

tive anaerobic bacteria were found to contain the enzyme, probably the iron form. This is considered to be the most primitive form of SOD. In general terms the distribution of SOD can be stated to be that the Cu/Zn enzyme is essentially a eukaryotic enzyme and the iron enzyme is essentially a prokaryotic enzyme. The manganese enzyme can also be considered to be a prokaryotic enzyme. It is, however, also found in mitochondria. Whilst certain species of bacteria have been found to have one form of SOD *i.e.*, either the Fe or the Mn form, there are species with both forms as well. It appears that which form of the enzyme is present depends on the growth conditions. The phototrophic purple sulfur bacterium *Thiocupsa roseopersicina* grown in the presence of acetate or glucose contains Fe-SOD. Growth on media with glucose but not acetate leads to the formation of Mn-SOD [1]. The synthesis of either Fe- or Mn-SOD with apparently identical protein moiety by *Photobacterium shermanii* was found to be conditional on the metal supply [2]. All eukaryotic species have the Cu/Zn and Mn form of the enzyme. Some plant species have the Fe form as well whilst two bacterial species have the Cu/Zn enzyme [3].

The Cu/Zn SOD is a homodimer of about 32,000 daltons. The molecular weight determined for the enzyme from a variety of sources are all substantially in agreement. By contrast, the Mn- and Fe-forms have a slightly higher molecular weight of about 40,000 daltons. Whilst all the Cu/Zn and the Fe-SODs have been shown to be dimeric, the manganese enzyme has been found to form tetramers. The extent of polymerisation does not apparently depend on the source of the enzyme.

The Cu/Zn SOD contain up to 2 g atoms of both Cu and Zn calculated on the basis of a molecular weight of 32,000 daltons. The values reported in the literature is 1.8 g atoms. The metal content of the Fe and Mn enzymes varies between 1 and 2 g atoms per dimer. Whether this variation is due to loss of metal content can only be determined once the number of metal binding sites is determined from the X-ray structure. Only preliminary data has so far been presented on the X-ray structure for this protein. The complete sequence has only been determined for the Mn enzyme from *E. coli* and *B. stearrowthermophilus* and yeast mitochondria [4–6]. The sequence homology is low compared to the homologies observed between the Cu/Zn enzyme. The nature of the Mn and Fe ligands is as yet unknown. Preliminary spectroscopy evidence, however, indicates that a tyrosine could not be a ligand.

The Cu/Zn SODs have been more extensively investigated than the Fe- and Mn-SODs. The primary structure has been determined for the enzyme from bovine [7] and human erythrocytes [8, 9], horse liver [10], yeast [11, 12], swordfish liver [13] and

the free living symbiotic bacterium *Photobacterium leiognathi* [14]. Extensive homology has been found to occur between the eukaryotic enzymes. The presence of a Cu/Zn SOD in *P. leiognathi* has led to speculation about a possible gene transfer from a eukaryotic to a prokaryotic species. However, the amino acid sequence only shows a 20–25% structural homology between this enzyme and the other eukaryotic SODs indicating an independent evolutionary line. All the Cu/Zn SODs have, however, a conserved metal binding site determined from the X-ray structure of the bovine enzyme. These are the histidines 47, 49, 76, 81, 134 for the Cu site and the histidines 76, 85, 94 and aspartate 97 for the zinc site. Arginine 157 claimed to be essential for activity is also conserved. However cysteine 125 is only present in the human enzyme.

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Iron and Manganese Superoxide Dismutases

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Fe and Mn SDs are enigmatic metalloproteins for several reasons: They are found in strict anaerobes as well as aerobes. In some cases the Fe and Mn ions

appear to be interchangeable while in others they are not. In some organisms the Mn-protein is strongly induced by O_2 while in others the Fe-protein is induced. The two distinct types of proteins are structurally related as judged from published amino acid sequences. The Fe-protein appears to have a non-covalently associated organic co-factor bound near the iron (see below).

