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An allosteric supramolecular hydrolase model: Combination of a guanidinium function with a metal center in cyclodextrin scaffold

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ABSTRACT

An allosteric hydrolase model (**1**) with cooperativity between a metal center and an allosteric site has been constructed through supramolecular assembly of an adamantanyl guanidinium and a copper (II) complex of tris(2-aminoethyl)amine modified cyclodextrin (tren-CD). The structure of the supramolecular inclusion has been determined by ^{1}H NMR and selective NOESY measurement. Furthermore, the allosteric role of the guanidinium group was unambiguously demonstrated by the catalytic behaviors of the allosteric model **1**, the kinetics studies for the allosteric model revealed that the allosteric guanidinium group strongly influences catalytic behaviors of the supramolecular catalyst in the cleavage of 4,4′-dinitrodiphenyl carbonate (DNDPC). For example, the turnover number k_{cat} of catalyst **1** was 20 min^{-1} and the second-order rate constant of 14,080 M^{-1} min^{-1} was observed for the hydrolysis of DNDPC, catalyst **1** exhibits higher activity than catalyst **2** without an allosteric site, and its activity is similar to that for copper (II) complex of tris(2-aminoethyl)amino-cyclodextrin (Cu-tren-CD) which possesses a free cavity as a substrate binding site. Obviously, this result revealed that the presence of guanidinium group led to change of the catalytic mechanism and efficiency in the hydrolytic process.

1. Introduction

In biological and artificial systems allosteric regulation controlled the structure and catalytic efficiency of many enzymes in their catalysis processes. For example, in the active site of *Escherischia coli* alkaline phosphatase that catalyzes the hydrolysis of phosphate monoesters, two zinc ions are essential for enzyme activity, and a Mg^{2+} ion is not involved directly in the hydrolysis process, but it acts as a strong allosteric activator. Replacement of Mg^{2+} ion by other metal ions alters or even inhibits catalytic activity [1,2]. In recent years, there is an increasing interest in the design of artificial allosteric catalysts, and construction of an effective allosteric enzyme system is an important topic not only to elucidate catalytic mechanism, but also to regulate the activity of artificial receptors [3–15].

Often, allosteric molecular recognition systems, in which metal ions or organic molecules act as important allosteric effectors, have been widely studied in supramolecular systems [16,17]. Whereas allosteric regulation is a nascent concept in synthetic catalysis, the reports on allosteric regulation of artificial catalysts are still rare. To our knowledge, mononuclear or multinuclear metalloenzymes containing various metal ions where metal ions play both roles for catalysis and allosteric action have been developed by Krämer and co-workers [18,19] for the hydrolysis of phosphate esters, a similar synthetic allosteric catalyst was reported by Takeuchi and co-workers [20]. Furthermore, a cyclodextrin (CD)-based allosteric enzyme model which promoted catalytic activity for the hydrolysis of esters and alkyl nitrites has also been reported [21,22].

Inspired by the concept of allosteric regulation in synthetic catalysts, we constructed an artificial allosteric hydrolase model **1** (see Scheme 1) for 4,4′-dinitrodiphenyl carbonate (DNDPC) cleavage through supramolecular inclusion of an adamantanyl guanidinium hydrochloride with copper complex of tris(2-aminoethyl)amine modified cyclodextrins (tren-CD). In our system, a guanidinium group acts as an efficient allosteric unit that recognizes the oxyanion of the tetrahedral transition state of DNDPC hydrolysis, Cu^{2+} ion acts as an important catalytic center. Moreover, the kinetic behaviors of this allosteric model have been investigated in detail.

