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COMMENTARY

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What is the ultimate fate of superoxide anion in vivo?

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Abstract For three decades, oxidative stress and the role of reactive oxygen species in biology have been extensively studied. Recently, a new interest in these areas has emerged with the discovery of superoxide reductases, a family of familiar bacterial metalloenzymes whose heretofore unknown function has now been apparently revealed. In a series of experiments utilizing genetic, molecular biological, and biochemical methods, these enzymes have been shown to be physiologically competent at removing superoxide. The role of these enzymes and their biological relationship to the well-known superoxide dismutases is discussed.

Keywords Superoxide dismutase · Superoxide reductase · Neelaredoxin · Desulfoferrodoxin · Rubredoxin

The biology of superoxide

Superoxide production

Superoxide anion, O_2^-, the single-electron reduction product of dioxygen, is one of the most abundant radicals produced in biological systems. It can be accidentally produced in vivo by several enzymatic systems. In bacteria, the primary source of superoxide comes from one-electron reduction of dioxygen via components of electron transport chains [1, 2]. Superoxide is also produced upon dioxygen reduction by enzymes containing reduced cofactors (e.g. flavin-containing oxidoreductases), by other redox components capable of univalent redox reactions (e.g. ascorbate, thiols, catecholamines, etc.), and by photochemical reactions [3]. In addition, a number of redox dyes can be used to artificially increase intracellular superoxide concentrations [4].

In eukaryotic organisms, superoxide production is compartmentalized. The mitochondrion is a major source of superoxide and it has been estimated that 1–3% of the oxygen reduced by the electron transport chain can form superoxide [5, 6, 7]. Another major source of superoxide in eukaryotic cells is NADPH oxidase, a vesicular enzyme abundant in phagocytic cells (neutrophils, eosinophils), whose purpose is to generate superoxide using NADPH as reductant for release into granules to aid in phagocytic killing of microbes [8, 9]. Whether any significant superoxide is produced in the cytoplasm remains an unanswered question. With a pK_a of 4.8 [10, 11], only a fraction of the superoxide produced within mitochondria or in phagosomes will traverse the membrane and make its way into the cytoplasm.

Adverse effects of superoxide

Small molecules, including polyphenols, catecholamines, α-tochopherol, ascorbate, and thiols, can rapidly react with superoxide and thus contribute to the propagation of radical chain reactions [6, 12]. Superoxide can inactivate some enzymes like catalase or glutathione peroxidase and can oxidize adrenaline, its small size making it difficult to be excluded from active sites [13, 14, 15]. Iron-sulfur clusters of dehydratase enzymes such as aconitase also constitute notable targets for superoxide. Indeed, the ratio of active and inactive aconitase provides a sensitive measure of the changes in steady state superoxide levels occurring in living cells under stress conditions [16, 17]. Superoxide, however, cannot traverse biological membranes except in its protonated state (vide supra); nor does it react very rapidly with polyunsaturated lipids or DNA [10, 18], although these two macromolecules represent some of the biological lesions associated with increased superoxide production.

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Actually, the deleterious effects of superoxide are mainly related to it being a precursor for more potent oxidants like H_2O_2 or hydroxyl radical. Because an increase of superoxide, directly or indirectly, results in an inhibition of cell growth, mutagenesis, and cell death, antioxidant systems are necessary to regulate the concentration of this reactive oxygen species (ROS).

Biological defenses toward superoxide

Superoxide dismutase

Superoxide itself undergoes spontaneous dismutation with a rate constant of 4×10^5 M^{-1} s^{-1} at pH 7.4. However, with an intracellular concentration of $\sim10^{-10}$ M, the half-life for dismutation is 3.5 h; the need for a defense mechanism is evident [7]! Since the discovery of the function of superoxide dismutase (SOD, EC 1.15.1.1) by Fridovich in 1969, enzymatic dismutation was believed to be the only way to metabolize the superoxide anion [19]. SODs, ubiquitous among aerobic organisms, provide a defense against oxidative stress by catalyzing the dismutation of superoxide into oxygen and hydrogen peroxide as shown in Eqs. 1, 2, 3. SODs react with superoxide with a rate constant close to the diffusion limit ($k=2\times10^9$ M^{-1} s^{-1} at pH 7.4) [6]:

