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Superoxide: a two-edged sword

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Abstract

Superoxide (O_2^-) is the compound obtained when oxygen is reduced by one electron. For a molecule with an unpaired electron, O_2^- is surprisingly inert, its chief reaction being a dismutation in which it reacts with itself to form H_2O_2 and oxygen. The involvement of O_2^- in biological systems was first revealed by the discovery in 1969 of superoxide dismutase, an enzyme that catalyzes the dismutation of O_2^-. Since then it has been found that biological systems produce a bewildering variety of reactive oxidants, all but a few arising ultimately from O_2^-. These oxidants include O_2^- itself, H_2O_2 and alkyl peroxides, hydroxyl radical and other reactive oxidizing radicals, oxidized halogens and halamines, singlet oxygen, and peroxynitrite. These various oxidants are able to damage molecules in their environment, and are therefore very dangerous. They are thought to participate in the pathogenesis of a number of common diseases, including among others malignancy, by their ability to mutate the genome, and atherosclerosis, by their capacity for oxidizing lipoproteins. Their properties are put to good use, however, in host defense, where they serve as microbicidal and parasiticidal agents, and in biological signalling, where their liberation in small quantities results in redox-mediated changes in the functions of enzymes and other proteins.

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- Regulation
- Host defense

Introduction

The reduction of a molecule of oxygen to two molecules of water is the major source of energy in aerobic biological systems. This reduction requires 4 electrons:

$$O_2 + 4\ e^- + 4\ H^+ \rightarrow 2\ H_2O$$

If these electrons are passed to oxygen one at a time, a series of partially reduced products is generated (1). The first of these is superoxide (O_2^-):

$$O_2 + e^- \rightarrow O_2^- \qquad E_o = -300\ mV$$

Reduction of O_2^- by the second electron yields hydrogen peroxide:

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

•OH (hydroxyl radical) plus the first molecule of water arise when the third electron is passed on to H_2O_2:

$$H_2O_2 + e^- + H^+ \rightarrow \bullet OH + H_2O$$

And finally, the fourth electron produces the second molecule of water from the hydroxyl radical:

$$\bullet OH + e^- + H^+ \rightarrow H_2O$$

Unlike most molecules with unpaired electrons, O_2^- is surprisingly inert. In aqueous systems, its principal reaction is with itself to generate a molecule of H_2O_2 and a molecule of oxygen in a dismutation reaction (2):

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

It is also a weak base, its conjugate acid being the much more reactive hydroperoxyl radical:

$$O_2^- + H^+ \rightarrow HO_2^{\bullet} \qquad pK_a = 4.8$$

It does, however, have certain other chemical properties that are important in a biological context. These include 1) its participation in the so-called Haber-Weiss reaction to generate •OH and in a closely related reaction to generate alkoxyl radical (RO•), reactions that are catalyzed by transition metals such as iron or copper (3-5):

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

$$Fe^{2+} + H_2O_2 \text{ (or ROOH)} \rightarrow Fe^{3+} + OH^- + \bullet OH \text{ (or RO}\bullet)$$

2) its ability to obtain Fe^{2+} needed for the Haber-Weiss reaction by liberating it from the iron storage protein ferritin and from iron-sulfur proteins such as aconitase (6,7); 3) its reaction with •OH to form singlet oxygen (8):

$$O_2^- + \bullet OH \rightarrow {}^1O_2 + OH^-$$

and 4) its reaction with nitric oxide to form peroxynitrite, a highly reactive oxidant that breaks down to produce a nitrating agent (9,10):

$$O_2^- + NO\bullet \rightarrow OONO^- + H^+ \rightarrow ? \; NO_2^+ + OH^-$$

In addition, the H_2O_2 produced by the dismutation of O_2^- is used by phagocytes to oxidize halide ions to the level of hypohalous acids (e.g., HOCl) (11), a group of highly reactive compounds which in turn react with amines to produce halamines (e.g., NH_2Cl), some of which are even more reactive than the hypohalous acids (12,13). In turn, the hypohalous acids can react with H_2O_2 to generate singlet oxygen (14,15). For example,

