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Roles of zinc(II) ion in zinc enzymes

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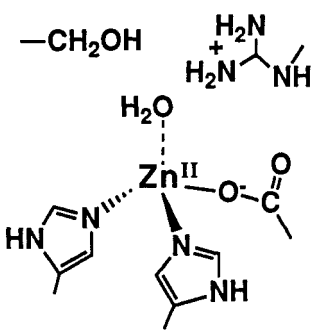
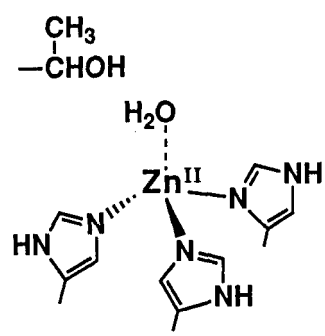
Abstract: Zinc(II) complexes of macrocyclic polyamines **1**, **2** and **3** make good models for zinc enzyme (e.g., carbonic anhydrase, alcohol dehydrogenase, phosphatase) active centers. Intrinsic acid properties of Zn^{II} pertinent to the biological functions are disclosed. Zinc(II) ion is not only important kinetically but it also plays an indispensable role thermodynamically.

I. INTRODUCTION

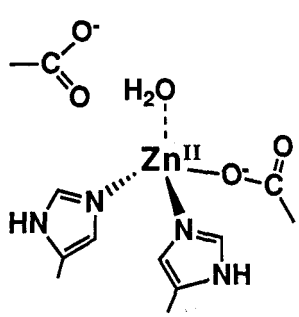
Zinc enzyme may be classified into (1) DNA and RNA polymerases, (2) alkaline phosphatases (AP), (3) peptidases, (4) carbonic anhydrases (CA) and (5) alcohol dehydrogenases (ADH). To spotlight the role of metal in hydrolytic enzymes, numerous models have been devised. While these studies have given us important roles of metal ions as Lewis acids, they fail to explain why the central metal ion must be zinc, but not more acidic metal ions such as Cu^{II}. It was surprising for me to find that how scanty our knowledge is of the intrinsic acid properties of Zn^{II} pertinent to the biological functions. The main reason lied in a lack of good ligands that (i) form stable and discrete complexes with Zn^{II} at physiological condition, (ii) leave



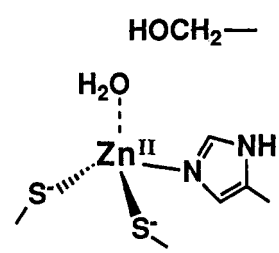
Alkaline Phosphatase



Carbonic Anhydrase



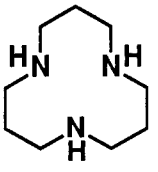
Carboxypeptidase



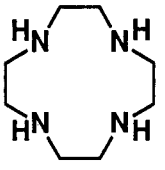
Alcohol Dehydrogenase

catalytic site open on Zn^{II} and (iii) offer structurally and/or functionally similar environments around the Zn^{II} as found in enzymes. The requirements (i) and (ii) are somewhat contradicting and were difficult to simultaneously meet with previously designed ligands.

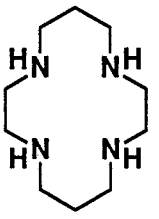
Since 1989 we have discovered that the Zn^{II} complexes with macrocyclic polyamines **1**, **2**, and **3**[1] can be good zinc enzyme models, by which not only deeper insight into the kinetic role, but also thermodynamic roles of Zn^{II} have been discovered.[2] It is now our contention that none but Zn^{II} ion can simultaneously achieve those essential functions.



1
[12]aneN_3



2
[12]aneN_4 (cyclen)

