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6-*O*-α-(4-*O*-α-D-glucuronyl)-D-glucosyl-β-cyclodextrin: solubilizing ability and some cellular effects

Sumitra Tavornvipas [a], Fumitoshi Hirayama [a], Hidetoshi Arima [a], Kaneto Uekama [a,*], Toshihiro Ishiguro [b], Masahide Oka [b], Kenichi Hamayasu [c], Hitoshi Hashimoto [c]

[a] *Faculty of Pharmaceutical Sciences, Kumamoto University, 5-1 Oe-honmachi, Kumamoto 862-0973, Japan*

[b] *Biotechnology Department, Pharmaceutical Research Division, Takeda Chemical Industries, Ltd., 2-17-85 Jusohonmachi, Yodogawa, Osaka 532-8686, Japan*

[c] *Bio Research Corporation of Yokohama, Yokohama Kanazawa High-Tech Center Techno-Core, Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan*

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Abstract

Some physicochemical and biopharmaceutical properties of a new branched cyclodextrin, 6-*O*-α-(4-*O*-α-D-glucuronyl)-D-glucosyl-β-cyclodextrin (GUG-β-CyD), were investigated. The interaction of GUG-β-CyD with drugs was studied by spectroscopic and solubility methods, and compared with those of parent β-CyD and 6-*O*-α-maltosyl-β-CyD (G_2-β-CyD). The hemolytic activity of GUG-β-CyD on rabbit erythrocytes was lower than those of β-CyD and G_2-β-CyD. GUG-β-CyD and G_2-β-CyD showed negligible cytotoxicity on Caco-2 cells up to at least 0.1 M. The inclusion ability of GUG-β-CyD to neutral and acidic drugs was comparable to or slightly smaller than those of β-CyD and G_2-β-CyD, probably because of a steric hindrance of the branched sugar. On the other hand, GUG-β-CyD showed greater affinity for the basic drugs, compared with β-CyD and G_2-β-CyD, owing to an electrostatic interaction of its carboxylate anion with positive charge of basic drugs. Thus, GUG-β-CyD may be useful as a safe solubilizing agent particularly for basic drugs.

Keywords: Branched cyclodextrin; Inclusion ability; Stability constant; Electrostatic interaction; Hemolysis; Cytotoxicity

1. Introduction

Extensive efforts have been directed to the development of safer and more functional cyclodextrin (CyD) derivatives as parenteral drug carriers (Duchêne, 1991; Loftsson and Brewster, 1996; Uekama et al., 1998; Szente and Szejtli, 1999). Among such CyD derivatives, 2-hydroxypropyl-β-CyD, sulfobutylether of β-CyD and branched CyDs seem to be the prospective drug carriers for parenteral preparations, because of their higher aqueous solubility and apparent lack

* Corresponding author. Tel./fax: +81-96-371-4160

E-mail address: uekama@gpo.kumamoto-u.ac.jp (K. Uekama).

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of toxicity (Pitha, 1985; Stella and Rajewski, 1997). Branched CyDs, in which mono- or disaccharides are introduced on one or two primary hydroxyl groups of CyDs through the α-1,6-glycosidic linkage, have received increasing attention in pharmaceutical and food fields (Okada and Koizumi, 1998). There are many advantages of branched CyDs over the parent CyDs, such as higher solubility in water, lower hemolytic activity and higher bioadaptability. Recently, 6-*O*-α-(4-*O*-α-D-Glucuronyl)-D-glucosyl-β-cyclodextrin (GUG-β-CyD, Fig. 1) has been prepared by the oxidation of 6-*O*-α-D-maltosyl-β-CyD (G_2-β-CyD) with *Pseudogluconobacter saccharoketogenes* (Ishiguro et al., 2001). Since GUG-β-CyD contains a carboxyl group (p*K*a 3.5, Ishiguro et al., 2001) in a molecule, which dissociates to negatively charged carboxylate anion depending on pH of solution, its inclusion ability may be different from that of non-ionized CyDs such as parent β-CyD and G_2-β-CyD. In this study, we investigated the inclusion complexation of GUG-β-CyD with various neutral, anionic and cationic drugs, in comparison with those of parent β-CyD and G_2-β-CyD. Further, some cellular effects of GUG-β-CyD were compared with those of β-CyD and G_2-β-CyD.

