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Macrocyclic Metal Complexes Built on Polyethylenimine

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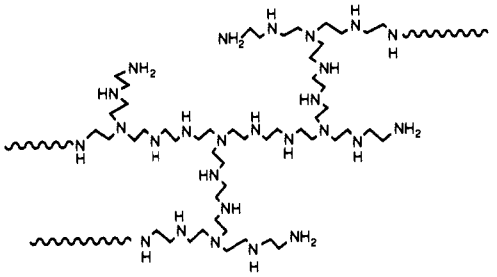
Abstract: Metal centers with macrocyclic ligands are created on polyethylenimine (PEI) by the condensation of dicarbonyl compounds with PEI in the presence of transition-metal ions. The macrocycle-containing PEIs possess fixed multivalent cationic centers and exhibit much greater affinity for benzoate anions compared with the unmodified PEI. Complex formation with anionic ester 2-nitro-4-carboxyphenyl acetate was reflected in the saturation kinetic behavior observed for the deacylation of the ester promoted by the macrocycle-containing PEIs. On the basis of the kinetic data, it is proposed that the anionic substrate is anchored by the metal center upon complexation with the macrocycle-containing PEI derivative and that the amine nitrogen atom located close to the metal center attacks the acyl carbon on the bound ester.

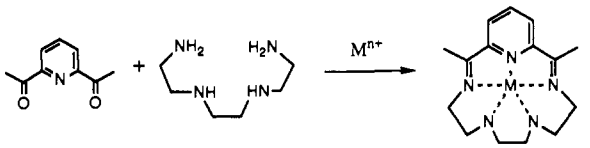
Polyethylenimine (PEI) and its derivatives have been used as synzymes (*syn*thetic en*zymes*),[1] since complex formation with substrates and catalytic turnovers of the bound substrates are achieved.[1-5] PEI is obtained by polymerization of ethylenimine and, thus, contain the ethylamine moiety as the repeating unit. About 25% of the nitrogen atoms of PEI are primary amines, 50% are secondary amines, and 25% are tertiary amines. The tertiary nitrogen atoms are the branching points on the polymer skeleton. Polycationic microenvironment is provided by the positive charges located on the nitrogen atoms of the polymer backbone, and hydrophobic microenvironment is obtained on the polymer domain by attaching alkyl chains to the nitrogen atoms. Some polar organic functional groups introduced to the polymer by alkylation or acylation of the nitrogens behave as catalytic groups.



PEI

Catalytic activity of macrocyclic metal complexes[6] has been intensively investigated. Aldehyde hydration,[7,8] ester hydrolysis,[9] phosphate hydrolysis,[10] and molecular recognition of small organic molecules[11] are among the reactions in which macrocyclic metal complexes mimic metalloenzymes. Recently, the utility of macrocyclic complexes became more versatile by combining macrocyclic complexes with cyclodextrin derivatives[12] or antibodies.[13] Redox reactions such as olefin epoxidation[14] and oxygen reduction[15] also have been subject to catalysis by macrocyclic complexes.

Many of the multiaza macrocyclic metal complexes are prepared by the condensation of carbonyl compounds with polyamines in the presence of metal ions,[6] as exemplified by eq 1.[16] In this



(1)

regard, PEI can be used as a synthon of the macrocyclic complex as well as the backbone of polymeric macrocycles. Since the ethylamine moiety is the repeating unit of PEI, it would be possible to prepare multiaza cyclic complexes with PEI. A typical commercial PEI has an average molecular weight of 60000, containing 1400 ethylamine moieties. Thus, a large number of macrocyclic

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domains can be built on PEI, resulting in polymeric macrocyclic complexes.

Efficient biomimetic systems might be obtained by combining the catalytic features of macrocyclic metal complexes with those of PEI, leading to the development of metallosynzymes. In this article, we report the first example of polymeric macrocyclic complexes built on PEI. In addition, deacylation of a complexed ester promoted by the macrocycle-containing polymers is described.

