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Attachment of 1,5,9-Triazacyclododecane and β-Cyclodextrin to Poly(ethylenimine) in Proximity by Site-Directed Functionalization

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A novel methodology for the site-directed introduction of a functional group in the vicinity of β-cyclodextrin (CD) on the backbone of branched poly(ethylenimine) (PEI) is developed. Site-directed or random attachment of 1,5,9-triazacyclododecane (TC) to CD-containing PEI followed by acetylation of the primary and secondary amines of the polymer backbone produced [TC–CD]^{SD}AcPEI or [TC–CD]^{Ran}AcPEI, respectively. By the addition of Ni(II), Cu(II), or Zn(II) ion, [M(II)TC–CD]^{SD}AcPEI or [M(II)TC–CD]^{Ran}AcPEI was obtained. The formation constant for the complexation of a hydrophobic ester revealed that the TC portion was indeed positioned in the vicinity of the CD cavity in [M(II)TC–CD]^{SD}AcPEI. Compared with [M(II)TC–CD]^{Ran}AcPEI, [M(II)TC–CD]^{SD}AcPEI appears to exert an extra stabilization effect ($-\Delta\Delta G^\circ$ = 1.0–1.3 kcal/mol) on the complexed ester by hydrogen bonding between the metal-bound water of the polymer and the carbonyl group of the ester.

INTRODUCTION

Branched poly(ethylenimine) (PEI) has been intensively exploited as macromolecular backbone of artificial enzymes (*1*). Among ca. 1400 amino groups of PEI (M_r ca. 60,000), ca. 25% are primary amines, ca. 50% are secondary amines, and the rest are tertiary amines representing branching points. One of the major obstacles faced in design of artificial enzymes with synthetic macromolecules has been lack of specific binding sites. In a previous study (*2*), we attached β-cyclodextrin (CD) to PEI as a binding site. Thus, the CD-containing PEI (CD–PEI) selectively recognized esters with t-butylphenyl moieties resulting in acylation of amino groups near the CD cavity as illustrated in I (*2*).

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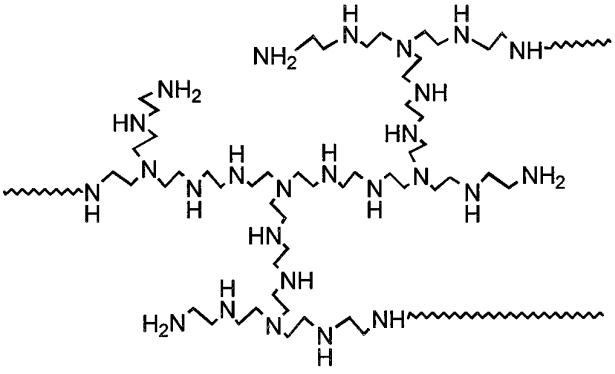
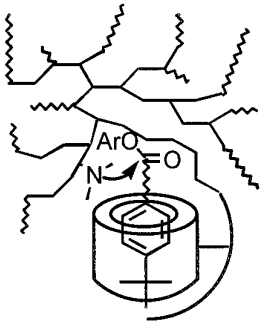


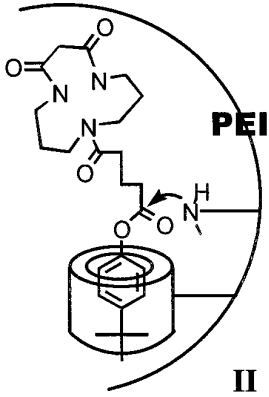
PEI



I

For designing effective artificial enzymes or receptors on synthetic macromolecules, it is necessary to develop a methodology to introduce an additional functional element in the vicinity of the functional group already present on the molecular backbone. In the case of biomimetic molecules based on CD–PEI, for instance, site-directed functionalization can lead to cooperative action by the CD moiety and the newly introduced functional element. Functionalization of CD–PEI can be also regarded as preparation of new derivatives (*3*) of CD.

