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ZINC METALLOENZYMES AND MODEL COMPOUNDS FOR CARBONIC ANHYDRASE: A REVIEW ARTICLE

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ABSTRACT

Zinc has been demonstrated to be essential for the growth, development and differentiation of all types of life, including microorganisms, plants and animals. After iron, zinc is the second most abundant trace metal in the human body A zinc-bound water molecule rapidly exchanges, because the ligand-zinc bond is kinetically labile. These features provide an effective hydrolysis function of the zinc complex through the activation of coordinated water. Therefore, it is of particular interest to study the metabolic conversion or degradation of biomolecules catalyzed by zinc enzymes. However, the mechanistic details of the enzyme function in the protein matrix are sometimes complicated, even though the structures of many zinc enzymes can be characterized by several different spectroscopies. On the other hand, a synthetic zinc complex related to the active site sphere will be a good tool with which to understand the role of the zinc ion and the complicated mechanism of catalytic reactions. A great deal of interest focused on the design of excellent functional or structural models on zinc enzymes. In this article, several representative zinc enzymes and related functional models of carbonic anhydrase will be discussed.

KEYWORDS: Zinc, carbonic anhydrase, metalloenzyme, zinc-binding sites, zinc enzyme, enzyme model, catalytic hydrolysis, zinc hydroxide, carboxypeptidase, carbonic anhydrase, alcohol dehydrogenase.

INTRODUCTION

Metals play roles in approximately one-third of the known enzymes. Metals may be a co-factor or they may be incorporated into the molecule, and these are known as metalloenzymes. Metalloenzymes are proteins which function as an enzyme and contain metals that are tightly bound and always isolated with the protein. In other metalloenzymes the metal is built into the structure of the enzyme molecule. The metal ion can not be removed with out destroying the structure of the enzyme. Metals are usually found in the active site of the enzyme. The metals resemble protons (H+) in that they are electrophiles that are able to accept an electron pair to form a chemical bond. In this aspect, metals may act as general acids to react with anionic and neutral ligands.[1] The structures of over 300 zinc-containing proteins were determined, where the zinc atom acts as a strong catalyst as well as regulates the physiological process or fixation of the unique structural conformation of bioactive species.[2,3,4] Zinc usually exists only in the dication state, $Zn^{2\ +}$ with a closed d^{10} electronic configuration, indicating that no reduction-oxidation process occurs in physiological systems, whereas it is well known that zinc is the most common Lewis acidic metal cation in bioinorganic chemistry.The first zinc metalloenzyme, carbonic anhydrase II was discovered in 1940 by Keilin and Mann. Since then,. 300 zinc enzymes covering all six classes of enzymes and in different species of all phyla have been discovered.[5,6,7] In most cases, the zinc ion is an essential cofactor for the observed biological function of these metalloenzymes. Furthermore, the biological functions of zinc, which are versatile and observed in many tissues, are most often associated with proteins.

Properties of zinc

- Unlike other first-row transition metals, the zinc ion (Zn^{21}) contains a filled *d* orbital (d^{10}) and therefore does not participate in redox reactions but rather functions as a Lewis acid to accept a pair of electrons.[8] This lack of redox activity makes Zn^{21} a stable ion in a biological medium whose potential is in constant flux. Therefore, the zinc ion is an ideal metal cofactor for reactions that require a redox-stable ion to function as a Lewis acid–type catalyst,[9] such as proteolysis and the hydration of carbon dioxide.
- Due to the filled *d*-shell orbitals, Zn^{21} has a ligand-field stabilization energy of zero,[10] in all liganding geometries, and hence no geometry is inherently more stable than another. This lack of an energetic barrier to a multiplicity of equally accessible coordination geometries can be used by zinc metalloenzymes to alter

the reactivity of the metal ion and may be an important factor in the ability of Zn^{21} to catalyze chemical transformations accompanied by changes in the metal coordination geometry.

