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An Effective Metallohydrolase Model with a Supramolecular Environment: Structures, Properties, and Activities

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Abstract: A supramolecular inclusion complex, $[Zn(L^1)(H_2O)_2(\beta\text{-CD})](ClO_4)_2\cdot 9.5\,H_2O$ (**1**) was synthesized and characterized structurally and its first-order active species for hydrolysis of esters, $[Zn(L^1)(H_2O)(OH)(\beta\text{-CD})](ClO_4)$ (**2**), was isolated (L^1 = 4-(4′-*tert*-butylbenzyl)diethylenetriamine; β-CD = β-cyclodextrin). The apparent inclusion stability constant of the host and the guest measured in aqueous solution was $(5.91\pm 0.03)\times 10^3$ for **1**. The measured values of the first- and second-order pK_a values of coordinated water molecules were 8.20 ± 0.08 and 10.44 ± 0.08, respectively, and were assigned to water molecules occupying the plane and remaining axial positions in a distorted trigonal bipyramid of the $[Zn(L^1)(H_2O)_2(\beta\text{-CD})]^{2+}$ sphere according to the structural analysis of $[Zn(L^2)(H_2O)\}_2(\mu\text{-OH})](ClO_4)_3$ (**3**) (L^2 = 4-benzyldiethylenetriamine). *p*-Nitrophenyl acetate (pNA) hydrolysis catalyzed by **1** at pH 7.5–9.1 and 25.0 ± 0.1 °C exhibited a first-order reaction with various concentrations of pNA and **1**, but the pH profile did not indicate saturated kinetic behavior. Second-order rate constants of 0.59 and $24.0\,\text{M}^{-1}\text{s}^{-1}$ were calculated for $[Zn(L^1)(H_2O)(OH)(\beta\text{-CD})]^+$ and $[Zn(L^1)(OH)_2(\beta\text{-CD})]$, respectively; the latter exhibited a potent catalytic activity relative to the reported mononuclear and polynuclear Zn^{II} species.

Keywords: carboxylic esters · cyclodextrins · hydrolysis · supramolecular chemistry · zinc

Introduction

Supramolecular assemblies of host cyclodextrins (CDs) and guest molecules have attracted increasing attention in recent years, because of their potential to serve as platforms for the construction of molecular machines, with functions including chiral discrimination, drug carrying, reaction mediation, and recognition of ionic species.[1–15] In particular, CD inclusion complexes serve as enzyme models to reveal enzyme–substrate interactions, and have potential applications as drug carriers. Solution studies of CD inclusion complexes and determination of bonding constants have provided thermodynamic data that are useful for chromatographic applications. In the solid phase, X-ray diffraction studies of inclusion complexes with guest molecules can provide direct information on the mechanism of the noncovalent interactions associated with the inclusion process. A recent study indicates that the coordination properties of the metal ion in a metal complex confined in a supramolecular environment as a guest molecule can undergo great change. For example, a stable 1:2 inclusion complex constructed from two β-CD derivatives and a porphinatoiron(III) derivative reported by Kano et al. shows a high selectivity toward the N_3^- ion.[16]

Recently, we reported an inclusion compound created by supramolecular assembly of an imidazolate-bridged homodinuclear copper complex and two β-CD molecules, $[\{Cu(L^1)(H_2O)(\beta\text{-CD})\}_2(im)][ClO_4]_3$, ($L^1$ = 4-(4′-*tert*-butyl)benzyldiethylenetriamine; im = imidazolate).[17] The UV/Vis study indicated that the imidazolate bridge in such an inclusion complex exhibits additional stability, even at the physiological pH. When copper ion was replaced by zinc, $[Zn(L^1)(H_2O)_2(\beta\text{-CD})]^{2+}$ and unexpected hydroxo-coordinated mononuclear zinc moieties, $[Zn(L^1)(OH)(H_2O)(\beta\text{-CD})]^+$, were isolated and characterized. Inclusion complexation weakened the formation of the hydroxo-bridged dimer and

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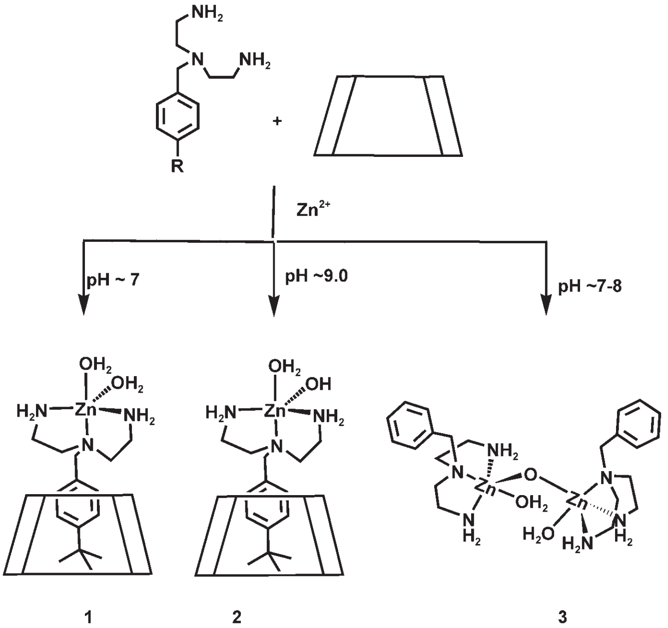
stabilized mononuclear hydroxyl species.[16] Since hydroxyl is the active species in the hydrolysis process,[18] such hydroxyl-coordinated species with additional stability engendered by the supramolecular environment may serve as an effective model for mononuclear metallohydrolases. Herein we report their supramolecular structures, thermodynamic properties, and metallohydrolase activities, evaluation of which was based on hydrolysis of *p*-nitrophenyl acetate.

