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# Manganese Superoxide Dismutase

by

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**Abbreviations:**

| | |
|---|---|
| CuZnSOD: | Copper zinc superoxide dismutase |
| FeSOD: | Iron superoxide dismutase |
| MnSOD: | Manganese superoxide dismutase |
| NBT: | Nitroblue tetrazolium |

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***Abstract***

MnSOD, as well as CuZnSOD and FeSOD, is a key component in controlling the free radical reactions related to superoxide generated within cells by catalyzing the dismutation of it. In eukaryotic cells, MnSOD is predominantly found in mitochondria. The structure of MnSOD is highly conserved in all species, indicating that there is a close relation between its structure and enzymatic activity. This paper will focus on the structures, catalytic mechanisms and assay of MnSOD.

## ***Introduction***

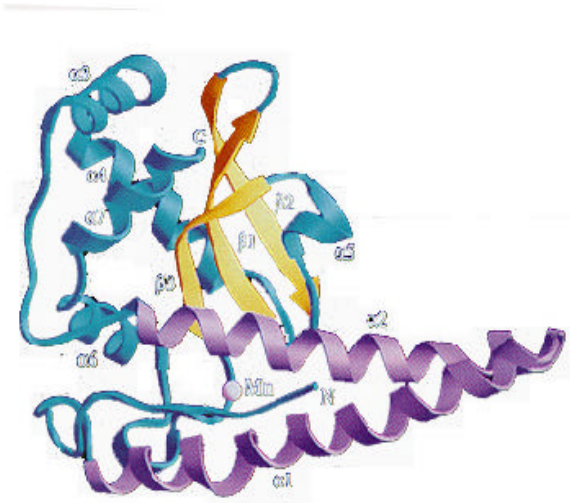
Superoxide ($O_2^{\bullet-}$) is a normal byproduct of aerobic metabolism and is produced in many reactions, such as oxidative phosphorylation and photosynthesis. Superoxide dismutase (SOD) is the primary cellular defense that protects cells from the deleterious effects of oxygen free radicals. In eukaryotic cells, there are three forms of SOD: CuZnSOD, MnSOD and FeSOD. Among them, CuZnSOD is located in the cytosol of animal cells; MnSOD is located in the mitochondria of bacteria, plants and animal cells; and FeSOD is found in some bacteria, algae and higher plants.

Due to the fact that the mitochondria consume more than ninety percent of the cell's oxygen and the respiratory chain of mitochondria is the main source of a large flux of oxygen radicals, it is reasonable to say that MnSOD is a critical component in the physiological protection from oxygen toxicity. This paper will focus on the physical and chemical characteristics of MnSOD, its structure and catalytic activity, and it synthesis and biological effects.

## ***Structure of MnSOD***

There are many x-ray crystal structures that have been reported, such as the bacterial MnSOD for *T. thermophilus* [1] and human mitochondrial MnSOD [2]. The structures of MnSOD from prokaryotic and eukaryotic enzymes are highly homologous. A whole human MnSOD protein is a homotetramer of 96 kDa. Each subunit consists of 198 residues. Two subunits pack tightly together participating in the formation of two active sites. Each subunit can be divided into two distinct domains: a N-terminal helical hairpin domain and a C-terminal $\alpha/\beta$ domain. The active site manganese is located between the helical hairpin and the $\beta$ sheet structural elements. In the resting state, the active site metal ion is in the trivalent state: $Mn^{3+}$.

Four amino acid residues, together with a water molecule, or a hydroxyl group ligate the manganese. The four amino acid residues belong to the two domains respectively: His 26 and His 74 from the N-terminal domain; Asp159 and His 163 from the C-terminal domain. The tetrameric interactions form a large solvent-filled tunnel that direct to the active site [2].



