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A Proposal for the Mechanism of Carbonic Anhydrase Action[1]

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A new catalytic mechanism is proposed for the hydration of CO_2 by the zinc metalloenzyme carbonic anhydrase. This mechanism identifies the group controlling catalytic activity as an active site histidine, in which the protonated imidazole ring coordinates to zinc, losing a proton. Geometric constraints on the histidine unit make the metal–ligand bond a strained and, therefore, labile one. In the hydration reaction, the metal-bound neutral histidine moiety serves as a proton acceptor for the transient ionization of metal-bound water. Zinc-bound hydroxide attacks the carbon of the substrate to generate metal-bound bicarbonate, and the system regenerates itself by losing the elements of carbonic acid.

Although carbonic anhydrase was initially purified almost 20 years ago and its reversible hydration of carbon dioxide involves only six substrate atoms, work by many investigators using often sophisticated methodologies has failed to produce a generally accepted proposal for its mechanism of catalysis (*1*). The catalytic activity of the enzyme is pH dependent, with the hydration of carbon dioxide proceeding optimally at pH values above neutrality, and is thought to reflect the ionization of a group at the active site controlling catalytic activity. Many physical properties of the enzyme, including nuclear magnetic relaxation of solvent water protons and the visible absorption spectra of the metal ion in the catalytically active cobalt enzyme, show similar pH dependencies. Many investigators favor the basic proposal that a zinc-bound water molecule at the active site of the enzyme ionizes near neutral pH, producing a metal-bound hydroxide ion that attacks the substrate in the hydration reaction (*1–4*). In this model the metal ion serves to lower the pK_a and promote the ionization of bound water. The participation of additional groups at the active site is not essential. Anion inhibitors are thought to compete with hydroxide for the metal, explaining the apparent increase in the pK_a of this group in their presence. Failure of chemical

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modification studies to identify additional essential groups at the active site provides indirect support for this model (*5–7*).

Despite its simplicity, the ionizable zinc-bound water molecule hypothesis faces several major difficulties: (1) there are no data to indicate that the pK_a of water can be reduced to less than 7 when bound to zinc (*8*); (2) there is no explanation for the marked differences in the visible spectra of the high-pH and anion-inhibited cobalt-substituted enzymes, both of which would have coordinated anions in this scheme (*9*); (3) the protein itself serves no role that could not be met by a simple metal–chelate complex (*8, 10*); and (4) nuclear magnetic relaxation studies of solvent water protons for both the manganese and cobalt-substituted enzymes suggest that there is no metal-bound hydroxide in the high-pH form of the enzyme (*11, 12*).

Several years ago we proposed an alternative model based on the anomalous behavior of the chemical shifts of a histidine residue at the active site observed by high-resolution proton magnetic resonance (pmr) spectroscopy (*10, 13*). X-Ray diffraction studies (*14*) of the high-pH form of the enzyme indicated that there are three histidine ligands to the metal. We suggested that one of these three histidines is not bound to the metal ion at low pH but that it simultaneously ionizes and coordinates to the metal with increasing pH. The configuration of the enzyme with respect to the metal ion at the active site makes this a strained, therefore labile, bond. This labile protein ligand was thought to be the active species in a concerted hydration reaction. The mechanism appeared to explain the pmr data, and the pK_a of metal-bound imidazole based on then available data appeared to be lower than that of metal-bound water (*10*). In agreement with the "entatic" or "strain" theory for the action of metalloenzymes proposed by Vallee and Williams (*15*), this model assigns to the protein the essential role of creating strain at the active site. The differences in the visible spectra of the anion-inhibited and high-pH forms of the cobalt enzyme occur because the ligand would be constrained by the protein in the latter, but not in the former, state. This mechanism is also consistent with the pmr relaxivity studies, since it does not require the presence of an hydroxide ion on the metal at high pH.

Subsequent work has failed to confirm that the pK_a of metal-bound imidazole is low enough to permit histidine to serve as the group controlling catalytic activity (*16*). In addition, studies by Koenig's group (*17*) suggest that the true pK_a of the group controlling enzymatic activity in carbonic anhydrase is closer to 5 than to 7 because of heretofore unappreciated large anion effects. Neither water nor imidazole when bound to zinc as neutral species would be expected to have a pK_a value this low.

Given this new information, we propose the catalytic mechanism shown in Fig. 1. This model incorporates features of both earlier proposals and identifies the group controlling catalytic activity as an active site histidine. The imidazole ring of this residue coordinates to zinc as a function of increasing pH as it loses a proton to form neutral imidazole. As with our previous model, geometric constraints on the histidine make the metal–ligand bond a strained and, therefore, labile one. In the hydration reaction this metal-bound neutral histidine serves as a proton acceptor for the transient ionization of metal-bound water and the zinc-

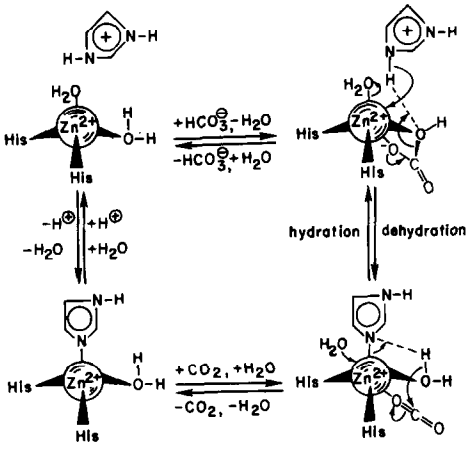

FIG. 1. Proposed catalytic mechanism for carbonic anhydrase.

bound hydroxide attacks the carbon of the substrate to generate metal-bound bicarbonate in a concerted mechanism. The system regenerates itself by losing the elements of carbonic acid.

Concerted deprotonation and metal-ion coordination of imidazole is chemically reasonable and is consistent with the recent finding of Koenig and co-workers (*17*) that the pK_a of the group controlling catalytic activity is much lower than it was previously believed to be. The protein itself serves to create a strained, labile species at the active site (*10*). It is this strain that is the driving force for catalysis.

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