ENRIQUE CADENAS
PSC 616

FREE RADICALS, OXIDATIVE STRESS, AND DISEASES

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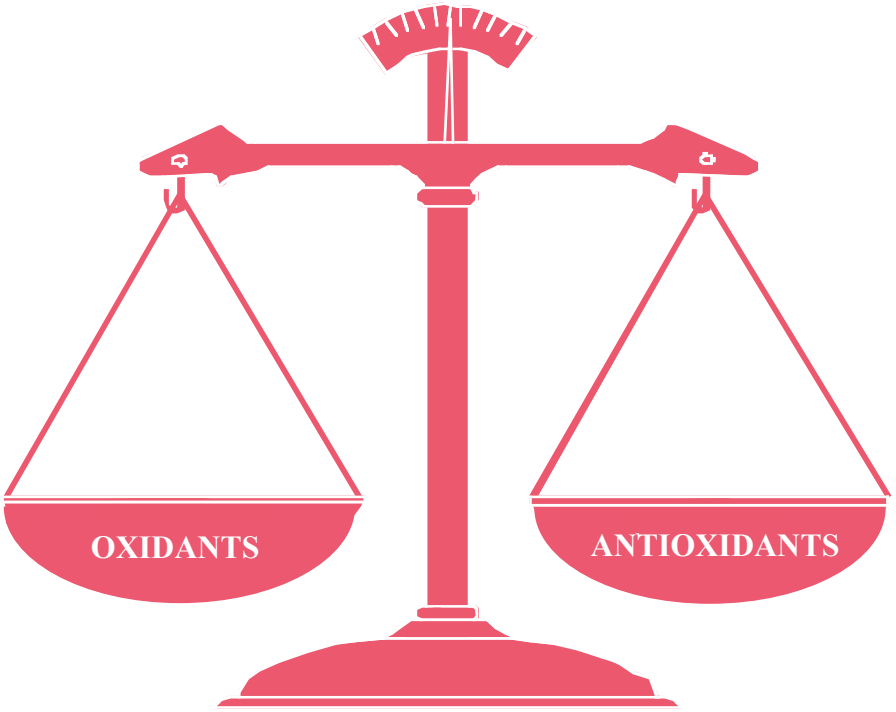
FREE RADICALS, OXIDATIVE STRESS, AND DISEASES

GENERAL INTRODUCTION

The ***formation of free radicals*** or oxidants is a well-established physiological event in aerobic cells, which convene enzymic and nonenzymic resources, known as ***antioxidant defenses***, to remove these oxidizing species. An imbalance between oxidants and antioxidants, the two terms of the equation that defines ***oxidative stress***, and the consequent damage to cell molecules constitutes the basic tenet of several pathophysiological states, such as neurodegeneration, cancer, mutagenesis, cardiovascular diseases, and aging.



INVOLVEMENT OF FREE RADICALS OR OXIDANTS IN DISEASES

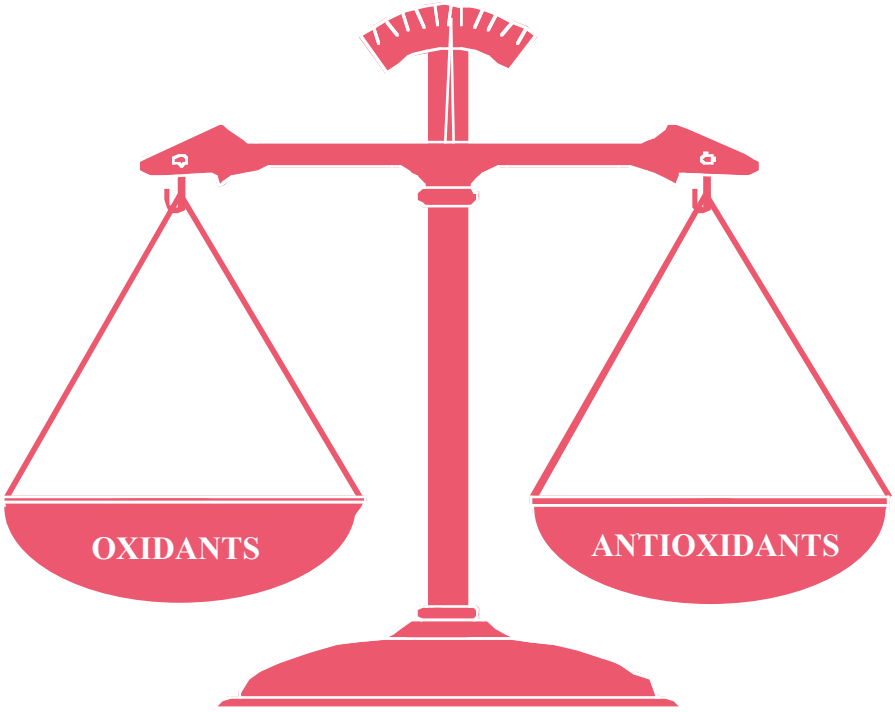
Oxygen is a relatively unreactive compound that can be metabolized *in vivo* to form highly reactive oxidants known as **oxygen free radicals**.

Increasing evidence suggests that the generation of these **oxygen free radicals** plays an important role in the pathophysiology of at least three disease states: *ischemia reperfusion injury, phagocyte-dependent inflammatory damage*, and *neurodegenerative disorders* as well as *aging*.

Other clinical conditions in which the involvement of oxygen radicals has been suggested are:

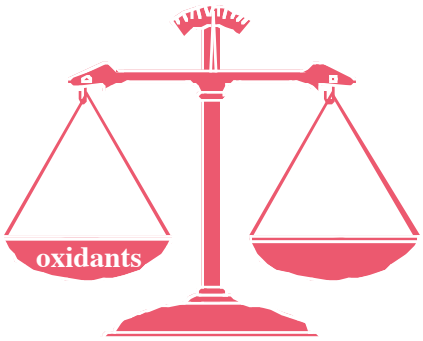
- **Skin**
  - porphyria
  - solar radiation
- **Eye**
  - cataract
  - retrolental fibroplasia
- **Cardiovascular system**
  - Keshan disease (selenium deficiency)
  - atherosclerosis
  - adriamycin cardiotoxicity)
- **Brain**
  - Parkinson's disease
  - Alzheimer's disease
  - Multiple sclerosis
  - neurotoxins
- **Inflammatory - immune injury**
  - rheumatoid arthritis
  - autoimmune diseases
- **Ischemia-reperfusion states**
  - myocardial infarction / stroke
  - organ transplantation
  - frostbite
- **Red blood cells**
  - hemolytic anemia
  - protoporphyrin photooxidation
  - lead poisoning
  - phenylhydrazine toxicity
  - primaquine and related drugs
- **Lung**
  - emphysema
  - bleomycin toxicity
  - paraquat toxicity
  - asbestos carcinogenicity

In order to evaluate the participation of oxygen free radicals in different toxicological, physiological, and pathological states, the following questions need to be considered:



1 What are oxygen radicals?
2 How reactive are oxygen radicals?
3 How are they generated in the cell?
4 How do they mediate cellular damage?
5 How do cells protect themselves against oxygen radicals?

1. WHAT ARE OXYGEN RADICALS OR REACTIVE OXYGEN SPECIES?

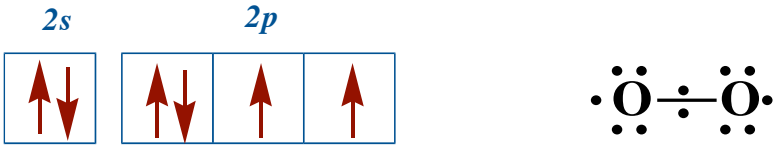


1. What are oxygen radicals
or reactive oxygen species?

The ability of oxygen metabolites to react with molecules that comprise cellular structures demands an understanding of the chemistry, biochemistry, and cell biology of oxygen in order to develop insight into the pathophysiology of oxygen in important processes, such as microcirculation, neurodegenerative diseases, and inflammation.

1.1. Definition of Free Radicals

A free radical is defined as any species that contains one or more *unpaired electron* occupying an atomic or molecular orbital by itself. The box diagram configuration for O_2 shows that in itself oxygen a diradical, because it possesses *two unpaired electrons*; the Lewis dot diagram on the right shows also the diradical character of molecular oxygen:



1.2. Mechanisms of Formation of Oxygen Free Radicals

Oxygen radicals or reactive oxygen species may be generated by

- **electron-transfer reactions**, and
- **energy-transfer reactions**.

Both types of reactions are important in a biological milieu and account partly for different types of cellular injury and toxicity.

The reactive oxygen species (not all of them are free radicals) generated by either process outlined above are:

• **electron–transfer reactions**	• **energy–transfer reactions**
- superoxide anion radical	- singlet oxygen
- hydrogen peroxide	- triplet carbonyl compounds
- hydroxyl radicals	
- lipid alkoxyl and peroxyl radicals	

1.3. Formation of Oxidants by Electron Transfer Reactions

The following scheme illustrates the sequential univalent reduction of oxygen to water with formation of different intermediates: superoxide anion radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($HO^{\bullet}$):

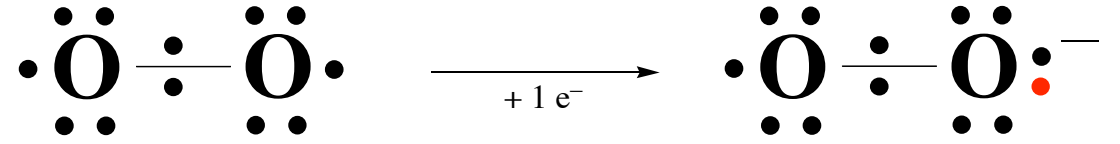
$$O_2 \longrightarrow O_2^{\bullet -} \longrightarrow H_2O_2 \longrightarrow HO^{\bullet} \longrightarrow H_2O$$

Molecular oxygen — superoxide anion — hydrogen peroxide — hydroxyl radical — water

1.3.a. Superoxide anion radical

The addition of one electron to molecular oxygen results in the formation of superoxide anion radical ($O_2^{\bullet -}$)

$$O_2 \xrightarrow[+\ 1\ e^-]{} O_2^{\bullet -}$$



molecular oxygen — superoxide anion

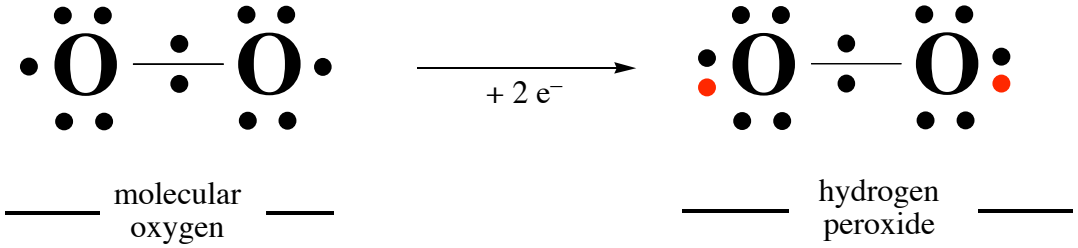
Superoxide anion radical is not a very reactive species and its chemical reactivity will depend on its site of generation in the cell, the possibility of being protonated to a stronger oxidant (perhydroxyl radical), and collision with suitable substrates.