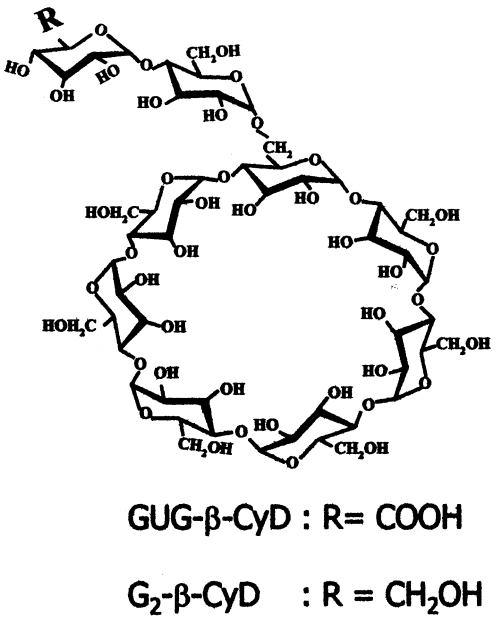

Fig. 1. Chemical structures of GUG-β-CyD and G_2-β-CyD.

2. Materials and methods

2.1. Materials

β-CyD was supplied from Nihon Shokuhin Kako Co. (Tokyo, Japan). GUG-β-CyD and its sodium salt and G_2-β-CyD were from Bio Research Corporation of Yokohama (Yokohama, Japan). The following drugs were used as supplied: betamethasone, diazepam, flurbiprofen and phenytoin from Wako Chemical Co. (Osaka, Japan), chlorpropamide and *p*-hydroxybenzoic acid esters (methyl, ethyl, propyl, butyl and hexyl) from Tokyo Kasei Co. (Tokyo, Japan), cinnarizine and indomethacin from Sigma Chemical Co. (MO, USA), digitoxin, prednisolone, progesterone and testosterone from Nacalai Tesque Co. (Kyoto, Japan) and furosemide and tolbutamide from Hoechst Marion Roussel Ltd (Tokyo, Japan). All other materials and solvents were of analytical reagent grade. Deionized, double-distilled water was used throughout the study.

2.2. Apparatus

Optical rotations were measured with a Jasco DIP-100 digital polarimeter (Tokyo, Japan). Melting points were measured with a Yanaco micro-melting points apparatus (Tokyo, Japan). Surface tension was measured with a duNouy surface tensiometer (Shimadzu, Kyoto, Japan). Ultraviolet (UV), fluorescence and circular dichroism (CD) spectra were measured at 25 °C using Hitachi U-2000 UV, F-4500 fluorescence spectrometers (Tokyo, Japan) and a Jasco J-720 polarimeter (Tokyo, Japan), respectively. ^{13}C-nuclear magnetic resonance (NMR) spectra were measured with a Jeol JNM-α 500 instrument (Tokyo, Japan), operating 126 MHz (^{13}C) at 25 °C, and chemical shifts were given as ppm downfield from that of tetramethylsilane, by referring to that of dioxane signal (67.4 ppm).

2.3. Solubility studies

The solubility studies were carried out according to the method of Higuchi and Connors (1965). Excess amounts of drugs were added to aqueous solutions containing various concentrations of CyDs, and the solutions were shaken at 25 °C. After equilibrium was attained, the solutions were centrifuged and an aliquot was taken through a cotton plug, diluted adequately and analyzed for drug by UV and HPLC methods: UV method: betamethasone ($\lambda = 239$ nm), chlorpropamide (232 nm), cinnarizine (254 nm), diazepam (231 nm), furosemide (276 nm), indomethacin (260 nm), digitoxin (215 nm), flurbiprofen (247 nm), phenytoin (258 nm), prednisolone (242 nm), progesterone (240 nm), testosterone (238 nm) and tolbutamide (228 nm). HPLC method: *p*-hydroxybenzoic acid esters (methyl, ethyl, propyl, butyl and hexyl) (detection 256 nm) and cinnarizine (254 nm). The HPLC conditions were as follows: a Jasco PU-1580 pump and a UV-970 detector (Tokyo, Japan), a YMC AM-312 column (6.0×150 mm^2) and a flow rate of 1.0 ml/min. The mobile phases for analysis of *p*-hydroxybenzoic acid esters and cinnarizine were methanol/water (7:3 v/v) and acetonitrile/0.01 M phosphate buffer (pH 2.5) (1:1 v/v), respectively. The stability constants ($K_{1:1}$) of 1:1 (guest:host) complexes were calculated from the slope and intercept of straight line of the phase solubility diagram according to the equation of Higuchi and Connors (Eq. (1), 1965);

