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

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The Catalytic Mechanism of Carbonic Anhydrase

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Steady-state and equilibrium kinetic studies of the CO_2–HCO_3^- interconversion catalyzed by human carbonic anhydrase II (or C) have led to the proposal of a mechanism scheme (Scheme 1) involving two ionizing groups [1–4]. One of these is probably a zinc-bound H_2O molecule ionizing to an OH^- ion which can react with CO_2 to form zinc-bound HCO_3^-. The other group is probably His-64 which is located in the hydrophilic part of the active site at some distance from the zinc ion. At low buffer concentrations the rate-limiting step in catalysis is the transfer of H^+ between His-64 and buffer molecules. At high buffer concentrations the rate-limiting step seems to be an intramolecular H^+ transfer between the two active-site groups:

$$His–E–Zn^{2+}–OH_2 \rightleftharpoons {}^{+}H–His–E–Zn^{2+}–OH^-.$$

When Scheme 1 was first proposed [1] it was assumed that the pK_a values of the two ionizing groups were identical and that there was no interaction between the groups. However, it has later been shown that the early results were affected by SO_4^{2-} ions which inhibit at low pH [5]. When the effects of SO_4^{2-} are taken into account it must be assumed that there is, indeed, an interaction between the two active-site groups so that they do not operate independently of one another. The rate equation describing the steady-state velocity resulting from Scheme 1 is exceedingly complex. Rather than use such an equation, we have computer simulated the kinetic behaviour predicted by Scheme 1 using various sets of rate constants. It will be shown by this method that Scheme 1 can describe satisfactorily the known kinetic behaviour of human carbonic anhydrase II. It will also be shown that Scheme 1 predicts the deviations from Michaelis–Menten kinetics observed for the low-activity human carbonic anhydrase I (or B) under certain conditions.

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Comparative Studies of Bovine and Human B Carbonic Anhydrases through their Cobalt(II) Substituted Derivatives

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A major physicochemical difference between the high activity bovine carbonic anhydrase B (BCAB) and the low activity human B isoenzyme (HCAB) resides in the pK_a s of the groups controlling the catalytic activity, which differ by at least one unit. Such a difference is maintained in the cobalt(II) substituted derivatives. The electronic spectra of the latter show in both cases a pH dependence which can be rationalized in terms of at least two acidic groups with close pK_a values [1].

Studies on the bovine isoenzyme and its adducts with inhibitors which act as metal ligands allowed us to propose a spectroscopic criterion to assign the coordination number of the metal, based on the combined use of electron spectroscopy, water proton NMRD, and ^1H NMR of the coordinated histidines [2–4]. With such a background, we turned to the investigation of the human B isoenzyme with the aim of giving the observed differences a more firm structural basis.

A careful examination of the electronic spectra of the low pH form of the latter derivative shows that the molar absorbance is considerably lower than that of the corresponding bovine isoenzyme; NMRD measurements extended down to 0.01 MHz indicate a substantially lower water proton relaxation