# Mechanism of Action of Biological Antioxidants (43429)

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*Abstract.* **More and more diseases have been proposed to have a radical or oxidant involvement. Although in most cases we do not know if this involvement is a cause or a result of the disease process, it is still valuable to learn about those compounds or enzymes that might block, inhibit, or prevent radical-initiated reactions. Therefore, it becomes increasingly important to understand which compounds can function as antioxidants, where they are located in the body, and what their mechanism of action might be. As we increase our knowledge in these areas, we will have a better opportunity to propose interventions that might suppress or even reverse some of the ravages of oxidant-based diseases in humans.** [P.S.E.B.M. 1992, Vol 200]

How can we best define the term antioxidant in the context of health and disease? According to a current dictionary (1), an antioxidant is "a chemical compound or substance that inhibits oxidation." Halliwell and Gutteridge (2) have defined an antioxidant as "any substance that, when present at low concentrations compared to those of an oxidizable substrate, significantly delays or inhibits oxidation of that substrate." I would propose broadening that definition by considering biological antioxidants to be "compounds that protect biological systems against the potentially harmful effects of processes or reactions that can cause excessive oxidations." Using the latter definition, we can begin describing the various types of biological antioxidants, their locations within or outside of cells, and their mechanisms of action.

## Types of Biological Antioxidants

**Enzymatic Processes.** Several enzymes have evolved whose primary functions appear to be to decrease the amount of oxidants or potential oxidants in the body and, therefore, to serve a protective function with respect to biological oxidants.

*Superoxide dismutase.* This enzyme, discovered by McCord and Fridovich (3), has allowed biologists and biochemists to enter into the free radical field, studying the formation and metabolism of radicals and oxidants such as superoxide ($O_2^-\cdot$), hydrogen peroxide ($H_2O_2$), and hydroxyl radical ($OH\cdot$). Superoxide dismutase catalyzes the following reaction:

$$2\ O_2^-\cdot + 2\ H^+ \rightarrow O_2 + H_2O_2$$

*Catalase.* One of the products of the action of superoxide dismutase is $H_2O_2$. This molecule can be detoxified by several enzymes such as catalase, which catalyzes the following reaction:

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

In the absence of these enzymes, and in the presence of transition metals such as iron or copper, the following reaction occurs which generates the extremely reactive hydroxyl radical $OH\cdot$.

$$O_2^-\cdot + Fe^{3+} \rightarrow O_2 + Fe^{2+}$$

$$H_2O_2 + Fe^{2+} \rightarrow OH\cdot + OH^- + Fe^{3+}$$

*Selenium glutathione peroxidase (Se-GSH-Px).* Although Se-GSH-Px was originally reported to reduce fatty acid hydroperoxides (4) in cell membranes, phospholipase $A_2$ ($PLaseA_2$) was later suggested to be required in liberating the free fatty acid hydroperoxide, which is the best substrate for Se-GSH-Px. Currently, $PLaseA_2$ and Se-GSH-Px are postulated (5) to act together in converting potentially harmful phospholipid hydroperoxides (PLOOH) to free fatty acid hydroper-

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0037-9727/92/2002-0248$3.00/0
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oxides (LOOH) and lysophospholipids, and then to harmless fatty acid alcohols (LOH).

$$\text{PLOOH} \xrightarrow{\text{PLaseA}_2} \text{Lysophospholipid} + \text{LOOH}$$

$$\text{LOOH} + 2\ \text{GSH} \xrightarrow{\text{Se-GSH-Px}} \text{LOH} + \text{GSSG} + \text{H}_2\text{O}$$

*Phospholipid hydroperoxide glutathione peroxidase (PLOOH-GSH-Px).* A relatively new enzyme (PLOOH-GSH-Px) has been described (6) that acts directly on phospholipid hydroperoxides, without the necessity of hydrolyzing the free fatty acid hydroperoxide from the phospholipid. The reaction catalyzed by PLOOH-GSH-Px is shown below:

$$\text{PLOOH} + 2\ \text{GSH} \xrightarrow{\text{PLOOH-GSH-Px}} \text{Phospholipid-LOH} + \text{GSSG} + \text{H}_2\text{O}$$

In this way, the membrane-perturbing properties of the phospholipid-LOOH are removed without the requirement for activating or mobilizing PLaseA2, which might liberate excessive amounts of substrates for prostanoid synthesis. In addition, PLOOH-GSH-Px can also reduce membrane-associated cholesterol hydroperoxides, thereby further diminishing the amount of potentially harmful lipid hydroperoxides (7).