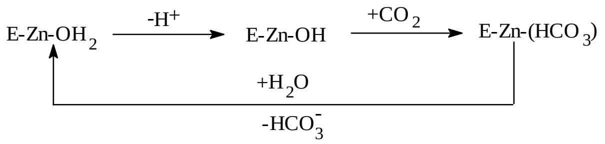
- A final important property of Zn^{21} that makes it well suited as a catalytic cofactor is that ligand exchange is rapid,[11] allowing for the rapid product dissociation required for efficient turnover.

Zinc is an essential element - one that is necessary for the occurrence of reactions that are required in the metabolic processes of living organisms. It is the second most abundant transition or post-transition metal, transported by proteins (macroglobulin, transferrin and albumin), stored in a protein (thionein) and bound to proteins. Pearson,[12] classified zinc as a “borderline” metal, meaning that Zn^{21} does not consistently act either “hard” (not very polarizable) or “soft” (highly polarizable) and does not have a strong preference for coordinating with either oxygen, nitrogen or sulfur atoms. In protein zinc-binding sites, the zinc ion is coordinated by different combinations of protein side chains, including the nitrogen of histidine, the oxygen of aspartate or glutamate and the sulfur of cysteine; among these, histidine is most commonly observed, followed by cysteine.[13]

Zinc-containing hydrolytic enzymes

There are several zinc-containing hydrolytic enzymes which catalyze addition or removal of water in a substrate molecule. Some of the zinc-containing metalloenzymes catalyzing hydrolytic and related reactions are given below-

***Carbonic anhydrase* (CA)** is a monomeric protein of molecular mass 30 kDa. It catalyzes the conversion of carbon dioxide to bicarbonate. The catalytic mechanism has been studied extensively and is generally accepted to consist of two steps (Scheme 2-1).

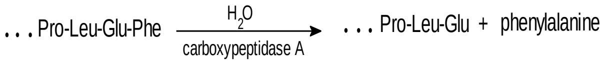


Scheme 2-1

Structural studies on the enzyme have identified that the zinc center is coordinated to three histidine imidazole groups and a water molecule, $[(His)_3Zn\text{-}OH_2]^{2+}$ (His = histidine). The catalytic mechanism of carbonic anhydrase activity has been the subject of both intense experimental,[14] and theoretical investigations and these studies have led to the proposal that a zinc bicarbonate complex $[(His)_3Zn\text{-}OCO_2H]^+$ may be a key intermediate. However, structural details of the proposed bicarbonate intermediate are not known and theoretical treatments have explored the possibilities of both unidentate and bidentate coordination modes.[15] The kinetics of the carbonic anhydrase reaction are pH-dependent, being faster at high pH and under the control of a group having an apparent pK_a value near 7.0. The simplest and most widely accepted interpretation of these observations is that this reaction involves formation of a zinc-bound hydroxide ion. This proposal is also supported by the observation that many anions inhibit the reaction, presumably by competing with water ligands for the open coordination site on zinc.

Human carbonic anhydrase has a molecular weight of ~ 30, 000 and occurs in two similar but not identical forms. The molecular structures of both are known from X-ray crystallography. The enzyme-catalyzed reaction rates are pH dependent in a way that indicates the existence of a group in the enzyme with a pK_a of ~ 7 that must be deprotonated to give that form of the enzyme E, which is required for hydration of CO_2. Conversely, an acid form EH^+ is required for the reverse reaction. It is necessary to assume that the substrate for dehydration is HCO_3^-, since the pH dependence of dehydration is the inverse of that for hydration. In effect, the enzyme makes the fast reaction $CO_2 + OH^- = HCO_3^-$, the major pathway of hydration at a pH of 7, whereas normally the slow reaction of CO_2 with H_2O would predominate at a pH of 7 and the reaction with OH^- would not become dominant until pH 10 or greater. The importance of a Zn–OH group in providing a potent attacking nucleophile is in harmony with the proposed importance of a similar feature of the carboxypeptidase mechanism. A difference between the two, however, is that here it must be assumed that hydrogen bonding to the two CO_2 oxygen atoms polarizes the C=O bonds so as to enhance the positive character of the carbon atom, whereas in carboxypeptidase, the zinc atom was also involved in the polarization of the C=O group.

***Carboxypeptidase** A*, a pancreatic enzyme, cleaves the carboxyl terminal amino acid from a peptide chain by hydrolyzing the amide linkage (Scheme 2-2).



