Synthesis and Reactivity in Inorganic and Metal-Organic Chemistry

ISSN: 0094-5714 (Print) 1532-2440 (Online) Journal homepage: http://www.tandfonline.com/loi/lsrt19

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To cite this article: Yongshu Xie , Qingliang Liu , Xiaolong Xu , Jun Yu & Ruisen Lin (2001) SYNTHESES OF A LINEAR PENTADENTATE LIGAND AND ITS ZINC(II) COMPLEX AND ITS PROMOTED HYDROLYSIS OF 4-NITROPHENYL ACETATE, Synthesis and Reactivity in Inorganic and Metal-Organic Chemistry, 31:2, 195-204, DOI: 10.1081/SIM-100002039

To link to this article: http://dx.doi.org/10.1081/SIM-100002039

SYNTH. REACT. INORG. MET.-ORG. CHEM., 31(2), 195–204 (2001)

SYNTHESES OF A LINEAR PENTADENTATE LIGAND AND ITS ZINC(II) COMPLEX AND ITS PROMOTED HYDROLYSIS OF 4-NITROPHENYL ACETATE

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ABSTRACT

A linear pentadentate ligand, N-(2-hydroxyethyl)-N″-(2-hydroxybenzyl)-diethylenetriamine (HL), and its zinc(II) complex were synthesized and characterized by IR spectra, ^{1}H NMR spectra and elemental analyses. By pH potentiometric titration, protonation constants of HL and stability constants of Zn(II) complexation with HL have been determined at $25 \pm 0.1^\circ$C and $I = 0.1$ mol/L (KNO_3). The kinetics of 4-nitrophenyl acetate (NA) hydrolysis catalyzed by the complex was determined spectrophotometrically at $25 \pm 0.1^\circ$C and $I = 0.1$ mol/L (KNO_3) in CH_3CN (10 % v/v) at pH 7.0–9.04 (50 mmol/L buffers), and the second-order rate constants k_c for NA hydrolysis were obtained. The experimental

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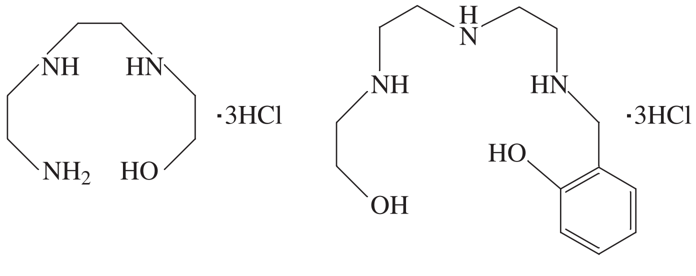
results indicate that Zn···⁻OR (R = alkyl) is a better nucleophile than Zn···⁻OAr (Ar = aryl). A good catalysis was realized with a k_c value of 8.5×10^{-2} $\mathrm{mol^{-1} \cdot L \cdot s^{-1}}$ at pH 9.04.

INTRODUCTION

Hydrolytic enzymes often use external H_2O or internal alcoholic residues (*e.g.* serine) as nucleophiles to react with electrophilic substrates (*e.g.* carboxyesters and phosphates), wherein the prior activation of the nucleophiles and/or electrophiles of the substrates is essential (1). For instance, in alkaline phosphatase (AP) (2), the Zn(II)-bound alcoholic OH (Ser_{102}) deprotonates to yield the good nucleophile Zn···⁻OR which initially attacks the phosphate substrates.

