Supplementary Material

Construction of a Tunable Metallohydrolase Center on an Invertible Molecular Pocket†

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Experimental section

Preparation of Zn(II)−CA-trimer complex

The trimer of cholate derivative **1** tri(1-cholic acidyl-4-methoxy-1,2,3-trizolyl) pentaerythritol was prepared according to a previously reported procedure (refs. 13 and 16). DMSO was purchased from Tianjin Guangfu Research Institute. PNPA were purchased from Acros. MES (pH 6.0-6.5), HEPES (pH 7.0-8.2) and CHES (pH 8.6-10.0) were purchased from Sigma, the concentrations of buffer solutions were all at 50 mM. Other reagents were of analytical grade and used as received. Water used in the experiment was of Milli-Q grade. The kinetic studies were done on a Shimadzu 2450 UV-vis spectrophotometer, the pH value of the buffer was measured with a Mettler Toledo 320 pH meter.

DMSO solution of **1** was added to the $ZnCl_2$ aqueous solution, which was sonicated for 30 min, to obtain the Zn(II)-CA-trimer complex solution.

The simulation of binding was carried out by Hyperchem soft according to the Benesi-Hildebrand equation with structures shown in Figure 1.

Determination of rate constant (k_{obs})

The rate constant of pseudo first order reaction (k_{obs}) is calculated according to

$$-\ln[(S_0 - S_t)/S_0] = k_{obs}t$$

The kinetic constant k_{cat} was calculated from the initial velocity of each reaction at a fixed concentration of the enzyme and with increasing concentrations of the substrate. The initial Michaelis-Menten equation

$$v = V_{max}[S]/(K_m+[S])$$

can be expressed as

$$1/v = (K_M/V_{max})·(1/[S]) + 1/V_{max}$$

and

$$[E]/v = (K_M/V_{max})\cdot([E]/[S]) + [E]/V_{max}$$

The initial velocity of each reaction at a fixed concentration of Zn−**1** complex and with increasing concentration of the substrate was collected. We determined the kinetic constant k_{cat} from the Michaelis-Menten equation in the form of a double-reciprocal plot, also known as the Lineweaver-Burk plot, with a slope $K_M[E]/V_{max}$ and an intercept of $[E]/V_{max}$. The reciprocal of the intercept of the line ($V_{max}/[E]$) is called the kinetic conversions k_{cat}. The Michaelis constant K_M (K_M equals to the product of the slope and k_{cat}) may be obtained from the equation above. The Michaelis-Menten equation above was used for the fitting of substrate saturation kinetics curve. The k_{obs} measurement of each reaction at different pH values were obtained in the same cuvette by adding different buffers. In the hydrolysis system (0.5 ml) the concentration of PNPA and Zn−**1** complex was kept constant while the pH was changed with the buffers.

Origin 7.5 was used for data analysis. The kinetic constant k_{cat} was calculated from the initial velocity of each reaction with 3 parallel experiments. The substrate was consumed within 5% for each experiment. Before the half-life of the reaction, the simulated kinetic constant k_{obs} for PNPA fits well with the experiment data, while certain deviation appeared afterwards.

Kinetics

Stock solution (10 mM) of the substrate PNPA was prepared in water. All reactions with these solutions were performed in a 1 cm^3 quartz cuvette, which was placed within a thermally-equilibrated cell compartment (25.0 ± 0.1 °C) of the spectrophotometer coupled to a thermostatted water bath. The appropriate volume of the stock solution was placed in the quartz cuvette, followed by the addition of the required volume of HEPES buffer and thermally equilibration for 1 minute. The reaction conditions: [Zn(II)-**1**] = 19.3 μM, [PNPA] = 10 μM, 25°C, and pH 7.0. DMSO (20%, v/v) was added as co-solvent because of the limited water solubility of PNPA. The reaction rate v was calculated with the help of Beer-Lambert's law

$$v = c/t = A/\varepsilon bt$$

The reaction was initiated by the addition of a stock solution of the required substrate (10 μM) by a microsyringe with stirring. The whole volume of hydrolysis system was limited to 0.5 mL. Nitrophenolate anion formation was monitored at a wavelength 400 nm for the substrate PNPA. At the assay pH, the extinction coefficient for the product p-nitrophenolate anion was 4200, 8700, 12800, 16200, 17300 and 17800 L·cm^{-1}·mol^{-1} at pH values of 6.5, 7.0, 7.5, 8.0, 8.5, and 9.0, respectively.

In this system, the k_{obs} of for PNPA hydrolysis catalyzed by Zn(II)−**1** complex was collected at different DMSO/H$_2$O ratios under the same condition as above. After 3 parallel measurement, the catalytic efficiency of Zn(II)−**1** complex in the different solvents was

obtained. With Zn(II) or the host only, no rate enhancement was observed for the uncatalyzed PNPA hydrolysis with varying amounts of DMSO in the time limit of our experiments.

Determination of coordination ratio of Zn(II)−1 complex

The k_{obs} of the Zn(II)−**1** complex for PNPA cleavage was collected at different Zn(II) concentrations under the same condition as mentioned above. After 2 parallel measurements the ratio of complexation of the Zn(II)−**1** complex was obtained.