2. Experimental

2.1. General materials and procedure

All reagents and solvents unless otherwise indicated were of the analytical grade commercially available and all the solvents used were dried by general procedure. Molecular weight was

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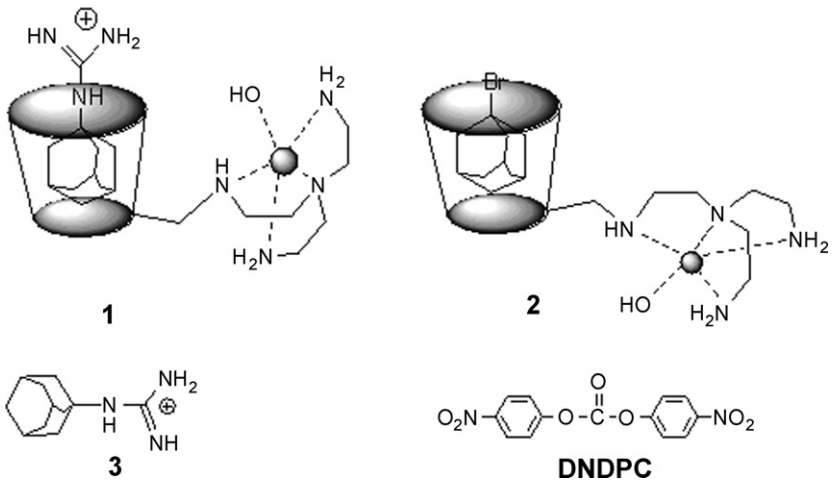


Scheme 1. Structure of the compounds.

obtained by the LDI-1700 MALDI-TOF-MS (Linear Scientific Inc., USA). The kinetic study was carried out using a Shimadzu 2450 UV–vis Recording Spectrophotometer. Data were analyzed by using Origin 7.5 software. Purification of cyclodextrin derivative was performed by G-15 Sephadex chromatography. ^{1}H NMR spectra were performed on a Bruker AVANCE 500. Selective ^{1}H-^{1}H NOESY spectra were measured using pulse sequences and standard procedures offered by Bruker.

2.2. Synthesis of 6-deoxy-6-[tris-(2-aminoethyl)]amino-cyclodextrin (tren-CD)

The compound was synthesized according to previous procedure [21,22]. Dried 6-Tso-β-CD (900 mg, 0.698 mmol) was dissolved in DMF (50 mL), and tris(2-aminoethyl)amine (6.98 mmol) and $(Et)_3N$ (6.98 mmol) were added to this solution. The mixture was stirred for 18 h at 353 K under N_2 atmosphere. After reaction acetone was added to this solution, the solid formed was collected by filtration. The crude residue was purified on a column of Sephadex G-15 chromatography followed by 0.1 M NH_4OH. The aim compound with 35% yield was obtained. ^{1}H NMR (500 MHz, D_2O): δ 2.50–3.29 (m, NH–CH_2), 3.46–3.59 (m, 2-H, 4-H), 3.74–3.92 (m, 3-H, 5-H, 6-H), 4.96-5.02 (m, 1-H); MALDI-MS: calcd 1263.2 found 1264.6 [M+H$^+$].

2.3. Synthesis of adamantanyl guanidinium (3)

The compound was synthesized by the literature method [23]. Adamantanamine (960 mg, 6.35 mmol), 1*H*-pyrazole-1-carboxamidine·HCl (926.9 mg, 6.35 mmol), and diisopropylethylamine (1.05 mL, 6.35 mmol) were suspended in dry DMF (40 mL). The reaction mixture was stirred for 24 h at room temperature under N_2, and ether was added dropwise. The precipitate was collected by filtration, washed with ether, and dried. The crude product was recrystallized from CH_3CH_2OH/ether and final product was obtained in 75% yield. ^{1}H NMR (500 MHz, DMSO): δ 1.56–1.58 (d, 3H), 1.65–1.67 (d, 3H), 1.78 (m, 6H), 2.08 (s, 3H), 7.99 (s, 3H).