The model studies (3,4) of Zn(II) complexes have indicated that Zn···⁻OAr can also act as nucleophiles to catalyze the hydrolysis of 4-nitrophenyl acetate. The model studies mainly focus on macrocyclic (5–7) and tripodal (8,9) ligands, but there are only a few works dealing with linear ligands (10–13). Herein we report the synthesis of a linear N-(2-hydroxyethyl)-N″-(2-hydroxybenzyl)-diethylenetriamine (HL) as shown in Figure 1. Its protonation and complexation equilibria with Zn(II) were studied by pH titration. Thus, a distribution diagram of the system at various pH values was obtained. The kinetics of the 4-nitrophenyl acetate hydrolysis catalyzed by the complex was investigated spectrophotometrically. Values of the rate constants (k_c) of the catalyzed hydrolysis reaction were obtained. Since HL contains both phenoxy and alkoxy groups, different nucleophiles, Zn···⁻OAr and Zn···⁻OR, may be yielded upon coordination with a Zn(II) ion. By comparing the k_c values with the distribution diagram, the relative nucleophility and the catalytic capacity for the various nucleophiles may be found. Based on these results, the mechanism of the hydrolysis reaction is discussed.



(L^1·3HCl) (HL·3HCl)

Figure 1. The structures of $L^1 \cdot 3HCl$ and $HL \cdot 3HCl$.

EXPERIMENTAL

Materials, Instruments, and pH Titration

N-(2-Hydroxyethyl)diethylenetriamine trihydrochloride ($L^1 \cdot 3HCl$) (Fig. 1) was prepared by a modified literature method (14). The other reagents used were of analytical grade. The buffers, MES (4-morpholinoethanesulfonic acid, Fluka) and HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, FARCO), were commercially available and used without further purification.

^1H NMR spectra were recorded on a Bruker AM-200 spectrometer. IR spectra were recorded on a Nicolet 170SXFT spectrophotometer (as KBr discs) and microanalyses of C, H and N were determined by a Perkin-Elmer 240C elemental analyzer.

Preparation of $L^1 \cdot 3HCl$

2-Chloroethanol (48.3 g, 0.60 mol), diethylenetriamine (61.9 g, 0.60 mol) and H_2O (60 mL) were added successively with stirring. The mixture was refluxed for 8 h, and then cooled to room temperature. Methanol (75 mL) and K_2CO_3 (51 g, 0.37 mol) were added to the reaction mixture and the resulted precipitate of KCl was filtered off. After the filtrate was freed of methanol, it was distilled under reduced pressure, bp 153–155°C (3 mm Hg). The trihydrochloride was obtained upon bubbling dry HCl into a CH_2Cl_2 solution of L^1, yield 8.3 g (54%), mp 172–174°C. *Anal.* Calcd. for $C_6H_{17}N_3O \cdot 3HCl$ ($M = 256.60$): C, 28.07; H, 7.86; N, 16.38%. Found: C, 27.84; H, 7.70; N, 16.08%. IR (cm^{-1}, KBr): 3432 br (ν (OH)); 2799 m ($\delta(NH_2)$), 1626 m, 1454 m ($\delta(NH_2)$); 1066 w (ν(C–N)); 1025 w (ν(C–O)). ^1H NMR (ppm, δ (D_2O), TMS): 3.80 (2H, m, $C\underline{H}_2OH$); 3.38 (8H, m, $(C\underline{H}_2)_2NH(C\underline{H}_2)_2$); 3.22 ($^2H$, m, $NHC\underline{H}_2$).

