BIOORGANIC CHEMISTRY **25,** 63–75 (1997)
ARTICLE NO. BH961054

Dendrimer Poly(ethylenimine)s Linked to β-Cyclodextrin

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Received November 20, 1996

β-Cyclodextrin was attached to two dendrimer poly(ethylenimine)s. The resulting cyclodextrin-containing dendrimers, CD-I and CD-II, can be considered either as dendrimers equipped with specific binding sites or as cyclodextrins containing amino groups around the cavities. Amines of CD-I and CD-II remarkably resisted protonation compared with those of the parent dendrimers. A compact conformation of CD-I or CD-II in which the dendrimer wraps itself around the cyclodextrin is proposed as a conformation consistent with the suppressed protonation. Esters containing *t*-butylphenyl groups were complexed by CD-I and CD-II and underwent fast deacylation. Kinetic data were obtained with several ester substrates, which revealed that two amino groups located in the vicinity of each cyclodextrin cavity of CD-I or CD-II participated as nucleophiles. In addition, optimum reactivity was attained when the spacer connecting the *t*-butylphenyl and the ester groups was $-O-CH_2-$ or $-CH=CH-$. Structures of the active sites for the accelerated deacylation of esters were elucidated on the basis of the kinetic data.

INTRODUCTION

Dendrimers are three-dimensional, highly ordered oligomeric and polymeric compounds formed by reiterative reaction sequences starting from smaller molecules (*1*, *2*). Dendrimers can mimic various properties of large biomolecules and, thus, numerous applications are conceivable (*1*). By attaching chemical moieties to dendrimers, a variety of biomimetic functional molecules can be designed.

Dendrimers with hydrophilic exteriors and hydrophobic interiors may be regarded as covalently fixed assemblies of amphiphiles (*3*). In view of intensive studies on catalytic reactions of micelles (*4*, *5*), dendrimers may be exploited as biomimetic catalysts. In addition, dendrimers are free from the basic instability elements of noncovalent self-assemblies like micelles and vesicles. Furthermore, dendrimers possess well-defined structures unlike micelles or vesicles. To construct artificial enzymes on dendrimers, creation of binding sites on the skeleton of a dendrimer is desirable. Without specific binding sites, major principles of enzymatic catalysis can be hardly reproduced.

Many cyclodextrin (CD) derivatives have been examined for their ability to recognize guest molecules and to catalyze chemical transformation of the included molecules (*6–10*). In order to devise effective biomimetic catalysts using CD deriva-

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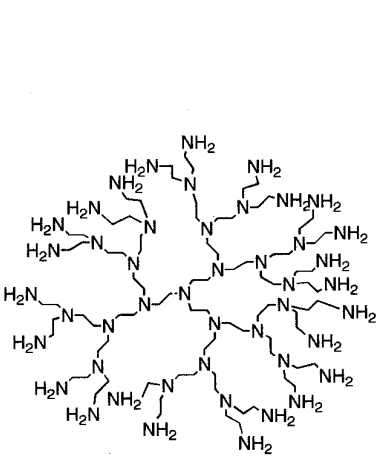
0045-2068/97 $25.00

tives, it is necessary to introduce catalytic groups to CDs in positions suitable for high catalytic efficiency. It is not easy to introduce a catalytic group in the productive position by using a short spacer to connect CD and the catalytic group. When a long spacer is used, however, conformational freedom of the resulting CD derivative should be suppressed to freeze the molecule in the productive conformation.

In an attempt to construct artificial enzymes by using dendrimers as the molecular skeleton, β-CD, a cyclic heptamer of α-D-glucose, was attached to dendrimer poly (ethylenimine)s I and II in the present study. Dendrimer I containing β-CD (CD-I) and dendrimer II containing β-CD (CD-II) can be regarded as either dendrimers equipped with specific binding sites or β-CD derivatives containing several amino groups around the CD cavities. Thus, reactions of compounds included in the CD cavity would be affected by the dendrimer moiety. In addition, the conformation and the chemical behavior of the dendrimer would be in turn influenced by the presence of β-CD in the molecular framework. In this paper, conformation of CD-I and CD-II in water and kinetic data for deacylation of carboxyl esters containing *t*-butylphenyl residues in the presence of CD-I and CD-II are presented.



