# Electrostatic and Hydrophobic Effects on Recognition and Deacylation of an Anionic Ester by Ni(II)-Macrocyclic Complexes Built on Poly(ethylenimine)

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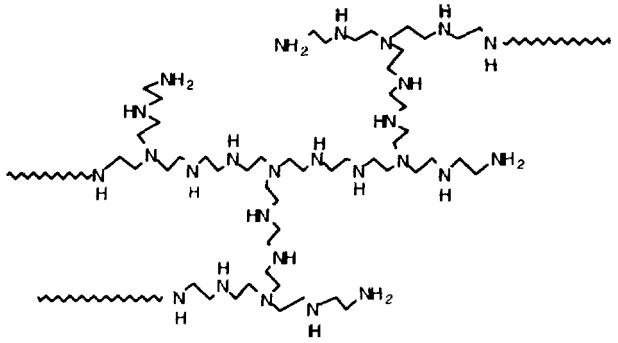
Three derivatives of poly(ethylenimine) (PEI) are prepared by Ni(II)-template condensation with glyoxal (GO): PEI[Ni(II)-GO]$_{0.08}$ (**1**), PEI[Ni(II)-GO]$_{0.03}$ (**2**), and lau$_{0.18}$PEI[Ni(II)-GO]$_{0.03}$ (**3**). The contents of Ni(II)-macrocyclic center of **1-3** are 8%, 3%, or 3%, respectively, of the monomer residues, and 18% of monomer residues for **3** are laurylated. The pH profiles for $k_{cat}$ and $K_m$ for the deacylation of 4-carboxy-2-nitrophenyl acetate are measured. The relative magnitude of the parameters for **1-3** and different shapes of the pH profiles for **1-3** are explained in terms of the electrostatic and the hydrophobic effects exerted by the metal centers and lauryl groups. For the artificial metalloenzymes built on PEI, therefore, the ionization of functional groups and the affinity toward counter-anions can be controlled by adjusting charge density and the content of hydrophobic groups.

## Introduction

Enzymatic catalysis is characterized by complex formation with substrates, various types of specificity, and high degree of acceleration. Synthetic catalysts manifesting these features can be considered as artificial enzymes.[1-9]

In order to design effective artificial enzymes, it is necessary to incorporate several catalytic features into the synthetic catalysts. In this regard, macromolecules have various advantages over small molecules.[7,8] Poly(ethylenimine) (PEI) derivatives have been developed as skeletons of artificial enzymes.[8-11] Each PEI (M.W. *ca.* 60,000) molecule contains *ca.* 1400 ethylamine moieties. About 25% of the amines are primary, 50% are secondary, and the remaining 25% are tertiary. The tertiary amine nitrogens represent branching points on the polymer backbone, and PEI is, therefore, a globular polymer.

In order to design artificial metalloenzymes, creation of



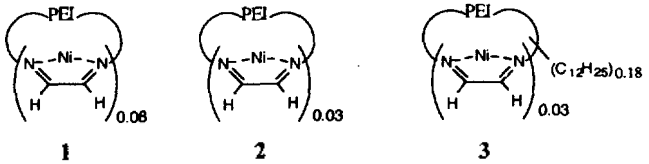
**PEI**

very tightly bound metal centers is needed. In this regard, we have reported construction of polyazamacrocyclic metal centers on PEI through condensation of PEI with dicarbonyl compounds in the presence of metal templates.[9,10] The metal ions of the macrocycles built on PEI do not dissociate even by repetitive dialysis. Benzoate anions are recognized by the metal centers created on PEI. Moreover, anionic esters are anchored by the metal centers and the nucleophilic attack by amine groups of PEI at the bound esters leads to efficient deacylation.

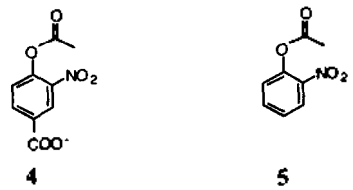
Incorporation of various catalytic elements to the macrocycle-containing PEIs is needed for improvement of their catalytic properties to obtain effective artificial metalloenzymes. In this paper, we report the effects of the electrostatic and the hydrophobic nature of microenvironments on the kinetic behavior of the macrocycle-containing PEIs.

## Results

Three different PEI-based Ni(II)-macrocyclic complexes were prepared in this study: PEI[Ni(II)-GO]$_{0.08}$ (**1**), PEI[Ni(II)-GO]$_{0.03}$ (**2**), and lau$_{0.18}$PEI[Ni(II)-GO]$_{0.03}$ (**3**). As noted previously,[10] **1-3** do not represent the correct structures of the macrocycles built on the polymer. Instead, they show the composition of the polymeric macrocycles. Thus, **1** and **2** were prepared by the Ni(II)-template condensation of PEI with glyoxal (GO) and **3** by that of lau$_{0.18}$PEI (18% of the amine nitrogens laurylated) with GO. The Ni(II) content is *ca.* 8% of the monomer residues of PEI in **1** and *ca.* 3% in **2** and **3**.



