Selective Molecular Recognition of Reactant, Product, and Transition State by Ni(II)-Macrocyclic Complexes Built on Poly(ethylenimine)

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Enzymatic catalysis may be described as the selective recognition and the consequent stabilization of transition states.[1] Catalytic antibodies are designed to recognize transition states selectively by using transition-state analogs as haptens.[2] Although recognition of stable guest molecules by synthetic host molecules has been extensively investigated,[3] recognition and stabilization of transition states by synthetic host molecules have been rarely achieved.[4]

Poly(ethylenimine) (PEI) derivatives have been developed as skeletons of artificial enzymes.[5-8] PEI is a globular polymer, and each PEI (MW ca. 60 000) molecule contains ca. 350 primary, ca. 700 secondary, and ca. 350 tertiary amino groups on the average. In a previous study, we reported construction of polyazamacrocyclic metal centers on PEI through condensation of PEI with dicarbonyl compounds in the presence of metal templates.[7] Benzoate anions were recognized by the metal centers created on PEI. Moreover, anionic esters were anchored by the metal centers and the nucleophilic attack by amino groups of PEI at the bound ester led to efficient deacylation. Incorporation of various structural elements to the macrocycle-containing PEIs is needed for improvement of their catalytic properties. In this paper, we report selective recognition of transition states, reactants, and products by closely related polymeric macrocyclic complexes.

Results and Discussion

The Ni(II)-template condensation of PEI with glyoxal, the simplest dicarbonyl compound, led to Ni(II)[PEI-GO] (A), whereas reduction of A with $NaBH_4$ in water produced Ni(II)[PEI-GO]H_2 (B).

Binding of benzoate anions C and D by A or B is illustrated in Figure 1, and the values of n (the number of C or D that can be bound per polymer molecule) and K_d (average dissociation constant of C or D bound to various binding sites of A or B) obtained therefrom are summarized in Table I. Much smaller K_d values obtained for A compared with B indicate that C and D are recognized by A better than by B.

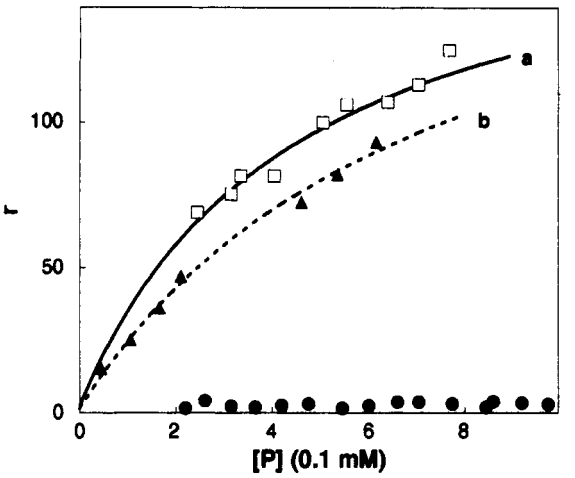
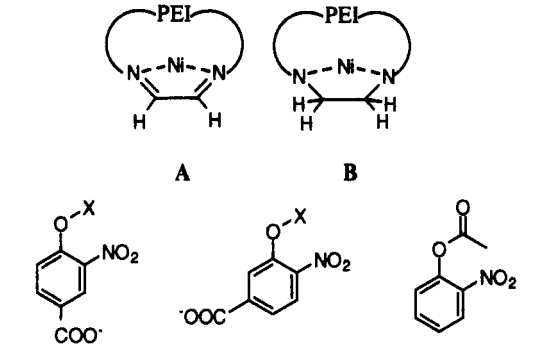


Figure 1. Plot[9] of r (number of anions bound per polymer molecule) against [P] (concentration of the anions unbound to the polymer) for binding of C (□: curve a) or D (▲: curve b) to A, and C (●) or D (data points are located close to ●) to B measured at 25 °C and pH 7.5.



