants to counteract these free radicals. But late several studies have indicated that antioxidants can also have deleterious effects on human health depending on dosage and bioavailability. This makes it essential to analyze the extent of utility of antioxidants in the improvement of human health. It is noteworthy that if the generation of free radicals exceeds the protective effects of antioxidants, this can cause oxidative damage which accumulates during the life cycle, and this has been implicated in aging and age-dependent diseases such as cardiovascular disease, cancer, neurodegenerative disorders, and other chronic conditions. This chapter highlights the main themes from studies on free radicals, antioxidants, and oxidative stress and effect of oxidative stress in diseases.

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## Keywords

Free radicals • Reactive oxygen species • Antioxidants • Oxidative stress • Diseases

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## 1 Introduction

In recent years, the role of free radical reactions in human disease, biology, toxicology, and food deterioration has become an area of intense interest. The free radicals have a special affinity for lipids, proteins, and DNA [1]. A free radical is an atom, molecule, or compound that is highly unstable because of its atomic or molecular structure (i.e., the distribution of electrons within the molecule). This instability makes free radicals very reactive, and they attempt to pair up with other molecules, atoms, or even individual electrons to create a stable compound. To achieve a more stable state, free radicals can “steal” a hydrogen atom from another molecule, bind to another molecule, or interact in various ways with other free radicals.

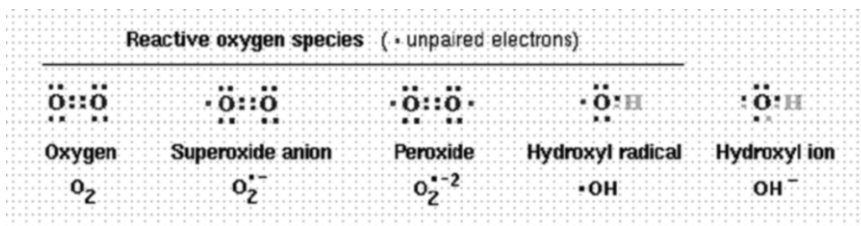
Free radicals can be defined as reactive chemical species having a single unpaired electron in an outer orbit and are continuously produced by the organism’s normal use of oxygen [2]. This unstable configuration creates energy that is released upon reaction with adjacent molecules, such as proteins, lipids, carbohydrates, and nucleic acids. The majority of free radicals that damage biological systems are derived from oxygen and more generally referred to as “reactive oxygen species.”

Oxygen ( $O_2$ ) is an essential element for cell function and life. It plays an important role in a series of biochemical reactions occurring in the respiratory chain, which is responsible for most of the production of adenosine triphosphate (ATP), which provides the energy required for a multitude of cellular reactions and functions. This process of

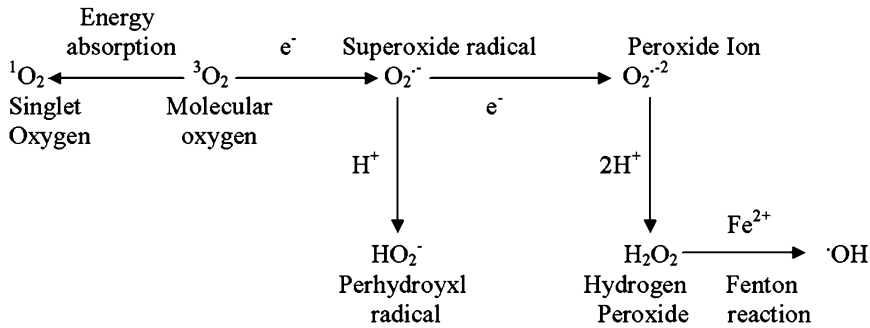
respiratory chain takes place in membrane-enclosed cell structures called mitochondria.

Molecular oxygen can accept a total of four electrons, one at a time, and the corresponding number of protons to generate two molecules of water. This process leads to the generation of different by-products that are generally ROS and reactive nitrogen species (RNS) [3]. Intracellular generation of ROS mainly comprises oxygen radicals, like superoxide ( $O_2^{\cdot-}$ ); peroxide ( $O_2^{\cdot\cdot}$ ), which normally exists in cells as hydrogen peroxide ( $H_2O_2$ ); and the hydroxyl radical ( $\cdot OH$ ) [4]. Superoxide, peroxide, and the hydroxyl radical are considered the primary ROS and have sparked major research on the role of free radicals in biology and medicine. Though only about 2–3% of the  $O_2$  consumed by the respiratory chain is converted to ROS [5], the toxic effects of oxygen in biological systems—such as the breakdown (i.e., oxidation) of lipids, inactivation of enzymes, introduction of changes (i.e., mutations) in the DNA, and destruction of cell membranes and, ultimately, cells—are attributable to the reduction of  $O_2$  to ROS [6–8].