Preparation of $HL \cdot 3HCl$

To a solution of N-(2-hydroxyethyl)diethylenetriamine trihydrochloride ($L^1 \cdot 3HCl$) (5.13 g, 0.02 mol) in ethanol (200 mL), salicylaldehyde (2.44 g, 0.02 mol) in ethanol (100 mL) was added dropwise with stirring. After stirring for *ca.* 2 h at room temperature, $NaBH_4$ (0.76 g, 0.02 mol) in ethanol (100 mL) was added. After stirring for another 2 h, HCl (5 mL, 6 M) was added to destroy excessive $NaBH_4$, and then the pH of the solution was adjusted to *ca.* 8. After it was evaporated effectively to dryness *in vacuo*, the residue was dissolved in H_2O (50 mL) and extracted five times with $CHCl_3$ (50 mL). The organic extracts were combined, washed with saturated Na_2CO_3 aqueous solution (50 mL), and then dried over Na_2SO_4. Na_2SO_4 used was filtered off; the filtrate was evaporated to dryness *in vacuo* and the product was converted into its trihydrochloride as mentioned above, yield 5.4 g (74%), mp 233–234°C. *Anal.* Calcd. for $C_{13}H_{23}N_3O_2 \cdot 3HCl$ (M = 362.73): C, 43.03; H, 7.23; N, 11.59%. Found: C, 42.95; H, 7.24; N, 11.45%. IR (cm^{-1}, KBr): 3399 br (ν(OH)); 2783 m (δ(NH2)); 1266 m (ν(C–O)); 1072 m (ν(C–N)); 1600 s, 762 s (δ(Ar-H)). ^1H NMR (ppm, δ (D_2O), TMS): 6.88–7.29 (4H, m, $\underline{\mathrm{Ar}}$); 4.22 (2H, s, $ArC\underline{H}_2$); 3.77 (2H, m, $C\underline{H}_2OH$); 3.44 (8H, s, $NH(C\underline{H}_2)_2$); 3.20 (2H, m, $NHC\underline{H}_2$).

Preparation of Zn(II) Complex with HL

$HL \cdot 3HCl$ (0.36 g, 1.00 mmol) suspended in methanol (50 mL) was added to $Zn(ClO_4)_2 \cdot 6H_2O$ (0.37 g, 1.00 mmol) in methanol (50 mL), and then an aqueous NaOH solution (1 mL, 5 M) was added to the reaction solution. After the mixture was refluxed for 2 h, most of the methanol was removed by a rotary evaporator to produce a white solid. It was filtered and washed successively with water, methanol and diethyl ether, and then dried *in vacuo*, yield 0.26 g (82%), d.p. 211–213°C. *Anal.* Calcd. for $C_{13}H_{21}N_3O_2Zn$ (M = 316.71): C, 49.30; H, 6.68; N, 13.27%. Found: C, 48.94; H, 6.54; N, 12.97%. IR (cm^{-1}, KBr): 3287 s (ν(N–H)); 3251 m (ν(N–H)); 1277 m (ν(C–O)); 1083 m (ν(C–N)); 1598 m, 758 s (δ(Ar)); 527 m (ν(Zn–O)); 467 m (ν(Zn–N)).

pH Potentiometric Titration

Standardization of KOH, HNO_3 and $Zn(NO_3)_2$ solutions and procedures of potentiometric pH-titration together with the nonlinear fitting program used to calculate the protonation and Zn(II) complexation constants employed the same methods as reported (3). Protonation equilibria were studied at an ionic strength of 0.10 M KNO_3 and at the ligand concentration 1.0×10^{-3} M at 25°C. The complex formation was studied by titrating 1.0×10^{-3} M ligand and equimolar $Zn(NO_3)_2$ at 25°C with 0.1314 M standardized KOH solution.

Kinetics

A kinetics study was carried out on a Shimadzu UV-240 spectrophotometer equipped with a thermostated cell (298 ± 0.1 K). The hydrolysis rate of 4-nitrophenyl acetate was measured by an initial slope method following the increase of the absorption at 400 nm of the released 4-nitrophenolate. The reaction solution was maintained at 298 ± 0.1 K and the ionic strength was adjusted to 0.10 with KNO_3. Buffers (50 mmol/L) were used to maintain the pH (HEPES 7–8, MES 8–9). To obtain a homogeneous solution, $CH_3CN–H_2O$ (10% v/v) was used as a solvent. For the initial rate determination, the following typical procedure was employed. After 4-nitrophenyl acetate and the complex solutions at a certain pH (with the reference solution containing no complex) were mixed, the UV absorption at 400 nm was recorded immediately at 5 sec intervals. The initial slope ($<5\%$ conversion) of a plot of the absorbance *vs*. time was determined (linear correlation coefficient >0.99). All experiments were run in triplicate and the average value of the parallel experiments was obtained as the result.

RESULTS AND DISCUSSION

Protonation and Complexation

Figure 2 A illustrates the titration curve for HL. The value of a is the ratio of moles of base to the moles of HL.