1.3.b. Hydrogen peroxide

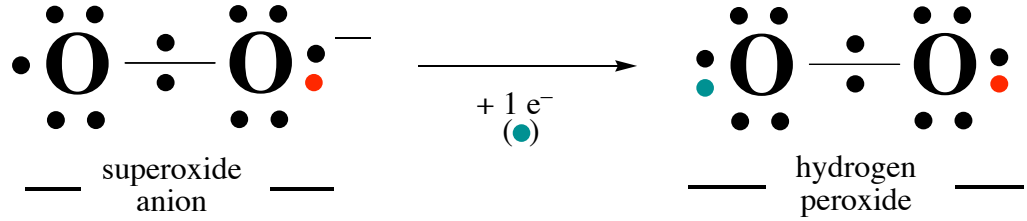
Hydrogen peroxide (H_2O_2) can be formed upon two-electron reduction of molecular oxygen or one-electron reduction of superoxide anion ($O_2^{\bullet -}$):

- *Two-electron reduction of molecular oxygen*

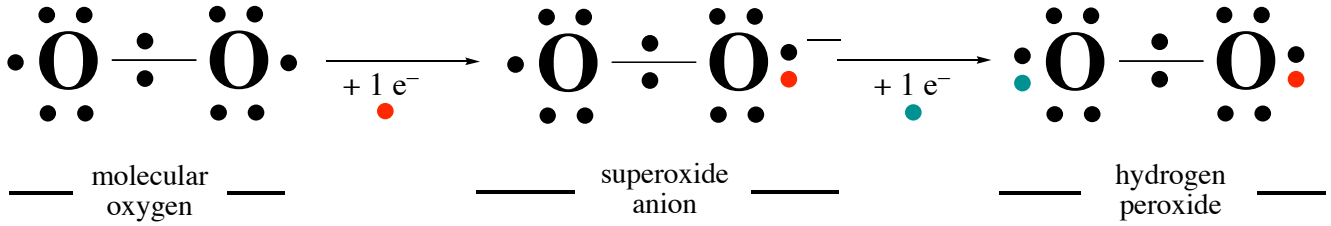
$$O_2 \xrightarrow[+\ 2\ e^-]{} H_2O_2$$



- *One-electron reduction of superoxide anion*

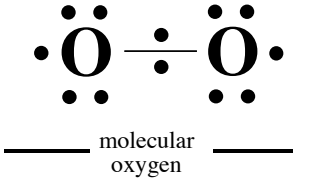


Therefore, the univalent reduction of molecular oxygen to hydrogen peroxide (H_2O_2) encompasses superoxide anion ($O_2^{\bullet -}$) as an intermediate:



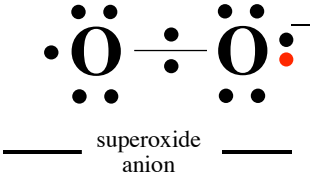
Remember the definition of a free radical as a species that contains one or more *unpaired electron* occupying an atomic or molecular orbital by itself. Therefore,

Lewis dot diagram



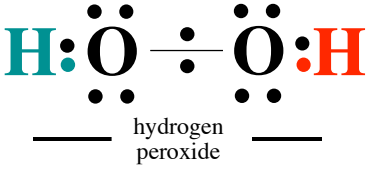
molecular oxygen

Molecular oxygen has two *unpaired electrons*. Hence, it is a *radical*



superoxide anion

Superoxide anion has one *unpaired electron* Hence, it is a *radical*

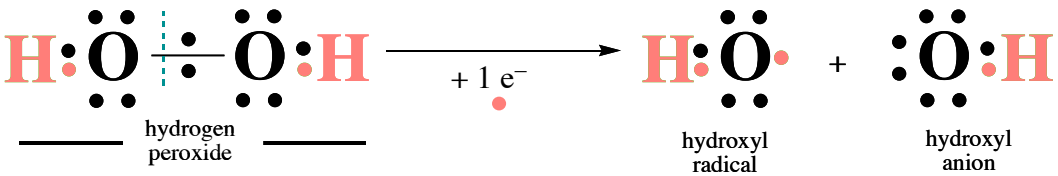


hydrogen peroxide

Hydrogen peroxide has no *unpaired electrons* Hence, it is not a radical

1.3.c. Hydroxyl radical

Hydroxyl radical ($HO^{\bullet}$) is the most reactive oxygen species originating from a reaction between superoxide anion radical ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2). Chemically, one-electron reduction of hydrogen peroxide yields **hydroxyl radical** and water (hydroxyl anion).



hydrogen peroxide — hydroxyl radical + hydroxyl anion

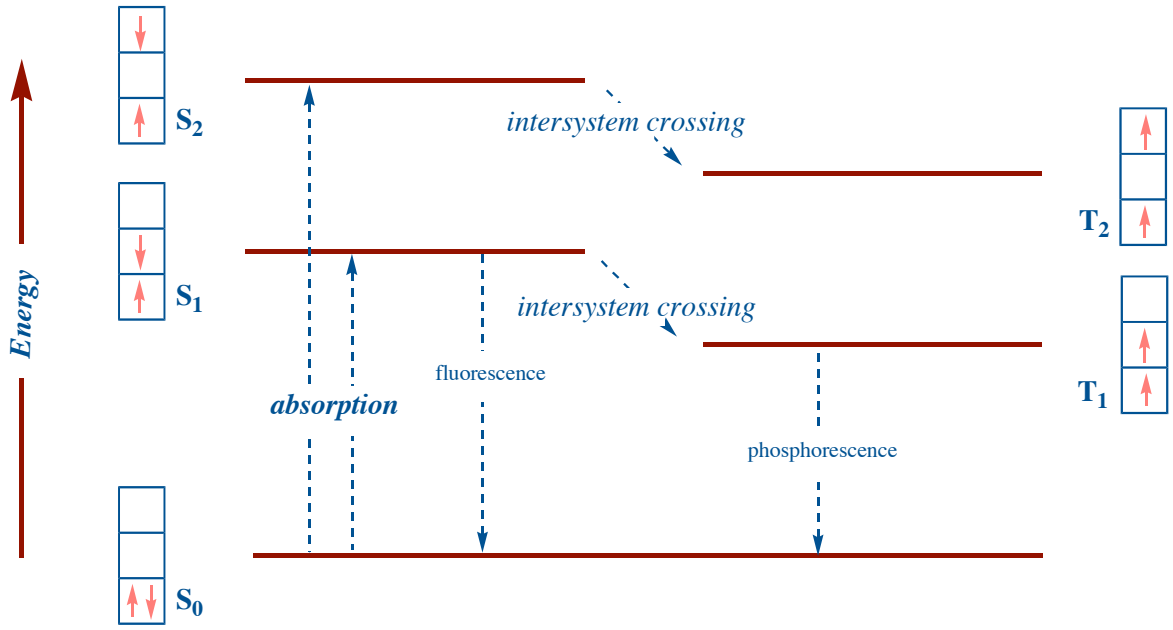
1.4. Formation of Oxidants by Energy Transfer Reactions

Transfer of energy from an excited sensitizer to ground state molecular oxygen is known as *photosensitization*. The sensitizer (S) absorbs energy upon irradiation and transfers it to molecular oxygen (O_2) with formation of singlet oxygen ($^1O_2$):

$$S + h\upsilon \rightarrow S^*$$
$$S^* + O_2 \rightarrow S + {}^1O_2$$

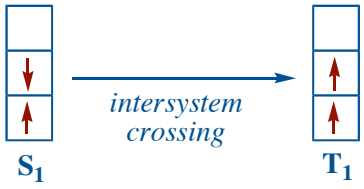
Examples of sensitizers are methylene blue, rose bengal, acridine orange, and several biological molecules, such as riboflavin, bilirubin, retinal, porphyrins, chlorophylls, etc.

Absorption of energy by a sensitizer in the ground state (S_0) is associated with promotion of an electron to the next energy level (box diagram), thereby yielding the excited state of the sensitizer (S_1). Depending on the amount of energy absorbed, the singlet states may be S_1, S_2, etc.

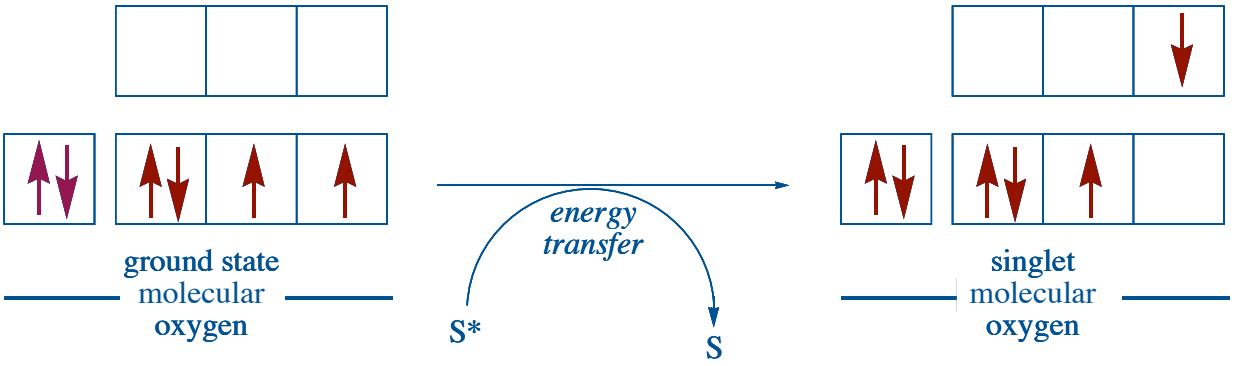


The electron promoted to the next energy level in a singlet state has a different spin. That promoted to the next energy level in a triplet state has the same spin.

Singlet states can release a modest amount of energy and be transformed into triplet states (which involves change of the electron spin). The process is known as intersystem crossing:



Molecular oxygen in the ground state is a triplet (in the ***p2*** boxes, two electrons have the same spin). Energy transferred to ground state molecular oxygen from an excited sensitizer is used to promote the electron to the next energy level as well as a change of spin, thereby the name *singlet oxygen*.



2. How Reactive Are Oxygen Radicals?

2. How reactive are oxygen radicals?

The chemical reactivity of a variety of reactive species, whether of free radical character or not, varies substantially; regardless of their source, it could be stated that in an appropriate setting virtually all cell components –lipids, nucleic acids, proteins, and carbohydrates– are sensitive to damage by reactive species (encompassing oxygen-, nitrogen-, carbon-, and sulfur-centered radicals).

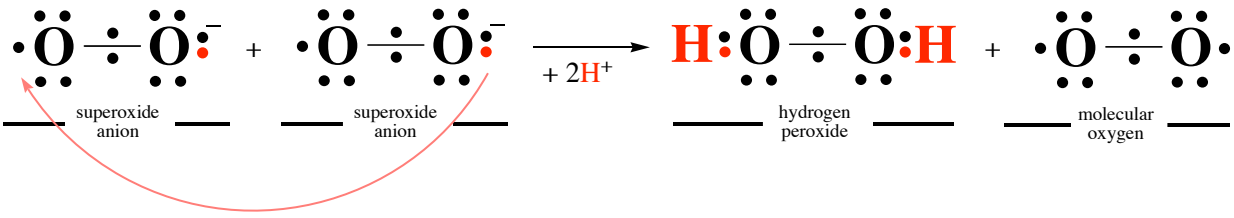
2.1. Reactivity of Superoxide Anion

The reactivity of superoxide radical is dependent on the cellular environment. Two reactions are important in a cellular setting, which change the chemical reactivity of superoxide anion ($O_2^{\bullet -}$):

2.1.a. Reactivity of superoxide anion with itself

Superoxide anion ($O_2^{\bullet -}$) is short lived and tends to react with itself, a process known as *dismutation* or *disproportionation* and which yields molecular oxygen and hydrogen peroxide (H_2O_2).