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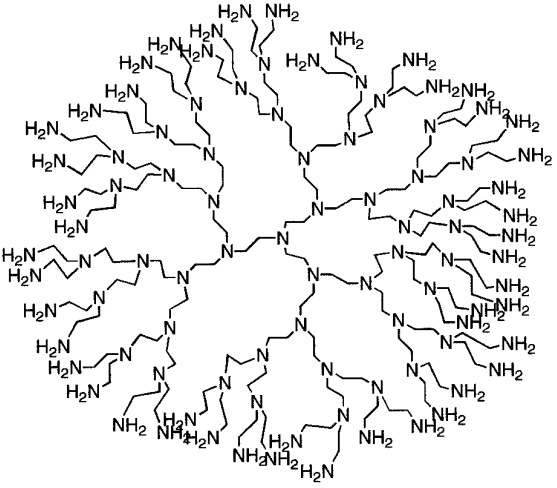
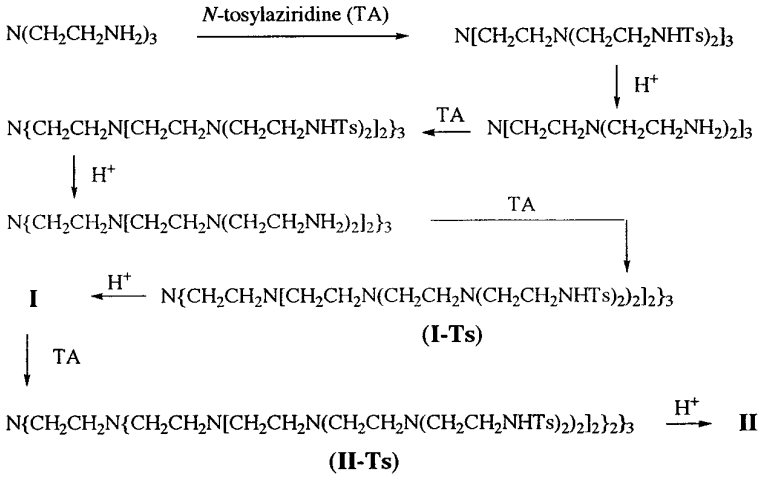


II

EXPERIMENTAL PROCEDURES

Synthesis of Dendrimers and Their Derivatives

Dendrimer poly(ethylenimine)s I and II were synthesized according to the procedure reported in the literature (*11*) as shown in Scheme I. Dendrimers I and II were examined by ^{1}H NMR microscopy and GPC. The ^{1}H NMR (300 MHz, DMSO-d_6) spectra of I and II were too broad to check the defect level. For the tosylated derivatives of I and II (I-Ts and II-Ts), however, the ratio of the signal integration of aromatic protons (δ 7.24–7.62) to ethylene protons (δ 2.26–2.64) agreed with



SCHEME I

the theoretical value within 10% error. GPC analysis showed the molecular weight distributions of M_w/M_n = 1.16 for I-Ts and M_w/M_n = 1.29 for II-Ts.

β-CD was linked covalently to I or II to obtain CD-I or CD-II, respectively, by the reactions of I (0.45 g, 0.23 mmol) or II (0.93 g, 0.23 mmol) with mono-6-(*p*-toluenesulfonyl)-β-CD (*12*) (0.30 g, 0.26 mmol) in 100 ml dimethyl sulfoxide (DMSO) at 60°C for 6 h followed by purification by ultrafiltration (Amicon Y1) and gel filtration (Sephadex G25). The ^{1}H NMR (300 MHz, D_2O) spectra of CD-I and CD-II showed that 0.87 and 1.05 residues, respectively, of CD were incorporated in CD-I and CD-II.

Preparation of Substrates

4-Carboxy-2-nitrophenyl 4-t-butylbenzoate **(S0-).** This compound was prepared according to the literature (*13*) by coupling the carboxylic acid and the phenol with *N*,*N*′-dicyclohexylcarbodiimide (DCC), mp 199–200°C (lit. 199–200°C (*10*, *13*)).

