Comments on Inorganic Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455155

Macrocyclic Polyamines as a Probe for Equilibrium Study of the Acid Functions of Zinc(II) Ion in Hydrolysis Enzymes

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To cite this Article Kimura, Eiichi and Koike, Tohru(1991) 'Macrocyclic Polyamines as a Probe for Equilibrium Study of the Acid Functions of Zinc(II) Ion in Hydrolysis Enzymes', Comments on Inorganic Chemistry, 11: 5, 285 — 301
To link to this Article: DOI: 10.1080/02603599108035829
URL: http://dx.doi.org/10.1080/02603599108035829

Macrocyclic Polyamines as a Probe for Equilibrium Study of the Acid Functions of Zinc(II) Ion in Hydrolysis Enzymes

Macrocyclic polyamine ligands have been used in studying intrinsic acid properties of Zn(II) ion to help elucidate the role of Zn(II) in Zn-enzymes such as carbonic anhydrase (CA), carboxypeptidase, etc. Among macrocyclic tri- and tetraamines, [12]aneN$_3$ is the most appropriate ligand that mimics the ligand field surrounding the Zn(II) in CA. In its 1:1 $[Zn^{II}L]^{2+}$ complex, the H_2O bound at the fourth coordination site deprotonates with a pK_a value of 7.30 at 25°C and $I = 0.1$, almost the same value reported for CA. Anion binding affinity to the $[Zn^{II}L]^{2+}$ is determined by pH-metric titration and inhibition kinetics of 4-nitrophenyl acetate hydrolysis. The order and magnitude, $OH^- >> HCO_3^- > CH_3COO^- > I^- > Br^- > Cl^- > F^-$, are almost comparable with the anion inhibition for CA. The pH-metric determination of the interaction of Zn(II) and Cd(II) with dissociable (acidic) hydrogen-containing macrocyclic polyamines has served to distinguish acid and coordination properties of these two metal ions. Thanks to macrocyclic stabilities the metal-promoted amide proton dissociations were observed for the first time, particular with the more acidic Zn(II). To yield the same complexes with less acidic Cd(II), higher pH was required. Thus, monooxocyclam is proven to be the first Zn(II)-selective chelating agent. Taking all of these equilibrium data (from our model complexes) into consideration, we find that the pK_a value for $Zn-OH_2 \rightleftarrows Zn-OH$, 1:1 anion affinity constants, blood pH, and the pK_a values for $H_2CO_3 \rightleftarrows HCO_3^- \rightleftarrows CO_3^{2-}$ are all ideally consistent for CA activities.

Key Words: *zinc-enzyme model, macrocyclic polyamine ligands, carbonic anhydrase, acid properties of Zn(II) and Cd(II), anion inhibition*

INTRODUCTION

Numerous Zn(II)-containing enzymes are known. In fact, metalloenzymes that associate with hydrolysis (of esters, amides or pep-

Comments Inorg. Chem.
1991, Vol. 11, Nos. 5 & 6, pp. 285–301
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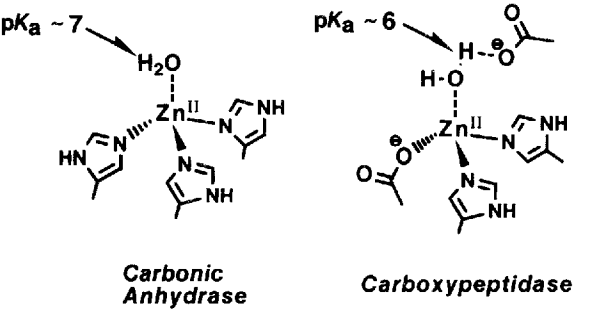
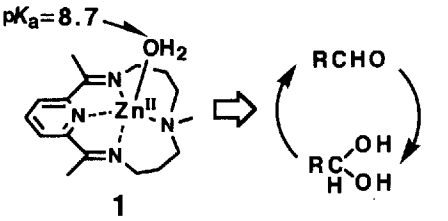
tides, phosphates) and hydration of biological molecules almost exclusively contain Zn(II) ion at the active center.[1] Then, the first and ultimate questions that naturally arise are why zinc(II)?; what are their special properties pertaining to their biological functions?; etc. Very often, the Zn(II) ion can be substituted without loss of enzymatic activity by other metal ions, typically cobalt(II), whose unfilled d electrons are adamantly taken advantage of in investigating the metal behaviors in the enzymes.[2] However, these questions still remain unanswered. Here, we come to realize how limited is our current knowledge of the intrinsic properties of Zn(II) up to now. Various hypotheses to attach special biological significance to Zn(II) have been presented,[3] but they are mostly based on literature surveys. Recently, theoretical approaches have been published.[4] However, the experiments specifically directed at those questions have rarely been reported.[5] The main reason lies in a lack of ligands that could provide appropriate ligand environments similar to those of enzymes and at the same time form rigid complex structures so as to define the unparallelled nature of the Zn(II) ion.



