Effect of Triamine Ligands (L) on the Acidity of L-Zn-OH_2 as a Model Compound of CA and AP

Yuki Fujii,* Toyofumi Itoh, Kazuhiko Onodera, and Toshiji Tada[†]
Department of Chemistry, Ibaraki University, 2-1-1, Bunkyo, Mito, Ibaraki 310
[†]*Analytical Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., 2-1-6, Kashima, Yodogawa-ku, Osaka 532*

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The acid dissociation constants of coordinated water of L-Zn-OH_2 (L= diethylenetriamine (**1**), *N*-(2-aminoethyl)-1,3-propanediamine (**2**), dipropylenetriamine (**3**), 1,3,5-triaminocyclohexane (**4**), and 1,5,9-triazacyclododecane (**5**)) and the hydrolysis rate constants of diphenyl 4-nitrophenyl phosphate (**6**) promoted by L-Zn-OH were found to correlate linearly with the enthalpy change (ΔH) for the formation of L-Zn-OH_2, which corresponds to the binding energy of Zn-L: the smaller the binding energy is, the stronger the Lewis acidity of zinc ion is and the faster the hydrolysis is.

Numerous model studies have been conducted to spotlight the role of metal ions in hydrolytic zinc enzymes such as carbonic anhydrase (CA) and alkaline phosphatase (AP), and it has been well established that zinc ion in the enzymes has extraordinarily strong Lewis acidity and thus the coordinated water on zinc ion is easily deprotonated to form active species Zn-OH (pK_a of CA~7) under biological condition (pH=7.4).[1] Strong anion affinity of CA is also explained in terms of the strong Lewis acidity of the zinc ion.[1-3] However, it is not clear why the zinc ion in enzymes is so acidic as compared with common zinc ion such as $[Zn(OH_2)_6]^{2+}$ (pK_a~9).[4] Further, it is also question why the Zn-OH in enzymes is so active for hydrolysis of substrates in spite of weak nucleophilicity of the OH group on strongly acidic zinc ion.[1,5]

In order to clarify the origin of the strong Lewis acidity of zinc ion in CA and AP and to explain the high substrate-hydrolysis ability of the zinc ion, we investigated the effects of supplemental ligand L (**1**-**5**) on the acid dissociation constant pK_a of model complexes L-Zn-OH_2 and on the hydrolysis rate constant *k* of ester **6** promoted by the model complexes.

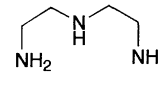
The pK_a values of coordinated water and the formation constants *K* for L-Zn-OH_2 were determined potentiometrically at I=0.1($NaClO_4$), and 288, 298, 308 K by applying the method in Ref.6, except for **3**. In the case of **3**, KCl was used as an electrolyte to prevent the precipitation of $[L\text{-}Zn\text{-}OH\text{-}Zn\text{-}L](ClO_4)_3$.[7] The complexes **1**~**5** were confirmed to exist as monomer in solution from the analysis of pH titration curves. The ΔH and ΔS values were obtained from the temperature dependency of *K*. The thermodynamic data were consistent with literature values. The hydrolysis rate constants k_{obs} were measured spectrophotometrically in 20%(v/v) CH_3CN-H_2O at pH=8.8, 8.6, 8.0, I=0.1($NaClO_4$), 50mM TAPS buffer (1M = 1mol dm^{-3}), λ=400 nm, and 298 K. From the pH dependency of k_{obs}, it was confirmed that active species is L-Zn-OH. Since the complex exists as an equilibrium mixture of L-Zn-OH_2 and L-Zn-OH under the pH condition employed, the hydrolysis rate constant k_{OH} per one mole of L-Zn-OH was calculated by the use of k_{obs} and pK_a. The thermodynamic and kinetic data are listed in Table 1.

