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Carboxy and diphosphate ester hydrolysis promoted by di- or tri-nuclear zinc(II) complexes based on β-cyclodextrin

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ABSTRACT

A new ligand (L), 6-mono-(2-(2-hydroxy-3-(hydroxymethyl)-5-methyl benzylamino)-ethylamino)-β-cyclodextrin, based on β-cyclodextrin derivatives with dinucleating units was synthesized and used to prepare a trimetallic bis-ligands zinc complex ($Zn_3(L^{2-})_2$). The esterase activity of the complex was investigated by the hydrolysis of two carboxylic acid esters, bis(4-nitrophenyl)carbonate (BNPC) and 4-nitrophenyl acetate (NA), and a DNA model bis(4-nitrophenyl)phosphate (BNPP) as a phosphate ester. The catalytic rate for BNPC was very high, which was found to be a 5.63×10^3-fold rate enhancement over uncatalyzed hydrolysis and 1.62×10^2-fold rate enhancement over uncatalyzed hydrolysis for NA hydrolysis at pH = 7.0. For the catalytic hydrolysis of BNPP, the initial first-order rate constant of 0.1 mM catalyst was 5.85×10^{-8} s^{-1} at pH = 8.50 and 35 °C, which is a 731-fold acceleration over uncatalyzed hydrolysis. The second rate constant (k_{BNPP}) was found to be 1.22×10^{-3} M^{-1} s^{-1} at pH = 10.0. According to the potentiometric titration study, the zinc complex exists in a dinuclear single ligand coordinated mode and a trinuclear bis-ligands system at pH ≥ 7.0. The ester hydrolysis activity was attributed to the cooperative interaction of the two metal centers and the hydrophobic cavity of β-cyclodextrin with substrates.

1. Introduction

Many metalloenzymes such as phospholipase C, nuclease P1 and exonuclease site of DNA polymerase use two or three metal ions in the active site to catalyze the hydrolytic cleavage of phosphate diester bonds in nucleotides (e.g., RNA and DNA). The metal ions act cooperatively as Lewis acid sites in the activation of a nucleophile and the substrate, and in the stabilization of the pentacoordinate phosphorus transition state and the leaving group. In the last two decades, many model systems for small molecule dinuclear or trinuclear hydrolytic metalloenzymes have been designed and studied for the hydrolysis of carboxylate esters and phosphate esters. Examples include macrocyclic polyamine dinuclear [1–7] and trinuclear metal complexes [8,9], rigid pyridine [10] or pyrazol [11–14] bridged polyamine ligands dinuclear metal complexes. Reinhoudt and co-workers reported synthetic dinuclear and trinuclear metallophosphodiesterases based on calix[4]arenes [15–18], which exhibited a very high catalytic activity in the transesterification of the RNA model substrate 2-hydroxypropyl-*p*-nitrophenyl phosphate. However, the complexes based on calix[4]arenas are not active in the hydrolysis of the phosphate triester diethyl *p*-nitrophenyl phosphate (DEPNP), the diester EPNP and the monoester *p*-nitrophenyl phosphate (PNP). Metalloenzyme models of mononuclear complexes based on cyclodextrin (CD) dimers exhibit high hydrolytic activities due to the cooperative binding of two β-CD cavities with the substrates. For example, Breslow and Zhang synthesized metallo-bisCDs bridged by a bipyridine unit with a N,N′-bidentate ligand as metallohydrolase models, which remarkably accelerated catalytic hydrolysis of carboxylic acid diesters and phosphate diesters [19,20]. β-Cyclodextrin dimer linked by telluroxides prepared by Liu et al. [21] exhibited good catalytic hydrolysis activity of carboxylic acid diesters.

We previously reported the zinc complexes of β-CD dimers, either linked by phenanthroline with tetradentate N_4 or pyridine with tridentate N_3 ligands, which demonstrated satisfactory activities for diester hydrolysis [22,23]. However, changing the bridge ligands from tetradentate to tridentate had no obvious rate enhancement in esters hydrolysis. Research on the hydrolytic activity influenced by poly nuclear metal complexes based on cyclodextrins will aid the design of highly active artificial hydrolase. Therefore, we designed and synthesized a dinucleating ligand based on a cyclodextrin, and prepared its trinuclear bis-ligand

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zinc complex. We also investigated the hydrolytic activities of this zinc complex affected by the cooperative action between metal centers and cooperative binding of two β-CD cavities with substrates. The investigation of esterase activity of the zinc complex was performed to promote hydrolysis of the carboxylic acid esters, bis(4-nitrophenyl)carbonate (BNPC) and 4-nitrophenyl acetate (NA), and phosphate ester, bis(4-nitrophenyl)phosphate (BNPP).