Active Centers of Some Zn(II) Enzymes

Among the past CA model systems, Woolley's [**1**] is probably the closest, which seems to successfully elucidate the reaction mechanism by which the Zn(II) catalyzes HCO_3^- dehydration, hydration of acetaldehyde, etc, reactions catalyzed by CA.[6] His model [**1**] supports the "Zn(II)–OH" mechanism, in which Zn(II)–OH functions as a nucleophile to the carbonyls. However, this model does not explain fully the properties of the Zn(II) in CA,

e.g., why the Zn (II) ion is ligated with three nitrogens (imidazole) instead of two or four; how the pK_a value of the Zn-bound H_2O is lowered to ca. 7 in CA, while those of $Zn(II)_{aq}$ and his complex are 9 and 8.7, respectively; whether the well-established anion inhibition in CA can be realized by such a simple model; or why it is that, while CA catalyzes ester hydrolysis (his model does, too), CA does not work on peptide hydrolysis, etc.



1

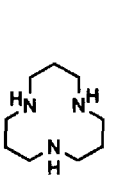
Zn(II)-*Carbonic Anhydrase* (CA) Model[6]

It would be of interest to point out here that most of the model studies so far have been concerned chiefly with kinetic aspects (e.g., rate enhancement effects) towards substrates, while paying less attention to the equilibrium aspects.

Recently, we have discovered that a macrocyclic triamine [12]aneN_3 [**2**] complex of Zn(II) provides a deeper insight into not only the kinetic but also the thermodynamic roles of the Zn(II) in CA active center.[7] Subsequently, we have found that the macrocyclic monooxopolyamines [**3**, **4**, **6**, and **7**] containing acidic (i.e., dissociable) hydrogens act as an extremely useful and versatile probe to spotlight the inherent acid properties of Zn(II) that are advantageous over those of Cd(II) in biological systems.[8]

ACID PROPERTIES OF Zn(II) IN MACROCYCLIC POLYAMINE COMPLEXES

Since it is now widely accepted that the Zn(II)-bound OH^- group plays the most important role in CA,[4g] carboxypeptidases,[4d] thermolysin,[9] etc., we were quite interested in how the primary donor structure affects the generation of the Zn(II)–OH species; i.e., how chelation influences the Zn(II) acidity that promotes the pro-



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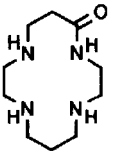
[12]aneN$_3$



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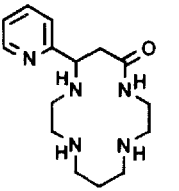
Monooxocyclam



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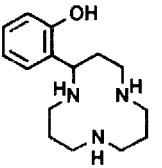
Pyridyl-pendant Monooxocyclam



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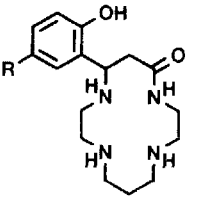
Phenol-pendant [12]aneN$_3$



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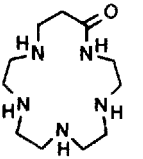
Phenol-pendant Monooxocyclam



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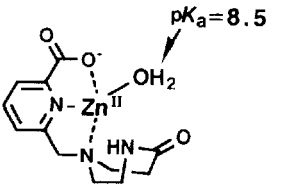
Monooxo[16]aneN$_5$

ton dissociation from the Zn(II)–OH$_2$. Of the past Zn(II)-enzyme model complexes, **1**[6] and **8**,[5d] where the Zn(II)–OH species are considered to be active nucleophiles, the pK_a values for the Zn–OH$_2$ ⇄ Zn–OH equilibrium are all above 8, which are higher than the reported pK_a values for CA (~7)[4g] and a carboxypeptidase A (~6).[10]



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We initiated our investigation with the pH-metric determination of the pK_a values of Zn(II)-bound H$_2$O in various macrocyclic polyamines.[7] The results are summarized in Fig. 1. Use of these macrocyclic polyamines as ligands for Zn(II) has various advantages as follows: (i) A systematic structural modification of Zn(II)

complexes is possible; (ii) the macrocyclic complexes in general are kinetically and thermodynamically stable, and thus rigid and well-defined complex structures are easily built; (iii) because of the extreme stabilities, macrocyclic polyamine complexation is complete at low pH and hence the measurements (at higher pH) of pK_a or anion affinity are easily conducted.

The most interesting result is that in the macrocyclic triamines with larger cavities such as [12]aneN$_3$ and iso[12]aneN$_3$, the Zn(II) ions are rendered surprisingly acidic so that they readily generate Zn(II)–OH species with pK_a values of 7.3. Note that aquated Zn(II) ion shows a pK_a of approximately 9.[10] Among the tetraamine complexes, [12]aneN$_4$ (cyclen) **12** offers the lowest pK_a value of 8.0. We must await a theoretical treatment to explain what factors of those triamines caused the Zn(II) ions to be so acidic. Significantly, *this pK_a of 7.3 is almost the same as that for CA* and