Results and Discussion

Supramolecular assembly: By mixing L^1, β-CD, and Zn^{2+} in aqueous solution at pH ≈ 7.0 and 9.0, two supramolecular inclusion complexes, $[Zn(L^1)(H_2O)_2(\beta\text{-CD})](ClO_4)_2 \cdot 9.5\,H_2O$ (**1**) and $[Zn(L^1)(OH)(H_2O)(\beta\text{-CD})]ClO_4$ (**2**) were obtained in moderate yields (Scheme 1). At pH > 9.5, elemental analysis showed that isolated precipitates contained hydroxides. No hydroxo-bridged dimers or carbonato-bridged polymers with or without β-CD were isolated at the pH values studied. However, the corresponding inclusion complex with β-CD could not be isolated from a mixed solution of L^2 (L^2 = 4-benzyldiethylenetriamine), β-CD, and Zn^{2+} at pH ≈ 7.0; instead, only a water-insoluble hydroxo-bridged dimer, $[\{Zn(L^2)(H_2O)\}_2(\mu\text{-}OH)][ClO_4]_3$ (**3**), was precipitated. Such dimerization behavior often takes place in many simple triamine–Zn^{2+} systems.[19] Experimental results showed that $[Zn(L^1)(H_2O)_2]^{2+}$ with a larger hydrophobic group is just able to undergo effective inclusion complexation with the β-CD molecule, and moreover this inclusion interaction can weaken the formation of the hydroxo-bridged dimer.[16] Complexes **1** and **3** were characterized structurally by X-ray crystallography;[20] crystal data and selected bond lengths and angles are listed in Tables 1–3.



Scheme 1. Synthesis of complexes **1**–**3**.

Table 1. Crystal data and structure refinement for **1** and **3**.

	1	**3**
formula	$C_{57}H_{120}Cl_2N_3O_{54.5}Zn$	$C_{22}H_{43}Cl_3N_6O_{15}Zn_2$
M_r	1855.83	868.71
T [K]	123(2)	298(2)
λ [Å]	0.71073	0.71073
crystal system	orthorhombic	monoclinic
space group	$C222_1$	$P2_1/c$
a [Å]	19.312(1)	17.777(1)
b [Å]	23.914(2)	11.905(7)
c [Å]	38.163(3)	17.786(1)
β [°]	90	109.19(1))
V [Å^3]	17624(2)	3555.0(4)
Z	8	4
ρ_{calcd} [g cm^{-3}]	1.381	1.623
μ [mm^{-1}]	0.444	0.493
$F(000)$	7864	7723
crystal size [mm^3]	0.44 × 0.36 × 0.20	0.50 × 0.40 × 0.34
θ range [°]	2.01–26.00	2.10–27.13
limiting indices	$-23 < h < 23$ $-28 < k < 29$ $-35 < l < 47$	$-9 < h < 22$ $-15 < k < 15$ $-22 < l < 19$
reflns collected	45987	20610
independent reflns	16976 ($R_{int} = 0.0309$)	5314 ($R_{int} = 0.0290$)
data/restraints/parameters	16976/239/1214	7723/277/545
goodness-of-fit on F^2	1.156	1.069
final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0978$ $wR_2 = 0.2632$[a]	$R_1 = 0.0569$ $wR_2 = 0.1465$[b]
R indices (all data)	$R_1 = 0.1079$ $wR_2 = 0.2752$	$R_1 = 0.0872$ $wR_2 = 0.1698$
largest diff. peak/hole [e Å^{-3}]	1.661/−0.680	0.716/−0.625

[a] $w = 1/[s^2F_o^2) + (0.0957P)^{2+} + 96.5262P]$; [b] $w = 1/[s^2F_o^2 + (0.0843P)^2 + 4.9328P]$; $P = (F_o^2 + 2F_c^2)/3)$.

Table 2. Selected bond lengths [Å] and angles [°] for **1**.

Zn1–N1	2.195(8)	Zn1–O1w	2.058(6)
Zn1–N2	2.047(8)	Zn1–O2w	1.94(4)
Zn1–N3	1.901(11)	Zn1–O2w′	2.104(11)
Zn1–N3′	2.001(12)		
N3-Zn1-N3′	119.3(10)	O2w-Zn1-O2w′	96.0(19)
N3-Zn1-O2w	113.0(2)	N2-Zn1-O2w′	124.8(6)
N3-Zn1-N2	107.8(5)	O1w-Zn1-O2w′	88.5(4)
N3′-Zn1-N2	131.9(10)	N3-Zn1-N1	83.1(4)
O2w-Zn1-N2	139.0(19)	N3′-Zn1-N1	90.9(5)
N3-Zn1-O1w	103.6(4)	O2w-Zn1-N1	93.0(11)
N3′-Zn1-O1w	87.3(4)	N2-Zn1-N1	85.0(4)
O2w-Zn1-O1w	86.0(11)	O1w-Zn1-N1	173.1(3)
N2-Zn1-O1w	91.2(3)	O2w′-Zn1-N1	98.5(5)
N3′-Zn1-O2w′	103.3(9)		

Symmetry code 1: $x, -y+1, -z+2$.