$$OBr^- + H_2O_2 \rightarrow {}^1O_2 + H_2O + Br^-$$

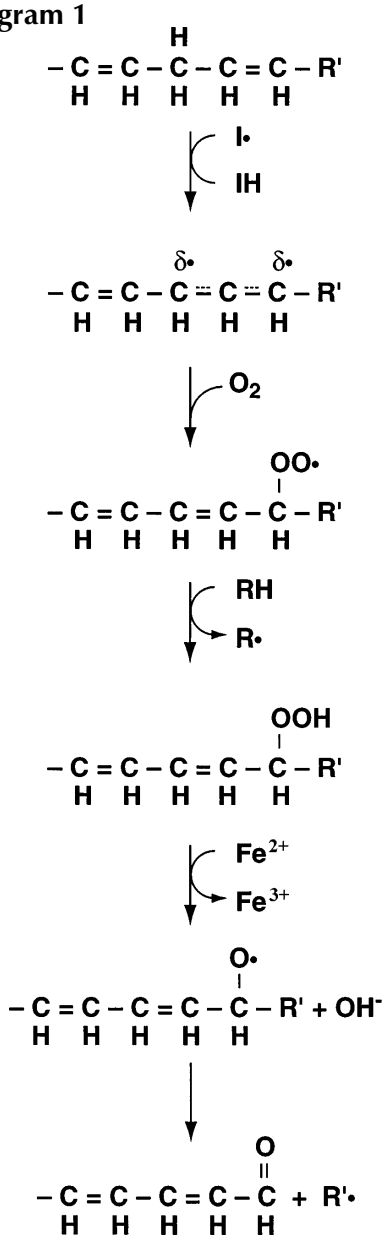
All this was of little interest to biologists, however, until 1969, when McCord and Fridovich (16) discovered superoxide dismutase. This enzyme destroys O_2^- by catalyzing the dismutation reaction described above. The ubiquitous occurrence of an enzyme that catalyzes the destruction of O_2^- implied that O_2^- had to be participating in an important way in the biological economy (1,17). Certain aspects of the nature of its participation are reviewed here.

Superoxide the evil

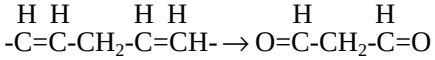
As the precursor of a large number of highly reactive oxidizing agents, including oxidizing radicals, singlet oxygen, peroxynitrite and oxidized halogens such as HOCl, O_2^- clearly had the potential to inflict considerable damage on biological systems. That O_2^- could realize this potential was shown in numerous experiments, at first indirectly by demonstrations, for example, that oxidative stress induced substantial increases in the superoxide dismutase concentration of *E. coli* (18), and later directly by genetic experiments with bacteria and eukaryotes containing no superoxide dismutase or excess superoxide dismutase (19-24). Damage to DNA (25), proteins (26) and lipids (27,28) have all been documented as consequences of exposure to O_2^- and its descendants. DNA damage may lead to the production of abnormal bases such as thymine glycol and 8-hydroxyguanine (25,29-31) or to strand breakage through a series of reactions initiated by the abstraction of a 4' hydrogen atom from a ribose residue (32-35). On proteins, susceptible residues such as cysteine and histidine are oxidized (36-38), leading in some cases to the production of oxo groups that can be assayed to provide an index of oxidative damage to proteins (36,39). In addition, tyrosine residues are nitrated, a consequence of the spontaneous decay of $ONOO^-$ into a nitrating agent of some sort (40,41). Hypohalous acids will decarboxylate α-amino acids to aldehydes (11), and will halogenate tyrosine and heterocycles (e.g., adenosine, NAD) (42-44). Polyunsaturated fatty acid

residues on phospholipids and triglycerides undergo peroxidation to form toxic alkyl hydroperoxides and aldehydes (45-47) (see Diagram 1).

Diagram 1

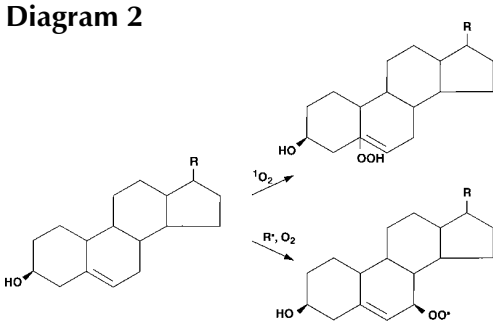


Among these is malondialdehyde, produced by the oxidation at the two double bonds flanking the methylene carbons of polyunsaturated fatty acids:



Because of its bifunctionality, malondialdehyde is an effective cross-linking agent, able to act on macromolecules such as DNA and proteins. In addition, malondialdehyde reacts with thiobarbituric acid to form a pink compound whose color has been used as a measure of lipid peroxidation. Cholesterol is oxidized at its susceptible 4- and 6-carbons (see Diagram 2).

