Zinc Complexes of Artificial Histidine-containing Dipeptides as Catalysts of Hydrolyses of *p*-Nitrophenyl Phosphates

Kazuhiko Ichikawa,* M. Khabir Uddin, and Kou Nakata
Graduate School of Environmental Earth Science, Hokkaido University, Sapporo 060-0810

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Zinc complexes of histidine-containing peptide derived from *N,N'*-dihistidylethylenediamine **L1** and im-bzl-*N,N''*-dihistidyldiethylenetriamine **L2** were designed and examined as catalysts for hydrolyses of bis(p-nitrophenyl)phosphate (BNPP) and p-nitrophenyl phosphate (NPP). The zinc complex of **L1** was inactive and another **L2** complex hydrolyzed efficiently BNPP and NPP: their pseudo-first-order rate constants are 1.1×10^{-5} s^{-1} and 2.1×10^{-5} s^{-1}, respectively.

The amino acid histidines ligate with zinc in the active center of many zinc-containing enzymes. So, histidine-or imidazole-containing zinc complexes have become the subject of growing interest nowadays.[1-11]The enzymes participate in many important biochemical transformations: alkaline phosphatase,[12-14] phosphotriesterase[15] and phospholipase C[16] hydrolyze the phosphoester; P1 nuclease[17] and polymerase I[18] cleave the phosphodiester backbone of DNA/RNA. The reactions of hydrolysis and cleavage for phosphoester have been established using artificial zinc complexes ;[19-23] but zinc complexes with artificial histidine-containing peptide are still very few.[22]

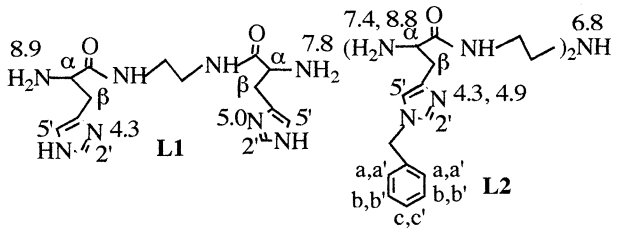
The roles of zinc complex in the above mentioned hydrolysis and cleavage reactions are Lewis acidic nature to stabilize its own phosphate-coordinated intermediate and nucleophile to promote the removal of phenolate from the intermediate.[19, 20]

$$[LZn(OH_2)]^{n+} = [LZn(OH)]^{(n-1)+} + H^+ \quad (1)$$

$$[LZn(OH)]^{(n-1)+} + BNPP \xrightarrow{k_{obsd}} [LZn(O_3POArNO_2]^{(n-2)+} + {}^-OArNO_2 \quad (2)$$

Our aim is to synthesize zinc complexes $[LZn(OH_2)]^{n+}$ of histidine-containing peptide **L** as well as to simulate the catalytic reactions eqs. (1) and (2) on hydrolyses of bis-(p-nitrophenyl)phosphate BNPP and sodium p-nitrophenyl phosphate NPP in aqueous solution. Macrocyclic amine and pyridine have been used as ligations in catalytically active zinc complexes.[19-21,23] From the view of native system, histidine -or imidazole-containing zinc complexes are more interesting.

N,N'-dihistidylethylenediamine **L1** and im-bzl-*N,N''*-dihistidyldiethylenetriamine **L2** were synthesized from ethylenediamine and diethylenetriamine, respectively,[24] as the artificial peptide-ligands by conventional solution phase methods using the racemization free and fragment condensation strategies.



Scheme 1. The numbers show pKa values.

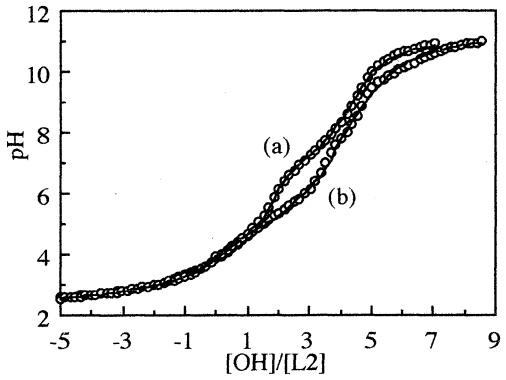


Figure 1. pH titration curves (O observed, −calculated) for 5.7×10^{-4} M **L2** in the presence of 5.7×10^{-3} M HNO_3 at I = 0.1 M $NaNO_3$ and 30 ° C. a) in the absence of zinc and b) in the presence of equimolar zinc.

