Characterization of Peracylated β-Cyclodextrins with Different Chain Lengths as a Novel Sustained Release Carrier for Water-Soluble Drugs

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A new series of peracylated β-cyclodextrins (β-CyDs) with different alkyl chains (acetyl—lauroyl) was prepared in high purity by acylating all hydroxyl groups of β-CyD using acid anhydrides in pyridine, and their physicochemical properties of solubility, hydrolysis and release and interaction capacity were evaluated. The solubility of peracylated β-CyDs in water decreased with lengthening alkyl chain, whereas that in ethanol/water increased with increase in ethanol concentration, but tended to decrease at higher ethanol concentration. The solubility parameter of peracylated β-CyDs was determined by analyzing the peak-solubility phenomenon by a modified Hildebrand equation. The alkaline hydrolysis rate of peracylated β-CyDs decreased with lengthening alkyl chain, and was about 4-fold faster than that of the corresponding fatty acid ethyl esters. The interaction of perbutanoyl-β-CyD (TB-β-CyD) with a water-soluble drug, molsidomine, in the solid state was investigated by differential scanning calorimetry (DSC). The analysis of DSC curves suggested that molsidomine and TB-β-CyD form a binary solid dispersion with a 2:1 (drug: TB-β-CyD) molar ratio. The rate of drug release was markedly retarded by the combination with peracylated β-CyDs in the increasing order of the hydrophobicity of host molecules.

Keywords acylated β-cyclodextrin; solubility; hydrolysis; sustained release; thermal analysis

Various kinds of cyclodextrins (CyDs) have been prepared in order to extend the physicochemical properties and inclusion capacities of parent CyDs as multifunctional drug carriers.[1-3] For example, hydrophilic CyD derivatives such as methylated, hydroxyalkylated and branched CyDs are useful to improve of low solubility, dissolution and bioavailability of poorly water-soluble drugs.[4] On the other hand, hydrophobic CyDs like ethylated derivatives have potential as sustained-release carriers for water-soluble drugs because of their less soluble complex formations.[5,6] In a previous paper,[7] we reported that peracylated β-CyDs decelerated the release rate of a water-soluble drug, molsidomine, in proportion to the lengthening of alkyl chain and suppressed the peak plasma level of the drug following oral administration of the peracylated β-CyD complexes in dogs. Among the peracylated CyDs, perbutanoyl-β-CyD (TB-β-CyD) showed the most prominent retarding effect owing to its superior mucoadhesive property and hydrophobicity.[7] The present paper deals with the preparation and characterization of a series of peracylated β-CyDs (acetyl—lauroyl), and their physicochemical properties of solubility, alkaline hydrolysis, and interaction with water-soluble drugs in the solid state were examined. The *in vitro* drug release behavior from the peracylated β-CyD complexes was also investigated, anticipating their use as a novel sustained release drug carrier.

Experimental

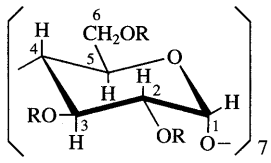
Materials β-CyD was supplied by Nihon Shokuhin Kako Co. (Tokyo, Japan). Molsidomine, salbutamol sulfate, and isosorbide dinitrate were donated by Takeda Chemical Industries (Osaka, Japan), Dainippon Pharmaceutical Co. (Osaka, Japan), and Toaeiyo, Ltd. (Tokyo, Japan), respectively. Propranolol hydrochloride was obtained commercially. Other chemicals and solvents were of analytical reagent grade, and deionized double-distilled water was used throughout the study.

Apparatus Nuclear magnetic resonance (NMR) spectra were taken on a JEOL JNM GX-400 spectrometer (Tokyo, Japan) operating at 399.65 and 100.40 MHz for protons and carbons, respectively, at 25 °C. Fast atom bombardment (FAB) mass spectra (MS) were measured in a positive ion mode at 25 °C by a JEOL JMS-DX 303 or a JMS-D300 mass spectrometer (Tokyo, Japan) using matrices: methanol+glycerol+*m*-nitrobenzyl alcohol (peracetyl (TA-) and perpropanoyl-β-CyDs (TP-β-CyDs)) and *m*-nitrobenzyl alcohol (TB- to perlauroyl-β-CyDs (TL-β-CyDs)). Specific rotation, $[\alpha]_D$, of the peracylated β-CyDs was measured at 25 °C using a Jasco DIP-360 polarimeter (Tokyo, Japan). Differential scanning calorimetry (DSC) was accomplished with a Rigaku TAS 100 (Tokyo, Japan) or a Perkin Elmer DSC-7 (Yokohama, Japan) operating at a scanning rate of 1.0 °C/min, and the sample amount was 5.0 mg.

