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Review

Superoxide dismutases: Ancient enzymes and new insights

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ABSTRACT

Superoxide dismutases (SODs) catalyze the de toxification of superoxide. SODs therefore acquired great importance as O_2 became prevalent following the evolution of oxygenic photosynthesis. Thus the three forms of SOD provide intriguing insights into the evolution of the organisms and organelles that carry them today. Although ancient organisms employed Fe-dependent SODs, oxidation of the environment made Fe less bio-available, and more dangerous. Indeed, modern lineages make greater use of homologous Mn-dependent SODs. Our studies on the Fe-substituted MnSOD of *Escherichia coli*, as well as redox tuning in the FeSOD of *E. coli* shed light on how evolution accommodated differences between Fe and Mn that would affect SOD performance, in SOD proteins whose activity is specific to one or other metal ion.

1. Introduction

Superoxide dismutases, or SODs, are enzymes that play a pivotal role in metabolizing $O_2^{\bullet-}$, preempting oxidizing chain reactions that cause extensive damage, and forestalling formation of a cascade of deleterious reactive oxygen species (ROS) including hydrogen peroxide (H_2O_2), hypochlorite (OCl^-), peroxynitrate (ONO_2^-) and hydroxyl radical ($HO^{\bullet}$). Aerobic metabolism makes some 18 times more energy available per glucose than does glycolysis, making possible large and complex organisms. Thus, ability to employ O_2 constitutes a decisive evolutionary advantage. Yet even now, equipped as we are with multiple SODs and numerous supporting antioxidant systems, it is inexorable damage due to ROS that eventually limits our lives.

Ability to survive O_2 is such a stringent selection criterion that very few organisms lacking SOD survived the transition from reducing to oxidizing environment brought about by the evolution of oxygenic photosynthesis some 2.4 billion years ago [1]. The evolutionary pressure to develop protection against superoxide was sufficiently intense that SODs evolved on at least three separate occasions. One of these was sufficiently ancient and important that this enzyme is found in all kingdoms of life, indicating that it evolved even before differentiation of eubacteria from archaea. Thus, the enzyme that keeps us young has been a spectator to much of the evolution of life.

The most primitive versions of SOD employ Fe, consistent with the prevalence of Fe and its ready availability in the reducing environment in which life is believed to have begun. However more modern organisms employ a version of this enzyme that requires Mn for activity, consistent with diminished bioavailability of Fe and increased Fe toxicity as O_2 levels rose. Thus, changes in the inorganic chemical makeup of the environment appear to have driven evolution of SOD. In what follows, I discuss chemical challenges inherent in the transition between catalysis based on Fe and catalysis based on Mn. I then review what has been learned about the occurrence and phylogeny of modern SODs. A growing wealth of such information is beginning to reveal the paths by which we have acquired and inherited the SODs we have today, from diverse ancestors.

2. Three distinct families of SODs

The name SOD denotes not one, but three unrelated enzymes. All three earned the name by virtue of ability to convert two molecules of superoxide to one each of dioxygen and hydrogen peroxide, with consumption of two equivalents of H^+.

One family of SODs employs a Ni ion to mediate the chemistry, another uses a Cu ion complexed with a Zn to do the same. The third family encompasses enzymes that use a Mn or an Fe as well as enzymes that can use either.[1] The different families of SODs differ not only with regard to the metal ion that supports activity, but also

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[1] We refer to enzymes active only with Mn bound as MnSODs, SODs requiring bound Fe for activity as FeSOD and SODs active with either Fe or Mn as Mn/FeSODs.

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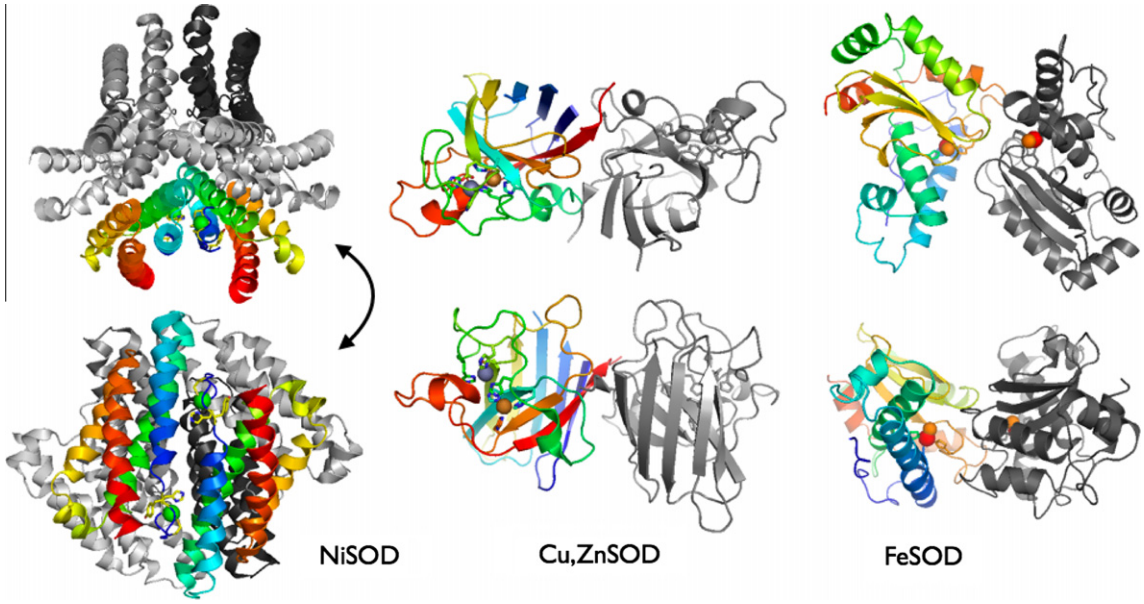