In this study, site-directed functionalization of CD–PEI was attempted with a t-butylphenyl ester containing a precursor of 1,5,9-triazacyclododecane (TC) by taking advantage of recognition of t-butylphenyl moiety by CD as illustrated in II. TC was chosen as the additional functional group in view of catalytic activity of its metal complexes (*4*).



II

EXPERIMENTAL PROCEDURES

Materials

4-t-Butylphenyl 5-(6,8-dioxo-1,5,9-triazacyclododec-1-yl)-5-oxopentanoate (**1**). Glutaric acid (1.0 g, 7.6 mmol) was dissolved in 150 ml dimethyl formamide : methylene chloride (1 : 5, v/v). 1,3-Dicyclohexylcarbodiimide (1.4 g, 6.8 mmol) and 4-t-

butylphenol (0.92 g, 6.1 mmol) were added, and the solution was stirred for 2 h at room temperature. After the solvent was evaporated under a reduced pressure, 1 N HCl aqueous solution (10 ml) was added, and oily 4-t-butylphenyl hydrogen glutarate obtained through extraction with ethyl acetate was purified with silica gel (1 : 3 ethyl acetate : *n*-hexane). ^{1}H NMR ($CDCl_3$) δ: 1.25–1.30 (s, 9H), 1.90–2.20 (m, 2H), 2.35–2.60 (t, 2H), 2.45–2.75 (t, 2H), 6.85–7.05 (d, 2H), 7.25–7.45 (d, 2H). 4-t-Butylphenyl hydrogen glutarate (1.0 g, 4.8 mmol), 1,3-dicyclohexylcarbodiimide (1.2 g, 5.8 mmol), and 4-dimethylaminopyridine (0.1 g) were dissolved in 150 ml methylene chloride. 1,5,9-Triazacyclododecane-2,4-dione (1.1 g, 5.5 mmol) prepared according to the literature (*5*) was added, and the solution was stirred at room temperature for 2 h. After the solution was washed with aqueous 1 M $NaHCO_3$ to remove unreacted 4-t-butylphenyl hydrogen glutarate, the product was extracted with methylene chloride. Compound **1** was purified with silica gel (10 : 1 methylene chloride : methanol), and recrystallized from methylene chloride–*n*-hexane, mp 205–206°C. ^{1}H NMR (DMSO-d_6) δ: 1.26 (s, 9H), 1.70–1.85 (m, 2H), 2.30–2.40 (t, 2H), 2.52–2.62 (t, 2H), 2.95 (s, 2H), 2.98–3.34 (m, 12H), 6.97–7.04 (d, 2H), 7.37–7.45 (d, 2H), 7.90–8.02 (t, 2H). ^{13}C NMR (DMSO-d_6) δ: 171.8, 170.5, 167.1, 167.0, 148.2, 148.0, 126.1, 121.1, 47.0, 36.0, 35.7, 34.2, 32.9, 31.2, 30.8, 27.3, 26.6. MS (FAB, glycerol) *m*/*e* 446 (M + H). *Anal.* Calcd for $C_{24}H_{35}N_3O_5$: C, 64.68; H, 7.92; N, 9.43. Found: C, 64.35; H, 8.00; N, 9.38.

Phenyl 5-(6,8-Dioxo-1,5,9-triazacyclododec-1-yl)-5-oxopentanoate (**2**). This compound was synthesized and purified as described above for **1** except that phenol was used instead of t-butylphenol, mp 198–200°C. ^{1}H NMR (DMSO-d_6) δ: 1.65–1.90 (m, 2H), 2.30–2.50 (t, 2H), 2.52–2.68 (t, 2H), 2.95 (s, 2H), 2.98–3.34 (m, 12H), 6.97–7.55 (m, 5H), 7.90–8.02 (t, 2H). MS (FAB, glycerol) *m*/*e* 390 (M + H). *Anal.* Calcd for $C_{20}H_{27}N_3O_5$: C, 61.67; H, 6.99; N, 10.79. Found: C, 61.50; H, 6.99; N, 10.72.