Diagram 2



These oxidation reactions are currently believed to participate in the pathogenesis of a number of important degenerative diseases, including atherosclerosis, in which the uptake of oxidized lipoproteins via the scavenger receptor of macrophages is thought to be an early step in the formation of the endothelial foam cells and lipid deposits characteristic of that condition (48,49); malignancies, some of which may arise as a result of oncogene mutations caused by oxidative damage to DNA (50,51); arthritis, due to joint damage inflicted in part by oxidants released at sites of inflammation (52), and possibly aging itself (53,54), not a disease but an inevitable consequence of living.

Because of the evil nature of these oxidants, many defenses have been erected against them. These defenses include both enzymes and low molecular weight compounds.

Antioxidant enzymes

Four enzymes comprise three enzyme-based antioxidant systems that deal with oxi-

dants formed by the partial reduction of oxygen:

1. *Superoxide dismutase* (2,55,56). Superoxide dismutase catalyzes the destruction of O_2^- by converting it to oxygen and H_2O_2:

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

The uncatalyzed reaction is very rapid, proceeding with a rate constant of ca. 10^6-10^7 M^{-1} sec^{-1} at the pH values prevailing in tissues. Superoxide dismutase, however, greatly accelerates the rate of destruction of O_2^-, in part by converting a second order reaction to a first order reaction. Because of the effect of superoxide dismutase, steady-state concentrations of O_2^- in tissues are many orders of magnitude lower than they would be if the elimination of O_2^- was solely dependent on its spontaneous dismutation.

Two forms of superoxide dismutase are present in eukaryotic cells: a form that contains Cu^{2+} and Zn^{2+}, the former serving as the redox center and the latter as a structural element, and a form that contains only one metal, namely Mn^{2+}, which functions as the redox center. The Cu^{2+}/Zn^{2+} form, a 32-kDa dimer, is found in the cytosol, while the Mn^{2+} form, an 80-kDa tetramer, is located in mitochondria. The Mn^{2+} form is also found in bacteria, as is a third form of superoxide dismutase containing Fe^{2+} as its redox element. The concentrations of the Cu^{2+}/Zn^{2+} and Fe^{2+} forms of superoxide dismutase are unaffected by oxidative stresses, but the Mn^{2+} form is inducible in both bacteria and eukaryotic cells, its activity increasing with oxidative stress.

Mutant forms of the Cu^{2+}/Zn^{2+} enzyme appear to explain the familial forms of a fatal neurological disease known as amyotrophic lateral sclerosis, or motor neuron disease (57). In this condition, the motor neurons in the patient's cerebral cortex and spinal cord degenerate over the course of a few years, leading to weakness and eventually paralysis, with death from pneumonia caused by the inability of the patient to clear respiratory secretions. The mutant enzymes dismute superoxide in a normal fashion, but they have excess peroxidase activity, an activity present in normal Cu^{2+}/Zn^{2+} dismutase to only a very limited extent (58). It is presently thought that the oxidative damage inflicted by the increased peroxidase activity of the mutant dismutase is responsible for the early death of these neurons.

2. *Catalase* (59,60). The H_2O_2 produced by the dismutation of O_2^- or generated by H_2O_2-generating oxidases (e.g., D-amino acid oxidase) is handled by two systems: catalase and a glutathione-dependent antioxidant system that reduces H_2O_2 to water at the expense of NADPH. Catalase is a tetrameric heme enzyme of 240-kDa mass that catalyzes the dismutation of H_2O_2 to oxygen and water:

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

Catalase is erroneously said to work only at high concentrations of H_2O_2, and to serve principally as a backup for the glutathione-dependent system to be discussed below, but the enzyme has a binding site for NADPH, and when this site is occupied, catalase operates at H_2O_2 concentrations in the vicinity of those at which the glutathione-dependent systems operate (61). It is therefore likely that some half the H_2O_2 produced in the cell is destroyed by catalase. Catalase deficiency exists, but is relatively innocuous; the Swiss type is asymptomatic, while the Japanese variety is associated only with ulcers of the oral cavity.

