Metal-Dependent SOD Mimics

by

Wenqing Sun

Free Radical & Radiation Biology Program

B-180 Medical Laboratories

The University of Iowa

Iowa City, IA 52242-1181, USA

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Abbreviations:

CuDIPS:	Cu(II) (3,5-diisopropylsalicylic acid)$_2$
Cu(I) (DMP)$_2$:	Bis (2,9-dimethyl-1,10- phenaanthroline)-Cu(I)nitrate
DF-Mn:	Desferrioxamine-Mn (III)
Fe-EDTA:	Fe-Ethylenediaminetetraacetic acid
Fe-TPAA:	Fe (III)-tris [N-(2-pyridylmethyl-2-aminoethyl] amine
Fe(III) TMPyP:	Fe (III)-tetrakis (4-N-methylpyridyl) porphine
Fe-TPEN:	Fe(II)-tetrakis-N,N,N',N' (2-pyridylmethyl) ethylenediamine
NTA:	Nitrilotriacetate
SOD:	Superoxide Dismutase

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Abstract

Superoxide dismutase is an important antioxidant to control the free radical reactions related to superoxide generated in biological system. However, its large molecule and short life-span *in vivo* limit its clinical use. Extensive studies have been carried out to find the suitable SOD-mimics to substitute it. In order to acts as SOD mimics, a compound should be non-toxic, stable, easy to reach its targets and retain high SOD activity *in vivo.* Many compounds have shown SOD-like activity *in vitro*, however, no metal-dependent SOD mimics can really replace it.

Introduction

Superoxide is a major factor in radiation damage, inflammation, tumor promotion, and re-perfusion injury [1]. Fortunately, we have evolved an effective defense system against the toxicity of $O_2^{\bullet-}$, such as superoxide dismutase and catalase. In 1969, McCord and Fridovich first reported that the erythrocyte protein functioned as superoxide dismutase enzyme [2]. Generally there are three groups of SOD; each has different metal in the active site. According to the metals, they are termed as CuZnSOD, MnSOD and FeSOD. Acting as one of the most important antioxidants, all SODs catalyze the dismutation of $O_2^{\bullet-}$ as:

$$O_2^{\bullet-} + 2H^+ \rightarrow H_2O_2 + O_2$$

SOD can greatly accelerate this process. Under physiological pH, the rate constant for uncatalyzed dismutation is 5 x 10^5 $M^{-1}s^{-1}$; for CuZnSOD, k = 1.6 x 10^9 $M^{-1}s^{-1}$; for MnSOD, k= 1.8 x $10^9 M^{-1}s^{-1}$ [3]. However, the therapeutic use of SOD is limited by the facts that SOD cannot penetrate across cell membranes and it can be rapidly cleared by the kidney [4].

So, it is important to find SOD mimics, which have the activity of SOD and at the same time, are stable, non-toxic and capable of crossing cell membrane. There are two kinds of SOD mimics: metal-dependent and metal-independent mimics. This paper will focus on the metal-dependent SOD mimics, their assays, chemical characters and usage.

Catalyst Mechanism of SOD and Its Mimics

SOD catalyze the dismutation of superoxide through a so called "Ping-Pong mechanism": First the metal cation (M^{n+}) in SOD is reduced to $M^{(n-1)+}$ and reoxidized back to M^{n+}:

$$M^{n+} + O_2^{\bullet-} \xrightarrow{k1} M^{(n-1)+} + O_2 \quad (1)$$

$$M^{(n-1)+} + O_2^{\bullet-} \xrightarrow{k2} M^{n+} + H_2O_2 \quad (2)$$

The net reaction is:

$$2O_2^{\bullet-} + 2H^+ \xrightarrow{kcat} H_2O_2 + O_2 \quad (3)$$

$$k_{cat} = 2\frac{k_1 k_2}{k_1 + k_2}$$

For a typical experiment system, where $O_2^{\bullet-}$ is generated by the oxidation of xanthine, the flux of $O_2^{\bullet-}$ production is about 1.0 μM/min and 10^{-8} μM of SOD has a protective effect.

