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WHEN DO METAL COMPLEXES PROTECT THE BIOLOGICAL SYSTEM FROM SUPEROXIDE TOXICITY AND WHEN DO THEY ENHANCE IT?

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Many copper and iron complexes can be reduced by O_2^- as well as by H_2O_2. According to the rates of reduction and the concentration of O_2^- and H_2O_2, the metal complexes may serve either as catalyst of O_2^- dismutation or as catalysts of the reaction between O_2^- and H_2O_2 to form $OH^\cdot$ radical (Haber-Weiss reaction). Various factors which influence whether metal complexes protect the biological systems from superoxide toxicity or enhance it are discussed.

Key words: SOD Activity, 'Haber-Weiss' Reaction, Copper Complexes, Superoxide Toxicity

INTRODUCTION

The toxicity of O_2^- in biological systems has attracted great interest in the last decade.[1-3] Many studies have concentrated not only on superoxide toxicity but also on the role of superoxide dismutase (SOD) in protecting biological systems from O_2^-.[4-5]

In many of the systems where O_2^- toxicity is observed or where SOD protects the systems, it was found that H_2O_2 is a prerequisite for the expression of O_2^- toxicity and therefore catalase has a protective effect.[6] Furthermore, in many of these systems it was shown that metals, such as copper or iron ions, or some of their complexes, participate in the expression of O_2^- toxicity.[7-12] By chelating copper with EDTA or iron with desferal the system can be protected from O_2^- toxicity.[7-8,11-12]

These observations led to the proposal of the following mechanism, often referred to as the 'Haber Weiss' mechanism.[7-12]

$$O_2^- + \text{Fe(III) or Cu(II)} \rightarrow O_2 + \text{Fe(II) or Cu(I)} \quad (1)$$

$$\text{Fe(II) or Cu(I)} + H_2O_2 \rightarrow \text{Fe(III) or Cu(II)} + OH^- + OH^\cdot \quad (2)$$

$$OH^\cdot + \text{biological target} \rightarrow \text{damage} \quad (3)$$

In this mechanism, O_2^- is a precursor of $OH^\cdot$, which is thus formed by the overall reaction:

$$O_2^- + H_2O_2 \rightarrow O_2 + OH^- + OH^\cdot \quad (4)$$

The radical $OH^\cdot$ is the toxic entity.

In many of these systems, $OH^\cdot$ scavengers are ineffective in protecting against damage.[13] It was suggested that reactions (1) and (2) occur with the metal bound to the target so that the $OH^\cdot$ is being formed in the vicinity of the target and reacts immediately with it.[7-8,13-15] In such a case it is expected that $OH^\cdot$ scavengers at moderate concentrations will be ineffective and protection might be observed only at very high scavenger concentrations.[13]

This modified Haber-Weiss mechanism leads to the following assumptions:

1) The metal or its complex is bound at or near the target;
2) The bound metal or complex is reduced by O_2^-;
3) The reduced bound metal or its complex is then reoxidized by H_2O_2 and forms $OH^\cdot$ at the binding site.

This will be described by the modified Haber-Weiss cycle. e.g. for copper,

$$\text{Biol} - Cu^{2+} + O_2^- \rightarrow \text{Biol} - Cu^+ + O_2 \quad (1a)$$
$$\text{Biol} - Cu^+ + H_2O_2 \rightarrow (\text{Biol} - Cu^{2+} \ldots OH^\cdot) + OH^- \quad (2a)$$
$$(\text{Biol} - Cu^{2+} \ldots OH^\cdot) \rightarrow \text{damage} \quad (3a)$$

The modified mechanism does not take into account another reaction, namely the reoxidation of the monovalent copper by O_2^-, which might take place in addition to reactions 1-3 or 1a-3a.

$$Cu^+ + O_2^- \xrightarrow{2H^+} Cu^{2+} + H_2O_2 \quad (5)$$
$$\text{Biol} - Cu^+ + O_2^- \xrightarrow{2H^+} \text{Biol} - Cu^{2+} + H_2O_2 \quad (5a)$$

If the mechanism can be described by reactions (1) and (5) or (1a) and (5a) and reactions (2) or (2a) can be neglected, the systems describe the case where copper compounds dismute O_2^- and no toxicity is expected as no $OH^\cdot$ is formed. In this case the decay of O_2^- is given by:

$$-\frac{d[O_2^-]}{dt} = (k_1[Cu(II)] + k_5[Cu(I)])[O_2^-] \quad (6)$$

Assuming steady state for $[O_2^-]$, rate equation (7) is obtained:

$$-\frac{d[O_2^-]}{dt} = \frac{2k_1k_5}{k_1+k_5}[Cu(II)]_{total}[O_2^-] = k_{cat}[\text{catalyst}]_0[O_2^-] \quad (7)$$

where the 'turnover' rate constant, k_{cat}, is defined:

$$k_{cat} = \frac{2k_1k_5}{k_1+k_5}$$

For Cu-Zn SOD $k_1 = k_5$ and the 'turnover' rate constant, $k_{cat} = (2.4 \pm 0.5) \times 10^9\ M^{-1}sec^{-1}$, is pH independent over the range 4.8 – 9.5.[16-18] The rate constants of the reaction between O_2^- and a large number of copper complexes have been determined.[19-27] The aquo complex[21,24] and the copper chelates of some amino acids,[19-21,23] salicylates[22] and phenanthrolines[26-27] exert almost the same catalytic activity as SOD, which means that k_1 and k_5 have similar values. If $k_1 \gg k_5$ or $k_1 \ll k_5$, k_{cat} approaches

twice the value of the lower rate constant and thus one has a substance that dismutes O_2^- very slowly. In the case where $k_1 \gg k_5$ the copper complex can serve as a scavenger of O_2^-, *but not as a catalyst for* O_2^- *dismutation.*