3. *The glutathione-dependent antioxidant system* (62-65). The glutathione-dependent antioxidant system consists of glutathione plus two enzymes: glutathione peroxidase and glutathione reductase. As this system operates, glutathione cycles between its oxidized and reduced forms. The reactions catalyzed by these enzymes are:

$$2\ GSH + H_2O_2 \xrightarrow{\text{GSH peroxidase}} GSSG + 2\ H_2O$$

$$GSSG + NADPH + H^+ \xrightarrow{\text{GSSG reductase}} 2\ GSH + NADP^+$$

Net:

$$H_2O_2 + NADPH + H^+ \rightarrow 2\ H_2O + NADP^+$$

Like other enzymes that catalyze the interconversion of sulfhydryl groups and disulfides, the 22-kDa glutathione reductase uses FAD as its cofactor. Glutathione peroxidase, another 22-kDa protein, is unusual, however, in that the redox element in its active site is selenocysteine. The selenocysteine is introduced into the protein by a special t-RNA that is initially charged with serine but undergoes a series of reactions that convert it to t-RNA^{selenoCys}. Selenocysteine is encoded by the triplet UGA, which ordinarily introduces a stop but in the context of the glutathione peroxidase mRNA is recognized by the selenocysteine-linked t-RNA (66). The antioxidant properties of selenium are explained by its occurrence in glutathione peroxidase.

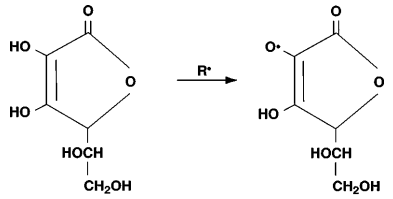
Families with inherited deficiencies of glutathione peroxidase (67,68) and glutathione reductase (69) have been reported. Affected members manifest a mild to moderately severe hemolytic anemia that is aggravated by infection and by oxidant drugs such as nitrofurantoin and certain sulfonamides. Selenium deficiency on a nutritional basis leads to a cardiomyopathy that may in part represent oxidative damage due to glutathione peroxidase deficiency but, in addition, is likely to reflect injury caused by the deficiency of other selenium-containing enzymes, because it is not seen in inherited glutathione peroxidase deficiency (70,71).

Lipid hydroperoxides, which are formed during the peroxidation of lipids containing unsaturated fatty acids, are reduced, not by the usual glutathione peroxidase, but by a special enzyme designed specifically to handle peroxidized fatty acids in phospholipids. This enzyme, known as phospholipid hydroperoxide glutathione peroxidase (72), is an 18-kDa protein that can reduce both H_2O_2 and lipid hydroperoxides to the corresponding hydroxides (water and a lipid hydroxide, respectively). In contrast to the phospholipid hydroperoxide glutathione peroxidase, ordinary glutathione peroxidase is unable to act on lipid hydroperoxides.

Low molecular weight antioxidants

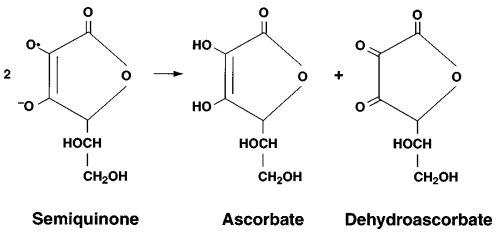
Many low molecular weight compounds can act as biological antioxidants, including among others carotenoids, bilirubin and uric acid. The most important of these, however, are two vitamins: ascorbic acid (vitamin C) (73-76), and α-tocopherol (vitamin E) (77-80). Ascorbic acid, a very water soluble compound, reacts with free radicals that arise in the aqueous compartments of tissues, forming the innocuous ascorbate semiquinone (81,82) (see Diagram 3).

Diagram 3



The semiquinone is consumed in a dismutation reaction in which two semiquinone molecules react to produce a molecule of ascorbate and a molecule of dehydroascorbate (see Diagram 4).

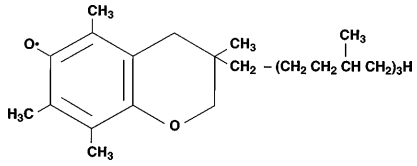
Diagram 4



The dehydroascorbate is then enzymatically reduced back to ascorbate by dehydroascorbate reductase (83).

α-Tocopherol, a highly lipophilic molecule, is the chief antioxidant in biological membranes. It reacts with free radicals to form the highly stable tocopherol semiquinone (84) (see Diagram 5).

