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THE UNIQUENESS OF SUPEROXIDE DISMUTASE (SOD) – WHY CANNOT MOST COPPER COMPOUNDS SUBSTITUTE SOD *IN VIVO*?

GIDON CZAPSKI and SARA GOLDSTEIN

Department of Physical Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

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It is shown that the copper zinc superoxide dismutase is unique in its ability to catalyze O_2^- dismutation *in vivo* in contrast to other copper compounds which have this feature only *in vitro*. The reasons for this difference are discussed in terms of kinetic and thermodynamic parameters.

KEY WORDS: SOD, O_2^-, dismutation, copper compounds.

INTRODUCTION

The participation of O_2^- in a number of biological processes has been firmly established since the discovery of SOD by McCord and Fridovich in 1969.[1] The dismutation of O_2^- in the presence of SOD has been found to be extremely efficient,[2-4] and since the concentration of SOD in cells reaches 10^{-5} M,[5] while that of O_2^- exceeds 10^{-11} M,[6] it was suggested that O_2^- is toxic and it must rapidly be removed from cells.

The toxicity of O_2^- and the role of SOD in protecting biological systems against O_2^- toxicity became very popular subjects for research, which attracted a lot of attention in the seventies. More recently, due to the possible role of superoxide and SOD in ischemic processes,[7] these subjects became again a focus of many studies.

Is O_2^- Toxic and how does SOD Protect against this Toxicity?

The main questions, which were the focus of the earlier research in this area, were directed to answer the following issues:

1) Is O_2^- toxic, and what is the mechanism of its toxic action?

2) Is SOD really an enzyme whose sole and main function is to catalyze O_2^- dismutation, and whether through this activity it protects against O_2^- toxicity?

Both of these issues were for many years the focus of controversy. The two principal antagonists in this dispute were on the one side Fridovich and coworkers, who discovered SOD, its activity and protective role, and on the other side Fee, who adopted the opposite approach.[8]

In our opinion, the fact that O_2^- is toxic is well established in the majority of these studies. It is also quite clear that in *most* cases O_2^- itself is not toxic but it serves as a precursor for a more toxic entity. The expression of the toxicity of O_2^- is apparently through its ability to reduce transition metal compounds (e.g., copper or iron com-

pounds), which are subsequently reoxidized by H_2O_2 yielding deleterious entities.[9] These entities can be $OH\cdot$ or $FeH_2O_2^{2+}$, Fe^{IV} or FeO^{2+} or the equivalent copper compounds ($CuH_2O_2^+$, Cu^{III}, CuO^+).[10–12] Due to the difficulty of $OH\cdot$ scavengers in protecting the cell against some of these processes,[13] it was suggested that the metal is bound to the biological target and that the deleterious entity is formed in a "site specific" manner.[13–16]

However, there are cases where SOD protects the systems while catalase and metal chelators do not. Recently, some of these cases were reviewed by Fridovich, who suggested that O_2^- finds critical targets in living cells, independent of its metal-catalyzed interaction with H_2O_2.[17]

THE MECHANISM BY WHICH SOD AND OTHER COPPER COMPOUNDS CATALYZE O_2^- DISMUTATION

SOD has a positive surface track that leads O_2^- into the active site,[18,19] where alternate reduction and oxidation of the copper takes place:[2–4,20]

$$Cu^{2+} + O_2^- \rightarrow Cu^+ + O_2 \quad (1)$$

$$Cu^+ + O_2 + 2H^+ \rightarrow Cu^{2+} + H_2O_2 \quad (2)$$

net:

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

It has been shown through the use of pulse radiolysis techniques that many copper compounds catalyze O_2^- dismutation with similar efficiency to that of the native SOD.[20–26] The determinations of the catalytic efficiency of SOD and various copper compounds were also carried out using indirect assay methods, where O_2^- was usually generated with the xanthine/xanthine-oxidase system, using cytochrome c or nitro-blue tetrazolium as O_2^- scavengers. Using indirect methods, the SOD activity of most copper compounds, but not of the native SOD, was orders of magnitude lower than that determined directly with the pulse radiolysis system.[20–27]

With indurect methods, low steady state concentrations of O_2^- are generated, similar or even higher than those generated in *in vivo* systems. Therefore, a copper compound which yields low SOD activity with an indirect assay method, would probably be an inefficient catalyst *in vivo*.