2.4. Supramolecular model 1

Firstly, the complex of tren-CD and adamantanyl guanidinium was prepared by the mixing of polyamine cyclodextrin ligand and compound **3** in molar ratio of 1:1, after ultrasonic for 30 min, the complex was purified on a column of Sephadex G-15. And supramolecular model **1** was obtained by mixing of above complex of $CuCl_2$ in neutral aqueous solution, after ultrasonic for 30 min, ethanol was added to this solution, the precipitate was centrifuged off and washed with 20 ml of ethanol and the product was obtained.^{1}H NMR (500 MHz, D_2O): δ 4.91–4.94 (m, 7H) 3.64–3.78 (m, 28H), 3.44–3.55 (m, 14H), 2.56–3.20 (m, 12H), 2.22 (s, 3H), 1.84 (m, 6H), 1.64–1.71 (m, 6H).

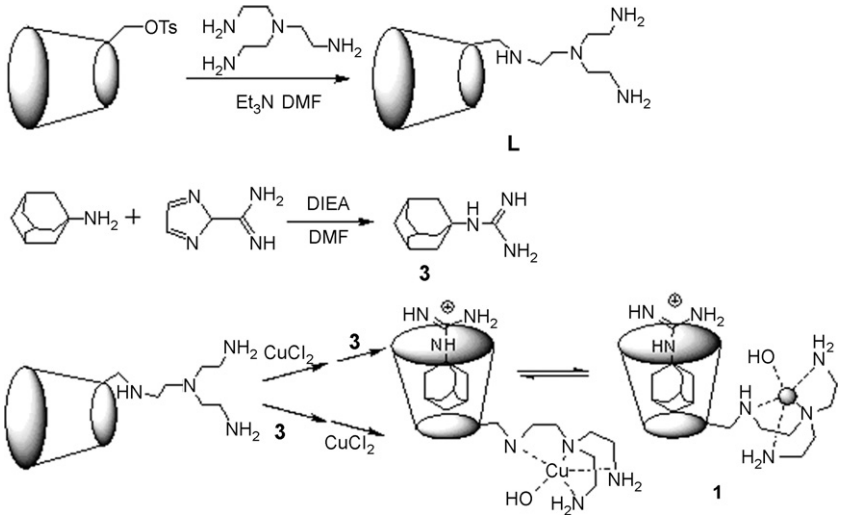
2.5. Hydrolysis of 4,4′-dinitrodiphenyl carbonate

The hydrolysis rate of DNDPC as measured by following the increase in absorption (400 nm, $\varepsilon = 8700\ M^{-1}\ cm^{-1}$, pH 7.0) of the released 4-nitrophenolate (NP) using a computer-linked UV-2450 spectrophotometer. The reaction solution was maintained at 298 K and pH 7.0 with 20 mM HEPES buffer and in 20% (v/v) acetonitrile (CH_3CN) aqueous solution, and the concentration of the substrate and the catalyst **1** were 20 and 500 μM, respectively.

3. Results and discussion

3.1. Synthesis and structure of the catalysts

Cyclodextrins are excellent host molecules, they have capacity to bind a variety of aromatic and aliphatic guest molecules and have been widely utilized to construct functional supramolecules in host–guest chemistry [23,24]. In this study, we employed β-CD appended a polyamine as a ligand, the hydrophobic cavity of CD was used to introduce an allosteric site by the complexation of CD with adamantanyl guanidinium **3**. Both ligands adamantanyl guanidinium hydrochloride **3** and 6-deoxy-6-[tris-(2-aminoethyl)] amino-cyclodextrin (tren-CD) were synthesized according to the literature methods, respectively [25,26]. The detailed synthetic routes were illustrated in Scheme 2. In the preparation process of model **1**, the order of adding $CuCl_2$ and adamantanyal guanidinium **3** did not influence the structure of the complex. This has been proved through investigation of the catalytic activity of the supramolecular hydrolase model **1** in the hydrolysis of DNDPC (see Fig. 1). To determine the structure of the inclusion complex between tren-CD and 3, selective NOESY measurement was carried out [27]. Fig. 2 shows the NOESY NMR spectrum of the complexation of compound **3**/tren-CD, the signals of H_b of compound **3** were observed when H3 of the tren-CD was selectively pre-saturated, this result indicated that an adamantanyl moiety inserts the cavity of CD from the end of the secondary side of tren-CD and the adamantanyl moi-



Scheme 2. The routes for the synthesis of ligand (L), compound **3** and complex **1**.