Structure of $[Zn(L^1)(H_2O)_2(\beta\text{-CD})](ClO_4)_2 \cdot 9.5\,H_2O$ (1): The 4-*tert*-butylbenzyl group of the $[Zn(L^1)(H_2O)_2]^{2+}$ moiety in **1** is inserted as a guest in the hydrophobic cavity of the β-CD host on the primary hydroxyl side to form a supramolecular unit (Figure 1), $[Zn(L^1)(H_2O)_2(\beta\text{-CD})]^{2+}$, in which the atoms of the guest are partially disordered (Figure S1).[17] Two units form a symmetrical head-to-head dimer through

Table 3. Selected bond lengths [Å] and angles [°] for **3**.

Zn1−N1	2.265(4)	Zn2−N4	2.272(4)
Zn1−N2	2.062(4)	Zn2−N5	2.045(4)
Zn1−N3	2.049(5)	Zn2−N6	2.039(5)
Zn1−O1	1.925(3)	Zn2−O1	1.935(4)
Zn1−O1w	2.286(4)	Zn2−O2w	2.286(4)
O1-Zn1-N3	119.56(18)	O1-Zn2-N6	123.09(18)
O1-Zn1-N2	116.74(19)	O1-Zn2-N5	116.74(17)
N3-Zn1-N2	123.7(2)	N6-Zn2-N5	120.0(2)
O1-Zn1-N1	106.95(15)	O1-Zn2-O2w	93.71(18)
N3-Zn1-N1	82.43(17)	N6-Zn2-O2w	90.7(2)
N2-Zn1-N1	82.71(17)	N5-Zn2-O2w	89.12(19)
O1-Zn1-O1w	91.33(15)	O1-Zn2-N4	101.59(16)
N3-Zn1-O1w	89.08(17)	N6-Zn2-N4	82.79(19)
N2-Zn1-O1w	88.67(17)	N5-Zn2-N4	82.16(17)
N1-Zn1-O1w	161.71(15)	O2w-Zn2-N4	164.56(17)

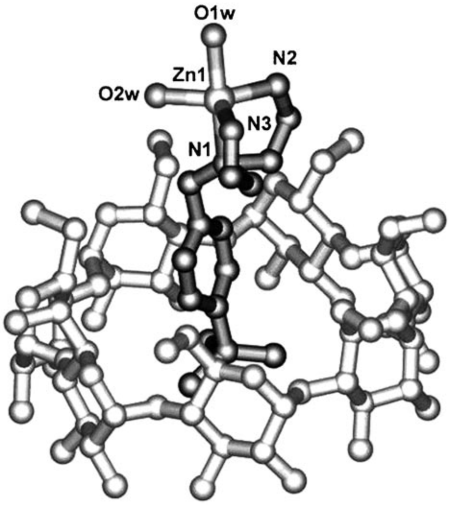


Figure 1. X-ray structure of $[Zn(L^1)(H_2O)_2(\beta\text{-CD})]^{2+}$ in **1** at 123 K showing the guest molecule inserted in β-CD by hydrophobic interaction. The ligand is doubly disordered, comprising two sets of structures, one of which is omitted for clarity.

hydrogen-bonding interaction of secondary hydroxyl groups. The zinc atom in the guest has a distorted trigonal-bipyramidal configuration. The degree of distortion (τ) between the trigonal bipyramid and the square pyramid can be estimated by a literature method.[21] For an ideal square-pyramidal geometry τ is zero, whereas it becomes unity for an ideal trigonal-bipyramidal geometry. The calculated τ value of 0.57 indicates that the geometry of coordination around the Zn atom is fairly close to a trigonal-bipyramidal sphere.

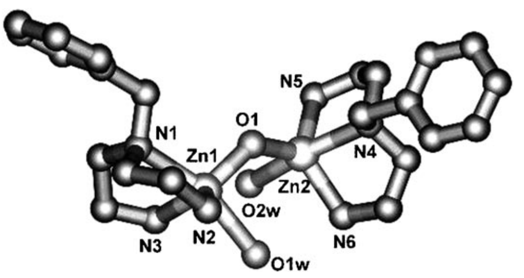
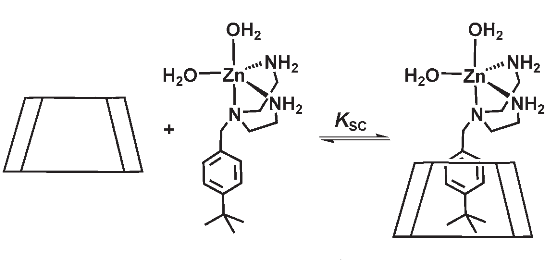


Figure 2. X-ray structure of $[\{Zn(L^2)(H_2O)\}_2(\mu\text{-OH})]^{3+}$ in **3** at 298 K.

Structure of $[\{Zn(L^2)(H_2O)\}_2(\mu\text{-OH})][ClO_4]_3$ (3): The cationic structure of **3** is a hydroxo-bridged binuclear Zn^{2+} compound (Figure 2). Each Zn^{2+} ion has a distorted trigonal-bipyramidal N_3O_2 coordination sphere, in which the terminal nitrogen atoms of L^2 and the hydroxyl group lie in the trigonal plane. The calculated τ values of 0.63 for Zn1 and 0.69 for Zn2 configurations indicate that both coordination geometries around Zn atoms are closer to trigonal-bipyramidal spheres.[21] The hydroxyl group is located in an equatorial position of each trigonal bipyramid in **3**, indicating that equatorial water molecules in the trigonal bipyramid have lower pK_a values than axial ones.