Preparation of Peracylated β-CyDs Peracylated β-CyDs (acetyl—lauroyl) were prepared using corresponding acid anhydrides and pyridine as a solvent. For example, dried β-CyD (10 g) was dissolved in anhydrous pyridine (100 ml), butyric anhydride (60.5 ml, about 42 times the molar quantity of β-CyD) was added dropwise for 2—3 h, and the mixture was stirred at 80 °C for 36 h. The reaction solution was poured into cold water and the resulting precipitate was filtered. In case of an oily product, the reactant was extracted with chloroform and the organic phase was washed with water, dried with Na_2SO_4 and evaporated. The crude products were applied to repeated silica gel column chromatography (eluant: chloroform and then chloroform/methanol (50:1, v/v)). The eluant containing TB-β-CyD was washed with 0.002 M aqueous sodium carbonate and then with water, and evaporated to dryness. The residue was recrystallized from methanol to give white crystals (16.54 g, yield 72%). The preparation conditions and identification of each peracylated β-CyD are described below, including the reaction time, the eluant for column chromatography, and some analytical data. NMR spectroscopic data of the peracylated β-CyDs are listed in Table I.

1. TA-β-CyD: Reaction time 6 h; eluant $CHCl_3$ to $CHCl_3/CH_3OH$ (30:1); yield 75%; white crystal with mp 201—202 °C; $[\alpha]_D^{25}$ +125° ($CHCl_3$); MS (positive FAB, methanol/glycerol/*m*-nitrobenzyl alcohol) *m*(nominal mass)/*z* 2017 $[M+H]^+$; *Anal.* Calcd for $C_{84}H_{112}O_{56}$: C, 50.00; H, 5.59. Found: C, 49.82; H 5.57. Rf=0.46 ($CHCl_3/CH_3OH$, 25:1).
2. TP-β-CyD: Reaction time 24 h; eluant $CHCl_3$ to $CHCl_3/CH_3OH$ (40:1); yield 62%; white crystal with mp 168—169 °C; $[\alpha]_D^{25}$ +106° ($CHCl_3$); MS (methanol/glycerol/*m*-nitrobenzyl alcohol) *m/z* 2311 $[M+H]^+$; *Anal.* Calcd for $C_{105}H_{154}O_{56}$: C, 54.54; H, 6.71. Found: C, 54.81; H 6.85. Rf=0.45 ($CHCl_3/CH_3OH$, 30:1).
3. TB-β-CyD. Reaction time 36 h; eluant $CHCl_3$ to $CHCl_3/CH_3OH$ (50:1); yield 72%; white crystal with mp 126—127 °C; $[\alpha]_D^{25}$ +100° ($CHCl_3$); MS (*m*-nitrobenzyl alcohol) *m/z* 2605 $[M+H]^+$; *Anal.* Calcd for $C_{126}H_{196}O_{56}$ C, 58.05; H, 7.58. Found: C, 58.10; H 7.49. Rf=0.56 ($CHCl_3/CH_3OH$, 40:1).

TABLE I. ^{1}H- and ^{13}C-NMR Chemical Shifts (δ, ppm)[a] and ^{1}H–^{1}H Coupling Constants (J, Hz) of Acylated β-CyDs[b] in $CDCl_3$ at 25 °C



R=$-CO(CH_2)_nCH_3$, n=0—10

	δ (ppm)							
	TA	TP	TB	TV	TH	TO	TD	TL
H1	5.06	4.99	4.98	5.04	5.00	5.05	5.04	5.04
H2	4.77	4.71	4.68	4.74	4.68	4.72	4.71	4.71
H3	5.27	5.25	5.24	5.32	5.27	5.32	5.32	5.31
H4	3.68	3.66	3.65	3.73	3.68	3.73	3.72	3.71
H5	4.12	4.06	4.04	4.08	4.03	4.07	4.07	4.07
H6a	4.54	4.46	4.44	4.51	4.47	4.52	4.51	4.51
H6b	4.25	4.24	4.23	4.29	4.24	4.28	4.28	4.27
CH_2[c]	—	2.1—2.4	1.5—2.3	1.3—2.4	1.2—2.4	1.3—2.4	1.2—2.4	1.2—2.4
CH_3[c]	2.0—2.1	1.0—1.1	0.82—0.89	0.86—0.96	0.80—0.86	0.85—0.90	0.84—0.91	0.85—0.89
C1	96.70	96.55	96.40	96.43	96.23	96.31	96.34	96.34
C2	70.35	70.14	70.02	70.32	70.26	70.41	70.44	70.44
C3	70.80	70.50	70.17	70.32	70.26	70.50	70.56	70.56
C4	76.70	76.33	76.24	76.18	76.00	76.03	76.06	76.06
C5	69.53	69.50	69.44	69.59	69.44	69.47	69.50	69.50
C6	62.43	62.25	62.09	62.19	62.00	62.09	62.13	62.13
CH_2[c]	—	27.11	18.1—35.6	22.1—33.7	22.1—33.9	22.6—34.1	22.7—34.2	22.7—34.4
CH_3	20.72	8.76	13.35	13.64	13.70	14.01	14.07	14.1
C=O	170.66	173.93	172.96	173.36	173.17	173.33	173.36	173.33
	170.35	173.51	172.54	172.87	172.69	172.81	172.84	172.81
	169.35	172.42	171.41	171.66	171.51	171.63	171.66	171.63

	J (Hz)							
	TA	TP	TB	TV	TH	TO	TD	TL
H1, 2	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7
H2, 3	9.5	9.6	9.9	9.9	10.1	9.9	9.9	10.3
H3, 4	8.8	8.8	8.8	8.8	8.8	9.2	9.2	8.8
H6, 6	−12.0	−11.7	−12.1	−11.7	−11.7	−11.4	−12.1	−11.7

a) Relative to external tetramethylsilane (TMS). *b*) The concentration of acylated β-CyDs was 5.0 w/v%. *c*) ^{1}H-signals of methyl and methylene groups could not be assigned accurately because of the close proximity or overlap of the C2, C3 and C6 acyl groups. The acyl groups gave a set of ^{13}C-resonance peaks corresponding to methylene moiety between about 18—35 ppm.

