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Stereochemistry of Cobalt(II) *in Cobalt Bovine Carbonic Anhydrase* and its Derivatives

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The *cobalt carbonic anhydrase* is a metallo enzyme in which the native zinc(II) ion has been substituted by cobalt(II) [1]. Its electronic spectrum shows that the enzyme is present as two forms in a pH dependent equilibrium, the bands of each species being well shaped and intense [2]. Analysis of the spectra leads to the conclusion that the cobalt ion is in an environment of low symmetry either tetracoordinated pseudotetrahedral or pentacoordinate [3]. Even further physical measurements (CD [4], MCD [5], esr [6], nmr [7], resonance Raman [8], *etc.*) could not allow to definitely discard one of the two possibilities.

It is also known that several ligands bind the cobalt in the cobalt enzyme in a 1:1 molar ratio. The electronic spectra and other physical data indicate that the new complexes are pseudotetrahedral [9]; however, we feel that in some cases a more careful analysis of the spectral data especially in the visible and near infrared region may provide useful information on the stereochemistry of this type of complexes [10]. As an example we would like to report here the electronic spectra between 8000 and 25000 cm^{-1} of the *bovine cobalt* enzyme at high and low pH values as well as of the halide, acetate, cyanate, and acetazolamide derivatives (Fig. 1). The latter spectra have been recorded in large excess of inhibitors and every time a check with a further addition of inhibitor was performed in order to make sure that the spectrum actually corresponds to the limit spectrum. The near infrared spectrum of the pure enzyme had been previously reported at high pH values [3, 9]; also the entire spectrum of the chloride derivative had been reported [9], however, the presence of a weak absorption at 13 500 cm^{-1} had been neglected. Such band is in general present in every spectrum of lower intensity than that of the pure enzyme and the less intense is the spectrum the more pronounced is the band.

The electronic spectra of the chloride derivative have been measured at temperatures of 5, 17, 26 and 35 °C, a temperature range in which the enzyme does not undergo substantial denaturation (Fig. 2). Although the temperature range is very limited, the

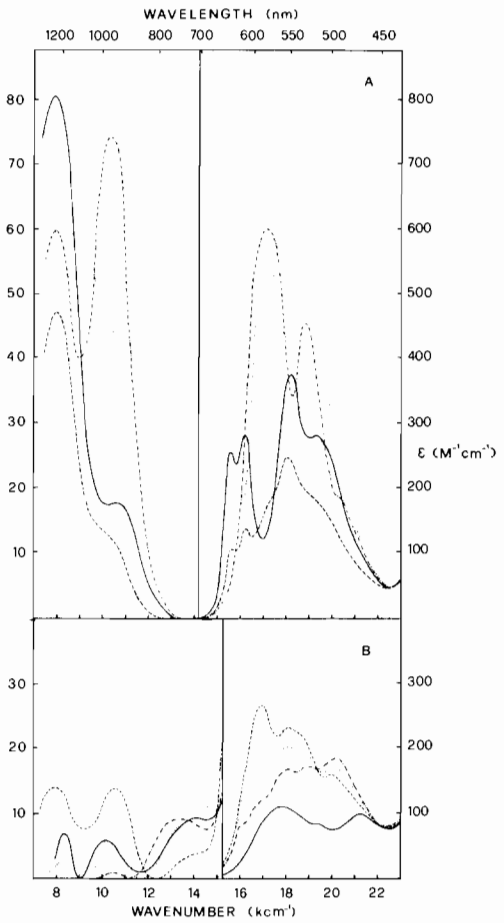

Fig. 1. A) Electronic spectra of *cobalt(II) bovine carbonic anhydrase* at pH 5.9 (– – –) and pH 8.0 (——), and limit electronic spectra of the acetazolamide derivative, pH 8.0 (· · · · ·), and cyanate derivative, pH 5.9 (– · – · –). B) Limit electronic spectra of chloride (– – –), bromide (· · · · ·), iodide (– · – · –), and acetate (——) derivatives, pH 5.9).