$$SOD_{ox} + O_2^- \rightleftarrows SOD_{red} + O_2 \quad (1)$$

$$SOD_{red} + O_2^- + 2H^+ \rightleftarrows SOD_{ox} + H_2O_2 \quad (2)$$

Net: superoxide dismutation:

$$2O_2^- + 2H^+ \rightleftarrows O_2 + H_2O_2 \quad (3)$$

In the presence of SOD the half-life of O_2^- is independent of $[O_2^-]$. Given that the typical intracellular [SOD]$\approx$10 μM, the cellular half-life of O_2^- is $\sim10^{-4}$ s, some 10^8-fold lower than the half-life for spontaneous dismutation.

Superoxide reductase

Surprisingly, anaerobic organisms, which very often lack antioxidant enzymes like SOD and catalase, have various degrees of tolerance to oxygen, ranging from extremely sensitive (e.g. methanogens) to the more aerotolerant (e.g. sulfate-reducing bacteria) organisms. In addition, some microaerophilic bacteria, which survive best in the presence of low partial pressures of oxygen (e.g. *Borrelia burgdorferi*, *Treponema pallidum*), also lack these classical ROS defense enzymes [20].

The discovery of superoxide reductase (SOR, EC 1.15.1.2) was a new step in the understanding of an anaerobe's defense against oxidative stress. SORs catalyze only one of the two reactions of SODs, i.e., the reduction of superoxide to hydrogen peroxide (Eq. 4):

$$SOR_{red} + O_2^- + 2H^+ \rightleftarrows SOR_{ox} + H_2O_2 \quad (4)$$

$$SOR_{ox} + Rd_{red} \rightleftarrows SOR_{red} + Rd_{ox} \quad (5)$$

Net: rubredoxin:superoxide oxidoreductase or superoxide reductase:

$$Rd_{red} + O_2^- + 2H^+ \rightleftarrows Rd_{ox} + H_2O_2 \quad (6)$$

With a few notable exceptions [21, 22, 23], most SORs are poor SODs [24, 25, 26] but rather function as oxidoreductases, deriving the reducing equivalents for superoxide reduction ultimately from reduced pyridine nucleotides via intermediate electron carriers. At least three SORs have been shown to utilize the one-iron Fe:S metalloprotein rubredoxin (Rd) as a competent electron donor (Eq. 5) [24, 27, 28].

SOR, like SOD, is physically configured so that the reaction with superoxide is virtually diffusion limited. The second-order rate constant for the reaction between SOR and superoxide is $\sim10^9$ M^{-1} s^{-1} [29, 30, 31]. It is also interesting to note that intracellular SOR concentrations are similar to those of SOD (the SOR from *T. pallidum* is present at an estimated 9500 monomers per cell [32]; given typical cellular dimensions of 0.2 μm diameter and a length from 5 to 20 μm, intracellular concentrations are estimated to be 25–100 μM!). In the case of SOR, however, questions remain about the reaction mechanism and the characterization of reaction intermediates. Since the reaction of SOR and superoxide is diffusion limited, the physiological rate-determining step for superoxide reduction will necessarily be governed by the rate of electron transfer from NAD(P)H to SOR. The fact that some SORs have been shown to restore efficient growth to an SOD-deficient *Escherichia coli* strain, an organism lacking the gene for Rd, suggests that an electron transport chain exists in *E. coli* that is kinetically competent (in a physiological sense) to donate electrons for superoxide reduction via SOR [24, 26].