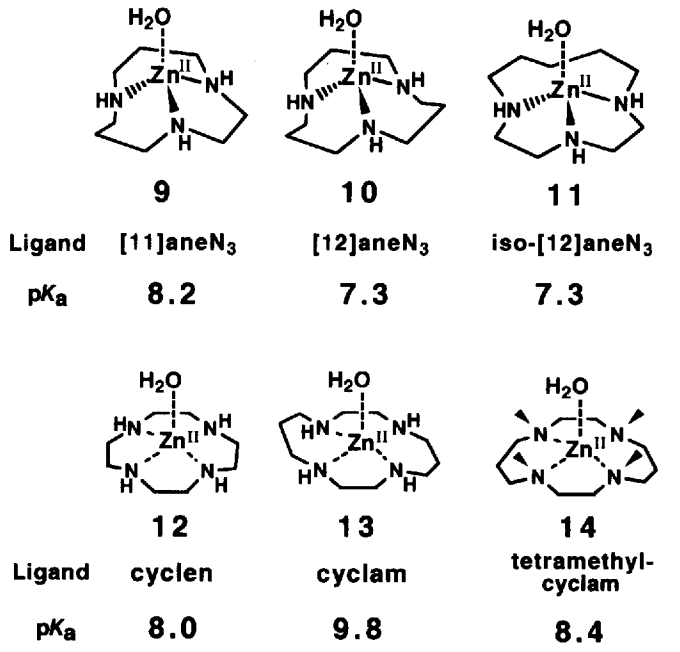


FIGURE 1 Comparison of pK_a values for Zn(II)–OH$_2$ ⇄ Zn(II)–OH with various macrocyclic polyamines (at 25°C and I = 0.1 (NaClO$_4$)).

TABLE I

1:1 Anion association constants, log $K(ZnL-A^-)$ at 25°C, for model complexes and CA

Anion(A^-)	Zn(II)–[12]aneN$_3$		Zn(II)–[12]aneN$_4$	Zn(II)–CA
OH^-	6.4 ± 0.1[a]		6.0 ± 0.1[b]	6.5[d]
HCO_3^-		3.1 ± 0.2[c]		1.6[d]
$CH_3CO_2^-$	2.6 ± 0.1[a],	2.5 ± 0.1[c]	1.7 ± 0.1[b]	1.1[d]
SCN^-	2.4 ± 0.1[a],	2.0 ± 0.1[c]	2.1 ± 0.1[b]	3.2[d]
I^-	1.6 ± 0.1[a]		1.0 ± 0.1[b]	1.2[d]
Br^-	1.5 ± 0.1[a],	1.5 ± 0.1[c]	1.0 ± 0.1[b]	1.1[d]
Cl^-	1.3 ± 0.1[b],	1.5 ± 0.1[c]	1.3 ± 0.1[b]	0.7[d]
F^-	0.8 ± 0.1[a]		0.7 ± 0.2[b]	−0.1[d]

[a] From Ref. 7 (determined by the pH-metric titration of Zn(II)–[12]aneN$_3$ in the presence of large excess A^-). $K(ZnL-A^-) = [ZnL-A^-]/[ZnL][A^-]$ (M^{-1}).

[b] Unpublished results (determined pH-metrically).

[c] Unpublished results (determined kinetically from the inhibition activities in 4-nitrophenyl acetate hydrolysis at pH 8.0 (100 mM HEPES buffer)).

[d] From Ref. 13b (determined kinetically from the inhibition activities in 4-nitrophenyl acetate hydrolysis).

hence these macrocyclic complexes offer a good model to compare the reactivity of the common Zn(II)–OH species with that of CA. The detailed kinetic study is described in Ref. 7.

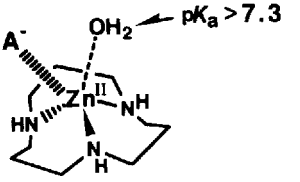
It is noteworthy that if triamines are nonmacrocyclic (e.g., **dien**), the Zn–N_3 complexes are much less stable, ready decomplexation at pH 7 ~ 9 tends to occur, and hence well-defined Zn(II)–OH species are difficult to generate in practice. Although a 9-membered triamine [9]aneN$_3$ forms a more stable 1:1 Zn complex (log $K(ZnL) = 11.6$)[11] than [12]aneN$_3$ (log $K(ZnL) = 8.8$),[7] precipitation occurs above neutral pH where pK_a for the H^+ dissociation is to be measured.

Anion and Acetazolamide Affinity to Zinc(II)-Macrocyclic Polyamine Complexes and CA. The pH-metric titration showed the Zn(II)–[12]aneN$_3$ complex **10** has anion affinity (see Fig. 1 in Ref. 7). Details of the calculations for the 1:1 anion affinity constants are compared with those for CA (Table I). The latter values were calculated from inhibition activities in 4-nitrophenyl acetate hydrolysis. By the same kinetic technique as for CA, the 1:1 anion association constants for HCO_3^- and several other anions were also obtained. *This is the first successful measurement of anion binding*

constants with the CA models. We are surprised to find that the order and magnitude of 1:1 association constants are very similar. With such a good agreement between our model and CA, we could extrapolate an interpretation based on the model data to the Zn(II)–A^- interaction in CA.

It is most significant that the potentially bidentate ligands $CH_3CO_2^-$ and HCO_3^- have appreciable affinity (relative to halogen ions) to the $Zn-N_3$ complex **10**, a fact suggesting the possible availability of more than one coordination site on the $Zn-N_3$ complexes. Quite interestingly, with the [12]aneN_4 (cyclen) complex **12**, the anion affinity trend changes; i.e., binding of $CH_3CO_2^-$, I^-, and Br^- ions decreases. This fact supports the above notion that the N_3 complex has more open space for the (potential) bidentate $CH_3CO_2^-$ or bigger sized halogen ions, whereas the N_4 complex has limited vacant space to allow only monodentate binding.

