

- 1 J. A. Tainer, E. D. Getzoff, K. M. Beem, J. S. Richardson and D. C. Richardson, *J. Mol. Biol.*, **160**, 181 (1982).
- 2 I. Fridovich, *Adv. Inorg. Chem.*, **1**, 67 (1979).
- 3 For a review of anion-binding to this protein, see J. S. Valentine and M. W. Pantoliano, in 'Copper Proteins', T. G. Spiro, Ed., Wiley, New York, p. 291 (1981).
- 4 G. Rotilio, L. Morpurgo, C. Giovagnoli, L. Calabrese and B. Mondovì, *Biochemistry*, **11**, 2187 (1972).
- 5 A. Rigo, P. Viglino, G. Rotilio and R. Tomat, *FEBS Lett.*, **50**, 86 (1975).
- 6 L. Calabrese, D. Cocco and A. Desideri, *FEBS Lett.*, **106**, 142 (1979).
- 7 K. G. Strothkamp and S. J. Lippard, *Biochemistry*, **20**, 7488 (1981).
- 8 M. E. McAdam, *Biochem. J.*, **161**, 697 (1977).
- 9 A. Cudd and I. Fridovich, *J. Biol. Chem.*, **257**, 11443 (1982).
- 10 O. Bermingham-McDonough, D. Mota de Freitas, A. Kumamoto, J. E. Saunders, D. M. Blech, C. L. Borders, Jr., and J. S. Valentine, *Biochem. Biophys. Res. Commun.*, **108**, 1376 (1982).

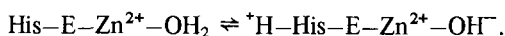
B38

The Catalytic Mechanism of Carbonic Anhydrase

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Steady-state and equilibrium kinetic studies of the $\text{CO}_2\text{-HCO}_3^-$ 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:



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.

- 1 H. Steiner, B.-H. Jonsson and S. Lindskog, *Eur. J. Biochem.*, **59**, 253 (1975).
- 2 H. Steiner, B.-H. Jonsson and S. Lindskog, *FEBS Lett.*, **62**, 16 (1976).
- 3 B.-H. Jonsson, H. Steiner and S. Lindskog, *FEBS Lett.*, **64**, 310 (1976).
- 4 I. Simonsson, B.-H. Jonsson and S. Lindskog, *Eur. J. Biochem.*, **93**, 409 (1979).
- 5 I. Simonsson and S. Lindskog, *Eur. J. Biochem.*, **123**, 29 (1982).

B39

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