Scheme 2-2

The enzyme consists of a protein chain of 307 amino acid residues plus one Zn(II) ion to yield a molecular weight of about 34,600. The molecule is roughly egg-shaped, with a maximum dimension of approximately 5000 pm and a minimum dimension of about 3800 pm. There is a cleft on one side that contains the zinc ion, the active site. The metal is coordinated approximately tetrahedrally to two nitrogen atoms and an oxygen atom from three amino acids (His 69, Glu 72, His 169) in the protein chain. The fourth coordination site is free to accept a pair of electrons from a donor atom in the substrate to be cleaved. The coordination geometry is completed by a water molecule, resulting in pentacoordinate Zn(II). In the presence of substrates or inhibitors, the Glu-72 carboxylate group becomes coordinated in a more nearly unidentate fashion. The ability of Glu or Asp residues to rearrange in such a manner, a process that has been termed the carboxylate shift, allows maintenance of a constant or nearly constant coordination number even when various forms of substrate are bound to the metal. There are hydrogen bonds between the coordinated water molecule and Glu-270. Several arginine and tyrosine residues are positioned in the active site in a way that allows them to participate in substrate binding and activation. The enzyme is thought to act through coordination of the zinc atom to the carbonyl group of the amide linkage. In addition, a nearby hydrophobic pocket envelops the organic group of the amino acid to be cleaved and those amino acids with aromatic side groups react most readily. Accompanying these events is a change in conformation of the enzyme. The arginine side chain moves about 200 pm closer to the carboxylate group of the substrate, and the phenolic group of the tyrosine comes within hydrogen bonding distance of the imido group of the C-terminal amino acid, a shift of 1200 pm.[16] The hydrogen bonding to the free carboxyl group (by arginine) and the amide linkage (by tyrosine) not only holds the substrate to the enzyme but helps break the N–C bond. Nucleophilic displacement of the amide group by an attacking carboxylate group from a glutamate group could form an anhydride link to the remainder of the peptide chain. Hydrolysis of this anhydride could then complete the cycle and regenerate the original enzyme. More likely, the glutamate acts indirectly by polarizing a water molecule that attacks the amide linkage.

Carboxypeptidase represents a classic case in bioinorganic chemistry of the application of metal-substitution probes. Because Zn(II) is magnetically and spectroscopically silent, extensive studies of various metal-substituted derivatives have been carried out. The cobalt(II) derivative, in particular, can be prepared from the apoprotein and is more active than the native enzyme in hydrolyzing peptides and equally good at ester hydrolysis.

Alkaline Phosphatase cleaves phosphate monoester non-specifically. This enzyme contains two zinc ions at the active site. The catalytic role of zinc ions is ascribed to the binding and activation of substrates, whereas deprotonation of co-ordinated water to produce a nucleophilic zinc hydroxide is also proposed as an essential catalytic hydrolysis function. Other function of this enzyme is to transfer a phosphate ester to the external alcohol.[17] In alkaline phosphatase, a pair of zinc(II) ions binds the terminal phosphate group of a substrate, typically a monoester such as p-nitrophenyl phosphate. A serine hydroxyl group at the active site then attacks the phosphoryl group, cleaving the ester functionality, in the process, the phosphate is transferred to the enzyme, forming a phosphorylserine residue. Hydrolysis of this phosphate ester by coordinated hydroxide ion completes the catalytic cycle. The pair of zinc ions serve as a general Lewis acid, polarizing the substrate and rendering it a better electrophile. Positioning of the phosphate ester at the active site by the arginine residue is analogous to Arg-145 facilitated terminal carboxyl orientation of substrate in carboxypeptidase. Interestingly, in alkaline phosphatase, there is a third metal, a magnesium ion, within ~ 5.0 Å of one zinc and 7.0 Å of the other. Although this metal ion does not appear to participate directly in the catalytic mechanism, it may contribute by shaping the structure and the electrostatic potential of the active site.

