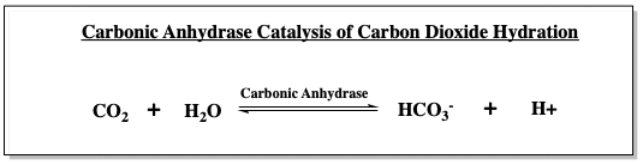
2.1: About Carbonic Anhydrase

Introduction

Carbon-dioxide (CO2) hydration and its mechanism in living systems are of fundamental importance for bioinorganic chemistry. In 1932 the existence of an enzyme catalyzing CO2 hydration in red blood cells was established,31 The enzyme was named carbonic anhydrase (abbreviated CA). In 1939 the enzyme was recognized to contain zinc (Zn).32 Because CO2 is either the starting point for photosynthesis or the endpoint of substrate oxidation, carbonic anhydrases are now known to be ubiquitous, occurring in animals, plants, bacteria, and fungi. Different enzymes from different sources, catalyzing the same reaction and usually having homologous structures, are termed isoenzymes. Thus far, a total of 7 distinct classes of CAs have been identified based upon organism: alpha, beta, gamma, delta, zeta, eta, and theta. Each class may contain multiple isoenzymes. Sometimes the same organism has more than one isoenzyme for a particular function, as is true for human carbonic anhydrase. Humans have 15 CAs that belong to the alpha class; these isozymes vary by location in the body and by catalytic activity. CA is a classic example of a hydrolytic enzyme, one that catalyzes addition or removal of water to a substrate molecule. More specifically, CA catalyzes the reversible conversion of carbon dioxide (CO2) to bicarbonate (HCO3-), also referred to as carbonic acid.



Although hydration of CO2 is spontaneous in water at pH 7, the reaction is kinetically slow (k = 10-1 s-1), too slow to convert all CO2 produced in respiration. Only above pH 9 does the uncatalyzed reaction become fast, owing to direct attack of OH-, which is a much better nucleophile than H2O (k = 104 M-1 s-1, where M-1 refers to the OH- concentration). The figure below compares nucleophilic attack of water versus hydroxide (OH-) on CO2.

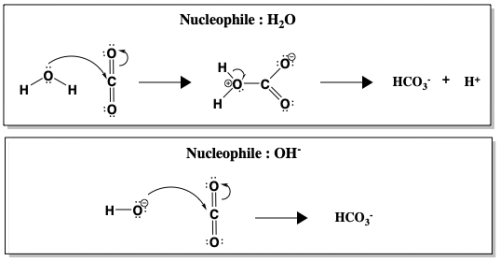


Figure: This figure depicts nucleophilic attack on carbon in carbon dioxide (CO2*) in the formation of bicarbonate (HCO3-). In the top panel, H2O is the nucleophile. The bottom panel shows the reaction with hydroxide (OH-) as the nucleophile. Since the OH- is a stronger nucleophile, HCO3- formation is faster when OH- is the nucleophile.*

Between H2O and OH-, formation of HCO3- occurs faster when OH- is the nucleophile. A faster reaction at higher pH, when more OH- ions are present, suggests OH- is involved in the rate determining step. However, realistically, the pH of human blood cannot be changed to speed up hydration of CO2. Instead, humans use carbonic anhydrase to catalyse the reaction. When CA is present, the reaction is sped up to a rate of k = 106 s-1.

The ubiquity of CA in different organisms reflects the importance of these enzymes in sustaining life. The speed of CA-catalyzed CO2 hydration is essential to meet the needs of living cells. Some physiological CA functions include pH regulation, electrolyte secretion, ion transport, and CO2 homeostasis. In the digestive tract, CAs plays a role in the secretion of acid and keep saliva neutral by modulating pH.G,H Among these functions, CA most notably plays a role in transport of CO2 and HCO3- related to respiration, the process of atmospheric oxygen and carbon dioxide exchange that occurs when humans inhale oxygen and exhale carbon dioxide. With low solubility, CO2 must be converted to a more soluble form, HCO3-, for transport throughout the body. Bicarbonate ion (HCO3-) eventually reaches the lungs, gets converted back to CO2, and exits the body through exhalation.C

Medical research revolving around CA focuses on the Zn-containing active site as a therapeutic target for various disease treatments; both CA inhibitors and activators are incorporated in drug design. CA inhibitors are used as treatment for epilepsy, ulcers, cancer, obesity, and other neurological disorders. In the eye, CA produces hydrogen ions that maintain optic pressure. However, too much pressure in the eye can damage the optic nerve and cause glaucoma. CA activity can create a concentration

gradient that drives the transport of water to the optical nerve. When too much water is around the optical nerve, pressure around the nerve increases causing damage. Inhibition of CA has become a key treatment of glaucoma.