$$K_{1:1} = \text{slope}/\text{intercept}(1\text{-slope}) \quad (1)$$

The A_p type diagrams were analyzed according to the method of Higuchi and Kristiansen (1970) to obtain the 1:1 and 1:2 ($K_{1:2}$, guest:host) stability constants.

2.4. Spectroscopic studies

The spectroscopic changes (UV, CD or fluorescence) of drugs in the presence of CyDs were analyzed at 25 °C in 0.1 M phosphate buffer. The stability constants of the 1:1 complexes ($K_{1:1}$) were obtained from the Scott equation (Eq. (2), 1956)

$$ab/d = 1/(K_{1:1}\varepsilon_c) + b/\varepsilon_c \quad (2)$$

where a is the total concentration of drug, b is the total concentration of CyDs, ε_c is the difference in molar absorptivities for free and complexed drugs, and d is the change in absorbance of drugs by the addition of CyDs. The stoichiometry of the complexes in isotonic phosphate buffer (pH 7.4) at 25 °C was determined by the continuous variation method (Job, 1928).

2.5. Hemolysis assays

From freshly drawn rabbit blood, erythrocytes were separated by centrifugation at $1000 \times g$ for 5 min, washed 3 times with isotonic phosphate buffer (pH 7.4) and resuspended in the buffer solution to give a hematocrit of 5%. The cell suspension (0.2 ml) was added to the buffer solution (2.0 ml) containing CyDs at various concentrations. The mixture was incubated for 30 min at 37 °C and then centrifuged at $1000 \times g$ for 5 min. The release of hemoglobin from the cells was measured spectrophotometrically at 543 nm. Results were expressed as percentages of the total efflux of hemoglobin that was obtained when water was used instead of the buffer solution.

2.6. Cytotoxicity

The intracellular enzyme activity was assayed by using a cell counting kit (WST-1 method, Anderberg et al., 1992) from Wako Pure Chemical Industries (Osaka, Japan). Caco-2 cells were seeded at 5×10^5 cells on 96-well microplates (Iwaki, Tokyo, Japan) and incubated for 1 d in a humidified atmosphere containing 5% CO_2 and 95% air at 37 °C. Cells were washed 3 times with Hank's-balanced salt solution (HBSS, pH 7.4) and then incubated for 1 h with 100 μl of HBSS containing CyDs or Tween 20 at various concentrations in a humidified atmosphere. After washing 3 times with HBSS to remove CyDs and Tween 20 again, fresh HBSS (100 μl) and WST-1 reagent (10 μl) were added to the plates and incubated for 2 h at 37 °C. The absorbance at 450 nm against a reference wavelength of 620 nm was determined

with a miniplate reader (Nalge Nunc International NJ-2300. Rochester, USA).

3. Results and discussion

3.1. Physico- and bio-chemical properties

Table 1 lists some physico- and bio-chemical properties of GUG-β-CyD, in comparison with those of parent β-CyD and G_2-β-CyD. The solubility of GUG-β-CyD (>2 g/ml) and G_2-β-CyD (>1.5 g/ml) was about 100 and 80 times, respectively, greater than that (0.019 g/ml) of β-CyD in water at 25 °C. Values of surface tension of these β-CyDs were about the same as that of water (71 mM/m), indicating negligible surface activity. This is in contrast to the case of alkylated CyD derivatives such as methylated and hydroxyalkylated β-CyDs that have slightly higher surface activity (Yoshida et al., 1989). Fig. 2 showed the hemolytic effects of GUG-β-CyD, G_2-β-CyD and parent β-CyD on rabbit erythrocytes in isotonic phosphate buffer. The hemolytic curve of GUG-β-CyD shifted to higher concentrations, indicating that its hemolytic activity is weaker than those of G_2-β-CyD and β-CyD, i.e. the concentration to induced 50% hemolysis (Table 1) was in the order of GUG-β-CyD (11.6 mM) > G_2-β-CyD (8.4 mM) > β-CyD (5.7 mM). Okada et al. (1988) investigated the relationship between aqueous solubility and hemolytic activity of doubly-branched CyDs, 6^1,6^n-di-*O*-α-D-glucopyranosyl-β-CyDs ($1,n$-$(Glc)_2$-β-CyD, $n=2$–4), and reported that the 1,4-isomer with low aqueous solubility has a stronger hemolytic activity than