One can recalculate the same pH-titration data and reinterpret with the raised pK_a values in the presence of anions; e.g., at 100 mM F^- (pK_a = 7.6), 100 mM I^- (8.0), 20 mM SCN^- (8.5), etc. Namely, in 5-coordinate (instead of 4-coordinate) complexes **15** with an extra anion ligand the dissociation of H^+ from the Zn–OH_2 becomes more difficult. The decreased concentration of the Zn(II)–OH species at physiological pH accounts for the decreased catalytic efficiency of CA in the presence of anion inhibitors.

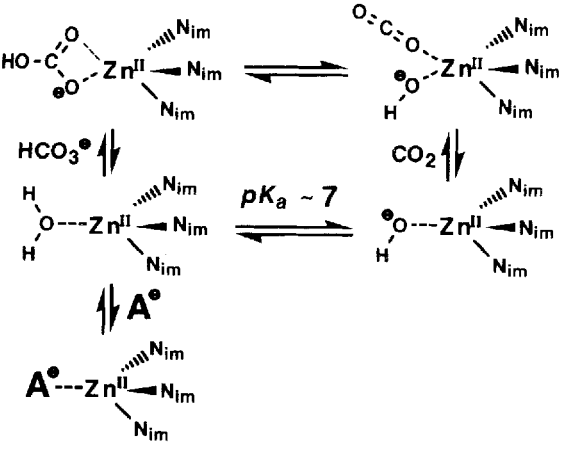


15

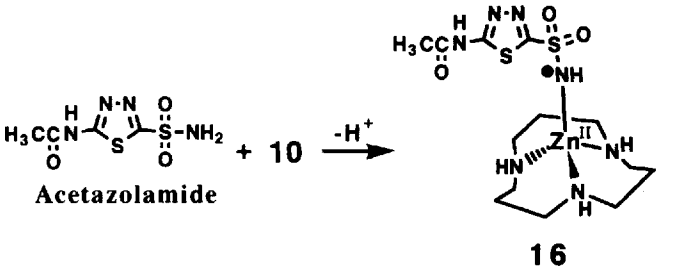
With the possible exception of HCO_3^- the good agreement for anion affinity in our model complex and CA verifies the previous theory that such anions bind to the vacant 4th coordination site of the Zn(II) in CA (Scheme I). The established order in the Zn(II)–A^- affinity constants is especially instructive for the fact that when CA catalyzes at high pH (~9) the CO_2 hydration, HCO_3^- dehydration, or a 4-nitrophenyl acetate hydrolysis, this anion inhibition is abolished.[12] This behavior is explained well by the extremely

strong OH^- ion affinity for Zn(II) in CA. With the pK_a value being 7.3 for generation of Zn(II)-bound OH^-, the other anions are unlikely to bind to Zn(II) at alkaline pH. Thus, (i) the competitive inhibition of HCO_3^- dehydration by anions is explained as a competitive binding of HCO_3^- and inhibitory anions to Zn; (ii) the CA activity toward HCO_3^- dehydration is greatly diminished at pH 8[13]; and (iii) HCO_3^- produced by hydration of CO_2 on the Zn(II) is favorably replaced by OH^-. We could also argue that prior to the HCO_3^- interaction for dehydration the Zn(II)–OH species must be converted to the Zn(II)–OH_2 species, which can be effectively achieved merely by slightly lowering pH to 6 ~ 7. However, for the reverse CO_2 hydration to occur, the Zn(II)–OH species must be restored in basic conditions. For both the forward and backward reactions conveniently to occur, the most useful media pH should be adjusted to the near pK_a value for Zn–OH_2 $\rightleftarrows$ Zn–OH, which is exactly the case in our body; e.g., blood pH is set at ~7.4!

Sulfonamides bind with CA in 1:1 as anionic forms, as monodentates (through the sulfonamide N^-) or bidentate ligands (through sulfonamide N^- and O).[4a] Treatment of a typical sulfonamide inhibitor, acetazolamide, with **10** in acetonitrile immediately yields



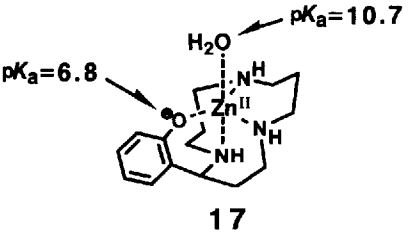
SCHEME 1



SCHEME II

precipitates, which was identified as a 1:1 complex **16** wherein the sulfonamide nitrogen is deprotonated (Scheme II).[7] It is of interest to point out that with other transition metal ions (e.g., Ni(II) ion)[14] acetazolamide binds via the thiazole N, but not via the deprotonated N^-. Such a different behavior of Zn(II) from that of transition metal ions best illustrates the *outstanding acid nature of Zn^{II} that favors anionic donors over neutral donors*. The pK_a value of 7.42 (25°C, I = 0.1) for acetazolamide is close to that for the H_2O in **10**. Thus, the easy deprotonation of sulfonamides with simultaneous coordination is facilitated.