2. Experimental

2.1. Materials

2-Hydroxy-5-methylisophthalaldehyde, NA, BNPC, and BNPP were purchased from Sigma Aldrich. Ethylene diamine was purchased from Acros. β-CD (reagent grade) was recrystallized twice from H_2O and dried in vacuo for 12 h at 100 °C. DMF was dried over CaH_2 for 2 days and then distilled under reduced pressure prior to use. Common organic reagents were reagent grade and redistilled before use. 6-Mono(*p*-toluenesulfonyl)-β-cyclodextrin was synthesized by a literature procedure [24]. Water used in all physical measurement experiments was Milli-Q grade. All compounds were confirmed by elemental analyses, ESI-MS and ^{1}H NMR spectroscopy.

2.2. Instrumentation

^{1}H NMR spectra were recorded on a Varian INOVA-300NB or Mercury plus 300 spectrometers. Elemental analyses were performed on a Perkin-Elemer 240 elemental analyzer. ESI-MS spectra were performed on a Thremo LCQ-DECA-XP spectrometer. UV–vis spectra were obtained on a Varian Cary 300 UV/Vis spectrophotometer equipped with a temperature controller (±0.1 °C).

2.3. Synthesis

2.3.1. Preparation of 6-mono-(N-aminoethyl) amino-β-cyclodextrin

A solution of 6-mono(*p*-toluenesulfonyl)-β-cyclodextrin (4.000 g, 3.10 mmol) in dry DMF (12 mL) was added to 10 mL ethylene diamine with stirring. The mixture was heated to 65 °C for 3 h under argon atmosphere. After cooling to room temperature, excess ethylene diamine was removed by evaporation under reduced pressure, and then 400 mL acetone was poured into the resulting residue to give a white precipitate. The crude product was recrystallized twice from H_2O to give the pure compound (3.898 g) in 98.4% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 5.70–5.69 (m, 14H; OH-2,3), 4.82–4.81 (m, 7H; H-1), 4.45–4.44 (m, 6H, OH-6), 3.31–3.62 (m, 46H; H-2,3,4,5,6; CH_2), MS (ESI, H_2O/CH_3OH): m/z: calcd: 1177.3 $[M+H]^+$; found: 1177.6; elemental analysis calcd (%) for $C_{44}H_{76}N_2O_{34}\cdot 7H_2O$: C, 40.55; H, 6.96; N, 2.15. Found: C, 40.69; H, 6.93; N, 2.22.

2.3.2. Preparation of 6-mono-(2-(2-hydroxy-3-(hydroxymethyl)-5-methylbenzylamino)-ethylamino)-β-cyclodextrin (L)

A solution of 2-hydroxy-5-methylisophthalaldehyde (0.210 g, 1.30 mmol) in 5 mL dry DMF was added to the solution of 6-mono-(N-aminoethyl) amino-β-cyclodextrin (1.521 g, 1.29 mmol) in 20 mL dry DMF and methanol with stirring. The mixture was heated to 50 °C for 48 h under argon. After cooling to room temperature, the mixture was chilled in an ice-water bath and added with $NaBH_4$ (0.284 g, 7.51 mmol), then allowed to slowly warm to room temperature and stirred overnight. A small amount of hot water was then added to the mixture and filtered. The filtrate was evaporated to dryness under reduced pressure. The resulting residue was dissolved in a small amount of hot water and poured into acetone (200 mL) to give a light-yellow precipitate. The crude product was dried and purified by column chromatography over Sephadex G-25 and eluted with distilled deionized water to give 0.450 g (26.3%) of the pure compound as white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 6.78–6.74 (m, 2H; phenyl-H), 6.58–6.61 (brs, 1H; phenyl-OH), 5.69–5.68 (m, 14H; OH-2,3), 4.82 (m, 9H; H-1, hydroxymethylene-CH_2), 4.56–4.46 (m, 6H; OH-6), 3.63 (m, 30H; H-3,5,6, pyridine–CH_2, methylene–CH_2), 3.36 (m, 18H; H-2,4, methylene–CH_2), 2.16(s, 3H; methyl–CH_3), 1.07 (brs, 2H; NH, CH_2OH). MS (ESI, H_2O/CH_3OH): m/z: calcd: 1327.3 $[L+H]^+$, 1349.3 $[L+Na]^+$; found: 1327.6, 1349.3; elemental analysis calcd (%) for $C_{53}H_{86}N_2O_{36}\cdot 10H_2O$: C, 41.98; H, 7.11; N, 1.85. Found: C, 41.57; H, 6.60; N, 2.08.