Required Qualifications of SOD Mimics

SOD mimics are those compounds that can function as SOD to catalyze the dismutation of superoxide. To act as a qualified SOD mimic *in vivo* conditions, a compound need to meet following qualities [5]:

1. The compound should not be toxic at the concentration needed for its SOD activity;
2. The compound should have a relatively long metabolic half-life for it to carry out its SOD activity;
3. The compound should be able to penetrate into the cells so to reach the target region;
4. The compound should retain its high SOD activity *in vivo.*

Assay of SOD Activity

To directly assay the SOD activity, we need to initially generate high concentration of $O_2^{\bullet-}$ and follow its decay by measuring the absorbance change at 250 nm in the absence and in the presence of a tested compound.

The most often used methods are "indirect assay". In an indirect assay, $O_2^{\bullet-}$ is generated with a constant flux (often by a mixture of xanthine oxidase and xanthine). Then $O_2^{\bullet-}$ reacts with detector molecules such as Fe (III)cytochrome *c* [6]:

$$\text{Cyt } c \text{ (Fe(III))} + O_2^{\bullet-} \rightarrow O_2 + \text{cyt } c \text{ Fe(II)} \quad (k = 2 \times 10^5\ M^{-1}s^{-1},\ pH = 7.8,\ 25°C) \qquad (4)$$

Fe(II)cytochrome *c* has an absorbance at 550 nm. SOD competes with Fe(III)cytochrome *c* for $O_2^{\bullet-}$. Since the ratio of the rate constant for $O_2^{\bullet-}$ dismutation for the catalysis by SOD and that for reduction of Fe(III)cytochrome *c* is $2 \times 10^9\ M^{-1}s^{-1}/2 \times 10^5 M^{-1}s^{-1}$, one part of SOD will

compete with 10,000 parts of cytochrome *c* and inhibit the change of absorbance. One unit of SOD activity is defined as the amount of SOD that inhibits the cytochrome *c* reduction by 50%. However, this assay can be interfered with by various factors, such as oxidization of cytochrome *c* by peroxynitrite, cytochrome oxidase and hydrogen peroxide [3]. Cytochrome *c* can be replaced by other molecules, such as nitroblue tetrazolium (NBT), adrenaline, and lluciferin.

Copper-containing complex

CuZnSOD contains two protein subunits; each of its active site contains one copper and one zinc ion. The zinc ion acts to stabilize the enzyme, while the copper ion acts as the functional metal.

Since copper acts as the active center in CuZnSOD, many copper complexes have been synthesized and tested. Research has shown that many complexes have SOD-like activities, such as Cu(II)(3,5-diisopropylsalicylic acid)$_2$ (CuDIPS) [7], Cu(II) histidine complexes [8], Cu(II) complexes of macrocyclic polyamine derivatives [9], Bis(2,9-dimethyl-1,10-phenaanthroline)-Cu(I)nitrate (Cu(I)(DMP)$_2$) [10] and Cu(II)-oligopeptide [11].

Table 1 [12] SOD-Like Activity, Percent Reactivity, and Rate of Superoxide Dismutation for some Copper complexes and CuZnSOD

Complex	Concentration (μM)	% reactivity	Rate (x10^9 $M^{-1}s^{-1}$)
CuZnSOD	0.02	100	1.3
Cu (II) (DIP)$_2$	2.9	0.70	1-2
Cu (II) (salicylate)$_2$	4.6	0.65	1.6

Since the dismutation reaction involves the redox cycle of Cu(II) and Cu(I), it is reasonable to expect that the redox potential of Cu(II)complex/Cu(I) complex can influence the SOD-like activity, while the ligand of the complex determines the redox potential [9]. It was shown that although His-Phe-Cu(II) complexes had a relatively high SOD activity (k = 3.57 x10^9 $M^{-1}s^{-1}$), Phe-His-Cu(II) displayed no SOD activity (k = 9.9 x10^4 $M^{-1}s^{-1}$) [13]. After comparing a

series of Cu(II)-macrocyclic polyamine derivatives, Kimura [9] proposed that the activity depended on ring size, type and subsistent on the macrocycles. By modifying their chemical structure, we can improve their biological activity.