4. Perpentanoyl β-CyD (TV-β-CyD): Reaction time 2 d; eluant *n*-hexane to *n*-hexane/ethyl acetate (9 : 1); yield 70%; white crystal with mp 54—56 °C; $[\alpha]_D^{25}$ +91° ($CHCl_3$); MS (*m*-nitrobenzyl alcohol) *m/z* 2899 $[M+H]^+$; *Anal.* Calcd for $C_{147}H_{238}O_{56}$: C, 60.85; H, 8.27. Found: C, 61.14; H, 8.45. *Rf*=0.88 ($CHCl_3$/CH_3OH, 40 : 1).

5. Perhexanoyl β-CyD (TH-β-CyD): Reaction time 4 d; eluant *n*-hexane to *n*-hexane/ethyl acetate (20 : 1); yield 80%; colorless oil; $[\alpha]_D^{25}$ +82° ($CHCl_3$); MS (*m*-nitrobenzyl alcohol) *m/z* 3193 $[M+H]^+$; *Anal.* Calcd for $C_{168}H_{280}O_{56}$: C, 63.14; H, 8.83. Found: C, 63.12; H, 9.00. *Rf*=0.35 (benzene/ethyl acetate, 15 : 1).

6. Peroctanoyl β-CyD (TO-β-CyD): Reaction time 4 d; eluant benzene to benzene/ethyl acetate (30 : 1); yield 78%; colorless oil; $[\alpha]_D^{25}$ +73° ($CHCl_3$); MS (*m*-nitrobenzyl alcohol) *m/z* 3781 $[M+H]^+$, *Anal.* Calcd for $C_{210}H_{364}O_{56}$: C, 66.64; H, 9.69. Found: C, 66.56; H, 9.95. *Rf*=0.48 (benzene/ethyl acetate, 15 : 1).

7. Perdecanoyl β-CyD (TD-β-CyD): Reaction time 5 d; eluant *n*-hexane to *n*-hexane/ethyl acetate (40 : 1); yield 51%; colorless oil; $[\alpha]_D^{25}$ +61°; MS (*m*-nitrobenzyl alcohol) *m/z* 4369 $[M+H]^+$; *Anal.* Calcd for $C_{252}H_{448}O_{56}$: C, 69.19; H, 10.32. Found: C, 69.02; H, 10.57. *Rf*=0.29 (*n*-hexane/ethyl acetate, 10 : 1).

8. TL-β-CyD: Reaction time 7 d; eluant *n*-hexane to *n*-hexane/ethyl acetate (50 : 1); yield 60%; colorless oil; $[\alpha]_D^{25}$ +57°; MS (*m*-nitrobenzyl alcohol) *m/z* 4957 $[M+H]^+$; *Anal.* Calcd for $C_{294}H_{532}O_{56}$: C, 71.14; H, 10.80. Found: C, 71.20; H, 11.18. *Rf*=0.32 (*n*-hexane/ethyl acetate, 10 : 1).

Solubility Measurements Excess amount of peracylated β-CyDs was added to an ethanol/water solution and the mixture was shaken at 25 °C. After equilibrium was attained (about 30 d), the mixture was filtered, and the filtrate was assayed for peracylated β-CyDs by high performance liquid chromatography (HPLC) under the following conditions: pump, Jasco BIP-1 (Tokyo, Japan); column, LiChrosorb Si 60-5 (4.6 × 250 mm, GL Sciences, U.S.A.); mobile phase, *n*-hexane/ethanol (20 : 1, v/v); flow rate, 1.0 ml/min; detection, 210 nm.

Kinetic Studies Alkaline hydrolysis of peracylated β-CyDs (1.0×10^{-4} M) was conducted in 0.05 M NaOH/80% ethanol–water solution at constant temperatures (15—50 °C). At timed intervals, the reaction solution (0.2 ml) was sampled and neutralized with 2.8 M HCl (0.01 ml) and the resulting aliphatic acids were determined by HPLC under the following conditions: pump, Hitachi 635A (Tokyo, Japan); column, YMC AM-312 (6.0 × 150 mm, Kyoto, Japan); mobile phase, 0.5% phosphoric acid/methanol (1 : 1, v/v); flow rate, 1.0 ml/min; detection, 210 nm.

