

Manganese Superoxide Dismutase (MnSOD)

by

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**For 77:222, Spring 2001
8. March, 2001**

Abbreviations:

MnSOD: Manganese superoxide dismutase

SP-1: specific protein-1

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Abstract

Manganese superoxide dismutase (MnSOD) is the primary antioxidant enzyme that protects cells from oxidative stress by catalyzing dismutation of superoxide ($O_2^{\bullet -}$) to hydrogen peroxide and oxygen in the mitochondria of eukaryotic cells. The rate constant of this dismutation reaction is near the diffusion limit. Human MnSOD is a homotetramer. The active site metal complex is formed from four protein side chains. Manganese in the active site carries out a one-electron transfer between two $O_2^{\bullet -}$ radicals. A number of residues that are strictly conserved in MnSOD families are essential for catalytic function. Many different kinds of tumors express low level of MnSOD. Overexpression of MnSOD suppresses tumorigenicity. Much evidence supports that MnSOD is a tumor suppressor. This paper will review the mechanism, molecular and chemical structure and biological significance of MnSOD.

Introduction

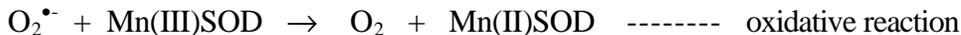
Manganese superoxide dismutase (MnSOD) is a very important antioxidant enzyme that catalyzes the conversion of superoxide radicals ($O_2^{\bullet-}$) to hydrogen peroxide and molecular oxygen in the mitochondria [1]. Under normal physiological conditions, mitochondria are the major source of $O_2^{\bullet-}$ production. Numerous studies have indicated that MnSOD plays an important role in preventing cells from oxidative stress and inhibiting tumorigenicity [2].

Phylogenetic Distribution and Subcellular Localization

Manganese superoxide dismutase is a ubiquitous metalloenzyme found in virtually all aerobic organisms from bacteria to humans, and even anaerobes [3]. Manganese superoxide dismutase is uniformly distributed throughout the cytoplasm in procaryotic cells [4]. For the highly partitioned eukaryotic cells, MnSOD is exclusively associated with mitochondria [1]. The mitochondrial enzyme is encoded by a nuclear gene and must be transported across two mitochondrial membranes to the matrix. This process involves translation of a proenzyme which in the case of the human MnSOD includes a 24-amino acid N-terminal leader peptide targeting the protein to the mitochondrion. The delivery of MnSOD to the mitochondrial matrix is essential for organelle function.

Kinetics and Mechanism

The elementary studies in mechanism of MnSOD catalysis have been elucidated from kinetic studies [5]. The enzymatic reaction consists of a bimolecular reaction with a catalytic cycle involving two distinct half-reactions, an oxidative reaction in which the substrate, $O_2^{\bullet-}$, is oxidized to dioxygen and a reductive half-reaction in which $O_2^{\bullet-}$ is converted to H_2O_2 .



These two reactions differ in the oxidation state of the metal ion and the involvement of proteins in the reductive half-reaction. The bimolecular rate constants for reaction of oxidized and reduced *T. thermophilus* MnSOD with $\text{O}_2^{\bullet-}$, measured by pulse radiolysis, are near the diffusion limit ($2.0 \times 10^9 \text{ M}^{-1}\text{S}^{-1}$, $2.2 \times 10^9 \text{ M}^{-1}\text{S}^{-1}$, respectively), making MnSOD among the fastest of all enzymes (Figure 1) [5,6].

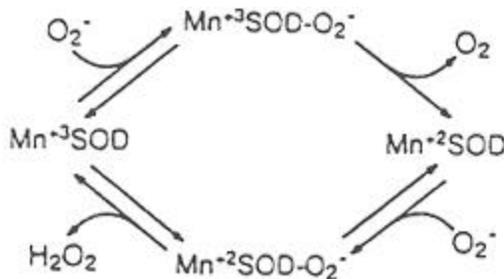


Figure 1. $\text{O}_2^{\bullet-}$ will be converted to the H_2O_2 and O_2 by MnSOD [From 5].

Under the conditions of stopped-flow kinetic measurements, a dead-end peroxide complex forms that effectively inactivates approximately 95% of the enzyme in the steady state. This peroxide complex is an isomer of the peroxy intermediate formed during turnover, and conversion to the dead-end complex exhibits a solvent isotope effect less than unity, suggesting that proton transfer to the productive peroxyanion complex prevents formation of the dead-end complex. The close balance between oxidative and reductive rates is expected to be important for efficient cycling of the enzyme during turnover [6].