Experimental Section

Materials. Fe^{III}[PEI-DAP]. To an ethanolic solution (40 mL) of 2,6-diacetylpyridine (0.4 g, 2.6 mmol), an aqueous solution (40 mL) of $FeCl_2 \cdot 4H_2O$ (1.99 g, 10 mmol) was added, and the mixture was warmed to 60 °C. To the resulting solution, anhydrous PEI (0.43 g, 0.01 residue mol) dissolved in 3.5 mL of ethanol and 0.3 mL of acetic acid were added, and the reaction mixture was refluxed for 2–3 days. Then, the product was purified by dialyzing the mixture against 1:1 (v/v) ethanol–water, 0.1 M NaCl solution in 1:1 (v/v) ethanol–water, and then 1:1 (v/v) ethanol–water. The dark red product was obtained after evaporation of ethanol in vacuo and removal of water by lyophilization. Although ferrous chloride was used in the condensation reaction, the metal ion in the polymer is assigned as Fe(III). This is because Fe(II) is oxidized to Fe(III) during the preparation of related macrocyclic complexes in the presence of air.[17] Anal.: C, 36.59; H, 7.54; N, 16.16; Cl, 8.88; Fe, 6.85.

Co^{II}[PEI-GA]. This dark purple polymer was prepared according to the general procedure described above by using glutaraldehyde (0.10 g, 1 mmol), $CoCl_2 \cdot 6H_2O$ (0.71 g, 3 mmol), and PEI (0.43 g, 0.01 residue mol). Anal.: C, 35.59; H, 8.40; N, 15.26; Cl, 16.79; Co, 4.99.

Zn^{II}[PEI-GA]. This pale yellow polymer was prepared according to the general procedure described above by using glutaraldehyde (0.10 g, 1 mmol), $ZnCl_2$ (0.54 g, 4 mmol), and PEI (0.43 g, 0.01 residue mol). Anal.: C, 37.72; H, 7.86; N, 16.52; Cl, 13.83; Zn, 9.75.

Ni^{II}[PEI-BD]. This pale brown polymer was prepared according to the general procedure described above by using 2,3-butanedione (0.086 g, 1 mmol), $NiCl_2 \cdot 6H_2O$ (0.71 g, 3 mmol), and PEI (0.43 g, 0.01 residue mol). Anal.: C, 33.93; H, 8.73; N, 17.56; Cl, 13.74; Ni, 7.64.

2-Nitro-4-carboxyphenyl acetate (NCPA) was prepared according to the literature,[18] mp 151–152 °C (lit.[18] 152 °C). **4-Hydroxy-3-nitrobenzoic acid** (HNB) and **2-nitrophenyl acetate** (NPA) were obtained from commercial sources and were used after recrystallization. PEI with an average molecular weight of 60 000 was a gift from Dow Chemical Co.

Measurements. Binding experiments of Ni(II) to PEI or those of HNB to PEI and macrocycle-containing PEIs were performed with an apparatus that consisted of two compartments which were separated by a dialysis membrane. Initially, the polymer was put in one compartment and Ni(II) ion or HNB was contained in the other. After equilibration was completed, the concentration of Ni(II) ion or HNB unbound by the polymer was determined. The concentration of HNB was measured spectrophotometrically. The concentration of Ni(II) was estimated by converting it into $Ni(CN)_4^{2-}$ with CN^- and then measuring the concentration of the complex ion spectrophotometrically. Kinetics of deacylation reactions were measured spectrophotometrically. Spectrophotometric measurements were performed with a Beckman Du-64 UV/vis spectrophotometer. pH was measured with a Dongwoo DF-215 pH meter. Buffers (0.05 M) used were 4-morpholineethanesulfonic acid (pH 6.5), *N*-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (pH 7–8), and borate (pH 8.5), and water was distilled and deionized. Thermodynamic and kinetic measurements were performed at 25 ± 0.1 °C (controlled with a Haake E12 circulator) and in the presence of 0.8 (v/v) acetonitrile which was used as the solvent for stock solutions of substrates. Elemental analysis was performed by Galbraith Laboratories, Inc., Knoxville, TN.