Indeed, the possibility that redox species other than superoxide could donate/receive electrons to/from the oxidized/reduced forms of SOD, i.e., whether SOD could function also as a SOR or superoxide oxidase (SOO), has been explored recently [25]. It was shown that the eukaryotic copper,zinc SOD (Cu,Zn-SOD) catalyzed oxidation of ferrocyanide or the reduction of ferricyanide by superoxide, i.e., Cu,Zn-SOD, could act as a both a SOO and SOR. This result begs the question: "What is the ultimate fate of superoxide in vivo? Is it dismutation, reduction, or oxidation?" Whether a given superoxide-metabolizing enzyme dismutates, reduces, or oxidizes superoxide depends upon a variety of factors, including the concentrations of superoxide, metabolizing enzyme, and alternate redox species, as well as the corresponding second-order rates constants governing each redox reaction (see [25] for a detailed discussion).

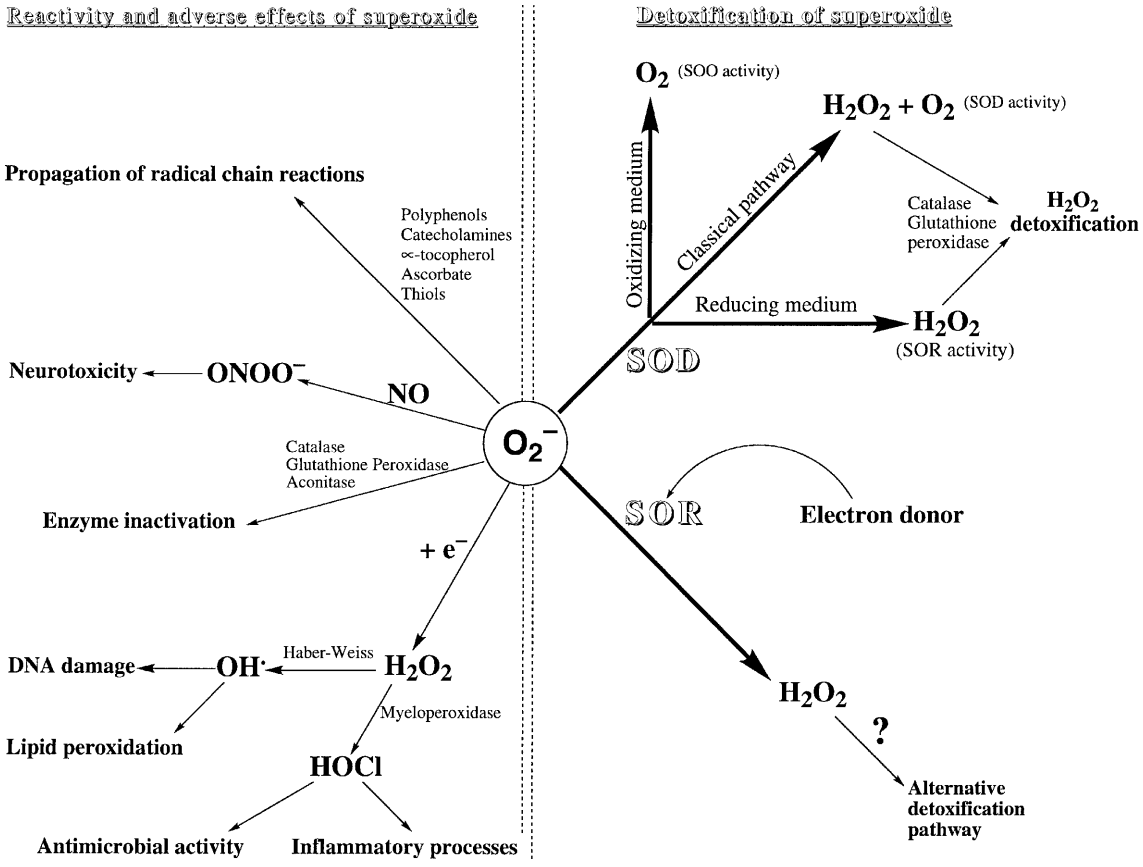

Fig. 1. The ultimate fate of superoxide in vivo: reactivity, adverse effects, and detoxification pathways