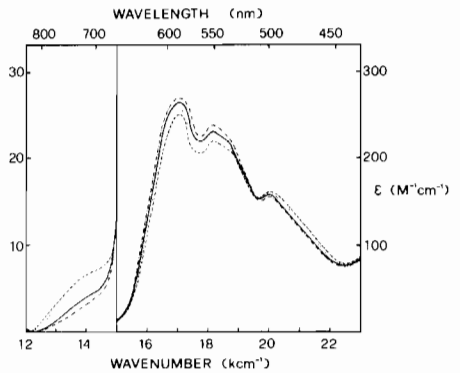

Fig. 2. Temperature dependence of the electronic spectrum of the chloride *cobalt(II) bovine carbonic anhydrase* (pH 5.9): 5 °C (– – –), 17 °C (· · · · ·), 26 °C (——), and 35 °C (– · – · –).

pattern, outside any experimental error, shows that at higher temperatures the band at 13 500 cm^{-1} decreases whereas the bands at 17000 and 18000 cm^{-1} increase. This means that there is a temperature dependent equilibrium between two species. It is interesting to note that the pattern obtained with decreasing temperature is the same obtained on going from the chloride to the iodide derivative.

The low intensity of the spectra and the presence of the band at *ca.* 13 500 cm^{-1} for halide and acetate derivatives are indicative of the presence of five coordinate species [10–12]. The shape of this band is typical of an F–F transition and tetrahedral complexes do not show transitions that high in energy. Furthermore octahedral species do not absorb in the range 10 000–16 000 cm^{-1}. Finally, five coordinate complexes have been reported to show spectra with intensity below 250 M^{-1} cm^{-1} in the F → P region [13] and well below 50 M^{-1} cm^{-1} in the infrared region [14]. These values are diagnostically different from those displayed by six coordinate and tetrahedral complexes [15]. The spectra of the cyanate and acetazolamide derivatives are assigned a tetrahedral stereochemistry on the grounds of the large absorption intensity and band positions as previously proposed.

The hypothesis that the weak band at 13 500 cm^{-1} is diagnostic of the presence of five coordinate species is in agreement with its temperature dependence, as found for the chloride derivative. In fact, if an equilibrium between five and tetracoordinate species is assumed, a decrease in temperature will shift the equilibrium towards the five coordinate species on the ground of entropic reasons.

When the pH of the solution containing the system enzyme–anions is increased, the spectrum of the pure enzyme at high pH is obtained. This confirms that only the low pH species reacts with the anionic inhibitors [1] and therefore its spectrum can be safely compared with that of the inhibited enzyme. From such a comparison it seems reasonable to infer that the low pH species is pseudotetrahedral.

The high and low pH spectra are significantly different, the major difference occurring in the F → P transitions region. Probably this is due to a change in the set of the donor groups and in the angle at the metal as well as in the metal donor distances. However, the lack of any absorption above 10,500 cm^{-1} leads again to the suggestion of a substantial tetrahedral stereochemistry.

References

1 S. Lindskog, L. E. Henderson, K. K. Kannan, A. Liljas, P. O. Nyman and B. Strandberg, *Enzymes, 3rd Ed., 5,* 587 (1971).
2 S. Lindskog, *J. Biol. Chem., 238,* 945 (1963).
3 A. E. Dennard and R. P. J. Williams, *Transition Met. Chem., 2,* 115 (1966); S. Lindskog, *Struct. Bonding (Berlin), 8,* 153 (1970).
4 J. E. Coleman, *Proc. Nat. Acad. Sci. U.S.A., 59,* 123 (1967).
5 J. E. Coleman and R. V. Coleman, *J. Biol. Chem., 247,* 4718 (1972).
6 J. S. Taylor, F. Mushak and J. E. Coleman, *Proc. Nat. Acad. Sci. U.S.A., 67,* 1410 (1970); E. Grell and R. C. Bray, *Biochim. Biophys. Acta, 236,* 503 (1971).
7 J. M. Fesando, *Biochemistry, 14,* 681 (1975).
8 K. Kumar, R. W. King and P. R. Carey, *FEBS Letters, 48,* 283 (1974).
9 S. Lindskog and A. Ehrenberg, *J. Mol. Biol., 24,* 133 (1967).
10 I. Bertini, C. Luchinat and A. Scozzafava, *J. Am. Chem. Soc., 99,* 581 (1977).
11 I. Bertini, C. Luchinat and A. Scozzafava, *Biochim. Biophys. Acta, 452,* 239 (1976).
12 I. Bertini, C. Luchinat and A. Scozzafava, *Bioinorg. Chem.,* in press.
13 R. C. Rosemberg, C. A. Root and H. B. Gray, *J. Am. Chem. Soc., 97,* 21 (1976).
14 R. Morassi, I. Bertini and L. Sacconi, *Coord. Chem. Rev., 11,* 343 (1973).
15 R. L. Carlin, *Transition Met. Chem., 1,* 1 (1966).