CD-PEI. PEI (M_r 50,000–60,000) purchased from Aldrich was purified by dialysis (cutoff M_r 12,000) to remove fractions of small molecular weights. CD–PEI was prepared as reported previously (*2*), and the content of CD in CD–PEI was 1.15 residue mol%.

[TC–CD]^{SD}AcPEI. To an aqueous solution (500 ml) of 0.215 resM CD–PEI, dimethyl sulfoxide (DMSO) (40 ml) was added and **1** (0.55 g, 1.24 mmol) dissolved in 10 ml DMSO was added dropwise over the period of 3 h at 50°C. After the mixture was stirred at 50°C for an additional 6 h, it was purified by dialysis against 40% aqueous ethanol (20 liters, twice), 0.1 M NaCl aqueous solution (20 liters, three times), and water (20 liters, three times). The resulting [dioxo TC–CD]^{SD}PEI was concentrated to 10 ml at <50°C, and was dissolved in 150 ml DMSO. Sodium borohydride (2.5 g, 0.066 mol) powder and methanesulfonic acid (2.0 ml, 0.031 mol) solution in 5 ml DMSO were added alternately in several small portions over the period of 3 h at room temperature (*6*). Then the reaction mixture was stirred for an additional 10 h. After 1 N NaOH aqueous solution (30 ml) was added to the solution, the mixture was stirred for 1 h. Then, 0.1 N HCl aqueous solution (30 ml) was added and the resulting mixture was stirred for 1 h. The resulting [TC–CD]^{SD}PEI was purified by dialysis against an aqueous HCl solution (pH ca. 5, 20 liters, twice) and water (20 liters, four times). Whether the dioxo form of TC (dioxoTC) in the PEI derivatives is completely reduced by the reduction procedure

(*6*) was not positively checked with [TC–CD]^{SD}PEI or [TC–CD]^{Ran}PEI due to the low content of TC. Instead, almost quantitative reduction of dioxoTC was confirmed by NMR in the case of a PEI derivative in which the content of dioxoTC was 10 residue mol%. The volume of [TC–CD]^{SD}PEI was adjusted to 150 ml. To the 75 ml solution of [TC–CD]^{SD}PEI, $NiCl_2$ (0.0546 M, 11.4 ml) was added, and the solution was stirred for 2 h. Then acetic anhydride (15 ml, 0.16 mol) was added dropwise with pH kept at 7.5–8.5 over the period of 3 h at room temperature. The resulting PEI derivatives was purified by dialysis against 1% (w/v) NaCN aqueous solution (20 liters, three times), water (20 liters, twice), 0.1 M NaCl aqueous solution (20 liters, three times), and water (20 liters, four times) to obtain [TC–CD]^{SD}AcPEI. ICP analysis of [TC–CD]^{SD}AcPEI indicated that more than 99% of Ni(II) ion was removed. As discussed later in this article, the content of TC for each CD in [TC–CD]^{SD}AcPEI is close to 1.0.

[*TC–CD*]^{Ran}*AcPEI*. To a 95% (v/v) DMSO solution (500 ml) of 0.215 resM CD–PEI, **2** (0.48 g, 1.24 mmol) dissolved in 5 ml DMSO was added, and the mixture was stirred at 50°C for 6 h. The resulting [dioxoTC–CD]^{Ran}PEI was purified by dialysis. Reduction of [dioxoTC–CD]^{Ran}PEI and the subsequent acetylation of [TC–CD]^{Ran}PEI were carried out by the method described above for [TC–CD]^{SD}AcPEI, and [TC–CD]^{Ran}AcPEI thus obtained was purified by dialysis. As discussed later in this article, the content of TC for each CD in [TC–CD]^{Ran}AcPEI is close to 1.0.

4-Nitrophenyl (*4-t-butylphenyl*)*acetate* (**3**). This compound was prepared according to the literature (*7*).

