A Structure-Based Mechanism for Copper-Zinc Superoxide Dismutase†,‡

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ABSTRACT:

A reaction cycle is proposed for the mechanism of copper-zinc superoxide dismutase (CuZnSOD) that involves inner sphere electron transfer from superoxide to Cu(II) in one portion of the cycle and outer sphere electron transfer from Cu(I) to superoxide in the other portion of the cycle. This mechanism is based on three yeast CuZnSOD structures determined by X-ray crystallography together with many other observations. The new structures reported here are (1) wild type under 15 atm of oxygen pressure, (2) wild type in the presence of azide, and (3) the His48Cys mutant. Final R-values for the three structures are respectively 20.0%, 17.3%, and 20.9%. Comparison of these three new structures to the wild-type yeast Cu(I)ZnSOD model, which has a broken imidazolate bridge, reveals the following: (i) The protein backbones (the “SOD rack”) remain essentially unchanged. (ii) A pressure of 15 atm of oxygen causes a displacement of the copper ion 0.37 Å from its Cu(I) position in the trigonal plane formed by His46, His48, and His120. The mutation of His48 to Cys, which does not bind copper, results in a five-coordinate square pyramidal, bridge-intact copper geometry with a novel chloride ligand. Combining results from these and other CuZnSOD crystal structures, we offer the outlines of a structure-based cyclic mechanism.

INTRODUCTION:

Higher organisms produce superoxide anion (O2-) as an occasional byproduct during the one-electron reduction of dioxygen that occurs in respiration and photosynthesis. Also, in animals, macrophages generate superoxide as part of the immune response. Organisms must therefore have ways to regulate superoxide concentrations since excess amounts can inactivate enzymes containing iron-sulfur clusters and can lead to the formation of highly oxidizing species (such as hydroxyl radical) damaging to other cellular constituents. Copper-zinc superoxide dismutase 1 (CuZnSOD) is a 32 kDa homodimeric protein in the cytoplasm of eucaryotic and bacterial cells that catalyzes the disproportionation of superoxide into dioxygen and hydrogen peroxide (2O2- + 2H+ f O2 + H2O2). Each monomer of the molecule binds one copper and one zinc ion and displays the Greek Key fold. The enzymatic mechanism proposed for CuZnSOD is reduction of the oxidized Cu(II) form of the enzyme by superoxide, releasing dioxygen.
(reaction 1), alternating with oxidation of the reduced Cu(I) form by another superoxide anion and two protons, generating hydrogen peroxide (reaction 2).

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\begin{align*}
\text{O}_2^- + \text{Cu(II)ZnSOD} & \rightarrow \text{O}_2 + \text{Cu(I)ZnSOD} \quad (1) \\
\text{O}_2^- + \text{Cu(I)ZnSOD} + 2\text{H}^+ & \rightarrow \text{H}_2\text{O}_2 + \text{Cu(II)ZnSOD} \quad (2)
\end{align*}
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FIGURE 1: Atomic model for the active site atoms of yeast wild-type CuZnSOD superimposed on 1.7 Å resolution electron density. The copper ion is 3.16 Å from the nitrogen atom of the bridging imidazolate, His63. It is held by a trigonal planar arrangement of histidine residues (His46, His48, and His120). Notice that the imidazolate bridge to the copper ion is broken.

FIGURE 2: Atomic model for the active site atoms of 15 atm of oxygen yeast CuZnSOD superimposed on 1.8 Å resolution electron density in the same orientation as in Figure 1. The copper ion has moved 0.37 Å approximately perpendicular to the trigonal plane relative to the wild-type Cu(I)ZnSOD copper position and shows two-state character suggestive of a mixture of oxidation states and binding geometries.