4-Nitrophenyl 4-t-butylbenzoate **(S0).** This compound was prepared by coupling the carboxylic acid and the phenol with DCC and purified by chromatography (silica, hexane/ethyl acetate = 5 : 1) and recrystallization from hexane-ethyl acetate, mp 124–126°C (lit. 123–125°C (*14*)).

4-Nitrophenyl (4-t-butylphenyl)acetate **(S1).** A solution of 4-*t*-butylbenzyl bromide (1.5 g, 6.4 mmol), potassium cyanide (1.3 g, 20 mmol), and 18-crown-6 (0.2 g) dissolved in tetrahydrofuran (30 ml) was refluxed for 1 day. The residue obtained after evaporation of the solvent under reduced pressure was extracted with hexane. The hexane solution was washed with water, dried with sodium sulfate, and evaporated. The mixture of the resulting (4-*t*-butylphenyl)acetonitrile and 50 ml 4 N NaOH was refluxed for 16 h and then acidified with 1 N HCl. The residue was extracted with diethyl ether and purified by chromatography (silica, hexane/ethyl

acetate = 10:1) to give (4-*t*-butylphenyl)acetic acid. EIMS m/e 192(M_+). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.29 (s, 9H), 3.56 (s, 2H), 7.18–7.34 (m, 8H).

A solution of (4-*t*-butylphenyl)acetic acid (0.34 g, 1.7 mmol), 4-nitrophenol (2.3 g, 1.7 mmol), DCC (0.38 g, 1.8 mmol), and 4-*N*,*N*′-dimethylaminopyridine (DMAP) (0.1 g) in methylene chloride (20 ml) was stirred for 2 h at room temperature. The solvent was evaporated under reduced pressure and the resulting mixture was treated with ethyl acetate. After removal of the undissolved residue by filtration, the product was purified by chromatography (silica, hexane/ethyl acetate = 20:1) and recrystallized from methylene chloride-hexane to give **S1,** mp 66–67°C. EIMS m/e 313(M_+). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.33 (s, 9H), 3.87 (s, 2H), 7.25–7.32 (m, 4H), 7.39–7.43 (m, 2H), 8.83–8.88 (m, 2H). *Anal.* Calcd for $C_{18}H_{19}N_1O_4$: C, 68.99; H, 6.11; N, 4.47. Found: C, 68.58; H, 6.07; N, 4.63.

4-Nitrophenyl 3-(4-t-butylphenyl)propionate **(S2A).** Ethyl *trans*-4-*t*-butylcinnamate (see below) (0.50 g, 2.2 mmol) was hydrogenated with H_2 (1 atm) and 10% Pd/C (0.1 g) for 16 h at room temperature. Pd/C was removed through celite and the filtrate was concentrated. The resulting ester and NaOH (170 mg, 4.3 mmol) were dissolved in methanol (20 ml)–water (10 ml). After reflux for 2 h, methanol was evaporated. The aqueous solution was washed with diethyl ether, acidified with 1 N HCl, and extracted with methylene chloride. The organic layer was dried with sodium sulfate and evaporated to give (4-*t*-butylphenyl)propionic acid, mp 116–117°C (lit. 116–117°C (*16*)). (4-*t*-Butylphenyl)propionic acid (100 mg, 0.49 mmol), 4-nitrophenol (68 mg, 0.49 mmol), and DCC (120 mg, 0.58 mmol) in methylene chloride (5 ml) were stirred for 6 h at room temperature. Hexane (20 ml) was added to the reaction mixture and the resulting solid was removed by filtration. The product thus obtained was purified by chromatography (silica, hexane/ethyl acetate = 5:1) and recrystallization from methylene chloride-hexane, to obtain **S2A,** mp 74–76°C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.30 (s, 9H), 2.85–3.11 (m, 4H), 7.15–8.23 (m, 8H). EIMS m/e 339(M_+). *Anal.* Calcd for $C_{19}H_{21}N_1O_4$: C, 69.71; H, 6.47; N, 4.28. Found: C, 69.82; H, 6.33; N: 4.39.

4-Nitrophenyl (4-t-butylphenoxy)acetate **(S2B).** This compound was prepared according to the literature (*15*), mp 79–80°C (lit. 79–80°C (*15*)).