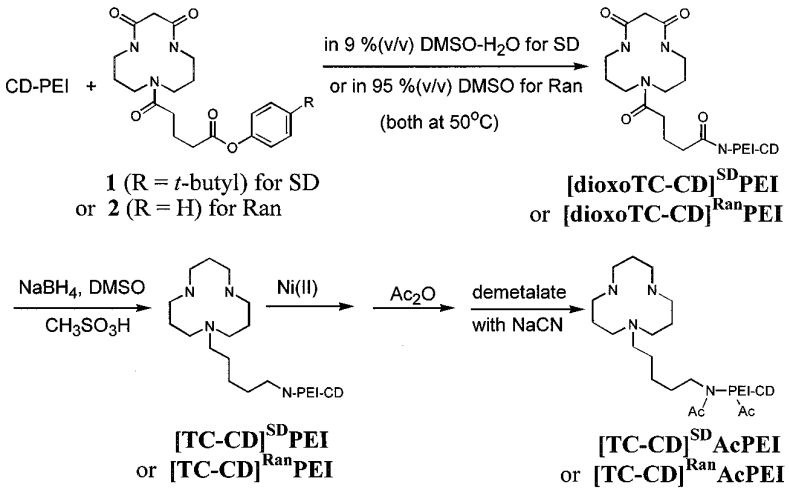
Measurements

Estimation of formation constants for complexes of PEI derivatives formed with Ni(II) ion was carried out by dialysis as reported previously (*8*). Reaction rates for the hydrolysis of **3** in the presence of the PEI derivatives were measured spectrophotometrically. Buffers (0.05 M) used were *N*-(2-hydroxyethyl)-1-piperazineethanesulfonate (pH 7–8) and boric acid (pH 9.1). Distilled and deionized water was used in dialysis and preparation of buffer solutions. ICP analysis of metal ions was carried out with a Perkin–Elmer Plasma 40 or a Shimadzu ICP S-1000IV. Spectrophotometric measurements were performed with a Beckman DU-68 UV/vis spectrophotometer or a Jasco FP-777. pH was measured with a Dongwoo DP880 pH/ion meter. Temperature was controlled with a Fisher Scientific 71 circulator.

RESULTS AND DISCUSSION

Synthetic routes for various PEI derivatives containing both CD and TC ([TC–CD]^{SD}PEI, [TC–CD]^{SD}AcPEI, [TC–CD]^{Ran}PEI, and [TC–CD]^{Ran}AcPEI)[2] are indicated in Scheme I. The content of CD in CD–PEI was 1.15 residue mol%.

[2] The abbreviated names of the PEI derivatives indicate the nature of the pendants (TC, CD, Ac, or M(II)) attached to PEI and whether TC is introduced in the vicinity of CD by site-directed or random (SD or Ran, respectively) functionalization. For example, [Cu(II)TC–CD]^{SD}AcPEI stands for the PEI derivative containing CD and acetyl groups as well as the Cu(II) complex of TC introduced by site-directed functionalization.



(SD: site-directed functionalization, Ran: random functionalization)

SCHEME I

The key step for site-directed functionalization of CD–PEI is acylation of CD–PEI with **1.** Because of the limited solubility of **1** in water, it was inevitable to add an organic solvent to the reaction mixture. Since complexation of CD with guest compounds takes place even in 60% (v/v) DMSO–water (*3b*), **1** was added to a solution of CD–PEI in 9% (v/v) DMSO–water in small portions. On the other hand, **2** was added to a solution of CD–PEI in 95% (v/v) DMSO for random functionalization of CD–PEI in view of the weaker affinity of CD toward phenyl derivatives than toward t-butylphenyl derivatives and in 95% (v/v) DMSO than in 9% (v/v) DMSO–water. The molar amount of **1** or **2** added to CD–PEI was the same as that of the CD moiety. TC was formed by reduction of its precursor (*6*). Intermediate PEI derivatives as well as the final products were purified by repetitive dialysis. To minimize complications due to amino groups of the PEI backbone, it is desirable to block the amino groups. Acetylation of PEI derivatives with acetic anhydride is known to acetylate the primary and secondary amino groups of PEI (*9*). To prevent secondary amino groups of the TC moiety from acetylation by acetic anhydride, the TC moiety was selectively protected with Ni(II) ion.

