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WHAT IS UNIQUE ABOUT SUPEROXIDE TOXICITY AS COMPARED TO OTHER BIOLOGICAL REDUCTANTS? – A HYPOTHESIS

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Usually the toxicity of superoxide is attributed to its ability to reduce metal ions and subsequently reoxidation of the metal by hydrogen peroxide yields deleterious oxidizing species. As many other nontoxic biological reductants reduce metal compounds, we suggest that part of the mechanism of superoxide toxicity results from its ability to oxidize metal ions bound to biological targets, which subsequently degrade the target via an intramolecular electron transfer reaction.

KEY WORDS: Superoxide, Haber-Weiss mechanism, OH·, Cu(III), Fe(IV).

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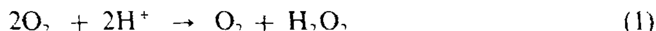
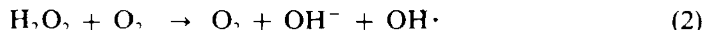
INTRODUCTION

Since the discovery of superoxide dismutase (SOD)[1] and the formation of superoxide anion radical (O_2^-) in biological systems,[2] it became clear that O_2^- may be very toxic in living systems.[3] It was assumed that SOD protects the living systems from the deleterious effects of O_2^- by catalyzing its dismutation:



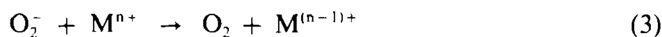
$$2O_2^- + 2H^+ \rightarrow O_2 + H_2O_2 \quad (1)$$

The superoxide radical is a relatively unreactive radical towards most biological molecules.[4,5] Therefore, its toxicity was related to the formation of the highly oxidizing OH· radical,[6] formed in the so-called Haber–Weiss reaction:[7]



$$H_2O_2 + O_2^- \rightarrow O_2 + OH^- + OH\cdot \quad (2)$$

However, O_2^- dismutases much faster than the formation of OH· through reaction (2) which is extremely slow.[8,9] Therefore, it was suggested that metal ions, such as copper(II) and iron(III), catalyze this reaction. The reaction mechanism proposed is given by the sequence of reactions (3) and (4):[8-14]



$$O_2^- + M^{n+} \rightarrow O_2 + M^{(n-1)+} \quad (3)$$

$$M^{(n-1)+} + H_2O_2 \rightarrow M^{n+} + OH^- + OH\cdot \quad (4)$$

This mechanism was based on the observations that SOD, catalase, metal chelators, such as desferal, and OH· scavengers protect the systems from the deleterious effect of O_2^-.[9-14] It is possible that other oxidizing species would be formed in reaction (4), such as $MH_2O_2^{(n-1)+}$ or $M^{(n+1)+}$, which are also highly oxidizing species.[15-17]

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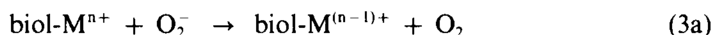
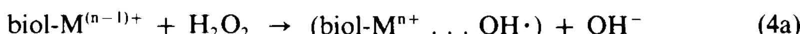
In later studies, it was observed that there are systems where $OH\cdot$ scavengers do not protect efficiently, in that one has to use relatively high concentrations of these scavengers in order to provide protection.[18] These observations led to modification of the reaction scheme given by reactions (3) and (4). The modification assumes that metal ions are bound to the biological target and reactions (3) and (4) occur with the metal bound to the target:[14,19]



$$\text{biol-M}^{n+} + O_2^- \rightarrow \text{biol-M}^{(n-1)+} + O_2 \quad (3a)$$



$$\text{biol-M}^{(n-1)+} + H_2O_2 \rightarrow (\text{biol-M}^{n+} \ldots OH\cdot) + OH^- \quad (4a)$$

$\downarrow$ damage

The sequence of reactions (3a) and (4a) describes the site specific mechanism, where the oxidizing species is formed at or near the target. Therefore, it would not be able to diffuse away from the target and will react with it within a very short time. As a result, very high concentrations of scavengers will be needed in order for the scavengers to be able to compete with the target for the oxidizing species.[18] The site specific mechanism given by reactions (3a) and (4a) is therefore consistent with the experimental observations.

WHAT IS UNIQUE ABOUT O_2^- TOXICITY?

