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Dendrimer Poly(ethylenimine)s Linked to β-Cyclodextrin

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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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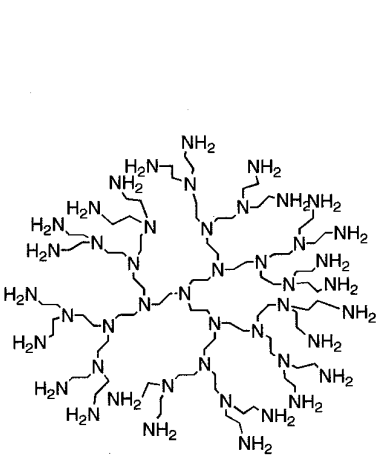
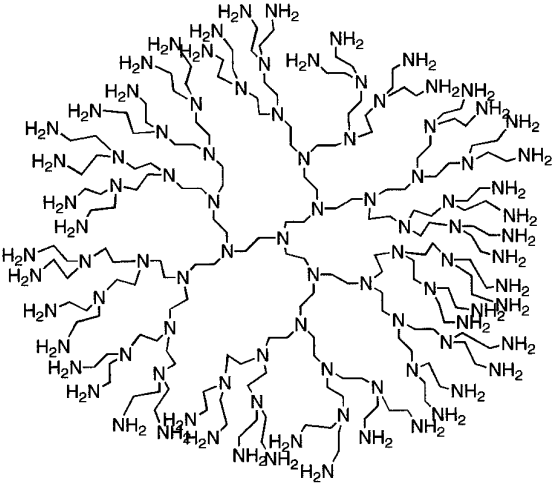
tives, it is necessary to introduce catalytic groups to CDs in positions suitable for high catalytic efficiency. It is not easy to introduce a catalytic group in the productive position by using a short spacer to connect CD and the catalytic group. When a long spacer is used, however, conformational 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.



I

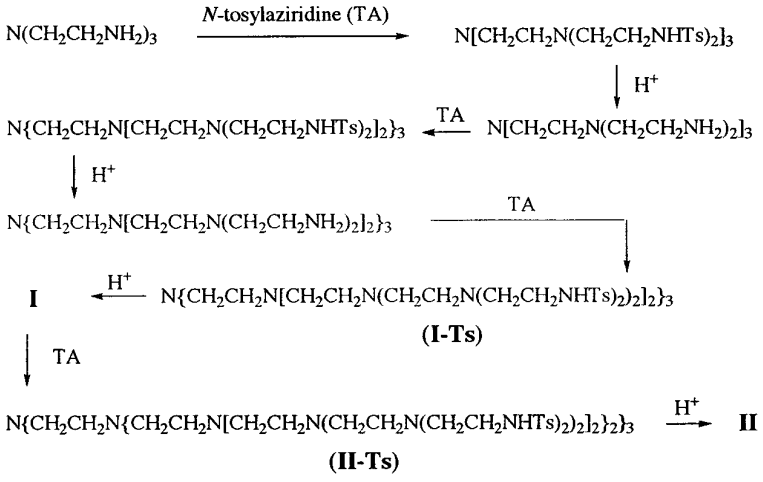


II

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

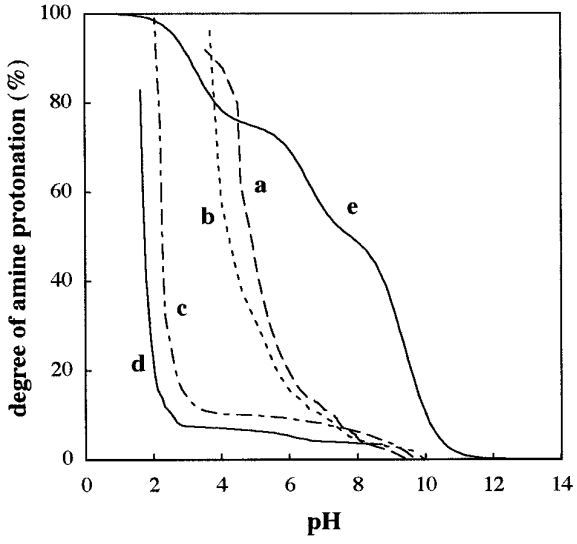


FIG. 1. Degree of protonation of amino groups at 25°C and various pH for I (a), II (b), CD-I (c), CD-II (d), and $NH_2(CH_2)_2NH(CH_2)_2NH(CH_2)_2NH_2$ (e). Concentrations of I, II, CD-I, and CD-II were ca. 50 mM in terms of individual amines.