When Ni(II), Cu(II), or Zn(II) ion was added ($[M(II)]_0 = [CD]_0$) to [TC–CD]^{SD}AcPEI or [TC–CD]^{Ran}AcPEI at pH 7.00 or 7.57 and the mixture was dialyzed for 1 week at 25°C, the metal ions were almost completely (92–99%) retained by the PEI derivative as checked by ICP analysis. When the amount of Ni(II) or Cu(II) added was raised up to four times of $[CD]_0$, about 1 eq (>90% of $[CD]_0$) of the metal ion was retained by the polymer even after three successive dialyses. Spectral titration of [TC–CD]^{SD}AcPEI or [TC–CD]^{Ran}AcPEI with Ni(II) (Fig. 1) also indicated that the content of strong binding sites for Ni(II) ion agrees with the content of the CD moiety within ±5%. The visible spectrum for the Ni(II) complex of [TC–CD]^{SD}AcPEI (λ_{max} = 607 nm, pale green, ε_{max} = 9.1 M^{-1} cm^{-1}) or [TC–

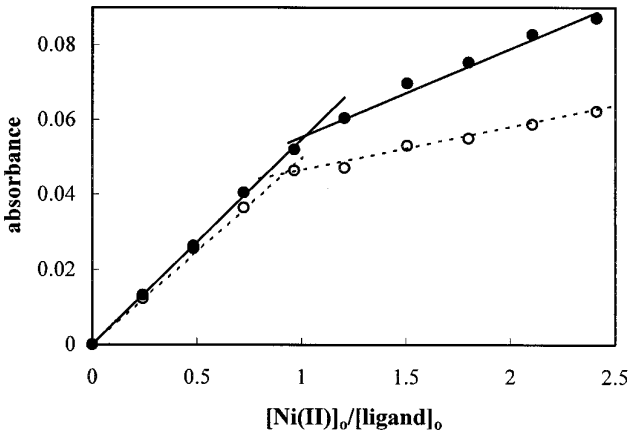


FIG. 1. Plot of absorbance at 606 nm against $[Ni(II)]_0/[CD]_0$ for $[TC–CD]^{SD}$AcPEI (○) or $[TC–CD]^{Ran}$AcPEI (●) at 25°C and pH 8.00. $[CD]_0$ was 5.89×10^{-3} M.

$CD]^{Ran}$AcPEI (λ_{max} = 606 nm, $\varepsilon_{max} = 1.0 \times 10^1$ M^{-1} cm^{-1}) was similar to that of TC (λ_{max} = 607 nm, $\varepsilon_{max} = 1.3 \times 10^1$ M^{-1} cm^{-1}) but was distinctly different from that[3] of PEI (λ_{max} = 543 nm, pale pink, $\varepsilon_{max} = 8.3$ M^{-1} cm^{-1}). This reveals that the primary Ni(II) binding site of $[TC–CD]^{SD}$AcPEI or $[TC–CD]^{Ran}$AcPEI is TC. It further indicates that TC is successfully protected by Ni(II) ion during the acetylation of $[TC–CD]^{SD}$PEI or $[TC–CD]^{Ran}$PEI. Although the PEI backbone contains ethylenediamine units which can act (*10*) as ligands for metal ions, metal binding by the backbone is suppressed upon acetylation of the primary and the secondary amines of PEI.

That TC is protected by Ni(II) ion during the acetylation step is confirmed again by measurement of formation constants (K_f) for the Ni(II) complex of the TC moiety and that of the PEI backbone. Estimation of the K_f values was carried out by dialyzing Ni(II) complex of the PEI derivative with a competing ligand by a method reported previously (*8*). Either nitrilodiacetic-3-propanoic acid (NDPA) (log K_f = 8.80 (*11*) for Ni(II)(NDPA) at pH 7.00 and 25°C) or nitrilotriacetic acid (NTA) (log K_f = 11.02 (*11*) for $Ni(II)(NTA)_2$ at pH 7.00 and 25°C) was used as the competing ligand, and the results obtained with CD–PEI and $[TC–CD]^{SD}$PEI are summarized in Table 1. When $[Ni(II)]_0$ was four times greater than $[TC]_0$, the limiting amount of Ni(II) ion that can be extracted by NDPA was 77 ± 2% of the Ni(II) ion added to $[TC–CD]^{SD}$PEI. When NTA, a stronger chelating agent than NDPA, was used as the competing ligand, all of the added Ni(II) ion was extracted by NTA at large concentrations of NTA even when $[Ni]_0 = 4[CD]_0$. Thus, the Ni(II) ion added in excess of $[TC]_0$ was associated with a weaker K_f value than that bound to the TC moiety. The TC moiety manifests about 10-fold greater affinity for Ni(II) ion compared with the PEI backbone.[4] Thus, Ni(II) can selectively block at least 90% of the TC moiety during the acetylation stage.