The pK_a of coordinated water was affected by the ligand L, and it decreased from 8.93 to 7.44 in the order of **1**~**2**>**3**>**4**>**5** (Table 1). This effect can be ascribed to the ligand-ligand interaction through L-Zn-O bond rather than that through space, because all the ligands belong to an analogous N_3 system which form five or six membered chelate rings so that the coordination structure of L-Zn-OH_2 is expected to be very similar to one another. Hence, the difference of basicity or coordination ability of the ligand is thought to be responsible for the difference of the pK_a of coordinated water. However, it is quite difficult to estimate the basicity or coordination ability of the tridentate ligand, because the pK_a of tridentate ligand used here is affected strongly with the structure of the ligand itself.[3,8-11] Thus, we compared the ΔH for the formation of L-Zn-OH_2 instead of the basicity or coordination ability of ligand. As shown in Figure 1A, the pK_a of coordinated water increases linearly as the increase of

Table 1. Thermodynamic and kinetic data for zinc-triamine complexes

Ligand L	Formation constants and deprotonation constants of L-Zn-OH_2[a]				Hydrolysis rate constants of diphenyl 4-nitrophenyl phosphate[b]	
	log *K*	$-\Delta H$ / kJ mol^{-1}	ΔS / J $mol^{-1}K^{-1}$	pK_a	k_{obs} / $10^{-2}M^{-1}s^{-1}$	k_{OH} / 10^{-2} $M^{-1}s^{-1}$
1	8.92 ± 0.01 (8.80[c])	28.9 ± 0.1 (27.2[c])	73.9 ± 0.1 (79.5[c])	8.93 ± 0.01	5.0 ± 0.1	12 ± 1
2	8.41 ± 0.01 (8.77[d])	27.9 ± 2.6	67.8 ± 8.9	8.90 ± 0.01 (8.86[d])	5.0 ± 0.5	11 ± 1
3	7.94 ± 0.05 (7.92[e])	22.0 ± 1.8 (22.6[f])	77.8 ± 6.3 (75.3[f])	8.58 ± 0.03 (8.56[e])	11.0 ± 0.8	18 ± 2
4	6.95 ± 0.01 (6.90[g])	3.4 ± 0.1 (0.2[h])	121.7 ± 0.2 (129.7[h])	7.95 ± 0.01 (8.00[g])	18.2 ± 0.1	21 ± 1
5	8.25 ± 0.01 (8.41[i])	-14.6 ± 0.1	207.1 ± 0.5	7.44 ± 0.01 (7.30[i])	32.0 ± 0.3	33 ± 1

[a] pH titration, [Zn(II)]=1mM, [Ligand]=1mM, 0.1M $NaClO_4$ (for Ligand 3, 0.1M KCl). [b] In 20%(v/v)CH_3CN-H_2O, [ZnL]=0.2~1.0M, 0.05M TAPS buffer, I=0.1 ($NaClO_4$), 25°C, pH=8.8. [c] Ref.15, 0.1M KCl. [d] Ref.9, 0.5M KCl. [e] Ref.10, 0.1M KCl. [f] Ref.16, 0.1M KCl. [g] Ref.11, 0.1M KCl. [h] Ref.17, 0.1M KCl. [i] Ref.3, 0.1M $NaClO_4$.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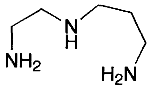
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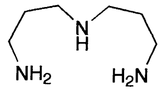
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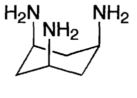
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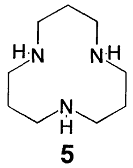
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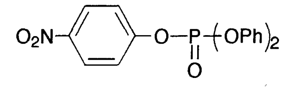
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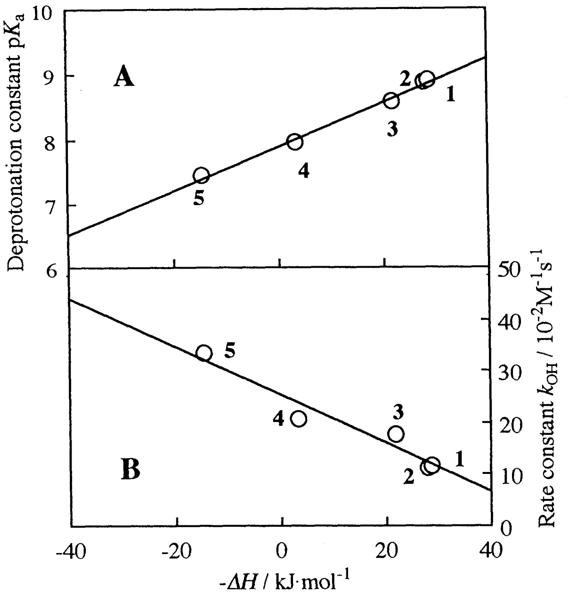

