Organic & Biomolecular Chemistry

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Cite this: *Org. Biomol. Chem.*, 2011, **9**, 8220

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COMMUNICATION

Construction of a tunable metallohydrolase center on an invertible molecular pocket†

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Received 15th July 2011, Accepted 19th September 2011
DOI: 10.1039/c1ob06174g

A trimer of cholic acid made by linking three cholic acid molecules with pentaerythritol *via* click chemistry serves as the center of an artificial metallohydrolase after the complexation of the triazole groups with a Zn^{2+} ion. The invertible amphiphilic cavity can respond to the change in polarity of the solvent media and efficiently modulate the catalytic activity of the artificial enzyme.

Enzymes evolve into sophisticated three-dimensional structures and catalyze diverse reactions with remarkably high rates. Considerable efforts have been devoted for several decades to the development of catalysts with enzyme-like tailored properties by rationally designing the host molecules.[1,2] The design of small molecule-based artificial enzymes has received much attention. Cholic acid has a facial amphiphilic structure (one face is hydrophilic, the other is hydrophobic) and has been widely used as a building block in the construction of molecular devices responding to the stimuli of solvents.[3–6] Oligomeric cholic acid derivatives have been investigated as molecular containers or carriers by forming a hydrophobic pocket in aqueous solutions.[3–6] Potential applications include delivery of molecules in the trans-bilayer movements,[7,8] formation of ion channels,[9–11] hydrogelator[12,13] and the detection of metal ions by oligo-foldamers.[1,14] However, the study of solvent-stimulated metallic complexes as enzyme models is rare.[15,16] Recently we have shown that a tripodal cholic acid derivative forms an invertible cavity depending on the polarity of the media.[17] The cholic acid trimers with 1,2,3-triazole groups can complex with multivalent metal ions.[18,19] Moreover, the trimeric cholic acid derivatives (the CA-trimer, as **1** and **2** in Fig. 1) have been shown to be excellent hosts, which can bind aromatic and aliphatic hydrophobic guest molecules. Therefore in host–guest chemistry, the CA-trimer has been widely used in the construction of functional supramolecular systems.[3–6]

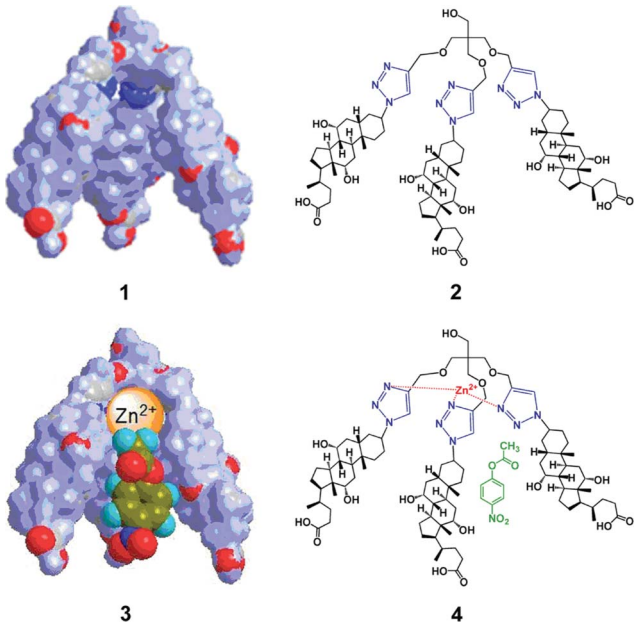

Fig. 1 The simulated structures and the chemical structures of the cholic acid trimer (CA-trimer) (**1** and **2**) and of the complex of Zn(II)–CA-trimer with the substrate PNPA (**3** and **4**).

It has been reported that 1,2,3-triazole could be used for general acid–base catalysis.[20,21] The 1,2,3-triazole group can provide an efficient coordination site to complex with metal ions; however, coordination chemistry of metallic ions with 1,2,3-triazole groups, especially those with substitution at positions 1 and 4, remains to be better understood.[22,23] Up to now, only very few results have been reported for coordination with Zn^{2+} ions to serve as catalysts.[24,25]