Fig. 1. Comparison of the ribbon structures that characterize the three families of SOD. In FeSOD and Cu,ZnSOD one monomer is coloured, for NiSOD two monomers are coloured. Each structure in the second row displays the view obtained by rotating the structure above by approximately 90° around a horizontal axis in the plane of the page, tipping what was the top of the figure in the upper row towards the back. The NiSODs are homohexamers of four-helix bundles of total molecular weight ≈ 80 kDa [7]. Each four-helix bundle binds a Ni ion (green sphere) at the N-terminus. However each Ni site also derives supporting hydrogen bonds from residues from the neighboring four-helix bundle in a reciprocal arrangement, so two four-helix bundles are colored and the other four are shown in greys. The Cu,ZnSODs are generally 32 kDa homodimers (or dimers of dimers), where each monomer is a flattened eight-strand beta barrel. The Cu and Zn ions (gold and silver spheres, respectively) are bound on the outside of the barrel by two loops, including a short helix. The Fe or MnSODs are 45 kDa homodimers (or dimers thereof) where each monomer includes an alpha-helical N-terminal domain and a C-terminal domain comprised of a three-stranded beta sheet surrounded by alpha helices. The Mn or Fe ion (red sphere) is bound between the two domains by two amino acids from each and a solvent molecule (orange sphere). Cartoons were made using Pymol [8] and the coordinates 1Q0D.pdb for NiSOD [3], 1HL5.pdb chains F and M [7,9] for Cu,ZnSOD, and 1ISB.pdb for FeSOD [10,11].

with regard to the protein fold (Fig. 1). Recent progress in understanding the mechanism of Ni-SODs has been described by [2,3], and ongoing advances in understanding the biological significance of Cu,Zn SODs are summarized in [4] while the mechanism is reviewed in [5,6]. The current review will concentrate initially on the Fe- and/or Mn-utilizing SODs and then place them in the context of the occurrence and possible evolution of all three SODs.

3. New SODs from old: differences required for Mn-based SOD activity vs. Fe-based activity

Diverse primitive organisms employ FeSOD, yet it is apparently absent from animals and fungi, and present in plants only in the chloroplasts. This suggests that ancient versions of this enzyme used Fe but that there was a switch to the use of Mn that occurred concurrent with evolution of eukaryotes. The switch could have occurred in response to diminished bioavailability of Fe once the atmosphere became oxidizing and Fe's tendency to engage in Fenton chemistry with superoxide, especially under conditions of oxidative stress. However the cost of Fe acquisition continues to be borne for production of hemes and numerous Fe_xS_x and Fe-containing enzymes. A second factor will be that for many Fe-dependent systems, the chemistry in question simply does not lend itself to Mn-mediated catalysis. Another factor was likely the ease with which evolution could execute a transition from Fe-based activity to activity based on Mn, without 'interruption of service'. Most simply, for evolution to be able to effect change, the enzyme should possess a selectable amount of the destination activity to serve as the raw material for the action of evolution. SOD meets both criteria, as witnessed by bacterial and archaeal SODs that can function with either Fe or Mn. Indeed, these 'cambialistic' SODs are often found in anaerobic organisms that are relatively primitive. Thus modern FeSODs and MnSODs could have evolved from a common ancestor via SODs able to use either metal.

In order to learn what distinctions might be involved in optimizing Mn-dependent as opposed to Fe-dependent SOD activity, we compared the MnSOD and FeSOD of *Escherichia coli*. As for FeSODs and MnSODs in general, those of *E. coli* share a conserved protein fold and a dimer interface that involves active site residues as well as the funnel by which substrate gains access to the catalytic metal ion (Fig. 2A). Both are dimers; they share 43% amino acid identity including the three His and one Asp^- that bind the metal ion in all Fe- and MnSODs, in addition to the conserved Tyr and a nearby Gln[2] (Fig. 2B). In both, the Fe or Mn ion also binds to a solvent molecule that in turn engages in hydrogen bonds (H-bonds) with the second sphere Gln. Thus, comparison of the two active sites does not suggest an obvious explanation for the different metal ion dependencies of activity. Moreover both sites are able to bind either metal ion in similar coordination geometries[3] [12] with similar electronic structures [13–15].

The very similar sites provide a conserved context in which to identify differences that could enable one protein to support Mn-based activity while the other supports only activity based on Fe. Fe and Mn have similar ionic radii and ligand preferences, and both cycle between their 2+ and 3+ oxidation states in the course of SOD turnover. However the oxidation states with the same charge correspond to different d-electron configurations for Mn and Fe, and this has consequences for both the ease with which the 3+ ions can be reduced to the 2+ state, and the tendency of each to coordinate a sixth ligand. Thus, the different metal ions possess different natural tendencies that may require different modulation and complementation on the part of the protein.

[2] This Gln is replaced by a His in *Mycobacterium tuberculosis* and its relatives.

[3] The active site pK of Fe^{3+}(Mn)SOD is considerably lower than that of Fe^{3+}SOD. Therefore the structure of Fe-substituted MnSOD (Fe(Mn)SOD) reveals a second coordinated OH^- in one active site that is absent from the structure of FeSOD [12].