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X-Ray Absorption Spectroscopy of 3-Fe Clusters in Fe–S Proteins

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Fe EXAFS and edge spectroscopy have been used to characterize the 3-Fe clusters in aconitase and *Azotobacter vinelandii* ferredoxin I (*Av* Fd I). Fe EXAFS of a frozen solution of oxidized (unactivated) beef heart aconitase indicates a 'compact' cluster structure with Fe–Fe distances of *ca.* 2.7 Å. In combination with independent iron and acid-labile sulfur determinations, these results allow us to propose a structure for the aconitase 3-Fe cluster with a stoichiometry of [3Fe-4S].

Single crystal polarized X-ray absorption spectroscopy was performed on *Av* Fd I crystals with the goal of distinguishing Fe–Fe scattering contributions from the 4- and 3-Fe clusters. Crystallographic results on this protein suggest an 'extended' structure for the 3-Fe cluster with *ca.* 4.1 Å Fe–Fe distances. Polarized spectra were recorded with the X-ray polarization vector both normal to and parallel to the average plane of the irons in the 3-Fe clusters. The Fourier transforms were compared and found to be identical in the region in which a 4.1 Å scattering peak would be expected. Thus, the long (*ca.* 4.1 Å) Fe–Fe distance cannot be detected in *Av* Fd I crystals at room temperature. Low-temperature solution work is planned to look for evidence of this interaction.

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A Water Proton and Anion Affinity Investigation of Zinc(II) Deprived Superoxide Dismutase

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In native superoxide dismutase, copper(II) is bound to four histidine nitrogens in a puckered square planar arrangement, the angles N–Cu–N for the two couples of trans nitrogen atoms being 160° and 230°, respectively. One of these nitrogens belongs to an histidinate residue bridging the zinc ion [1]. The EPR spectrum shows a large rhombic component [2]. Anions like CN^-, N_3^-, NCS^-, NCO^- substitute in our opinion for a histidine nitrogen [3, 4].

Zinc deprived SOD shows an axially symmetrical EPR spectrum [5] indicating that the removal of the bridging requirement leads to a less distorted chromophore. Water proton NMRD of solutions containing SOD have indicated the presence of a coordinated axial water molecule [6]. The same measurements performed on the zinc deprived derivative (Fig. 1) have shown that water is still present, although loosely bound as in the native SOD.

The affinity constants of the anions N_3^- and NCS^- at pH 6.0 for zinc deprived SOD (160, 26 M^{-1}) closely compare with those for the native enzyme (90,

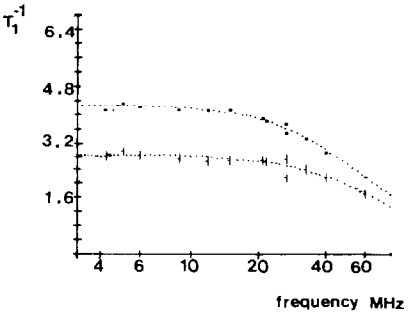

Fig. 1. Magnetic field dependence of the proton relaxation rate of aqueous solutions of native (■) and zinc deprived (+) SOD. The samples were 5.6×10^{-4} M protein, pH 6.0.