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Synergistic effect of cyclodextrin-based binuclear complexes in the hydrolysis of amide

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Abstract

Novel cyclodextrin-based binuclear metal complexes have been prepared and proved to be far more efficient than their mononuclear complexes in promoting amide hydrolysis.

Keywords: cyclodextrin; binuclear metal complex; amide hydrolysis.

Amides are resonance-stabilized entities and difficult to hydrolyze. The half-life is estimated to be hundreds of years for the hydrolysis of a typical peptide bond around neutral pH.[1] In nature, various enzymes use two metal ions in their active center to cooperatively catalyze the hydrolysis of their substrates.[2,3] It is of particular interest to develop artificial models of these binuclear metalloenzymes because it cannot only promote the understanding of the enzymatic chemistry but also provide potential applicability in biotechnology. Therefore, a number of binuclear metal complexes have been synthesized by linking two metal-centers to a molecular scaffold,[4,5] mainly focused on phosphate hydrolysis and sparsely aimed at amide hydrolysis.[6,7] These complexes are developed to mimic the active sites of some binuclear metalloenzymes but most of them lack a hydrophobic substrate-binding site. The first example of a catalytic binuclear metal complex with hydrophobic binding appeared in 1997. The calixarene-based binuclear zinc complex showed a 2300-fold rate acceleration in phosphate diester transesterification.[8–10] A cyclodextrin (CD)-based bis-tren-Zn(II) complex was also reported, but it appeared that the two metal centers showed poor cooperation in the phosphate hydrolysis.[11] We found that the β-CD-based binuclear Zn(II) and Cu(II) complexes of **2** have high catalytic activity and the latter has significant cooperation between the two metal centers in the hydrolysis of amide **4**. In this paper, we describe the preliminary results on the synthesis and catalytic behavior of these complexes.

Ligand **2** was prepared by reductive alkylation of CD diamine **1**[12,13] (Scheme 1). Thus, $NaBH_3CN$ (0.2 g) in MeOH (100 ml) was added dropwise to a MeOH (200 ml) solution containing **1** (0.5 g) and 2-pyridinecarboxaldehyde (0.38 g) at rt overnight, and the solution was stirred at rt for another 2 days. After evaporation of the solvent, the residue was dissolved in a 10% ammonia solution (100 ml), and

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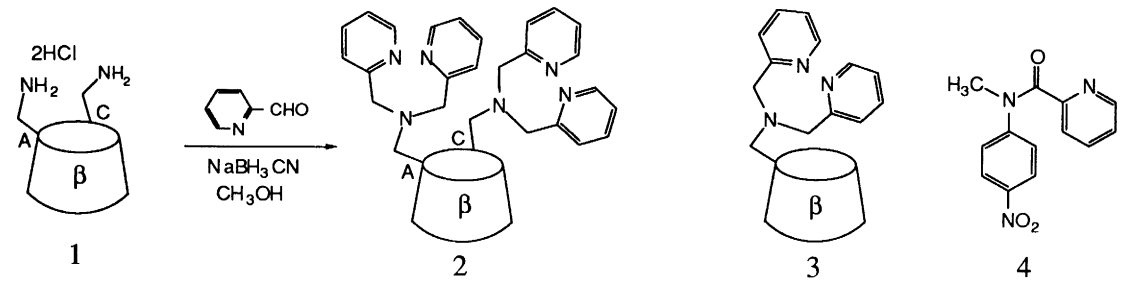

Scheme 1.

stirred at rt for 1 day. Chromatography of the reaction mixture afforded **2** in 74% yield. By a similar procedure, ligand **3** was synthesized for comparison.†