Phenol is another inhibitor of CA.[15] A question may be raised if it may also be deprotonated for coordination with Zn(II). A possible model for metal-induced deprotonation of a phenol (pK_a = 6.8) is demonstrated by another complex **17** of a phenol-pendant [12]aneN_3 **5**.[16] Its crystal structure is trigonal bipyramidal with an extremely short phenolate–Zn(II) bond distance (1.93 Å). The H_2O bonding to the Zn(II) is lengthened (distance 2.22 Å) to occupy an apical position and its pK_a value is raised to 10.7.[17]



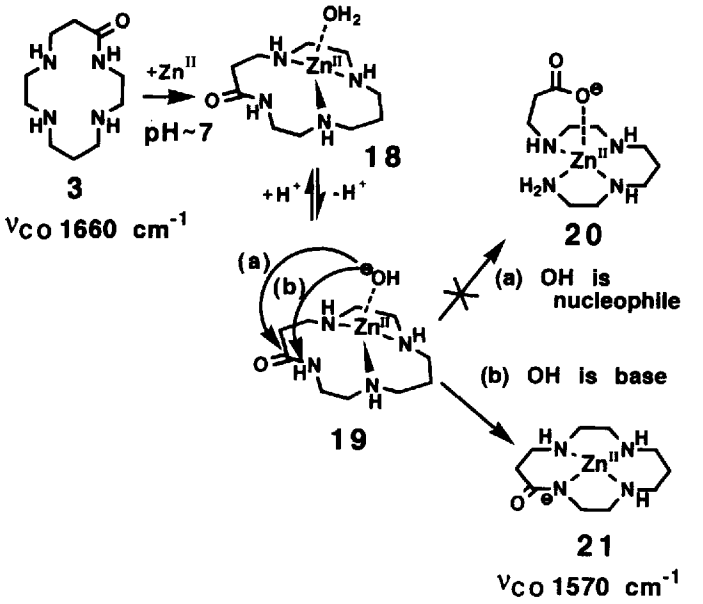
ACID PROPERTIES OF ZINC(II) AND CADMIUM(II) IONS TOWARD AMIDE-CONTAINING MACROCYCLIC POLYAMINES[8]

One aspect of the acid nature of Zn(II) has been characterized by the use of [12]aneN_3. Next, we demonstrate that different types of macrocyclic polyamines, **3**, **4**, **6**, and **7**, containing dissociable, acidic hydrogen(s), work as suitable ligands to reveal other acidic properties of Zn(II). Since Zn(II) (d^{10}) has no specially distorted or directional ligand field, use of such ligands, even though equipped with donors from various directions, poses little disadvantage in this study.

Recently, iodoacetamide and ethyl carbamate were shown to be the CA inhibitors, which are proposed to bind with the Zn(II) ion.[2b] Metal-induced deprotonation of the Zn(II)-bound amides were indicated by kinetics and visible spectral studies. However, there has been no report of the Zn(II)-deprotonated amide coordinating complexes. In this connection, it should be recalled that while CA has esterase activities, it has no peptidase activity. It is suspected that the Zn(II)–OH in CA acts on amides as a base (rather than as a nucleophile) to produce amide anions that form stable bonds with the Zn(II) ion, resulting in no further amide bond cleavage reaction.[4a]

Interaction of Zn(II) and Cd(II) with Monooxocyclam, **3**. First, we have tested the interaction of Zn(II) with monooxocyclam **3** to see if the possible intermediate N_3–Zn(II)–OH **19** acts as a nucleophile (path **a** to **20** in Scheme III) or a base (path **b** to **21**).

The result from the pH-metric titration proved that **b** is the exclusive pathway. The product Zn(II)-inclusion complex was indeed isolated as a perchlorate salt, whose IR spectrum at $\nu_{C=O}$ 1570 cm^{-1} supports the deprotonated amide structure **21**. Although an otherwise unstable Zn(II)-amide anion bond should be rendered possible by the macrocyclic N_4-coordinate structure, this is the first direct observation of the Zn(II)-induced deprotonation of an amide, a fact implying feasibility of the deprotonated amide coordination of amide inhibitors to the Zn(II) in CA, where collaboration of neighboring peptide residues would also contribute to stabilizing the anionic amide binding.