Release Studies The kneading method was employed to prepare the sample powders (TA—TV-β-CyDs) and oil (TH-β-CyD) of drugs/peracylated β-CyDs in a 1 : 1 molar ratio, as reported previously.[8] For example, molsidomine (1.0 g) and TB-β-CyD (10.76 g) were dissolved in ethanol (about 10 ml), kneaded thoroughly for about 40—60 min and dried under a reduced pressure for about 3 d. The release behavior

of sample powders (sieved through 100 mesh) or the viscous oil was examined employing the dispersed amount method.[9] The samples were put into 25 ml water which was kept at 37 °C and stirred at 57 rev/min. Samples (0.5 ml) were withdrawn with a cotton plug, diluted with water and assayed spectrophotometrically at the following wavelengths: 290 nm (propranolol hydrochloride),[10] 245 nm (diltiazem hydrochloride),[11] 313 nm (molsidomine),[12] and 210 nm (isosorbide dinitrate).[13] Salbutamol was determined by a fluorescence spectrometer ($\lambda_{emit}=310$ nm, $\lambda_{excit}=273$ nm).[14]

Results and Discussion

NMR and Mass Spectroscopic Characterization of Peracylated β-CyDs The ^{1}H-NMR spectra of peracylated β-CyDs in $CDCl_3$ were unequivocally assigned by ^{1}H correlation spectroscopy (COSY) (see Table I). For example, the peak areas of methyl and ethylene signals in TB-β-CyD were 9- and 12-fold larger than that of the anomeric proton (H1), respectively, indicating that all hydroxyl groups of β-CyD were substituted by butanoyl groups. As shown in Table I, the coupling constants of the peracylated glucose unit ($J_{1,2}=3.7$ Hz, $J_{2,3}=9.9$ Hz, $J_{3,4}=8.8$ Hz) were almost the same as those of diethyl-β-CyD,[15] suggesting little change in the $^4C_1$ conformation of the glucose unit. The ^{13}C-NMR spectrum of TB-β-CyD showed six signals assigned to the glucose unit, three signals assigned to carboxyl groups of the butanoyl moiety introduced at 2, 3 and 6 positions of the glucose unit, and three signals assigned to the propyl groups which were magnetically equivalent between the 2, 3 and 6 positions. Figure 1 shows a typical example of the FAB-MS (positive mode) of TB-β-CyD, which gave the m/z peak at 2605 $[M+H]^+$ from which the butanoyloxyl moiety was eliminated stepwise (2517, 2429, 2341). The purity of peracylated β-CyDs was confirmed further by TLC, HPLC and elemental analysis. These results indicate that all the hydroxyl groups of β-CyD are acylated, maintaining a macrocyclic structure in the peracylated β-CyDs.

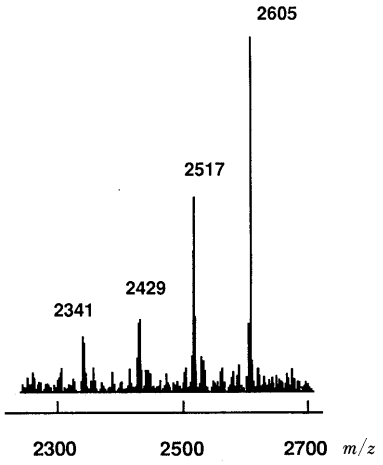


Fig. 1. Positive Ion FAB Mass Spectrum of TB-β-CyD
Matrix: *m*-nitrobenzyl alcohol.

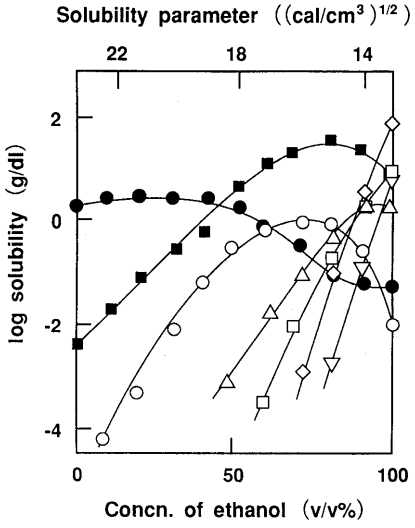


Fig. 2. Solubility Profiles of β-CyD Derivatives as a Function of Ethanol Concentration in Water at 25 °C or Solubility Parameter of Solvent

●, β-CyD; ■, DE-β-CyD; ○, TA-β-CyD; △, TP-β-CyD; □, TB-β-CyD; ◇, TV-β-CyD; ▽, TH-β-CyD.

Solubility Behavior of Peracylated β-CyDs in Ethanol/Water Mixture TA-, TP-, TB- and TV-β-CyDs were isolated as white crystals, the melting point decreasing in that order (201—202, 168—169, 126—127, 54—56 °C, respectively), whereas the homologs higher than TH-β-CyD occurred as a colorless oil. Figure 2 shows solubility curves of the peracylated β-CyDs as a function of ethanol concentration in water or solubility parameter of the solvent mixture. The solubility in water of peracylated β-CyDs decreased with increasing alkyl chain length, whereas that in ethanol showed a maximum at TV-β-CyD and decreased with further chain elongation. The solubility of peracylated β-CyDs in ethanol/water increased with increasing ethanol concentration, but tended to decrease with further increase in ethanol concentration. Since these peak-solubility phenomena may result from the solute–solvent interaction, the solubility curves were analyzed by the modified Hildebrand equation, using solubility parameter (δ) of the solvent mixture in various volume fractions.[16,17] The polynomial regression equations for solubilities (S) of parent β-CyD, heptakis(2,6-di-*O*-ethyl)-β-CyD (DE-β-CyD) and peracylated β-CyDs were as follows:

For parent β-CyD,

$$\log S = 356.1 - 92.95\delta + 9.390\delta^2 - 0.4624\delta^3 + 0.01117\delta^4 - 0.0001064\delta^5$$
$$n=11,\quad r^2=0.9959$$

For DE-β-CyD,

$$\log S = -62.24 + 10.60\delta - 0.5583\delta^2 + 0.009128\delta^3$$
$$n=11,\quad r^2=0.9786$$

For TA-β-CyD,

$$\log S = -77.67 + 12.26\delta - 0.6188\delta^2 + 0.009804\delta^3$$
$$n=11,\quad r^2=0.9938$$

For TP-β-CyD,

$$\log S = -15.80 + 2.625\delta - 0.1060\delta^2$$
$$n=7,\quad r^2=0.9730$$

For TB-β-CyD,

$$\log S = -10.65 + 2.401\delta - 0.1155\delta^2$$

$$n=5,\quad r^2=0.9976$$

For TV-β-CyD,

$$\log S=-6.392+2.319\delta-0.1298\delta^2$$
$$n=4,\quad r^2=0.9999$$

Parent β-CyD required a polynomial in the fifth degree, whereas for DE-β-CyD and peracylated β-CyDs, the polynomial in the second or third degree was enough to reproduce the experimental solubility curves. Then, the solubility parameters of peracylated β-CyDs were calculated by differentiating the above equations [$d(\log(S))/d\delta=0$], because the δ value of solvent mixture at a peak corresponds to that of solute. The values thus obtained were as follows: 20.65 (β-CyD), 15.03 (DE-β-CyD), 15.94 (TA-β-CyD), 12.39 (TP-β-CyD), 10.40 (TB-β-CyD), 8.93 (TV-β-CyD). Obviously, the δ value of peracylated β-CyDs decreased with lengthening of the alkyl chain. It is also predictable that methanol ($\delta=14.5$) and propylene glycol ($\delta=14.8$) are suitable solvents for dissolution of DE- and TA-β-CyDs because of their comparable δ value, whereas TV-β-CyD is more soluble in benzene (9.1) or chloroform (9.3).[18,19]

Hydrolysis Behavior of Acylated β-CyDs in Alkaline Solution Hydrolysis behavior of TP-, TB- and TV-β-CyDs was investigated, using 80% ethanol/water as a solvent, because they were hardly soluble in water. The hydrolysis rate was monitored by measuring the liberated aliphatic acids at an early stage (within about 15 min) of the reaction by HPLC, because it was difficult to quantify a number of positional isomers of partially hydrolyzed acylated β-CyDs, and parent β-CyD and its complexes with aliphatic acids precipitated as the reaction proceeded. The hydrolysis rate constant was determined to be a pseudo first-order rate constant (k) with respect to the concentration of peracylated β-CyDs, from the linear plot according to Eq. 1, because the alkaline concentration (5.0×10^{-2} M) was much higher than that of the substrate (1.0×10^{-4} M):

$$\log((21\cdot C_0-C)/21\cdot C_0)=-2.303\cdot k\cdot t+1 \quad (1)$$

In Eq. 1, C is the concentration of liberated aliphatic acids at time t and the initial concentration of these acids was assumed to be 21 times the concentration (C_0) of peracylated β-CyDs because they have 21 acyl groups in a molecule. The typical first-order plots according to Eq. 1 are shown in Fig. 3, where the deviation from the linear plot was due to the precipitation of parent β-CyD and its solid complex with aliphatic acids. No concentration-dependence of the rate constant ($5.6\pm0.4\times10^{-4}$ s^{-1}) was confirmed over the concentration range of $5.0-15\times10^{-5}$ M of TB-β-CyDs in 0.05 M NaOH/80% (v/v) ethanol–water (pH meter reading: about pH 13.8) at 25 °C. Figure 4 shows the first-order dependence of k of TB-β-CyD on hydroxide ion concentration ([OH]) in 0.01—0.15 M NaOH/80% ethanol–water at 25 °C. The k value increased linearly with increasing [OH] concentration at a slope of k_{OH}, second order rate constant, and an intercept of k_0, spontaneous rate constant, as expressed by Eq. 2. The second term of Eq. 2 is negligible compared with the first term (*e.g.*, $|1.20\times10^{-2}\times0.05|>>|4.26\times10^{-6}|$ in 0.05 M NaOH), suggesting that a specific base-catalysis is predominant in the hydrolysis of peracylated β-CyDs.