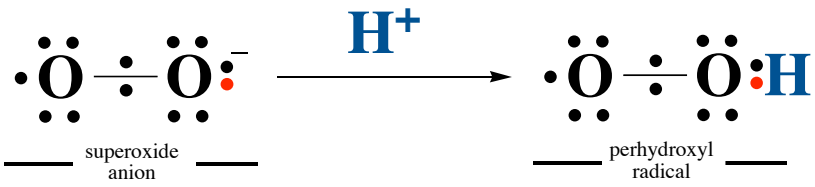
$$O_2^{\bullet -} + O_2^{\bullet -} + 2H^+ \rightarrow H_2O_2 + O_2$$



2.1.b. Protonation of superoxide anion

This process may take place in the vicinity of membranes (with an increased proton gradient). The *protonation* of superoxide anion ($O_2^{\cdot -}$) yields *perhydroxyl radical* ($HO_2^{\cdot}$), which is an extremely reactive species.

$$O_2^{\cdot -} + H^+ \rightarrow HO_2^{\cdot}$$



Hence, the above two process are important to understand the chemical reactivity of superoxide anion radical in cells:

- its *dismutation* yields non-radical products: oxygen and hydrogen peroxide (H_2O_2), thereby decreasing the reactivity of superoxide radical
- its *protonation* increases the reactivity by generating perhydroxyl radical.

2.2. Reactivity of Hydrogen Peroxide

As mentioned above, hydrogen peroxide (H_2O_2) is not a free radical, but it may be considered as an oxidant. *Per se*, hydrogen peroxide (H_2O_2) is little reactive. Its reactivity in biological systems depends on two properties:

- first, it can diffuse long distances crossing membranes
- second, it reacts with transition metals by a homolytic cleavage yielding the highly reactive hydroxyl radical ($HO^{\cdot}$).

2.3. Reactivity of Hydroxyl radical

Hydroxyl radical ($HO^{\cdot}$) is the most powerful oxidant and unlike superoxide anion ($O_2^{\cdot -}$) and hydrogen peroxide (H_2O_2), it indiscriminately reacts with almost all biological compounds. The extremely reactive nature of hydroxyl radical ($HO^{\cdot}$) suggests that it will only mediate direct effects close to its site of generation (it cannot diffuse long distances), that is, once generated, hydroxyl radical ($HO^{\cdot}$) will not diffuse large distances within the cell, but it will damage the first molecule it collides with.

The chemical reactivity of hydroxyl radical ($HO^{\cdot}$) may be assumed to encompass two main reactions:

• *Hydrogen abstraction*

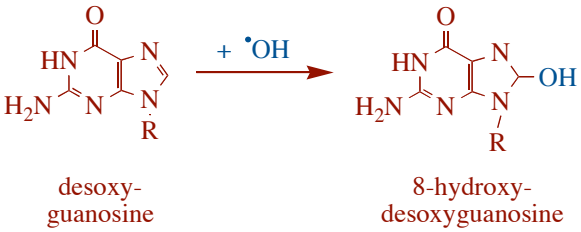
Hydroxyl radical ($HO^{\cdot}$) may react with almost any compound abstracting a hydrogen and yielding a free radical species of the compound. Abstraction of a hydrogen by hydroxyl radical ($HO^{\cdot}$) results in its reduction to water:

$$RH + HO^{\cdot} \rightarrow R^{\cdot} + H_2O$$

Again, because of its reactivity, RH could be any type of molecule within the cell, for example polyunsaturated fatty acids, DNA, glutathione, and certain amino acids.

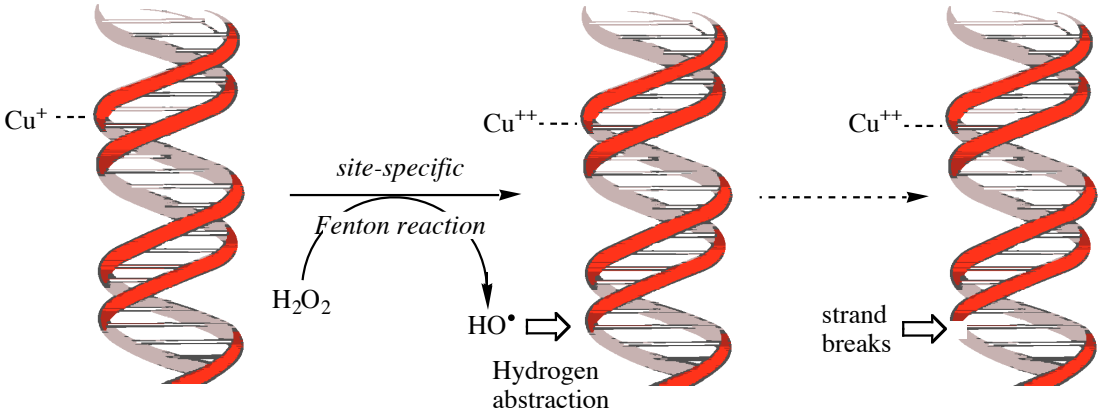
• *Addition reactions*

One of the most important addition reactions of hydroxyl radical ($HO^{\cdot}$) pertains the generation of 8-hydroxy-desoxyguanosine (a DNA base). Oxidation of this base, which can be detected *in vivo*, is a fingerprint of free radical attack to informational molecules.

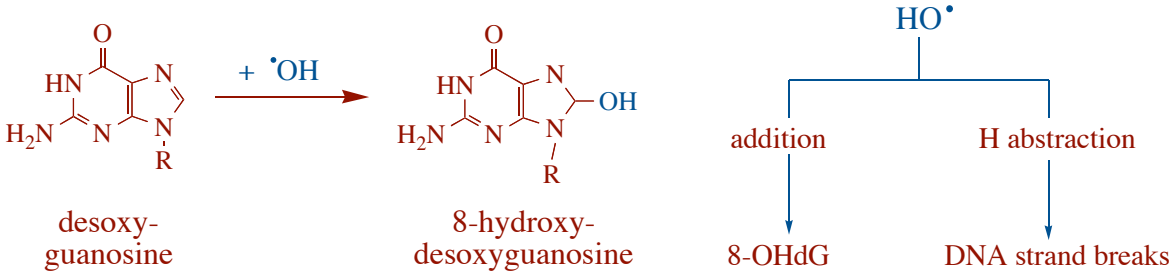


Remember the two type of products observed upon hydroxyl radical ($HO^{\cdot}$) attack on DNA:

Hydrogen abstraction reactions lead to *DNA strand breaks*



Addition reactions lead to accumulation of base oxidation as *8-hydroxydesoxyguanosine*.

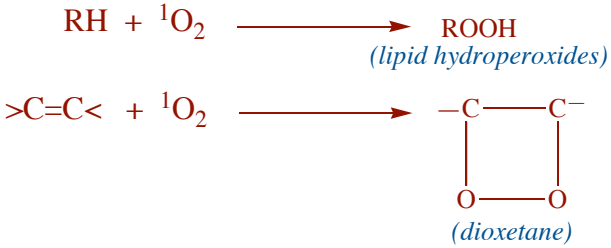


2.4. Reactivity of Singlet Oxygen

Singlet oxygen reacts efficiently with several molecules of biological importance:

- vitamin E or α-tocopherol
- vitamin C or ascorbic acid
- bilirubin
- DNA
- cholesterol
- β-carotene
- tryptophan
- methionine
- cysteine
- NADPH
- polyunsaturated fatty acids

The chemical reactivity of singlet oxygen is rather specific comprising five types of reactions, of which *ene* addition to fatty acids and *dioxetane* formation are of biological interest:



In the *ene addition* a lipid hydroperoxide is formed (RH, unsaturated fatty acid; ROOH, unsaturated fatty acid hydroperoxide). Dioxetanes are formed when singlet oxygen adds across a double bond (also important in fatty acid oxidation).

3. How Are Oxygen Radicals Generated in the Cell?

3. How are oxidants generated in the cell?

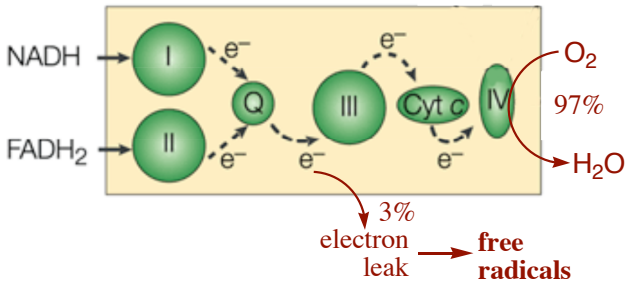
In cells, there are two main sources of superoxide anion ($O_2^{\cdot -}$) and hydrogen peroxide (H_2O_2). Hydroxyl radical ($HO^{\cdot}$) is generated from superoxide anion ($O_2^{\cdot -}$) and hydrogen peroxide (H_2O_2).

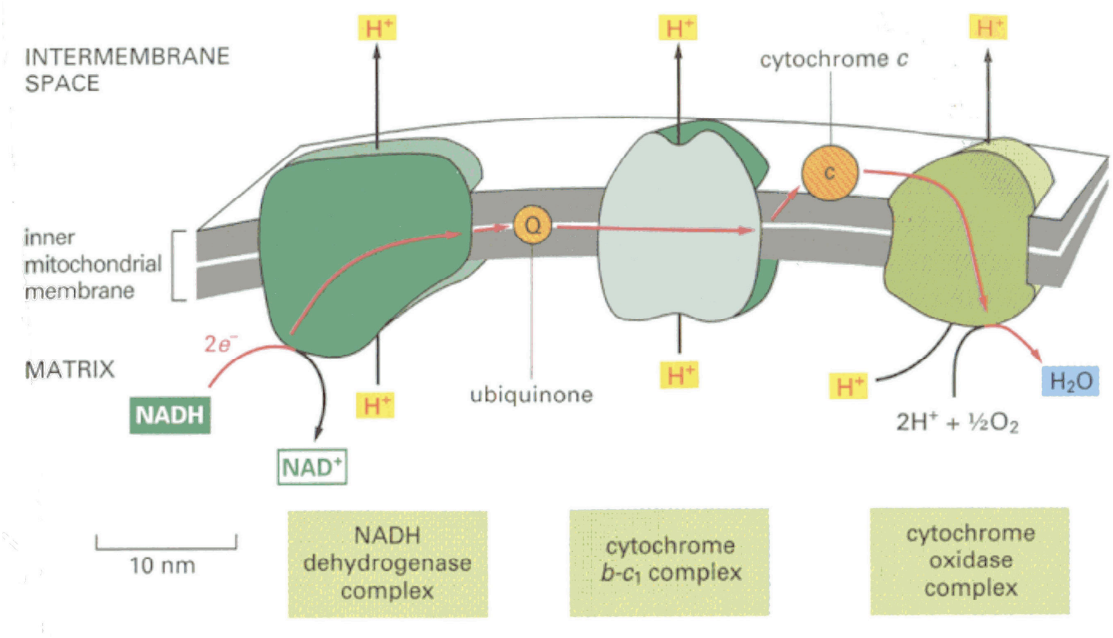
3.1. Sources of Superoxide Radical

The following table lists the most important reactions within the cell that generate superoxide anion ($O_2^{\cdot -}$).