Their pKa values, which were obtained from the simulation of the observed potentiometric pH titration curves, showed that **L1** and **L2** at pH = 7 have two and three ligating sites for zinc, respectively, as shown in Figure 1 and scheme 1.

The ratio of zinc and ligands for the peptide zinc-complexes was obtained from zinc titrations using ^{1}H NMR technique as a function of R = $[Zn^{2+}]/[L]$, where **L** is **L1** or **L2.** Figure 2 shows that the imidazole protons 2' were shifted to

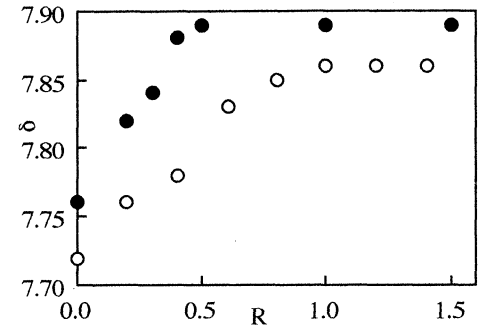


Figure 2. Zinc titration curves of 3.4×10^{-4} M **L1**= (●) and 1.9×10^{-3} M **L2**= (O) as a function of R, at I = 0.1 M $NaNO_3$ in D_2O, at pH 7 and 30 °C using 2'-^{1}H NMR technique.

downfield and two **L1**s bind zinc in a 2:1 complex and a **L2** binds zinc in a 1:1 complex. The simulation of zinc-titration curve concluded that Kst of equimolar zinc-complex with **L2** is at least above 1×10^5 M^{-1}. Among chemical species in equimolar **L2** and zinc solution, their distribution obtained by simulation of pH-titration curve (Figure 1) showed the equimolar zinc-complex with tridentate **L2** as a major species. The distribution of the sum of $[HL2Zn(OH_2)]^{3+}$ and $[H_2L2Zn(OH)]^{3+}$ has a peak around pH = 7: pKa and pKst of $[HL2Zn(OH_2)]^{3+}$ are 8.26 and 6.15, respectively. The zinc complex of **L2** was prepared from $Zn(ClO_4)_2$ and **L2** at pH = 7 was determined as a 1 : 1 zinc complex with **L2** **2**.[25] The steric hindrance of benzyl groups in **L2** prevented the formation of octahedral zinc complex which consists of two **L2**s and the fourth coordination site around zinc is occupied by a water molecule or a hydroxide ion.[10, 11]

The hydrolyses of BNPP (~290 nm), and NPP (~305 nm) were monitored by the UV appearance of p-nitrophenolate (~400 nm). The hydrolysis of BNPP was carried out in aqueous solution including 3.6×10^{-3} M **L2** and equimolar zinc nitrate (R = 1) or **2** at 50 °C and 35 °C, and pH = 7. Under experimental condition of the ratio of BNPP to **2** , 1 : 100, k_{obsd} defined in eq. (2) can be given by

$$\ln [BNPP]_o/[BNPP]_t = k_{obsd}\ t \qquad (3)$$

where k_{obsd} stands for pseudo-first-order rate constant, $[BNPP]_o$ and $[BNPP]_t$ are the initial concentration and the concentration at time t of BNPP, respectively . The observed rate constants are shown in Table 1 . The complex **2** hydrolyzed more efficiently

Table 1. Observed rate constants $10^5 \times k_{obsd} / s^{-1}$

complex	BNPP		NPP	
	50 °C	35 °C	50 °C	35 °C
2	1.1	0.07	2.1	0.10
C1[a]	0.34		0.28	
C2[a]	0.64		0.06	
none	b		b	

[a] C1 and C2 were the zinc complexes of **7** and **9** in ref. 19, and the highest rate constants at 55 °C .
[b] No hydrolysis reaction upto 5 days in this work.

