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REQUIREMENTS FOR SOD MIMICS OPERATING *IN VITRO* TO WORK ALSO *IN VIVO*

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When an efficient SOD mimic operating *in vitro* is introduced into cells, the following requirements are needed in order that this compound will catalyze O_2^- dismutation efficiently: it should be non toxic, stable, has a long metabolic half life, does not form ternary complexes with the cell components, its reduced form reacts slowly with molecular oxygen, should be able to cross cell membranes and also to reach lipophilic or hydrophobic regions. Thus, it seems that finding an efficient compound that has high SOD-like activity *in vivo* will not be easily achieved.

KEY WORDS: SOD, SOD-mimics, O_2^- dismutation, copper complexes, catalysis.

INTRODUCTION

The toxicity of O_2^- and its role in many deleterious processes in biological systems is not questioned any more, and the proceeding of this conference includes many examples. The ability of superoxide dismutase (SOD) to catalyze O_2^- dismutation was first discovered by McCord and Fridovich in 1969,[1] and it is believed that it protects the cells from superoxide toxicity.[2,3]

Native SOD has a few drawbacks in its use as a drug in those cases where it is believed to protect against O_2^- toxicity. The main problems arising with the use of this enzyme as a drug are due to its short metabolic half life, which is about 10 minutes, and its inability to penetrate into the cells and also to reach lipophilic regions.[4]

There are numerous copper complexes including Cu_{aq}^{2+}, which were found to be almost as efficient as SOD in catalyzing O_2^- dismutation in a pulse radiolysis system.[5] However, many of these compounds lose their activity *in vivo*, and some of them even enhance O_2^- toxicity.[5]

It is the purpose of this communication to point out the requirements needed from a compound so that it will be able to mimic SOD efficiently *in vivo*.

DISCUSSION

A) Mechanism of SOD and SOD Mimics

The mechanism of SOD as well as many other copper complexes is assumed to proceed via the so called the 'ping-pong' mechanism, where copper oscillates between Cu(II) and Cu(I):[5]

$$\text{Cu(II)} + O_2^- \rightarrow \text{Cu(I)} + O_2 \qquad (1a)$$

$$Cu(I) + O_2^- + 2H^+ \rightarrow Cu(II) + H_2O_2 \quad (2a)$$

Other alternative mechanisms have been also suggested for the catalysis of O_2^- dismutation by copper compounds were,

$$Cu(II) + O_2^- + 2H^+ \rightarrow Cu(III) + H_2O_2 \quad (1b)$$

$$Cu(III) + O_2^- \rightarrow Cu(II) + O_2 \quad (2b)$$

or

$$Cu(II) + O_2^- \rightleftharpoons CuO_2^+ \quad (1c)$$

$$CuO_2^+ + O_2^- + 2H^+ \rightarrow Cu(II) + H_2O_2 \quad (2c)$$

or

$$Cu(II) + O_2^- \rightleftharpoons CuO_2^+ \quad (1d)$$

$$CuO_2^+ \rightleftharpoons Cu(I) + O_2 \quad (3)$$

$$CuO_2^+ + Cu(I) + 2H^+ \rightarrow 2Cu(II) + H_2O_2 \quad (4)$$

The various schemes describe the dismutation of O_2^- yielding hydrogen-peroxide and molecular oxygen:

$$2O_2^- + 2H^+ \rightarrow O_2 + H_2O_2 \quad (5)$$

The first three mechanisms, provided back reaction (1) is negligible, cannot be distinguished kinetically and they all yield the following rate equation:

$$-d[O_2^-]/dt = k_{cat}[Cu(II)]_0[O_2^-] \quad (6)$$

where

$$k_{cat} = 2k_1k_2/(k_1 + k_2)$$

The higher the values of k_1 and k_2 are, the better the catalyst is. If k_1 and k_2 differ substantially, k_{cat} approaches the value of the slower of the rate constants. When $k_1 = k_2$, the catalyst is more efficient and $k_{cat} = k_1 = k_2$. Provided $k_1 = k_2 = (2\text{–}3) \times 10^9\ M^{-1}s^{-1}$, k_{cat} reaches the values determined for the enzyme.[6]