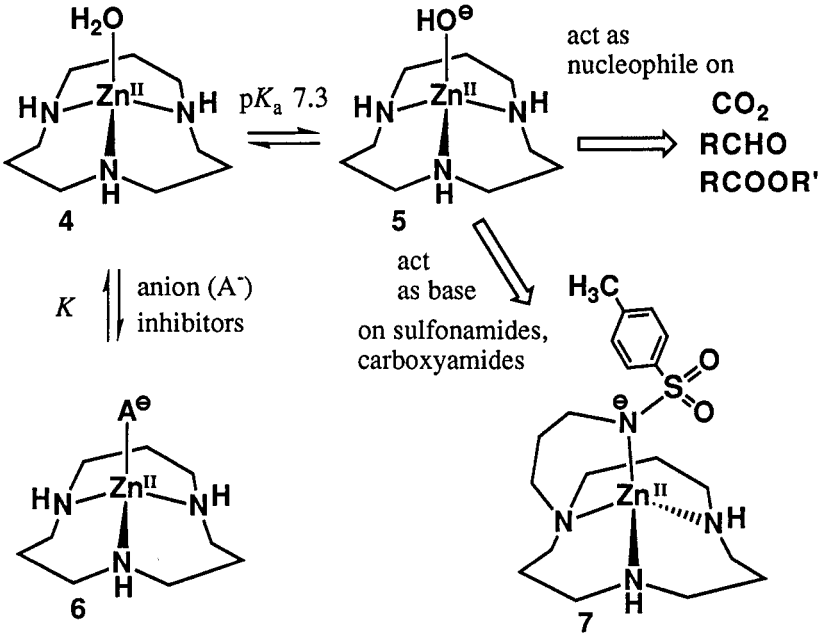


3
[14]aneN_4 (cyclam)

We have taken a different approach from most of the previous strategies which had mostly pursued the kinetic aspect, i.e., how much rate enhancement is achieved by using model complexes? Ours were (a) whether Zn^{II}-binding H_2O really dissociates a proton with pK_a ~7, as found in CA and ADH; (b) what determines the anion affinities found for CA; i. e., $OH^- > HCO_3^- > I^- > Br^- > Cl^- > F^-$; (c) do aromatic sulfonamides intrinsically bind with Zn^{II} as amide anions at physiological pH as found for CA?

II. CARBONIC ANHYDRASE (CA) MODEL[2-9]

The macrocyclic triamine **1** is the most appropriate ligand that mimics the LF surrounding Zn^{II} in CA. In its 1:1 Zn^{II}-**1** complex **4**, H_2O bound at the fourth coordination site deprotonates with pK_a value of 7.3 (25 °C and I = 0.10), almost the same value reported for CA.[2] The conjugate base **5** (isolable) has strong nucleophilicities to CO_2 (to yield HCO_3^-),[3] aldehydes (to hydrates),[2] and carboxyl esters (to carboxylates),[2] which are identical behaviors with CA. Moreover, the second order rate constants vs. pH plots show similar sigmoidal curves as reported for CA.[2,3]

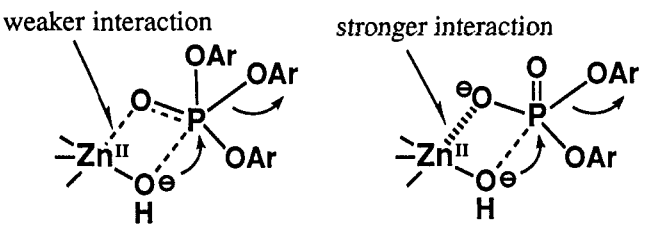


The nucleophilicity of **5** is inhibited by the presence of anions, which was proven to be due to the 1:1 anion complex **6** formation.[2] The order and magnitude (log K);[2,5] OH^- (6.4) > HCO_3^- (2.8) > I^- (1.6) > Br^- (1.5) > Cl^- (1.3) > F^- (0.8) are almost comparable with the reported anion inhibition of CA; OH^- (6.5) > HCO_3^- (1.6) > I^- (1.2) > Br^- (1.1) > Cl^- (0.7) > F^- (-0.1). From our model study, all the equilibrium values involved in the CA activities (pK_a for Zn^{II}-OH_2 $\rightleftharpoons$ Zn^{II}-OH^-, 1:1 anion affinity constants, blood pH ~7, and pK_a values for H_2CO_3 $\rightleftharpoons$ HCO_3^- $\rightleftharpoons$ CO_3^{2-}) can be beautifully rationalized for the effective CA catalysis.[4]