The term ROS is often used to include not only the radicals  $\cdot OH$ ,  $RO_2\cdot$ ,  $NO\cdot$  and  $O_2\cdot^-$  but also the non-radicals  $HOCl$ ,  $^1O_2$ ,  $ONOO^-$ ,  $O_3$ , and  $H_2O_2$  [9]. However, the biological system undergoes damage mainly by radicals generated from oxygen. Oxygen has two unpaired electrons in separate orbitals in its outer shell. This electronic structure makes oxygen especially susceptible to radical formation. Sequential reduction of molecular oxygen (equivalent to sequential addition of electrons) leads to formation of a group of reactive oxygen species: superoxide anion, peroxide (hydrogen peroxide), and hydroxyl radical (Fig. 1).



**Fig. 1** Structure of reactive oxygen produced in biological systems derived from oxygen. Note the notation used to denote them and the difference between hydroxyl radical and hydroxyl ion, which is not a radical



**Fig. 2** Schematic representation of ROS generation. The single-electron reduction of  $\text{O}_2$  results in the generation of the  $\text{O}_2^{\cdot-}$ . At low pH, dismutation of  $\text{O}_2^{\cdot-}$  is unavoidable, with one  $\text{O}_2^{\cdot-}$  giving up its added electron to another  $\text{O}_2^{\cdot-}$  and then with protonation resulting in the generation of  $\text{H}_2\text{O}_2$ . Again,  $\text{O}_2^{\cdot-}$  can be protonated to form the  $\text{HO}_2^{\cdot}$ .

Additionally, in the presence of transition metals such as copper and iron, further reactions take place, e.g., through the Haber–Weiss mechanism or the Fenton reaction to give up  $\cdot\text{OH}$ .  $\text{O}_2^{\cdot-}$  can also react with another very influential signaling-free radical species,  $\text{NO}^{\cdot}$ , to give up peroxynitrite ( $\text{OONO}^-$ )

Another radical derived from oxygen is singlet oxygen, designated as  ${}^1\text{O}_2$  (Fig. 2). This is an excited form of oxygen in which one of the electrons jumps to a superior orbital following absorption of energy, thereby freeing oxygen from its spin-restricted state. Further, the singlet state can release a modest amount of energy and get transformed to a triplet state which involves change of electron spin.

## 2 Sources of ROS Generations

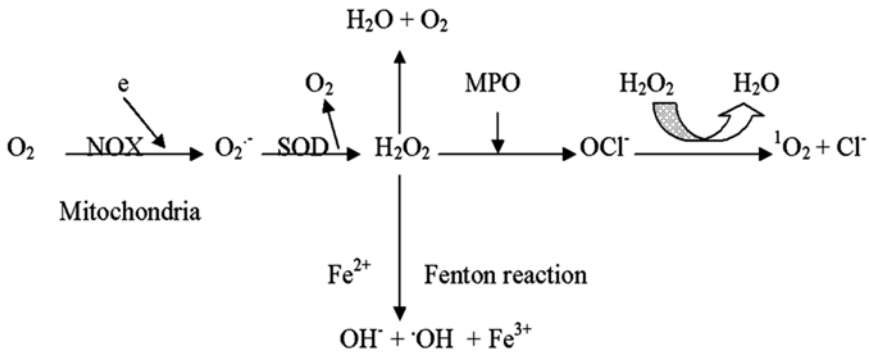
The amount of free radical production is determined by the balance of many factors, and ROS are produced both endogenously and exogenously. The endogenous sources of ROS include mitochondria, cytochrome P-450 metabolism, peroxisomes, and inflammatory cell activation [5]. In general, ROS can be (i) generated during UV light irradiation and by X-rays and gamma rays; (ii) produced during metal-catalyzed reactions; (iii) present in the atmosphere as pollutants; (iv) produced by neutrophils, eosinophils, and macrophages during inflammation; and (iv) by-products of mitochondrial-catalyzed electron transport reactions and various other mechanisms [3, 4].

There are numerous cellular systems that can produce ROS. The major source of ROS production in the cell is the mitochondrial respiratory

chain that utilizes approximately 80–90 % of the  $\text{O}_2$  a person consumes and generates most of the ROS produced in the body.

Another major source of ROS, especially in the liver, is a group of enzymes called the cytochrome P-450 mixed function oxidases. There are many variants of these iron-containing enzymes, some of which are responsible for removing or detoxifying a variety of compounds present in our environment and ingested (e.g., foods or drugs), including alcohol [10]. Some cytochrome P-450 enzymes also are important for metabolizing substances that naturally occur in the body, such as fatty acids, cholesterol, steroids, or bile acids [11]. The cytochrome P-450 molecules in their biochemical reactions catalyzed use  $\text{O}_2$ , and during these reactions small amounts of ROS are generated. The extent of ROS generated varies considerably depending on the compound to be degraded and on the cytochrome P-450 molecule involved. One type of cytochrome molecule that is especially active in producing ROS is known as CYP2E1, whose activity increases after heavy alcohol exposure [12].