Physiological implications

Aerobes and anaerobes thus seem to have adopted different strategies for coping with the oxidative stress of superoxide, using two enzymes structurally and biochemically different, but assuming the same function (Fig. 1). Although some microaerophiles like *T. pallidum* are exposed to oxygenated environments during their in vivo propagation, how does one explain that strict anaerobes need to scavenge the superoxide anion? The exact amount of O_2^- in anaerobes is still unknown, but the presence of an antioxidant defense in such organisms suggests that they periodically encounter oxygen and must defend themselves against ROS. Moreover, it is interesting to note that facultative anaerobes prefer using the classical SOD system and synthesize SOD in anaerobic habitats in anticipation of transient exposure to oxygen. This is a good example of the adaptability of bacteria to various growth conditions.

It is also of interest to try to explain the existence of two different detoxification pathways and the potential advantages of each method, especially the advantages that superoxide reduction can provide compared to its dismutation. The reaction catalyzed by SOD generates one molecule each of O_2 and H_2O_2 per mole of superoxide consumed, whereas the reaction catalyzed by SOR only produces one molecule of hydrogen peroxide. In view of that, aerobic organisms may prefer the SOD pathway, while oxygen-sensitive organisms may choose the latter to avoid regenerating oxygen. Nevertheless, the relative toxicity of H_2O_2 and O_2 for anaerobic organisms needs to be considered. Indeed, oxygen can more rapidly inactivate some enzymes involved in anaerobic fermentations pathways but hydrogen peroxide is a stronger oxidant. Considering that anaerobes very often lack catalase, these bacteria would also have to find an alternative way to eliminate hydrogen peroxide. The SOR pathway, which necessitates the use of endogenous reductants, may also be facilitated in anaerobes where cellular reducing agents are abundant. Indeed, the choice of one system or the other has to take account of the composition of the cellular medium, the derived oxidized products, and the requirement to detoxify these products efficiently.

It is interesting to note that some organisms appear to have genes for both defense systems. Thus, if SODs have the potential to function as SORs, what growth conditions define the use of SOR versus SOD [25]? For example, the sulfate reducer *Desulfovibrio gigas* uses both the classical antioxidant strategy, involving SOD and catalase, in addition to a SOR system. The choice of different SODs can be explained by the organism's adaptation regarding the bioavailability of specific metals, but the selection of having two enzymatic systems assuming the same theoretical function is relatively

unclear. Nevertheless, cells may utilize SOR in reducing compartments and SOD in other locations like the periplasm in order to optimize the efficiency of the two enzymatic systems. Indeed, when necessary, *Mycobacterium tuberculosis* is capable of exporting SOD to the extracellular medium during the host-pathogen interaction [33]. Moreover, when microbes are starved and have a lower reducing capacity, it could be useful to use SOD instead of SOR, because the latter consumes reducing equivalents.

We can thus finally ask whether the reduction of superoxide is the real function of SOR or is an adventitious secondary effect of an unknown function, as was initially suggested for SOD [34]? The fact that the active sites of both SOD and SOR are designed to allow reaction with superoxide with a rate constant close to the diffusion limit is strong evidence for a physiological role in superoxide metabolism. Nevertheless, other possible functions for SODs are becoming apparent, as recently described [34]. SOR may also represent a more primitive enzymatic system for metabolizing superoxide, with the aquisition of SODs ultimately replacing SORs progressively through an evolutionary selection processes [25]. Alternatively, SOR and SOD may represent examples of convergent evolution in which Nature solved the challenge of detoxifying the superoxide anion via two independent mechanisms.

The presence of these two different detoxification pathways could also suggest unknown deleterious effects of the superoxide anion, in agreement with the fact that some phenotypes are not yet completely elucidated. According to this hypothesis, superoxide could have additional routes of toxicity. Interesting information could therefore come from an identification of the damage incurred by ROS in SOR mutants, i.e., anaerobes may represent good models for the study of protective mechanisms against oxygen toxicity.