Beyond pharmaceutical applications, CA has also been investigated for its utility in carbon capture and carbon sensor applications. Carbon capture and storage happens as CAs convert CO2 to bicarbonate. Increased availability of bicarbonate in the presence of calcium ions (Ca2+) causes precipitation of calcite (CaCO3). This process, called bio-mineralization, may be a viable mode of long term storage of CO2 in calcite to mitigate CO2 emissions.

Details about the structure and function of CA's Zn-containing active site have been elucidated over 80 years of research. The current article delves into the metalloenzyme active site using bioinorganic concepts.

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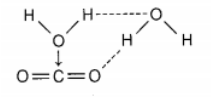
Carbon-dioxide hydration and its mechanism in living systems are of fundamental importance for bioinorganic chemistry. In 1932 the existence of an enzyme catalyzing CO_2 hydration in red blood cells was established,[31] The enzyme was named carbonic anhydrase (abbreviated CA). In 1939 the enzyme was recognized to contain zinc.[32] Because CO_2 is either the starting point for photosynthesis or the endpoint of substrate oxidation, carbonic anhydrases are now known to be ubiquitous, occurring in animals, plants, and several bacteria. Different enzymes from different sources, catalyzing the same reaction and usually having homologous structures, are termed isoenzymes. Sometimes the same organism has more than one isoenzyme for a particular function, as is true for human carbonic anhydrase.

CO_2 gas is relatively soluble in water (3 x 10^{-2} M at room temperature under p_{CO2} = 1 atm), equilibrating with hydrogen carbonate at pK_a 6.1:

$$CO_2 + H_2O \rightleftharpoons HCO_3^- + H^- \tag{2.6}$$

The uncatalyzed reaction is kinetically slow around physiological pH ($k \simeq 10^{-1}\ s^{-1}$), whereas, in the presence of the most efficient isoenzyme of CA, the maximal CO_2 turnover number (i.e., the number of substrate molecules transformed per unit time by each molecule of enzyme)[33] is $\simeq 10^6\ s^{-1}$. The uncatalyzed attack by water on CO_2 may be facilitated by two hydrogen-bonded water molecules, one of which activates the carbon by means of a hydrogen bond to a terminal CO_2 oxygen, the other of which binds the carbon atom via oxygen: [34,35]



(2.7)

Only above pH 9 does the uncatalyzed reaction become fast, owing to direct attack of OH^-, which is a much better nucleophile than H_2O ($k \simeq 10^4\ M^{-1}s^{-1}$, where M^{-1} refers to the OH^- concentration):

$$CO_2 + OH^- \rightleftharpoons HCO_3^- \tag{2.8}$$

On the other hand, the rate constant in the presence of the enzyme, called k_{cat}, is pH-independent above pH 8 in every CA isoenzyme (Figure 2.2).[33,36]

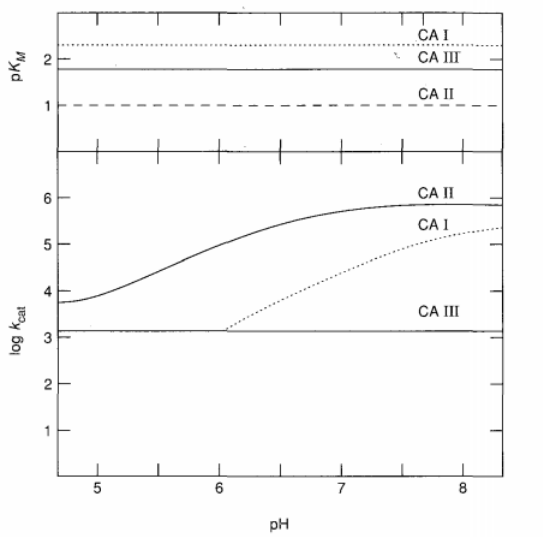


Figure 2.2 - pH dependence of k_{cat} and K_m values for CO_2 hydration catalyzed by carbonic anhydrase I, II, and III isoenzymes.[33,36]