The bacterial ***phosphotriesterase*** (PTE) catalyzes the hydrolysis of organophosphates with turnover numbers that approach the diffusion-controlled limit for the best substrates. The structure of this protein has been determined at high resolution by X-ray crystallography. A coupled binuclear metal complex of two zinc ions has been shown to be required for the delivery of the hydroxide to the phosphorus center during substrate turnover. Site directed mutagenesis has been used to systematically alter the substrate and stereochemical specificity of this enzyme. The wild-type enzyme hydrolyzes the P-F bond of diisopropyl fluorophosphate (DFP) with a turnover number of 41 per second. Mutagenesis of two phenylalanine residues within the leaving group pocket to histidine residues and replacement of the Zn(II) with Co(II) enhances the turnover of DFP by a factor of 80. Ethyl p-nitrophenyl phosphate is hydrolyzed by the native enzyme 100,000 times more slowly than the triester, diethyl p-nitrophenyl phosphate. The diminished activity of the diester can be partially rescued by the addition of small alkyl amines to the reaction medium. These results suggest that the amine binds to the active site and neutralizes the negative charge of the diester.

The biological importance of phosphate transfer, specifically by zinc enzymes, has attracted many coordination chemistry research groups. Numerous mechanistic studies.[18] and model complexes.[19] have been published. Similar studies related to the metabolism of phosphate-bearing substrates have been less popular among chemists so far.

Reaction mechanisms of hydrolytic metalloenzymes (e.g., carbonic anhydrase (CA), carboxypeptidase, phosphatase) and the role of the metal ions in their active centers have constantly been interesting bioinorganic subjects.[20,21] Literature also revealed that in most of the zinc-containing hydrolytic enzyme the mononuclear hydroxo is the active species. As one of the approaches, various types of metal complexes have been designed to account for or mimic the functions played by the central Zn(II) ions, a typical central metal ion.

Several model complexes have also been reported by various workers and their catalytic activity for the hydration of cabon dioxide have been proposed. Gultneh et al.[22] synthesized a hydroxo-bridged dinuclear Zn(II) complex as possible model for the hydrolytic zinc enzymes. They also reported that the synthesised complex reacts reversibly in acetonitrile solution with CO_2. Rombach et al.[23] reported that $Tp^{Ph,Me}Zn$-H and $Tp^{Ph,Me}Zn$-OH undergo insertion reactions with CO_2. Allred et al.[24] reported the chemistry of a binuclear cadmium(II) hydroxide complex and its reactivity towards CO_2. Bergquist and Parkin.[25] described the protonation of the hydroxide ligand in a synthetic analogue of carbonic anhydrase, $[Tp^{tBu,Me}]$ ZnOH and demonstrated that the protonation inhibits the reactivity towards CO_2. Looney et al.[26] reported the tris(pyrazolyl)hydroboratozinc hydroxide complexes as functional models for carbonic anhydrase and performed the study on the nature of the bicarbonate intermediate. Nakata et al.[27] reported the synthesis of water soluble zinc complex and used it in the study of CO_2 hydration. Doring et al.[28] described bi- and tetranuclear Zn complexes of the tridentate N,N,O ligand [{[2-(2-pyridyl)ethyl]imino}methyl]phenol (HL) and their enzyme-like reactions are described. The coordinated H_2O molecules in a dizinc complex of this ligand can be deprotonated reversibly similar to those in the resting states of Zn enzymes. The resulting tetranuclear complex bearing bridging hydroxide ions allows the reversible uptake of CO_2 whereas the coordination of H carbonate was not observed. Also some synthesised hydroxo complexes of zinc (II) were used for the reaction with various nucleic acid bases and their nucleosides.[29-33]

CONCLUSION

The above reported literatures revealed that in most of the hydrolytic enzymes, the mononuclear hydroxo species are involved at some stages of their catalytic cycle. However the structurally well characterised mononuclear Zn(II) hydroxo complexes are limited.[34-36]

These studies demonstrate that the chemical nature of the direct ligands and the structure of the surrounding hydrogen bond network are crucial for both the activity of carbonic anhydrase and the metal ion affinity of the zinc-binding site. An understanding of naturally occurring zinc-binding sites will aid in creating de novo zinc-binding proteins and in designing new metal sites in existing proteins for novel purposes such as to serve as metal ion biosensors.

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