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Comparative studies on hydrolysis of bis(*p*-nitrophenyl) phosphate catalyzed by short- and long-alkyl-multiamine metallomicelles

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ABSTRACT

Four short- and long-alkyl-multiamine ligands L^1–L^4 have been synthesized and characterized. The catalytic efficiency of complex CuL^1 and functional metallomicelles CuL^2–CuL^4 were comparatively investigated for the hydrolysis of bis(*p*-nitrophenyl) phosphate (BNPP) in buffered solution at 30 °C. The ternary kinetic model for metallomicellar catalysis was suggested to analyze the experimental data. The kinetic and thermodynamic parameters k'_N, K_T and pK_a were obtained. The results indicated that the complexes with 1:1 ratio of ligands L^2–L^4 to copper(II) ion were the kinetic active catalysts, and the deprotonized Cu(II) complex formed by activated water molecule was the real active species for BNPP catalytic hydrolysis. The real rate constant of the reaction catalyzed by CuL^1–CuL^4 was 4.00×10^{-6}, 7.44×10^{-5}, 1.42×10^{-4} and $4.10 \times 10^{-4}\ s^{-1}$, respectively. The effects of ligand and microenvironment on the hydrolytic reaction of BNPP have been discussed in detail.

1. Introduction

In live bodies, phosphodiester bond has close relation to information store and conduction, energy transfer and protein phosphoacylation. The mechanisms of phosphodiester bond hydrolysis, both enzymatic and nonenzymatic catalysis, have been a subject of continuing interest to chemists and biologists. In general the hydrolysis of phosphodiester bond is very difficult. The spontaneous first-order rate constants for the hydrolysis of a phosphodiester bond under physiological conditions have been estimated to be 1×10^{-10} and $1 \times 10^{-11}\ s^{-1}$ for single-strand and double-strand DNA, respectively [1–3]. In last decades, a variety of organic and inorganic catalysts for the hydrolysis of phosphoesters were designed, and the catalytic mechanisms were studied. These studies showed that metal ions combined with different ligands could act as catalysts for the hydrolysis of DNA and RNA. Most of those focus on the studies of the catalytic function of metal ions and metal complexes [4–10]. As new catalytic systems, metallomicelles have attracted considerable interest for recent years. The micellar medium promotes the electrophilicity of metal ions toward micellar-bound substrates in the positively charged stern layer and the metallomicellar function group mimics the structure of the metalloenzymatic active center and the hydrophobic microenvironment [11–15].

In previous studies, we found that the hydrophobic microenvironment of micelle played an important role in activating substrates and H_2O, as well as in stabilizing the intermediate, and therefore, change the catalytic activity of catalysts [16–18]. In order to better clarify the influence of hydrophobic microenvironment on the hydrolysis of bis(*p*-nitrophenyl) phosphate (BNPP), we synthesized four different length alkyl-multiamine ligands. These functional metallomicellar systems showed excellent catalytic activity on the hydrolysis of phosphate diester.

2. Experimental

2.1. Instruments

^1H spectra were obtained on a Brucker DPX-300FXNMR spectrometer. Mass spectrometry was performed using a Finnigan MAT 45001 mass spectrometer. Elemental analyses were performed with a Carlo Erba 1106 instrument. Infrared spectra were performed by a PE983IFS FIOIP spectrometer. Critical micellar concentration (cmc) was measured by model DDS-307 conductometer instruction (Shanghai Precision & Scientific Instrument CO., Ltd.). Kinetic studies were carried out by UV–vis methods with a TU-1901 UV–vis spectrophotometer equipped with a thermostatic cell holder (Beijing Purkinje General Instrument CO., Ltd.).

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2.2. Materials

$Cu(NO_3)_2·6H_2O$, $CuSO_4·5H_2O$, K_2CO_3, KNO_3, Na, Na_2SO_4, chloroform, diethyl malonate, absolute ethanol, 1-bromohexadecane, 1-bromobutane, 1-bromooctane, ethylenediamine purchased from Kelong Corp., BNPP purchased from Sigma Corp., were analytical grade commercial products. BNPP stock solution for kinetics was prepared in distilled water. Tris(hydroxymethyl) aminomethane (Tris) buffer was used in all cases and its pH was adjusted by adding analytically pure nitric acid.