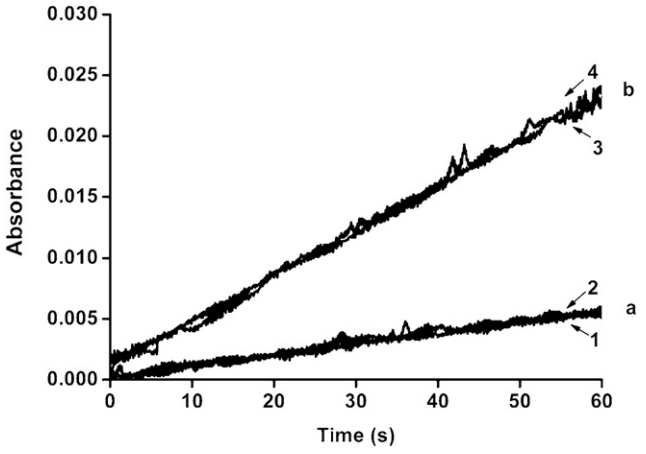


Fig. 1. Plots of absorbance against time for the hydrolysis of DNDPC ([S] = 20 μM) promoted by model **1** with different order of adding $CuCl_2$ and adamantyl guanidinium hydrochloride **3** at pH 7.0 and 298 K. (a) Concentration of catalyst 1: 10 μM. (b) Concentration of catalyst **1**: 0.5 mM; (1 and 3) Compound **3** was added prior to $CuCl_2$; (2 and 4) $CuCl_2$ was added prior to compound **3**.

ety was shallowly included in the cavity of tren-CD. Some steric hindrance and weak coordination of both copper ion and guanidinium group in neutral aqueous solution may lead to this structure [28].

3.2. Kinetic study of catalysts

The catalytic capacity of these compounds was evaluated by investigating the hydrolysis of substrate DNDPC. We selected carbonate DNDPC as a substrate, since the guanidinium group has an important function to bind the oxyanion of the tetrahedral transition state of carbonate hydrolysis [29]. The hydrolysis reaction rates of the ester bond cleavage of DNDPC were measured by an initial slope method following the increase in 400 nm absorption of the released 4-nitrophenolate (ε = 8700 M^{-1} cm^{-1}, pH 7.0) [30] in a Tris–HCl buffer solution containing 0.1 M KCl and 20% (v/v) CH_3CN at 298 K. Firstly, the kinetic behavior of complexes **1** and **2** has been studied and the results were shown in Fig. 3. The plot of initial rate of DNDPC hydrolysis reveals that the enhancement of the catalytic activity in the case of complex 1 with guanidinium group is obviously higher than that for complex **2** determined under identical conditions. The comparison of the hydrolysis ability of complexes