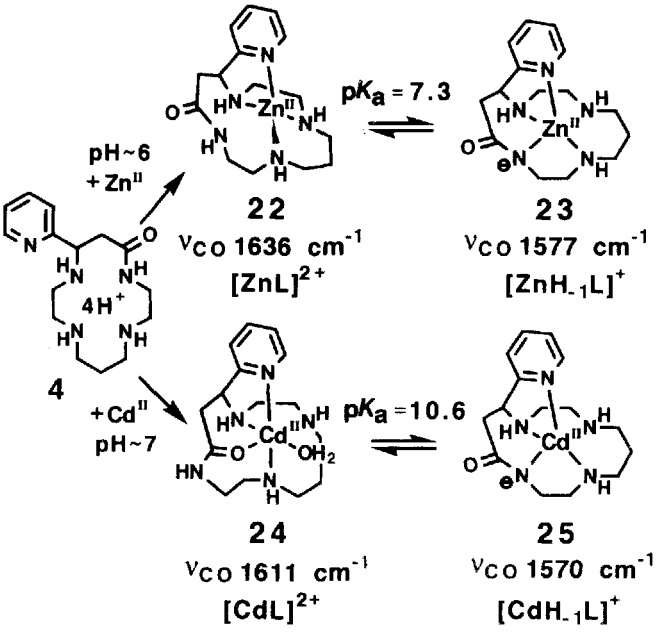


SCHEME III

By comparison, the amide dissociation from dioxocyclam to a doubly deprotonated product (as seen with Cu(II), Ni(II), or Pt(II))[18] did not take place with Zn(II).[8]

Acid properties of Cd(II) were compared using the same macrocyclic ligand. Cd(II) ion has weaker interaction with the secondary amine of monooxocyclam below pH 8 than Zn(II) ion (see Fig. 1 of Ref. 8). As the pH is raised, precipitation occurs before the amide proton dissociation. This result may be interpreted that N_3–Cd(II) interaction is weaker (or Cd(II) is less acidic than Zn(II)) and hence stability of N_3–Cd(II)–OH (like **19**) cannot beat $Cd(OH)_2$ precipitation. At any rate, monooxocyclam **3** turned out to be the first chelating agent to selectively recognize Zn(II) over Cd(II). In previously synthesized ligands,[19] such a clear-cut host molecule for Zn(II) ion against Cd(II) ion has been unknown.

Interaction of Zn(II) and Cd(II) with a Pyridylmonooxocyclam, **4**. The interaction modes of Zn(II) and Cd(II) at various pH are



SCHEME IV

summarized in Scheme IV. Zn(II) ion below pH 6 binds with three amines and a pyridyl N to yield $[ZnL]^{2+}$, **22**, while larger Cd(II) ion gives a similar $[CdL]^{2+}$ complex, **24** only at higher pH ~7. The Cd(II) complex **24** crystallized and its X-ray analysis shows an octahedral structure with an additional amide oxygen ($\nu_{C=O}$ 1611 cm^{-1}) and a water coordination. Such an expanded six-coordinate geometry of **24** is in strong contrast to the four-coordinate **22** ($\nu_{C=O}$ 1636 cm^{-1} indicating noninteraction of the amide with Zn(II)), which reflects the different metal ion size; larger Cd(II) vs. smaller Zn(II). In fact, the average Cd–N (secondary) bond distance of ca. 2.3 Å in **24** is much longer than the average Zn–N distance of ca. 2.1 Å in **22**.

The weaker acidity (due to the larger ion size) of Cd(II) in comparison to Zn(II) is well demonstrated in the subsequent processes of metal-induced amide deprotonation with concomitant metal-inclusion to give $[ML_{-1}L]^+$, **23** and **25**, which occur at much higher pH for Cd(II). The pK_a for $[ML]^{2+} \rightleftarrows [MH_{-1}L]^+$ are 10.6 for

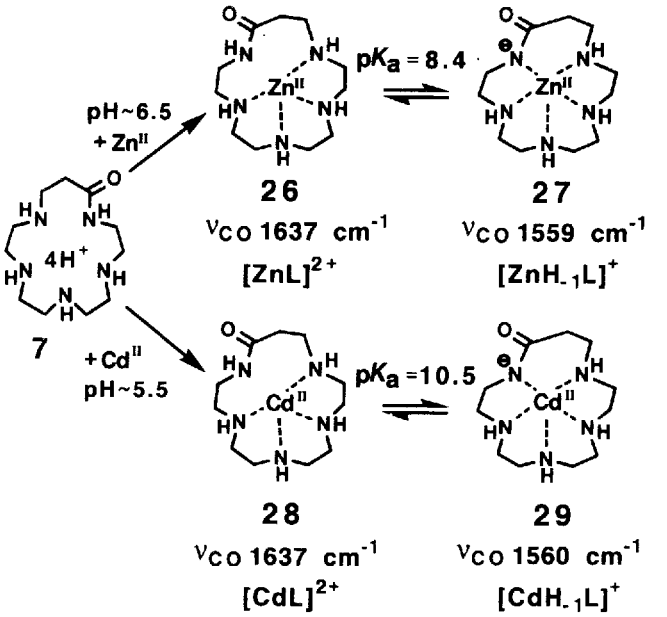
Cd(II) and 7.3 for Zn(II). The final five-coordinate zinc complex, **23** as $[ZnH_{-1}L]\cdot ClO_4\cdot 3H_2O$ was isolated and X-ray analyzed. As anticipated, the Zn(II) stays in the $N_3(N^-)$ square plane and the Zn–N^- (amide anion) bonding is stronger (2.04 Å) than the Zn–NH bondings (ca. 2.1 Å). The similar $\nu_{c=o}$ for **23** (1577 cm^{-1}) and **25** (1570 cm^{-1}) implies the similar complex structure for the Cd(II) complex.

