and relate structural information about the iron sites in the cluster. In a related study, we have obtained the iron K-edge EXAFS of the 3Fe ferredoxin II of *Desulfovibrio gigas* in the oxidized and reduced states [2]. For both states, interpretation of the EXAFS data suggests that the Fe–S distance is near 2.25 Å, in agreement with crystallographic studies of model compounds and proteins containing 2Fe-2S and 4Fe-4S centers, as well as with a recent crystallographic study of *Azotobacter vinelandii* ferredoxin I (D. Ghosh, W. Furey, Jr., S. O'Donnell and C. D. Stout, *J. Biol. Chem., 256,* 4185 (1981).). The Fe–Fe distance of 2.7 Å, however, agrees with similar distances observed in other Fe–S centers, but disagrees with the 3Fe cluster in the *Azotobacter vinelandii* ferredoxin I structure, for which an Fe–Fe distance of 4.2 Å was reported. We conclude that the two 3Fe ferredoxins may have substantially different core dimensions, a possibility apparently unique to 3Fe centers among known Fe-S systems in proteins. The implication of such structural variation of 3Fe centers in the nitrogenase problem will be discussed.

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EXAFS Studies of Copper and Zinc in Metallothionein and Bovine Superoxide Dismutase

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We have accomplished the measurement and interpretation of the copper and zinc K-edge EXAFS for these elements in two protein environments; metallothionein, from a variety of sources, and bovine erythrocyte superoxide dismutase.

Metallothioneins are a ubiquitous class of proteins which are believed to play important roles in the control of zinc and copper metabolism and in the detoxification of metals, such as cadmium and mercury. Each of these proteins is characterized by its low molecular weight (~6,000), a high cysteinyl content, and the ability to bind substantial amounts of metal ions, such as Cd, Zn, Cu, Ag, Co and Hg. Results obtained from a variety of spectroscopic studies clearly indicate that the metal ions are bound to the protein *via* the sulphur atoms of the cysteinyl residues. However, a definitive structural characterization of a metallothionein has not yet been achieved. To provide further information concerning the details of metal attachment to metallothionein, we have recorded the copper and zinc K-edges for pig liver metallothionein containing copper and zinc, and the K-edge of zinc for sheep liver metallothionein 2 containing zinc, and rabbit liver metallothionein containing zinc or zinc and cadmium. Freeze-dried samples were employed and X-ray absorption spectra were recorded for these materials at room temperature and 77K.

For zinc, the EXAFS data are consistent with back-scattering from, essentially, a single shell of our sulphur atoms at *ca.* 2.28 Å. The chemical interpretation of this information is that zinc is bound by the sulphur atom of four cysteinyl groups, as $Zn(Scys)_4$. Similar conclusions have been reached for the immediate environment of the copper atoms in pig liver metallothionein containing *ca.* six copper and two zinc atoms per protein molecule. The Cu–S distance is *ca.* 2.22 Å and there appear to be three sulphur atoms at this distance.

An assessment of the quality of the interpretation of the EXAFS data obtained for zinc and copper in metallothionein has been obtained by graphically displaying the variation of the function

$$r_g = 1/\Sigma_k[k^2(\psi_T(k) - \psi_E(k))]^2$$

for different values of the parameters used to interpret the EXAFS data. The reciprocal of the simple least-squares function was chosen since it produces clearer visual displays than the function itself; thus, the optimum interpretation of the experimental data is when r_g is maximised. An iterative procedure was adopted, refining (R and E_0) and (N and α) sequentially, until reasonable self-consistency was obtained. The final value of R (Zn–S) was obtained by plotting a slice through the r_g *versus* (R, E_0) profile at the optimum value of E_0; the uncertainty in this value being taken as the width of this peak at 70% height.

Bovine erythrocyte dismutase has been the subject of a large number of investigations and much of this work has been stimulated by the determination of the crystal structure to 3 Å resolution. However, this structure alone does not contain the detailed information needed to explain aspects of the enzyme's mechanism as the resolution is too low to detect the subtle structural features which presumably control the activity. Therefore, we have undertaken an X-ray absorption spectroscopic study of the metal centres in this superoxide dismutase, in its native, reduced, and azide bound forms. Data was recorded for freeze dried material and aqueous solutions of the protein.

The similarities in the profile of the copper K-edge EXAFS of native superoxide dismutase and those obtained for $[Zn(imidazole)_4][ClO_4]_2$ and $[Cu(imidazole)_4(NO_3)_2]$, indicate that, in this form of the enzyme, the copper is co-ordinated by four essentially equivalent imidazole groups, with $Cu-N_\alpha$ distances of *ca.* 2.00 Å. The $CU\cdots C_\beta$ and $Cu\cdots C_\gamma(N_\gamma)$ distances follow from the $Cu-N_\alpha$ value. A similar environment is found about copper in the azide bound form but reduction causes a significant structural change at this atom. The EXAFS data obtained for the zinc site of native superoxide dismutase are reasonably reproduced by an environment comprising one oxygen atom, presumably from the asparate carboxylate group, at *ca.* 1.92 Å and one and two imidazole groups with Zn–N distances of *ca.* 2.06 and 1.97 Å, respectively. The geometry about this atom is not changed in any significant respect in the azide bound and reduced forms of the enzyme.

These results will be discussed with reference to EXAFS data for a variety of copper and zinc complexes of a known structure. Attempts to determine the geometry of the sulphur atoms about the metal atoms of metallothionein from XANES data will also be described.