**1** **2** **3**



**4** **5**

The kinetics of deacylation of **4** was measured under the conditions of $C_o$ (initially added concentration of **1-3**; taken as the concentration of metal centers) $\gg S_o$ (initially added concentration of esters). The pseudo-first-order rate constants thus obtained were analyzed according to the scheme of Eq. (1) and the corresponding rate expression of Eq. (2).[10]

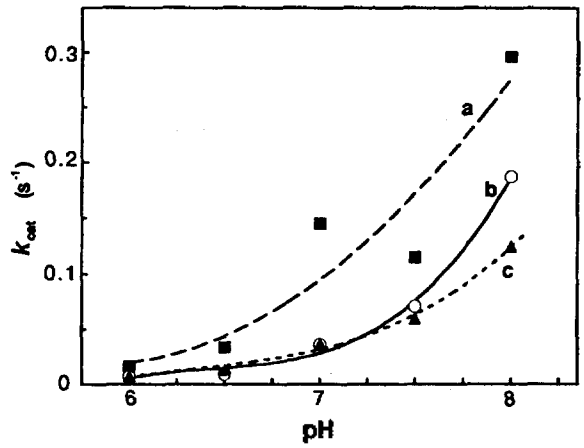



**Figure 1.** pH profile of $k_{cat}$ for the deacylation of **4** by **1** (curve **a**) **2** (curve **b**), and **3** (curve **c**) at 25℃.

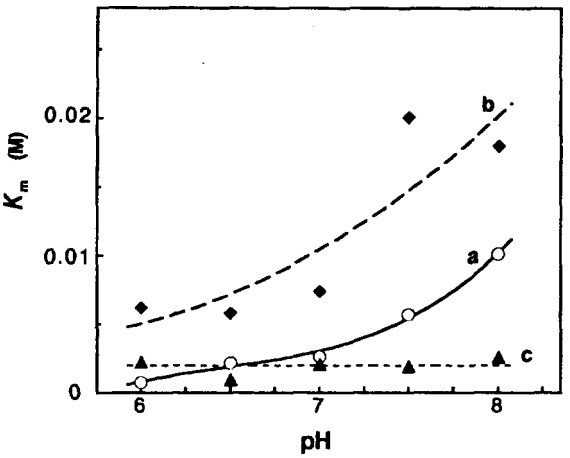



**Figure 2.** pH profile of $K_m$ for the deacylation of **4** by **1** (curve **a**) **2** (curve **b**), and **3** (curve **c**) at 25℃.

The scheme of Eq. (1) is similar to the Michaelis-Menten scheme of enzymatic kinetics. The values of $k_{cat}$ and $K_m$ measured at pH 6-8 for **1-3** are illustrated in Figures 1 and 2, respectively.

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

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

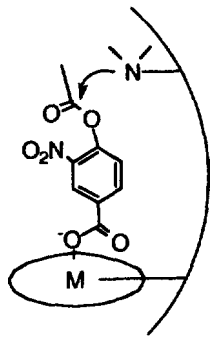
Deacylation of **4** in the presence of **1-3** under the conditions of $C_o<S_o$ manifested biphasic kinetic behavior with fast initial step followed by slow second phase. The second phase was attributable to spontaneous deacylation. 4-Carboxy-2-nitrophenol released during the initial burst stage corresponded to $C_o$ irrespective of $S_o$, as reported previoulsy for other derivatives.[10] Deacylation of **5**, the neutral analog of **4**, by **1-3** was much slower than that of **4** when measured under the conditions of $C_o \gg S_o$.

## Discussion

Much faster deacylation by **1-3** of **4** compared with **5** indicates that the metal centers of **1-3** recognize the anionic ester.[10] Results of the biphasic kinetis measured with **4** show that acylation of amino groups inactivates the metal centers. These confirm the mechanism (**6**) proposed previoulsy.[10] Here, the metal center anchors the benzoate anion of **4** and a proximal primary or secondary amino group located on the polymer backbone makes nucleophilic attack at the bound ester.

Parameter $K_m$ can be taken as the dissociation constant of complex CS and $k_{cat}$ the first-order rate constant for the deacylation within complex CS. Greater $K_m$ values indicate weaker complexation between C and S, whereas greater $k_{cat}$ correspond to faster attack of the amino group at the complexed ester.

The pH profile of $k_{cat}$ (Figure 1) shows that $k_{cat}$ increases




**6**

as pH is raised of **1-3** and that $k_{cat}$ of **1** is considerably greater than those of **2** or **3**. The smaller $k_{cat}$ at lower pH is consistent with the protonation of nucleophile amines. The magnitude of $k_{cat}$ for **2** compared with those of **1** and **3** is, however, rather unexpected in view of its greater content of unmodified amines. Primary amines are condensed with ketones to form stable imines and laurylation of PEI is known[12] to occur mainly on the primary amines. In **1** and **2**, 16% and 6%, respectively, of the total amines are condensed with GO. In **3**, 6% are condensed with GO and 18% are laurylated.