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

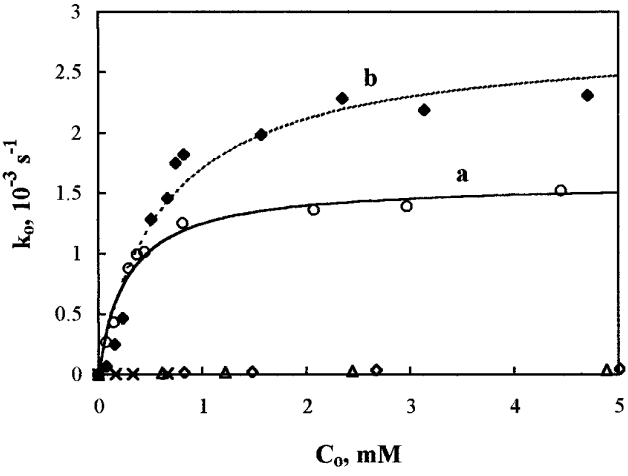


FIG. 2. Plot of k_o (pseudo-first-order rate constant) against C_o for deacylation of **S0-** in the presence of CD-I (○; curve a), CD-II (◆; curve b), I (△), II (◇), or CD (×) under the condition of $C_o \gg S_o$ at pH 7.57 and 25°C. For CD-I, CD-II, and CD, C_o was expressed in terms of the concentration of CD moiety. For I or II, C_o was corrected so that it corresponds to the concentration of I or II in CD-I or CD-II. Curves a and b were obtained by fitting the data to Eq. [2].

pH 3, almost all of the amino groups were protonated for I and II whereas less than 10% were protonated for CD-II.

Several nitrophenyl esters **(S0-, S0, S1, S2A, S2B, S2C, S3, S4, S5,** and **S2C*)** containing various structural features were prepared. As illustrated in Fig. 2, deacylation of **S0-** was much faster in the presence of CD-I or CD-II compared with that in the presence of CD, I, or II. Deacylation by CD-II was considerably faster with **S2C** than with **S2C*** at low C_o concentrations (Fig. 3).

Saturation kinetic behavior observed with deacylation by CD-I or CD-II was consistent with complex formation between the ester and CD-I or CD-II. The pseudo-first-order rate constants (k_o) measured under the conditions of S_o (initially added substrate concentration) $\ll C_o$ (initially added concentration of CD-I or CD-II) were analyzed according to Eqs. [1] and [2] by analogy with the Michaelis–Menten scheme. In this paper, C_o is expressed in terms of the concentration of the CD moiety for CD-I or CD-II unless noted otherwise. The values of k_{cat} and K_m estimated for deacylation of the ester substrates by CD-I and CD-II are summarized in Table 1.