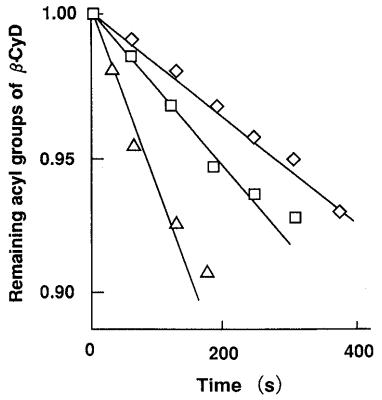


Fig. 3. Semi-logarithmic Plots for Hydrolysis of Peracylated β-CyDs in 0.05 M NaOH/80% Ethanol–Water Solution (pH Meter Reading: about 13.8) at 25 °C

△, TP-β-CyD; □, TB-β-CyD; ◇, TV-β-CyD.

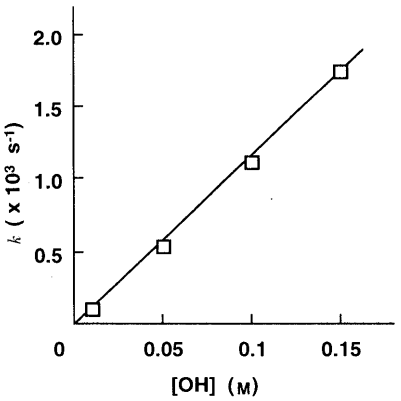


Fig. 4. First-Order Dependence of Hydrolysis Rate of TB-β-CyD on Hydroxide Ion Concentration in 80% Ethanol–Water Solution at 25 °C

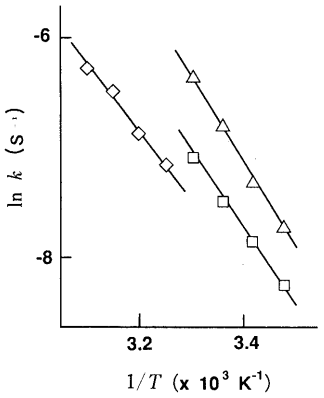


Fig. 5. Arrhenius Plots for Alkaline Hydrolysis of Acylated β-CyDs (1.0×10^{-4} M) in 0.05 M NaOH/80% Ethanol–Water Solution (pH Meter Reading: about 13.8)

△, TP-β-CyD; □, TB-β-CyD; ◇, TV-β-CyD.

$$k=k_{OH}[OH]+k_0=1.20\times10^{-2}\cdot[OH]-4.26\times10^{-6}\quad (r=0.9989) \quad (2)$$

Figure 5 shows Arrhenius plots for the hydrolysis rate of TP-, TB- and TV-β-CyDs over the temperature range of 15—50 °C. Table II summarizes their thermodynamic

TABLE II. Thermodynamic Activation Parameters[a] for Alkaline Hydrolysis of Acylated β-CyDs[b] and Fatty Acid Esters[c] in 0.05 M NaOH/80% Ethanol–Water Solution[d]

Compound	$k^{e)}$ ($\times 10^4\,s^{-1}$)	E_a (kJ/mol)	ΔH^* (kJ/mol)	ΔS^* (J/mol·K)
TP-β-CyD	16.4±1.4	64.8± 5.7	62.3± 5.7	−92±19
TB-β-CyD	8.3±0.4	57.6± 3.2	55.1± 3.2	−122±11
TV-β-CyD	5.8±0.4	49.3± 3.8	46.7± 3.8	−153±12
Ethyl *n*-propionate	4.0±0.1	52.9± 1.8	50.4± 1.8	−144± 6
Ethyl *n*-butanoate	2.2±0.1	57.6± 2.9	55.1± 2.9	−134± 9
Ethyl *n*-pentanoate	2.0±0.3	63.2± 9.1	60.7± 9.1	−116±16
n-Propyl *n*-butanoate	1.6±0.2	63.8± 6.8	61.3± 6.8	−115±13
Isopropyl *n*-butanoate	0.4±0.1	65.5±10.2	63.0±10.2	−122±34

a) The thermodynamic activation parameters were calculated using the following equations: $\Delta H^* = E_a - RT$, $k = \kappa T/h(\exp(-\Delta G^*/RT))$, $\Delta G^* = \Delta H^* - T\Delta S^*$ where E_a, R, T, κ and h are activation energy, gas constant, absolute temperature, Boltzmann constant and Planck constant, respectively. *b*) The concentration of acylated β-CyD was 1.0×10^{-4} M. *c*) The concentration of fatty acid esters was 2.1×10^{-3} M. *d*) pH meter reading was about 13.8. *e*) At 303 K.