Although *in vitro* studies show promising results of these copper complexes, *in vivo* experiments are often disappointing. The first reason is that many complexes undergo dissociation *in vivo*, yielding copper ions that subsequently combine with serum components and lose their SOD-like activity. The second reason relates to the reoxidation of the reduced compound by O_2. Unlike SOD (k_{-1} = 0.44 $M^{-1}s^{-1}$), most copper compounds have a k_{-1} at the range of 10^3-10^5 $M^{-1}s^{-1}$ [14]. At physiological situation, the concentration of $O_2^{\bullet-}$ is about 10^{-11} M. If k_{-1} $[O_2]$> k_2 $[O_2^{\bullet-}]$, the rate constant of metal compounds to catalyze $O_2^{\bullet-}$ dismutation would be [14]: $k_{real} = 2k_1k_2 \big/ (k_1 + k_2 + k_{-1}[O_2]/[O_2^{\bullet-}])$

So, these compounds can mimic SOD only when k_1 < 10 $M^{-1}s^{-1}$. Otherwise, they can only act as scavengers.

Iron-containing compounds

FeSOD is found in bacteria, algae and higher plants. During the catalytic cycle, the iron "oscillates" between Fe(III) and Fe(II) state. Some useful iron complexes with SOD-like activity include: Fe-ehylenediaminetetraacetic acid (Fe-EDTA), Fe(III)-tetrakis (4-N-methylpyridyl) porphine (Fe(III)TMPyP), Fe(II)-tetrakis-N,N,N',N'(2-pyridylmethyl)ethylenediamine (Fe-TPEN) and Fe(III)-tris [N-(2-pyridylmethyl-2-aminoethyl] amine (Fe-TPAA). At pH 10.1, Fe(III)-TMPyP can catalyze dismutation with a rate constant of 3 x 10^7 $M^{-1}s^{-1}$ [15]. Nagano [16] reported that in the xanthine oxidase-cytochrome *c* assay, 0.8 μM Fe-TPEN and 7.5 μM Fe-TPAA were equivalent to 1 unit of SOD activity. Paraquat can be reduced to monocation radical ($PQ^{\bullet+}$), which then reacts rapidly with molecular oxygen to yield $O_2^{\bullet-}$. Unlike copper-containing

complexes, which cannot suppress paraquat toxicity, both Fe-TPEN and Fe-TPAA can block the toxic effect of paraquat on *E. coli* growth and survival [16]. Some other TPAA-analogues liganding with Fe or Cu also show high SOD-like activity. It is suggested that the pyridine rings of TPAA might provide the metal an environment similar to that of native SOD [17].

However, the activity *in vitro* does not always correspond to those *in vivo*. *In vitro*, the SOD-like activity of Fe-TPEN is 100 times higher than that of Fe-TPAA; *in vivo*, Fe-TPAA has a much higher level of protection (10 fold) than Fe-TPEN [16]. As a potential Fenton catalyst, in the presence of H_2O_2, Fe(II)-TPEN reacted with H_2O_2 to generate hydroxyl radical and Fe(III)-TPEN; Fe (III)-TPEN then undergoes reducing by GSH or ascorbate [18]

$$\text{Fe(II)-TPEN} + H_2O_2 \rightarrow \text{Fe(III)-TPEN} + OH^- + OH^{\bullet} \quad (5)$$

So, the *in vivo* use of Fe(II)-TPEN as a SOD mimic is severely impaired by its capacity to generate $^{\bullet}OH$.