Diagram 5



The semiquinone is then reduced back to the alcohol by ascorbic acid (85-87).

A small antioxidant with special properties is taurine (2-aminoethyl sulfonic acid). This compound reacts with hypohalous acids and halamines (77-80,88-91), which are routinely generated by phagocytes for use as microbicidal killing agents (see below, Superoxide the good) but whose great reactivity allows them to inflict major damage in the tissues in which they are released. The product of the reaction between an oxidized halogen and taurine, however, is a halamine with exceptionally low reactivity for this class of compounds - such low reactivity, in fact, that it is harmless to tissues (92). An example is the reaction between taurine and hypochlorous acid to form taurine chloramine:

$$^{=}O_3S\text{-}CH_2\text{-}CH_2\text{-}NH_2 + HOCl \rightarrow {}^{=}O_3S\text{-}CH_2\text{-}CH_2\text{-}NHCl + H_2O$$

Taurine is therefore able to detoxify these very reactive and dangerous oxidized halogens by converting them to innocuous compounds.

Superoxide the good

O_2^- production by phagocytes

The consequences of O_2^- production in tissues are not all bad. In fact, the production of O_2^- can be lifesaving. This was first demonstrated by the surprising finding that O_2^- is produced in large quantities by stimulated phagocytes (93,94), and that individuals with chronic granulomatous disease, an inherited disorder in which phagocytes are unable to manufacture O_2^-, are highly susceptible to very dangerous bacterial and fungal infections that formerly killed most of the patients before they reached their tenth birthday (95), though recently the advent of newer modes of treatment has greatly improved their outlook (96). The O_2^- produced by these cells is made by the leukocyte NADPH oxidase (97), a membrane-associated enzyme, that catalyzes the one-electron reduction of oxygen at the expense of NADPH:

$$2\ O_2 + NADPH \rightarrow 2\ O_2^- + NADP^+ + H^+$$

The oxidase is dormant in resting cells, but develops catalytic activity when the cells encounter a microorganism or are exposed to any of several soluble stimuli, including N-formylated peptides, the complement polypeptide C5a or leukotriene B_4.

The O_2^- produced by these cells is only weakly microbicidal, though it can inactivate bacterial iron-sulfur proteins such as aconitase. The major microbicidal oxidant of phagocytes is HOCl, produced by the H_2O_2 generated by the dismutation of O_2^- (98-100). HOCl production is catalyzed by myeloperoxidase, which is abundant in neutrophils and monocytes and catalyzes the two-electron oxidation of the chloride ion by hydrogen peroxide (12,101):

$$Cl^- + H_2O_2 \rightarrow OCl^- + H_2O$$

Myeloperoxidase can also oxidize Br^- and I^- to the corresponding hypohalous acids (102). An enzyme in eosinophils, the eosinophil peroxidase, catalyzes the same reaction, except that Cl^- is not a substrate; the chief product of the eosinophil peroxidase reaction is HOBr (102,103). The hypohalous acid will react with any of the hundreds of

amines present in the cell to form a vast array of halamines (12,88,102,104) whose toxicities range from none to extreme, the degree of toxicity roughly correlating with the lipid solubility of the halamine.

Recent work with *E. coli* has revealed a mechanism by which oxidized halogens can kill a microorganism. It has been observed that the lethal action of HOCl against *E. coli* takes place on the membrane. This membrane contains a binding site to which the origin of replication of the *E. coli* genome (oriC) has to attach before the DNA can be copied during bacterial replication. HOCl is able to inactivate this binding site, and experiments have shown that the fraction of bacteria killed by HOCl at a given time is virtually identical to the fraction of binding sites that have been inactivated (105). These results imply that the destruction of this binding site is tantamount to the destruction of the microorganism itself.

The oxidizing radicals produced from the O_2^- in the Haber-Weiss and related reactions also participate in the oxidative killing of microorganisms, but principally as a backup system. This is shown by the observation that patients with myeloperoxidase deficiency have little or no problem with infections (106,107), in contrast to patients with chronic granulomatous disease, whose very high susceptibility to such infections was discussed above. This indicates the occurrence in phagocytes of a backup microbicidal system that is dependent on oxygen and is active in patients whose neutrophils and monocytes are unable to manufacture oxidized halogens (i.e., cells deficient in myeloperoxidase). This system is highly likely to employ the oxidizing radicals known to be produced by these phagocytes.