**Nonenzymatic Processes.** A relatively large number of compounds have been shown to possess some measure of antioxidant activity, usually determined by their ability to prevent lipid peroxidation or metal-catalyzed radical reactions. One convenient method of characterizing them is by their solubility in either lipid solvents or in aqueous systems (8). The structures of several of these biological antioxidants are shown in Figure 1.

*Lipid-soluble antioxidants.* Major lipid-soluble antioxidants include the tocopherols, the carotenoids, and the quinones.

*Tocopherols.* The tocopherols, such as $\alpha$-tocopherol or vitamin E, have proven to be effective inhibitors of the propagation step of lipid peroxidation (9, 10). Each tocopherol molecule can react with two peroxyl radicals, as shown below:

$$\alpha\text{-tocopherol} + \text{LOO}\cdot \rightarrow \alpha\text{-tocopherol}\cdot + \text{LOOH}$$

$$\alpha\text{-tocopherol}\cdot + \text{LOO}\cdot \rightarrow \text{LOO-}\alpha\text{-tocopherol}$$

The first product is the $\alpha$-tocopheroxyl radical ($\alpha$-tocopherol$\cdot$), which is a resonance-stabilized, oxygen-centered radical. The $\alpha$-tocopheroxyl radical can react with another peroxyl radical to form a stable adduct, which has been isolated (11). The relative effectiveness of $\alpha$-tocopherol as an antioxidant in liposome and membrane preparations is only 1–2% of that in homogeneous solutions. This finding has been attributed to a lower mobility of $\alpha$-tocopherol in the membranes and to the greater probability of chain propagation in the more tightly structured environment of the membranes (12).

Recently, interest has begun to focus on the tocotrienols, which have now been shown to be more effective antioxidants than $\alpha$-tocopherol in solution and in biological membranes (13).

*Carotenoids.* As summarized recently (14, 15), most members of this large family of conjugated polyenes have very similar biological antioxidant activity. Carotenoids are bleached when exposed to radicals such as those that arise during lipid peroxidation (16), which indicates that these pigments must also intercept active oxygen species. Their long, conjugated, double-bond systems make them excellent substrates for radical attack. For example, carotenoids are very rapidly bleached (17) when exposed to the trichloromethylperoxyl radical ($CCl_3OO\cdot$), which is generated during the pulse radiolysis of chloroform in the presence of oxygen.

The effectiveness of $\beta$-carotene as an antioxidant at various oxygen tensions was evaluated by Burton and Ingold (18), who reported that $\beta$-carotene was a better antioxidant at 15 torr (2% oxygen) than at 150 torr (20% oxygen), and that it acted as a prooxidant at 760 torr. These authors suggested that $\beta$-carotene (CAR) might react directly with the peroxyl radical (LOO$\cdot$) to form a resonance-stabilized, carbon-centered radical, as shown below:

$$\text{CAR} + \text{LOO}\cdot \rightarrow \text{LOO-CAR}\cdot$$

If this, indeed, was the case, carotenoids should be able to quench at least two peroxyl radicals (14), as does $\alpha$-tocopherol.

$$\text{LOO-CAR}\cdot + \text{LOO}\cdot \rightarrow \text{LOO-CAR-OOL}$$

However, the quenching ability need not stop here, but could continue with the formation of multiple resonance-stabilized, carbon-centered radicals on a single carotene molecule, followed by radical-radical quenching with the addition of another peroxyl radical, as shown below:

$$\text{LOO-CAR-OOL} + \text{LOO}\cdot \xrightarrow{+\text{LOO}\cdot} (\text{LOO})_2\text{-CAR-(OOL)}_2$$

Although we know very little about the interaction of $\beta$-carotene and radicals, some of the products formed from the reaction of peroxyl radicals with $\beta$-carotene have recently been described (19–21). The products are primarily carbonyl derivatives of $\beta$-carotene, along with some epoxides.