Given the particular amphiphilic structure of the cholic acid molecule and taking advantage of its good response to the surrounding media, we designed a metallohydrolase model based on the complex of a trimeric cholic acid derivative (CA-trimer) and Zn(II) ion with an invertible amphiphilic cavity. We used the tripodal cholic acid derivative linked through triazole groups to a pentaerythritol center as the host molecule, and a Zn^{2+} ion is introduced to the triazole ligand to serve as the catalytic metal center (shown as **3** and **4** in Fig. 1). The novel metallohydrolase model exhibits a strong capacity to catalyze the hydrolysis of a hydrophobic substrate, *p*-nitrophenyl acetate (PNPA) in mixtures

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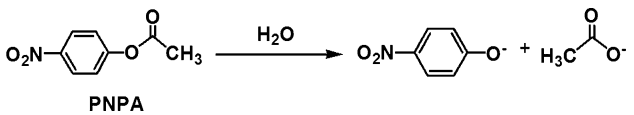
† Electronic supplementary information (ESI) available: Experimental details. See DOI: 10.1039/c1ob06174g

of water and DMSO. More interestingly, the amphiphilic molecule can respond to the polarity of the solvent. By changing the polarity of the solvent media, the catalytic activity can be tuned through observing the obvious change of the apparent rate constant k_{obs}. The development of an artificial enzyme with an amphiphilic pocket represents the first example of an invertible artificial enzyme model with media response.

To evaluate the catalytic capacity of the Zn(II)–CA-trimer complex, a typical substrate of hydrolase, PNPA, was selected as the substrate due to its neutrality (avoiding electrostatic interaction in the reaction system) (Scheme 1). High catalytic activity of the Zn(II)–CA-trimer complex for the hydrolysis of PNPA was observed. As shown in Fig. 2, the slopes of lines b and c are steeper than that of the line a, indicating that the hydrolysis rate of PNPA in the presence of the Zn(II)–CA-trimer complex is higher than its spontaneous hydrolysis under the same condition. In the presence of the Zn(II)–CA-trimer complex at 19.3 μM, the hydrolysis rate of PNPA became higher when the concentration of PNPA increased from 10 to 25 μM.



Scheme 1 The hydrolysis of the substrate *p*-nitrophenyl acetate (PNPA).

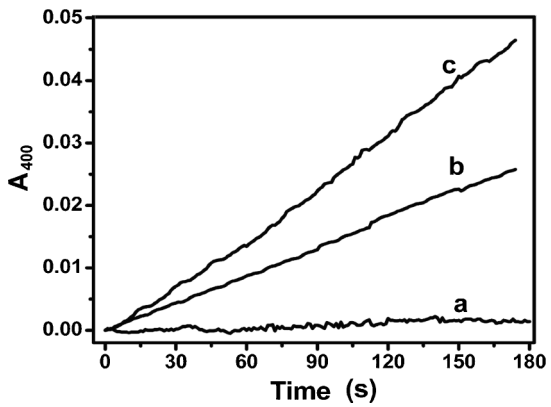


Fig. 2 The absorbance at 400 nm plotted as a function of time for the hydrolysis of PNPA assisted by 19.3 μM Zn(II)–CA-trimer complex (b and c) in a DMSO/HEPES mixture (20 : 80 v/v) at 25 °C and pH 7.0. (a) Control experiment, [PNPA] = 10 μM, no Zn(II)–CA-trimer complex; (b) [PNPA] = 10 μM; and (c) [PNPA] = 25 μM.

Fig. 3 shows that the catalytic activity of the Zn(II)–CA-trimer complex in the hydrolysis of PNPA is very different at different DMSO/water ratios. When DMSO content is less than 30 vol%, the rate constant k_{obs} of the Zn(II)–CA-trimer complex for the hydrolysis of PNPA is larger than that at other DMSO contents even though an abnormal rise is observed at *ca.* 90 vol% of DMSO, which is probably due to the presence of a limited buffer microenvironment surrounded by an excess of DMSO. The conformation of this "molecular basket"[13] made of three cholic acid residues is influenced by the polarity of the surrounding media. At a lower DMSO content, the hydrophobic sides of the cholic acid units point inward and its conformation would be similar to that of a mono-molecular micelle (Scheme 2, right). At