Results and Discussion

Binding of Ni(II) to PEI. Since PEI contains repetitive ethylamine units, PEI itself can act as a multidentate ligand for metal ions. A part of the nitrogen atoms of PEI are protonated unless pH is very high and, therefore, PEI is a cationic polyelectrolyte. The electrostatic repulsion between PEI and metal ions, however, would suppress metal binding to PEI. In addition, metal ions bound to PEI would exert unfavorable electrostatic effects on one another.

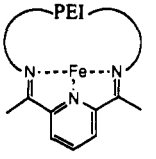
In order to gain insights into the interaction of metal ions with PEI, the binding constant for Ni(II) ion to PEI was measured at pH 7.0 and 25 °C. After equilibrium was attained between PEI and Ni(II) ion, the values of r (number of metal ions bound to each polymer) and [L] (the concentration of the metal ion uncomplexed by the polymer) were measured. A plot of $1/r$ against $1/[L]$ (range of [L] used in the plot: 1.1×10^{-5} to 2.8×10^{-4} M) according to eq 2[19] led to K_d (dissociation constant

$$1/r = 1/n + (K_d/n)(1/[L]) \qquad (2)$$

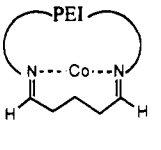
of the bound ligand ion from the polymer) = $(7.6 \pm 1.0) \times 10^{-5}$ M^{-1} and n (number of binding sites per polymer molecule) = 17.5 ± 2.4. The linear relationship between $1/r$ and $1/[L]$ indicates that the bound metal ion exerts no electrostatic influence on the succeeding binding when the number of bound metal ions per polymer molecule is less than n.[19]

On the basis of the K_d value, it is expected that more than 20% of the initially bound Ni(II) ion dissociates spontaneously when the concentration of the PEI-bound Ni(II) ion is 1×10^{-4} M. When the Ni(II)-containing PEI is dialyzed several times, Ni(II) ion dissociates completely. Thus, although Ni(II) ion is bound fairly strongly by PEI, it does not create a fixed multivalent cationic center on the polymer.[20] To devise a metallosynzyme with tightly bound metal ions, therefore, it is necessary to create new chelating sites on PEI.

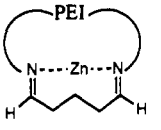
Preparation of PEI-Based Macrocyclic Complexes. Polymeric macrocyclic complexes were prepared by condensation of PEI with diketones in the presence of various metal ions. The macrocycle-containing PEIs were purified by repetitive dialysis. The PEI-based macrocyclic complexes prepared in the present study are indicated by A–D. Each of A–D show the nature of the metal



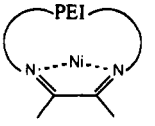
A (Fe(III)[PEI-DAP])



B (Co(II)[PEI-GA])



C (Zn(II)[PEI-GA])



D (Ni(II)[PEI-BD])

ion and the diketone used in the condensation, instead of the exact structure of the complex. Thus, A is prepared by the metal-template condensation of PEI and 2,6-diacetylpyridine, B and C by that of PEI and glutaraldehyde, and D by that of PEI and butanedione. On the other hand, the extent of hydration of the imine bonds leading to the formation of carbinolamines, the number of the nitrogen atoms interacting with the metal ions, and the size of the chelate ring on the side of the PEI backbone are not known. In addition, the macrocyclic metal centers may contain more than one dicarbonyl moiety.

The contents of the metal ions in A–D were calculated on the basis of the results of elemental analysis (C, H, N, Cl, and metals). The amount of the metal ion per monomeric ethylamine unit was estimated as 12% for A, 7.8% for B, 13% for C, or 10% for D.[21] The PEI-based macrocycles manifested IR absorption peaks at 1620–1660 cm^{-1}, indicating the presence of imine double bonds.

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(21) The relative error of the metal content calculated for A–D may be estimated as ±20%, considering the uncertainties involved in the elemental analysis.