References

1. Imlay JA, Fridovich I (1991) J Biol Chem 266:6957–6965
2. Imlay JA (1995) J Biol Chem 270:19767–19777
3. De Groot H, Littauer A (1989) Free Rad Biol Med 6:541–551
4. Hassan HM, Fridovich I (1979) Arch Biochem Biophys 196:385–395
5. Cadenas E, Boveris A, Ragan CI, Stoppani AO (1977) Arch Biochem Biophys 180:248–257
6. Fridovich I (1983) Annu Rev Pharmacol Toxicol 23:239–257
7. Halliwell B, Gutteridge JM (1999) Free radicals in biology and medicine. Oxford University Press, Oxford, pp 31–32
8. Babior BM (1999) In: Gilbert DL, Colton CA (eds) Reactive oxygen species in biological systems: an interdisciplinary approach. Kluwer/Plenum, New York, pp 503–526
9. Klebanoff SJ (1988) Inflammation: basic principles and correlates. Raven, New York, p 451
10. Sawyer DT, Valentine JS (1981) Acc Chem Res 14:393–400
11. Behar MG, Severinghaus JW (1970) J Appl Physiol 29:413
12. Mashino T, Fridovich I (1987) Arch Biochem Biophys 254:547–551
13. Blum J, Fridovich I (1985) Arch Biochem Biophys 240:500–508
14. Kono Y, Fridovich I (1982) J Biol Chem 257:5751–5754
15. Odajima T, Yamazaki I (1972) Biochim Biophys Acta 284:360–367
16. Flint DH, Tuminello JF, Emptage MH (1993) J Biol Chem 268:22369–22376
17. Gardner PR (1997) Biosci Rep 17:33–42
18. Bielski BH, Arudi RL, Sutherland MW (1983) J Biol Chem 258:4759–4761
19. McCord JM, Fridovich I (1969) J Biol Chem 244:6049–6055
20. Radolf JD, Steiner B, Shevchenko D (1999) Trends Microbiol 7:7–9
21. Abreu IA, Saraiva LM, Carita J, Huber H, Stetter KO, Cabelli D, Teixeira M (2000) Mol Microbiol 38:322–334
22. Romão CV, Liu MY, LeGall J, Gomes CM, Braga V, Pacheco I, Xavier AV, Teixeira M (1999) Eur J Biochem 261:438–443
23. Silva G, Oliveira S, Gomes CM, Pacheco I, Liu MY, Xavier AV, Teixeira M, LeGall J, Rodrigues-Pousada C (1999) Eur J Biochem 259:235–243
24. Jenney FE Jr, Verhagen MFJM, Cui X, Adams MWW (1999) Science 286:306–309
25. Liochev SI, Fridovich I (2000) J Biol Chem 275:38482–38485
26. Pianzzola MJ, Soubes M, Touati D (1996) J Bacteriol 178:6736–6742
27. Coulter ED, Kurtz DM Jr (2001) Arch Biochem Biophys 394:76–86
28. Hazlett KRO, Cox DL, Auchere F, Rusnak F, Radolf JD (2002) Methods Enzymol 353 (in press)
29. Coulter ED, Emerson JP, Kurtz DM Jr, Cabelli DE (2000) J Am Chem Soc 122:11555–11556
30. Lombard M, Houée-Levin C, Touati D, Fontecave M, Nivière V (2001) Biochemistry 40:5032–5040
31. Nivière V, Lombard M, Fontecave M, Houée-Levin C (2001) FEBS Lett 497:171–173
32. Jovanovíc T, Ascenso C, Hazlett KR, Sikkink R, Krebs C, Litwiller R, Benson LM, Moura I, Moura JJ, Radolf JD, Huynh BH, Naylor S, Rusnak F (2000) J Biol Chem 275:28439–28448
33. Harth G, Horwitz MA (1999) J Biol Chem 274:4281–4292
34. Liochev IL, Fridovich I (2001) J Biol Chem 276:35253–35257