4-Nitrophenyl trans-4-t-butylcinnamate **(S2C).** A solution of 4-*t*-butylbenzaldehyde (1.2 g, 7.5 mmol) and (carboethoxymethylene)triphenylphosphorane (2.9 g, 8.3 mmol) in 20 ml of toluene was stirred for 6 h at room temperature. After concentration, the residue was dissolved in diethyl ether (10 ml) and hexane (30 ml) was added slowly to precipate triphenylphosphine oxide. The resulting solid was removed by filtration through celite. The product thus obtained was purified by chromatography (silica, hexane/ethyl acetate = 30:1) to give ethyl *trans*-4-*t*-butylcinnamate. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.30 (s, 9H), 1.40 (t, 3H), 4.25 (q, 2H), 6.37 (s, 1H), 6.43 (s, 1H), 7.35–7.72 (m, 4H).

A solution of ethyl *trans*-4-*t*-butylcinnamate (0.50 g, 2.2 mmol) and NaOH (0.17 g, 4.3 mmol) in methanol (20 ml)–water (10 ml) was refluxed for 4 h. After methanol was removed by evaporation, the aqueous solution was washed with diethyl ether, acidified with 1 N HCl, and extracted with methylene chloride. The organic layer was dried with sodium sulfate and evaporated under reduced pressure. To a solution of the resulting *trans*-4-*t*-butylcinnamic acid (100 mg, 0.49 mmol) in 10 ml methylene

chloride, 4-nitrophenol (68 mg, 0.49 mmol), DCC (120 mg, 0.59 mmol), and DMAP (10 mg) were added. After stirring for 6 h at room temperature, the reaction mixture was evaporated under reduced pressure. After the resulting mixture was treated with diethyl ether, the undissolved portion was removed by filtration. The product thus obtained was purified by chromatography (silica, hexane/ethyl acetate = 5:1) and recrystallization from methylene chloride–hexane to give **S2C.** mp 152–154°C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.35 (s, 9H), 6.54 (s, 1H), 6.62 (s, 1H), 7.30–8.35 (m, 8H). EIMS m/e 337 (M_+). *Anal.* Calcd for $C_{19}H_{19}N_1O_4$: C, 70.14; H, 5.89; N, 4.30. Found: C, 70.29; H, 5.99; N, 4.38.

4-Nitrophenyl (*4-t-butylbenzyloxy*)*acetate* **(S3).** To a solution of 4-*t*-butylbenzyl alcohol (3.3 g, 20 mmol) and NaH (1.3 g, 53 mmol) in 200 ml ethanol was added chloroacetic acid (2.0 g, 21 mmol). After reflux for 6 h, the reaction mixture was acidified with 3 N HCl and extracted with methylene chloride. The organic layer was dried with sodium sulfate and evaporated under reduced pressure. The resulting residue was recrystallized from ethanol to give (4-*t*-butylbenzyloxy)acetic acid. A solution of (4-*t*-butylbenzyloxy)acetic acid (0.59 g, 2.6 mmol), 4-nitrophenol (0.30 g, 2.6 mmol), DCC (0.65 g, 3.2 mmol), and DMAP (0.1 g) in tetrahydrofuran (20 ml) was stirred for 5 h at room temperature. The residue obtained after evaporation of the solvent was treated with ethyl acetate. After removal of the undissolved portion by filtration, the solvent was evaporated. The resulting residue was recrystallized from diethyl ether-hexane to obtain **S3,** mp 87–88°C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.33 (s, 9H), 2.60–2.85 (t, 2H), 3.40–3.80 (q, 2H), 6.34 (s, 1H), 7.16–8.30 (m, 8H). *Anal.* Calcd for $C_{19}H_{21}N_1O_5$: C, 66.46; H, 6.16; N, 4.08. Found: C, 66.68; H, 6.07; N, 3.93.