Manganese-Containing Compound

MnSOD is mainly located in the mitochondria. The "resting" enzyme contains Mn(III) at its active site. At pH 7.0, the rate constant of MnSOD catalyzed $O_2^{\bullet-}$ dismutation is similar to that of CuZnSOD, but it decreases at alkaline conditions.

The ligands for manganese-containing SOD mimics usually have a macrocycle structure, such as TMPyP, EDTA and NTA.

Like its Fe-containing counterpart, Mn(III)-TMPyP also effectively catalyzes the dismutation of $O_2^{\bullet-}$ and acts against paraquat [19]. Desferrioxamine-Mn(III) (DF-Mn) is another low-molecular-weight SOD mimic that protects cells from oxidative damage. In addition to facilitating the removal of $O_2^{\bullet-}$, DF-Mn can also oxidize reduced metals, such as DNA-bound Fe(II) or Cu(I), so DF-Mn can protect cells through $O_2^{\bullet-}$–independent mechanism [20].

Unfortunately, *in vivo*, the resultant Mn(II) complex can be reduced at a rate constant of 4 x 10^9 $M^{-1}s^{-1}$ [21], which means *in vivo*, Mn-porphyrins only act as a NADPH/GSH: $O_2^{\bullet-}$ oxido-reductase rather than $O_2^{\bullet-}$ dismutase.

Pharmaceutical Usage of SOD Mimics

Superoxide is deleterious *in vivo.* The direct targets of $O_2^{\bullet-}$ include many enzymes such as ribonucleotide reductase, which is responsible for DNA synthesis and 6-phosphogluconate dehydratase. In addition, $O_2^{\bullet-}$ can generate more reactive species, including $^{\bullet}OH$, $^{\bullet}NO_2$.

Acting as SOD mimics, many metal complexes have shown promising clinical effects. Oberley [22] proposed that copper complexes were effective in anti-inflammation, anti-ucler and anti-diabetic. Pretreatment with CuDIPs can inhibit TPA-induced carcinogenesis in mouse skin [23]. A Mn(II) complex with bis (cyclohexylpyridine)–substituted macrocyclic ligand has been proved to be stable *in vivo* and can increase the survival from ischemia-reperfusion injury [24].

Summary

Compared with SOD, metal-dependent SOD mimics have several advantages. First, they are easy and cheap to synthesize; second, as low-molecular-weight-molecules, they can across the cell membrane easily; third, they are easily modified in the lab.

However, they also have several shortcomings. The use of copper complexes *in vivo* is often severely limited by inactivation on processes due to chelating agents normally found in living cells. Although manganese and iron complexes are less influenced by chelating agents; some other limiting factors have to be taken into account. So, there still a long way for us to find the suitable nontoxic, stable and effective SOD mimics.

Reference

1. Goldstein S, Czapski G. (1991) Comparison between different assays for superoxide dismutase-like activity. *Free Rad Res Comm*. **12-13:** 5-10

2. McCord JM, Fridovich I. (1969) Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). *J Biol Chem.* **244:**6049-2055

3. Halliwell B, Gutteridge JMC. (1993) *Free Radicals in Biology and Medicine*. 3rd Ed. New York: Oxford University Press; pp107-130

4. Beakman JS, Robert L, Minor J, White CW, Repine JE, Rosen GM, Freeman BA. (1988) Superoxide dismutase and catalase conjugated to polyethlene glycol increases endothelial enzyme activity and oxidant resistence. *J Bio Chem.* **263:** 6884-6892

5. Czapski G, Goldstein S. (1990) Superoxide scavengers and SOD or SOD mimics. In: Emerit I Ed. *Antioxidants in Therapy and Preventive Medicine*. New York: Plenum Press; pp 45-50

6. Fridovich I. (1985) Cytochrome *c*. In: CRC Handbook of Methods for Oxygen Radical Research. Boca Raton: CRC Press; pp213-215

7. Kensler TW, Trush M. (1983) Inhibition of oxygen radical metabolism in phorbol ester-activated polymorphonuclear leukocytes by an antitumor promoting copper complex with superoxide dismutase-mimetic activity. *Beiochem Pharm.* **32:** 3485-3487