A Zn^{II} complex **7** has been synthesized and characterized as a model for aromatic sulfonamide inhibition of CA.[5] Potentiometric titration disclosed the facile deprotonation of the sulfonamide at pH <7 (25 °C and I = 0.10) to bind strongly to the Zn^{II}. The N^- binding in **7** inhibits formation of the Zn^{II}-OH^- and hence causes loss of the nucleophilicity to carbonyl compounds. Intermolecular interaction of **4** (or **5**) with CA inhibitors ($ArSO_2NH_2$) and substrate (HCO_3^-) was determined by inhibition kinetics in 4-nitrophenyl acetate hydrolysis.[5] The apparent 1:1 association constants (log K_i) at pH 8.4 are acetazolamide, a potent CA inhibitor drug (3.6) > HCO_3^- (2.8) > 4-nitrobenzenesulfonamide (2.6) > *p*-toluenesulfonamide (2.4). These constants are parallel with the reported ones for CA and thus our model study provides the first chemical evidence for strong acetazolamide binding to the Zn^{II} in CA.

III. PHOSPHATASE MODEL[10]

The Zn^{II} complexes of **1** and **2** promote the hydrolysis of tris(4-nitrophenyl)phosphate, TNP^0 (a neutral phosphotriester), and bis(4-nitrophenyl)phosphate, BNP^- (a monoanionic phosphodiester). Kinetic studies show that the reactive species are commonly L-Zn^{II}-OH^- (**5** for L = **1**) and the kinetically obtained pK_a values are almost identical to those obtained thermodynamically by pH-metric titration. The comparative reactivities of OH^-, **2**-Zn^{II}-OH^- and **5** with the conjugate acid pK_a values of 15.7, 8.0 and 7.3, respectively, have been studied to understand the role of Zn^{II} in alkaline phosphatases. For the neutral TNP^0, the second-order rate constants (M^{-1} sec^{-1}) at 25 °C, I = 0.2 are 10.7, 7.0, and 3.7, respectively, indicating free OH^- ion (the most basic OH^- species) is the best promoter. On the other hand, toward the anionic BNP^-, **5** becomes more efficient than free OH^-. The second-order rate constants (x 10^5 M^{-1} sec^{-1}) are 2.4, 8.5, and 2.1, respectively, at 35 °C and I = 0.2. A bifunctional mechanism of Zn^{II} toward phosphate (especially the anionic phosphate), in which the Zn^{II}-bound OH^- acts as a nucleophile, while the vacant Zn^{II} coordination site anchors the substrate P=O or P-O^- group, is well disclosed. Phosphate anion affinity constants K (M^{-1}) with **4** determined by pH-metric titration are $10^{3.5}$ for phenyl phosphate, PP^{2-}, and $10^{3.1}$ for 4-nitrophenyl phosphate, NP^{2-}, which are 25 times larger than those with less acidic aqueous Zn^{II} ion.



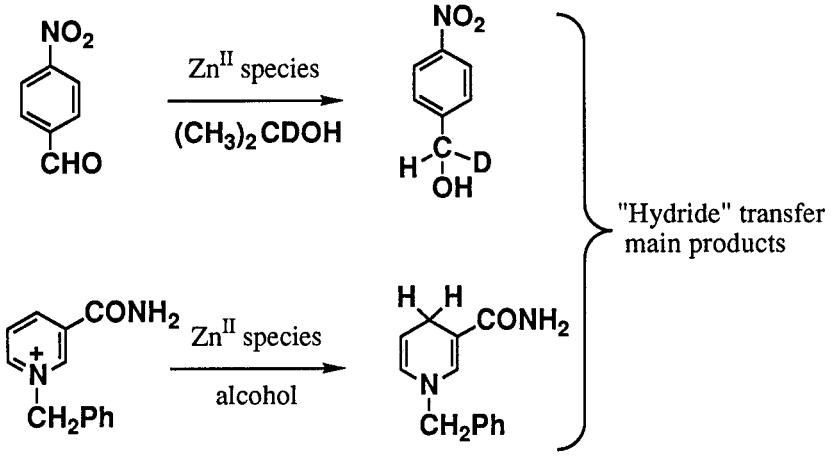
Phosphotriester hydrolysis
Zn^{II}-less bifunctional role

Phosphodiester hydrolysis
more bifunctional role

IV. ALCOHOL DEHYDROGENASE (ADH) MODEL[11]

The role of Zn^{II} in ADH has been well-defined by the comparative studies of Zn^{II} complexes of **1**, **2**, **3** and Zn^{II}_{aq}. Variations in Zn^{II} acidity and coordination environment in these results in varying degrees of catalytic activity in the reduction of 4-nitrobenzaldehyde and an NAD^+ model compound with alcohols as "hydride" sources to 4-nitrobenzylalcohol and the corresponding NADH model (1,4-adduct), respectively.