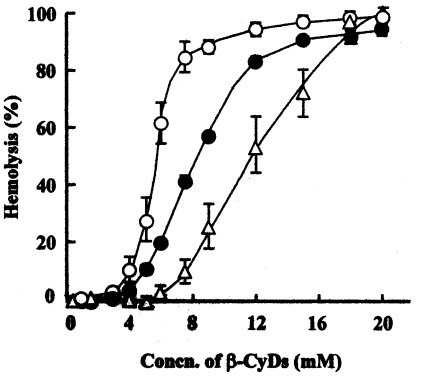

Fig. 2. Hemolysis effects of β-CyDs on rabbit erythrocytes in isotonic phosphate buffer (pH 7.4) at 37 °C. Each point represents the mean ± S.E. of 6–8 experiments. (○) β-CyD; (●) G_2-β-CyD; (△) GUG-β-CyD.

1,2- and 1,3-isomers with higher solubility. Therefore, the weak hemolytic activity of GUG-β-CyD, compared with β-CyD and G_2-β-CyD, may be ascribed partly to its higher solubility. Further, we found that the inclusion ability of GUG-β-CyD to drugs having steroidal skeletons is smaller than that of G_2-β-CyD. These results suggest that the lower hemolytic activity of GUG-β-CyD may be due to the minimal capacity to solubilize cholesterol from the lipid membrane of erythrocytes, reflecting in the gradual increase of hemolytic curve, compared with the steep ones of β-CyD and G_2-β-CyD. The cytotoxicity of GUG-β-CyD, G_2-β-CyD and parent β-CyD toward Caco-2 cells was studied by measuring intracellular dehydrogenase activity (WST-1), and the results were shown in Fig. 3. A non-ionic surfactant, Tween 20, was used as a positive control. GUG-β-CyD

Table 1
Some physicochemical properties of β-CyD, G_2-β-CyD and GUG-β-CyD

CyD	Glucose unit	Molecular weight	$[\alpha]_D$[a]	Solubility (mg/ml)[a]	Surface tension (dyne/cm)[a,b]	Hemolytic active (mM)[c]
β-CyD	7	1135	158	19	75	5.7
G_2-β-CyD	9	1459	166	>1500	72	8.4
GUG-β-CyD	9	1473	155	>2000	73	11.6

[a] At 25 °C in water.
[b] Concentration of CyDs was 0.1% w/v.
[c] The concentration of CyDs to induce 50% hemolysis of rabbit erythrocytes.

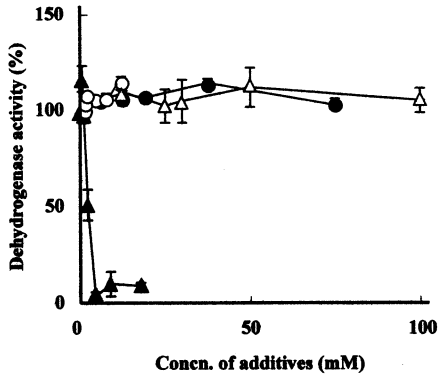

Fig. 3. Effects of additives on intracellular dehydrogenase activity of Caco-2 cells at apical side after incubation for 1 h in HBSS (pH 7.4) at 37 °C. Each point represents the mean ± S.E. of 6 experiments. (○) β-CyD; (●) G_2-β-CyD; (△) GUG-β-CyD, (▲) Tween 20.

and G_2-β-CyD showed negligible cytotoxicity on Caco-2 cells up to at least 100 mM, and parent β-CyD up to its solubility limit (15 mM), whereas Tween 20 markedly decreased the viability of Caco-2 cells even below 5 mM. These results indicate that GUG-β-CyD is useful as a parenteral carrier from a safety viewpoint.