Interaction of Zn(II) with 4-Nitrophenol-Pendant Monooxocyclam, **6** *(R = NO_2).* When a highly dissociable group (as 4-nitrophenol, pK_a ~7) is attached to monooxocyclam, Zn(II) ion first binds to the phenolate O^- rather than the amide anion. This fact vividly proves that Zn(II) behaves as a cationic acid in preference to seeking the macrocyclic $N_3(N^-)$ ligand field stabilization. Eventually, at higher pH, the amide proton dissociates to give the Zn(II)–$N_3(N^-)$ inclusion complex $[ZnH_{-2}L]$, whereupon the Zn(II)–O^- bonding appreciably weakens.[8] Cd(II) ion does not form stable complexes with this ligand.

Interaction of Zn(II) and Cd(II) with Monooxo[16]aneN_5, **7**. A larger-sized monooxopentaamine **7** containing five N donors in a ring was finally tested (Scheme V). In contrast to the preceding N_4 system, the initial interaction to $[ML]^{2+}$ complexes, **26** and **28**, *is more favorable with Cd(II) ion than with Zn(II) ion*; i.e., log *K*(ML) = 11.6 for Cd(II) and 10.7 for Zn(II). This is evidently due to the fact that the larger Cd(II) ion fits better with the larger N_4 macrocycle than the smaller Zn(II) ion. As the pH is raised, however, Zn(II) yields a more stable deprotonated amide coordinating complex **27**, $[ZnH_{-1}L]^+$ ($\nu_{c=o}$ 1559 cm^{-1}) than Cd(II) does to $[CdH_{-1}L]^+$, **29** ($\nu_{c=o}$ 1560 cm^{-1}). The amide deprotonation constants (pK_a) are 8.4 for Zn(II) and 10.5 for Cd(II). *Thus, the Zn(II) and Cd(II) complex stability order reverses at the stage of the amide deprotonation.*

CONCLUDING REMARKS ON THE PRESENT EQUILIBRIUM STUDY IN RELEVANCE TO ZINC-ENZYMES

The acidity of Zn(II) ion can be enhanced in artificial triamine ligand fields, so as to promote the deprotonation from its bound



SCHEME V

H_2O to pK_a 7.3 at 25°C, $I = 0.1$, an almost equivalent value being reported for the Zn(II)–OH_2 in CA, where the Zn(II) is coordinated by three imidazole donors (His 94, His 96, and His 119) that might be aligned like our macrocycle N_3 ligand field. If that were the case, we might not need to invoke contributions from neighboring amino acid residues (e.g., imidazole, carboxylate) to account for a pK_a value of ~7 for CA. Rather, the role of such functional amino residues may be more important in the subsequent catalytic cycles. When Zn(II) is bound to tetraamine ligands, the fifth coordinating H_2O becomes more difficult to deprotonate and hardly generates the Zn(II)–OH species at physiological pH (in the absence of any assistance from the neighboring groups).

Moreover, the triamine complexes are more unsaturated than the tetraamine complexes and thus the former have more open space for incoming substrate donors. This is extremely advantageous in the interaction of Zn(II)–OH with CO_2 for the subsequent hydration or in the interaction of Zn(II) with another substrate

HCO_3^- (a potential bidentate ligand) over other abundant monodentate anions such as Cl^-.

The pK_a value of 7.3 for the $Zn-OH_2 \rightleftarrows Zn-OH$ is very close to physiological pH, which is most essential for the maximum catalytic fulfillment of the $CO_2 \rightleftarrows HCO_3^-$ reaction. If our physiological pH is far less than 7.3, the hydration process of CO_2 requiring the Zn–OH species at the initial step would not work, and if our pH is far higher than 7.3, the reverse reaction, i.e., the dehydration of HCO_3^- requiring the $Zn-OH_2$ species, would not occur.

When Zn(II) is bound with anions A^-, Zn(II) acidity is weakened and Zn(II)–A^- can practically no longer accommodate an additional OH^- group at physiological pH. Under such circumstances, the Zn(II) complex could not yield an efficient nucleophile for carbonyl substrates. We predict that carboxypeptidase (where Zn(II) is bound with two imidazole and a carboxylate anion) has difficulty generating the Zn–OH species without the assistance from proximate imidazole and carboxylate.

The Zn(II)–OH species in CA, like our monooxocyclam–Zn(II)–OH species **19**, would act as a good base rather than a nucleophile toward amide (or peptide) substrates, so as to produce amide anions that become donor ligands to Zn(II). Therefore, CA does not show peptidase activity. This is further extrapolated to permit an argument that for the substrate peptides effectively to be hydrolyzed by the Zn(II)–OH group, special devices to prevent the occurrence of such a pathway would be needed, such as making the amide proton transfer (to Zn(II)–OH) difficult by mutually longer distance or by the presence of other proton supply sources.

With Zn(II)–[12]aneN_3, the order of anion affinity is: $OH^- >> HCO_3^-$ or $CH_3COO^- > I^-, Br^- Cl^-$, which is almost overlapping with CA. The 1:1 association constants are also similar. The only disagreement lies in the magnitude for the HCO_3^- affinity to the Zn(II) in [12]aneN_3 and CA. At any rate, we believe that this equilibrium sequence is extremely important for the CA catalytic cycle. The products formed (either HCO_3^- from CO_2 or CH_3COO^- from CH_3COOR) can be theremodynamically readily displaced by OH^- and removed from the Zn(II)-coordination sites. The Zn(II)–OH is of course readily convertible to the $Zn(II)-OH_2$ with pK_a of 7.3 in blood. A substrate HCO_3^- has a high affinity to the Zn(II)

at relatively lower pH, which is a prerequisite for the dehydration to follow.