NMR titration of the ligand solution with $Zn(NO_3)_2$ showed that ligand **2** can easily form a binuclear complex with Zn(II). In the ^{1}H NMR spectrum (Fig. 1, top), ligand **2** showed two doublets around 8.37 ppm for 6-H, two doublets around 7.44 ppm for 3-H, and multiplets at 7.65 and 7.17 ppm for 4- and 5-H of the pyridine residues. When Zn(II) was added and the Zn(II)/**2** increased to 1, these signals decreased and were finally replaced by a new set of signals resonating in the range from 6.9 to 8.7 ppm. The signals at relatively higher fields gradually diminished when the Zn(II)/**2** increased from 1 to 2. Thereafter, further addition of Zn(II) no longer caused obvious spectral changes. This result implies that the complex **2**-$[Zn(II)]_2$ can be easily obtained by mixing **2** with two equivalents of Zn(II). Examination of the NMR spectra of **2**-$[Zn(II)]_2$ (Fig. 1, bottom) gave important structural information. In complex formation, three pyridyl groups showed larger downfield shifts ($\Delta\delta$ 0.3–0.5 ppm) for their 4- and 5-H, smaller downfield shifts ($\Delta\delta$ 0.2–0.3 ppm) for their 6-H but no obvious shift for their 3-H protons. The methylene groups on these pyridine rings were greatly deshielded and shifted downfield by up to 0.7 ppm. The last one

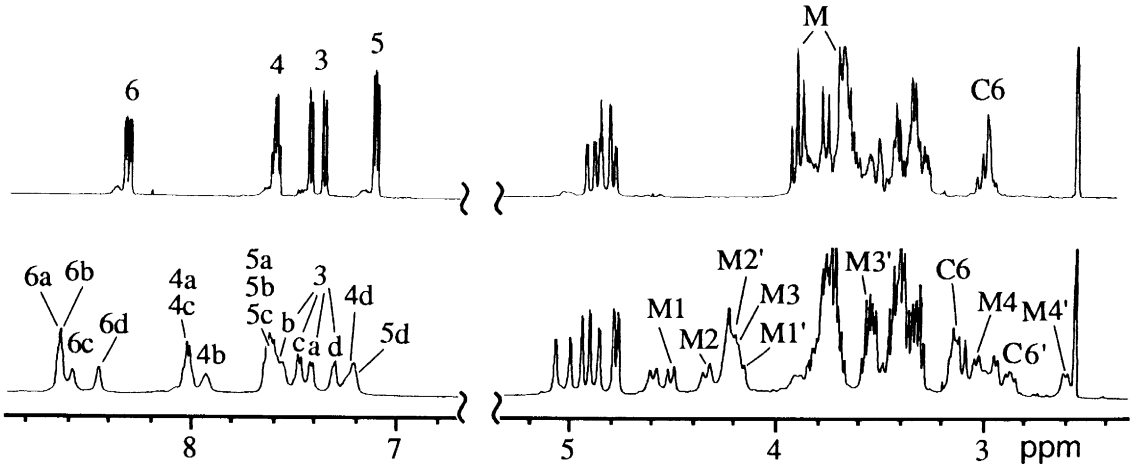

Fig. 1. 500 MHz ^{1}H NMR spectra of the ligand **2** (top) and its complex **2**-$[Zn(II)]_2$ (bottom) in DMSO[d_6], assigned based on 2D COSY spectra. The pyridyl protons are numbered according to their attached positions and letters a–d stand for different pyridine rings. M and C6 represent the methylene protons of the pendant groups and modified sugar units, respectively. The pyridylmethylene groups are differentiated with numeral numbers. The primed numbers denote the geminal protons resonating at higher fields

† Ligands **2** and **3** were characterized by FAB MS, ^{1}H and ^{13}C NMR spectra.

of the four pyridylmethylene residues, however, demonstrated quite a different shift pattern. Instead of large downfield shifts, very large upfield shifts were observed for its 4-H and methylene protons ($\Delta\delta$ up to 0.5 and 1.3 ppm, respectively), indicating that this residue is under a strong shielding of other pyridyl rings. The remarkable shifts for all the four pyridylmethylene residues suggests that both amino groups participated in chelating Zn(II), which was ascertained by the moderate downfield shifts ($\Delta\delta$ 0.1–0.4 ppm) for the 6- and 5-H protons of the two functional subunits.

Upon addition of $CuCl_2$ to a solution of **2**, a shoulder peak appeared around 290–360 nm. The absorbance at 320 nm increased with increasing concentration of Cu(II) and reached a plateau when 2 equivalents of $CuCl_2$ were added, which indicated the formation of complex **2**-$[Cu(II)]_2$. Titration of the **2**-$[Cu(II)]_2$ with the NaOH solution gave an apparent pK_a of 8.8, which was almost the same as that of **3**-Cu(II) (pK_a=8.9).