If however, reaction (1) is also going backwards, rate equation (7) is obtained:

$$-d[O_2^-]/dt = k_r[Cu(II)]_0[O_2^-] \quad (7)$$

where

$$k_r = 2k_1k_2/(k_1 + k_2 + k_{-1}[O_2]/[O_2^-])$$

Thus, if $k_{-1}[O_2]/[O_2^-] \gg (k_1 + k_2)$, then $k_{cat} > k_r$. In other words, the faster the reoxidation of Cu(I) by oxygen, the poorer is the SOD mimic. It turns out that most SOD mimics that have similiar k_1 and k_2 values to that of the enzyme are reoxidized quite fast by oxygen, while the reduced enzyme reacts extremely slowly with oxygen ($k_{-1} = 0.44\ M^{-1}s^{-1}$[7]). In air-saturated living cells the steady state concentration of O_2^- is lower by about 5–6 orders of magnitude than that generated initially in the pulse radiolysis experiments. This explains part of the uniqueness of SOD as compared to SOD-mimics, which have a high SOD-like activity in the pulse radiolysis system but are not effective *in vivo*. Table I demonstrates the relative values of k_{cat} for SOD and

TABLE I
Relative SOD activity of metal compounds as a function of $[O_2^-]$ and k_{-1} in air-saturated solutions

sys.	$[O_2^-]$, M	k_{-1}, $M^{-1}s^{-1}$					
		1 SOD	10	10^2	10^3	10^4 most SOD	10^5 mimics
p.r.	$10^{-6} - 10^{-4}$	1	1	1	1	1	1
γ.r.	10^{-8}	1	1	1	1	0.9	0.5
x/xod	10^{-9}	1	1	1	0.9	0.5	0.1
cell	10^{-10}	1	1	0.9	0.5	0.1	0.013
cell	10^{-11}	1	0.9	0.5	0.1	0.013	0.0013

p.r. - pulse radiolysis
γ.r. - gamma radiolysis
x/xod - xanthine/xanthine oxidase

SOD-mimics for which $k_1 = k_2 = 2 \times 10^9 M^{-1}s^{-1}$ as a function of k_{-1} and $[O_2^-]$ in air-saturated solutions. The table shows why *in vivo*, as well as in the xanthine/xanthine oxidase system, k_{cat} for SOD-mimics is much lower as compared to the native enzyme, and why in pulse radiolysis system both SOD and its mimics may have comparable efficiency.

B) Superoxide Scavengers and Dismutation Catalysts

In order to protect the biological target (T) against O_2^- toxicity, one can either use an O_2^- scavenger (S) or a catalyst for its dismutation reaction (cat). In both cases the scavenger or the catalyst compete with the deleterious reaction (8) through reactions (5) and (9).

$$O_2^- + T \rightarrow \text{damage} \tag{8}$$

$$O_2^- + S \rightarrow P \tag{9}$$

$$2O_2^- + 2H^+ \xrightarrow{\text{cat}} O_2 + H_2O_2 \tag{5}$$

None of the above processes are necessarily single step processes. In order to acheive protection against O_2^- toxicity, $k_9[S]$ or k_{cat} [cat] should exceed $k_8[T]$.

What is the difference between an O_2^- scavenger and a catalyst for its destruction? The definition of a reactant's scavenger is clear and unambiguous - it is a substance reacting with it stoichiometrically. The definition and common accepted meaning of a catalyst are not as unique. In 1836, Berzelius defined a catalyst as a substance influencing the reaction rate and remaining unchanged at the end of the reaction.[8] Bell's definition was different.[9] He defined a catalyst as a substance whose concentration appearing in the reaction rate equation is in a higher power than it does in the stoichiometric equation. This definition seems to discriminate between an O_2^- scavenger and a catalyst for its destruction. Generally, a scavenger, present in excess concentrations as compared to the reactant, is capable of reacting with it fast enough to prevent alternative processes where the reactant may participate. A catalyst is generally regenerated in contrast to a scavenger, and is present at much lower concentration than that of the reactant.