Spectral characterization of $[Zn(L^1)(OH)(H_2O)(\beta\text{-CD})]ClO_4$ (2): The crystals of complex **2** were too small for X-ray crystallography and therefore its composition was confirmed by elemental analysis, IR spectroscopy, and scanning electron microscopy (SEM). Complex **1** exhibited a strong, wide IR band at 1028–1155 cm^{-1} but **2** gave a strong peak only at 1081 cm^{-1}; these were assigned to ClO_4^- ions (Figure S2). The narrower band in **2** implies that there are fewer ClO_4^- ions in **2** than in **1**. The molar ratio Zn/Cl detected by SEM-EDA (energy dispersive analysis) was 49.39:50.61. To balance the two positive charges of the zinc ion, a coordinated water molecule must be deprotonated: the coordination ion of **2** has to include a coordinated hydroxyl group, $[Zn(L^1)(OH)(H_2O)(\beta\text{-CD})]$, rather than a free hydroxyl group. The pure $[Zn(L^1)(OH)_2(\beta\text{-CD})]$ species was not isolated because of the presence of hydroxide groups.

Stability constant of the inclusion complex: To understand better the guest–host inclusion complexation, we investigated the stability constants of the inclusion complexes by following the changes in absorbance at 263 nm by UV/Vis spectroscopy (Scheme 2). Zn^{II} with complex L^1 was titrated with various concentrations of β-CD according to a previously described method.[22,23] The K_{sc} value calculated for $[Zn(L^1)(H_2O)_2(\beta\text{-CD})]^{2+}$, $5.91 \pm 0.03 \times 10^3$, is slightly higher



Scheme 2. Inclusion equilibrium of the Zn^{2+} complexes with β-CD in aqueous solution.

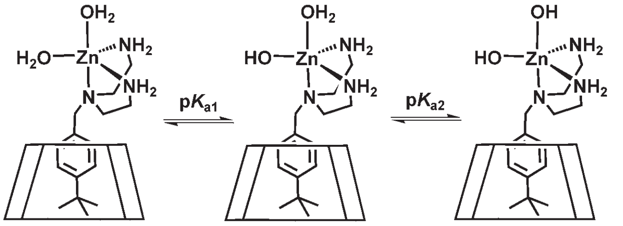
than that of the butyl *p*-hydroxybenzoate-β-CD inclusion complex (2.56×10^3).[24] For a cyclodextrin, which is fairly rigid, the guest molecule must fit snugly into the cavity. Because the combined reduction in cavity-water and insert-water surface areas is the driving force for complexation, the ideal situation for complexation is a snug fit of the nonpolar portion of the guest molecule into the nonpolar cyclodextrin cavity.[25] This would remove a maximum amount of water from the cavity and from the molecule surface.

Table 4. Equilibrium constants of ligands and metal complexation in the presence of β-CD.

Chemical equilibrium	Parameter	Equilibrium constant	
		dien[a]	L^1 + β-CD
$H_2L^{2+} = HL^+ + H^+$	pK_1	9.78 ± 0.01	9.67 ± 0.05
$HL^+ = L + H^+$	pK_2	8.99 ± 0.03	9.03 ± 0.04
$[Zn(H_2O)_6]^{2+} + L = [Zn(L)(H_2O)_2]^{2+}$	$\log K_{ML}$	8.92 ± 0.01	8.74 ± 0.06
$[Zn(L)(H_2O)_2]^{2+} = [Zn(L)(H_2O)(OH)]^+ + H^+$	pK_{a1}	8.93 ± 0.01	8.20 ± 0.08
$[Zn(L)(H_2O)(OH)]^+ = [Zn(L)(OH)_2] + H^+$	pK_{a2}	–	10.44 ± 0.08

[a] dien = diethylenetriamine.

Complexation and deprotonation constants and species distribution: Since the dimer with L^2 is precipitated easily under weak alkaline conditions, the protonation constant (K_n) of L^1 and its complex formation constant (K_{ML}) and the deprotonation constant (pK_a) of the coordinated water molecule (Scheme 3) were determined by potentiometric pH titration in the presence of β-CD at 25 ± 0.1 °C.[26,27] The pH profiles of the titration curves, including the distribution curves of the Zn^{2+} species as a function of pH (Figure 3), were analyzed; the calculated results are summarized in Table 4. A marked change is that the pK_{a1} of $[Zn(L^1)(H_2O)_2(\beta\text{-CD})]^{2+}$ (8.20 ± 0.08) is approximately 0.7 pH unit lower than that of $[Zn(dien)(H_2O)_2]^{2+}$ (8.93), an analogue of **1** (dien = diethylenetriamine).[28] This reduction is probably due to the effect of both the hydrophobic environment around the coordinated water molecule and weak interactions after complexation in **2**, similar to those in carbonic anhydrase or alkaline phosphatase.[18] Values for the pK_{a2} of water molecules coordinating the same metal ion such as that determined for $[Zn(L^1)(H_2O)_2(\beta\text{-CD})]^{2+}$, 10.44 ± 0.08, have not been reported for previously documented models. These results indicate that the strong hydrophobic interaction between the β-CD and the zinc complex can effectively weaken or prevent the formation of hydroxo-bridged dimers or carbonato-bridged polymers which occur in other simple mononuclear complex systems.[29]



Scheme 3. Deprotonation equilibria of Zn^{2+}-coordinated water in aqueous solution.