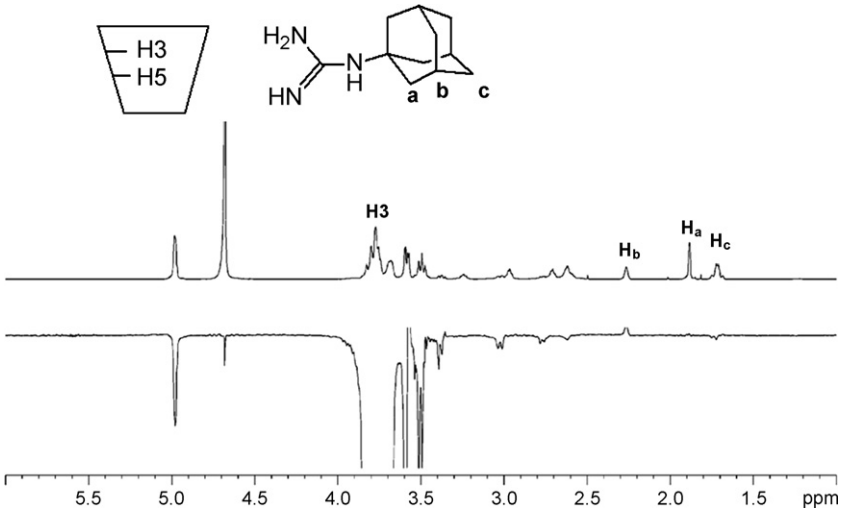


Fig. 2. NMR spectra of the inclusion of tren-CD and compound **3** in D_2O at 298 K. ^1H NMR (upper), and NOESY (lower), the H3 of tren-CD was irradiated.

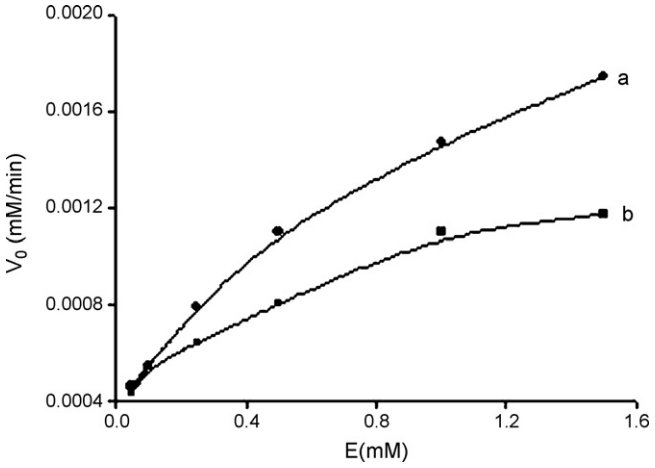


Fig. 3. Plots of initial rate for the hydrolysis of DNDPC ([S] = 20 μM) at various concentrations of complexes **1** and **2** in 20% CH_3CN/Tris buffer at pH 7.0, t = 298 K. (a) Complex **1** and (b) complex **2**.

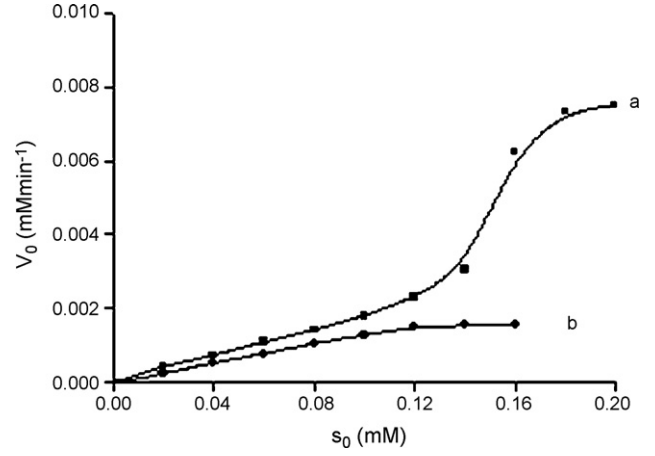


Fig. 4. Diagram of saturation kinetics of the hydrolysis of DNDPC by the supramolecular model **1** and control **2** in buffer solution (Tirs/MeCN 80:20), pH 7.0 at 298 K. (a) Model **1**. (b) Control **2**.

1 and **2** suggest that guanidinium group plays an important role in catalysis.

In order to evaluate the contribution of the guanidinium group to the hydrolysis of DNDPC, we have undertaken a detailed kinetic study for complexes **1** and **2** and the copper complex of tren and tren-CD. By varying the initial substrate concentration a kinetic profile of the reaction towards nonlinear saturation kinetic was observed for complex **1**, a plot of initial reaction rates against substrate concentration was listed in Fig. 4. As with the behaviors of natural allosteric enzymes, the catalysis of DNDPC hydrolysis by complex **1** shows Michaelis–Menten kinetics with a sigmoidal curve typically as an allosteric enzyme [1]. The Michaelis–Menten kinetics parameters in presence of different catalysts were shown