Molecular and Chemical Structures

Crystal structures have been solved at high resolution for MnSOD [6]. *E. coli* MnSOD is a homodimer, while both human and *T. thermophilus* MnSOD are homotetramers (dimers of

dimers). In each case, the subunits are composed of two domains, an all- α N-terminal domain and an α/β C-terminal domain (Figure 2).



Figure 2. *E. coli* MnSOD is a homodimer [From 6].

There are a number of residues that appear to be strictly conserved in MnSOD families which are expected to represent groups essential for catalytic function [7]. Four of these (H26, H81, D167, and D171 in *E. coli* MnSOD sequence numbering) are metal ligands, two more (H30 and Y34) form the gateway to the active site, and another (E170) lies in the outer sphere of the metal binding site (Figure 3) [7]. The active site metal complex in MnSOD is formed from four protein side chains and a molecule of coordinated solvent (water or hydroxide, depending on metal oxidation state) for overall five-coordination at the metal center (Figure 3). The only nonprotein ligand for the active site Manganese ion is the coordinated solvent, which, unlike solvent associated with other catalytic metalloenzyme complexes, is not the substrate binding site. Other important outer-sphere interactions radiate from the solvent

pocket, including a conserved interaction with a glutamine residue (Q146 in *E. coli* MnSOD), which arises from a different region of the polypeptide chain in Manganese proteins. This hydrogen bonding interaction extends to a conserved tyrosine (Y34) which, together with a histidine residue (H30), forms the gateway to the active site metal complex [Figure 3]. The hydrogen bonding chain Y34-Q146-OH has been identified as a relay pathway for proton transfer in and out of the solvent pocket [8]. The gateway formed by Y34 and H30 controls the coordination chemistry of the metal complex. These residues lie at the base of a substrate funnel that extends out to the surface of the globular protein that restricts access to the active site by small molecules.

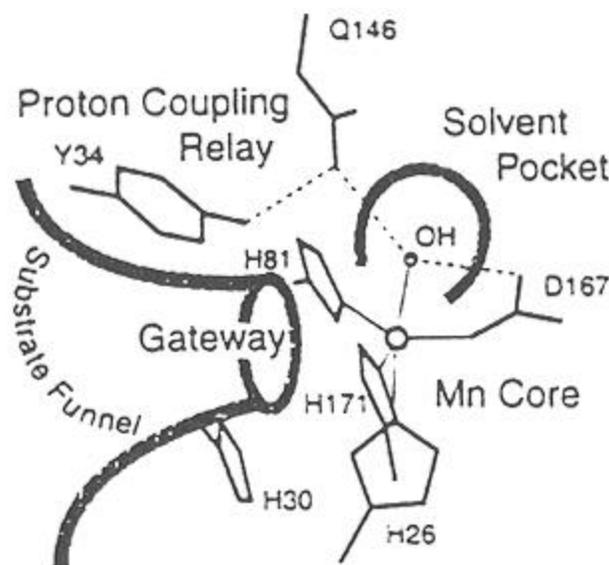


Figure 3. The active site of MnSOD contains four metal ligands [From 7].

The E170 residue is part of a pattern that is highly conserved over all MnSOD protein sequences ($D_{167}XWE_{170}H_{171}XXY_{174}$) including two metal ligands (D167 and H171) which form a DX_3H metal-binding motif, and two residues (E170, Y174) that span the dimer interface, bridging between active sites in opposite halves of the dimer (Figure 4) [9].

The ligands form a nearly perfect trigonal bipyramidal coordination polyhedron for the bound metal ion, and two imidazoles 81 and 171 comprising the equatorial ligand set and the remaining imidazole and solvent aligned axially [Figure 4]. The complex has approximate site symmetry with the mirror plane bisecting the equatorial pair of histidines. In the outer sphere of the complex, the E170 glutamate residue is hydrogen-bounded to the noncoordinating nitrogen of the H171 ligand and contributes to charge compensation for the buried metal ion, effectively neutralizing the single unit of residual charge unbalanced by anionic ligands in the inner sphere [10].