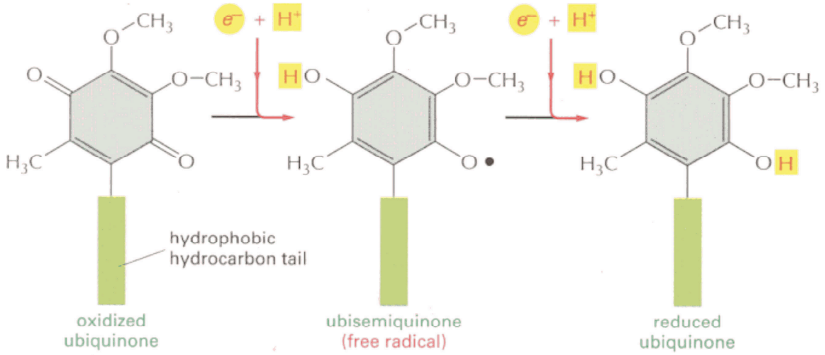
Source	*Pathophysiological Significance*
• *Enzymic reactions*	
- xanthine oxidase	Intestinal ischemia/reperfusion
- NADH oxidase	Present in leukocytes: bactericidal activity
- NADPH-cytochrome P450 reductase	
• *Cellular sources*	
- leukocytes and macrophages	Bactericidal activity
- mitochondrial electron transfer	
- microsomal monooxygenase	
• *Environmental factors*	
- ultraviolet light	
- X rays	
- toxic chemicals	
- aromatic hydroxylamines	
- aromatic nitro compounds	
- insecticides, such as paraquat	
- chemotherapeutic agents, such as quinones	

Mitochondria are major cellular sources of reactive oxygen species. Mitochondria consume oxygen associated with the process of oxidative phosphorylation. Under normal conditions, approximately 95-97% of the oxygen is reduced to water; a small fraction of the oxygen consumed (3-5%) is reduced univalently to superoxide anion ($O_2^{\bullet -}$). Coenzyme Q or ubiquinone is a mobile electron carrier in the respiratory chain and it collects electrons from complex I and complex II. The coenzyme Q pool faces both the intermembrane space and the mitochondrial matrix (outer coenzyme Q pool (Q_O) and inner coenzyme Q pool (Q_I), respectively). Coenzyme Q or

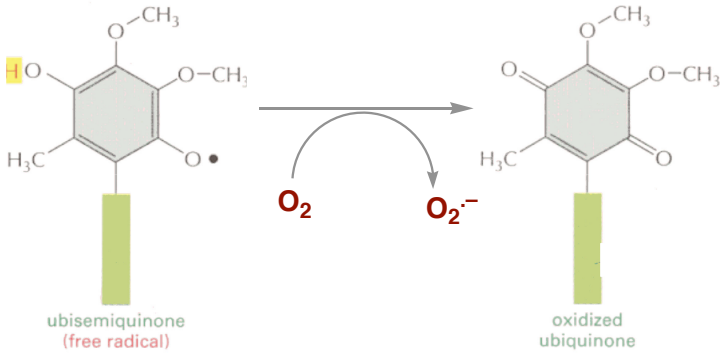




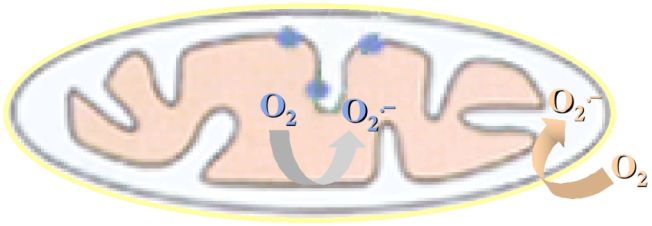
ubiquinone is reduced by Complex I and Complex II and donates electrons to complex III (the bc_1 segment). Because of these redox transitions, ubiquinone exists as a quinone (fully oxidized), semiquinone, and hydroquinone (fully reduced):

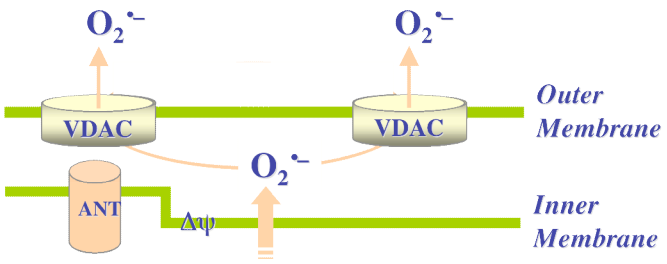


Electron leakage, accounting for about 3-5% of the total oxygen consumed by mitochondria, is associated with the generation of oxygen radicals: Ubisemiquinone donates one electron to molecular oxygen yielding superoxide anion and ubiquinone; this is known as *autoxidation of ubisemiquinone*.



Ubisemiquinone autoxidation is the major source of superoxide anion in mitochondria and because the ubiquinone or coenzyme Q pool faces both the intermembrane space and the mitochondrial matrix, superoxide anion ($O_2^{\cdot -}$) is vectorially released into both compartments. $O_2^{\cdot -}$ released in the intermembrane space can cross the outer mitochondrial membrane into cytosol through a voltage-dependent anion channel (VDAC).





3.2. Sources of Hydrogen Peroxide

Hydrogen peroxide (H_2O_2) is generated within the cell by two distinct processes: *nonradical or enzymic generation* and *radical or from superoxide anion disproportionation*.

• *Nonradical or enzymic generation*

The following enzymes do generate hydrogen peroxide (H_2O_2) upon reduction of their co-substrate, molecular oxygen:

glycolate oxidase	D-amino acid oxidase	urate oxidase
acetyl-CoA oxidase	NADH oxidase	**monoamine oxidase**

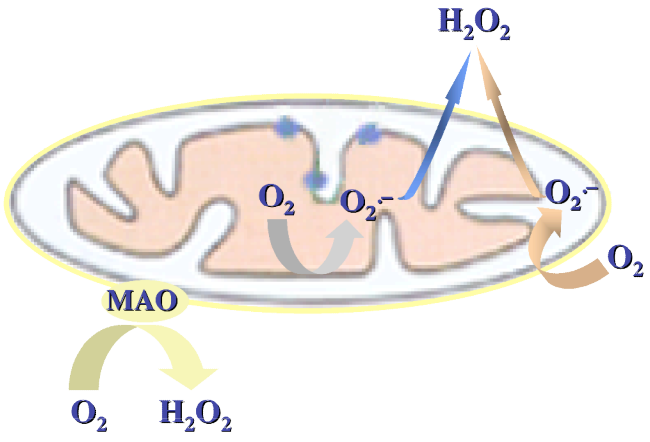
The latter enzyme, monoamine oxidase (MAO) occurs in two forms A and B and it catalyzes the oxidative deamination of biogenic amines. It is present in the outer mitochondrial membrane.

• *Radical generation or from superoxide anion disproportionation*

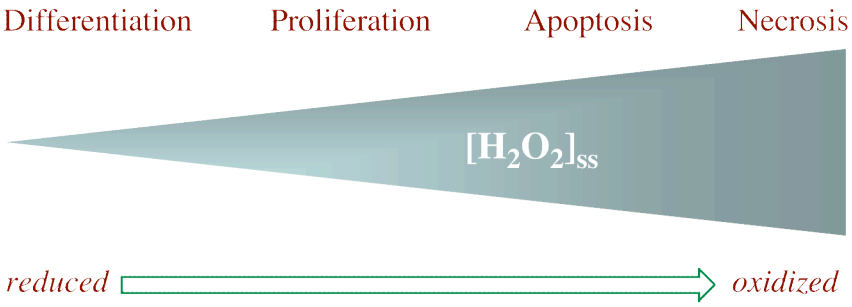
This is achieved upon dismutation or disproportionation of superoxide anion ($O_2^{\cdot -}$), according to the reaction mentioned before:

$$O_2^{\cdot -} + O_2^{\cdot -} + H^+ \rightarrow H_2O_2 + O_2$$

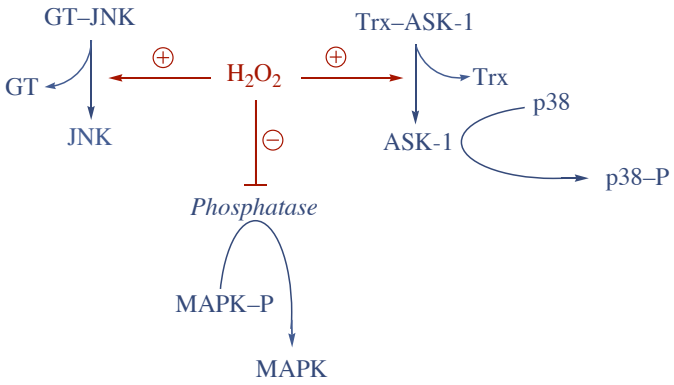
As mentioned above, ***mitochondria are major cellular sources of oxyradicals*.** Superoxide anion ($O_2^{\cdot -}$), generated upon autoxidation of ubisemiquinone, is vectorially released into the intermembrane space and the mitochondrial matrix. In the latter compartment, $O_2^{\cdot -}$ dismutates to H_2O_2. H_2O_2 is a freely diffusible species that can cross membranes. Hence, mitochondria have two major sources of H_2O_2: on the one hand, H_2O_2 generated by disproportionation of superoxide anion in the mitochondrial membrane and, on the other hand, the oxidative deamination of biogenic amines by the outer mitochondrial membrane-bound monoamine oxidase activity.



Mitochondrion-generated H_2O_2 is involved in the redox regulation of cell signaling pathways. The steady-state levels of H_2O_2 ($[H_2O_2]_{ss}$) determine the cellular redox status and the transition from proliferation to apoptosis and necrosis.



Mitochondrial H_2O_2 was demonstrated to regulate MAPK activity at multiple levels. JNK and p38 kinase are activated by H_2O_2. Under normal conditions, thioredoxin (Trx) is bound to and inhibits the activity of apoptosis signal-regulating kinase-1 (ASK-1), a MAPKKK involved in both JNK and p38 kinase activation. However, oxidative stress (H_2O_2) dissociates the thioredoxin-ASK-1 complex leading to activation of p38. A similar mechanism may function at the level of JNK: under non-stressed conditions, glutathione transferase (GT) binds to JNK and inhibits its activation, but this interaction is disrupted by oxidative stress (H_2O_2). Alternatively, JNK activation by H_2O_2 may occur in part through suppression of phosphatases involved in JNK inactivation.



3.3. Sources of Hydroxyl Radical

Most of the hydroxyl radical ($HO^{\cdot}$) generated *in vivo*, except for that during excessive exposure to ionizing radiation, originates from the breakdown of hydrogen peroxide (H_2O_2) *via* a *Fenton reaction*.

The Fenton reaction entails a metal-dependent reduction of hydrogen peroxide (H_2O_2) to hydroxyl radical ($HO^{\cdot}$). *Transition metals*, such as copper (Cu), iron (Fe), and cobalt (Co), in their reduced form catalyze this reaction:

$$Fe^{++} + H_2O_2 \rightarrow Fe^{+++} + HO^- + \mathbf{HO^{\cdot}}$$

As indicated above, the Fenton reaction requires the transition metal in its reduced state. Reduction of the transition metal may be accomplished by superoxide anion ($O_2^{\cdot -}$), as in the following example with Fe^{+++}:

$$Fe^{+++} + O_2^{\cdot -} \rightarrow Fe^{++} + O_2$$

The overall reaction, involving iron reduction by superoxide anion ($O_2^{\cdot -}$) and iron oxidation by hydrogen peroxide (H_2O_2), is as follows:

$$Fe^{+++} + O_2^{\cdot -} \rightarrow Fe^{++} + O_2$$
$$Fe^{++} + H_2O_2 \rightarrow Fe^{+++} + HO^- + \mathbf{HO^{\cdot}}$$

$$O_2^{\cdot -} + H_2O_2 \rightarrow O_2 + HO^- + \mathbf{HO^{\cdot}}$$

The latter reaction ($O_2^{\cdot -} + H_2O_2 \rightarrow O_2 + HO^- + \mathbf{HO^{\cdot}}$), is known as the *Haber-Weiss reaction*. This reaction, as such, proceeds at very slow rates. The *Fenton reaction*, that is, metal-catalyzed reduction of hydrogen peroxide (H_2O_2), prevails in a biological environment.

It is worth noting that, at variance with superoxide anion ($O_2^{\cdot -}$) and hydrogen peroxide (H_2O_2), there is *no direct generation of* hydroxyl radical ($HO^{\cdot}$) *in the cell. Both, superoxide anion and hydrogen peroxide are required to form the highly reactive hydroxyl radical ($HO^{\cdot}$).*

3.4. Singlet Oxygen

Singlet oxygen is a reactive oxygen species that can be formed not only by energy transfer (as mentioned above), but also by electron-transfer reactions.