ROS also are produced by a variety of oxidative enzymes present in cells, such as xanthine oxidase. Xanthine oxidase under normal physiological conditions acts as a dehydrogenase, wherein it removes hydrogen from xanthine or hypoxanthine and attaches it to NAD, thereby



**Fig. 3** Generation of ROS in neutrophils. The generation of superoxide ( $\text{O}_2^-$ ) from oxygen ( $\text{O}_2$ ) is mediated either by the NADPH oxidase complex (NOX) or in mitochondria by cytochrome c peroxidase or xanthine oxidase. Superoxide will be converted to hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) either spontaneously or mediated by superoxide dismutase (SOD). Hydrogen peroxide can be converted to

$\text{H}_2\text{O} + \text{O}_2$  in the presence of catalase and glutathione (GSH) peroxidase, or hydrogen peroxide can act as a source of hydroxyl radical ( $\cdot\text{OH}$ ) via the Fenton reaction. Myeloperoxidase (MPO) uses hydrogen peroxide as substrate for the formation of halogenated ROS such as hypochlorite ( $\text{OCl}^-$ ). Reaction of hypochlorite with hydrogen peroxide results in the formation of singlet oxygen ( ${}^1\text{O}_2$ )

generating NADH. However, under certain conditions, such as the disruption of blood flow to a tissue, xanthine dehydrogenase is converted to a ROS-producing oxidase form [13].

Other sources of ROS in the body are two types of immune cells called macrophages and neutrophils, which defend the body against invading microorganisms (Fig. 3). ROS production here is beneficial and even essential to the organism as it functions to destroy foreign pathogens [14]. Macrophages and neutrophils contain a group of enzymes called the NADPH oxidase complex, which, upon activation generates superoxide radicals and hydrogen peroxide. Hydrogen peroxide then interacts with chloride ions present in the cells to produce hypochlorite, which in turn destroys the pathogen. The NADPH oxidase complex and the resulting ROS production are critical to the body's defense against all kinds of diseases, as is evident in patients with a condition called chronic granulomatous disease, in which ROS production by the NADPH oxidase complex is drastically reduced. Patients with this condition are highly sensitive to infections and usually die at an early age [15].

Another peroxidase enzyme that is abundantly expressed in neutrophils is myeloperoxidase (MPO) (Fig. 3). MPO in the presence of heme as

a cofactor produces hypochlorous acid ( $\text{HOCl}$ ) from hydrogen peroxide and chloride anion (or the equivalent from a non-chlorine halide) [16]. It also oxidizes tyrosine to tyrosine radical in the presence of hydrogen peroxide. Both  $\text{HOCl}$  and tyrosine radical are cytotoxic and are used by the neutrophil to kill pathogenic organisms [17].

Humans are constantly exposed to environmental free radicals, including ROS, in the form of radiation, UV light, smog, tobacco smoke, and certain compounds called as redox-cycling agents, which include some pesticides as well as certain medications used for cancer treatment. The toxicity of these medications against tumor cells (as well as normal body cells) results because of their modification by cellular enzymes to an unstable intermediate that then reacts with molecular oxygen to produce the original product plus a superoxide radical. Thus, a vicious cycle of chemical reactions involving these compounds continually produces ROS [11].

### 3 Role of Metals

Earlier studies suggested the possibility that the superoxide radicals or hydrogen peroxide radicals in ROS production process interact with each

other to produce the most reactive ROS, the hydroxyl radical ( $\cdot\text{OH}$ ). Direct interaction between these two radicals under normal physiological conditions does not play a significant role in generating hydroxyl radicals. However, in the presence of certain metals, particularly free iron or copper ions, a sequence of two reaction steps can occur that results in hydroxyl radical generation. In the first step, hydrogen peroxide can produce the hydroxyl radical as the primary oxidant by removing an electron from the participating metal ion [18]. In the second step, the original metal ions are regenerated involving the superoxide radical ( $\text{O}_2^-$ ), so that they are again available for reaction with the hydrogen peroxide. This combination of two chemical reactions appears to account for most of the hydroxyl radical production in biological systems and supports the role of metals such as iron and copper to produce oxidative stress and ROS-induced injury in cells. Because of iron's critical contribution to hydroxyl radical formation, anything that increases the levels of free iron in the cells promotes ROS generation and oxidative stress [19, 20].

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#### 4 Oxidative Stress

The formation and removal of free radicals are balanced in a normal cell. However, with more formation of free radicals or when levels of antioxidants are diminished, the cell enters a state called as "oxidative stress." This state if prolonged can cause cell damage and death. Oxidative stress plays a major role in the development of chronic and degenerative diseases such as cancer, arthritis, aging, autoimmune disorders, and cardiovascular and neurodegenerative diseases [21]. In mammalian cells, oxidative stress leads to increase in intracellular levels of free  $\text{Ca}^{2+}$  and iron, and excessive rises in intracellular free  $\text{Ca}^{2+}$  may cause DNA fragmentation by activating endonucleases [22]. Endonuclease activation results in single- and double-strand DNA breaks that subsequently lead to increased levels of nuclear proteins

which are essential in DNA repair, p53, and poly-ADP ribosylation. Intranuclear  $\text{Ca}^{2+}$  fluctuations can also affect chromatin organization, induce gene expression, and influence protease and protein kinase activities. The induced kinases like MAP kinase and calmodulin kinases are involved in the activation of transcription factors that initiate transcription of early response genes, for example, c-fos and c-jun, and also activation of phospholipase  $\text{A}_2$  which results in permeabilization of the plasma membrane and of intracellular membranes such as the inner membrane of mitochondria leading to apoptosis [23].