*Quinones.* Coenzyme Q (ubiquinone, UQ), in its

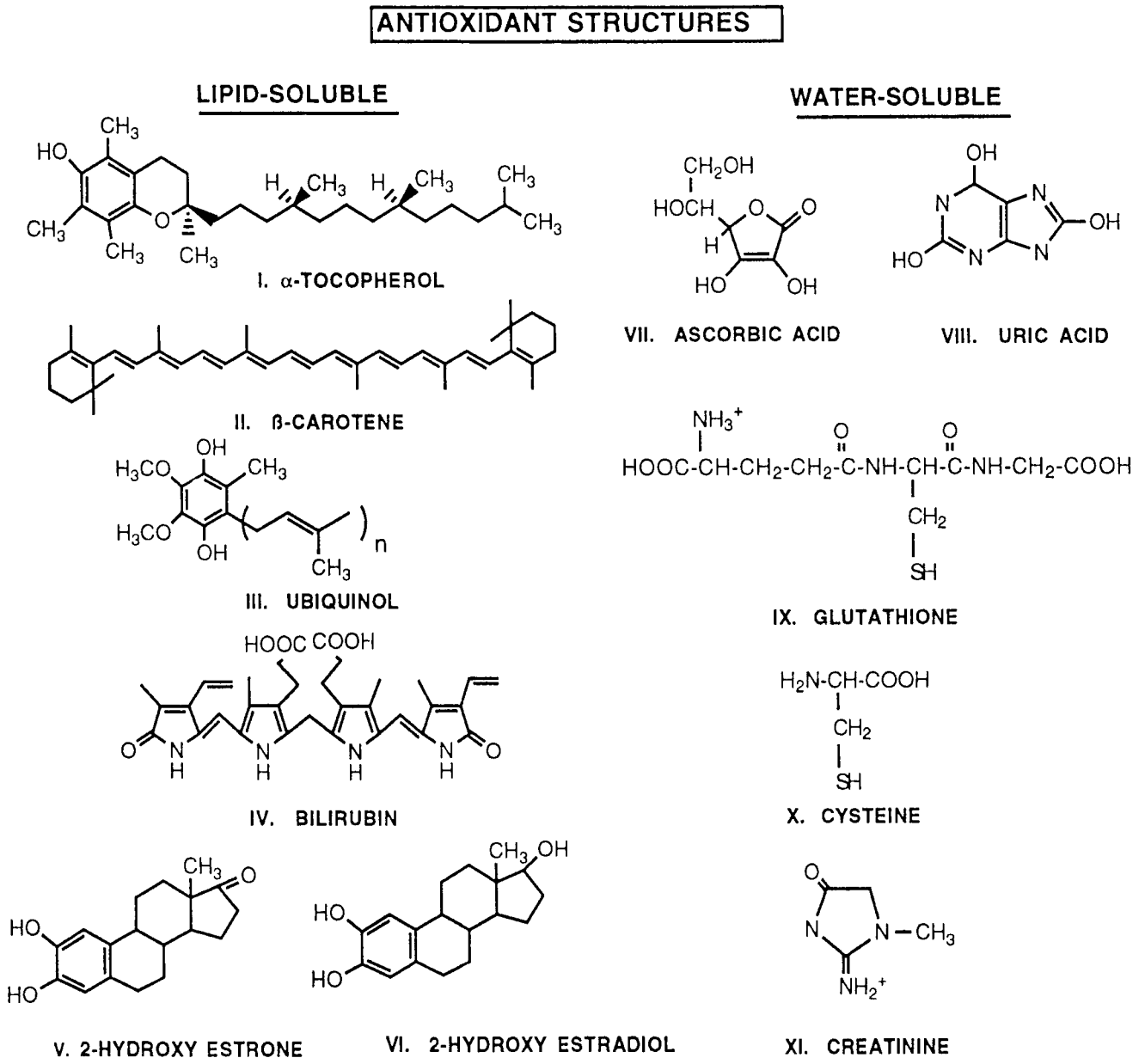


**Figure 1.** Structures of common lipid-soluble and water-soluble biological antioxidants.

reduced form ($UQH_2$), has long been known to inhibit lipid peroxidation (22, 23). Although $UQH_2$ might function by reducing the α-tocopheroxyl radical back to α-tocopherol (24), $UQH_2$ can also function in the absence of α-tocopherol, presumably by acting directly on either peroxyl or alkoxyl radicals (23).

*Bilirubin.* This compound, although primarily considered as an end product of heme metabolism, can effectively inhibit lipid peroxidation in both homogeneous solution and liposomes. Bilirubin is almost as effective an antioxidant as α-tocopherol in the latter system (25).

*Water-soluble antioxidants.* Major water-soluble antioxidants include ascorbic acid, uric acid, metal-binding proteins, and binding proteins for heme and heme-containing proteins.