In the titrating pH range, four protonation equilibria can be seen corresponding to the phenolate and the three amino groups. The conjugate base of HL with the phenol deprotonated can be symbolized as L^-. The protonation constants β_{01h} and K_{01h} are defined as following:

$$L^- + hH^+ \rightleftharpoons H_hL^{h-1} \quad \beta_{01h} = [H_hL^{h-1}]/([L^-][H^+]^h) \qquad (h = 1, 2, 3 \text{ and } 4) \quad (1)$$

$$H_hL^{h-1} \rightleftharpoons H_{h-1}L^{h-2} + H^+ \quad K_{01h} = [H^+][H_{h-1}\ L^{h-2}]/[H_hL^{h-1}] \qquad (h = 1, 2, 3 \text{ and } 4) \quad (2)$$

The values obtained for $\log\beta_{010} - \log\beta_{014}$ are 10.55(2), 19.78(3), 27.80(3) and 31.86(5), respectively. The $pK_{011} - pK_{014}$ values are 10.55, 9.23, 8.02 and 4.06,

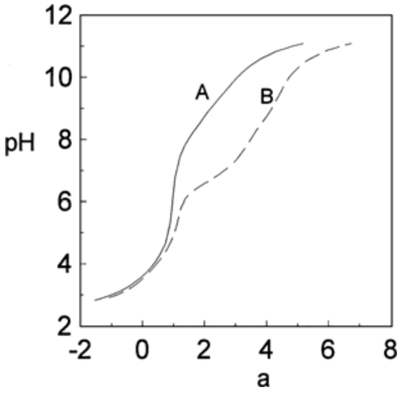


Figure 2. Titration curves for $HL \cdot 3HCl$ and its Zn(II) complex A) 1 mmol/L $HL \cdot 3HCl$; B) A + 1 mmol/L $Zn(NO_3)_2$.

respectively, where the first value corresponds to protonation of the phenolate and the next three correspond to that of the amino nitrogens.

Curve B in Figure 2 shows the titration curve for the complexation of HL with equimolar $Zn(NO_3)_2$. From the tiration curve it was shown that the complex began to form at pH 4. Five protons are dissociated upon coordination with Zn^{2+} and the data were treated as the following equation:

$$Zn^{2+} + L^- + hH^+ \rightleftharpoons ZnH_hL^{h+1} \quad \beta_{11n} = [ZnH_hL^{h+1}]/([Zn^{2+}][L^-][H^+]^h) \tag{3}$$

The values obtained for log β_{111}, log β_{110} and log β_{11-1} are 17.88(8), 11.89 (3) and 2.72(6), respectively. Upon coordination, the phenol and alkoxyl groups are activated and deprotonate, as represented by the following equations:

$$ZnHL^{2+} \rightleftharpoons ZnL^+ + H^+ \qquad K_{a_1} = [ZnL^+][H^+]/[ZnHL^{2+}] = \beta_{110}/\beta_{111} \tag{4}$$

$$ZnL^+ \rightleftharpoons ZnH_{-1}L + H^+ \qquad K_{a_2} = [ZnH_{-1}L][H^+]/[ZnL^+] = \beta_{11-1}/\beta_{110} \tag{5}$$

where $ZnH_{-1}L$ denotes that the coordinated alcoholic OH deprotonates to yield $Zn(II)\cdots{}^{-}OR$. The distribution diagram for this system as a function of pH is displayed in Figure 3. The obtained values of pK_{a_1} and pK_{a_2} are 5.99 and 9.17, respectively.