Figure 1. Relationships between deprotonation constants pK_a of coordinated water and $-\Delta H$ (**A**) and between hydrolysis rate constants k_{OH} of **6** and $-\Delta H$ (**B**). The number **1**~**5** corresponds to triamine complexes in Table 1.

$-\Delta H$. Since the ΔH corresponds mainly to the binding energy of Zn-L[12] it becomes clear that the weaker the binding energies is, the easier the deprotonation of coordinated water is. That is, as the Zn-L bond becomes weak, the Zn-OH$_2$ bond becomes strong, resulting in the promotion of deprotonation of coordinated water. Therefore, it can be concluded that the acidity of zinc ion depends linearly on the coordination ability (ΔH) of supplemental ligand L and the weaker the coordination ability of L, the higher the acidity of zinc ion. Interestingly, the ΔH of [12]aneN$_3$-Zn-OH$_2$, which is the most excellent model compound,[1,3] is positive (14.6kJ/mol). This suggests the binding energy (ΔH) of zinc ion in the hydrolytic zinc enzymes being very small, too. For the ΔS, the compensation effect by ΔH was observed.

The hydrolysis rate constant k_{OH} also depends on the ΔH (Figure 1B). In this case, the faster the substrate-hydrolysis is, the smaller the ΔH is. This corresponds to that the higher the acidity of zinc ion is, the higher the hydrolysis ability of the zinc ion is. This supports that the substrate-hydrolysis proceeds via an intermediate in which substrate coordinates to zinc ion with oxygen atom of P=O group, proposed recently by Kimura.[1] Because, the higher the acidity of zinc ion is, the easier the coordination of substrate is, and the coordination of substrate increases the polarization of P=O group. In fact, it is well known that the coordination number of zinc ion varies easily from four to five or six, it is four in [12]aneN$_3$-Zn-OH [3] but five in [12]aneN$_3$-Zn-(NCS)$_2$.[13] The X-ray crystal structure analysis for complexes **1**, **3**, and **4** is under investigation.

In the last, it should be noted that the similar relationships between the pK_a, k_{OH} and ΔH to Zn-N$_3$ system can be obtained in Zn-N$_4$ system (N$_4$=triethylenetetramine, *N*,*N*,*N*-triaminoethylamine, 1,4,7,10-tetraazacyclododecane, 1,4,8,11-tetraazacyclotetradecane).

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12 Generally, ΔG of complex formation in water is consist of ΔG_r (reaction) and ΔG_h(hydration): $\Delta G = \Delta G_r + \Delta G_h = \Delta H_r - T\Delta S_r + \Delta H_h - T\Delta S_h$. And if $\Delta G_r \gg \Delta G_h$, $\Delta G \sim \Delta G_r = \Delta H_r - T\Delta S_r$.[14] In the case of L-Zn-OH$_2$, the ΔS increases in the order of **1** ~ **2** ~ **3** < **4** < **5**, and this order coincides with the increasing order of chelate effect (ΔS_r) of L. Hence, the formation of L-Zn-OH$_2$ can be regarded as "reaction control" rather than "hydration control" and thus the ΔH corresponds mainly to the ΔH_r, namely the binding energy of Zn-L.
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