Kinetic data (Figure 2) for deacylation of anionic esters AcC and AcD measured in the presence of A and B were analyzed according to the scheme of eq 1, which is

$$C + S \underset{K_m}{\rightleftharpoons} CS \xrightarrow{k_{cat}} \text{products} \quad (1)$$

$$k_o = k_{cat}C_o/(K_m + C_o) \quad (2)$$

analogous to the Michaelis-Menten scheme of enzymatic kinetics, and the corresponding rate expression of eq 2 derived under the condition of C_o (the initially added concentration of the macrocyclic metal center) $\gg S_o$.[7,10] Here, K_m is the dissociation constant for the substrate bound to the catalyst and k_{cat} the reactivity of the substrate which is completely bound to the catalyst.

Although saturation kinetic behavior is seen with A, k_o is proportional to C_o in the presence of B over the

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(10) The reactivity of neutral ester AcE relative to AcC or AcD, when analyzed as previously reported,[7] indicates that deacylation of AcC or AcD in the presence of A or B proceeds through complexation with the polymers, instead of direct collision with the polymers.

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Table I. Parameter Values Estimated from Binding and Rate Data Illustrated in Figures 1 and 2

compd	polymer	parameter	compd	polymer	parameter
C	A	$n = 184 \pm 16$	D	A	$n = 196 \pm 31$
C	A	$K_d = (4.47 \pm 0.81) \times 10^{-4}$ M	D	A	$K_d = (7.31 \pm 1.95) \times 10^{-4}$ M
C	B	$K_d \gg 10^{-3}$ M	D	B	$K_d \gg 10^{-3}$ M
AcC	A	$K_m = (2.18 \pm 0.22) \times 10^{-3}$ M	AcD	A	$K_m = (5.29 \pm 1.48) \times 10^{-4}$ M
AcC	A	$k_{cat} = (3.39 \pm 0.12) \times 10^{-3}$ s^{-1}	AcD	A	$k_{cat} = (4.51 \pm 0.32) \times 10^{-4}$ s^{-1}
AcC	B	$k_{cat}/K_m = 0.636 \pm 0.114$ s^{-1} M^{-1}	AcD	B	$k_{cat}/K_m = 0.440 \pm 0.027$ s^{-1} M^{-1}
AcC	B	$K_m \gg 0.01$ M	AcD	B	$K_m \gg 0.01$ M
AcC	B	$k_{cat} \gg 6.4 \times 10^{-3}$ s^{-1}	AcD	B	$k_{cat} \gg 4.4 \times 10^{-3}$ s^{-1}

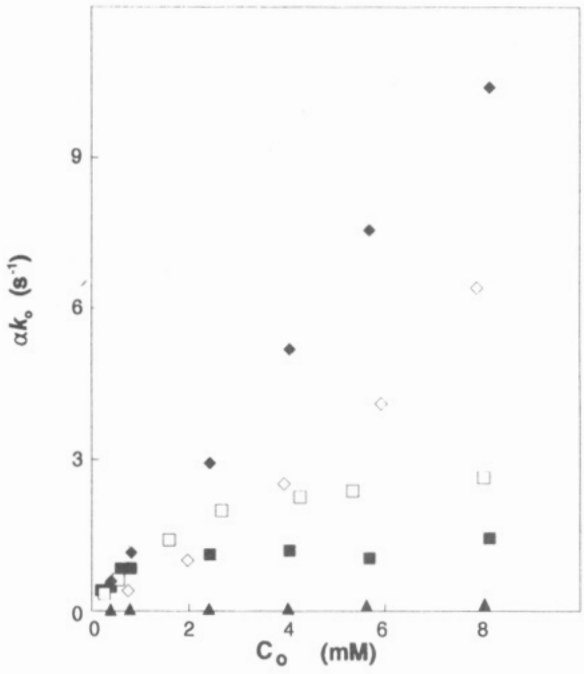


Figure 2. Plot of k_o (pseudo-first-order rate constant) against C_o measured under the condition of $C_o \gg S_o$ at 25 °C and pH 6.5 for deacylation of AcC (□), AcD (■), or AcE (▲) in the presence of A and AcC (◇), AcD (◆) or AcE (data points are located close to ▲) in the presence of B. Different α values are used for A (α = 1000) and B (α = 3000) in order to put their kinetic data on the same graphic scale.

concentration range examined. This indicates much greater K_m (Table I) for B compared with A.[11] Thus, the substrates are recognized by A much better than by B.