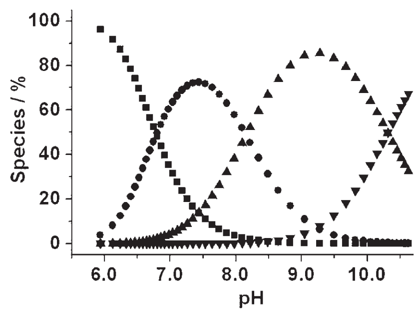


Figure 3. Distribution plots of species with ligand as a function of pH in the presence of β-CD at 0.1 M $NaClO_4$ and 25 ± 0.1 °C: (■) $[Zn(H_2O)_6]^{2+}$; (●) $[Zn(L^1)(H_2O)_2(\beta\text{-CD})]^{2+}$; (▲) $[Zn(L^1)(H_2O)(OH)(\beta\text{-CD})]^+$; (▼) $[Zn(L^1)(OH)_2(\beta\text{-CD})]$.

On the basis of the X-ray structure of **3**, which showed that the bridged hydroxyl group is located in an equatorial position of each distorted trigonal bipyramid, one can conclude that an equatorially coordinated H_2O molecule has a lower pK_a than an axially coordinated one. Therefore, the water molecule with pK_{a1} occupies the equatorial position and the water molecule with pK_{a2} is in an axial position of the trigonal bipyramid in **1**. In summary, the supramolecular complexation brings several unexpected properties, such as weakening or preventing formation of dimers, decreasing pK_a values, and stabilizing mononuclear hydroxyl species, which are very desirable properties for model compounds of mononuclear zinc enzymes.

Titration of the Zn^{2+} species of L^1 in the absence of β-CD failed due to precipitation at pH ≈ 8. Elemental analysis confirmed the white precipitate obtained to be the hydroxo-bridged zinc(II) dimer.

***p*-Nitrophenyl acetate hydrolysis**: The hydrolysis of *p*-nitrophenyl acetate (pNA) has been used widely for examining catalytic abilities of various mimics of metallohydrolase.[30–37] The initial rate was determined by monitoring formation of the *p*-nitrophenolate anion at 400 nm. Under the experimental conditions used, the initial hydrolysis rate increased linearly with the increase in [pNA] or **1** (Figure 4). The first-order rate constant obtained (v_{ic}, ic = inclusion complex) is one order of magnitude higher than that of $v_{\beta\text{-CD}}$ at pH 7–9, and therefore the contribution of β-CD can be neglected. Since the substrate concentration was essentially constant during the measurement, the initial first-order rate constant (k_{in}; in = initial) of the total inclusion complex was calculated as $v_{ic}/[pNA]_{total}$.[33]

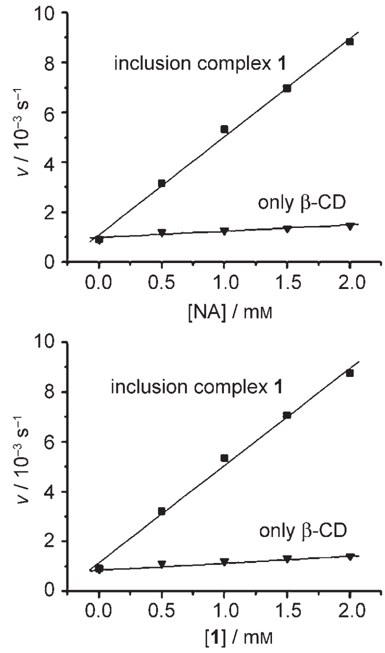


Figure 4. Dependence of initial rates on a) [pNA] and b) [**1**] for pNA hydrolysis catalyzed by (■) **1** and (▼) β-CD at pH 8.33.

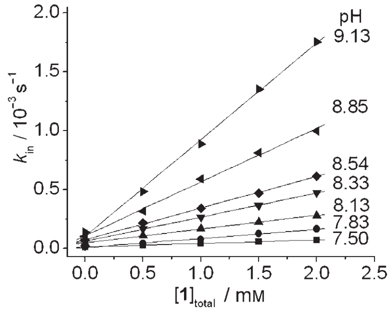


Figure 5. Dependence of k_{in} on $[\mathbf{1}]_{total}$ at pH 7.50–9.13, 0.1 M $NaClO_4$, 25 ± 0.1 °C in the presence of 10% (v/v) CH_3CN.

At a given pH value, the k_{in} values were measured at different concentrations of **1**. For each kinetic measurement, the k_{in} value increased linearly with the total concentration of **1**. The linear plots were extended to a wide pH range (7.5–≈9.1) (Figure 5). Thus, the slope of k_{in} versus [inclusion species]$_{total}$ resulted in the observed second-order rate constant k_{obs}.[32] The rate constants (k_{obs}) were found to increase exponentially with the increase in pH (Figure 6), in good agreement with the calculated percentage of $[Zn(L^1)(OH)(H_2O)(\beta\text{-CD})]^+$ and $[Zn(L^1)(OH)_2(\beta\text{-CD})]$ species (Figure 3), which suggests that the kinetic process is controlled by an acid–base equilibrium. These results indicate that these hydroxyl complexes are the kinetically active species. A complete rate can therefore be expressed as Eq. (1).