The main reason for the unique ability of the native SOD to catalyze O_2^- dismutation with similar efficiency using both pulse radiolysis and indirect assays methods will be discussed in the following section.

WHY MOST COPPER COMPOUNDS CANNOT REPLACE SOD IN VIVO AND WHAT IS UNIQUE ABOUT SOD?

The question, what is so unique about SOD in its function to catalyze O_2^- dismutation, was raised by Fee, who claimed that many copper compounds have similar catalytic efficiencies to that of the native SOD. Therefore, the ability of SOD to catalyze O_2^- dismutation is not unique to this enzyme. The possible necessity of SOD was argued to be due to the fact that free copper is not present in living cells. However, this argument is not valid for there are many copper complexes which are stable in

the living organism and have SOD activity *in vitro*, e.g., copper histidine, copper salicylate and copper 3,5-diisopropylsalicylato complexes.[24,26,28]

A possible explanation for the inability of copper compounds to catalyze O_2^- dismutation *in vivo*, although these compounds have an excellent SOD activity in pulse radiolysis systems, can be traced directly to the formation of ternary complexes with biological macromolecules, such as DNA.[29] We have shown that the ternary complexes of DNA and some copper complexes react extremely slowly with O_2^-.[29]

Beyond this last argument, we can show the uniqueness of SOD as compared to many other copper compounds such as (salicylate)$_2$Cu(II), (tyrosine)$_2$Cu(II), etc. The mechanism of the catalysis of O_2^- dismutation by the various copper compounds is through the "ping pong" mechanism described for the action of SOD (reactions (1) and (2)), and for SOD and for many copper compounds, k_1, $k_2 = 10^8$–$10^9\,M^{-1}s^{-1}$. However, the rate of the reoxidation of the reduced compound by O_2 (reaction (-1)) can differ substantially. For SOD, k_{-1} is very small ($0.44\,M^{-1}s^{-1}$),[2] while for most of the other copper compounds $k_{-1} = 10^3 - 10^5\,M^{-1}s^{-1}$.[24–25,30–33]

Under the condition where $k_{-1}[O_2] << k_2[O_2^-]$, the "turnover" rate constant, k_{cat}, would be given by equation (4):

$$k_{cat} = 2k_1k_2/(k_1 + k_2) \tag{4}$$

Knowing the values of k_2 and k_{-1} for SOD, we calculate that as long as $[O_2^-]/[O_2] > 10^{-10}$, and at aerated solutions as long as $[O_2^-] > 2 \times 10^{-14}\,M$, which is the case in most living cells, the efficiency of SOD to catalyze O_2^- dismutation would be given by equation (4). However, if SOD would be replaced by other copper compounds for which $k_{-1}[O_2] > k_2[O_2^-]$, the efficiency of the compound to catalyze O_2^- dismutation would deviate from equation (4) and it would be given by k_{real}:

$$k_{real} = 2k_1k_2/(k_1 + k_2 + k_{-1}[O_2]/[O_2^-]) \tag{5}$$

In pulse radiolysis experiments, where $[O_2^-] = (0.1 - 2) \times 10^{-5}\,M$, $[O_2] = (0.24 - 1.2) \times 10^{-3}\,M$, and $k_1, k_2 = 10^8 - 10^9\,M^{-1}s^{-1}$, as long as $k_{-1} < 10^6\,M^{-1}s^{-1}$, the compound would have SOD activity and k_{cat} would be given by equation (4). This condition is fulfilled for SOD as for most copper compounds. However, in biological systems, where normally $[O_2^-] = 10^{-11}\,M$,[6] only those compounds for which $k_{-1} < 10\,M^{-1}s^{-1}$, would mimic SOD. In cases where $k_{-1} > 10\,M^{-1}s^{-1}$, the SOD activity would be lower, $k_{real} << k_{cat}$ and in extreme cases this activity would be too low to compete with the self dismutation of O_2^-. For example, for (phenanthroline)$_2$Cu(II), $k_1 = 1.9 \times 10^9\,M^{-1}s^{-1}$, $k_2 = 3 \times 10^8\,M^{-1}s^{-1}$ and $k_{-1} = 5 \times 10^4\,M^{-1}s^{-1}$.[25] Therefore, at high concentrations of O_2^-, as in pulse radiolysis experiments, $k_{cat} = 5.1 \times 10^8\,M^{-1}s^{-1}$, while under physiological conditions $k_{real} = 10^6\,M^{-1}s^{-1}$ (and about an order of magnitude higher if $[O_2^-] = 10^{-10}\,M$). In the case of (histidine)$_2$Cu(II), $k_1 = k_2 = 3.4 \times 10^8\,M^{-1}s^{-1}$, $k_{-1} = 4 \times 10^4\,M^{-1}s^{-1}$,[24] and therefore $k_{cat} = 3.4 \times 10^8\,M^{-1}s^{-1}$ while $k_{real} = 2.4 \times 10^5\,M^{-1}s^{-1}$.

From the thermodynamic point of view, we can calculate the minimum redox potential of a copper compound which is required in order that it would be capable of catalyzing O_2^- dismutation under physiological conditions. The calculations were based on Nernst equations using $E^0 = -0.16\,V$[34] for the couple O_2/O_2^-, $[O_2] = 0.24\,mM$, $[O_2^-] = 10^{-8} - 10^{-11}\,M$, and different ratios of $[Cu(I)]_{s.s}/$

TABLE I
Minimum redox potential of a copper compound required in order that it would be able to catalyze O_2^- dismutation under low steady state concentrations of O_2^-

E^0, mV

	$[Cu(I)]_{s.s}/[Cu(II)]_{s.s}$				
$[O_2^-]$, M	0.01	0.1	1.00	10	100
10^{-11}	157	216	275	334	393
10^{-10}	98	157	216	275	334
10^{-9}	39	98	157	216	275
10^{-8}	−20	39	98	157	216

$[O_2] = 0.24\,mM$, $E^0 = -0.16\,V$ for O_2/O_2^-

$[Cu(II)]_{s.s}$, based on the assumption that when this ratio exceeds 100 or is lower than 0.01 the compound would be a poor catalyst (Table I). From this table it is understandable why many copper compounds such as $Cu(II)_{aq}$, $(bipyridine)_2Cu(II)$, $(salicylate)_2cu(II)$, $(tyrosine)_2Cu(II)$, etc., do not have efficient SOD activity under physiological conditions or in systems where low concentrations of O_2^- are generated.

CONCLUSIONS

One of the main arguments of Fee was that there is nothing unique about SOD in its ability to catalyze O_2^- dismutation, as many other copper compounds have identical properties. We have shown that many of these compounds, which *in vitro* exhibit SOD activity, would not be efficient *in vivo*. The uniqueness of SOD turned out to be due to its low reactivity towards oxygen, while the majority of the other copper compounds show a relatively high reactivity towards oxygen. Therefore, Fridovich is right when he claims that SOD is unique in protecting against O_2^- toxicity *in vivo*, at least in those processes where O_2^- concentrations are low. In strong inflammatory processes or in phagocytosis, where local concentrations of O_2^- can be rather high, copper compounds may replace SOD. This conclusion has no implication on the other question raised, how is O_2^- toxic and what is unique about O_2^- as compared to other reductants in biological systems.

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