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

$$k_o = k_{cat} C_o/(K_m + C_o) \qquad [2]$$

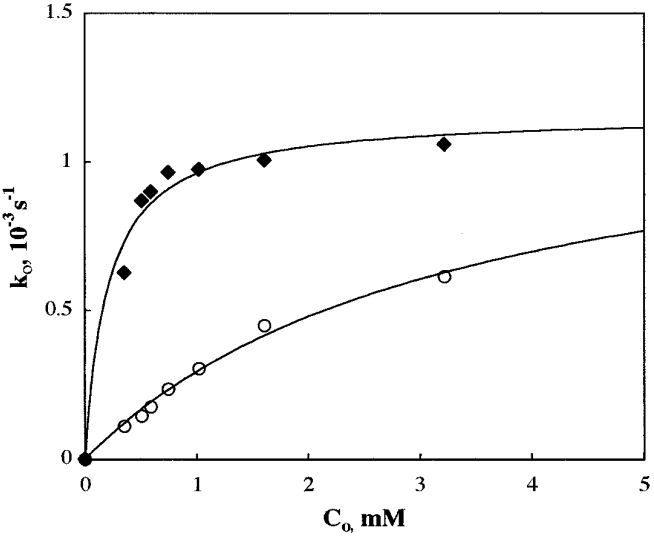
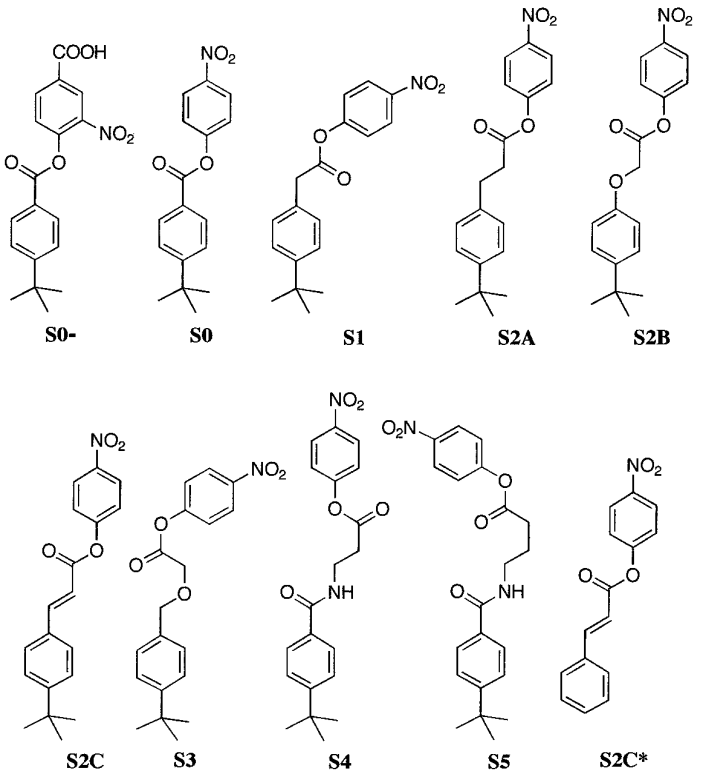


FIG. 3. Plot of k_o against C_o for deacylation of **S2C** (♦) and **S2C*** (○) in the presence of CD-II under the conditions of $C_o \gg S_o$ at pH 7.57 and 25°C. The curves were obtained by fitting the data to Eq. [2].

TABLE 1
Values of Kinetic Parameters for Deacylation by *n*-Butylamine (BA) at pH 10.74 and by CD-I and CD-II at pH 7.57 and 25°C[a]

	BA	CD-I		CD-II	
ester	k_{am} (10^{-3} M^{-1}s^{-1})	k_{cat} (10^{-3} s^{-1})	K_m (10^{-4} M^{-1})	k_{cat} (10^{-3} s^{-1})	K_m (10^{-4} M^{-1})
S0-	4.18	1.59	2.72	2.78	6.36
S0	0.742			0.541	6.74
S1	1.07	0.858	7.08	1.67	3.77
S2A	1.65	1.27	1.88	5.01	6.27
S2B	0.232	1.36	6.70	2.76	5.78
S2C	0.182	1.43	1.88	1.16	2.08
S3	1.39			1.09	4.46
S4	1.03			1.04	5.96
S5	1.18	0.996	7.44	0.921	4.88
S2C*	0.179			1.28	33.1

[a] See text for details of reaction conditions. Standard deviations were 1–3% of the parameter values for k_{am}, 1–10% of the parameter values for k_{cat}, and 3–15% of the parameter values for K_m. The values of k_{am} were obtained by normalizing the rate data to fully unprotonated BA.