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#### 5 Antioxidants and Prevention of Oxidative Damage

Uncontrolled generation of ROS can lead to their accumulation causing oxidative stress in the cells. The human body has several mechanisms to counteract oxidative stress by producing antioxidants which are either naturally produced in the body or externally supplied through foods and/or supplements. Antioxidants are those molecules that are present at low concentrations and significantly delays or prevent oxidation of the oxidizable substrate [24]. Endogenous and exogenous antioxidants are effective as free radical scavengers by donating their own electrons to ROS and thereby neutralize adverse effects of the latter [4]. Thus, they can enhance the immune defense and lower the risk of cancer and degenerative diseases [25]. In general, an antioxidant in the body may work at three different levels: (a) **prevention**, maintains formation of reactive species to a minimum level, e.g., desferrioxamine; (b) **interception**, scavenges reactive species either by using catalytic and non-catalytic molecules, e.g., ascorbic acid and alpha-tocopherol; and (c) **repair**, repairs damaged target molecules, e.g., glutathione [23].

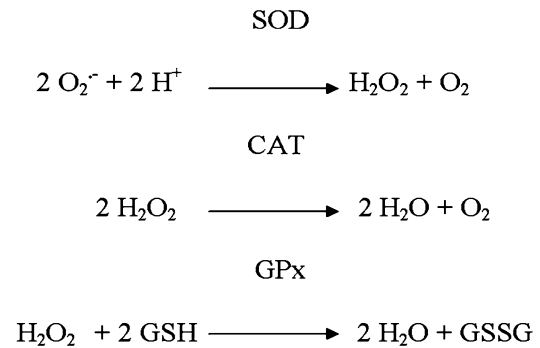
The antioxidant systems are classified into two major groups, protective or enzymatic antioxidants and nonenzymatic antioxidants.

## 6 Enzymatic Antioxidants

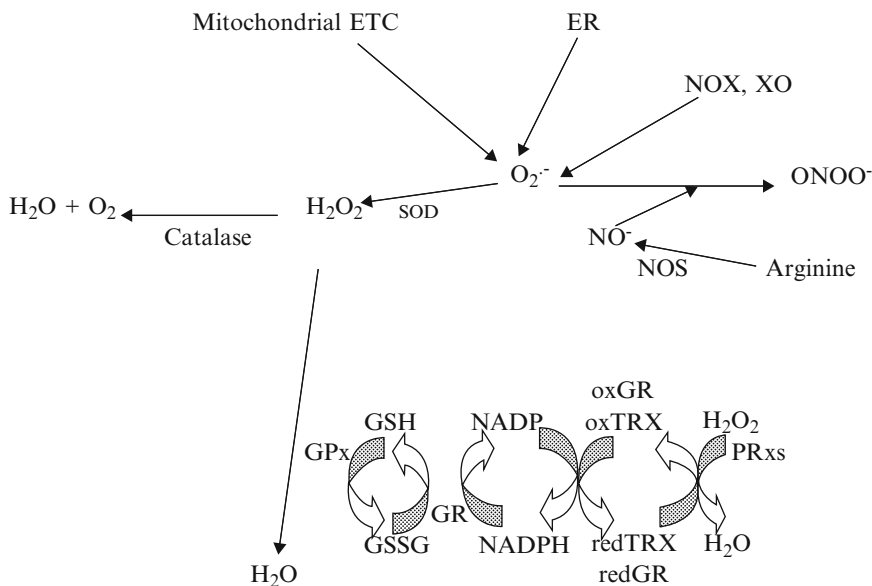
Enzymatic antioxidants involved in the elimination of ROS include superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) (Figs. 4 and 5) [26]. There are different types of SODs that are differentially located in the mammalian cells and requirement of metal ions as cofactors for their function. For example, a copper-zinc SOD is present in the cytosol and in the space between two membranes surrounding mitochondria. Again, manganese-containing SOD is present in the matrix of mitochondria [27].

The enzymes, catalase, and glutathione peroxidase help to remove hydrogen peroxide. Catalase is an iron-containing enzyme found primarily in the small membrane-enclosed cell components called peroxisomes and detoxifies hydrogen peroxide by catalyzing a reaction

between two hydrogen peroxide molecules, resulting in the formation of water and O<sub>2</sub>. In addition, catalase can promote the interaction of hydrogen peroxide with compounds that can



**Fig. 4** The antioxidant enzymes and the reactions they catalyze. Note the notations used: *SOD* for superoxide dismutase, *CAT* for catalase, *GPx* for glutathione peroxidase, *GSH* for reduced glutathione (monomeric glutathione), *GSSG* for oxidized glutathione (glutathione disulfide)