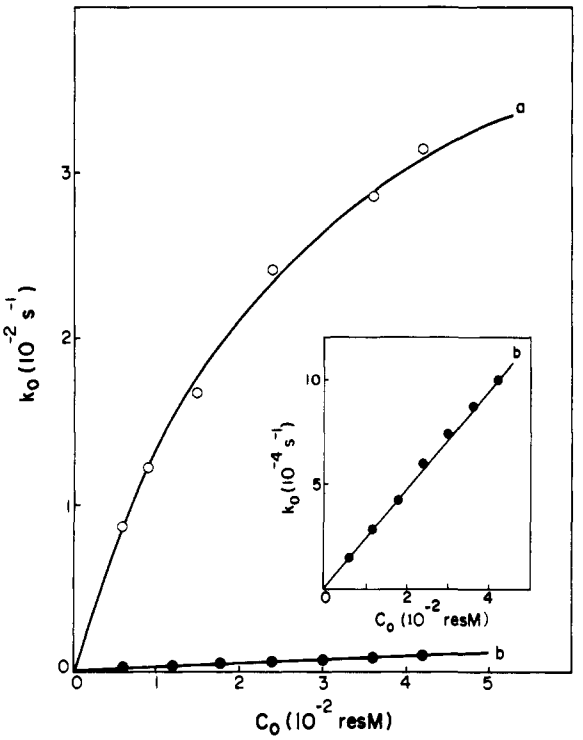
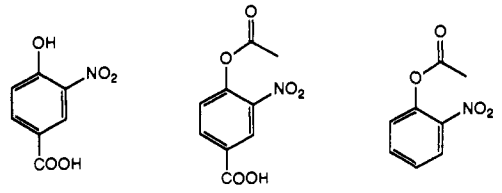


Figure 1. Plot of k_0 against C_0 for the deacylation of NCPA (line a, O) or NPA (line b, ●) in the presence of D at pH 7.5. The inserted diagram is an expanded view of line b.

The metal ions of the PEI-based macrocycles are very tightly bound to the polymer, as they do not dissociate during repetitive dialysis. In addition, the number of metal centers attached to each PEI molecule is fairly large. For example, when the content of the metal ion is 10% per monomeric ethylamine unit, about 140 metal ions are bound to each PEI molecule. Both the formation constant of the metal ions and the population of the metal ions are much greater for the macrocycle-containing PEI compared with unmodified PEI. These are attributable to the formation of macrocyclic complexes on the polymer.

Binding of HNB Anion to PEI and Macrocycle-Containing PEIs. The PEI derivatives containing macrocyclic metal complexes possess permanent cationic centers with multiple charges. In the unmodified PEI, however, ammonium ions are the only cationic center, which can be destroyed by proton transfer. In order to gain insights into the electrostatic effects exerted by the metal centers of the macrocycle-containing PEIs, binding of the anion of 4-hydroxy-3-nitrobenzoic acid (HNB) to the polymers was investigated.



HNB NCPA NPA

Analysis of the concentration of HNB anion (L of eq 2) bound to C and the initially added concentrations of HNB anion and the polymer according to eq 2 led to $K_d = (1.81 \pm 0.56) \times 10^{-4}$ M and $n = 17.8 \pm 2.4$ at pH 8.0. In the presence of the unmodified PEI ($[Pol]_0 = 5.5 \times 10^{-5}$ M), binding of HNB was not detected when the concentration of HNB was varied as $(0.3-5) \times 10^{-4}$ M, indicating that the affinity of HNB to PEI ($K_d > 5 \times 10^{-3}$ M) is much weaker than that to C. The polycationic environment provided by the macrocyclic metal centers of C, therefore, is considerably more effective than that by the protonated amines of PEI in attracting the HNB anion to the polymer surface.

Table I. Values of Kinetic Parameters for Deacylation of NCPA and NPA Promoted by PEI, Macrocycle-Containing PEI Derivatives, and Ethylenediamine at pH 7.5

substrate	nucleophile	k_{cat}, 10^{-2} s^{-1}	K_m,[a] 10^{-2} res M	K_m,[b] 10^{-3} M	k_2, 10^{-2} res M^{-1} s^{-1}
NCPA	PEI				88 ± 1
	A	2.5 ± 0.2	2.2 ± 0.2	(2.6 ± 0.2)	
	B	3.0 ± 0.4	2.7 ± 0.3	(2.1 ± 0.2)	
	C	2.3 ± 0.1	2.0 ± 0.1	(2.6 ± 0.1)	
	D	5.4 ± 0.3	3.1 ± 0.2	(3.1 ± 0.2)	
	ethylene-diamine				45 ± 2[c]
NPA	PEI				6.0 ± 0.6
	A				1.2 ± 0.1
	B				1.3 ± 0.1
	C				0.95 ± 0.03
	D				2.4 ± 0.1
	ethylene-diamine				13 ± 1[c]

[a] Estimated on the basis of C_0. [b] Estimated on the basis of $[Mac]_0$. [c] Unit: 10^{-2} M^{-1} s^{-1}.