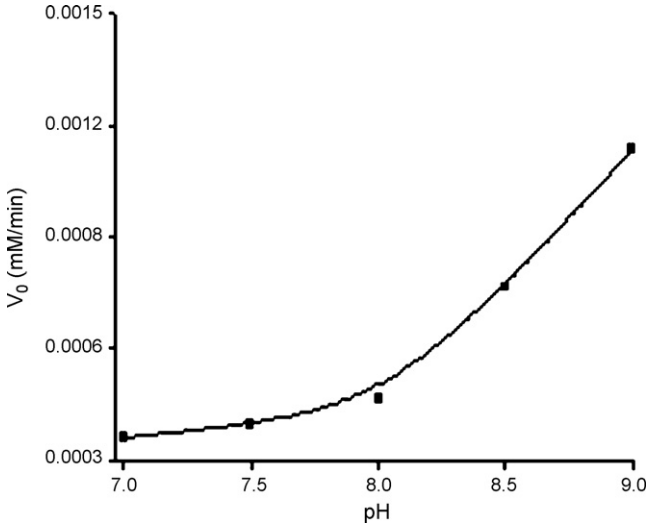
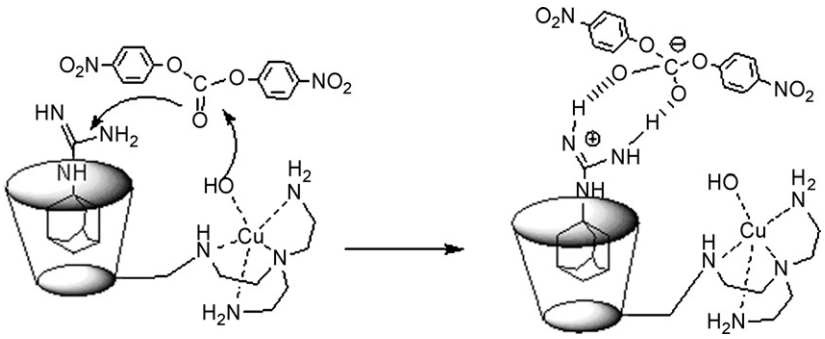


Fig. 5. Dependence of v_0 on pH for the hydrolysis of DNDPC ($S_0 = 2 \times 10^{-2}$ mM) catalyzed by supramolecular hydrolase **1** ($E = 5 \times 10^{-3}$ mM) at 298 K.

in Table 1. These Michaelis–Menten kinetic values were obtained from Lineweaver–Burk plots. The turnover number k_{cat} = 20 min^{-1} and the Michaelis constant K_m = 1.42 mM were derived for DNDPC hydrolysis catalyzed by the allosteric model **1**. A remarkable rate enhancement of 4760-fold (k_{cat}/k_{uncat}) for the hydrolysis of DNDPC by **1** compared to the uncatalyzed hydrolysis in the same solution was observed. However, for the complex **2** without the regulatory site, it showed a low k_{cat} = 1.37 min^{-1}, and the k_{cat}/k_{uncat} was found to be only 163, which is 30-times lower than that for complex 1 catalysis. Furthermore, in the case of compound Cu-tren, very low k_{cat} value of 0.165 min^{-1} and only a 39-fold rate enhancement over the uncatalyzed reaction were observed. It is shown that k_{cat} value is about two orders lower than that for the complex **1**. Comparison of Michaelis constant K_m with complex 1 and Cu-tren indicates that the rate enhancement of the hydrolysis was induced by the guanidinium group of complex **1**, and not by the change in substrate affinity. Furthermore, a much better catalytic efficiency k_{cat}/K_m for complex 1 compared to complex **2** and Cu-tren was obtained (see Table 1). The catalytic proficiency can also be described by the quantity K_{tx}^{-1}, where $K_{tx}^{-1} = k_{cat}/K_m/k_{uncat}$. The K_{tx} is interpreted as the dissociation constant for the complex formed between the transition state and the enzyme, and it can reflect the hypothetical binding affinity of an enzyme for the transition state [31]. To complete a catalytic cycle, enzymes must first recognize and bind their substrates to setup the correct geometry then stabilizes the transition state for a particular reaction, and the binding process is of importance in the development of an enzyme mimic. The comparison of K_{tx}^{-1} for **1** and **2** catalyses unambiguously demonstrated that the guanidinium group is an important factor for stabilizing the transition-state of DNDPC hydrolysis by complex **1**. Interestingly, the complex **1** and Cu-tren-CD showed similar catalytic efficiencies by the comparison of the second-order

