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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

capability of the chromophore [5], while T_1 measurements of the 4H signal of the coordinated His 119 show that the lower nuclear relaxing efficiency is due to a shorter electronic relaxation time [6]. All of these data are taken as evidence for a large percentage of five coordinate cobalt(II) in the low pH form of the human isoenzyme. The metal donor set would then be constituted by three histidine nitrogens and two water oxygens.

There are convincing experimental results and arguments to indicate that the main activity-linked acid base group is a coordinated water molecule. The existence of five coordinated chromophore in the low pH form of CoHCAB, as opposed to the mainly four-coordinate CoBCAB, for the first time satisfactorily accounts for the difference in pK_a of the coordinated water.

CoBCAB and CoHCAB are further differentiated by the higher affinity for imidazole and related ligands of the human isoenzyme [7]. The pH dependence of the affinity of such ligands for the enzyme is accounted for.

At low pH the behavior of the two isoenzymes towards anionic inhibitors appears to be different. While the spectra of the adducts with CoBCAB are pH insensitive in the range of existence of the complexes, the human isoenzyme shows an increase in molar absorbance at low pH. Such behavior is shown particularly by thiocyanate. Whereas the affinity of the inhibitor for the isoenzyme is governed by a pK_a of ~7.5, that of the change in molar absorbance is governed by a pK_a of 6.5.

Such differences are reminiscent of the different behavior of the copper derivatives with respect to sulfonamides [8].

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Mechanistic Aspects of Coordination, Catalysis and Control in Carbonic Anhydrase

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Among the most significant developments in the bioinorganic chemistry of carbonic anhydrase during the past few years are those associated with the role of coordinated zinc(II) ion in the activation of H_2O and bicarbonate. Illustrative of the catalytic processes encompassed by these developments are many hydration–dehydration processes and a variety of hydrolysis reactions. Contributing to the intensive interest and research that this field has generated is the unusual catalytic efficiency connected with the physiologically important process, eqn. 1:

$$CO_2 + H_2O \rightleftharpoons H^+ + HCO_3^- \quad (1)$$

The problems of elucidating the details of the enzymatic pathway are compounded not only by its multistep character but also by the fact that each of the intermediates in the proposed catalytic cycle coexists in several forms related not only through proton transfers but also via ligand addition and dissociation processes.

Substitution of an alkyl group (methyl through n-pentyl) for the proton of bicarbonate dramatically alters the properties of the resultant alkyl carbonate esters, $ROCO_2^-$, toward the enzyme. While bicarbonate is the natural substrate of various carbonic anhydrases with turnover numbers of $\sim 10^6$ s^{-1}, the alkyl carbonates show no detectable activity as substrates. The alkyl carbonates, however, bind efficiently to the various carbonic anhydrases and act as typical anionic inhibitors of enzyme catalyzed CO_2 hydration and HCO_3^- dehydration, with K_i values comparable to those of the corresponding RCO_2^- anions. It appears that the substitution of an alkyl group inhibits a proton transfer essential in the enzyme-catalyzed dehydration of HCO_3^-, and further that the bicarbonate proton permits a unique binding interaction with carbonic anhydrase.

Examination of the kinetic features (temperature-jump, stopped-flow and NMR) of the catalytic system emphasizes a number of additional points, notably: (a) The requirement of several binding sites. (b) Non-protein ligand lability during the interconversion of ES, EP and ESP. (c) Accessibility of two different coordination numbers for Zn(II)-, Co(II)- and Mn(II)-carbonic anhydrases. (d) Accessibility of two or more protonation states in the active site. (e) The potentially important role of the