Amide **4** was used as a substrate to probe the catalytic properties of the metal complexes of **2**. Initial rates were detected by following the release of *N*-methyl-4-nitroaniline at 400 nm, and the kinetic parameters were listed in Table 1. This amide is inherently stable at pH 8.4 and 25°C and the reaction half-life is over 1 year. Ligand **2** actually stabilizes the substrate by 10 times. However, when **2**-$[Zn(II)]_2$ was used, the reaction was accelerated by at least two orders of magnitude. Greater rate enhancement was obtained in the case of **2**-$[Cu(II)]_2$. When 6.0×10^{-5} M amide **4** was mixed with 3.0×10^{-4} M ligand **2** and 6.0×10^{-4} M Cu(II) at pH 8.4 and 25°C, the pseudo first order rate constant was 8.5×10^{-6} s^{-1}, corresponding to a half-life of 23 h. Under the same reaction conditions, the mononuclear complex **3**-Cu(II) was 50 times less effective than **2**-$[Cu(II)]_2$.

Table 1
Pseudo first order rate constants[a] of the hydrolysis of **4** (6.00×10^{-5} M)

catalyst	[catalyst] (10^{-4} M)	pH	k_{obs}(10^{-6} s^{-1})	half-life
none		8.4	0.014	1.6 years
2	3.00	8.4	0.0012	18 years
3-Zn(II)	3.00	8.4	0.13	1.5×10^3 h
2-$[Zn(II)]_2$	3.00	8.4	0.47	4.1×10^2 h
3-Cu(II)	6.00	8.4	0.15	1.2×10^3 h
2-$[Cu(II)]_2$	3.00	8.4	8.5	23 h
2-$[Cu(II)]_2$	3.00	7.0	0.44	4.3×10^2 h
2-$[Cu(II)]_2$	3.00	7.5	0.49	3.9×10^2 h
2-$[Cu(II)]_2$	3.00	8.0	4.9	39 h
2-$[Cu(II)]_2$	3.00	8.2	7.7	25 h
2-$[Cu(II)]_2$	3.00	8.6	7.1	27 h
2-$[Cu(II)]_2$	3.00	8.8	5.3	36 h
2-$[Cu(II)]_2$	3.00	9.0	2.5	77 h
2-$[Cu(II)]_2$	3.00	9.3	0.98	2.0×10^2 h

[a] Metal complexes were prepared *in situ* by mixing the ligands with stoichiometric amounts of $Zn(NO_3)_2$ or $CuCl_2$. The kinetics measurements were performed at 25 °C by following the absorption change at 400 nm of the reaction solutions, which were buffered with HEPES for pH 7-8, TAPS for pH 8.2-8.8 and CHES for pH 9-10 and remained clear during the measurements.

The above results reveal that the binuclear complex **2**-$[Cu(II)]_2$ is an efficient catalyst for the amide hydrolysis and the two metal centers can cooperate in catalysis and are essential for obtaining greater rate acceleration. This synergistic effect was further proved by the pH-rate behavior of the reaction. It was reported that in the pH range of 5–9, the uncatalyzed hydrolysis of peptide bonds was predominated by the nucleophilic attack of H_2O and was, therefore, independent of pH.[1] Nevertheless, the **2**-$[Cu(II)]_2$-mediated hydrolysis of **4** was remarkably influenced by pH variation from 7.5 to 9.3. The reaction rate increased rapidly when pH was increased from 7.5 to 8.4, and reached an optimum at pH 8.4. Thereafter, the rate decreased with increasing pH. This pH-rate dependence indicates that a general acid–base bifunctional catalysis works in the hydrolysis of **4**.

By measuring the initial rate at different substrate concentrations, saturation kinetics were observed for the **2**-$[Cu(II)]_2$-mediated hydrolysis of **4**. The Michaelis–Menten constant K_M was 1.2 mM, the same magnitude by which a nitrophenyl species binds to β-CD.

In conclusion, the new CD-based binuclear complex **2**-$[Cu(II)]_2$ binds and cleaves the amide **4** efficiently. The good synergistic action of the two metal centers makes the binuclear complex much more efficient than their corresponding mononuclear complex.

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