2.3. Synthesis of ligands and Cu(II) complex

2.3.1. 6-Hexadecyl-1,4,8, 11-tetraazaundecane-5,7-dione (**L⁴**)

After dissolving 2.3 g (0.1 mol) of Na in 250 ml of anhydrous EtOH, 16 g (0.1 mol) of diethyl malonate were added dropwise under argon. The solution was stirred at 50 °C for 1 h. Then 30.6 g (0.1 mol) of 1-bromohexadecane was added and the mixture was stirred at 50 °C for 15 h. The solution was concentrated to dryness and washed with hot $CHCl_3$. The precipitate of NaBr was filtered and the solution was dried over Na_2SO_4. After evaporation, an yellow oil was obtained and distilled to give 24 g (62%) of $\mathbf{L^{4a}}$.

4.25 g of $\mathbf{L^{4a}}$ was added dropwise to 1.32 g of ethylenediamine under argon. The mixture was stirred at room temperature for 3 days. After concentration, a white precipitate was obtained, washed by water and ethyl acetate to remove traces of unreacted ethylenediamine and ester. 3.50 g (77%) of $\mathbf{L^4}$ was obtained.

The syntheses of *6-butyl-1,4,8,11-tetraazaundecane-5,7-dione* ($\mathbf{L^2}$) and *6-octyl-1,4,8,11-tetraazaundecane-5,7-dione* ($\mathbf{L^3}$) were similar to that of $\mathbf{L^4}$ [19].

2.3.2. N,N′-Di-(2-aminoethyl) malondiamide ($\mathbf{L^1}$)

Diethyl malonate (10 ml) was added slowly to ethylenediamine (40 ml) at 0 °C. The liquids were thoroughly mixed, kept at 0 °C for 1 h, and left for 2 days at room temperature. The excess of ethylenediamine and methanol was removed under reduced pressure, leaving a pale pink solid which was washed with about 20 ml of cold absolute ethanol, filtered, and dried (10 g).

Complex Cu $[CH_2(CONCH_2CH_2NH_2)_2]·H_2O$: the diamine (1.10 g) was dissolved in water (15 ml), and to the stirred solution, $CuSO_4·5H_2O$ (0.71 g) dissolved in water (4 ml) was added dropwise. As each drop entered the solution, a blue precipitate formed which gradually dissolved to give a deep red-purple solution again. The solution was stirred for a further 1 h, and then the solvent was removed under reduced pressure until about 4 ml of solution and purple plate-like crystals remained. The crystals were filtered off, washed with a little water, and dried (0.63 g, 82% based on Cu) (found: C, 31.4; H, 5.8; N, 21.05. $C_7H_{16}CuN_4O_3$ requires C, 31.4; H, 6.0; N, 20.9%) [20].

6-Hexadecyl-1,4,8,11-tetraazaundecane-5,7-dione($\mathbf{L^4}$): mp, 120 °C. Ir (KBr): $\upsilon_{CO} = 1660\ cm^{-1}$. ^1H NMR ($CDCl_3$) δ_H: 0.85 (t, 3H, CH_3, $J = 6$ Hz); 1.220 (s, 30H, $(CH_2)_{15}$); 1.81 (m, 4H, $(NH_2)_2$); 2.95 (t, 1H, H_α, $J = 6$ Hz); 2.79 (t, 4H, $(CH_2\ NH_2)_2$, $J = 6$ Hz); 3.26 (t, 4H, $CONHCH_2$, $J = 6$ Hz); 7.23 (m, 2H, $(NH)_2$).

6-Octyl-1,4,8,11-tetraazaundecane-5,7-dione ($\mathbf{L^3}$): mp, 112 °C. Ir (KBr): $\upsilon_{CO} = 1660\ cm^{-1}$. ^1H NMR (DMSO-d_6) δ_H: 0.86 (t, 3H, CH_3, $J = 6$ Hz); 1.23 (s, 12H, $(CH_2)_6$); 1.66 (m, 2H, $CH_{2\beta}$); 2.50 (m, 4H, $(NH_2)_2$); 2.63 (m, 4H, $(CH_2NH_2)_2$, $J = 6$ Hz); 3.10 (m, 5H, $(CONHCH_2)_2$ and $CH_{\underline{\alpha}}$); 7.88 (t, 2H, $(NH)_2$, $J = 6$ Hz).