*Ascorbic acid.* This compound has been reported to act synergistically with α-tocopherol in preventing lipid peroxidation in a micellar preparation of linoleic acid or methyl linoleate (26). This synergistic mechanism may well explain the action of many other water-soluble antioxidant compounds with respect to the inhibition of lipid peroxidation (8).

*Uric acid.* Uric acid, although primarily considered as the waste product of purine metabolism, shows strong antioxidant activity with respect to water-soluble free-radicals at a physiological concentration (27). It is ineffective, however, with lipid-soluble radical generators (28).

*Metal-binding proteins.* Several metal-binding proteins reduce the effective concentration of transition metals that are capable of reacting with hydroperoxides (LOOH), as follows:

$$LOOH + Fe^{2+} \rightarrow LO\cdot + OH^- + Fe^{3+}$$

Transferrin normally carries only 20–30% of its full capacity of iron. Because of its very high affinity with iron, the free iron concentration in human plasma is kept at a vanishing low level (29). Lactoferrin, which is made in neutrophils but released into plasma, has very similar properties to transferrin (30). Ceruloplasmin appears to have two antioxidant properties. First, it binds copper ions and, therefore, prevents this transition metal from catalyzing hydroperoxide decomposition to radicals. Second, ceruloplasmin oxidizes $Fe^{2+}$ to $Fe^{3+}$ and concomitantly converts $O_2$ to water. Unlike the nonenzymatic process described above, however, radical species are not formed in this reaction (30). Albumin can also bind copper ions, thereby preventing them from initiating radical-generating reactions (31).

*Binding proteins for heme and heme-containing proteins.* Both free heme and heme proteins such as hemoglobin or myoglobin are pro-oxidants. They can react with $H_2O_2$, forming a ferryl species that can initiate lipid peroxidation (32). In addition, $H_2O_2$ can cause the release of free iron from either heme or heme proteins, which in turn can also stimulate peroxidative reactions. Two serum proteins with similar properties protect against such extracellular events. Haptoglobin binds hemoglobin with high affinity, whereas hemopexin binds free heme. Both complexes are then rapidly cleared from the circulation (33).

## Location of Biological Antioxidants

Every body compartment has some type of antioxidant defense, although some extracellular fluids appear to be quite deficient in some of these defenses, as described in the following sections. What appears to be most important, with respect to antioxidant defenses, is what assay is chosen by a given investigator. This problem is discussed carefully by Halliwell and Gutteridge (30), who point out that assays that solely detect peroxyl radical processes may very well not measure the antioxidant activity of metal binders or chelators.

**Antioxidants in Extracellular Fluids.** The major extracellular antioxidants are listed in Table I, along with their specific locations and mechanism of action. As can be noted, many of these compounds seem to function by decreasing the concentration of metal ions that catalyze reactions that generate highly reactive radical species (30).

**Intracellular Antioxidants.** Intracellular antioxidants tend to be the lipid-soluble compounds associated with cell membranes. As yet, very little information has accumulated with respect to the specific localization of these lipid-soluble compounds.

## Mechanisms of Action

**Effects on Radical-Initiated Processes.** Radical processes have been extensively studied in biological systems because of the ease of initiating lipid peroxidation from polyunsaturated fatty acids. As almost all biological lipids contain some polyunsaturated fatty acids present in cholesteryl esters, triacylglycerols, and phospholipids, or even as free fatty acids, radical-initiated lipid peroxidation is a rather common process. The remarkable thing is that we are all not turning rancid from this pervasive peroxidative process. Our

**Table I.** Extracellular Antioxidants

| Antioxidant | Location | Mechanism of action |
|---|---|---|
| Albumin | Plasma | Binds copper ions and bilirubin |
| Ascorbic acid | Plasma, synovial fluid, cerebrospinal fluid | Scavenges both radicals and $^1O_2$; reduces $\alpha$-tocopheroxyl radical back to $\alpha$-tocopherol |
| Ceruloplasmin | Plasma | Binds copper ions and prevents reinitiation reactions; oxidizes $Fe^{2+}$ to $Fe^{3+}$, thereby inhibiting iron-dependent lipid peroxidation |
| Haptoglobin | Plasma | Binds free hemoglobin, preventing it from reacting with $H_2O_2$ to accelerate lipid peroxidation |
| Hemopexin | Plasma | Binds free heme and functions similarly to haptoglobin |
| Lactoferrin | Plasma (from leukocytes) | Similar to transferrin |
| Transferrin | Plasma | Removes free iron from solution, thereby preventing reinitiation reactions |
| Uric acid | Plasma | Radical scavenger |