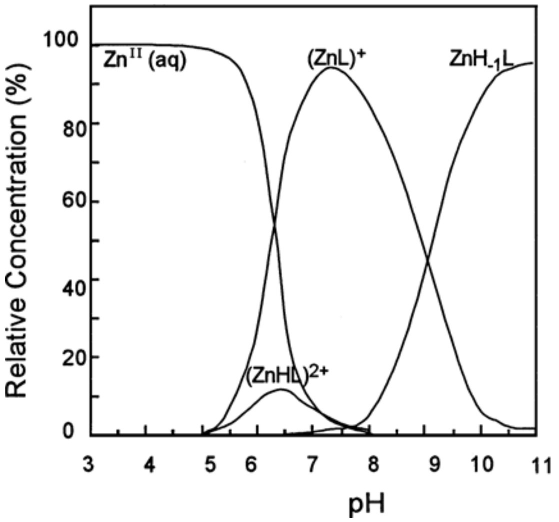
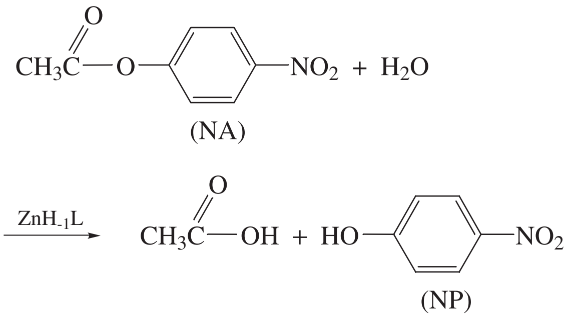


Figure 3. Distribution curves of Zn(II) complexes of HL · 3HCl at 25 ± 0.1°C, I = 0.10 mol/L KNO_3.

Kinetics

The hydrolysis process may be represented as the following equation:



$$\upsilon = -\mathrm{d[NA]/dt} = \mathrm{d[NP]/dt} = \mathrm{dA}/\varepsilon \mathrm{dt}$$

where A and ε represent optical density and molar extinction coefficient, respectively. The following kinetic equation is obtained by a method similar to the literature (3):

$$\upsilon = (k_c[\mathrm{Zn(II)}] + k_{\mathrm{OH}}[\mathrm{OH}^-])[\mathrm{NA}] + k_0$$

where k_c, k_{OH} and k_0 are the catalytic rate constants of the zinc(II) complex, OH^- and water, respectively. The obtained k_{OH} and k_0 values are:

$$k_0 = 1.14 \times 10^{-5} s^{-1}, \quad k_{\mathrm{OH}} = 7.85\,\mathrm{M}^{-1} \cdot \mathrm{s}^{-1}.$$

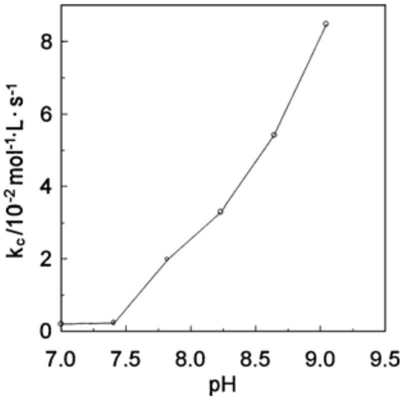


Figure 4. Rate-pH profile for the second-order rate constants of NA hydrolysis catalyzed by Zn(II) complexes of HL · 3HCl at 25 ± 0.1°C, and I = 0.01 mol/L $\mathrm{KNO_3}$ in $\mathrm{CH_3CN}$ (10% v/v).

The k_{OH} value is nearly the same as the literature value (3). When k_c is plotted versus pH (Fig. 4), it is not a typical sigmoid curve.

As is shown in Figure 3, $[(ZnL)^+]$ increases with the increase of pH until it reaches a maximum at pH 7.5, and then it begins to decease. On the other hand, $[ZnH_{-1}L]$ increases continuously. k_c has the same trend as $[ZnH_{-1}L]$, indicating that the catalysis is predominantly contributed not by $(ZnL)^+$ but by $ZnH_{-1}L$. It is indicative of a kinetic process controlled by an acid-base equilibrium (due to the deprotonation of the alkoxyl proton).