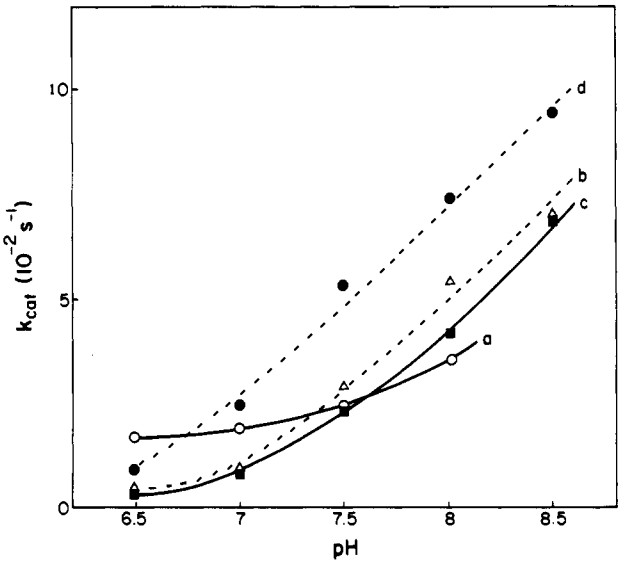


Figure 2. pH profile of k_{cat} for the deacylation of NCPA in the presence of A (line a, O), B (line b, Δ), C (line c, ■), or D (line d, ●).

Ester Deacylation Promoted by PEI-Based Macrocycles. Kinetics of the deacylation of 2-nitro-4-carboxyphenyl acetate (NCPA), the acetyl ester of HNB, and 2-nitrophenyl acetate (NPA) were measured in the presence of various macrocycle-containing PEIs.

The kinetics for the reaction of NCPA in the presence of the macrocycle-containing PEIs manifested saturation kinetic behavior, as exemplified in Figure 1, under the condition of $C_0 \gg S_0$ (C_0, total concentration of the catalyst;[22] S_0, initially added concentration of the substrate).[23] The dependence of k_0 on C_0

(22) Concentration of PEI and the macrocycle-containing PEIs are expressed in this paper as $[Pol]_0$ (polymer concentration calculated on the basis of the average molecular weight of 60 000 for PEI; unit is M), C_0 (residue molar concentration calculated on the basis of the monomer molecular weight of 43 for PEI; unit is res M), or $[Mac]_0$ (concentration of the macrocyclic metal center; unit is M). The C_0 concentration is $1400 \times [Pol]_0$. The metal content per monomeric ethylamine unit corresponds to $[Mac]_0/C_0$. The $[Pol]_0$ concentration was used in the estimation of K_d according to eq 2. Here, K_d stands for the dissociation constant for the complex of L formed with the polymer. In the analysis of kinetic data obtained with the PEI derivatives in terms of eqs 3–10, C_0 or $[Mac]_0$ were used, since the number (m) of the catalytic sites per monomer residue were unknown. The K_m values estimated from the kinetic data, therefore, stand for K_d/m.[2] The m value may be estimated by using the kinetic data measured under the condition of $C_0 \gg S_0$ and those measured under the condition of $C_0 \ll S_0$.[2] It was not possible, however, to obtain kinetic data under the latter condition due to the slow rates at low C_0 concentrations.