activation parameters, together with those of fatty acid esters determined under the same conditions (the initial concentration of fatty acid esters was 21 times that of peracylated β-CyDs). The hydrolysis rate of peracylated β-CyDs decreased with lengthening of the alkyl chain, and was faster by a factor of 3—4 than that of corresponding fatty acid ethyl esters. This deceleration with the chain elongation was thermodynamically different between the CyD and ethyl ester systems, *i.e.*, the activation entropy term (ΔS^*) contributed to the deceleration of peracylated β-CyDs, in contrast to the case of ethyl esters. In general, ΔS^* change can be ascribed to the molecular motions affecting the frequency factor of Arrhenius equation.[20] The increase in ΔS^* of the ethyl esters with the chain elongation may have been due to the suppression of molecular motions, facilitating attacks of hydroxide ion, whereas such ΔS^* contribution is counteracted by the increase in ΔH^* due to the formation of an energetically unfavorable tetrahedral intermediate having a longer hydrophobic chain. In the case of peracylated β-CyDs, particularly TV-β-CyD, access of the hydroxide ion to the carbonyl group seemed to be difficult because the hydrophobic chains are condensed in one molecule, which may result in a large decrease in ΔS^*. This ΔS^* contribution was partly cancelled out by the decrease in ΔH^*, probably due to hydrogen bonding of the hydroxyl group of the tetrahedral intermediate with carbonyl groups of neighboring acyl moieties. To gain insight into the reaction site of peracylated β-CyDs, the hydrolysis behavior of *n*-propyl butanoate and isopropyl butanoate was investigated, these being models for the esters of primary alcohol (6 position of glucose in β-CyD) and secondary alcohol (2 and 3 positions of glucose in β-CyD), respectively. As shown in Table II, the hydrolysis rate of *n*-propyl butanoate was about 4-fold faster than that of iso-propyl butanoate, suggesting that acyl groups at the primary alcohol site may be preferably hydrolyzed.

Interaction Behavior of TB-β-CyD in Solid State We recently reported that TB-β-CyD maintained sufficient plasma levels of molsidomine, a peripheral vasodilator, for a long period of time after oral administration in dogs.[7] Therefore, the molsidomine–TB-β-CyD system was selected as a model system, and the interaction behavior in the solid state was investigated using DSC, since quantitative study in the aqueous solution was difficult due to the low solubility of peracylated β-CyDs in water. Figure 6A shows DSC thermograms of the simple physical mixtures of TB-β-CyD and molsidomine in various molar ratios. The melting peaks of TB-β-CyD (126 °C) decreased with increasing amount of molsidomine, and were significantly broadened. On the other hand, the melting point (139 °C) of molsidomine disappeared below a mixing ratio of about 2 : 1 (drug: TB-β-CyD), whereas it remained above this ratio, suggesting that at least two drug molecules dissolved in one TB-β-CyD molecule. To further clarify the stoichiometry, molsidomine/TB-β-CyD dispersions

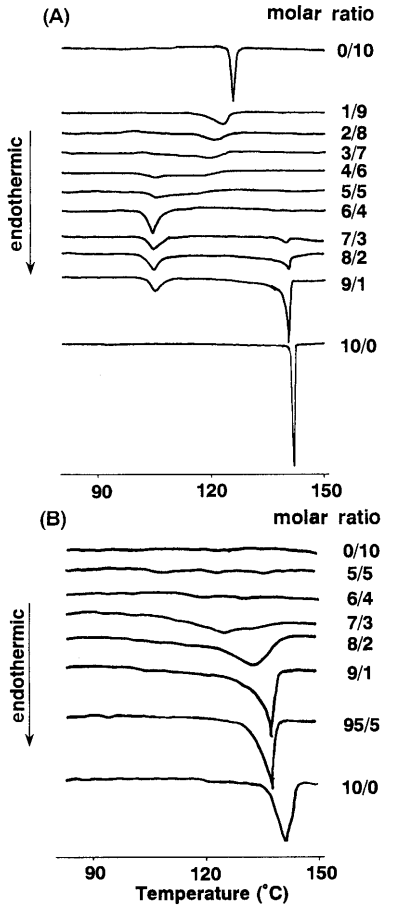


Fig. 6. DSC Thermograms of Molsidomine/TB-β-CyD Physical Mixtures (A) and Their Fusion Products (B) Prepared in Various Molar Ratios (Molsidomine/TB-β-CyD)

with various compositions were prepared by the fusion method and their thermal behavior was investigated. For example, the simple physical mixture of molsidomine and TB-β-CyD was heated to 160 °C and kept at this temperature for 10 min, the fusion product was allowed to cool down to 40 °C, and DSC was measured. As shown in Fig. 6B, the melting peak of TB-β-CyD disappeared with the fusion, because its crystallization from the molten state was significantly slow, in contrast to the rapid crystallization of molsidomine. The melting peak of molsidomine disappeared below about 2:1 molar ratio (molsidomine: TB-β-CyD), whereas it was present above this ratio. These facts indicate that two molecules of molsidomine were dispersed in one TB-β-CyD molecule to form the solid dispersion and an excess amount of molsidomine crystallized in the TB-β-CyD matrix. Giordano *et al.*[21] reported that the stoichiometry of solid CyD complexes can be determined by DSC measurements of the complexes containing excess amounts of guest, where the free mole fraction of guest (FGMF) is expressed by Eq. 3:

$$\mathrm{FGMF} = \mathrm{TGMF}(1+R) - R \qquad (3)$$

where TGMF and R stand for the total mole fraction of guest and the stoichiometry (guest/host) of the complex, respectively. Therefore, DSC curves (Fig. 6B) of the molsidomine/TB-β-CyD system were analyzed by Eq. 3. Figure 7 shows the theoretical lines of Eq. 3 assuming $R=1:1$, 2:1 and 3:1, together with FGMF values obtained from the fusion enthalpy of molsidomine. The fusion enthalpy (0.512 J/mol) of molsidomine was assumed not to be significantly changed by the addition of TB-β-CyD, although its melting point decreased slightly (about 5 °C). It is apparent that the experimental data fitted well to the theoretical line of $R=2$, indicating a 2:1 composition of the solid dispersion.[22,23] This composition may become larger as the alkyl chain lengthens, because of the elongated hydrophobic environment. The higher-order complexation can be expected from the construction of a Corey–Pauling–Koltun molecular model of peracylated β-CyDs.

