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Proton Relaxation of Water Solutions Containing Copper Carbonic Anhydrase

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When the zinc(II) ion of the enzyme Carbonic Anhydrase is replaced by copper(II), the resulting compound does not show any appreciable enzymatic activity, although the latter ion is believed to occupy the same position of zinc within the molecule [1]. Whereas the native enzyme has been deeply investigated [2], relatively few data are available concerning the copper derivatives [3–5] so that little is known with respect to the stereochemistry and the donor groups. In order to investigate the possibility of the existence of donor groups containing protons which rapidly exchange with water protons, proton longitudinal relaxation times and linewidths of water in H_2O–D_2O (fifty–fifty) solutions of copper enzyme $\simeq 10^{-3}$ *M* have been measured. T_1 values have been obtained through the well known 180–τ–90-delay technique in Fourier Transform with a Varian CFT-20 spectrometer operating at 15 °C, whereas T_2 values were determined by linewidth measurements. The copper derivative of the carbonic anhydrase has been obtained through the usual treatment of the native bovine enzyme (Sigma Co.) with 1,10-phenanthroline and addition of slightly less than the stoichiometric amount of copper sulfate [3].

Solutions containing copper carbonic anhydrase 10^{-3} *M* display T_1's one order of magnitude shorter than native enzyme solutions do (Fig. 1). This means that there are protons close to the metal ion which rapidly exchange with water protons so that the effect of the paramagnetic center, *i.e.* T_1 shortening and linewidth increase, is averaged over the solvent protons. The T_1 values depend also on pH outside the experimental indetermination, showing that minor copper–hydrogen interactions occur in the pH range 7–9 (Fig. 1). Addition of acetate and iodide ions, which bind to the metal ion as shown by the electronic spectra of the adducts [4] (Fig. 2), do not affect the T_1 values nor the linewidth. On the contrary, the oxalate ion causes an increase of T_1 and T_2 almost up to the diamagnetic values. It can be concluded therefore that a group with mobile hydrogens, say R, interacts with the copper atom. Acetate and iodide bind the metal at a site different from that involved with the R group whereas oxalate, capable

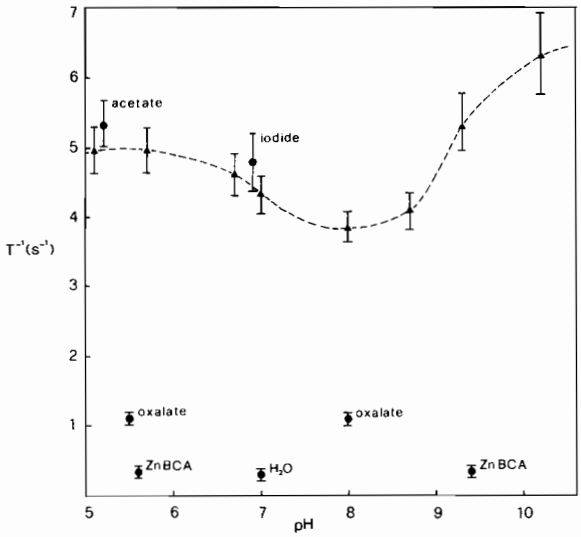

Fig. 1. T_1^{-1} values of the ^1H NMR signal of unbuffered water solutions ($^1H_2O/^2H_2O$ 1/1) of Copper(II) Bovine Carbonic Anhydrase 1.0×10^{-3} *M versus* pH (▲). The values of the acetate, iodide, and oxalate derivatives, as well as the native enzyme (ZnBCA) are also reported. pH values are given as the uncorrected pH-meter readings.

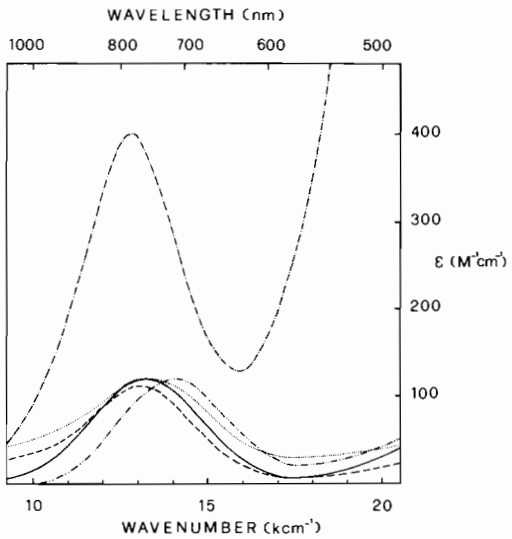

Fig. 2. Electronic spectra of Copper(II) Bovine Carbonic Anhydrase at unbuffered pH 5.5 (——) and 9.3 (·····), and of its adducts with iodide (– · – · –), acetate (– – –), and oxalate (– · · – · · –).

of acting as bidentate, can compete both with R and at the site at which acetate and iodide compete. Indeed the substitution of the iodide ion by the oxalate ion can be easily demonstrated by the disappearance of the characteristic charge transfer bands of the copper carbonic anhydrase–iodide adduct [4] as oxalate, in an oxalate–iodide ratio 1:1, is added. Although more information on the R group is

needed, the present data might be reasonably consistent with R being a water molecule as previously proposed by Koenig [6]. These results are quite different from those obtained on the cobalt [7] and manganese [8] enzymes. In the latter cases the proton relaxivity at low pH values indicates that water is either unbound or it exchanges slowly on the NMR time scale.

Metal ions in carbonic anhydrase are known to be bound to three histidine nitrogens [1, 2]; in the case of zinc and cobalt derivatives a fourth ligand is assumed to be bound to the metal giving rise to a pseudotetrahedral structure [9, 10]. Iodide, acetate, and oxalate give rise to five coordinate adducts [10, 13]. In the case of the copper enzyme the electronic spectra are consistent with a pseudotetrahedral or a five-coordinate structure, since tetragonal species either planar or six-coordinate absorb at much higher energies [14]. Iodide, acetate, and oxalate derivatives are very similar to each other and to the spectrum of the pure enzyme, the frequency of the maxima falling in the range 13,000–14,000 cm^{-1} (Fig. 2). The higher molar absorbance of the iodide adduct is presumably due to intensity borrowing from the near charge transfer bands. Therefore the geometry should be substantially the same among the various derivatives. Since acetate and iodide do not affect the T_1, they either substitute one of the three histidine nitrogens or a fourth donor group different from R In the former case the chromophores of both the pure enzyme and its derivatives are pseudotetrahedral, in the latter five-coordinate. We feel that the hypothesis of five-coordination is more consistent with the common chemical sense; furthermore, the high apparent affinity constants for the copper enzyme are strongly indicative of a competition with a weakly bound group. It is not uncommon in copper(II) complexes that a fifth donor is farther from the metal than the other four donors [14].

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