(23) Since the number of catalytic sites per polymer molecule (or per monomeric residue is not known, the concentration of the catalytic site is not correctly estimated. Whether the condition of $C_0 \gg S_0$ is met was tested with the dependence of k_0 on S_0, since k_0 is independent of S_0 only when $C_0 \gg S_0$.

was analyzed in terms of the scheme of eq 3 and the corresponding

$$S + C \underset{K_m}{\rightleftharpoons} CS \xrightarrow{k_{com}} \text{products} \quad (3)$$

$$k_0 = k_{cat}C_0/(K_m + C_0) \quad (4)$$

$$k_{cat} = k_{com} \quad (5)$$

rate expression of eqs 4 and 5, leading to the values of k_{cat} and K_m. The values of k_{cat} and K_m measured at pH 7.5 for the deacylation of NCPA in the presence of various polymeric macrocycles are summarized in Table I. The pH profiles of k_{cat} and K_m are illustrated in Figures 2 and 3, respectively.

The kinetics of NCPA in the presence of PEI and that of NPA in the presence of PEI or macrocycle-containing PEIs did not manifest saturation kinetic behavior when measured up to C_0 = 0.08 res M. Instead, k_0 was proportional to the polymer concentration (Figure 1). This proportionality, however, does not exclude the possibility of complexation between the substrate and the polymer. For the scheme of eq 3, k_0 is proportional to C_0 ($k_0 = k_{cat}C_0/K_m$) when $K_m \gg C_0$. The proportionality, therefore, indicates that $K_m \gg 0.08$ res M. The second-order rate constants (k_2) obtained as the proportionality constant (k_0/C_0) between k_0 and C_0 observed for these reactions are summarized in Table I. The k_2 values obtained for the reactions of NCPA and NPA with ethylenediamine are also indicated in Table I.

The values of k_2 for the reaction of ethylenediamine with NCPA and NPA reflect the relative intrinsic reactivity of NCPA and NPA toward aminolysis. The reaction of NCPA or NPA in the presence of PEI mostly leads to the acetylation of the amines of PEI.[24] The anionic ester NCPA might form a complex with a cationic polymer PEI, whereas the neutral ester NPA might not. This may lead to faster rates for NCPA. Toward PEI, NCPA is 15-times more reactive than NPA. On the other hand, NCPA is 3.5-times more reactive than NPA toward ethylenediamine. This difference may be taken to indicate that complexation between NCPA and PEI occurs, but the complexation is not very significant.

Some of the nitrogen atoms of PEI are protonated at neutral pHs. Although PEI is polycationic, complex formation with anionic substrate NCPA is weak. Since complexation of benzoate anion NCPA to the macrocycle-containing PEIs is very efficient as revealed by the kinetic data, the polycationic microenvironment created by the macrocyclic complexes on the macrocycle-containing PEIs is much stronger than that created by multiple ammonium cations on PEI.

Although the saturation kinetic behavior observed for the reaction of NCPA in the presence of macrocycle-containing PEIs does indicate the complexation between the ester and the polymer, it does not necessarily show that the observed deacylation is due to the reaction of the complex. For example, saturation kinetic behavior is also expected if the substrate is deacylated by direct attack of the polymer and if the substrate is totally protected from deacylation upon complexation with the polymer (scheme of eq 6 and the corresponding rate expression of eq 7).

$$\text{products} \xleftarrow{k_2} S + C \underset{K_m}{\rightleftharpoons} CS \quad (6)$$

$$k_0 = k_2K_mC_0/(K_m + C_0) \quad (7)$$

When the scheme of eq 3 is combined with that of eq 6, the scheme of eq 8 is obtained and the rate expression is derived as eqs 9 and 10.

$$\begin{array}{ccc} S + C & \underset{K_m}{\rightleftharpoons} & CS \\ \Big\downarrow k_2 & & \Big\downarrow k_{com} \\ \text{products} & \longleftarrow & \end{array} \quad (8)$$