THE RELATION BETWEEN SOD ACTIVITY OF COPPER COMPOUNDS AND THE TOXICITY OF O_2^- IN THE PRESENCE OF THESE COMPOUNDS

In order that the toxicity of O_2^- in the presence of copper compounds should be expressed, it is necessary that the rate of reactions (5) or (5a) be small as compared with the rates of reactions (2) and (2a). If the contrary is true, the copper compounds will catalyze O_2^- dismutation and thus will protect from O_2^- toxicity rather than being toxic. For these compounds, $k_2[O_2^-]$ must exceed the value of $k_5[H_2O_2]$.

In normal cells the steady state concentrations of both O_2^-, ($[O_2^-]_{s.s}$), and H_2O_2, ($[H_2O_2]_{s.s}$), are very low. The estimates for $[O_2^-]_{s.s}$ can be calculated from the oxygen consumption assuming that 1–5% yield O_2^- and from the SOD levels in cells. These assumptions yield steady state concentrations of O_2^- which are in the range of $10^{-10} - 10^{-11}$ M. The steady state concentrations of H_2O_2 in most normal cells are below 10^{-8} M. Thus usually in such cells $[H_2O_2]_{s.s}/[O_2^-]_{s.s} < 1000$.[28-29] These assumptions do not apply to some extreme cases such as phagocytosis where $[O_2^-]_{s.s}$ and $[H_2O_2]_{s.s}$ exceed the above mentioned values.[30]

Around neutral pH, for SOD $k_1 = k_5 = (2.4 \pm 0.5) \times 10^9\ M^{-1}sec^{-1}$ and k_2 is very small. H_2O_2 very slowly deactivates SOD with a rate constant of 6.7 $M^{-1}sec^{-1}$,[31] but this reaction was shown to be the reduction of Cu(II) in SOD. Therefore there is no appreciable oxidation of the reduced copper in SOD by H_2O_2[14] and SOD has its catalytic activity and does not rapidly inactivate itself.

The protection of various chelating agents like detapac, desferal phenanthroline or substituted phenanthroline and other chelates, can be explained by different reasons:

1) In some cases the complexation prevents the reduction of the metal ion by O_2^- and therefore no OH˙ can subsequently be formed. This can be due to a change in the redox potential of the metal ion couple which prevents reaction (1) on thermodynamic grounds, or due to a change in the rate of this reaction.[32]
2) In other cases where reaction (1) occurs, reaction (2) may not proceed or be too slow on the same grounds as mentioned above. In an extreme case with neocuproine (2,9-dimethyl-phenanthroline), H_2O_2 does not oxidize the cuprous complex (reaction (2)). On the contrary, H_2O_2 reduces the cupric complex into its monovalent oxidation state.
3) There is also the possibility that chelation transforms the deleterious metal ion into a complex which has SOD activity. This will happen if $k_5[O_2^-] \gg k_2[H_2O_2]$. In such a case the chelation will inhibit the toxicity of the metal ion.

   These hypotheses do not explain why in some systems with iron, a chelator like EDTA is a protector while in others it is a sensitizer. One possibility which seems unlikely, is that EDTA is a weaker complexant that some of the biological targets and therefore its addition will not have any effect. Another possibility is that EDTA pulls iron off biological binding sites. If the result of this action is that the Haber-Weiss reaction takes place in the bulk and this produces the OH˙ radicals far from the biological target, then EDTA would serve as a protector. If on the

other hand EDTA transfers iron from 'safe sites' to 'ill placed sites' as proposed by Willson,[33] then EDTA would serve as a sensitizer and would enhance the deleterious effects. There are cases where iron ions precipitate as phosphate, and EDTA or ADP sensitization is due to solubilization of the iron.[34]

4) Another possibility is that a ternary complex is formed between the metal copper ion, the chelator and the biological target (e.g. a cuprous-1, 10-phenanthroline-DNA complex). In such a case the absolute and the relative values of the rate constants of reactions (1), (2) and (5) for various ternary complexes will differ. Therefore, the same chelator may show different effects with various biological systems or targets.

In some cases a copper-chelate complex dismutes O_2^- and may form a ternary complex which will initiate $OH^\cdot$ formation and hence this chelator will show toxicity, while in other biological systems the same chelator forms a ternary complex that still dismutes O_2^- and is not toxic. We have shown earlier that copper 1, 10-phenanthroline, $(op)_2Cu^{2+}$, is an efficient catalyst for dismutation of O_2^-,[26-27] while it has been shown that this compound is efficient in initiating DNA cleavage.[35-36] We believe that this behaviour can be explained by the different properties of cupric-phenanthroline as compared with those of cupric-phenanthroline-DNA and cuprous-phenanthroline-DNA in reactions (1), (2) and (5).

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