In vitro, carbonic anhydrase is quite versatile, catalyzing several reactions that involve both OH^- and H^+, such as the hydrolysis of esters and the hydration of aldehydes. The various isoenzymes have been characterized to different degrees of sophistication. High-activity forms are labeled II ($k_{cat} \simeq 10^6\ s^{-1}$ at 25 °C); low-activity forms I ($k_{cat} \simeq 10^5\ s^{-1}$), and the very-low-activity forms III ($k_{cat} \simeq 10^3\ s^{-1}$).[37] X-ray structural information at nominal 2 Å resolution is available for HCA I[38] and HCA II,[39] where H indicates human. The structure of HCA II has been refined recently.[40] High-resolution structures of mutants and of their substrate and inhibitor derivatives are being reported.[211] All isoenzymes are single-chain polypeptides, with M.W. about 30 kDa and one zinc ion per molecule. They have the shape of a rugby ball with a crevice 16 Å deep running through the south pole (Figure 2.3 See color plate section, page C-2.). At the bottom of the crevice, the zinc ion is anchored to the protein by three histidine nitrogen atoms and is exposed to solvent. Two histidines (His-94 and His-96, HCA I numbering) are bound to zinc via their Nϵ2 atoms, whereas one (His-119) is bound via its Nδ1 atom (Figure 2.4). It is quite general that histidines bind zinc equally well by either of the two histidine nitrogens, the preference being probably dictated by the steric constraints imposed by the protein folding. The three histidine NH protons are all engaged in H-bonding (Figure 2.4). Histidine-119 is involved in H-bonding with a glutamate residue. As mentioned, this could be a way of controlling the basicity of the metal ligands. A solvent molecule bound to zinc is involved in an H-bond with Thr-199, which in turn is H-bonded to Glu-106. This H-bonding network is important for understanding the subtle structural changes that occur with pH changes; these could, in principle, account for the pH-dependent properties. Although the structure of crystals grown at pH 8 in sulfate-containing buffer gives some indication of a single solvent molecule bound to zinc (Figures 2.3 and 2.5 See color plate section, pages C2, C3.), theoretical studies indicate that two water molecules can be at bonding distances.[42] Such a finding is consistent with spectroscopic studies on other derivatives and with the concept that attachment and detachment of substrates occur through five coordination.

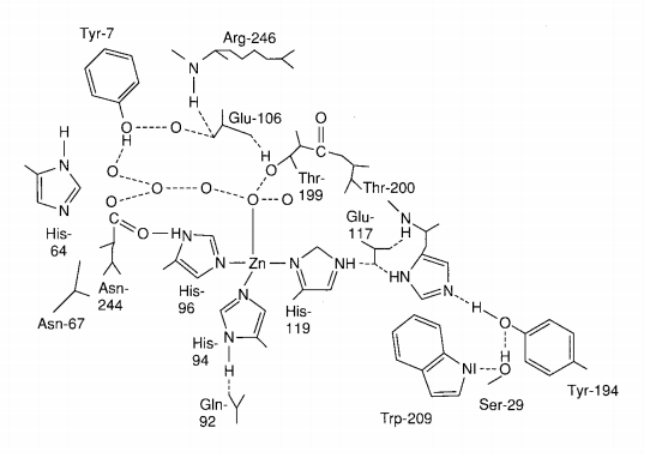


Figure 2.4 - Schematic representation of the active site of human carbonic anhydrase II. Hydrogen bonds (---) and ordered water molecules (o) are indicated.[41]

Just as is true for every zinc enzyme in which zinc is at the catalytic site, activity is lost if the metal is removed, and is restored by zinc uptake. The tertiary structure of carbonic anhydrase is maintained in the absence of zinc; even the denatured apoprotein can refold spontaneously from a random coil to a native-like conformation. Although such a process is accelerated by zinc,[43,44] the presence of the metal does not seem to be an absolute requirement for the correct folding of CA, whereas it is an absolute requirement for several other metalloproteins.[23,29,30]

Anions are attracted in the metal cavity by the positive $Zn(N_3OH_2)^{2+}$ moiety, and are believed to bind to zinc in carbonic anhydrase very effectively; so their use should be avoided as much as possible if the goal is to study the enzyme as it is. When the protein is dialyzed against freshly doubly distilled or carefully deionized water under an inert atmosphere, the pH of the sample approaches the isoelectric point, which is below 6 for HCA I and bovine (BCA II) enzymes. The pH can then be adjusted by appropriate additions of NaOH. All the measurements reported in the literature performed in acetate, phosphate, imidazole, or tris sulfate buffers are affected by the interference of the anion with the metal ion. However, buffer species containing large anions like Hepes (4[(2-hydroxyethyl)-l-piperazinyl]ethanesulfonic acid) can be used,[45] since these anions do not enter the cavity.

There are many indications that zinc in the high-pH form of CA is four-coordinate with an OH group in the fourth coordination site. At low pH the enzyme exists in a form that contains coordinated water; the coordination number can be four (one water molecule) or five (two water molecules). Of course, the occurrence of the low-pH species depends on the pK_a's of the complex acid-base equilibria.