**Fig. 5** Reactions of enzymatic antioxidants. Major sites of cellular ROS generation include mitochondrial electron transport chain (ETC), endoplasmic reticulum system (ER system), and NAD(P)H oxidase (NOX) complex. Nitric oxide synthases (NOS) are key enzymes for production of NO. GSH and NADPH play roles in maintaining reduced cellular state. Symbols used: *GPx*, glutathione peroxidase;

*GR*, glutathione reductase; *oxTRX*, oxidized thioredoxin; *redTRX*, reduced thioredoxin; *oxGR*, oxidized glutaredoxin; *redGR*, reduced glutaredoxin; *H<sub>2</sub>O<sub>2</sub>*, hydrogen peroxide; *NO•*, nitric oxide; *ONOO•*, peroxynitrite; *SOD*, superoxide dismutase; *GSH*, reduced glutathione; *GSSG*, oxidized glutathione; *NADPH*, reduced nicotinamide adenine dinucleotide phosphate; *XO*, xanthine oxidase; *PRxs*, peroxiredoxins

serve as hydrogen donors so that the hydrogen peroxide can be converted to one molecule of water, and the reduced donor becomes oxidized (a process sometimes called the peroxidatic activity of catalase) [28].

The glutathione peroxidase system consists of several components, including the enzymes glutathione peroxidase and glutathione reductase and the cofactors monomeric glutathione (GSH) and reduced nicotinamide adenosine dinucleotide phosphate (NADPH) [29]. Glutathione peroxidase contains an amino acid that is modified by addition of a molecule of the metal selenium; therefore, low amounts of selenium are critical for the body's antioxidant defense. Together, these molecules effectively remove hydrogen peroxide. GSH serves as a cofactor for the enzyme glutathione transferase, which helps to remove certain drugs and chemicals as well as other reactive molecules from the cells. GSH can also interact directly with certain ROS (e.g., the hydroxyl radical) to detoxify them, as well as performing other critical activities in the cell [4].

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## 7 Nonenzymatic Antioxidants

GSH due to its functions is probably the most important antioxidant present in cells. Therefore, enzymes that generate GSH are critical for the body's ability to protect itself against oxidative stress. NADPH is involved in a much more diverse range of reactions in the cell than GSH, and due to its role played in the glutathione peroxidase system, NADPH or the enzymes that generate this compound are sometimes considered antioxidants [30].

In addition to GSH and NADPH, numerous other nonenzymatic antioxidants are present in the cells, most prominently vitamin E ( $\alpha$ -tocopherol) and vitamin C (ascorbic acid). Vitamin E is a major antioxidant found in the lipid phase of membranes and, like other chemically related molecules, acts as a powerful terminator of lipid peroxidation. During the reaction between vitamin E and a lipid radical, the vitamin E radical is formed, from which vitamin E can be

regenerated in a reaction involving GSH and ascorbate (Fig. 6) [31].

Ascorbic acid can exert its antioxidant activity both directly and also in concert with vitamin E and glutathione. The hydrogen atoms produced in the oxidation of ascorbic acid first to ascorbyl radical and then to dehydroascorbate react with a polyunsaturated fatty acid and/or phospholipid peroxy radical (PUFA-OO $\cdot$ ) to form the non-radical product, PUFA-OOH. Trapping of PUFA-OO $\cdot$  prevents this radical from attacking polyunsaturated fatty acid side chains and other lipoproteins in the plasma membrane and thus breaks the chain reaction of lipid peroxidation. Dehydroascorbic acid is reduced back to ascorbic acid by glutathione and enzymatic reduction. Ascorbic acid also helps  $\alpha$ -tocopherol in trapping aqueous radicals.  $\alpha$ -Tocopherol also traps peroxy radicals before they propagate radical chain reactions leading to lipid peroxidation and regenerate  $\alpha$ -tocopherol by reducing the  $\alpha$ -tocopheroxy radical produced in this trapping reaction (Fig. 6) [32].