BNPP and NPP compared to Chapman and Breslow's zinc complex.[19] The complex **2** has Lewis acidic nature for coordinated water-molecule enhanced by zinc ion. The 2 : 1 zinc complex with **L1** **1**, free **L1**/**L2**, or zinc(II) shows no hydrolysis effect on BNPP and NPP even after 5 days at 50 °C. Since zinc in **1** is surrounded by two **L1**s, it has no space to bind water molecule as nucleophile which accelerates the hydrolysis reaction . The pH dependence of k_{obsd} for **2** showed a bell-shaped profile around pH = 7. The profile of k_{obsd} was similar to that of pH-dependence of the distribution for the sum of $[HL2Zn(OH_2)]^{3+}$ and $[H_2L2Zn(OH)]^{3+}$. There exist no zinc complexes with coordinated hydroxide -ion under pH ~ 6 and much less zinc complexes with coordinated water-molecule above pH ~ 9. Thus, the catalytic hydrolyses of phosphate esters take place under the cooperative contribution from the above-mentioned coordinated OH^- and H_2O zinc complexes with **L2**: where a coordinated H_2O at one zinc complex may be substituted by BNPP or NPP and a coordinated OH^- at the other acts on its phosphorus as nucleophile. Thus, hydrolysis mechanism may be analogous to double zinc model proposed for the action of alkaline phosphatase.[12-14,19]

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24 Syntheses scheme of **L1** and **L2**:

Nt-BOC-im-bzl-L-His —DCC/HOBT→ (Nt-BOC-im-bzl-L-His)$_2$en

(Nt-BOC-im-bzl-L-His)$_2$en —liq.NH_3/Na, $NH_2(CH_2)_2NH_2$, en→ —CF_3COOH→ **L1**• $4CF_3COOH$

L1 —anion exchange , OH^-→ **L1**

Nt-BOC-im-bzl-L-His —DCC/HOBT→ (Nt-BOC-im-bzl-L-His)$_2$dien

(Nt-BOC-im-bzl-L-His)$_2$dien —$NH_2(CH_2)_2NH(CH_2)_2NH_2$, dien, CF_3COOH→ **L2**• $5CF_3COOH$ —anion exchange, OH^-→ **L2**

All the intermediates were characterized by ^1H NMR, TLC and FAB-mass. Elemental analysis data of $C_{30}H_{39}N_9O$ (=**L2**) •$5CF_3COOH$•H_2O (557.7 + 570.1 + 18.0): Calcd. C 41.9, H 4.1, N 11.0; Found C 42.2, H 4.3, N 11.5. The trifluoroacetic acid salt of **L2** was passed through an anion exchange column (Dowex) with water to obtain **L2** as a colorless liquid. **L1** and **L2** were fully characterized by ^1H NMR. ^1H NMR of **L1** in D_2O: 2.46 (t, 4H, CH_2-CH_2), 2.78(d, 4H, $C_\beta H2$), 3.51(t, 2H, $C_\alpha H$), 7.05(s. 2H, Im H-5') and 7.77(s, 2H, Im H-2').^1H NMR of **L2** in D_2O: 2.34 - 2.44(m,4H, -CH_2 -NHCO), 2.76 (d, 4H, $C_\beta H_2$), 3.03 (t, 4H, -NH-CH_2 -)3.53 (t, 2H, $C_\alpha H$), 4.97 (s, 4H, CH_2-benzyl), 6.73 (s. 2H, Im H-5'), 6.79 - 7.12(m, 4H, C_6H_5 H-a,a'), 7.21 - 7.28(m, 6H, C_6H_5 H-b,b',c,c') and 7.55(s, 2H, Im H-2').

25 Preparation of equimolar zinc complex **2** with **L2**: After the $Zn(ClO_4)_2$•$6H_2O$ was added to **L2,** white precipitate of zinc complex of **L2** was produced, filtered by membrane filter, and washed out by ether. The ratio of zinc and **L2** was determined by atomic absorption with standard zinc-solution and ^1H NMR with known concentration of 3-(trimethylsilyl)-1-propane sulfonate sodium salt, and the concentrations of zinc and L2 in sample solution were 0.75 and1.49 ppm, i.e., 2.52×10^{-6} and 2.67×10^{-6} mmol., respectively.