Table 1
Kinetic parameters for the hydrolysis of DNDPC in presence of supramolecular hydrolase **1**, control complex **2**, Cu-tren-CD and Cu-tren

Catalyst	k_{cat} (min^{-1})	K_m (mM)	k_{cat}/k_{uncat}	k_{cat}/K_M (min^{-1} M^{-1})	K_{tx}^{-1}(M^{-1})
1	20	1.42	4760	14,080	3352
2	1.37	0.40	163	3,390	407
Cu-tren-CD	10	0.73	2380	13,700	3260
Cu-tren	0.165	1.17	39	141	33



Scheme 3. Representation of possible mechanisms for DNDPC hydrolysis by complex **1**.

rate constants of their catalysis, the hydrophobic cavity of Cu-tren-CD for substrate recognition is responsible for this high activity of Cu-tren-CD catalysis, however, for complex **1** catalysis it can be attributed to a favorable contribution of the guanidinium moiety for transition-state recognition, because in this case the hydrophobic cavity of CD in **1** has been occupied by adamantane moiety. Apparently, the high catalytic efficiency of our enzyme model **1** is due to synergetic action of the catalytic center and the allosteric site which tuning the structure and catalytic efficiency in hydrolysis process.

3.3. Mechanism of hydrolysis reaction

The pH-rate profiles for DNDPC cleavage catalyzed by complex **1** are depicted in Fig. 5. The rate of DNDPC cleavage increases with increasing pH under the same condition. The result suggested a general basic catalysis mechanism for hydrolysis of DNDPC [32]. From the kinetic results, the mechanism of DNDPC hydrolysis catalyzed by allosteric complex **1** was conjectured as shown in Scheme 3. Firstly, a nucleophilic attack by the Cu(II)-OH on ester carbonyl carbon generates a tetrahedral transition state, the guanidinium group of the supramolecular enzyme model stabilized the oxyanion of the tetrahedral intermediate formed by the attack on the scissile carbonyl bond through electrostatic interactions, and finally the product was obtained.

4. Conclusions

In summary, we have successfully constructed an allosteric hydrolase model by supramolecular combination of a catalytic metal center and an allosteric site on cyclodextrin. In this allosteric model guanidinium group acts as an allosteric site and copper (II) ion as a catalytic center. Taking advantage of the microenvironment provided by guanidinium group, this supramolecular catalyst demonstrates strong transition state binding ability and high catalytic efficiency for the hydrolysis of a carbonate substrate. Analysis of the catalysis kinetics indicates that the cooperativity between the guanidinium group and metal center is an important factor for the rate enhancement of carbonate hydrolysis. In artificial enzyme design, although synthetic organic chemistry has had tremendous success in past years, an attractive alternative is the development of self-assemblied supramolecular catalyst which can model specific aspects of biological catalysis. Supramolecular catalyst is an increasing new field in biomimetic chemistry due to its benefits: the high degree of assembly usually makes the preparation easier than that of the covalently synthesized analogue. The highly precise assembly processes lead to self-controlled and equilibrium-controlled structures and these structures often show interesting dynamic behaviors, for example, the allosteric property in our model. It is possible to create supramolecular catalysts with distinct properties that meet the requirements of a given reaction. With this, the large potential of supramolecular assemblies as synthetically useful tools in biomimetic chemistry becomes apparent.

Acknowledgments

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