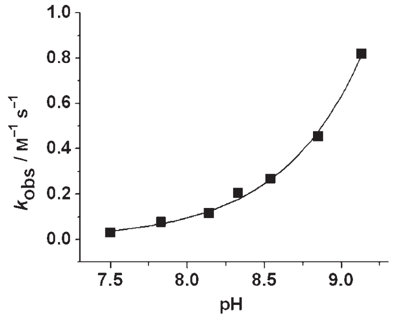


Figure 6. Dependence on pH of the second-order rate constants for pNA hydrolysis catalyzed by **1**.

$$\begin{aligned} k_{obs} &= (k_{cat}{}^{I}[LZnOH] + k_{cat}{}^{II}[LZn(OH)_2] + \ldots)/ \\ [Zn^{2+}\ \text{species}]_{total} &= (k_{cat}{}^{I}K_{a1}[H^+] + k_{cat}{}^{II}K_{a1}K_{a2})/ \\ ([H^+]^2 &+ K_{a1}[H^+] + K_{a1}K_{a2}) \end{aligned} \qquad (1)$$

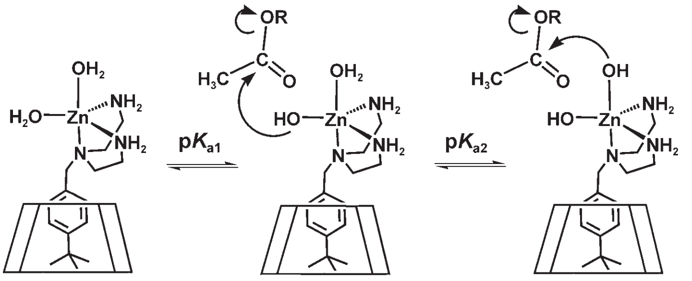
Subsequently, the k_{cat} and pK_a values (Table 5) were found by curve-fitting to be 0.59 $M^{-1}s^{-1}$ and 8.47 for $[Zn(L^1)(OH)(H_2O)(\beta\text{-CD})]^+$, and 24.0 $M^{-1}s^{-1}$ and 10.5 for $[Zn(L^1)(OH)_2(\beta\text{-CD})]$, respectively ($R = 0.994$).

Table 5. Rate of pNA hydrolysis for **1** and other reported Zn^{II} complexes.

Catalyst	pK_a	k_{cat} ($M^{-1}s^{-1}$)	Ref.
$[Zn([12]aneN_3)(OH)]^+$ [a]	7.30	0.041	[32a]
$[Zn(cyclen)(OH)]^+$ [b]	7.90	0.11	[32b]
$[Zn(Mecyclen)(OH)]^+$ [c]	7.68	0.047	[32c]
$[Zn([15]aneN_3O_2)(OH)]^+$ [d]	8.8	0.6	[35a]
$[Zn(dien)(OH)]^+$ [e]	8.93	0.08	[34]
$[Zn(L)(OH)]^+$ [f]	9.39	2.16	[35d]
$[Zn(L)(OH)]^+$ [g]	8.0	0.71	[32e]
$[Zn(L)(OH)]^+$ [h]	8.74	0.934	[32f]
$[Zn(L)(H_2O)(OH)(\beta\text{-CD})]^+$	8.47	0.59	this work
$[Zn(L)(OH)_2(\beta\text{-CD})]$	10.5	24.0	this work
$[Zn_2([15]aneN_3O_2)(OH)_2]^{2+}$ [d]	9.2	1.3	[35a]
$[Zn_2(L)(OH)_2]^{2+}$ [i]	9.36	3.5	[35b]
$[Zn_3\{([12]aneN_4)_3\text{-tren}\}(OH)_3]^{3+}$ [j]	9.7	4.2	[35c]
$[Zn_3\{([14]aneN_4)_3\text{-tren}\}(OH)_3]^{3+}$ [k]	8.9	3.7	[35c]
Zn(CA)OH [l]	7.0	400	[38]

[a] $[12]aneN_3$ = 1,5,9-triazacyclododecane. [b] cyclen = 1,4,7,10-tetraazacyclododecane. [c] Mecyclen = 1-methyl-1,4,7,10-tetraazacyclododecane. [d] $[15]aneN_3O_2$ = 1,4-dioxa-7,10,13-triazacyclopentadecane. [e] dien = diethyltriamine. [f] L = 2,5,8-trisaza[9]-10,23-phenanthrolinophane. [g] L = 1,1,1-tris(aminomethyl)ethane. [h] L = *N*,*N*′-dialkyl-1,10-phenanthroline-2,9-dimethanamine. [i] L = 1,4,7,10,19,22,25,28-octaaza-13,16,31,34-tetraoxacyclotriacontane. [j] $([12]aneN_4)_3$-tren = tris(2-(1,4,7,10-tetraazacyclododecan)ethyl)amine. [k] $([14]aneN_4)_3$-tren = tris(2-(1,4,8,12-tetraazacyclododecane)ethyl)amine. [l] CA = bovine carbonic anhydrase.