The rate for the deacylation reaction is considerably faster for B compared with A. The faster rate indicates greater stabilization of the transition state by B. The difference in the pseudo-first-order rate constant becomes greater as C_o is increased. This is due to the much greater k_{cat} for deacylation by B compared with that by A (Table I).[12]

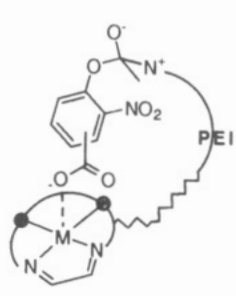
When the kinetics of the deacylation reaction of AcC were examined for A and B under the condition of $S_o > C_o$, biphasic behavior was observed, as noted previously for other macrocycle-containing PEI derivatives.[7] The amount of C released during the initial burst stage was identical with C_o, being independent of S_o, indicating that the acetylation of the amine residue located in the vicinity of the metal center inactivates the polymer.

(11) In the plot of k_o against C_o, curvature corresponding to half-saturation is to be seen at $C_o = K_m$. The linearity observed for k_o plotted against C_o indicates that K_m is much greater than the largest C_o concentration (eq 2).

(12) For AcD, k_o is ca. eight times greater for B compared with A at C_o = 8 mM (Figure 1). For A, k_{cat} is similar to k_o measured at this C_o. On the other hand, k_{cat} for B is much greater than the k_o measured at this C_o. The difference in k_{cat} is, therefore, much greater than 8-fold.

Selective recognition of the substrates and the products by A is consistent with the greater Lewis acidity of the metal centers of A compared with B due to the weaker basicity of the imine nitrogen ligands of A compared with the amine nitrogen ligands of B. In addition, the dπ–pπ back-bonding between Ni(II) and the two imine bonds in A may further enhance the Lewis acidity of the metal centers of A.

The greater stabilization of the transition state by B in spite of much poorer recognition of the substrate is not easy to explain rigorously, since various unknown types of polyazamacrocyclic centers are present on A and B. Selective binding of anionic esters by A and B and the stoichiometric acetylation of the A and B revealed by the burst kinetic behavior indicates that the deacylation of AcC or AcD proceeds through the nucleophilic attack of the amino group of PEI backbone at the ester anchored by the metal center, as proposed[7] previously. Thus, cyclic transition states are involved in the deacylation reactions. A simple explanation of the selective stabilization of the transition state by B is the reduced strain in the cyclic transition state. Different structures around metal centers of A and B may lead to relief of the strain of the cyclic transition state to different degrees (e.g., F and G), resulting in greater stabilization of the transition state by B. In F and G, C=N bonds of A are shorter than C–N bonds of B, and the dπ–pπ back-bonding in A may shorten the Ni(II)–N (imine nitrogen) bonds.



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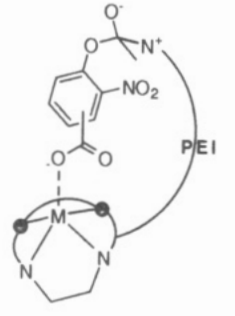

G

Analogous results have been observed with a purely organic host system, β-cyclodextrin (CD) derivatives. Breslow and co-workers were able to adjust the depth of the CD cavity through attachment of various bottoms to CD.[13] For the deacylation of an ester, K_m was ca. five times smaller for the native CD compared with the CD derivative with a shallower cavity, indicating better recognition of the substrate by the native CD. On the other hand, k_{cat} was ca. 18 times greater for the CD with a shallower cavity compared with the native CD, indicating better stabilization of the transition state. This has been

(13) Breslow, R.; Czarniecki, M. F.; Emert, J.; Hamaguchi, H. *J. Am. Chem.* **1980**, *102*, 762.

explained in terms of a better fit of the substrate to the CD with a deeper cavity and a smaller strain in the transition state for the CD with a shallower cavity.