6-Butyl-1,4,8,11-tetraazaundecane-5,7-dione ($\mathbf{L^2}$): mp, 110 °C. Ir (KBr): $\upsilon_{CO} = 1660\ cm^{-1}$. ^1H NMR (DMSO-d_6) δ_H: 0.90 (t, 3H, CH_3, $J = 6$ Hz); 1.25 (m, 4H, $(CH_2)_2$); 1.70 (m, 2H, $CH_{2\beta}$); 2.07 (m, 4H, $(NH_2)_2$); 2.60 (t, 4H, $(CH_2NH_2)_2$, $J = 6$ Hz); 3.10 (m, 5H, $(CONHCH_2)_2$ and CH_α); 7.95 (m, 2H, $(NH)_2$).

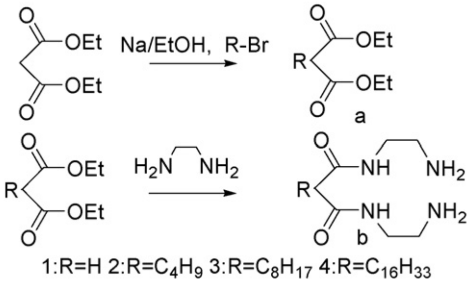
2.4. Kinetics

Kinetic measurements were made spectrophotometrically at 30 °C and different pH values, employing a TU-1901 UV–vis spectrophotometer with a thermostatic cell compartment. Reactions were initiated by injecting 30 μl of a $0.005\ mol\ L^{-1}$ stock solution of BNPP into 3 ml of buffer solution containing the desired reagents. The reaction rate was followed by monitoring the liberation of *p*-nitrophenol at 400 nm under the conditions of excess catalyst over substrate (at least 10-folds). The apparent first-order rate constants k_{obsd} were obtained by least-square method using $\ln(A_\infty - A_t) - \ln(A_\infty - A_0) = -k_{obsd}t$. Kinetic runs, carried out in triplicate, gave rate constants with uncertainly of less than 3%.

3. Results and discussion

3.1. Apparent rate constants of hydrolysis of BNPP at pH 7.50 and 30 °C

Ligands L^1–L^4 were synthesized according to the procedures outlined in Scheme 1. Ligands L^1 and L^2 were water-soluble, ligands L^3 and L^4 were hardly water-soluble, however, their complexes of Cu^{2+} were soluble in buffered solution. Table 1 shows the pseudo-first-order rate constants of BNPP hydrolysis at pH 7.50 and 30 °C. From Table 1 it can be found that three metallomicelle systems display significant catalytic activity for the hydrolysis of BNPP. Compared with the spontaneous hydrolysis rate constant of BNPP (by P–O cleavage) $k = 1.1 \times 10^{-11}\ s^{-1}$ [8], the Cu^{2+} ion and the metal complex CuL^1 show certain rate-enhancing effect. Rate acceleration can be clearly found in the metallomicelle systems. And an interesting phenomenon can be discovered that k_{obsd} increased with the increasing length of alkyl R. The most remarkable rate acceleration was observed in CuL^4-catalyzed system. Compared with the spontaneous hydrolysis of BNPP, the k_{obsd} values increase by 2.82×10^5, 3.21×10^6, 3.46×10^6 and 2.94×10^7-fold for ligands L^1–L^4, respectively. The order of catalytic activity is $CuL^4 > CuL^3 > CuL^2 \gg CuL^1$, which shows that the catalytic efficiency changes with the length of hydrocarbon chain of ligands for the same active center, which can be rationalized by the following kinetic and thermodynamic



Scheme 1. The synthesis of ligands.

Table 1
Apparent first-order rate constants of BNPP hydrolysis catalyzed by metallomicelles pH 7.50 and 30 °C[a]

Systems	10^5k_{obsd} (s^{-1})
Buffer + Cu^{2+}	0.12
CuL^1	0.31
CuL^2	3.53
CuL^3	3.81
CuL^4	32.37

[a] In $0.01\ mol\ L^{-1}$ Tris–HNO_3 buffer, $\mu = 0.1$ (KNO_3); [BNPP] = $5 \times 10^{-5}\ mol\ L^{-1}$; [ligand] = [$Cu^{2+}$] = $5 \times 10^{-3}\ mol\ L^{-1}$.