The k_{cat} of the first-order monohydroxyl form of **1** fell within the normal range, with similar pK_a values.[31] Surprisingly, the k_{cat} of the second-order dihydroxyl form is 300 times higher than that of its simple unassembled analogue $[Zn(dien)(OH)(H_2O)]^+$ ($k_{cat} = 0.08\ M^{-1}s^{-1}$),[34] and is also at least fivefold higher than those of other reported binuclear and trinuclear complexes (Table 5). It is possible that the hydroxyl group in an equatorial position of the trigonal bipyramid attacks the carboxylic carbon directly at low pH, and the axial one with higher nucleophilicity attacks the carboxylic carbon at high pH (Scheme 4).



Scheme 4. Possible intermediates involving nucleophilic attacks by the first and second hydroxyl groups.

Interestingly, the pK_a dependence of the second-order rate constants for pNA hydrolysis catalyzed by **1**, **2**, and other reported complexes exhibits exponential growth rather than a linear increase (Figure 7). Therefore, the kinetic behavior of dihydroxyl species of **2** cannot be explained simply by reference to changes in pK_a. Formation of dihydroxyl species led to a decrease in the positive charge distribution of the whole nucleophilic agent, which is propitious to nucleophilic attack on the carboxylic carbon. Compared with monohydroxyl species, dihydroxyl species have a lower oxidation number and more nucleophilic groups, and the second nucleophilic group has higher nucleophilicity. All of these factors have significant effects on their catalytic activity for hydrolysis of the carboxylic ester. The effect of pK_a on nucleophilicity is only one of the factors that affect the catalytic activity.

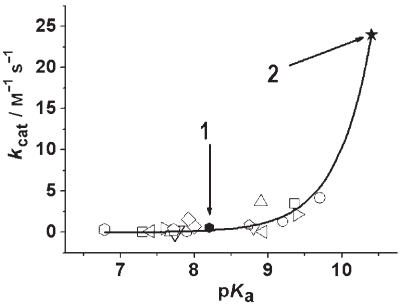


Figure 7. Dependence on pK_a of the second-order rate constants for pNA hydrolysis catalyzed by the zinc complexes listed in Table 5.

The potent catalytic activity of the dihydroxyl species may be applied to elucidate the previously reported pH profile of the second-order rate constant for hydrolysis of methyl 4-nitrophenyl carbonate catalyzed by bovine carbonic anhydrase.[38] In the pH profile, the rate constant of the enzyme increases with the increase from pH 6 to pH 11. The rapid increase in the rate constant at pH 6–8 was attributed to the formation of first-order monohydroxyl species of the enzyme, such as active (His)$_3$ZnOH species (pK_a = 7.0). However, no explanations were given for the continual growth of the rate constant at pH 8–11. Based on our results, the increase in the rate constants in this pH range may be due to the contribution from second-order dihydroxyl forms of the active site of the enzyme, for example, active [(His)$_3$Zn(OH)$_2$] species.

Conclusion

A Zn^{2+}–triamine complex with pendant 4-*tert*-butylbenzyl can be assembled effectively with a β-CD molecule to form a supramolecular inclusion complex. This inclusion complexation can lower the pK_a values of Zn^{2+}-coordinated water molecules, weaken or prevent the formation of hydroxo-bridged dimers of the zinc moiety, and stabilize hydroxyl species in solution. Such an inclusion complex is an ideal model for mononuclear metallohydrolases and has potential in the development of an effective model for metalloenzymes; of all the model compounds, its double-hydroxyl species exhibits the highest catalytic activity for pNA hydrolysis found so far.

Experimental Section

Materials: β-Cyclodextrin and *p*-nitrophenyl acetate were purchased from Aldrich Chemical Company. Common organic reagents were reagent grade and redistilled before use. The water used in all physical measurements was Milli-Q grade. The ligands were prepared as described and were confirmed by their elemental analyses and NMR spectra.[17]

[Zn(L^1)(H$_2$O)$_2$(β-CD)](ClO$_4$)$_2$·9.5H$_2$O (1): β-CD (0.567 g, 0.50 mmol) was added to a solution of L^1·3HClO$_4$·3H$_2$O (0.328 g, 0.50 mmol) and NaOH (0.060 g, 1.5 mmol) in water (10 mL) with magnetic stirring until the solution became clear. This solution was then added dropwise to a solution of Zn(ClO$_4$)$_2$·6H$_2$O (0.187 g, 0.50 mmol) in water (10 mL) with stirring for 30 min at room temperature, the pH was adjusted to 7, and the solution was filtered, and then allowed to stand to evaporate slowly in a desiccator charged with P$_2$O$_5$. Several days later, colorless cubic crystals suitable for crystal analysis were obtained in 46% yield. Elemental analysis calcd (%) for C$_{57}$H$_{115}$N$_3$Cl$_2$O$_{52}$Zn: C 37.81, H 6.40, N 2.32; found: C 37.71, H 6.21, N 2.35 (the crystals effloresced easily and lost 2.5H$_2$O).

[Zn(L^1)(OH)(H$_2$O)(β-CD)]ClO$_4$ (2): NaOH (0.5 M) solution was added to a solution of **1** (0.090 g, 0.050 mmol) in water (5 mL) until a pH ≈ 9.0 was reached. The solution was filtered and the filtrate was allowed to stand to evaporate slowly at room temperature. Several days later, colorless crystals were obtained (yield 31%). Elemental analysis calcd (%) for C$_{57}$H$_{100}$N$_3$ClO$_{41}$Zn: C 43.21, H 6.36, N 2.65; found: C 43.89, H 6.74, N 2.65; molar ratio Zn/Cl: 49.39:50.61.