- *Electron transfer reactions*. Of biological interest, the enzyme *myeloperoxidase*, present in the neutrophil, can catalyze the formation of hypochlorite from Cl^- and H_2O_2. The further re-

$$Cl^- + H_2O_2 \xrightarrow[myeloperoxidase]{} HOCl + H_2O$$

action of hydrogen peroxide (H_2O_2) with formed HOCl yields singlet oxygen ($^1O_2$):

$$HOCl + H_2O_2 \rightarrow Cl^- + H_2O + H^+ + \mathbf{^1O_2}$$

- *Energy transfer reactions*. This is another way to generate singlet oxygen as it is comprised in the photosensitization of different chemotherapeutic agents. The chemotherapeutic agent (or sensitizer = S) absorbs energy upon irradiation and transfers this energy to molecular oxygen with formation of singlet oxygen ($^1O_2$).

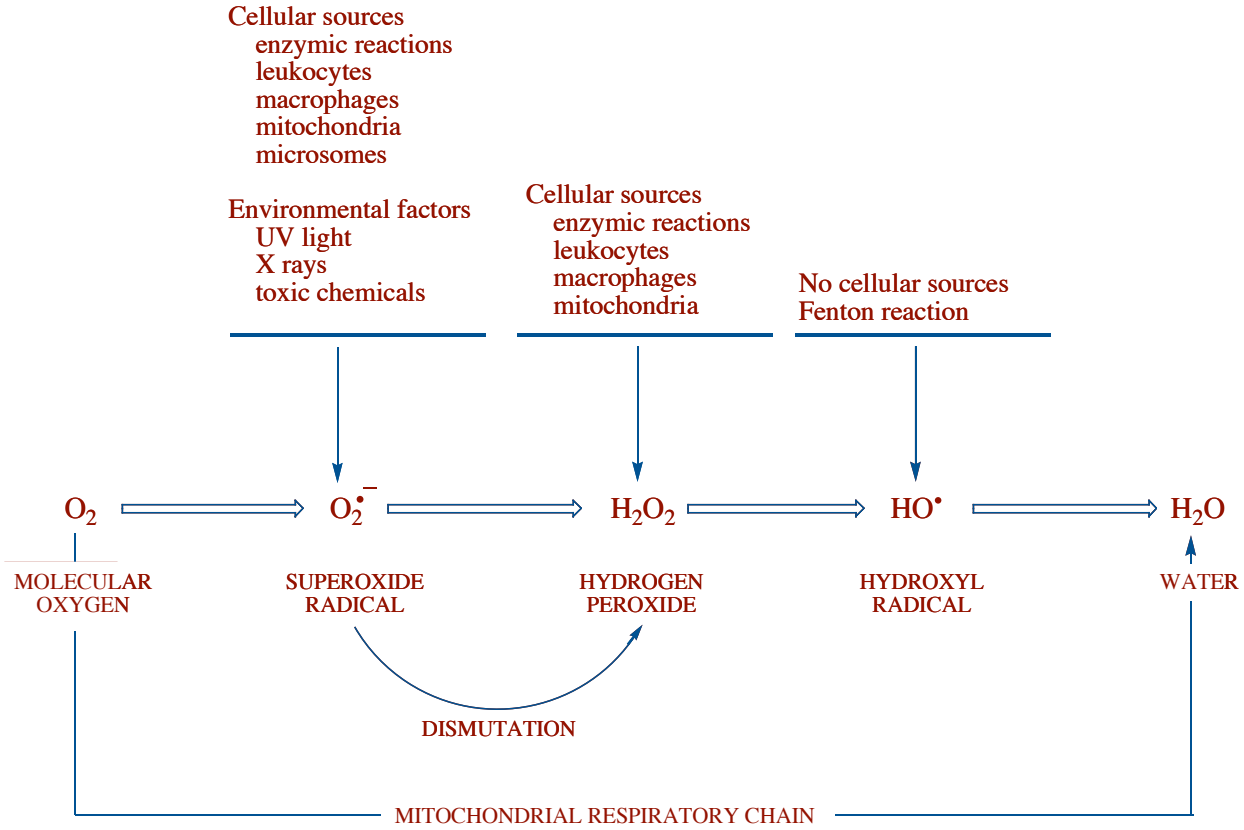
$$S + h\upsilon \rightarrow S^*$$

$$S^* + O_2 \rightarrow S + {}^1O_2$$

As mentioned before, singlet oxygen ($^1O_2$) is a reactive species that reacts with molecules, such as vitamin E, vitamin C, DNA, cholesterol, carotenoids, polyunsaturated fatty acids in membranes, and certain amino acids.

3.5. Summary of Cellular Sources of Oxygen Radicals

Actual sources of reactive oxygen species exist for superoxide anion ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) –and probably for singlet oxygen. Hydroxyl radical ($HO^{\bullet}$) formation requires a cellular steady-state level of both superoxide anion ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2), precursors of hydroxyl radical ($HO^{\bullet}$) *via* a Fenton reaction.



4. How Do Oxidants Mediate Cellular Damage?

4. How do oxidants mediate cell damage?

Given the wide spectrum of oxidants that can be generated in the cell and in the microcirculation (as that triggered by neutrophils), it is clear that no intracellular or extracellular molecules are invulnerable to free radical attack. It can be considered that superoxide anion ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) are less reactive than hydroxyl radical ($HO^{\bullet}$) and singlet oxygen. However, in a suitable biological setting the two first species may display considerable chemical reactivity leading to damage of various biomolecules.

4.1. Lipid peroxidation

Biomembranes and subcellular organelles are particularly sensitive to oxidative attack due to the presence of polyunsaturated fatty acids (PUFA) in their membrane phospholipids. Lipid peroxidation consists of three steps: initiation, propagation, and termination.

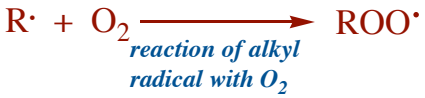
- *Initiation*

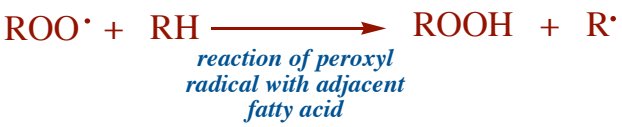
Lipid peroxidation of biomembranes can be initiated by the interaction of a sufficiently reactive oxidant, such as hydroxyl radical ($HO^{\bullet}$), with a fatty acid (RH) to generate a fatty alkyl free radical:

$$RH + HO^{\bullet} \longrightarrow R^{\bullet} + H_2O$$

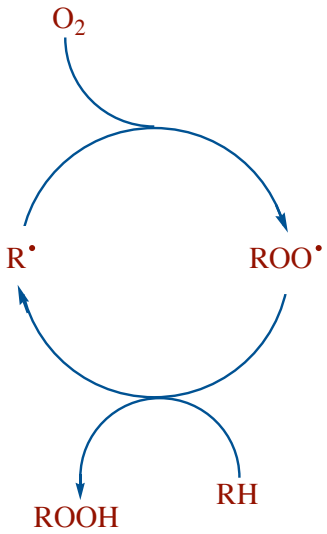
- *Propagation*

The fatty alkyl free radical ($R^{\bullet}$) reacts very rapidly with molecular oxygen(diffusion-controlled rates) to form a fatty peroxyl radical ($ROO^{\bullet}$). This species has sufficient oxidizing potential to attack a neighboring unsaturated fatty acid (RH) in the membrane to form hydroperoxides and a new fatty alkyl radical ($R^{\bullet}$)





In this manner, an autocatalytic cycle or chain reaction is initiated that will *propagate* until the free radical chain is terminated.

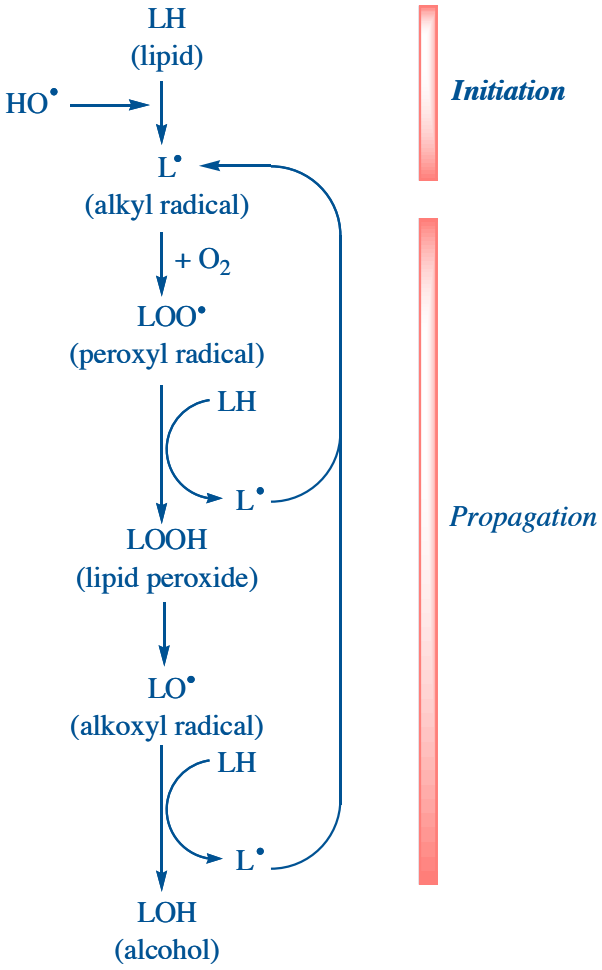


• *Termination*

The autocatalytic chain referred to above is terminated by the collision of two radical species to form nonradical products. The contribution of the termination reactions outlined below depends on the intracellular oxygen concentration.

Oxidative impairment of biomembranes of lipoproteins can initiate a complex cascade of events leading to the formation of reactive, unstable oxidants, long-lived toxic by-products or

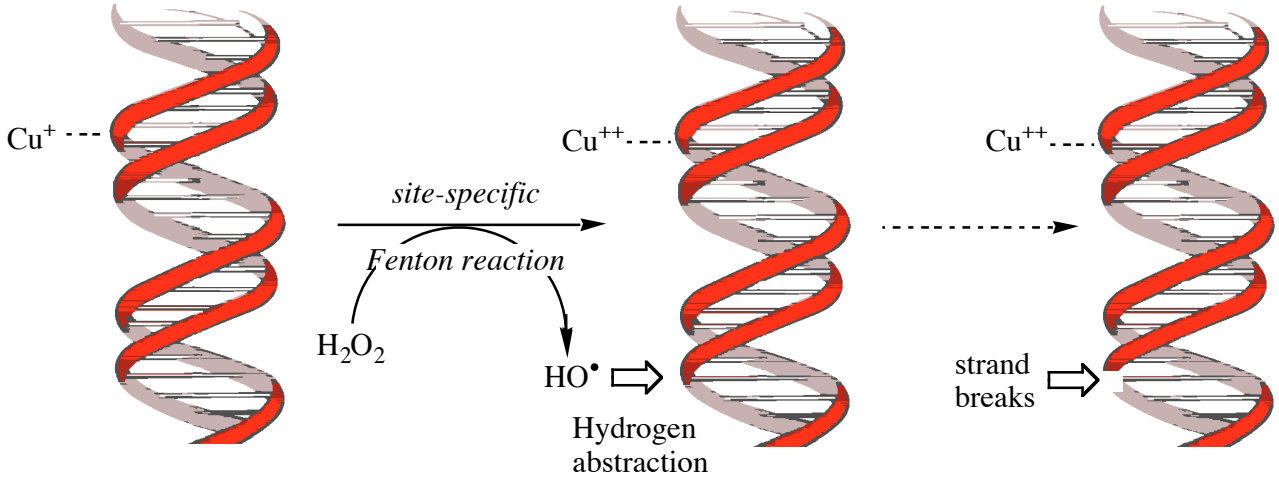
biologically active inflammatory mediators that have the potential of propagating damage beyond the confines of the original focus. Free radical attack of unsaturated fatty acids in membranes or lipoproteins is, of course, associated with important functional changes that can result in cell dysfunction or cell death.