The toxicity of O_2^-, as believed by now and described through reactions (3) and (4) or (3a) and (4a), is due primarily to the reductive properties of O_2^-. This radical is not a highly reducing entity. Its redox potential under standard conditions of 1 M O_2^- and 1 M oxygen is only -0.16 V,[20] and its concentration in cells is in the range of 10^{-9}–10^{-11} M.[21] The superoxide radical is not the most abundant reducing entity in the cell and reductants such as glutathione ($E^0 = -0.23$ V), NAD(P)H ($E^0 = -0.32$ V) and vitamin C (-0.058 V) are present in the cells in concentrations exceeding that of O_2^- by several orders of magnitude.[22–24] If the deleterious effect of O_2^- is due to reaction (3) or (3a), it is rather puzzling how we survive in the presence of other reductants in the cells which are also capable of reducing metal ions, some of which can substitute O_2^- in reaction (3) or (3a) very efficiently. This, in our opinion, is the most serious question on O_2^- toxicity, which challenges the mechanism of its toxicity given by reactions (3) and (4) or (3a) and (4a). There are some cases where O_2^- reacts with a few biological targets directly,[25–31] but these cases do not seem to explain why O_2^- is so toxic and why SOD is present in most cells at rather high concentration (10^{-5} M).[32] Recently, Fridovich reviewed the literature and pointed out several cases where O_2^- toxicity apparently does not involve H_2O_2 and possibly does not involve metal ions.[33]

It seems that there has to be some reason for the unique toxicity of O_2^-, which is not given by the Haber–Weiss reaction catalyzed by metal ions. In the next paragraph we will propose a working hypothesis which may account for the majority of the features of the toxicity of O_2^- and the protective effects observed, and which does not incorporate the Haber–Weiss mechanism. This working hypothesis may account for the unique toxicity of O_2^- as compared to other reductants.

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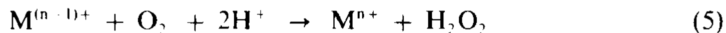
THE POSSIBLE TOXICITY OF O_2^- AS AN OXIDANT OF Cu(II), Fe(III) OR Mn(II) COMPOUNDS

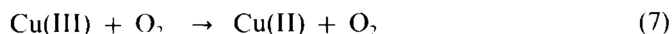
The chemistry of copper and iron compounds in the catalysis of O_2^- dismutation or in the participation of O_2^- toxicity assumes that the first stage is the reduction of the metal by O_2^-. Then, reoxidation of the metal by O_2^- (reaction (5)) or by H_2O_2 (reaction (4)) occur in the protective pathway or in the toxic pathway, respectively.



$$M^{(n-1)+} + O_2^- + 2H^+ \rightarrow M^{n+} + H_2O_2 \quad (5)$$

The mechanism of O_2^- dismutation catalyzed by Cu, Zn-SOD, as well as by many other copper and iron compounds is assumed to involve alternate reduction and oxidation of the metal through reactions (3) and (5).[34-39] However, it was already pointed out by Klug-Roth and Rabani[37] that the kinetics of the decay of O_2^- in the presence of copper compounds would be indistinguishable whether copper oscillates between Cu(II) and Cu(I) (reactions (3) and (5)) or Cu(II) and Cu(III) (reactions (6) and (7)).

$$Cu(II) + O_2^- + 2H^+ \rightarrow Cu(III) + H_2O_2 \quad (6)$$

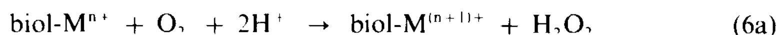


$$Cu(III) + O_2^- \rightarrow Cu(II) + O_2 \quad (7)$$

The same would be valid for iron compounds, where iron may oscillate either between Fe(III) and Fe(II) or Fe(III) and Fe(IV), but not for Mn(II) compounds as the formation of Mn(I) is improbable.[40]

In some later studies, it was suggested that for some macrocyclic polyamine complexes of copper(II) and nickel(II) the higher oxidation state of the metal might be involved in the mechanism of O_2^- dismutation.[41,42] The possible involvement of Cu(III) and Fe(IV) in the mechanism of O_2^- dismutation is not surprising as the redox potential of the couple O_2^-/H_2O_2 is 0.87 V, which implies a strong oxidizing capability. For many complexes with ligands which are good sigma donors, the redox potential of Cu(III)/Cu(II) and Fe(IV)/Fe(III) is lower than that of the free ions,[16,41-43] so that from the thermodynamic point of view reaction (6) might occur. Furthermore, stabilization of some Cu(III) complexes has been demonstrated by showing that the Cu(II) complexes are even air-oxidized at physiological pH.[42,43]

In contrast to the accepted mechanism, where O_2^- is the precursor for OH• radical, the mechanism described by reaction (6) or (6a) assumes that O_2^- is the precursor for Cu(III), Fe(IV) or Mn(III), which are highly oxidizing species which might cause damage.