$$k_0 = (k_2K_m + k_{com})C_0/(K_m + C_0) = k_{cat}C_0/(K_m + C_0) \quad (9)$$

$$k_{cat} = k_2K_m + k_{com} \quad (10)$$

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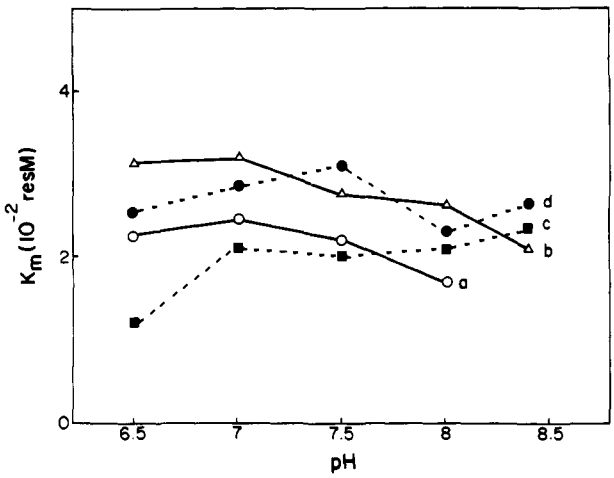
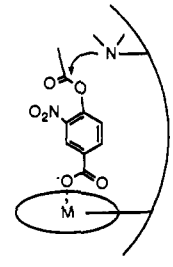


Figure 3. pH profile of K_m for the deacylation of NCPA in the presence of A (line a, ○), B (line b, △), C (line c, ■), or D (line d, ●).

The k_2 value measured for the direct attack of ethylenediamine at NCPA is 3.5-times greater than that at NPA. The k_2 value for the direct attack of the macrocycle-containing PEIs at NCPA also may be approximated to be similarly greater than that of NPA. On the basis of the k_2 value estimated in this way and the K_m value determined experimentally, the contribution of the k_2K_m term to the observed k_{cat} is estimated to be less than 5%. Therefore, the deacylation of NCPA in the presence of the macrocycle-containing PEIs occurs mainly through the reaction (k_{com} path) of the substrate complexed to the polymer.

To obtain information on the nature of the nucleophile that attacks at the complexed NCPA, release of HNB was followed in the reaction of D with excess NCPA. When $S_0 = (2.3\text{–}4.2) \times 10^{-4}$ M and $C_0 = 1.2 \times 10^{-3}$ res M ($[Mac]_0 = 1.2 \times 10^{-4}$ M), biphasic kinetic behavior was observed, with the first process being much faster than the second process. The rate constant of the second step was almost identical with the pseudo-first-order rate constant for the spontaneous hydrolysis of NCPA. The amount of HNB released during the fast step was estimated by extrapolation of reaction curve of the second process to the initial mixing time. The amount (average value of three measurements at different S_0 concentrations) of HNB released during the initial burst period was $(1.1 \pm 0.1) \times 10^{-4}$ M. Because the deacylation of the substrate inactivates the reaction center, the nucleophilic species on the polymer is assigned as the amine nitrogen. Since the number of the reaction site is very close to the number of the macrocyclic metal center, it is highly likely that the amine nitrogens located in the vicinity of the reaction site are the nucleophiles. It is proposed that the metal center anchors the anionic substrate and the amine located close to the ester linkage of the bound substrate attacks at the acyl carbon atom of the substrate (E) and that the acetylation of the amine inactivates the polymer.



E

The pH profiles of k_{cat} (Figure 2) reveal that k_{cat} measured at pH 8.5 is 10–15-times greater than that at pH 6.5 for the macrocycle-containing PEIs examined. The pH dependence of k_{cat} represents the relative concentration of the unprotonated amines of the complex E at various pHs. Unlike k_{cat}, K_m is not affected considerably by the pH change. Over the pH range investigated, the concentration of the macrocyclic metal centers does not change.

The pH profiles of K_m are consistent with the complexation mode indicated by E where the anion of NCPA is bound to the macrocycle-containing PEIs mainly through interaction with the macrocyclic centers, instead of the ammonium cationic centers.

Since each macrocyclic metal center appears to represent the reaction site for NCPA,[25] the K_m values (listed in parentheses in Table I) calculated on the basis of $[Mac]_0$ concentrations correspond to K_d. The K_d values measured for HNB and NCPA in the presence of C indicate that HNB forms a considerably stronger complex than NCPA. Spectral titration (not shown) indicated the $pK_a = 6.3$ for the phenol group of HNB. The major portion of HNB is present as a dianion at pH 7-8, and, therefore, would manifest greater affinity toward the polymer compared with the monoanionic NCPA.