Deacylation of **S0-** by CD-I and CD-II was examined under the conditions of $S_o > C_o$. As illustrated in Fig. 4 (not shown for CD-I), rapid initial burst followed by slow release was observed for the appearance of 4-hydroxy-2-nitrobenzoate during the deacylation of **S0-** by Cd-I or CD-II. The amount (Δ_i of Fig. 4) of 4-hydroxy-2-nitrobenzoate released during the initial burst stage can be estimated by extrapolation of the curve. The value of Δ_i corresponds to 1.8 and 2.2 times C_o for CD-I and CD-II, respectively. Since ratio S_o/C_o was 4.5 or 7.3 for CD-I or CD-II, respectively, data of the initial burst experiment indicate that about 2 molecules of the ester were deacylated for each CD residue of CD-I or CD-II.

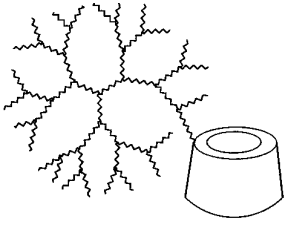
In order to estimate the intrinsic reactivities of the ester substrates for aminolysis, kinetics of deacylation of the esters ($1–5 \times 10^{-5}$ M) were measured in the presence of *n*-butylamine (0.025–0.1 M) at pH 10.74. At this pH, *n*-butylamine was half-protonated as checked by potentiometric titration. The bimolecular rate constants (k_{am}) estimated for the aminolysis of the esters are summarized in Table 1.

DISCUSSION

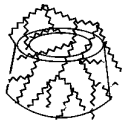
In polyamines such as I and II, protonation of an amino group would suppress subsequent protonation due to electrostatic interactions. In addition, the ethylene moieties of the dendrimers may exert hydrophobic effects leading to suppression

of ionization of amines. The fractions of amino groups protonated at various pH values are calculated for $NH_2(CH_2)_2NH(CH_2)_2NH(CH_2)_2NH_2$ by using the pK_a values of the amines reported in the literature (*18*) and are illustrated in Fig. 1 together with those measured with I, II, CD-I, and CD-II.

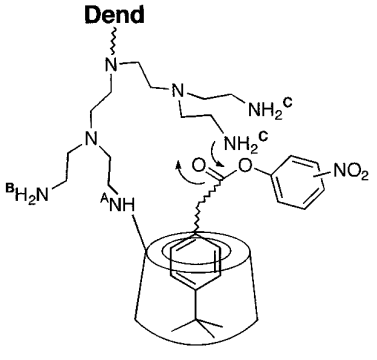
Amines of CD-I and CD-II show remarkably lower basicity compared with I and II. Attachment of CD to I or II appears to induce changes in the conformation of I or II, placing amino groups either in more hydrophobic microenvironments or in closer proximity to other amino groups compared with those of I or II. If some of the amino groups of CD-I or CD-II are hydrogen-bonded to hydroxyl groups of CD, their basicity would be also reduced considerably. Although the conformations of CD-I and CD-II are not clearly determined at present, the dendrimer conformation is considerably altered upon attachment of CD. If CD does not affect conformation of the dendrimer considerably (III), attachment of CD to the dendrimers would not influence the protonation of amino groups of I or II appreciably. The compact conformation (IV) of CD-I or CD-II in which the dendrimer wraps itself around CD is one of the possible conformations consistent with the results of Fig. 1.



III



IV



V

Deacylation of **S0-** is much faster in the presence of by CD-I or CD-II compared with CD, I, or II. Comparison of deacylation of **S2C** and **S2C*** indicates that the