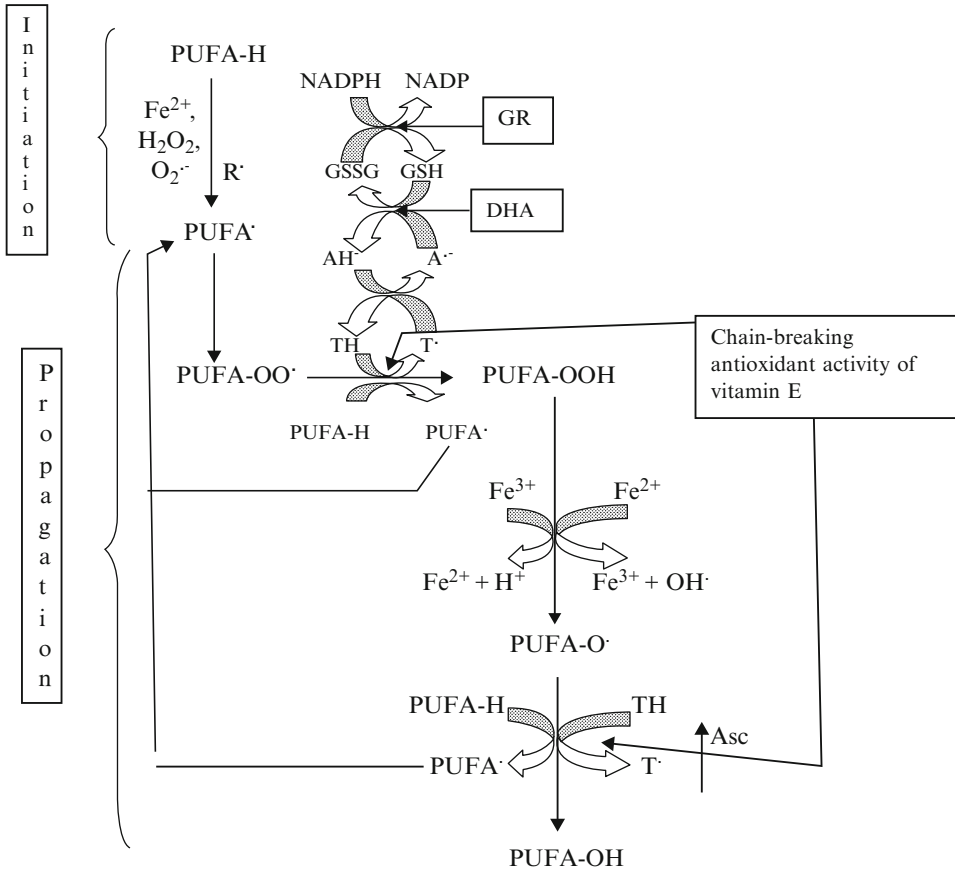
Under certain conditions, ascorbic acid may work as a prooxidant rather than as an antioxidant by reducing transition metal ions, which in turn drives the Fenton reaction potentially resulting in oxidative stress. A few ions of transition metal elements have redox transitions with potentials of a magnitude that allows the catalytic decomposition of hydroperoxides. The redox couples of most importance to biological systems are Cu $^+$ /Cu $^{2+}$  and Fe $^{2+}$ /Fe $^{3+}$ . The one electron redox cycle results in the formation of peroxy and alkoxy radicals, the latter of which can rearrange and react with oxygen to form peroxy radicals [33].

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## 8 ROS Toxicity

ROS may be toxic to cells because they can react with most cellular macromolecules, including proteins, lipids, and DNA and generate different types of secondary radicals like lipid radicals, sugar- and base-derived radicals, amino acid radicals, and thiol radicals. These radicals in the presence of oxygen are converted to peroxy radicals, which can induce chain reactions [34]. The





**Fig. 6** Main peroxidative reaction on PDFA.  $\alpha$ -Tocopherol (TH, Vitamin E) efficiently transfers a H-atom to a lipid free radical peroxy (PUFA-OO $\cdot$ ), alkoxy (PUFA-O $\cdot$ ), and carbon-centered (PUFA $\cdot$ ) radicals giving the corresponding nonradical product of PUFA (PUFA-OOH, PUFA-OH, and PUFA-H) and an  $\alpha$ -Tocopheroxyl radical (T). T once formed reacts with the second free radical (PUFA-OO $\cdot$ , PUFA-O $\cdot$ , and PUFA $\cdot$ ), or each other to make a nonradical product (T-OOL, T-OL, or T-T). Symbols used: R, a radical;

O<sub>2</sub><sup>-</sup>, superoxide anion; PUFA-H, polyunsaturated fatty acid; PUFA $\cdot$  carbon-centered PUFA radical (lipid alkyl radical); PUFA-OO $\cdot$  hydroperoxyl radical; PUFA-OO $\cdot$ , hydroperoxide; PUFA-O $\cdot$ , alkoxy radical; PUFA-OH, hydroxylated PUFA; AH, Vitamin C or ascorbic acid; ZA<sup>-</sup>, ascorbyl radical/Ascorbate; DHA, dehydroascorbate; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione

biological implication of such reactions primarily depends on several factors like site of generation, nature of the substrate, reactivation mechanisms, and redox status [25, 31].

The ROS-induced oxidation of proteins can lead to changes in the proteins' three-dimensional structure as well as fragmentation, aggregation, or cross-linking of the proteins [35]. The side chains of all amino acid residues of proteins, in particular tryptophan, cysteine, and methionine residues, are susceptible to ROS oxidation. Protein oxidation products are usually carbonyls such as aldehydes and ketones. Finally, protein

oxidation often will make the marked protein more susceptible to degradation by cellular systems responsible for eliminating damaged proteins from the cell [36].

Membranes that surround the cells as well as other cellular structures, such as the nucleus and mitochondria, contain high concentration of unsaturated fatty acids in their lipid components. The complete degradation (i.e., peroxidation) of membrane lipids is a hallmark of oxidative damage [35] and can result in the formation of lipid hydroperoxide (PUFA-OOH) which can further breakdown to aldehydes such as malonaldehyde



and 4-hydroxynonenal (4-HNE) or form cyclic endoperoxide, isoprotans, and hydrocarbons. The lipid peroxidation can lead to cross-linking of membrane proteins, change in membrane fluidity, and formation of lipid–protein and lipid–DNA adducts which may be detrimental to cell functions [31].