**The binding experiment between 1 and the substrate PNPA**

In the model system the change of absorbance of UV-vis area (200-700 nm) was recorded on the UV-vis spectrophotometer. The binding between **1** and PNPA was studied by keeping the PNPA concentration constant while varying the concentration of **1**. The reaction condition is the same as mentioned above. Three parallel measurements were done in the study of the binding between **1** and PNPA.
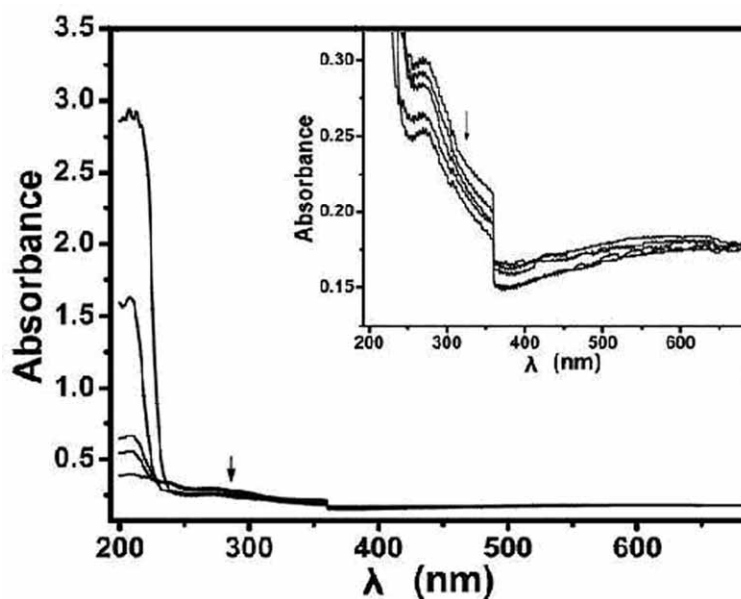
Figure S1. The UV-vis spectral change during the binding of the substrate PNPA by the CA-trimer (19.3 μM) in the DMSO/HEPES mixture (20:80, v/v) at 25 °C and pH 7.0 (The arrows indicate the PNPA concentration change from 2 to 20 μM).
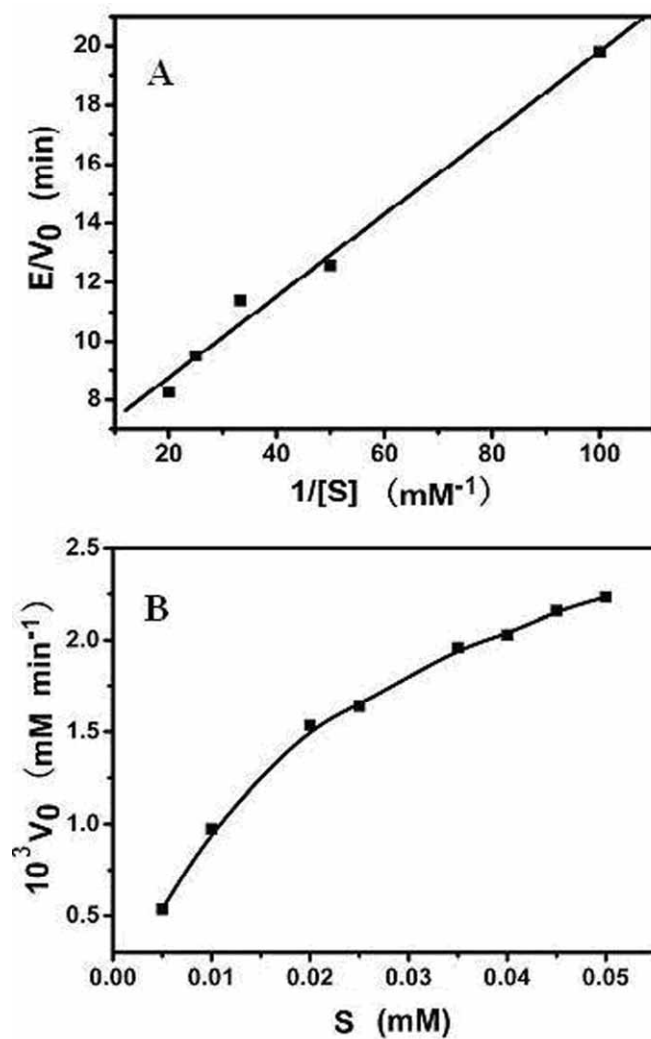
Figure S2. (A) Double-reciprocal plots and (B) diagram of saturation kinetics for the hydrolysis of PNPA catalyzed by the Zn(II)−CA-trimer complex (19.3 μM) the DMSO/HEPES (20:80, v/v) mixture at 25 °C and pH 7.0.
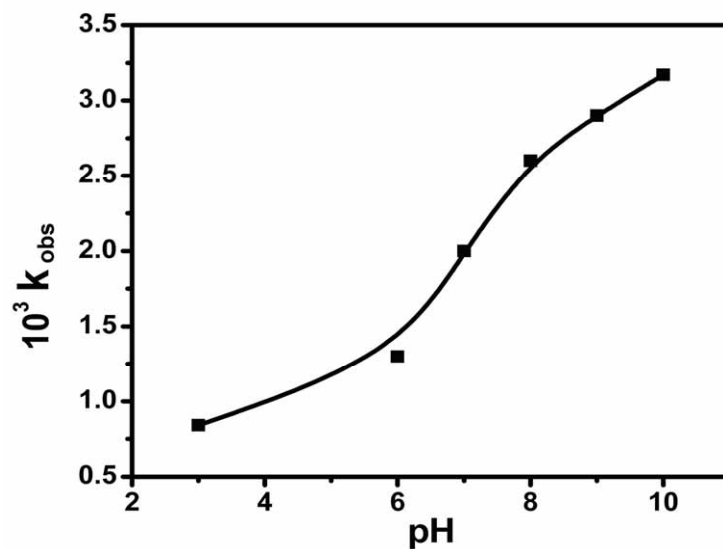
Figure S3. The dependence of the rate constant k_{obs} (s^{-1}) on the pH for the hydrolysis of PNPA (10 µM) catalyzed by the Zn(II)−CA-trimer complex (19.3 µM) in the DMSO/HEPES mixture (20:80, v/v) at 25 °C and pH 7.0.