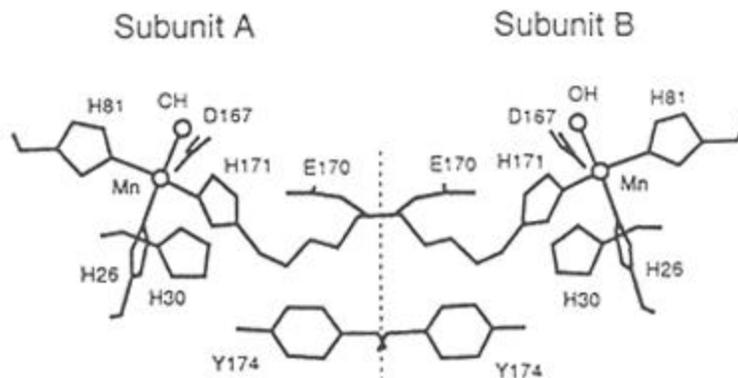


Figure 4. A double bridge is formed by two E170 and two Y174 between two active sites in MnSOD of all species [From 9].

Thus, the glutamate residue lying in the outer sphere of the Manganese complex in one subunit arises from the polypeptide chain of the other subunit [9]. As a result of the twofold symmetry of the dimeric protein, corresponding glutamates in the two subunits form a double bridge between the active sites. The dimeric protein has an irregular form that leaves a hemicylindrical groove between the subunits as a conserved feature of MnSOD structure [9]. The diameter of the cleft is close to the diameter of B-form DNA, suggesting that this feature may be involved in the nonspecific association with DNA [4]. Molecular dynamics modeling

allows B-form DNA to be docked within the cleft with only minor rearrangement of amino acid side chains (Figure 5).

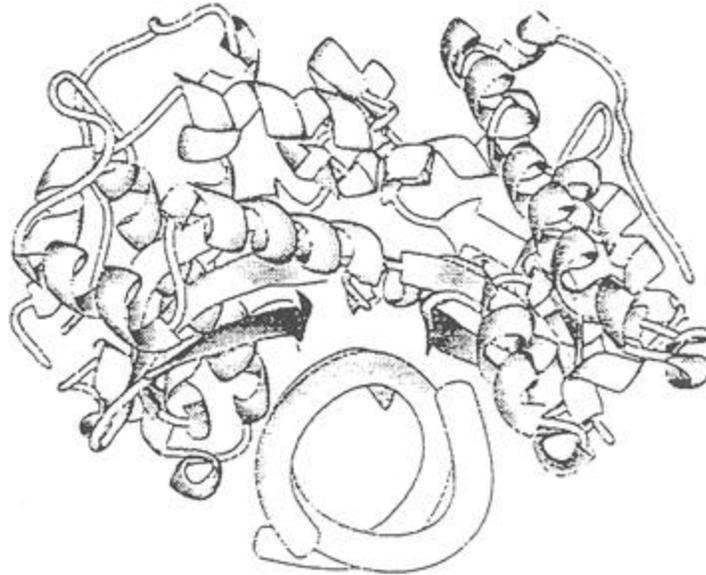


Figure 5. Molecular dynamics adapted structure of *E. coli* MnSOD-DNA complex [From 9].

Biological Significance

Manganese superoxide dismutase plays a key role in protecting cells against oxidative damage and regulating cellular concentration of $O_2^{\bullet-}$, which is an extremely oxidant and an unwanted byproduct of cellular metabolism [11]. Alterations in MnSOD levels have been associated with a number of neurodegenerative diseases, including Parkinson's disease, Duchenne muscular dystrophy, Charcot-Marie-Tooth disease, and Kennedy-Alter-Sung syndrome [12]. Many different types of tumors have been demonstrated to have low MnSOD activity [2]. Overexpression of MnSOD suppresses the tumorigenicity of human melanoma cells, breast cancer cells and glioma cells, suggesting that MnSOD is a tumor suppressor gene in a wide variety of cancers [13,14,15]. For example, St. Clair *et al* reported that suppression of tumor metastasis by MnSOD is associated with reduced tumorigenicity and elevated fibronectin

[16]. They found that the median tumor growth times for the tumors derived from all MnSOD-transfected cells were longer than those of the parental cells. They also found increased extracellular matrix fibronectin levels in MnSOD transfected cell lines. Since oxidative stress can inactivate the promoter of human fibronectin gene containing SP-1 binding sites and antioxidants support the transcription activity of SP-1 proteins, they speculated that the expression of MnSOD provides an antioxidant environment which supports the activity of SP-1, leading to an increased level of fibronectin in MnSOD-transfected cells [16, 17].

Summary

Manganese superoxide dismutase is the primary antioxidant enzyme which can protect cells from oxidative damage by catalyzing dismutation of $O_2^{\bullet-}$ to H_2O_2 and O_2 . The most promising role of MnSOD is associated with its inhibition of tumorigenicity. Many studies suggest that MnSOD may function as a general tumor suppressor gene. It is believed that MnSOD will be applied to cancer therapy in the near future.

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