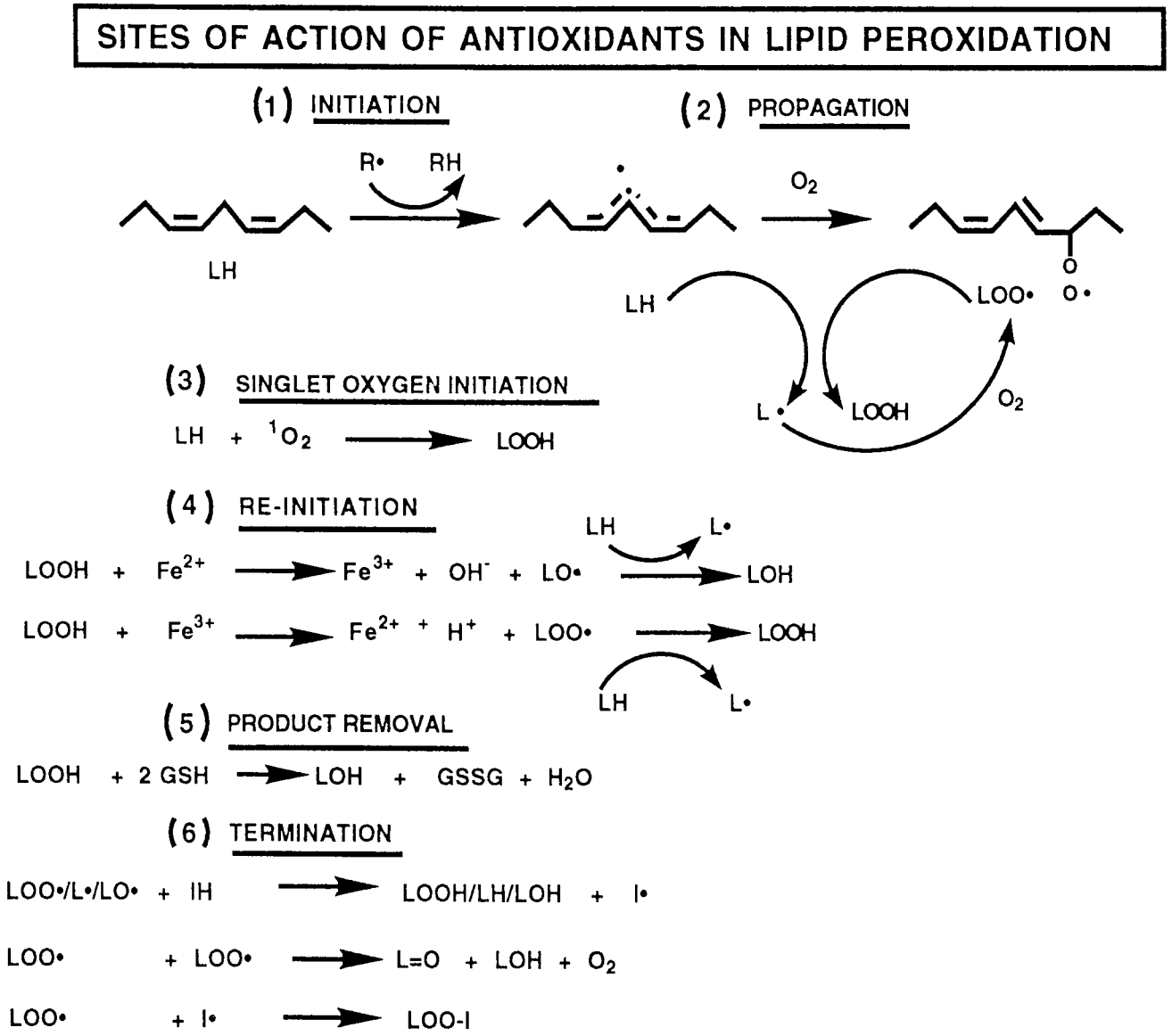


**Figure 2.** Sites of action of antioxidants in lipid peroxidation.

salvation is that a wide variety of antioxidants have evolved along with, or possibly one step ahead of, the factors that are likely to cause lipid peroxidation. Antioxidant effects on lipid peroxidative reactions are graphically described in Figure 2. The numbered steps displayed in Figure 2 are discussed individually below.