4.2. DNA Oxidation

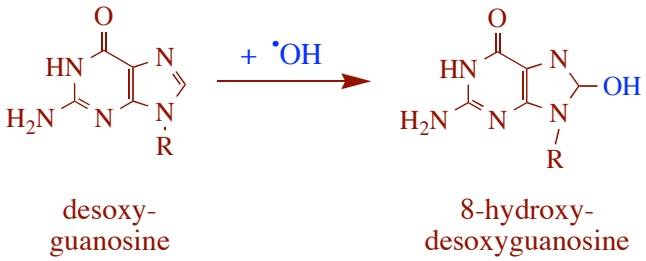
Hydroxyl radical (HO·) is endowed with unique properties: due to a combination of high electrophilicity, high thermochemical reactivity, and a mode of production that can occur in the vicinity of DNA (site specific mechanism), it can both *abstract* H atoms from the sugar in the DNA helix and *add* to DNA bases, leading to *single strand breaks* and *nucleobase (8-hydroxydesoxyguanosine) oxidation*, respectively.

- *Hydrogen abstraction - DNA strand breaks*



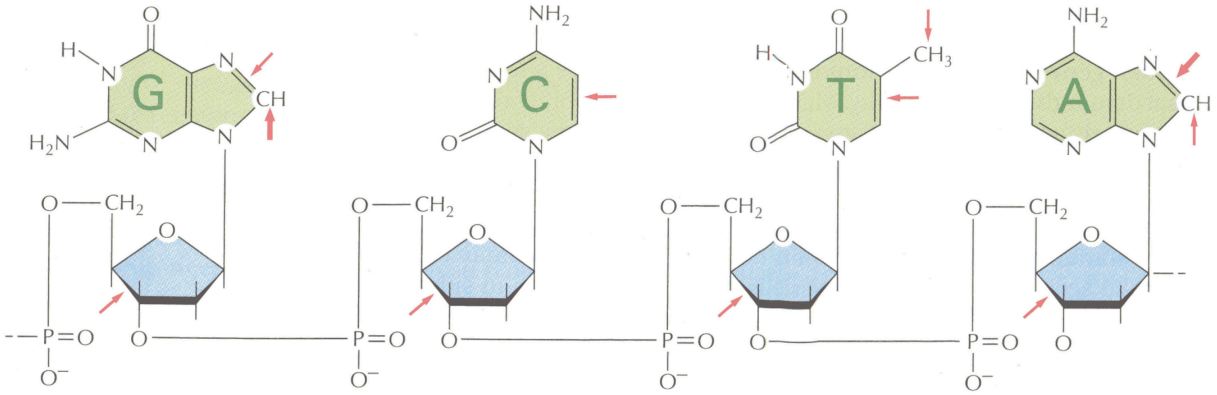
- *Addition - Nucleobase oxidation*

Hydroxyl radical (HO·) addition to bases such as guanine, proceeds very rapidly and leads to the formation of 8-hydroxydesoxyguanosine, which is used as a fingerprint of nucleobase oxidative damage.

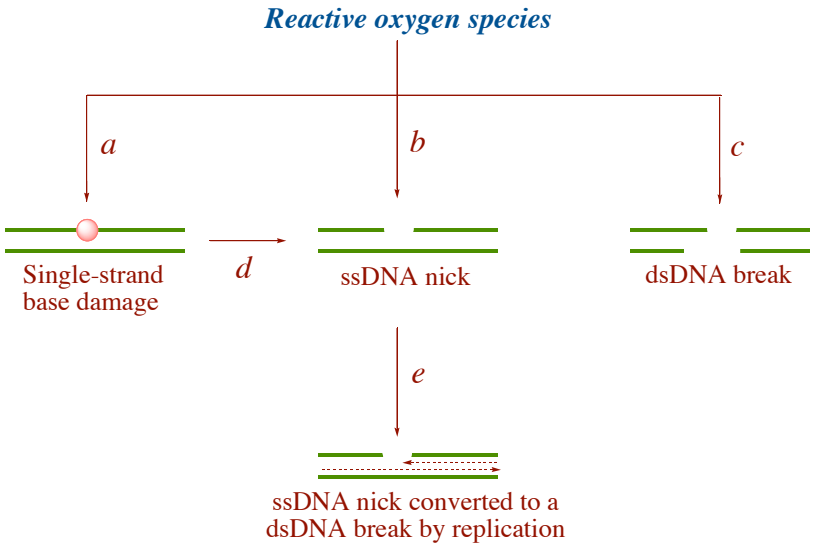


DNA is susceptible to changes that would lead to mutations. For example, DNA bases are damaged by an encounter with free radicals or environmental chemicals. Hydroxyl radical-mediated damage on sugars (deoxyribose) is part of the known C'4 mechanism and leads to

strand breaks. Oxidative damage of bases, usually leads to adduct formation, as exemplified above with 8-hydroxydesoxyguanosine.



Reactive oxygen species can damage DNA at different levels: hydroxyl radical through addition reactions can cause single-strand base damage (e.g., formation of 8-hydroxydeoxyguanosine) and through H abstraction single strand DNA nick (ssDNA nick) or double strand DNA break (dsDNA break). Upon replication, ssDNA nick can be converted to a dsDNA break:



5. How Do Cells Protect Themselves Against Oxidants?

5. How do cells protect themselves against oxidants?

Mammalian cells are not defenseless in the face of oxidant attack, but they are endowed with complex sets of protective mechanisms, which have evolved in cells and are designed to *prevent*, *limit*, or *repair* oxidative damage.

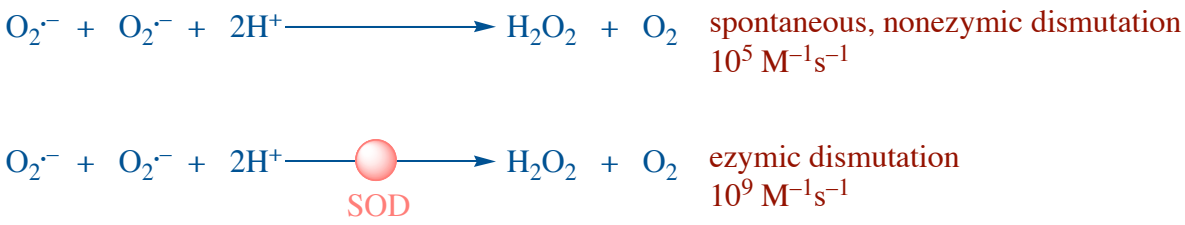
On the one hand, the cell convenes *specific enzymic defenses* against oxygen radical attack, which can be considered *preventive antioxidants*. On the other hand, there exist *small antioxidant molecules*, which can react with a variety of free radicals and that may be considered as *chain-breaking antioxidants*.

5.1. Specific enzymic defenses or preventive antioxidants

Mammalian cells contain specific enzymes, which remove either superoxide anion or hydrogen peroxide, the two required precursors of hydroxyl radical ($HO^{\cdot}$).

Removal of Superoxide Anion: Superoxide Dismutases

Superoxide anion radical is formed by different nonenzymic and enzymic reactions within the cell. Superoxide dismutases (abbreviated SOD) catalyze the rapid dismutation of superoxide radical to hydrogen peroxide and oxygen. The rate of this reaction is 10,000-fold higher than that of the spontaneous dismutation.



All superoxide dismutases are metalloproteins containing Cu,Zn, or Mn. There are three types of superoxide dismutases in humans:

- Cu,Zn-superoxide dismutase ..... cytosol
- Mn-superoxide dismutase ..... mitochondrial matrix
- Cu,Zn-superoxide dismutase ..... mitochondrial intermembrane space
- Cu,Zn-superoxide dismutase ..... extracellular space

The content of Cu,Zn-superoxide dismutase in human tissues is illustrated in the table below:

Tissue	Cu,Zn-SOD μg/mg protein
Liver	4.7
Cerebral gray matter	3.7
Testis	2.2
Renal cortex	1.9
Cardiac muscle	1.8
Renal medulla	1.3
Pituitary	1.0
Lung	0.5

Removal of Hydrogen Peroxide: Catalase and Glutathione Peroxidases

The product of the superoxide dismutase-catalyzed reaction above is hydrogen peroxide (H_2O_2). Although the latter is less reactive than superoxide anion radical, it is still a strong oxidant and a precursor of hydroxyl radical ($HO^{\cdot}$) via a Fenton reaction.

The cell possesses mechanisms by which hydrogen peroxide (H_2O_2) is readily reduced to water. The enzymes catalyzing this reaction are catalase and glutathione peroxide.

Catalase. This enzyme is located in the peroxisomes and catalyses the following reaction:

$$H_2O_2 + H_2O_2 \rightarrow 2H_2O + O_2$$

Glutathione Peroxidase. The enzyme occurs in cytosol and the mitochondrial matrix and it requires *glutathione*, a tripeptide present in high concentrations in most mammalian cells. During this reaction hydrogen peroxide (H_2O_2) is reduced to water and glutathione (GSH) is oxidized to glutathione disulfide (GSSG).

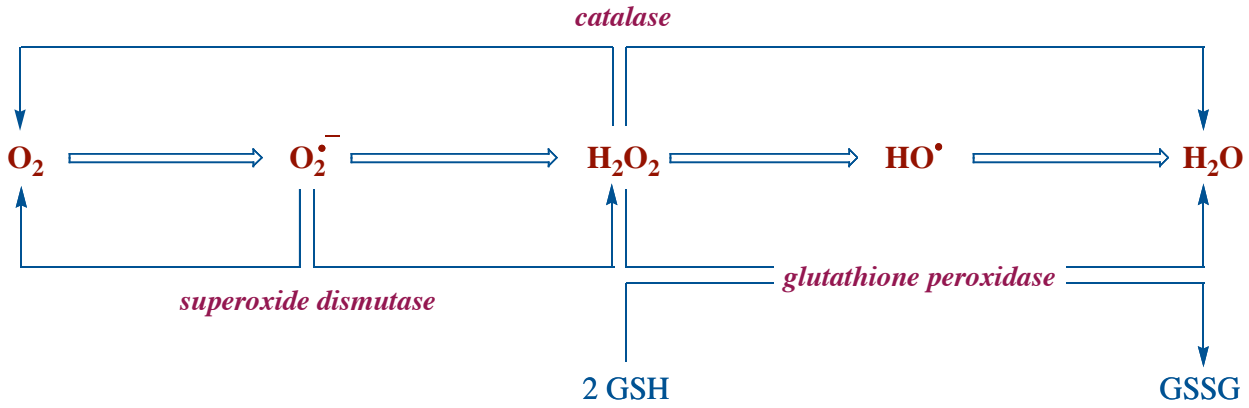
$$H_2O_2 + 2GSH \rightarrow H_2O + GSSG$$

The activities of catalase and glutathione peroxidase in normal human tissues is listed below:

Tissue	catalase (units *per* mg protein)	GPX
Liver	1300	190
Erythrocytes	1000	19
Kidney cortex	430	140
Lung	210	53
Pancreas	100	43
Heart	54	69
Brain	11	79

Summary of Specific Enzymic Antioxidant Enzymes

The generation of free radicals described above along with the specific enzyme systems designed to protect the cell against oxygen radical attack are summarized in the following scheme:



It is to be noticed that whereas there are *specific enzymic defenses against superoxide anion and hydrogen peroxide*, the cell lacks a specific system to remove or scavenge hydroxyl radical ($HO^{\bullet}$). Because the formation of hydroxyl radical ($HO^{\bullet}$) requires both superoxide anion ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2), the scavenging of these species by superoxide dismutase and catalase/glutathione peroxidase, respectively, is expected to prevent hydroxyl radical ($HO^{\bullet}$) formation.