It is concluded from the comparative study that the most acidic and coordinatively least saturated Zn^{II} in **1** catalytically generates Zn^{II}-alkoxide complex to facilitate the "hydride" transfer to the H^- acceptors on the Zn^{II} coordination sphere. This study provides the first chemical model illustrating the significance of the Zn^{II} acidity and the steric requirement around Zn^{II} in the "hydride" transfer reaction (from alcohol =CHOH) catalyzed by Zn^{II}-containing ADH.



V. EXTENSION FROM THE MODEL STUDY OF Zn^{II}-MACROCYCLIC POLYAMINE COMPLEXES

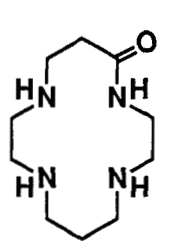
1. Inversion of DNA helicity induced by Zn^{II}-macrocyclic polyamines[12]

Exposure of synthetic polynucleotide poly(dG-dC)·poly(dG-dC) to a Zn^{II} complex of macrocyclic tetraamine **2**, produces a dramatic change in its circular dichroism (CD) in H_2O at pH 7.2 and 24° C: the CD spectrum of the initial B form changes to the spectrum characteristic of Z form (or a non-Z structure with left-handed helix) at very low concentrations ([Zn^{II}]/[base pair] in molar basis < 1). By contrast, Zn^{II}-**1** and Zn^{II}-**3** do not significantly affect such a topological change of the polynucleotide even at much higher concentrations. An increase in Na^+ ionic strength nullified the effect of Zn^{II}-**2** on the CD spectrum, indicating an outside interaction (electrostatic and/or hydrogen bonding) of the DNA model. This study illustrates the significance of the macrocyclic ligand structure around Zn^{II} ion for the specific interaction with DNA.

2. Selective uptake of Zn^{II} over Cd^{II} by macrocyclic polyamines[4, 13]

The pH-metric titration study of the interaction of Zn^{II} and Cd^{II} ion with dissociable (acidic) hydrogen-containing macrocyclic polyamines (**8**, **9** and **10**) has served to distinguish inherent acid and coordination properties of these two metal ions. In complexation with **8** below pH 8, Zn^{II} ion can replace the amide hydrogen and forms a planar 1:1 Zn^{II} complex **11** containing the deprotonated amide N^--Zn^{II} coordination, while Cd^{II} ion does not yield such a complex. A square-pyramidal Zn^{II} complex **12** (with amide deprotonated form of **9**) is crystallized at pH <8, as confirmed by an X-ray structure analysis. In

contrast, the larger and less acidic Cd^{II} displaces the amide proton of the same ligand at pH >10. The intermediate six-coordinate complex Cd^{II}-**9** containing a Cd^{II}-O(amide) bond was isolated at pH ~6 and characterized by an X-ray structure analysis. A larger sized 16-membered macrocyclic monooxopentaamine **10**, initially (pH <6) bind more strongly with Cd^{II} than with Zn^{II} using the four secondary nitrogen donors to form M^{II}-**10**. At higher pH, however, the more acidic Zn^{II} yields a more stable 5-coordinate amide-deprotonated complex **13** than the less acidic Cd^{II} does.



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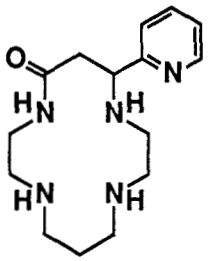

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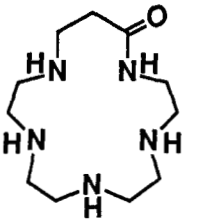

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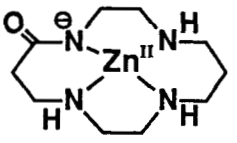

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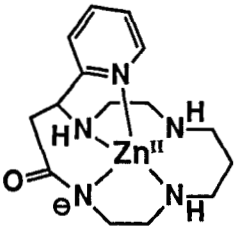

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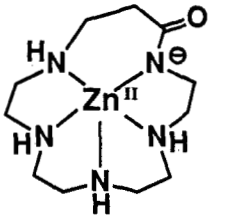

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