[3] The relative amount of Ni(II) ion added to PEI was 1.15 residue mol% and ε_{max} was based on $[Ni(II)]_0$.

[4] The log K_f for $[Ni(II)TC–CD]^{SD}$AcPEI (12.38) is greater than that (10.93) reported (*12*) for the Ni(II) complex of TC. This may be attributed to favorable conformational and/or medium effects exerted by $[Ni(II)TC–CD]^{SD}$AcPEI.

TABLE 1
Formation Constants for the Ni(II) Complex of PEI Derivatives Measured with Various $[Ni(II)]_0$ at pH 7.00 and 25°C[a]

PEI derivative	$[Ni(II)]_0/[CD]_0$	Competing ligand	log K_f
CD–PEI	1	NDPA	11.48 ± 0.22
[Ni(II)TC–CD]^{SD}PEI	1	NTA	12.38 ± 0.06[b]
[Ni(II)TC–CD]^{SD}PEI	4	NTA	12.00 ± 0.08[c]
[Ni(II)TC–CD]^{SD}PEI	4	NDPA	11.38 ± 0.06[d]

[a] $[CD]_0 = (0.5–1.0) \times 10^{-4}$ M.
[b] Represents metal affinity of TC portion.
[c] Represents average metal affinity of both TC portion and PEI backbone.
[d] Represents metal affinity of PEI backbone.

By adding metal ions such as Ni(II), Cu(II), or Zn(II) ion to [TC–CD]^{SD}AcPEI or [TC–CD]^{Ran}AcPEI ($[M(II)]_0 = [TC]_0$), [M(II)TC–CD]^{SD}AcPEI or [M(II)TC–CD]^{Ran}AcPEI was obtained. That the TC group is positioned near the CD moiety in [M(II)TC–CD]^{SD}PEI was confirmed by measuring kinetics of deacylation of **3** promoted by these PEI derivatives. Kinetics of reactions catalyzed by PEI derivatives follow the scheme of Eq. [1] which is analogous to Michaelis–Menten scheme (*1*). In the present study, C_0 (the initially added concentration of the catalyst) is expressed in terms of the concentration of CD moieties. Under the conditions of $C_0 \gg [CS]$, pseudo-first-order reactions were observed as expected from Eq. [1]. Saturation kinetic behavior was observed for the plot of k_0 (pseudo-first-order rate constant) against C_0 as illustrated in Fig. 2. The values of k_{cat} and $1/K_m$ estimated from the kinetic data are summarized in Fig. 3.

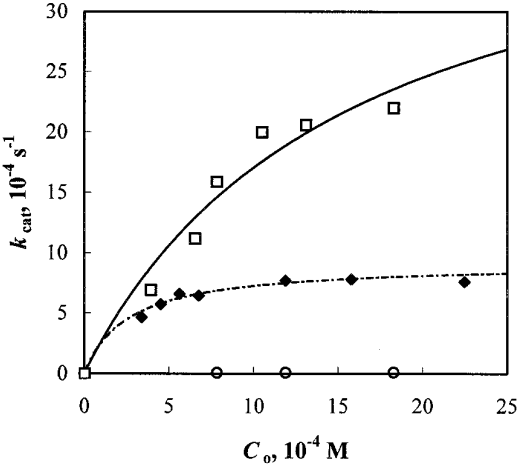


FIG. 2. Pseudo-first-order rate constants obtained for hydrolysis of **3**(5.00×10^{-5} M) in the presence of [Cu(II)TC–CD]^{SD}AcPEI (◆), [Cu(II)TC–CD]^{Ran}AcPEI (□), [TC–CD]^{SD}AcPEI (○), or acetylated CD–PEI (close to those marked by ○) at 25°C and pH 7.57.