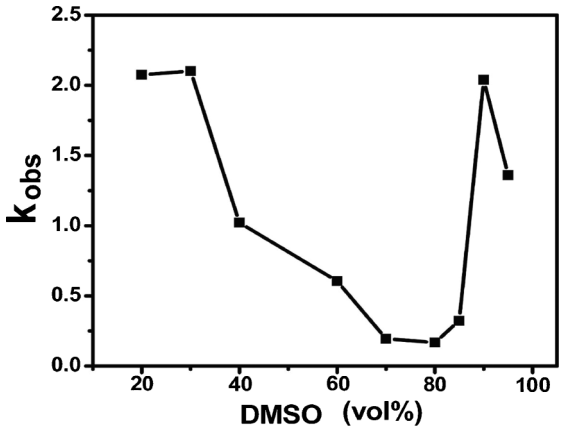
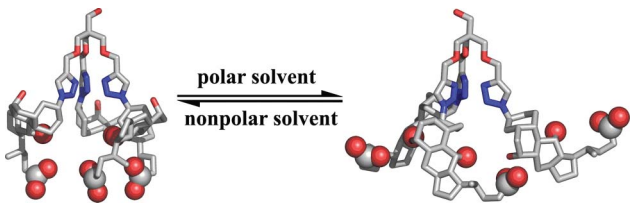


Fig. 3 The variation of the rate constant k_{obs} for the hydrolysis of PNPA (10 μM) catalyzed by the Zn(II)–CA-trimer complex (19.3 μM) at 25 °C and pH 7.0 with varying DMSO contents in the DMSO–water mixture (the concentrations cited here are the final concentrations in the DMSO–water mixture).



Scheme 2 The possible conformer of CA-trimer formed in polar solvent and a nonpolar solvent.

higher DMSO content the hydrophilic sides of the cholic acid units would now turn inward, and the conformation would be similar to that of a reverse micelle (Scheme 2, left). In comparison with more complicated multi-component constructs, this mono-component system has two obvious advantages: (1) a high catalytic capacity endowed by this simple host molecule; and (2) a better-controlled profile of the conformation of the complex due to the amphiphilic structure of the pocket in different solvent media. This new hydrolase model system works efficiently as a solvent-dependent metallic artificial enzyme, and exhibits excellent flexibility with an invertible catalytic compartment.

The apparent rate constant k_{obs} for the hydrolysis of PNPA by the Zn(II)–CA-trimer complex is obtained at various Zn^{2+} concentrations (Fig. 4). The value of k_{obs} increases with increasing Zn^{2+} concentration until the molar ratio of CA-trimer to Zn^{2+} approaches 1 : 1. This indicates that Zn^{2+} coordinates with the CA-trimer to form an equimolar complex. Unlike the findings by others with the 1,2,3-triazole substituents as ligands in catalysis,[21] each Zn^{2+} ion here is only involved in the complexion with one CA-trimer and there is no interference from the 1,2,3-triazole groups of any other trimer molecules.

As illustrated in Fig. 1, the hydrophobic cavity formed by the three cholic acid units of the CA-trimer favors the incorporation of the substrate to interact with the metal ion that forms a complex with the triazole groups of the trimer. In order to further understand the catalysis, a binding experiment was carried out. Fig. S1 (Supplementary Material†) shows that the absorbance of the complex of the CA-trimer and the substrate PNPA increases as the concentration of PNPA is raised. The results indicate

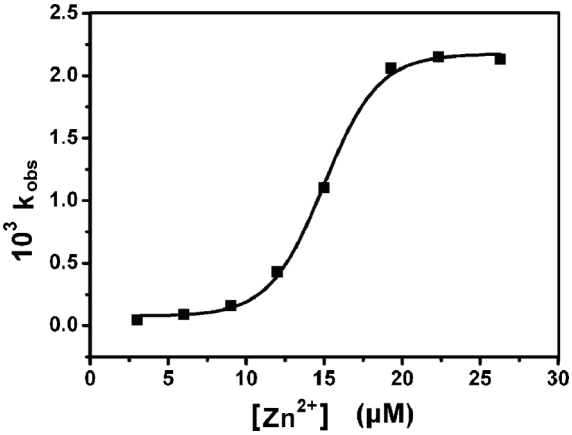
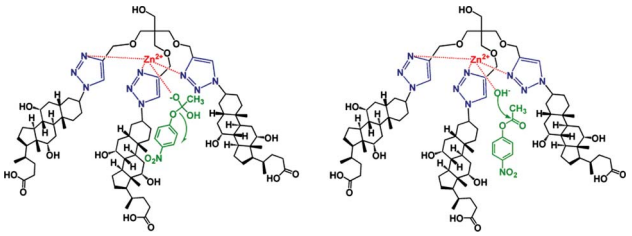


Fig. 4 Plots of k_{obs} for the hydrolysis of PNPA (10 μM) by the Zn(II)–CA-trimer complex (19.3 μM) as a function of Zn^{2+} concentration in the DMSO/HEPES buffer (2: 8 v/v) mixture at 25 °C and pH 7.0.

that the CA-trimer can bind to the substrate PNPA, leading to a facilitated hydrolysis of the substrate. To further demonstrate this binding capacity, a computer simulation was carried out and the oligomeric cholic acid derivatives showed an obvious binding capacity for PNPA with a ratio of 1 : 1 when the concentration of CA-trimer attained a certain value and the solvent was in appropriate proportions (Fig. 1).