8. Weinstein J, Bielski BHJ. (1979) Reaction of superoxide radical with copper (II)-histidine complexes. *J Am Chem Soc.* **102:** 4916-4919

9. Kimura E, Sakonaka A, Nakamoto M. (1981) Superoxide dismutase activity of macrocyclic polyamine complexes *Biophy Acta.* **678:** 172-179

10. Bijloo GJ, Goot H, Bast A, Timmerman H. (1990) Copper complexes of 1,10-phenanthroline and related compounds as superoxide dismutase mimetics. *J Inorg Biochem.* **40:** 237-244

11. Ueda J, Ozawa T, Miyazaki M, Fujiwara Y. (1994) Activation of hydrogen peroxide by coppper (II) complexes with some histidine-containing peptides and their SOD-like activities. *J Inorg Biochem.* **55:** 123-130

12. Sorenson JRJ. (1984) Bis (3,5-diisopropylsalicylato) copper (II), a potent radioprotectant with superoxide dismutase mimetic activity. *J Med Chem.* **27:** 1747-1749

13. Amar C, Vilkas E, Foos J. (1982) Catalytic activity studies of some copper (II)-histidine-containing dipeptide complexes on aqueous superoxide ion dismutation. *J Inorg Ciochem.* **17:** 313-323

14. Czapski G, Goldstein S. (1988) The Uniqueness of superoxide dismutase (SOD) – why cannot most copper compounds substitute SOD *in vivo*? *Free Rad Res Comms.* **4:** 225-229

15. Pasternack RF, Halliwell B. (1979) Superoxide dismuatse-activities of an iron porphyrin and other iron complexes. *J Am Chem Soc.* **101:** 1026-1030

16. Nagano T, Hirano T, Hirobe M. (1989) Superoxide dismutase mimics based on iron *in vivo. J Bio Chem.* **264:** 9423-9449

17. Nagano T, Hirano T, Hirobe M. (1991) Novel iron complexes behave like superoxide dismutase *in vivo. Free Rad Res Comms.* **12-13:** 221-227

18. Iuliano L, Pedersen JZ, Ghiselli A, Pratico D, Rotilio G, Violi F. (1992) Mechanism of reaction of a suggested superoxide dismutase mimic Fe (II)-N,N,N',N'-tetrakis (2-pyridylmethyl) ethylenediamine. *Arch Biochem Biophy.* **293:** 153-157

19. Pasternack RF, Banth A, Pasternack JM, Johnson CS. (1981) Catalysis of the disproportionation of superoxide by metalloporphyrins. III. *J Inorg Biochem.* **15:**261-267

20. Nahn SM, Krishna CM, Samuni A, Mitchell JB, Russo A. (1991) Mu (II)-Desfemioxamine superoxide dismutase-mimic: alternative models of action. *Arch Biochem Biophy.* **288:** 215-219

21. Faulkner KM, Liochev SI, Fridovich I. (1994) Stable Mn (III) porphyrins mimic superoxide dismutase in vitro and substitute for it *in vivo. J Bio Chem.* **269:** 23471-23476

22. Sorenson JRJ, Oberley LW, Crouch RK, Kensler TW, Kishore V, Leuthauser SWC, Oberley TD, Pezeshk A. (1983) Pharmacologic Activities of copper compounds in chronic diseases. *Bio Trace Element Res.* **5:** 257-273

23. Egner PA, Kensler T. (1985) Effects of a biomimetic superoxide dismutase on complete and multistage carcinogenesis in mouse skin. *Carcinogenesis.* **6:** 1167-1172

24. Salvemini D, Wang ZQ, Zweier JL, Samouilov A, Macarthur H, Misko TP, Currie MG, Cuzzocrea S, Sikorski JA, Riley DP. (1999) A nonpeptidyl mimic of superoxide dismutase with therapeutic activity in rats. *Science.* **286:** 304-6