Hence, the primary device of the cell is to use superoxide dismutase, catalase, and glutathione peroxidase to prevent superoxide anion ($O_2^{\bullet -}$) or hydrogen peroxide (H_2O_2) from participating in reactions that generate more reactive oxidants, such as hydroxyl radical ($HO^{\bullet}$).

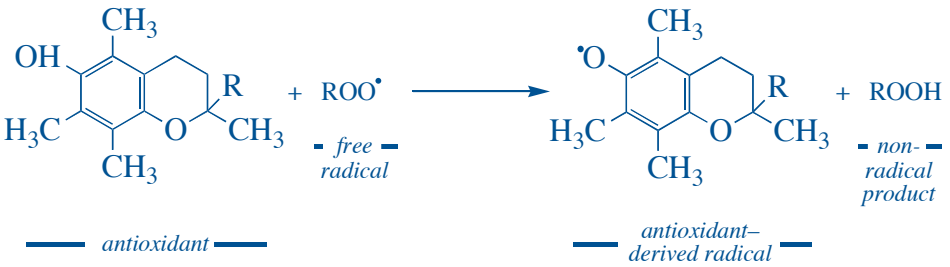
5.2. Nonspecific antioxidant molecules

The major task confronting the cell is the necessity to limit or repair the damage caused when highly reactive oxidants, such as hydroxyl radical (HO•), breach specific intracellular defenses. A second line of defense against free radical attack is constituted by small antioxidant molecules, such as vitamin E, vitamin C, ubiquinone or Coenzyme Q, carotenoids. Some of these compounds are considered chain-breaking antioxidants because they effectively interrupt free radical propagation reactions (as described for lipid peroxidation).

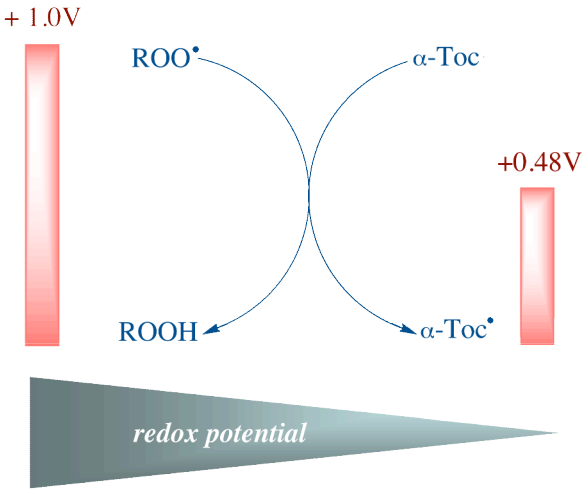
Vitamin E or tocopherols

Natural vitamin E is a mixture of tocopherols (α, β, and γ) and tocotrienols (α, β, and γ). It is a lipid soluble vitamin, which concentrates mainly in the interior of membranes and blood proteins. It is the *major lipid soluble antioxidant in human blood plasma*.

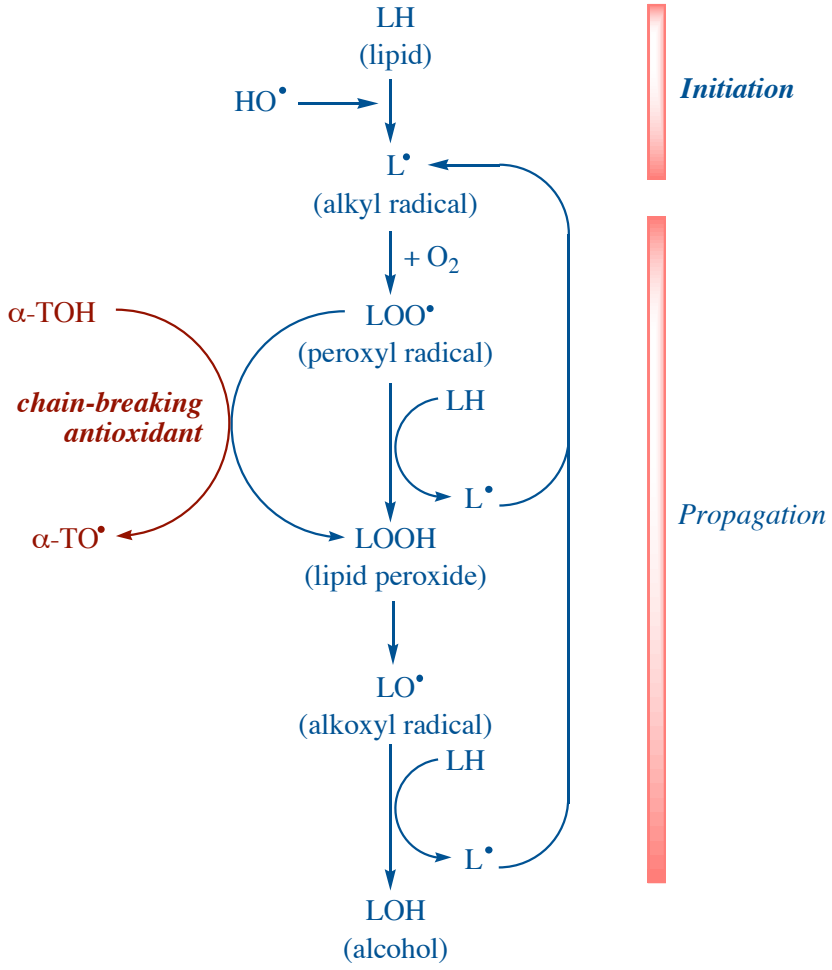
Vitamin E reacts at considerable rates with a variety of free radical species, with emphasis on lipid peroxyl radicals formed during lipid peroxidation.



During the course of this reaction, *as in any other antioxidant mechanism*, a free radical form of vitamin E is formed. The new radical species, the α-tocopheroxyl radical, has a chemical reactivity lower than the original free radicals quenched. When comparing the reduction potential of the original radical with that of the *antioxidant-derived radical*, it is clear that a 'less reactive species' has been formed. Hence, the *transfer of the radical character* proceeds toward creating less oxidizing species:



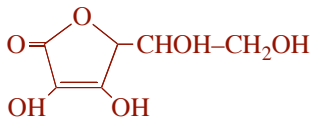
The following scheme illustrates the chain-breaking antioxidant activity of vitamin E:



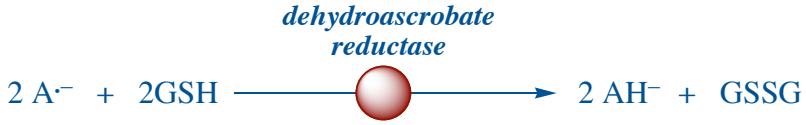
Vitamin C

Vitamin C or ascorbic acid (AH^-) is a water-soluble vitamin that reacts with several radical species producing semidehydroascorbic acid or ascorbyl radical ($A^{\bullet -}$).

$$AH^- + HO^{\bullet} \rightarrow A^{\bullet -} + H_2O$$



Animals contain two enzymes that can reduce the ascorbyl radical or semidehydroascorbate radical back to ascorbate: dehydroascorbate reductase and NADH-semidehydroascorbate reductase. The former enzyme reduces ascorbyl radical back to ascorbate whilst oxidizing GSH to GSSG:

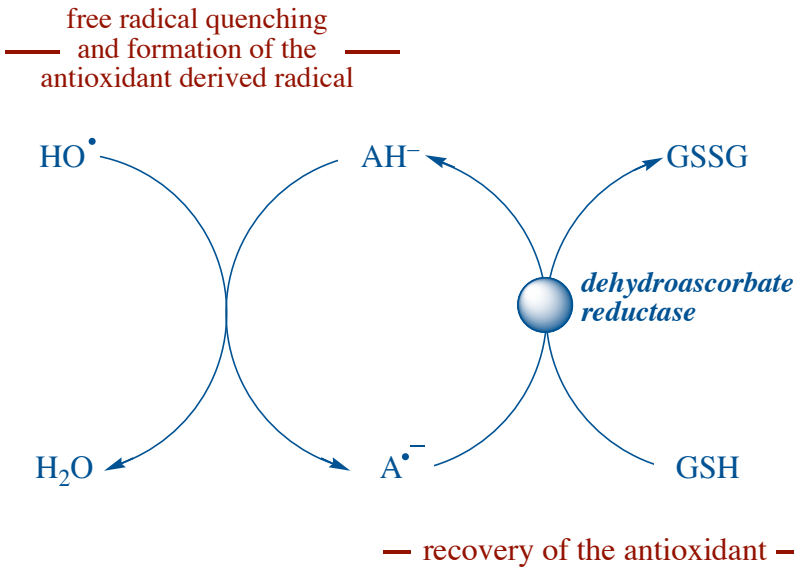


The latter enzyme, NADH-semidehydroascorbate reductase, reduces the ascorbyl radical back to ascorbate whilst oxidizing NADH to NAD^+.

Content of semidehydroascorbate reductase in human tissues

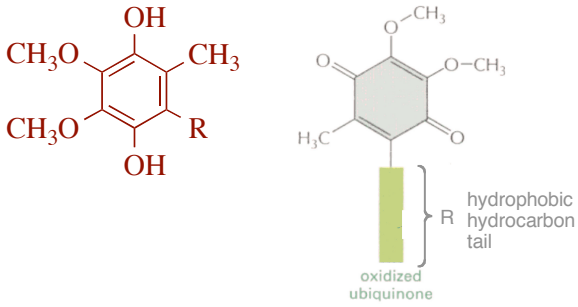
Tissue	Relative enzyme activity
adrenal cortex	50
kidney	49
liver	31
pancreas	16
testis	11
brain	9
lung	9
heart	0
skeletal muscle	0

The primary quenching of radicals by ascorbic acid or vitamin C yields, therefore, a nonradical product and the antioxidant-derived radical, ascorbyl radical. The latter can be recovered back to vitamin C by means of the GSH-dependent dehydroascorbate reductase activity:

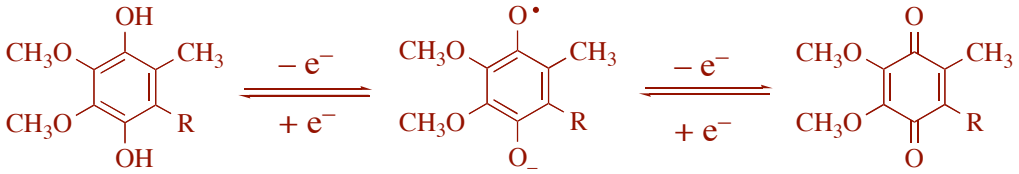


Ubiquinone or Coenzyme Q

Coenzyme Q or ubiquinone-10 is a redox component present in all mammalian cell membranes: in the inner mitochondrial membrane, ubiquinone plays a key role shuttling electrons from complexes I and II to complex III (bc_1 segment) of the respiratory chain. In extramitochondrial membranes, ubiquinone may function in its reduced form (ubiquinol) as an antioxidant protecting unsaturated fatty acids from peroxidative damage.

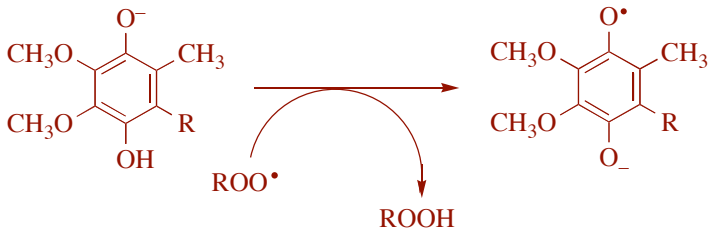


Like all quinones, ubiquinones are endowed with a main chemical property that underlies their biological functions: their ability to undergo reversible one-electron transfers with intermediate formation of a semiquinone species. The presence of isoprenoids substituents (R) in ubiquinones hinders nucleophilic attack across the double bond.