[{Zn(L^2)(H$_2$O)}$_2$(μ-OH)][ClO$_4$]$_3$ (3): A mixture of L^2·3HClO$_4$·H$_2$O (0.256 g, 0.5 mmol) and NaOH (0.06 g, 1.5 mmol) were dissolved in water (10 mL) containing β-cyclodextrin (0.567 g, 0.5 mmol) and stirred at room temperature until the solution became clear. This solution was added dropwise to an aqueous solution of Zn(ClO$_4$)$_2$·6H$_2$O (0.187 g, 0.5 mmol, 10 mL) with stirring, then stirring was continued for 30 min. The white precipitate (0.182 g) formed was collected by filtration. Colorless cubic crystals of **3** suitable for X-ray analysis were obtained by dissolving this precipitate in hot water and cooling to room temperature. Elemental analysis calcd (%) for $C_{22}H_{43}N_6Cl_3O_{15}Zn_2$: C 30.42, H 4.99, N 9.67; found: C 30.14, H 4.96, N 9.45.

Caution: *Perchlorate salts of organic compounds are potentially explosive; these compounds must be prepared and handled with great care!*

General methods: ^{1}H NMR spectra were recorded on a Varian INOVA-500NB or Mercury Plus 300 spectrometer, IR spectra on a Bruker FTIR Equinox 55 spectrometer, and ESI-MS spectra on a Thermo LCQ-DECA-XP spectrometer. Elemental contents were determined with a Perkin–Elmer 240 elemental analyzer.

X-ray crystallography: Single-crystal X-ray data of two complexes were collected on a Bruker Smart Apex CCD diffractometer at 123 and at 298 K, with graphite-monochromated $Mo_{K\alpha}$ radiation ($\lambda = 0.71073$ Å). The reflections were corrected for Lorentz and polarization effects and empirical absorption corrections were applied using the SADABS program. The space groups were determined from systematic absences and confirmed by refinement results. The structures of **1** and **3** were solved by direct methods using the SHELXTL software suite.[20] The data were refined by the full-matrix least-squares method on F^2. All the non-hydrogen atoms except some of the badly disordered ones were refined with anisotropic displacement parameters. All the hydrogen atoms of the organic ligands were placed in idealized positions and refined as riding atoms. The host β-CD molecules are quite normal, with only a few terminal groups showing slight disorder, whereas the guest molecules of the metal complexes exhibit serious disorder, a common feature of host–guest structures with large cavities. The perchlorate anions were badly disordered; each was refined with a fractional occupancy, with the sum of site occupancy factors equal to unity. The structural refinements were carried out with a few restraints owing to the serious disorder phenomenon. However, not all seemingly abnormal bond lengths and angles were modeled, because they do not affect our general understanding of the host–guest chemistry. The crystallographic data are listed in Table 1.

UV/Vis spectroscopy: Inclusion stability constants were determined with a Varian Cary 300 UV/Vis spectrophotometer equipped with a temperature controller (±0.1 °C), by monitoring the changes in UV absorption of solutions of metal complexes (0.1 mM) in a tris(hydroxymethano)aminomethane (Tris) buffer upon addition of different molar ratios of β-CD at pH 8.33, 25±0.1 °C, and $NaClO_4$ (0.1 M).

Potentiometric titration: An automatic titrator (Metrohm 751GPD Titrino) coupled to a Metrohm electrode was used and calibrated according to the Gran method.[26,27] The electrode system was calibrated with buffers and checked by titration of $HClO_4$ with NaOH (0.1 M). All titrations were carried out under an N_2 atmosphere, at 25.0±0.1 °C and 0.10 M $NaClO_4$. Titrations were performed on the following aqueous solutions with standard NaOH solution ((0.1 M; $V_{tot} = 25$ mL, [species] = 1.0 mM): a) $L^1{\cdot}3\,HClO_4 + \beta$-CD; b) $L^1{\cdot}3\,HClO_4 + Zn^{2+} + \beta$-CD. Duplicate measurements were performed, for which the experimental error was below 1%. The titration data were fitted with the PSEQUAD program to calculate the ligand protonation constants K_n, the complex formation constant K_{ML}, and the deprotonation constants of the coordinated water molecules in the absence and in the presence of β-CD.

Kinetics of *p*-nitrophenyl acetate hydrolysis: The hydrolysis rate of pNA in aqueous solution was measured by following the increase in absorption at 400 nm of the *p*-nitrophenolate released.[31] At this wavelength, the absorbance of the ester substrate was negligible. The reaction solution was maintained at 25±0.1 °C and the ionic strength was adjusted to 0.10 with $NaClO_4$. Tris (50 mM; pH 7.5–8.5) and *N*-(1,1-dimethyl-2-hydroxyethyl)-3-amino-2-hydroxypropane sulfonate (AMPSO; pH 8.8–9.1) were used as buffers. The solutions used in kinetic measurements contained 10% (v/v) CH_3CN. Aqueous solutions of the inclusion compounds were prepared by dissolving their crystals in buffer solutions of different pH values and oscillating them ultrasonically for 30 min before testing. The results of the kinetic measurements could be duplicated within ±5%. The molar absorbance values of NA at different pH values were measured for calculation.[30–37]

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