Recently, the three dimensional structures of FeSD from *E. coli* and *Ps. ovalis* have been elucidated in the laboratories of M. Ludwig and G. Petsko. The FeSD molecule is composed of a dimer of identical subunits related within the crystal by a dyad axis. The distance between the Fe atoms is 18 Å and each is close to the subunit interface. Access to the Fe appears to be from a region near the interface. Each monomer has two distinct structural domains, connected by a single strand and the Fe is bound at the interface of the two domains receiving two ligands from each. The Fe is surrounded by four ligands from the protein and probably a water molecule. The geometry, at 3 Å resolution has the appearance of a flattened pyramid. According to present interpretations of the electron density map the four protein ligands are His-26, residue 69, and residues 148 and 152. The spectral properties of the protein would appear to exclude tyrosine as a ligand to the Fe. A region of unconnected electron density is found in the region of the molecule separating the two domains and about 6 Å from the Fe. This is a dominant feature in FeSD from both sources and may represent an organic co-factor. To date we have not isolated and identified this material.

We are involved in a program to correlate structural and functional properties of the FeSD from *E. coli*. The presentation will be concerned with redox probes of the Fe, steady state and transient kinetic studies of superoxide dismutation, and relations between anion inhibition and binding to the Fe. If time allows a comparison will be made between the mechanism of FeSD action and the weak superoxide dismutase activity of Fe-EDTA.

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Phosphate is an Inhibitor of Copper, Zinc Superoxide Dismutase

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Bovine erythrocyte Cu, Zn superoxide dismutase (SOD) in its oxidized form has been shown by X-ray crystallography [1] to be a dimer of two equiv-

alent subunits, with one Cu(II) and one Zn(II) ion per subunit. The Cu ion is bound to four histidyl imidazole ligands and a water molecule making the overall geometry five-coordinate. One of these imidazoles is deprotonated and acts as a bridging ligand between Cu and Zn. The remaining ligands to Zn are two additional histidyl imidazoles and aspartyl carboxylate, forming a distorted tetrahedral geometry. Cu, Zn SOD's are found to be extremely efficient catalysts of the disproportionation of superoxide ($2O_2^- + 2H^+ \rightarrow O_2 + H_2O_2$) and it has been proposed that this activity is their primary biological function *in vivo* [2].

A number of anions have been observed to bind to Cu, Zn SOD e.g. CN^- , N_3^- , SCN^- , OCN^- , and halides. Each of these anions binds to copper(II) in the enzyme, as indicated by the pronounced spectral shifts observed upon binding [3]. Inhibitory effects of these anions have been attributed to this binding [3]. There have been several observations reported in the literature that suggest that phosphate also interacts with Cu, Zn SOD. For example, it has been mentioned in passing that phosphate interferes with the binding of CN^- to the enzyme [4] and that it has an inhibitory effect on the SOD activity [5]. In addition, it has been reported that phosphate influences the mode of binding of cobalt to the apoprotein [6] and that it interacts with the four-copper derivative (where Cu has been substituted for Zn in the native protein) [7].

We have found no visible or ESR spectral changes in solutions of Cu, Zn SOD at high concentrations of phosphate, suggesting strongly that any interaction of phosphate with the enzyme does not occur by binding of that anion to Cu(II). Contrary to the reports of McAdam [8] and Cudd and Fridovich [9], who assumed that the inhibitory effect of phosphate on the SOD activity could be entirely attributed to ionic strength effects, we found that the SOD activity of native bovine Cu, Zn SOD measured at constant ionic strength decreased significantly in the presence of increasing concentrations of phosphate. In addition, we have titrated the native enzyme with NaN_3 in differing concentrations of phosphate at constant ionic strength and thereby demonstrated that the presence of phosphate decreased the affinity of the enzyme for the azide anion. This result is reminiscent of the decrease in binding affinity of this protein when arginine-141 is chemically modified with phenylglyoxal [10]. Arginine-141 may well be the site of phosphate binding to this protein, since the binding of phosphate to the arginine-modified protein is apparently not affected by the presence of phosphate. Spectroscopic evidence concerning the nature of the interaction between phosphate and Cu, Zn SOD has also been obtained.