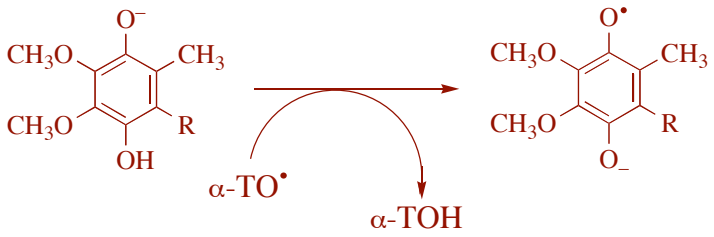


The ubiquinol ⇔ ubisemiquinone redox transition. The two major antioxidant properties of ubiquinol are linked to *reduction of peroxyl radicals* and *α-tocopheroxyl radicals* within the ubiquinol ⇔ ubisemiquinone transition.

- The former reaction implies that ubiquinol functions as *chain-breaking antioxidant* reducing peroxyl radicals to hydroperoxides;



- The latter, suggests the participation of ubiquinol in a concerted mechanism encompassing the reduction of the α-tocopheroxyl radical to tocopherol.



(In these reactions, ubiquinols are depicted in the monoanion form (UQH^-), because deprotonation of hydroquinones is a requisite condition for electron transfer. In fact, the reaction sequence involving deprotonation → electron transfer → deprotonation

$$QH_2 \rightarrow QH^- + H^+$$

$$QH^- - e^- \rightarrow QH^{\cdot}$$

$$QH^{\cdot} \rightarrow Q^{\cdot -} + H^+$$

is implicit to all mechanistic models for oxidation of ubihydroquinone in the respiratory chain and, likely, in extramitochondrial membranes).

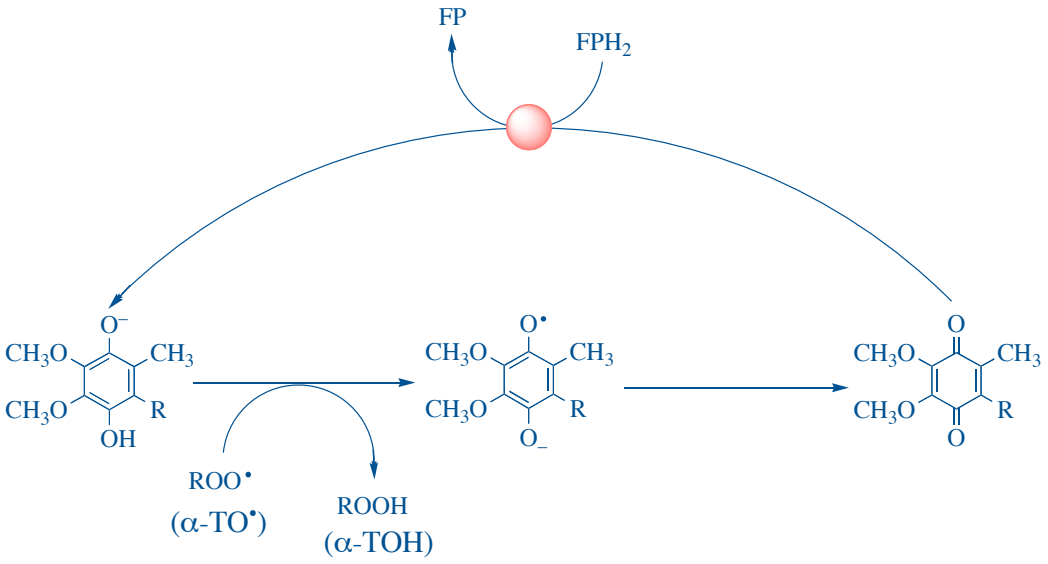
Content of Coenzyme Q (UQ_{10}) in different membranes

Membrane	Coenzyme Q (nmol/mg protein)
Golgi apparatus	3.3
Inner mitochondrial membrane	2.3
Lysosomes	2.3
Mitochondria	1.8
Plasma membrane	0.8
Peroxisomes	0.4
Endoplasmic reticulum	0.2

Distribution and Redox State of UQ_{10} in human tissues

Tissue	Amount	% Reduced State
Heart	114.0	61
Kidney	66.5	75
Liver	54.9	95
Muscle	39.7	65
Brain	13.4	23
Lung	7.9	25

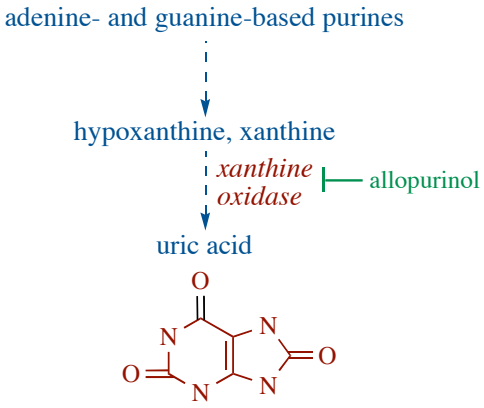
Once ubiquinone or coenzyme Q reacts with a free radical species, it forms the *antioxidant-derived radical*. The previous table indicates that in extramitochondrial membranes, coenzyme Q is largely in the reduced state. The question arises as to the mechanism(s) underlying the maintenance of ubiquinone in its reduced state. An enzyme, NADPH-ubiquinone reductase, present in liver cytosol, appears to keep ubiquinone as ubiquinol in extramitochondrial membranes.



Hence, following the primary interaction of ubiquinol with peroxyl radicals (chain-breaking activity) or vitamin E radical (α-tocopheroxyl radical = α-TO·), the antioxidant-derived radical (ubiquinone) is recovered back to ubiquinol by the action of the flavin-linked enzyme (FPH_2), NADPH-ubiquinone reductase.

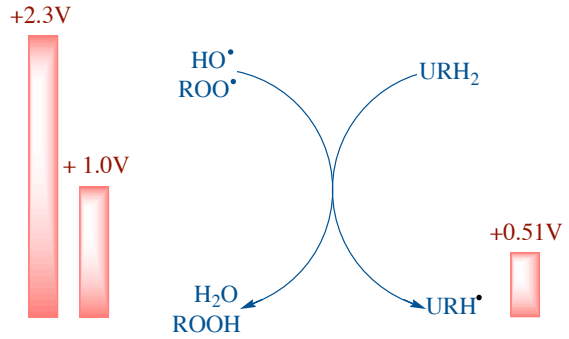
Uric acid

Uric acid is produced by the oxidation of hypoxanthine and xanthine by *xanthine oxidase* and dehydrogenase enzymes. In most species, the peroxisomal enzyme *urate oxidase* converts urate into allantoin, which is further converted into gloxylate plus urea, all products more soluble in water than is urate.



However, humans lack *urate oxidase* and, hence, urate accumulates in human blood plasma to concentrations normally in the range of 0.2-0.4 mM and is excreted in the urine. Urate is also present intracellularly and in all other body fluids, usually at somewhat lower levels (for example, 0.1-0.2 mM in saliva). Because urate has limited solubility in water, the excess production in vivo can lead to its crystallization out of solution, as it occurs in *gout*, a disease often treated with an inhibitor of xanthine dehydrogenase, *allopurinol*.

Strong oxidants, such as $HO^{\cdot}$ and $ROO^{\cdot}$ oxidize urate (UrH_2) to the urate free radical ($UrH^{\cdot-}$). The redox potential of urate/urate radical is approximately +0.59 V. That of the radicals it urate scavenges is much higher (*i.e.*, $HO^{\cdot}/HO^{-}$ = +2.3 V; $ROO^{\cdot}/ROOH$ = 1.0 V). Hence, the *transfer of the radical character* proceeds from a strong oxidizing radical to a weak oxidizing *antioxidant-derived radical*). In addition to $HO^{\cdot}$ and $ROO^{\cdot}$, urate is also a powerful scavenger of $^1O_2$, ozone (O_3), and nitrogen dioxide ($NO_2^{\cdot}$).



The antioxidant-derived radical, urate radical, may not be biologically innocuous, for it has been shown *in vitro* to lead to inactivation of at least two proteins: alcohol dehydrogenase and α_1-antiproteinase.

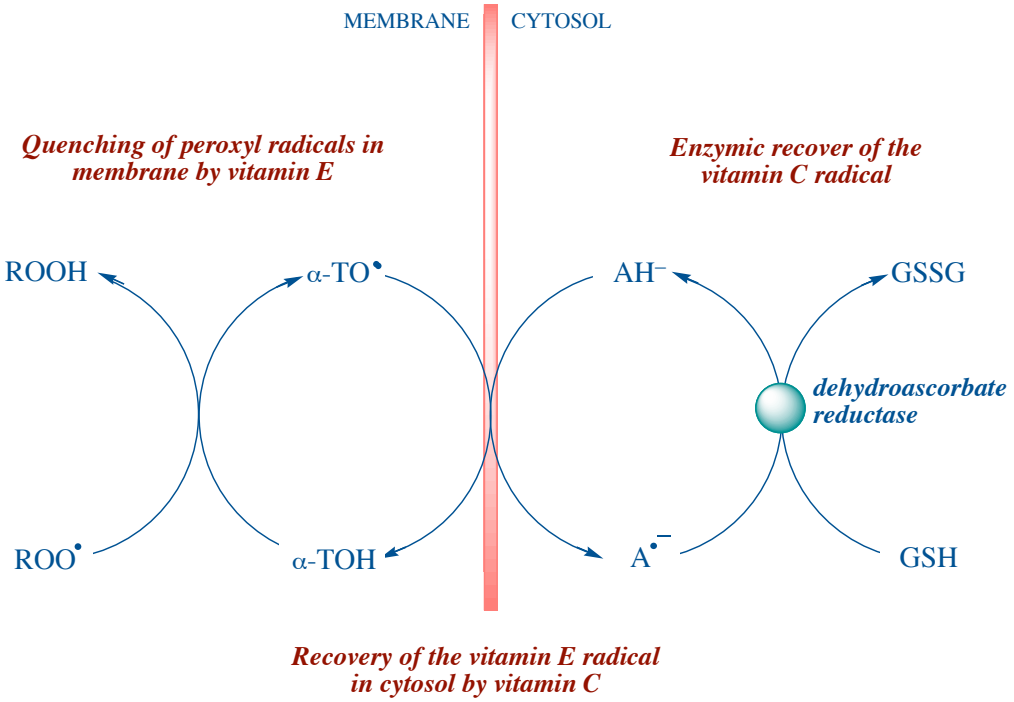
Synergism between vitamin E and vitamin C

- *Vitamin E* is a *lipid-soluble vitamin and antioxidant* (a chain-breaking antioxidant reacting mainly with peroxyl radicals formed during lipid peroxidation). Vitamin E is present in *membranes*. The reactivity of vitamin E with lipid peroxyl radicals at the membrane site

yields the corresponding antioxidant-derived radical, vitamin E radical or tocopheroxyl radical.

- *Vitamin C* is a *water-soluble vitamin and antioxidant* that reacts with a variety of free radical species; the antioxidant-derived radical, ascorbyl radical, is recovered *via* dehydroascorbate reductase. Vitamin C is present in the *cytosol*.

The different compartmentalization of vitamins E and C provides a synergistic antioxidant mechanism by which the free radical character is transferred from the lipid phase (membrane) to the cytosol according to the following scheme:



5.3. Summary of Antioxidant Defenses