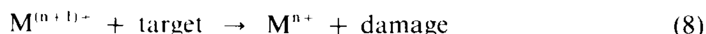


$$\text{biol-}M^{n+} + O_2^- + 2H^+ \rightarrow \text{biol-}M^{(n+1)+} + H_2O_2 \quad (6a)$$

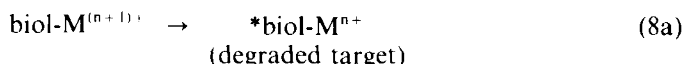
Free or bound $M^{(n+1)+}$ might be reduced by O_2^- and/or by other reducing/protecting agents, or might react directly with the target. In the first case the metal would catalyze O_2^- dismutation through reactions (6) and (7) or (6a) and (7a).

$$\text{biol-}M^{(n+1)+} + O_2^- \rightarrow \text{biol-}M^{n+} + O_2 \quad (7a)$$

In the second pathway, damage might occur either through the reaction of $M^{(n+1)+}$ with the target or through an intramolecular electron transfer reaction when the metal is bound to the target. In this latter case O_2^- would be toxic.



$$M^{(n+1)+} + \text{target} \rightarrow M^{n+} + \text{damage} \quad (8)$$



$$\text{biol-}M^{(n+1)+} \rightarrow {}^*\text{biol-}M^{n+} \text{ (degraded target)} \quad (8a)$$

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In several studies it has has been demonstrated that some Cu(III) compounds, especially peptide compounds, the antibiotic drug bleomycin, and Fe(IV) aminopolycarboxylate complexes undergo intramolecular electron transfer reactions yielding degraded ligand.[43-49] It has already been demonstrated that O_2^- oxidizes Mn(II) compounds to either Mn(III) or MnO_2^+, depending on the conditions[40,50-52] and that these species can, in turn, oxidize other targets.[53] Fridovich and co-workers studied the effect of vanadate on the oxidation of NAD(P)H by O_2^-, and it appears that an oxidant is formed in the reaction between vanadate and O_2^-, which then oxidizes NAD(P)H.[54-56] In these systems SOD inhibited the vanadate-stimulated oxidation of NAD(P)H, while catalase had no effect.[55,56]

The mechanism involving Cu(III), Fe(IV) or Mn(III) may also explain the inability of OH· scavengers to exhibit protection in several cases, as Cu(III), Fe(IV) and Mn(III) are expected to react with OH· scavengers with different relative rates yielding different products as compared to OH radicals. In some cases Cu(III) or Fe(IV) may dissociate to yield OH· radicals and one would expect that OH· scavengers would protect the systems, but relatively high concentrations of these scavengers would be needed as compared to the case where OH· is formed directly.
reactions (6) or (6a) as compared to reactions (3) or (3a), as well as the relative rates of these reactions.

The mechanism involving Cu(III), Fe(IV) or Mn(II) may also explain the inability of OH· scavengers to exhibit protection in several cases, as Cu(III), Fe(IV) and Mn(III) are expected to react with OH· scavengers with different relative rates yielding different products* to yield OH· radicals and one would expect that OH· scavengers would protect the systems, but relatively high concentrations of these scavengers would be needed as compared to the case where OH· is formed directly.

What is not clear enough is the role of H_2O_2 in these processes, as in many systems catalase as well as metal chelators have protective effects, although there are many cases where they do not inhibit the damage.[33,54-57] It is plausible that $M^{(n+1)+}$ forms with H_2O_2 complexes which are probably very potent oxidizing, and therefore toxic, agents. It is possible that in some systems the damage occurs partially through $M^{(n-1)+}$ (Haber–Weiss Mechanism) and partially through $M^{(n+1)+}$. In such a case the role of H_2O_2 is clear, but one should observe only partial inhibition of the damage by catalase.

CONCLUSIONS

We conclude that in systems where O_2^- is unique in causing damage as compared to other biological reductants, it may not reduce the metal ion but rather oxidizes it to form a highly oxidizing entity. This entity might decompose either via an intramolecular electron transfer reaction causing degradation of the ligand attached to the metal or it might react directly with the biological target. However, this proposed mechanism does not eliminate the formation of OH· radicals or other oxidizing species through the Haber–Weiss reaction catalyzed by metal ions. The latter case accounts also for damage initiated by some other reductants, while the first one does not. It is possible that in some systems the damage occurs through the Haber–Weiss mechanism and in others through our proposed mechanism or through both mechanisms.

*As compared to OH· radicals. In some cases Cu(III) or Fe(IV) may dissociate.

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