Overall, we conclude that all the relevant pK_a and pH values as well as anion binding constants are too perfectly consistent for CA activities, as if controlled by the hand of God: pK_a of ca. 7 for $Zn-OH_2$, pH ca. 7.4 for blood, and pK_a ca. 4 for H_2CO_3 and ca. 10 for HCO_3^- (i.e., at physiologicala pH, HCO_3^- is an exclusive carbonate species).

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References

1. I. Bertini, C. Luchinat, W. Maret and M. Zeppezauer (Eds.), in *Zinc Enzymes* (Birkhäuser, Boston, 1986).
2. Recent articles: (a) I. Bertini and C. Luchinat, Acc. Chem. Res. **16,** 272–279 (1983); (b) J. I. Rogers, J. Mukherjee and R. G. Khalifah, Biochemistry **26,** 5672–5679 (1987).
3. E. Ochiai, J. Chem. Educ. **65,** 943–946 (1988).
4. (a) J.-Y. Liang and W. N. Lipscomb, Biochemistry **28,** 9724–9733 (1989); (b) R. S. Alexander, Z. F. Kanyo, L. E. Chirlian and D. W. Christianson, J. Am. Chem. Soc. **112,** 933–937 (1990); (c) D. W. Christianson and R. S. Alexander, J. Am. Chem. Soc. **111,** 6412–6419 (1989); (d) D. W. Christianson and W. N. Lipscomb, Acc. Chem. Res. **22,** 62–69 (1989); (e) I. Bertini, C. Luchinat, M. Rosi, A. Sgamellotti and F. Tarantelli, Inorg. Chem. **29,** 1460–1463 (1990); (f) K. M. Merz, R. Hoffman and M. J. S. Dewar, J. Am. Chem. Soc. **111,** 5636–5649 (1989); (g) D. N. Silverman and S. Lindskog, Acc. Chem. Res. **21,** 30–36 (1988).
5. Recent articles: (a) S. H. Gellman, R. Petter and R. Breslow, J. Am. Chem. Soc. **108,** 2388–2394 (1986); (b) R. Breslow, D. Berger and D.-L. Huang, J. Am. Chem. Soc. **112,** 3686–3687 (1990); (c) Y. Pocker and N. Janjić, J. Am. Chem. Soc. **111,** 731–733 (1989); (d) J. T. Groves and R. M. Dias, J. Am. Chem. Soc. 1033–1035 (1979).
6. P. Woolley, Nature (London) **258,** 677–682 (1975).
7. E. Kimura, T. Shiota, T. Koike, M. Shiro and M. Kodama, J. Am. Chem. Soc. **112,** 5805–5811 (1990).
8. E. Kimura, T. Koike, T. Shiota and Y. Iitaka, Inorg. Chem. **29,** 4621–4629 (1990).
9. M. C. Izquierdo and R. L. Stein, J. Am. Chem. Soc. **112,** 6054–6062 (1990).
10. W. L. Mock and J. Tsay, J. Biol. Chem. **263,** 8635–8641 (1988).
11. R. Yang and L. Zompa, Inorg. Chem. **15,** 1499–1502 (1976).
12. Y. Pocker and T. L. Deits, J. Am. Chem. Soc. **104,** 2424–2434 (1982).
13. (a) Y. Pocker and S. Sarkanen, Advance in Enzymology **47,** 149–274 (1978);

(b) Y. Pocker and J. T. Stone, Biochemistry **7,** 2936–2945 (1986).
14. (a) S. Ferrer, J. Barrás, C. Miratvilles and A. Fuertes, Inorg. Chem. **28,** 160–163, (1989); (b) S. Ferrer, J. Barrás, C. Miratvilles and A. Fuertes, Inorg. Chem. **29,** 206–210 (1990).
15. (a) I. Simonsson, B. Jonsson and S. Lindskog, Biochem. Biophys. Res. Commun. **108,** 1406–1412 (1982); (b) W. L. Mock and J. Tsay, Biochemistry **25,** 2920–2927 (1986).
16. E. Kimura, M. Yamaoka, M. Morioka and T. Koike, Inorg. Chem. **25,** 3883–3886 (1986).
17. E. Kimura, T. Koike and K. Toriumi, Inorg. Chem. **27,** 3687–3688 (1988).
18. (a) E. Kimura, J. Coord. Chem. **15,** 1–28 (1986); (b) E. Kimura, S. Korenari, M. Shionoya and M. Shiro, J. Chem. Soc., Chem. Commun. 1166–1168 (1988).
19. K. R. Adam, K. P. Dancey, A. J. Leong, L. F. Lindoy, B. J. McCool, M. McPartlin and P. A. Tasker, J. Am. Chem. Soc. **110,** 8471–8477 (1988), and references therein.