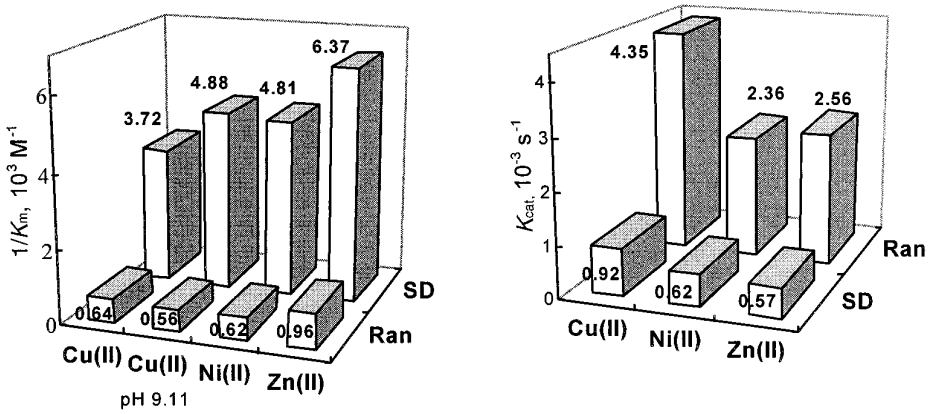
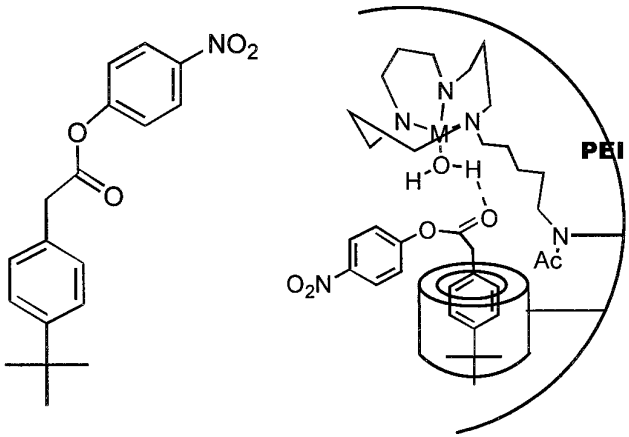


FIG. 3. Values of $1/K_m$ and k_{cat} for the hydrolysis of **3** catalyzed by [M(II)TC–CD]^{SD}AcPEI and [M(II)TC–CD]^{Ran}AcPEI at 25°C in the presence of 5.9% (v/v) CH_3CN. pH is 7.57 unless noted otherwise. Kinetic data were obtained with $C_0 = 2–25 \times 10^{-4}$ M and $S_0 = 5 \times 10^{-5}$ M. At pH 9.11, k_{cat} is 1.09×10^{-2} and 2.74×10^{-2} s^{-1} for [Cu(II))TC–CD]^{SD}AcPEI and [Cu(II)TC–CD]^{Ran}AcPEI, respectively. Relative standard deviations are 10–20% for $1/K_m$ and 5–10% for k_{cat}.

$$C + S \overset{K_m}{\rightleftharpoons} CS \xrightarrow{k_{cat}} C + \text{products} \qquad [1]$$



3 **III**

Deacylation of **3** is very slow in the presence of [TC–CD]^{SD}AcPEI or acetylated CD–PEI (Fig. 2). This excludes the possibility of nucleophilic attack at **3** by the hydroxyl groups of CD or the tertiary amino groups of the PEI backbone in the deacylation by [M(II)TC–CD]^{SD}AcPEI or [M(II)TC–CD]^{Ran}AcPEI.