A possible antioxidant activity of O_2^- itself

There appears to be an optimum for the intracellular concentration of superoxide dismutase. Too much superoxide dismutase can be just as harmful as too little (108-111). This finding has given rise to the speculation that a little intracellular O_2^- is necessary for the welfare of the cell. O_2^- is chemically able to reduce potentially dangerous semiquinones that might arise in the course of a cell's metabolic activities (112,113):

$$O_2^- + Q\bullet \gg O_2 + QH_2$$

and interference with the detoxification of such semiquinones due to a reduction in the steady-state O_2^- concentration within the cell has been proposed as the basis for the harmful effects of too much superoxide dismutase. On the other hand, the Cu^{2+}/Zn^{2+} dismutase is also a peroxidase, so the possibility remains that the harm caused by excess superoxide dismutase could be a result of peroxidation (114).

Regulation by O_2^- and H_2O_2

It has become increasingly apparent over the last few years that O_2^- and H_2O_2 are signalling molecules, changing the behavior of proteins as diverse as transcription factors and membrane receptors by virtue of their ability to undergo redox reactions with the proteins with which they interact, converting -SH groups to disulfide bonds, for example, and changing the oxidation states of enzyme-associated transition metals. As signalling molecules, O_2^- and H_2O_2 are manufactured by several types of cells, including fibroblasts, endothelial and vascular smooth muscle cells, neurons, ova, spermatozoa and cells of the carotid body. All these cell types appear to use an NAD(P)H oxidase similar to the classical leukocyte NADPH oxidase to produce these oxidants. The stimuli that elicit oxidant production, however, and the purposes for which the oxidants are employed, vary from cell to cell.

1. *Fibroblasts.* Fibroblasts manufacture small but significant amounts of O_2^- in response to inflammatory mediators such as N-formylated peptides and interleukin-1

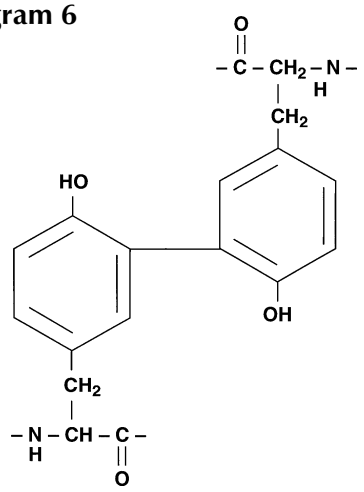
(115-117). The O_2^- produced by these cells has been postulated to function as a signalling molecule. Optical spectroscopy has shown that fibroblast membranes contain a heme protein that is different from the flavocytochrome subunit of the leukocyte NADPH oxidase but has properties very similar to those of the leukocyte protein (115,118). This heme protein has been suggested as the source of the O_2^- made by these cells.

2. *Endothelial and vascular smooth muscle cells*. These cells use an NAD(P)H oxidase to produce O_2^- in response to angiotensin II, a peptide hormone that increases blood pressure (119,120). This increase in blood pressure appears to be due to the consumption by O_2^- of the NO• that is generated on a continuing basis by the endothelial cells. The resulting fall in NO• concentration raises blood pressure by attenuating or eliminating the vasodilatory effect of NO• that normally prevails in the vascular tree.

3. *Neurons*. A recent study has shown that neuronal cells in culture produce oxidants when exposed to amyloid ß-peptide, found in amyloid deposits seen in the brains of patients with Alzheimer's disease, or related peptides from other amyloid diseases. The possibility that this O_2^- is produced by an NADPH oxidase is suggested by the observation that flavoprotein inhibitors known to act on the leukocyte NADPH oxidase also inhibit oxidant production in this system (121). The production of oxidants may be part of a defense used by the neuron against the peptide, with these oxidants perhaps reacting with the peptide to render it susceptible to proteolytic cleavage.

4. *Ova*. At the moment of fertilization, a membrane NADPH oxidase in sea urchin ova is activated to produce large amounts of H_2O_2 (122). This oxidant cross-links the proteins of the fertilization membrane by forming dityrosyl bridges (see Diagram 6), making the membrane impermeable to spermatozoa and thereby preventing polyspermy.