4-Nitrophenyl N-(*4-t-butylbenzoyl*)*-3-aminopropionate* **(S4).** β-Alanine methyl ester (1.1 g, 11 mmol) was added to a solution of *t*-butylbenzoic acid (1.9 g, 11 mmol), DCC (2.2 g, 11 mmol), and DMAP (0.1 g) in methylene chloride (100 ml). After the mixture was stirred for 2 h at room temperature, precipitates formed by addition of hexane were removed by filtration. The product thus obtained was purified by chromatography (silica, hexane/ethyl acetate = 4:1) and recrystallization from ethyl acetate–hexane to give methyl *N*-(4-*t*-butylbenzoyl)-3-aminopropionate. Following the procedure described above for **S2C,** the methyl ester was hydrolyzed and then coupled with 4-nitrophenol to obtain **S4** which was purified by recrystallization from ethyl acetate–hexane (mp 98–99°C). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.33 (s, 9H), 2.58–2.87 (t, 2H), 3.38–3.81 (q, 2H), 7.16–8.30 (m, 8H). *Anal.* Calcd for $C_{20}H_{22}N_2O_5$: C, 64.86; H, 5.98; N, 7.56. Found: C, 64.93; H, 6.03; N, 7.47.

4-Nitrophenyl N-(*4-t-butylbenzoyl*)*-4-aminobutyrate* **(S5).** This compound was prepared according to the literature (*15*), mp 103–104°C (lit. 103–104°C (*15*)).

4-Nitrophenyl trans-cinnamate **(S2C*).** This compound was prepared by using DCC, mp 146–147°C (lit. 146.5–147.5°C (*17*)).

Measurements

All the kinetic measurements and titration were carried out at 25°C. Reaction rates were measured spectrophotometrically with a Beckman DU 650 UV/Vis spectrophotometer. pH measurements were carried out with a Dong-Woo pH meter

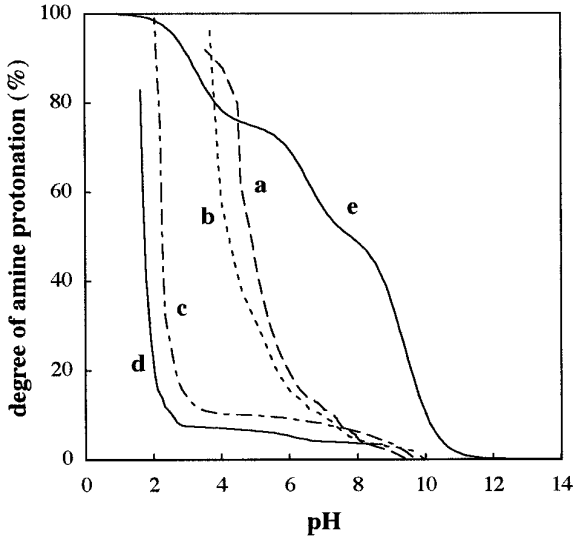


FIG. 1. Degree of protonation of amino groups at 25°C and various pH for I (a), II (b), CD-I (c), CD-II (d), and $NH_2(CH_2)_2NH(CH_2)_2NH(CH_2)_2NH_2$ (e). Concentrations of I, II, CD-I, and CD-II were ca. 50 mM in terms of individual amines.

DP 880. Stock solutions of ester substrates were made in acetonitrile and the buffer solutions for deacylation of the esters contained 9.8% (v/v) acetonitrile. Buffer (0.020 M) used at pH 7.57 was Na_2HPO_4.

RESULTS

One of the primary hydroxyl groups of β-CD was tosylated, and the resulting tosyl derivative was used in the alkylation of the amino groups of dendrimers I and II to obtain CD-I and CD-II. On the average, CD-I and CD-II contained 0.87 and 1.05 residue of CD, respectively. It is expected that the primary amines, instead of the tertiary amines, of I and II were alkylated due to the steric hindrance involved in the alkylation of tertiary amines.

In order to obtain information on microenvironments of amino groups of the dendrimers, basicity of the amino groups was examined by titration with HCl. As summarized in Fig. 1, both I and II manifested large buffer capacity at pH 5–6. On the other hand, CD-I and CD-II showed considerably weaker buffer capacity at pH 3–8. At pH 5, about 40% of the amino groups of I and II were protonated whereas only 5–10% of the amino groups of CD-I and CD-II were protonated. At