In summary, macrocycle-containing PEIs are newly prepared in the present study by the condensation of PEI with dicarbonyl compounds in the presence of transition-metal ions. As a consequence of the condensation reaction, the metal ions are very tightly bound to the polymer. The polycationic environment created by the metal ions attracts benzoate anions. When an anionic ester is used, the anionic portion is anchored by the macrocyclic metal center of the polymer and a nearby amine of the polymer makes a nucleophilic attack at the bound ester linkage.

To develop artificial metalloenzymes that are capable of both recognition of the substrate structure and highly efficient catalytic conversion, it is desirable to incorporate several catalytic groups in planned positions. Efficient metallosynzymes may be obtained by tailoring PEI through creation of metal centers on the polymer and introduction of additional functional groups in proximal positions. This may be achieved by acylation of the nearby amines by using the metal centers as anchors. The macrocycle-containing PEIs may be viewed as polymers of macrocyclic metal complexes. For catalytic processes in which cooperation among multiple metal centers is necessary, the macrocycle-containing PEIs might lead to effective catalysts.

(25) The number (n) of binding sites estimated according to eq 2 represents the number of molecules of L that can be bound to the polymer without affecting binding of another L. It is possible that more than n molecules of L can be bound with less efficient K_d values. The n value of NCPA might not differ considerably from that of HNB. Even after an NCPA molecule acetylates a nitrogen atom of the polymer, however, the nearby metal centers can still bind NCPA leading to deacylation of NCPA, although K_d might be affected. This is why the number (ca. 140) of reaction sites for NCPA is much greater than n (ca. 18) for HNB.

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Solvation Effects in Solid-Phase Peptide Synthesis

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Abstract: The success of solid-phase peptide synthesis is highly dependent on the accessibility of the growing resin-bound peptide chain to reagents. To maximize this accessibility, the relationship between solvent properties and peptide–resin solvation has been explored. The tridecapeptide [Lys_9]-α-conotoxin G I (from *Conus geographus*), which contains nine side-chain-protected residues, was used to study solvation effects of protected peptide–resins. Efficient solvation of (aminomethyl)copoly(styrene–1% DVB) and peptide–copoly(styrene–1% DVB) could be directly correlated to solvent Hildebrand and hydrogen-bonding solubility parameters (δ and δ_h, respectively). Solvation was also highly dependent on the side-chain protecting group strategy (benzyl (Bzl), *tert*-butyl (tBu), or *p*-methoxybenzyl (Mob)) utilized. The most efficient solvation by a single solvent occurred with NMP, regardless of side-chain protection, although the relative solvation is greater for the Bzl-based versus tBu-based side-chain-protected conotoxin–resin. Mixed-solvent systems with optimized δ and δ_h values, such as 45% THF/NMP and 20% TFE/DCM, offered greater solvation than single solvents for the Bzl and tBu side-chain-protected conotoxin–resins. Solvation results for Mob side-chain-protected conotoxin–resin suggested that replacement of the tBu side-chain protecting group by the Mob group improves solvation by single solvents, such as NMP, while still providing the weak acid lability desired for side-chain deprotection following solid-phase peptide synthesis utilizing Fmoc chemistry.

Introduction

Since its inception in the early 1960s,[1] solid-phase peptide synthesis (SPPS) has become one of the most important methodologies in bioorganic chemistry. The success of this technique is highly dependent upon the accessibility of the free amino termini of the resin-bound peptide chains,[2] as the diffusion of reagents to resin reactive sites is not rate-limiting.[3-7] Therefore, efficient SSPS requires a thorough understanding of the physicochemical properties of the peptide–resin. ^{13}C and ^1H nuclear magnetic resonance (NMR) spectra of $CDCl_3/CHCl_3$-solvated copoly(styrene–1% divinylbenzene (DVB)) resins[8-10] and pulsed-field-gradient spin-echo NMR experiments of toluene-solvated copoly(styrene–5.7, 10, 20, or 40% DVB) resins[11] have shown that the linear chains are as accessible as if free in solution. This accessibility depends on the solvent and degree of DVB cross-

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