Functions of synthetic molecules may be greatly improved through minute structural alterations. Fine structural adjustment of cyclic transition states is important in designing artificial enzymes. This is accomplished in this study by changing the geometry around the metal center. In this regard, coordination compounds can be useful in the design of effective biomimetic catalysts.

Experimental Section

PEI (MW 50 000–60 000, purchased from Aldrich or Sigma) was purified by ultrafiltration on a PM-30 (Amicon) membrane to remove portions with low molecular weights (<30 000). To an aqueous solution (50 mL) of glyoxal (1.81 g, 40% solution, 12.5 mmol) was added $NiCl_2 \cdot 6H_2O$ (9.7 g, 40 mmol) dissolved in 100 mL water, and the solution was stirred for 2 h at 55–60 °C. To the resulting mixture, kept at 55–60 °C, was added PEI (5.24 g, 122 residue mmol) dissolved in 20 mL of water over a period of 1 h, and the mixture was stirred for a further 20 min. Then, 2.5 mL of acetic acid was added to the mixture, which was refluxed for 72 h. The product (A: Ni(II)[PEI-GO]) was purified by dialysis against 12 L of water (once), 12 L of 0.1 N NaCl (three times), and 12 L of water (three times).

To an aqueous solution of Ni(II)[PEI-GO] (0.246 residue M, 100 mL) was added $NaBH_4$ (3.65 g) over a period of 1 h. An additional portion of $NaBH_4$ was added, when necessary, until no UV–vis spectral change was observed. The product (B: Ni(II)[PEI-GO]H_2) was purified by dialysis as described above.

Polymers A and B were obtained as light brown and light violet powders, respectively, upon freeze-drying the purified solutions. The Ni(II) ion was not removed by repetitive dialysis of A and B, indicating formation of tight metal centers. The amine nitrogens of PEI should be the ligands of Ni(II) ions in A and B, although the exact structures of the macrocyclic centers are not known as they are built on a polymer.[7,14] The C=N bond in A is reflected in the ir peak at 1680 cm^{-1}, which is not seen with B. ICP analysis of A and B indicated that the Ni(II) content is 6.6% of the monomer residues of PEI. Burst kinetic studies with AcC revealed the Ni(II) content of A and B of 7.5%.

4-Carboxy-2-nitrophenyl acetate (AcC) and 5-carboxy-2-nitrophenyl acetate (AcD) were obtained as described in the literature,[7,15] and 2-nitrophenyl acetate (AcD), 4-hydroxy-3-nitrobenzoic acid (C), and 3-hydroxy-4-nitrobenzoic acid (D) were obtained from commercial sources and used after recrystallization.

Binding studies and the kinetic measurements were carried out at 25 ± 0.1 °C with 0.05 M buffer (4-morpholineethanesulfonate at pH 6.5 and *N*-(2-hydroxyethyl)-1-piperazineethanesulfonate at pH 7.5) in the presence of 1.2% (v/v) acetonitrile which was added as the solvent for the stock solutions of substrates. For the binding of C or D to A or B, a solution of both the polymer and the benzoate anion contained in a dialysis tube was equilibrated against a solution containing only the benzoate. Narrow and tall dialysis tubes were used to make the concentration changes due to osmotic pressure negligible. By measuring the decrease in the concentration of the benzoate outside the dialysis tube after equilibrium was reached, the amount of the benzoate bound to the polymer was calculated. Rates of deacylation of AcC or AcD were measured spectrophotometrically by following the release of the nitrophenols.

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(14) As indicated previously,[7] the exact structure of the macrocycles formed on PEI (such as the number of nitrogen atoms interacting with the metal ions, the size of the chelate ring on the side of the PEI backbone, the extent of hydration of the imine bonds leading to the formation of carbinolamines, etc.) is not known. Ni(II) ion is bound to A very tightly, and the metal-binding imine linkages of A are not hydrolyzed appreciably over extended periods. These indicate that macrocyclic complexes are formed with both of the imine nitrogen atoms, originating from condensation with a glyoxal molecule, being coordinated to the same metal ion. Since B is obtained by the reduction of A, the macrocyclic structures of A would be conserved in B.

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