Release Behavior of Water-Soluble Drugs from Peracylated β-CyD The release behavior of the water-soluble drugs, isosorbide dinitrate, molsidomine, propranolol hydrochloride, and salbutamol sulfate from peracylated β-CyDs was investigated, anticipating a retardation effect depending on the acyl chain length. Figure 8 shows the release profiles of molsidomine, as an example, from powder samples (TA-β-CyD—TV-β-CyD) or the viscous oil (TH-β-CyD) of kneading mixtures in a 1:1 molar ratio. The 1:1 composition was chosen for convenience, since the composition may be changed depending on the length of the alkyl chain. The dissolution rate of drug itself was very fast due to its high solubility in water, whereas the release from peracylated β-CyDs was markedly retarded. The released percentage of molsidomine from TB-β-CyD after 12 h was about 40% under the present conditions. The retarding effect of peracylated β-CyDs was greater with lengthening of the acyl chain, and was dependent on not only the hydrophobicity of peracylated β-CyDs but also the solubility of the drug employed. As shown in Fig. 9, there was a linear relationship between the release rate and solubility parameter of the drugs in each peracylated β-CyD system, indicating that the higher the solubility of a drug in water, the faster is the release from peracylated β-CyDs. In Fig. 9, the release rate constant was calculated

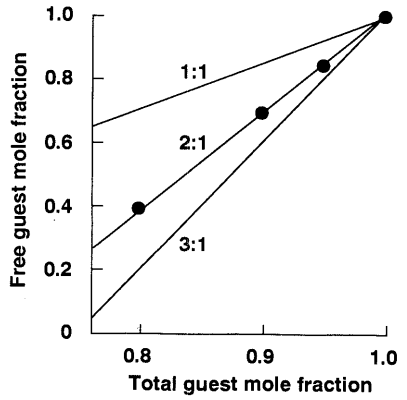


Fig. 7. Plot According to Eq. 3 for Molsidomine/TB-β-CyD System

The solid lines are theoretical ones calculated on the basis of 1:1, 2:1 and 3:1 (molsidomine: TB-β-CyD) stoichiometries, and the closed circles are experimental data.

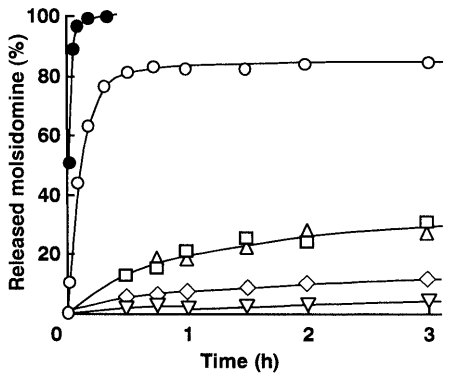


Fig. 8. Release Profiles of Molsidomine from Its Acylated β-CyD Complexes in Water at 37 °C, Measured by Dispersed Amount Method

●, drug alone (dissolution); ○, TA-β-CyD complex; △, TP-β-CyD complex, □, TB-β-CyD complex, ◇, TV-β-CyD complex, ▽, TH-β-CyD complex.

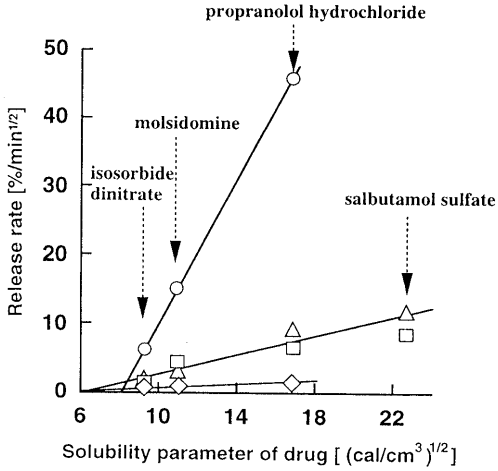


Fig. 9. Plots of Release Rate of Drugs from Their Acylated β-CyD Complexes *versus* Solubility Parameter of Drugs

○, TA-β-CyD; △, TP-β-CyD system; □, TB-β-CyD system; ◇, TV-β-CyD system.

from Higuchi's square root equation,[24] and the solubility parameter of a drug was determined by measuring the maximal solubility of the drug in an ethanol/water solution at various concentrations. From these data, the release rate of water-soluble drugs from their peracylated β-CyD complexes is predictable using the solubility parameter of the drugs.

In conclusion, the present results suggest that peracylated β-CyDs have the potential to modify the release rate of various water-soluble drugs. In particular, TB- and TV-β-CyDs having mucoadhesive and film-forming properties[7] will have broad applicability in the development of transdermal and transmucosal dosage forms; this will be reported elsewhere.

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