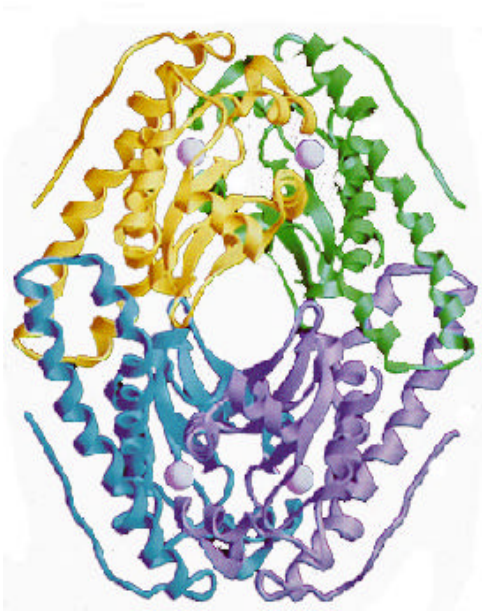



**Figure 1.** Human MnSOD subunit fold and active site.

**Figure 2.** Assembly of human MnSOD Tetramer

**Figure 1**[2]: RIBBONS diagrams of human MnSOD subunit fold and active site geometry. This is a single MnSOD subunit. The N-terminal domain (bottom) is made up of the N-terminal loop and two long α-helices ($\alpha_1$ and $\alpha_2$). The C-terminal α/β domain (top) is composed of five α-helices ($\alpha_3$-$\alpha_7$) and three β-strands ($\beta_1$-$\beta_3$). The manganese lies between the two domains.

**Figure 2** [2]: RIBBONS diagrams of human MnSOD tetramer. The dimer at the top and the dimer at the bottom of the figure each form a crystallographic asymmetric unit. The apparent central cavity is occluded by the adjacent side chains of the cross-cavity interface. The active site manganese atoms are located near the dimer interface.

## ***Relation of Structure and Dismutase Activity***

Some amino acids of MnSOD are highly preserved in both bacteria and human, indicating their importance in maintaining structure stability and catalytic activity.

The two dimeric subunits of MnSOD form a channel through which the superoxide anion may approach the manganese ion that lies in the bottom of the active site. An examination of the structural on *T. thermophilus* MnSOD suggests that Arginine 189 is on one of the side chain that composes the channel wall. Arginine is positively charged and provides a diffuse electrostatic attractive force to guide superoxide to the catalytic metal center. By treating with phenylglyoxal, an arginine-specific reagent, Borders [3] reported that the MnSOD from *E. coli* was extensively inactivated.

The activity of MnSOD can be inhibited in many pathophysiological conditions. It is reported that [4] during renal allograft rejection, mitochondrial MnSOD is subject to inactivation by tyrosine nitration. As a mediator in transplantation rejection, nitric oxide ($^{\bullet}$NO) reacts with superoxide to form peroxynitrite (ONOO). At high cumulative concentrations of peroxynitrite, tyrosine 34, 45 and 193 in MnSOD were nitrated. Among them, tyrosine 34 is the most susceptible residue to peroxynitrite-mediated nitration [5].

Tyrosine 34, which resides within a few angstroms of the active site, is conserved in all of the MnSODs and FeSODs [6] and plays an essential role in the catalytic activity of superoxide dismutase. Azide anion, an inhibitor of SOD, forms a hydrogen bond to the hydroxyl group of tyrosine 34 and acts as sixth ligand with the metal [7].

Glutamine 143 is located 4.6 Å away from the manganese and 2.7 Å from the hydoxyl group of tyrosine 34. After replaced glutamine 143 by asparagine, Hsieh [6] found the tyrosine 34 side-chain hydroxyl moved to become 0.6 Å more distant from the metal, as a result, the overall catalytic activity of mutant MnSOD was decreased 2-3 orders of magnitude compared with wild-type MnSOD.

***Synthesis of MnSOD***

Unlike CuZnSOD and FeSOD, which are constitutively produced *in vivo*, MnSOD is absent during anaerobic conditions and is rapidly synthesized upon exposure to oxygen. Oberley [8] pointed out that x-irradiation can induce MnSOD amount and activity in a dose and time dependent manner. Many proinflammatory mediators [9], such as tumor necrosis factor alpha (TNFα) and interferon gamma (INFγ), can up-regulate MnSOD synthesis dramatically by increasing the rate of transcription [10].

Iron supplementation can increase lipid peroxidation; increased oxidative stress can consequently increase MnSOD protein level and activity [11].