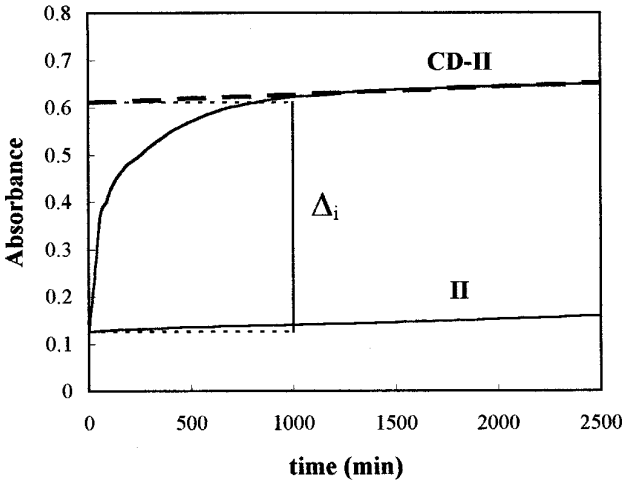


FIG. 4. Release of 4-hydroxy-2-nitrobenzoate during the deacylation of **S0-** in the presence of CD-II under the condition of S_o (3.86×10^{-4} M) > C_o (5.31×10^{-5} M) at pH 7.57 and 25°C. A control reaction was carried out with II. Δ_i is the absorbance increase due to 4-hydroxy-3-nitrobenzoate released during the initial burst stage. See Fig. 2 for definition of C_o.

t-butylphenyl portions of the esters are recognized by CD-I or CD-II. It is highly likely that the *t*-butylphenyl portions are included in the cavity of CD portions of CD-I or CD-II. If the esters are complexed to CD-I or CD-II of the extended conformation such as III, amino groups on the dendrimer portion would have limited access to the complexed ester. If the esters are complexed to CD-I or CD-II of the compact conformations such as IV, some amino groups would be located close to the ester linkage of the complexed substrate. Fast deacylation of the complexed esters is, therefore, consistent with the compact conformation.

Nonpolar species tend to aggregate in water solution so as to decrease the hydrocarbon–water interfacial area (hydrophobic effect) (*19*). Substrate binding into the CD cavity is driven by the hydrophobic effect (*19*). For example, inclusion of *t*-butylphenyl rings of the ester substrates examined in the present study protects *t*-butylphenyl rings from water. Since the exterior wall of CD is as hydrophobic as the interior wall of CD, wrapping of CD with dendrimers I and II can also reduce the interfacial area between the nonpolar surface and water. The hydrophobicity of the dendrimer portion would decrease as the amino groups are protonated. At pH 7.5, the dendrimer portions of CD-I and CD-II are mostly unprotonated and would exhibit near maximal hydrophobicity. Wrapping of CD with the dendrimer may involve hydrogen bonding between the amino groups of the dendrimer and the hydroxyl groups of CD and/or hydrophobic interaction between the dendrimer and CD.

Results of the initial burst experiment (Fig. 4) indicate that CD-I or CD-II is deactivated when about two molecules of **S0-** are deacylated for each CD moiety. As illustrated in V, CD is attached to a peripheral amino group (^{A}NH) of the