**Initiation (Step 1).** The first step in the initiation of lipid peroxidation from a polyunsaturated fatty acid is attack by a radical species (R·) capable of abstracting one of the doubly allylic hydrogen atoms on the carbon atom between two double bonds (34). A wide variety of radical species, such as hydroxyl radical (OH·), peroxyl radical (LOO·), alkoxyl radical (LO·), or alkyl radical (L·), can initiate this reaction (35). Therefore, any compound that can react with these initiating radicals without generating a reactive radical species can be considered to be an antioxidant acting by inhibiting initiation.

**Propagation (Step 2).** The product of the radical attack upon a polyunsaturated fatty acid (LH) is a delocalized pentadienyl radical (L·), which can react extremely rapidly with oxygen to form the peroxyl radical LOO·. The peroxyl radical can then extract a hydrogen atom from an unsaturated fatty acid to yield another free radical (L·) and a peroxide (LOOH). Any process or reaction that decreases the local concentration of oxygen would be expected to decrease the formation of peroxyl radicals in lipid peroxidation.

**Singlet Oxygen Initiation (Step 3).** Singlet oxygen, which can initiate lipid peroxidation from unsaturated fatty acids, may represent one of the ways that this electronically excited species of oxygen is toxic to biological systems. Although the effects of singlet oxygen

are usually limited to photosensitized reactions (36), singlet oxygen may be generated either in eosinophils (37) or from the reaction of ozone with biological material (38). Thus, this oxidant may play a broader role in biological systems than originally thought (39).

Carotenoid pigments are excellent quenchers of singlet oxygen, inasmuch as they react at a diffusion-controlled rate without being consumed in the process (40). Carotenoids readily accept the energy of singlet oxygen, and then dissipate this energy to the solvent system, as seen below:

$$^1O_2 + \text{carotenoid} \rightarrow {}^3O_2(\text{ground state oxygen}) + {}^3\text{carotenoid}$$

$$^3\text{carotenoid} \rightarrow \text{carotenoid} + \text{heat}$$

Sum: $^1O_2 \rightarrow {}^3O_2 + \text{heat}$

Carotenoids not only can quench singlet oxygen (41), but also can react directly with radicals involved in lipid peroxidation (42).

**Reinitiation (Step 4).** One of the most common ways of initiating lipid peroxidation is by means of the metal-catalyzed breakdown of peroxides already present in the system. Both oxidized and reduced transition metals, such as iron or copper, can catalyze the decomposition of peroxides to form either alkoxyl, alkyl, or hydroxyl radicals. All of these species can then initiate the peroxidative process, as described in Step 1 above. Under these circumstances, any compound or process that can decrease the free concentration of iron or copper in biological systems will effectively act as reinitiation inhibitors. Although the free transition metal ion may initiate cleavage, as shown in Figure 2, uncertainty still exists about the actual species involved (43, 44).

**Product Removal (Step 5).** Reinitiation reactions can also be prevented by removal of the hydroperoxide LOOH, the product of the first three steps of lipid peroxidation. As already mentioned, this process involves the use of glutathione-dependent selenoperoxidases that catalyze the reduction of the hydroperoxide to the corresponding alcohol, LOH. Although glutathione peroxidase has long been considered to be the primary enzyme involved in this process, a newly characterized selenoperoxidase, phospholipid hydroperoxide glutathione peroxidase, appears to be even more important, since it can react directly with membrane phospholipid hydroperoxides (7, 45).

**Termination (Step 6).** Compounds that react with the chain propagating radical species, such as the peroxyl or alkoxyl radicals, and result in the formation of species no longer capable of hydrogen abstraction are considered chain-breaking antioxidants. A variety of compounds, such as phenols, aromatic amines, and conjugated polyenes, can function as chain-breaking antioxidants. The phenol, $\alpha$-tocopherol, has been proposed as the major lipid-soluble, chain-breaking antioxidant in human plasma (46), although other compounds in plasma, such as carotenoids (47), ubiquinol (48), and bilirubin (25), are also active as lipid-soluble antioxidants. Recently, it was suggested that ubiquinol-10 protects human low density lipoprotein more effectively than does $\alpha$-tocopherol (49).

## Summary

Various types of biological antioxidants, including enzymes, other proteins, and smaller molecules, have been described, and their location within the body has been discussed. For many of these compounds, we have some idea of their mechanism of action. For many others, we are still in the process of developing and testing hypotheses that will help us to understand how biological antioxidants function. This understanding should prove to be valuable in more effectively treating diseases that have as their basis some oxidant or radical involvement.

This work was partially supported by National Institutes of Health Grant CA-51506.

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