***Mechanism of Catalytic Activity***

Once produced, MnSOD disposes of the highly reactive superoxide anion by a two-step dismutation reaction. In this process, $Mn^{3+}$ is reduced and then reoxidized:

$$Mn^{3+} + O_2^{\bullet-} \rightarrow Mn^{2+} + O_2 \quad (1)$$

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

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

The rate constant for MnSOD catalyzed dismutation depends on pH. At pH 7.0, k is similar to that of CuZnSOD, *i.e.* $5 \times 10^9\ M^{-1}s^{-1}$. While at pH 7.8, $k = 1.8 \times 10^9\ M^{-1}s^{-1}$, and at pH 10.2, k is only $0.33 \times 10^9\ M^{-1}s^{-1}$ [12].

The rate constant of these reactions depends on the reaction driving force (redox potential) and on the electron self-exchange rate constant for both the oxidant and reductant [13]. The redox potential for *T. thermophilus* MnSOD is + 0.06 V [14], the redox potential for *E. coli* MnSOD is + 0.31V, and for *B. stearothermophilus*, it is 0.26 V [15].

***Assay of MnSOD***

Assays of MnSOD include assays for MnSOD protein and for the catalytic activity of MnSOD.

By immunohistochemical methods, we can detect CuZnSOD and MnSOD proteins in animal tissues. These two enzymes are so different that the antibodies do not cross-react.

The most often used methods to measure the activity of SOD are indirect. In an indirect assay, $O_2^{\bullet-}$ is generated with a constant flux, and then reacts with detector molecules.

One of the detector molecules that often used is ferricytochrome *c*.

Cyt *c* (Fe(III)) + $O_2^{\bullet-}$ ? $O_2$ + cyt *c* (Fe(II)) (k = 2 x $10^5$ $M^{-1}s^{-1}$ pH 7.8, 25C) [16](4)

Ferrocytochrome *c* has an absorbance at 550 nm. In the presence of SOD, less ferrocytochrome *c* will be formed. From equations 1 and 2, it can be show that [17]:

$$\frac{dO_2^{\bullet-}}{dc^{2+}} = \frac{k_4[c^{3+}] + k_{cat}Mn^{2+}}{k_4[c^{3+}]} \qquad (5)$$

Where $k_{cat} = \frac{2k_1k_2}{k_1 + k_2}$, $c^{2+}$ is ferrocytochrome *c*, and $c^{3+}$ is ferricytochrome *c*.

Another detector molecule commonly used is nitroblue tetrazolium (NBT). In NBT assay, the reaction course is followed by the increase in absorbance at 560 nm as NBT was converted to formazan. The apparent rate constant for the reaction of $O_2^{\bullet-}$ with SOD, $k_{SOD}$, can be obtained as [18]:

$$k_{SOD} = \frac{k_N[NBT]}{[SOD]}(1/? - 1) \qquad (6)$$

Where $k_N$ is the rate constant for the reaction of NBT with $O_2^{\bullet-}$; ? is the ratio of observed rate constants in the presence and absence of the SOD.

There are several means to separate MnSOD from its copper and iron counterparts. For example, $H_2O_2$ can inhibit the activity of CuZnSOD and FeSOD, while it has no effect on that of MnSOD. In addition, CuZnSODs are inhibited by $CN^-$, whereas FeSOD and MnSOD are not, so $CN^-$ can therefore be used to distinguish MnSOD from CuSOD in assays of tissue homogenates or on polyacrylamide gels.

***Biological Effects***

Although according to rate constant, MnSOD is less efficient than CuZnSOD and FeSOD, it still has some unique advantages, which make MnSOD more suitable for medical uses. First, unlike its copper and iron counterparts, MnSOD is not inhibited by hydrogen peroxide. Also, MnSOD has a half-life in sera of 5-6 hours compared with 6-10 minutes for CuZnSOD [19].

Many experiments support the roles of MnSOD in aging, cancer and various neurodegeneration diseases. By transfecting MnSOD cDNA into melanoma cell lines, Church [20] reported that increased MnSOD expression could alter the phenotype of melanoma and repress their clonal forming activity.

***Summary***

MnSOD is a primary antioxidant enzyme that protects cells from oxidative damages by rapidly converting superoxide radicals into hydrogen peroxide, which is further detoxified by other enzymes. The structure of MnSOD has very close relationship with it catalytic function. Further studies about its structure can help us understand its enzymatic mechanism better and found potential mimics for clinical use.

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