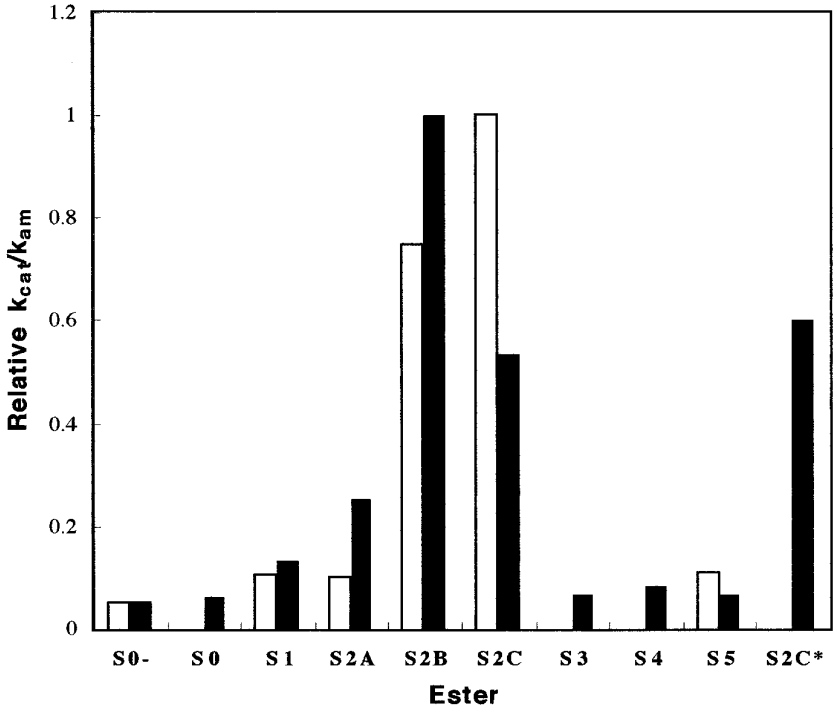


FIG. 5. Relative magnitude of k_{cat}/k_{am} for the ester substrates in the deacylation by CD-I (light bars) and CD-II (dark bars). The values of k_{cat} and k_{am} are listed in Table 1.

dendrimer. Examination by the CPK space filling model and by a molecular mechanics calculation indicated that considerable amount of strain is introduced when the nearest neighboring primary amine ($^{B}NH_2$) makes nucleophilic attack at the included ester. When the next neighboring primary amines ($^{C}NH_2$) are the nucleophiles, the strain is not significant. As the number of $^{C}NH_2$ is two, results of the initial burst experiment are consistent with the mechanism of V.

The K_m value for the deacylation of **S2C** by CD-II is much smaller than that of **S2C*,** in agreement with the inclusion of the cinnamoyl portions of the two esters in the cavity of CD. Despite the large difference in K_m values, k_{cat} for **S2C** is not much different from that of **S2C*.** Once the ester is bound to the host molecule, therefore, the reactivity is not much different.

Reactivities of the ester substrates complexed to CD-I or CD-II are reflected by k_{cat}. Apparently, k_{cat} varies widely for the ester substrates examined in the present study (Table 1). To consider the differences in intrinsic reactivity toward aminolysis of the ester substrates, kinetic data for aminolysis by *n*-butylamine were obtained. The relative reactivities of the nucleophilic amino group toward various ester substrates bound by CD-I or CD-II are best estimated by the values of k_{cat}/k_{am}, which

are compared in Fig. 5.[2] Each ester substrate contains a different spacer connecting the *t*-butylphenyl portion and the ester group. The data of Fig. 5 reveal that the relative reactivity is considerably greater for **S2B** and **S2C** for CD-I and CD-II. The spacer contained in **S2B** is $-O-CH_2-$ and that in **S2C** is $-CH=CH-$. As the spacer becomes shorter or longer, the relative reactivity is reduced considerably. Deacylation of the esters by CD-I and CD-II, therefore, manifests appreciable selectivity with regard to the substrate structure with the optimum geometry of the transition state being provied by **S2B** or **S2C.**

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[2] To achieve the deacylation rate (k_{cat}) of **S2B** complexed to CD-II, 12 M of unprotonated *n*-butylamine is needed in water. The values of k_{am} measured for aminolysis of **S2A** by *n*-butylamine at 25°C were $(8.13 \pm 0.03) \times 10^{-4}$ $M^{-1}s^{-1}$ in methanol, $(5.52 \pm 0.10) \times 10^{-4}$ $M^{-1}s^{-1}$ in ethanol, and $(1.94 \pm 0.18) \times 10^{-4}$ $M^{-1}s^{-1}$ in *n*-propanol, being smaller than that in water (Table 1). Thus, the aminolysis became slower as the solvent was made less polar. Although the exact microenvironment of the active site for ester deacylation in CD-I or Cd-II is not precisely known, it would be appreciably less polar than water. If the microenvironment of the nucleophilic amino group ($NH_2^{\S}$ of structure V) is similar to methanol or *n*-propanol, its effective molarity (A. J. Kirby (1980) *Adv. Phys. Org. Chem.* **17,** 183) toward the bound ester would be ca. 24 or 100 M, respectively.