The relative size of $k_{cat}$ for **1-3** is accounted for by considering the microenvironments on **1-3** as well as the nucleophilic attack by both primary and secondary amines of PEI. The magnitude of cationic charges in **1** is considerably greater than that in **2**. Thus, it is expected that protonation of amines would be more suppressed on **1** than on **2**. Although the Ni(II) content of **3** is very similar to that of **2**, the microenvironments on **3** would be much more hydrophobic compared with those on **2**, again suppressing protonation of amines. Suppression of the protonation of amine nitrogens on the polymer backbone can raise $k_{cat}$.

Dissociation constant of CS, $K_m$, is considerably greater for **2** compared with **1** or **3**. The content of macrocyclic metal centers is smaller for **2** than for **1**. This would lead to smaller affinity (greater $K_m$) of **2** toward anionic ester **4**. Although the content of the metal center is almost the same for **2** and **3**, the hydrophobic environments on **3** would facilitate electrostatic interaction between the metal center and the anionic substrate. Thus, much smaller $K_m$ (stronger binding) for **3** compared with **1** is attributable to the hydrophobic microenvironments on **3**.

The $K_m$ for **1** or **2** decreases as pH is lowered, indicating

that protonation of amines further assists complexation of the cationic polymers with the anionic ester. On the other hand, $K_m$ for **3** is independent of pH. This may be again attributed to the hydrophobic environments. On the hydrophobic surface of **3**, electrostatic interaction between the metal centers and the anionic substrate could be strong enough and formation of additional positive charges by amine protonation may not affect the binding constants.

In summary, an increase in the content of macrocyclic metal centers on PEI results in greater cationic charge density, suppressed protonation of the amine moieties, and enhanced affinity toward the anionic substrate. Introduction of hydrophobic groups to the PEI-based macrocyclic complexes also leads to suppressed amine protonation and the enhanced binding of the anionic substrate. For the artificial metalloenzymes built on PEI, therefore, the ionization of functional groups and affinity toward counter-anions can be controlled by adjusting the charge density and the content of hydrophobic groups.

## Experimental Section

PEI (M.W. 50,000-60,000) was purchased from Aldrich or Sigma and was purified by ultrafiltration with a PM-30 membrane (Amicon) to remove smaller (<30,000) portions. PEI was laurylated according to the literature[13] to obtain $lau_{0.18}$ PEI (18% of the monomer residues laurylated).

The Ni(II)-template condensation of PEI or $lau_{0.18}$PEI with glyoxal was carried out as follows. To an aqueous solution (50 m*l*) of glyoxal (1.81 g, 40% solution, 12.5 mmol), $NiCl_2 \cdot 6H_2O$ (9.7 g, 40 mmol) dissolved in 100 m*l* water was added and the solution was stirred for 2 h at 55-60℃. To the resulting mixture kept at 55-60℃, PEI (5.24 g, 122 monomer residue mmol) dissolved in 20 m*l* water was added over a period of 1 h and the mixture was stirred for further 20 min. Then, 2.5 m*l* acetic acid was added to the mixture, which was refluxed for 72 h. After purification by dialysis against 12 L water (once), 12 L, 0.1 N NaCl (3 times), and 12 L water (3 times), $PEI[Ni(II)\text{-}GO]_{0.08}$ (**1**) (Ni content, 7.6% of the monomer residues) was obtained.

When the condensation was carried out with 22 mmol glyoxal, 67 mmol $NiCl_2 \cdot 6H_2O$, and 446 monomer residue mmol PEI, $PEI[Ni(II)\text{-}GO]_{0.03}$ (**2**) (Ni content, 2.9% of the monomer residues) was obtained. When the condensation was carried out with 7.8 mmol glyoxal, 24 mmol $NiCl_2 \cdot 6H_2O$, and 160 monomer residue mmol $lau_{0.18}$PEI, $lau_{0.18}PEI[Ni(II)\text{-}GO]_{0.03}$ (**3**) (Ni content, 2.9% of the monomer residues) was obtained.

The C=N bonds of the PEI-based macrocyclic complexes are reflected by IR peaks at 1650-1680 $cm^{-1}$. After lyophilization of the polymer solutions, light brown (**1** and **2**) or light yellow (**3**) powders were obtained. The Ni contents of the polymers were estimated both from the ICP analysis of Ni and from the amount of 4-carboxy-2-nitrophenol released during the initial burst stage in the polymer-promoted deacylation of 4-carboxy-2-nitrophenyl acetate (**4**) under the condition of $S_o > C_o$. Esters **4** and **5** (2-nitrophenyl acetate) were obtained as reported previously.[10]

Kinetic measurements were carried out spectrophotometrically as reported previously[10] at 25± 0.1℃ with 0.05 M buffer (4-morphoineethanesulfonate at pH 6-6.5 and *N*-(2-hydroxyethyl)-1-piperazineethanesulfonate at pH 7-8) in the presence of 1.2% (v/v) acetonitrile which was added as the solvent for the stock solutions of substrates.

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