As indicated in Fig. 3, k_{cat} for [M(II)TC–CD]^{SD}AcPEI is smaller than that for [M(II)TC–CD]^{Ran}AcPEI by 3.9–4.7 times, whereas $1/K_m$ for [M(II)TC–

CD]^{SD}AcPEI is greater than that for [M(II)TC–CD]^{Ran}AcPEI by 5.8–8.7 times. The physical meanings of k_{cat} and K_m for the Michaelis–Menten scheme are not straightforward, especially when stable intermediates are involved in (*13*). It is not likely that any covalent intermediates accumulate in the hydrolysis of nitrophenyl esters investigated in the present study with the PEI derivatives. In enzymatic reactions, conversion of the Michaelis complex to intermediates or products is very fast and the Michaelis complex is not in equilibrium with the reactants. For the hydrolysis of **3** by the PEI derivatives, the catalytic step is much slower than the enzymatic processes. Then, the *CS* complex of Eq. [1] could be in equilibrium with *C* and *S* and, therefore, $1/K_m$ might be taken as a measure of formation constant of the most stable complex formed between the polymer and **3**. The greater $1/K_m$ for [M(II)TC–CD]^{SD}AcPEI compared with [M(II)TC–CD]^{Ran}AcPEI may be taken to indicate that an extra binding force is present in the complex formed between **3** and [M(II)TC–CD]^{SD}AcPEI. Similar values of $1/K_m$ were observed for [Cu(II)TC–CD]^{SD}AcPEI at pH 7.57 and 9.11. Thus, the extra binding interaction is not affected by the pH change. On the other hand, the smaller k_{cat} for [M(II)TC–CD]^{SD}AcPEI compared with [M(II)TC–CD]^{Ran}AcPEI indicates that the bound ester is hydrolyzed more slowly by the PEI derivatives prepared by the site-directed functionalization.

The stronger but less productive binding of **3** by [M(II)TC–CD]^{SD}AcPEI compared with [M(II)TC–CD]^{Ran}AcPEI suggests the binding mode of III. Interaction of the metal-bound water molecule of TC complex with the carbonyl group of the bound ester facilitates the complexation. On the other hand, the assembly of III may sterically protect the ester linkage from attack by nucleophiles. Similar $1/K_m$ values for [Cu(II)TC–CD]^{SD}AcPEI at pH 7.57 and 9.11 are also consistent with III. When the central metal ion of III has additional coordination sites, they may be occupied by water. For [Cu(II)TC–CD]^{SD}AcPEI, the extra water ligand may change its ionization state upon raising pH from 7.57 to 9.11. Even if this happens, it would exert only secondary effects on binding of the ester.

Unlike $1/K_m$, k_{cat} values for both [Cu(II)TC–CD]^{SD}AcPEI and [Cu(II)TC–CD]^{Ran}AcPEI are 11 and 6 times, respectively, greater at pH 9.11 than at pH 7.57. This suggests that hydroxo ligand attached to the metal center may be the nucleophile attacking the bound ester. Mechanism of the catalyzed hydrolysis, however, is not certain at present.

The difference in $1/K_m$ between [M(II)TC–CD]^{SD}AcPEI and [M(II)TC–CD]^{Ran}AcPEI corresponds to a decrease in the free energy for complexation of 1.0–1.3 kcal/mol ($-\Delta\Delta G_f$). Hydrogen bond energies in an enzyme–substrate complex have been estimated by mutagenesis (*14*). Fersht and co-workers estimated 0.5–1.5 kcal/mol as the energy for hydrogen bond between a good hydrogen-bond donor on the enzyme and an uncharged group on the substrate. The value of 1.0–1.3 kcal/mol for $-\Delta\Delta G_f$ estimated in this study agrees with the existence of one extra hydrogen bonding in III which is absent in [M(II)TC–CD]^{Ran}AcPEI complexed with **3.**

Introduction of functional groups in planned positions on backbones of synthetic polymers is important for design of polymer derivatives performing various functions. The novel methodology reported here can be extended to construction of

functional sites comprising several groups on synthetic polymers as well as design of multiply functionalized derivatives of CD.

ACKNOWLEDGMENTS

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