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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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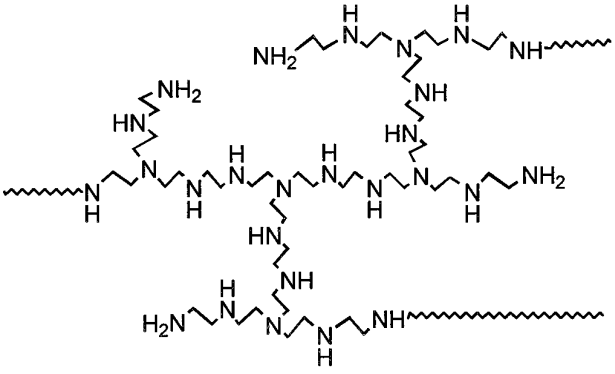
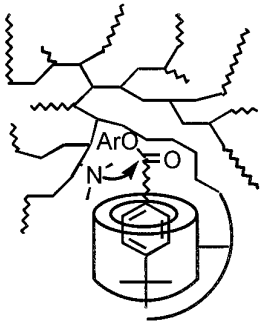


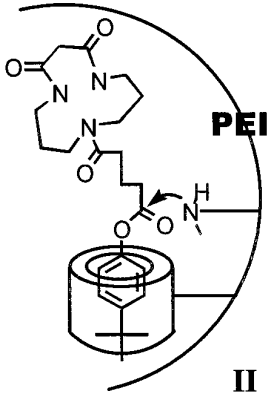
PEI



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*).

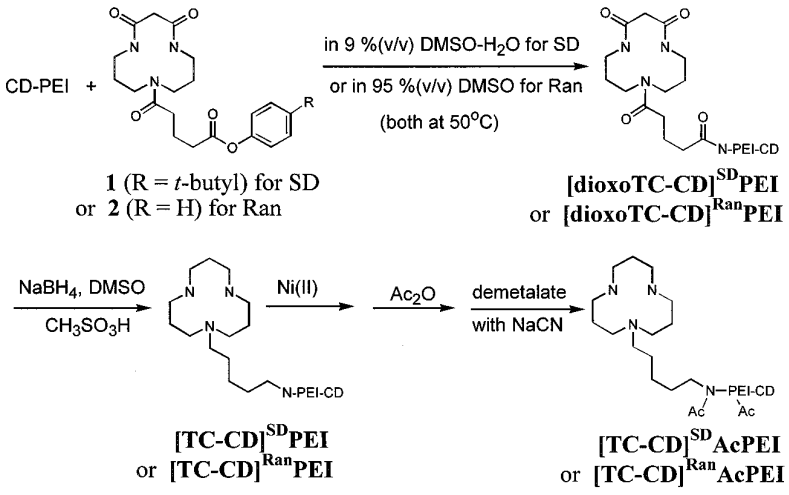
Measurements

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–