DNA is the cell's genetic material, and any permanent damage to the DNA can result in malfunctions of the proteins or complete inactivation of the affected proteins. Thus it is essential for the viability of individual cells or even the entire organism that the DNA remains intact. ROS are a major source of DNA damage, such as modification of DNA bases, single- and double-strand DNA breaks, loss of purines (apurinic sites), damage to the deoxyribose sugar, DNA–protein cross-linkage, and damage to the DNA repair system [37]. Of the reactive oxygen species, hydroxyl ( $\cdot\text{OH}$ ) radical is one of the potential inducers of DNA damage. A variety of adducts are formed on reaction of  $\cdot\text{OH}$  radical with DNA. The  $\cdot\text{OH}$  radical can attack purine and pyrimidine bases to form  $\cdot\text{OH}$  radical adducts, which are both oxidizing and reducing in nature which in turn can induce base modifications and sometimes release of bases. Some of the important base modifications include 8-hydroxydeoxyguanosine (8-OHdG), 8 (or 4-, 5-)-hydroxyadenine, thymine peroxide, thymine glycols, and 5-(hydroxymethyl) uracil [38]. Free radicals can also attack the sugar moiety to produce sugar peroxy radicals leading to strand breakage. Although cells have developed repair mechanisms to correct naturally occurring changes in the DNA, additional or excessive changes caused by ROS or other agents can lead to permanent changes or damage to the DNA with potentially detrimental effects like cell death, mutagenesis, carcinogenesis, and aging of the cell.

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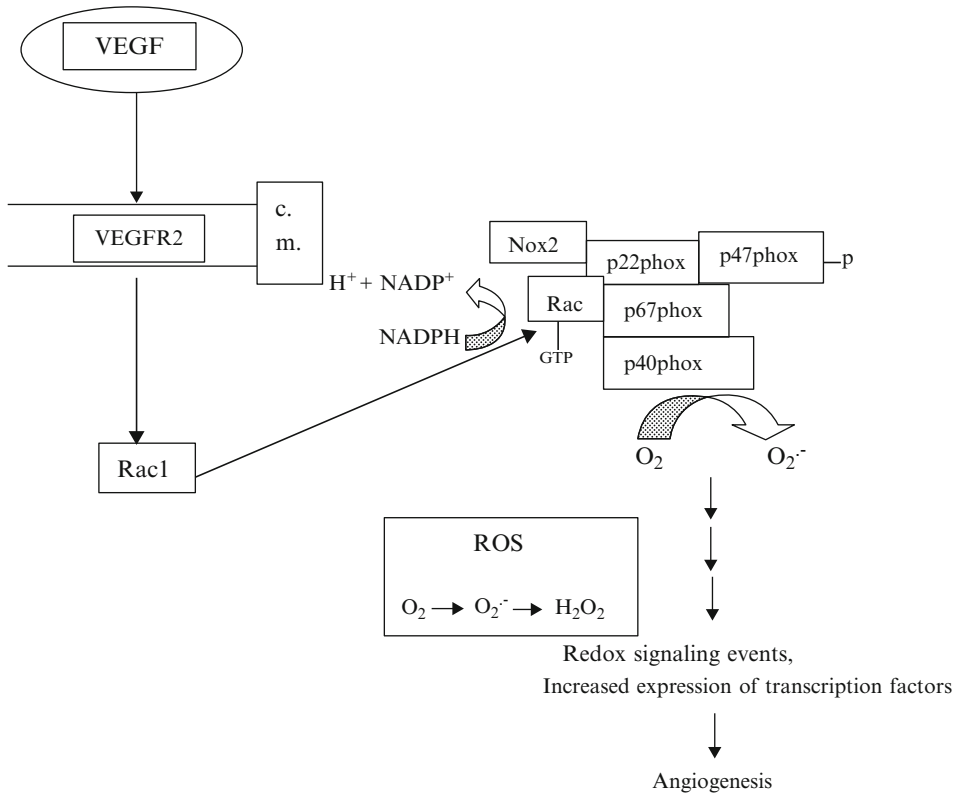
## 9 ROS in Normal Physiology

ROS act as stimulating agents for biochemical processes within the cell. It plays a role in low concentration for normal physiological functions like gene expression, cellular growth, and defense against infection by induction of transcription factors such as nuclear factor-kappa B (NF- $\kappa$ B)

and activator protein-1 (AP-1) and activation of signal transduction pathways [39, 40]. A particular example of signal transduction molecules activated by ROS is the mitogen-activated protein kinases (MAPKs).