Diagram 6



5. *Spermatozoa*. O_2^- appears to be necessary for the normal function of spermatozoa. When stimulated by a calcium ionophore, normal spermatozoa generate a 3- to 5-min burst of O_2^- (123). The O_2^- produced in this reaction is involved in capacitation of the spermatozoa, because the acrosomal response to a number of stimuli is suppressed by superoxide dismutase (124). On the other hand, spermatozoa that produce O_2^- without stimulation are functionally abnormal, perhaps because of a generalized disruption in their signalling machinery.

6. *The carotid body*. The carotid body is a small organ located at the bifurcation of the common carotid artery that measures the oxygen tension of the blood (125). This organ manufactures H_2O_2 on a continuing basis, and immunological analysis has shown that its cells contain all 4 of the specific subunits of the leukocyte NADPH oxidase, or proteins very closely related to those subunits (126,127). It has been postulated that a carotid body NADPH oxidase very similar or identical to the leukocyte NADPH oxidase is a key component of the oxygen-measuring apparatus of the carotid body.

As to the effects of these oxidants on cellular function, there is a truly astounding number of proteins whose operation appears to depend on the redox state of the cell. Examples include the general transcription

factors NF-kappa B (128-130) and AP-1 (jun/fos) (131), as well as several transcription factors that induce the synthesis of proteins that protect against oxidative stress (e.g., soxR (132,133), soxS (134), oxyR (135)). Membrane receptors and transporters, including, for example, the insulin receptor and receptors for certain neurotransmitters (136-138), are regulated by the redox state of the cell. A very large number of enzymes are also regulated by the cell's redox state. A partial list of proteins whose function is regulated by oxidation-reduction is presented in Table 1. These oxidants generally act by effecting alterations in iron-sulfur clusters (7,139-141) or by inducing the formation or rupture of disulfide bonds (142-145) on whose status the function of the protein depends. It can be postulated that at least for proteins regulated by sulfhydryl-disulfide equilibria, the effects of the oxidants are mediated through alterations in the ratio of oxidized to reduced glutathione, though this hypothesis is very difficult to prove experimentally, at least in intact cells.

Table 1 - Some proteins whose function is regulated by the redox state of the cell.

References are given within parentheses.

Enzymes
Collagenase (146,147)
p21Ras guanine nucleotide-binding protein (148)
Protein tyrosine phosphatase (149)
p56Lck protein tyrosine kinase (150)
Glycogen phosphorylase phosphatase (151)
Glycogen synthase (151)
Phosphofructokinase (151)
Fructose-1,6-bisphosphatase (151)
Hexokinase (151)
Pyruvate kinase (151,152)
Glucose-6-phosphate dehydrogenase (151)
3-Hydroxy-3-methylglutaryl CoA reductase (151)
Serotonin N-acetyltransferase (151)
Guanylate cyclase (151)
Medium-chain fatty acyl CoA dehydrogenase (153)
Xanthine dehydrogenase (154)
Chloroplast NADP-linked glyceraldehyde-3-phosphate dehydrogenase (155)
Chloroplast NADP-linked malate dehydrogenase (155)
Chloroplast sedoheptulose bisphosphatase (155)
Fructose bisphosphatase (155)
NADP-malic enzyme (156)
3α-Hydroxysteroid dehydrogenase (157)
DsbA protein disulfide isomerase from *E. coli* (158)
Creatine kinase (152)
Sarcoplasmic reticulum Ca^{2+}-ATPase (152)
Transcription factors
NF-kappa B (128-130)
AP-1 (jun/fos) (131)
SoxR (132,133)
SoxS (134)
OxyR (135)
Hypoxia-inducible factor 1 (159)
Thyroid transcription factor I (160)
Glucocorticoid receptor (161)
Sp1 (161,162)
Receptors
NMDA receptor (163)
Insulin receptor
NMDA receptor (164,165)
Ryanodine receptor (166)
HoxB5 (167)
c-Myb (167,168)
v-Rel (167)
p53 (169)
Isl-1 (170)
Others
Erythropoietin RNA-binding protein (171)

Conclusion

The discovery of superoxide dismutase by McCord and Fridovich (16) has revolutionized the way biologists think about oxygen. They have come to recognize oxygen as a dangerous gift: indispensable for energy production at the level needed for living at any but the most sluggish pace, but the cause of damage that accumulates slowly over a lifetime, damage that is at least in part responsible for most of the chronic illnesses that develop with age. The challenge for the future is to develop ways to attenuate the damage inflicted by oxygen, O_2^- and their many descendants when they assume their evil forms.

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