It is not necessary that the concentration of the catalyst should be lower than that of the reactant, but it should be lower than the total amount of the reactant reacting over the time, otherwise the 'catalyst' practically will operate only through reaction

(1) and it will serve as a scavenger. Thus, for superoxide, which is present in living cells at very low steady state concentrations of about $10^{-10} - 10^{-11}$ M,[10] the concentration of SOD exceeds that of O_2^- sometimes by several orders of magnitude. Still, it is not required that $[SOD] < [O_2^-]_{s.s.}$ but rather that $[SOD] < [O_2^-]_T$ were $[O_2^-]_T$ = flux × t (t is the reaction time).

The lower the value of k_{cat} is, one needs higher concentration of the catalyst in order that $k_{cat}[cat] > k_8[T]$. For the typical experiments where O_2^- is generated by the xanthine/xanthine oxidase with a flux of about 1 μM/min in the presence of a detector molecule,[11,12] 10^{-8} M SOD should be sufficient to compete with the detector molecule for O_2^-. In such systems, if one would replace SOD with SOD mimics for which $k_{cat} = 10^3 - 10^4\,M^{-1}s^{-1}$, as in the case of the nonmetal SOD mimic OXANO found by Samuni *et al.*,[13] one would require a catalyst concentration of the order of 6 mM. Even after an hour less than 0.1 mM of O_2^- would be formed, and if it would react with OXANO, assuming $k_1 = k_2$, paractically all O_2^- will react via reaction (1), and thus the OXANO would in reality serve as a scavenger, rather than a catalyst.

c) Additional Problems Arising With the Use of SOD-Mimics in Vivo

When an efficient SOD mimic operating *in vitro* is introduced into the cells, the metal may be sequestered out of the complex by the cell components, which are present at relatively high concentrations. The new formed complexes may not be able to catalyze O_2^- dismutation or may even be toxic. If the stability constant is high, so that sequestering of the metal will not occur, still the possibility that this compound can form ternary complexes with the cell components, which do not have SOD-like activity exist.

Several mimics, which were found to be very efficient in catalyzing O_2^- dismutation in the pulse radiolysis system, e.g., phenanthroline: Cu(II),[14] failed to show this activity *in vivo*.[15] On the contrary, this complex was shown to enhance O_2^- toxicity. The reason for this behaviour cannot be attributed to reaction (-1), but to the fact that the cupric as well as the cuprous complexes form ternary complexes with DNA, which react extremely slowly with O_2^-, and lose their SOD-like activity.[16] Furthermore, the ternary cuprous complex reacts with H_2O_2, which is present at relatively higher concentration than superoxide in living cells,[10] to yield oxidizing species which can be either OH· radicals or the metal at its higher valence state (Cu(III)).[16] Either of these entities can subsequently react with DNA causing its degradation at the binding site.

CONCLUSIONS

Modified enzyme overcomes the short metabolic half life of the native enzyme, but it is not superior to SOD in the ability to penetrate into the cells. Synthetic low molecular weight SOD-mimics may have the advantage in this direction, and with appropriate ligands they may also be directed to lipophilic or hydrophobic regions.

In order that a compound with a high SOD-like activity *in vitro* would be able to mimic SOD *in vivo*, it is required that:

(i) It should be non toxic.
(ii) It should have a long metabolic half life.
(iii) It should have a high cell permeability.
(iv) It should have a high stability constant.

(v) Its reduced form should not be reoxidized fast by oxygen or H_2O_2.
(vi) It should not form ternary complexes with the cell components, or alternatively, the ternary complexes should retain most of the above mentioned properties.
(vii) It should be able to be directed preferentially either to a lipophilic or hydrophobic regions according to the demand.

These reqirements suggest that finding a SOD mimic to operate *in vivo* will not be an easy task.

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