Research studies show that ROS molecules modulate multiple redox-sensitive intracellular signaling pathways in mammalian cells generated from NADPH oxidase (Nox), which include inhibition of protein tyrosine phosphatases, activation of certain redox-sensitive transcription factors, and modulation of the functions of some ion channels [41]. A prominent feature of Nox/ROS-mediated signaling is the heterogeneity of its activating stimuli, and at the cellular level, Nox can be activated by a large collection of chemical, physical, environmental, and biological factors [42]. For example, growth factor-mediated responses in endothelial cells (EC) leading to angiogenesis are signaled by ROS such as superoxide and  $\text{H}_2\text{O}_2$ . The Nox acts as major source of ROS in endothelial cells, which consists of Nox2 (gp91phox) and the homologues Nox1 and Nox4 with p22phox, p47phox, p67phox, and the small G protein Rac1 as subunits {"phox" stands for phagocytic oxidase and "Rac" stands for Rho-related C3 botulinum toxin substrate}. Vascular endothelial growth factor (VEGF), a key angiogenic growth factor along with angiopoietin-1, activates the endothelial Nox. Consequently, ROS derived from this Nox stimulate diverse redox-signaling pathways leading to angiogenesis-related gene induction as well as EC migration and proliferation that may contribute to postnatal angiogenesis in vivo (Fig. 7) [43].

ROS also appear to serve as secondary messengers in many developmental stages; for example, in sea urchins ROS levels are elevated during fertilization. Also, prenatal and embryonic development in mammals has also been suggested to be regulated by ROS [44]. Apart from these, ROS also participate in the biosynthesis of molecules such as thyroxine and prostaglandin that accelerate developmental processes [3]. In thyroid cells, regulation of  $\text{H}_2\text{O}_2$  concentration is critical for thyroxine synthesis, as it is needed to catalyze the binding of iodine atoms to thyroglobulin [45]. It is noteworthy that ROS are also used by the immune system, like ROS have been shown to



**Fig. 7** Schematic role of ROS derived from NADPH oxidase in angiogenesis binding of vascular endothelial growth factor (VEGF) to VEGF receptor VEGFR2 stimulates ROS ( $O_2^-$ ,  $H_2O_2$ ) production via Rac1-mediated NADPH oxidase activation. NADPH oxidase enzyme complex in endothelial cells consists of a flavocytochrome b558 reductase composed of a “Nox2” catalytic subunit bound to a smaller subunit, p22phox (maturation and stabilization partner); regulatory subunits including the cyto-

solic organizer proteins, p47phox and p40phox; cytosolic activator proteins, p67phox; and the small molecular weight G protein Rac1. The enzyme catalyzes transfer of electrons from NADPH to molecular oxygen to form  $O_2^-$  across the membrane, which in turn can be spontaneously or catalytically converted to  $H_2O_2$ . The ROS generated induces downstream signaling events which converge and integrate to induce angiogenesis

trigger proliferation of T cells through NF- $\kappa$ B activation. Macrophages and neutrophils generate ROS in order to kill the bacteria that they engulf by phagocytosis. Furthermore, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) mediates the cytotoxicity of tumor and virus-infected cells through ROS generation and induction of apoptosis [14].

## 10 Diseases Involving Excessive ROS Levels

Overproduction of ROS generates oxidative stress which reflects an imbalance between the systemic manifestation of reactive oxygen species and a

biological system’s ability to readily detoxify the reactive intermediates or to repair the resulting damage. Disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals [45]. Excess of free radicals damages essential macromolecules of the cell, leading to abnormal gene expression, disturbance in receptor activity, proliferation or cell dynamics, immunity perturbation, mutagenesis, and protein deposition and damages all components of the cell, including proteins, lipids, and DNA [46]. ROS has been implicated in many major diseases that plague humans [44]. Some of these noteworthy diseases due to oxidative stress-induced disruptions in normal mechanisms

of cellular signaling include cancer [47, 48], inflammatory diseases [49–51], cardiovascular diseases [52, 53], respiratory diseases [54], diabetes [55, 56], male infertility [57], aging process [58, 59], neurological diseases [59–61], etc.

## 11 Antioxidants and Their Supplementation

Antioxidants are the substances that may protect cells from the damage caused by free radicals. Antioxidants interact with and stabilize free radicals and may prevent some of the damage that free radicals might otherwise cause. However, these antioxidants whenever are consumed in large doses can act as prooxidants [28]. The antioxidants depending on their source of availability can be endogenous or exogenous in nature. The endogenous antioxidants can either be enzymatic, like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GRx) [26], or nonenzymatic in nature. The nonenzymatic antioxidants can be further grouped to metabolic antioxidants, such as lipoic acid, glutathione, L-arginine, uric acid, bilirubin [62], and nutrient antioxidants. Some of the nutrient antioxidant can be exogenous in nature as they cannot be produced in the body and must be provided through foods such as vitamin E, vitamin C, carotenoids, and trace elements (Se, Cu, Zn, Mn) [63–65].

## 12 Conclusion

Free radical formation is a continuous process in humans and can be involved in development of diseases. Although cells are rich in antioxidant enzymes as well as small antioxidant molecules, these agents may not be sufficient enough to normalize the redox status during oxidative stress, and there is a need of antioxidant supplementation. The health benefits of administering antioxidants such as vitamins E and C or other compounds are the subject of current research, and clinical trials employing antioxidants in the treatment of various conditions are under way.

However, there are many unsolved questions about antioxidant supplementation in disease prevention. This throws the future direction for research if this supplementation can be recommended as an adjuvant therapy.

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