The main differences between $(ZnL)^+$ and $ZnH_{-1}L$ is summarized as following: the phenol in $(ZnL)^+$ coordinates to Zn(II), yielding $Zn\cdots{}^-OAr$, and the coordinated phenol deprotonates with a pK_a value of 5.99, while the alkoxyl in $ZnH_{-1}L$ coordinates more weakly to Zn(II), yielding a nucleophile, $Zn\cdots{}^-OR$. Similar to that of alcohol-pendant [12]aneN_3^{5}, the mechanism of the hydrolysis of NA ester catalyzed by $ZnH_{-1}L$ may be tentatively proposed as following:

The nucleophile $Zn\cdots{}^-OR$ attacks the carbonyl of the NA ester, liberating a 4-nitrophenolate anion and yielding an acetyl intermediate A. The previously coordinated alkoxyl has combined with the acetyl, leaving a vacant site on Zn(II) ion, which is then occupied by a water molecule, yielding $Zn\cdots{}^-OH$. Thereafter, $Zn\cdots{}^-OH$ acts as a nucleophile to attack the carbonyl of the acetyl group, completing the catalytic cycle. As shown in Figure 4, k_c reaches $8.5 \times$

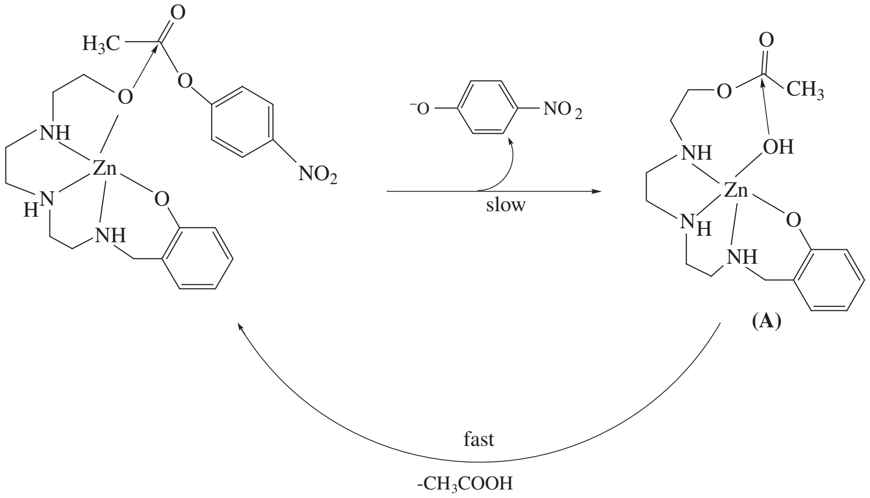


Figure 5. Proposed mechanism of NA hydrolysis catalysed by $ZnH_{-1}L$.

10^{-2} $mol^{-1} \cdot L \cdot s^{-1}$ at pH 9.04, suggesting good catalysis contributed by $Zn\cdots{}^{-}OR$ (pK_a = 9.17). Similarly, in alkaline phosphatase (AP), the Zn(II)-bound alkoxyl of serine residue was activated to deprotonate (pK_a *ca.* 7.5), yielding the good nucleophile $Zn\cdots{}^{-}OR$, which attacks the phosphate substrates. As described above, $(ZnL)^+$ is a much poorer catalyst than $ZnH_{-1}L$, and, therefore, it is reasonable to say that $Zn\cdots{}^{-}OAr$ is a poorer nucleophile than $Zn\cdots{}^{-}OR$. This is probably related to the difficulty for the strongly coordinated ^{-}OAr to leave the Zn(II) ion and to attack the substrate. As seen from the above results, the nucleophiles originate from the deprotonation of suitable groups coordinated to Zn(II). The pK_a value is essential to its catalysis. It must not be too low, otherwise the nucleophile generated may be too weak to give good catalysis. On the other hand, if the pK_a value is too large, the concentration of the nucleophile may be too low, and also only poor catalysis will be obtained. This subtle relationship between the strength of coordination (*i.e.* pK_a value) and its catalysis must be considered in designing the complexes mimicking the structures and activities of metallohydrolases.

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Received January 5, 2000
Accepted August 31, 2000

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