2.3.3. Preparation of trinuclear zinc complex ($Zn_3(L^{2-})_2$)

A solution of L (0.047 g, 0.035 mmol) in water (2 mL) was added dropwise to a dilute aqueous solution of $Zn(ClO_4)_2\cdot 6H_2O$ (0.020 g, 0.054 mmol) with stirring at room temperature. The mixture was adjusted to pH = 9–10 and stirred for 2 h, and then concentrated. Acetone was added to precipitate the product, which was collected by centrifugation and washed with 100 mL acetone, followed by drying in vacuo to give the pure complex as a white solid (0.030 g, 47.6%). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 6.86 (m, 2H; phenyl–H), 6.65 (m, 2H; phenyl–H), 5.82–5.64 (m, 28H; OH-2,3), 4.82 (m, 18H; H-1, hydroxymethylene–CH_2), 4.56–4.46 (m, 6H; OH-6), 3.62 (m, 60H; H-3,5,6, pyridine–CH_2, methylene–CH_2), 3.32 (m, 36H; H-2,4, methylene–CH_2), 2.12 (s, 3H; methyl–CH_3), 2.07 (s, 3H; methyl–CH_3); elemental analysis calcd (%) for $C_{106}H_{232}Cl_2N_4O_{112}Zn_3$: C, 35.15; H, 6.46; N, 1.55. Found: C, 34.74; H, 6.33; N 1.63. ICP, Zn, calcd: 4.98%; found: 5.33%.

CAUTION: Zinc perchlorate is potentially explosive. It should be prepared in small quantities and handled with care.

2.4. Potentiometric titration

An automatic titrator (Metrohm 702GPD Titrino) coupled to a Metrohm electrode was used and calibrated according to Gran's method [25], then checked by titrating $HClO_4$ with NaOH solution (0.10 M). The thermostated cell contained 25 mL of 1.00 mM species in aqueous solutions with the ionic strength maintained at 0.10 M by sodium perchlorate. All titrations were carried out in the aqueous solutions under argon atmosphere at 25 ± 0.1 °C, and initiated by adding fixed volumes of 0.10 M standard NaOH in small increments to the titrated solution. Duplicate measurements were performed and the experimental error was below 1%. The titration data were fitted from the raw data using the Hyperquad 2000 program to calculate the ligand protonation constants K_n, the complex formation constant K_{ML}, and the deprotonation constants of the coordinated water pK_a.

2.5. Kinetics of BNPC and NA hydrolysis

The hydrolysis rates of BNPC and NA in the presence of zinc complex were measured by an initial slope method that follows the increase in absorption at 400 nm of the released 4-nitrophenolate [22]. 50 mM Tris–HCl (pH = 7.00) buffers were used; the ionic strength was adjusted to 0.10 M with $NaClO_4$ and the reaction solution was maintained at 25 ± 0.1 °C. In a typical experiment, after substrate (NA or BNPC), zinc(II) cation and ligand in 10% (v/v) CH_3CN solution at pH = 7.0 were mixed, the UV absorption decay was recorded immediately and monitored until 2% decay of 4-nitrophenyl acetate. Errors on k_{obs} values were about 5%.

2.6. Kinetics of BNPP hydrolysis

The rate of hydrolysis of BNPP to give mono(4-nitrophenyl)phosphate and 4-nitrophenolate was measured