In order to further elucidate the catalytic behavior of the Zn(II)–CA-trimer complex, the kinetics of the catalytic hydrolysis of PNPA by Zn(II)–CA-trimer were studied in detail. The experimental results are presented as typical double reciprocal plots and saturation kinetics plots, which show that the catalytic kinetics correspond well to the Michaelis–Menten equation.[26] These results indicate that Zn(II)–CA-trimer complex shows remarkable enzyme-like properties (Supplementary Material, Fig. S2A†). The kinetic parameters were calculated to be 0.1667 min^{-1} (k_{cat}) and 23.82 mM (K_M) when the concentration of the Zn(II)–CA-trimer complex was kept at 19.3 μM. The rate enhancement (k_{cat}/k_{uncat}) for the catalytic hydrolysis of PNPA by the Zn(II)–CA-trimer complex was found to be 1048. 10 turnover per cavity has been observed in one hour. The Zn(II)–CA-trimer complex shows a saturation behavior at pH 7.0 (Supplementary Material†, Fig. S2B). We found that at a higher concentration of PNPA, the reaction rate did not increase further, which is an indication that the catalytic center had been saturated by the substrate.

Fig. S3 (Supplementary Material†) shows that the observed rate constants for the hydrolysis of PNPA by the Zn(II)–CA-trimer complex increased with increasing pH values. The result shows that the mechanism of the hydrolysis of PNPA by the Zn(II)–CA-trimer complex is a general acid–base catalysis.[27] Under acidic conditions, the triazole groups can be easily protonated, preventing them from forming stable complexes. At higher pH values, the triazole groups become deprotonated and water molecules begin to coordinate with Zn^{2+} forming Zn–OH bonds which may attack the substrate to accelerate the hydrolysis of PNPA. In the neutral pH range, the possible mechanism of the hydrolysis of PNPA catalyzed by the Zn(II)–CA-trimer complex is shown in Scheme 3. The catalytic site of the Zn(II)–CA-trimer complex, Zn–OH, acts as general base or nucleophilic reagent to activate the C–O carbonyl bond to generate a tetrahedral transition state. The bond becomes



Scheme 3 Representation of the possible mechanisms for the hydrolysis of PNPA by the Zn(II)–CA-trimer complex.

easier to cleave and the product (*p*-nitrophenol salt) is then released.

In summary, a trimer of cholic acid linked to pentaerythritol through triazole groups has been designed to complex with a metal ion (Zn^{2+}) and to incorporate a model substrate (PNPA). This system has been shown to mimic the catalytic function of the metallohydrolase in the hydrolysis of the substrate. In comparison with the uncatalyzed control reaction, the rate enhancement (k_{cat}/k_{uncat}) for the PNPA hydrolysis catalyzed by this model enzyme was over 10^3 fold. Saturation kinetic studies showed that the catalytic process of the constructed metallohydrolase mimic follows the Michaelis–Menten equation, just as natural enzymes. The amphiphilic trimer of cholic acid can form a hydrophobic pocket in aqueous environments and invert its pocket in response to the polarity change of the media. The kinetic analysis showed that when the DMSO/H_2O ratio changed, the solvent surrounding the cholic acid unit had an obvious impact on the structure and the catalysis of the Zn(II)–CA-trimer complex and caused significant changes in catalytic rate. The results of this work show that the designed cholic acid trimer with an invertible amphiphilic pocket is a promising candidate for the design of tunable artificial enzymes.

We acknowledge the financial support from the Natural Science Foundation of China (No: 91027023, 20874036, 20921003, 21004028), the NSFC for Outstanding Young Scientists (No. 20725415), the National Basic Research Program (2007CB808006) and Key Laboratory of Rare Earth Chemistry and Physics, Changchun Institute of Applied Chemistry (RERU2011008), NSERC of Canada and the Canada Research Chair program. JZ thanks the China Scholarship Council for supporting her stay at U de M.

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