3.2. Solubilizing ability of GUG-β-CyD

Aqueous solubility of various poorly water-soluble drugs in the absence and presence of GUG-β-CyD was measured, in comparison with G_2-β-CyD and parent β-CyD, to gain insight into their solubilizing abilities. Table 2 shows the enhancement in solubility of various drugs by the addition (15.0 mM) of GUG-β-CyD, G_2-β-CyD and parent β-CyD in water at 25 °C. It was apparent that GUG-β-CyD and G_2-β-CyD have a larger solubilizing ability to drugs having steroidal skeletons, such as betamethasone, digitoxin, prednisolone, progesterone and testosterone, and the affinity of these steroidal drugs to GUG-β-CyD was only slightly weaker than that to G_2-β-CyD. The solubility of flurbiprofen was significantly increased (by ~45 times) by the addition of the branched β-CyDs, whereas it increased only 3-fold by parent β-CyD. The higher solubilizing effect of the branched β-CyDs may be attributable to the formation of soluble complexes, i.e. they give A_L and A_P type solubility diagrams with no precipitation of solid complexes, whereas parent β-CyD forms solid complexes with flurbiprofen and steroids at higher CyD concentrations, giving B_S type diagrams. The solubilizing effect of the three

Table 2
Enhancement of solubility of poorly water-soluble drugs by complexation with β-CyDs (15 mM) at 25 °C

Drug	Solubility (S_o) in water (mM)	Solubility (S_c) in CyD solution (mM)		
		β-CyD	G_2-β-CyD	GUG-β-CyD
Betamethasone[a]	0.27	3.23 (12)	7.27 (27)	5.98 (22)
Chlorpropamide	0.75	2.19 (2.9)	2.24 (3.0)	2.42 (3.2)
Diazepam	0.19	0.70 (3.6)	0.73 (3.8)	0.65 (3.4)
Digitoxin[a]	0.09	0.84 (9.5)	3.91 (44)	3.75 (42)
Flurbiprofen	0.14	0.42 (2.9)	6.63 (46)	6.18 (43)
furosemide	0.23	0.41 (1.8)	0.36 (1.5)	0.73 (3.1)
Indomethacin	0.06	0.15 (2.5)	0.18 (3.1)	0.29 (5.0)
Phenytoin	0.10	1.29 (13)	1.22 (13)	1.20 (12)
Prednisolone[a]	0.68	9.98 (15)	11.5 (17)	10.1 (15)
Progesterone[a]	0.03	0.12 (4.5)	5.20 (200)	4.80 (185)
Tolbutamide	0.43	0.94 (2.2)	1.58 (3.7)	1.68 (3.9)

Average of the values for 2–3 experiments, with coincided with each other within ±1%. The value in parenthesis represents the ratio of S_c to S_o.
[a] Steroidal skeletron drugs.

β-CyDs on other drugs seemed to be comparable, although no quantitative comparison was conducted.

3.3. Interaction of GUG-β-CyD with neutral, acidic and basic drugs

The interaction of GUG-β-CyD with neutral, acidic and basic drugs was investigated, in comparison with those of parent β-CyD and G_2-β-CyD, by the solubility and spectroscopic methods, to gain insight into the inclusion property of GUG-β-CyD. *p*-Hydroxybenzoic acid esters with different alkyl chains were chosen as neutral guest molecules, flurbiprofen as an acidic guest molecule, and chlorpromazine and cinnarizine as basic guest molecules.

3.3.1. Neutral drugs

The interaction of the three β-CyDs with *p*-hydroxybenzoic acid esters was investigated by the solubility method in pH 7.4 phosphate buffer, where GUG-β-CyD is in anionic form due to the dissociation of its carboxylic acid (p*K*a 3.5, Ishiguro et al., 2001), while β-CyD and G_2-β-CyD are in neutral form. Apparent stability constants of the complexes and types of the solubility diagrams were summarized in Table 3. The phase solubility diagrams of GUG-β-CyD and G_2-β-CyD with *p*-hydroxybenzoic acid esters of short alkyl moieties (methyl to butyl) showed A_L type diagrams, where the solubility of guests increased linearly with CyD concentrations. The hexyl ester gave a A_P type diagram, where the solubility deviated positively from a straight line at higher CyD concentrations, indicating a formation of 1:1 and 1:2 (guest:host) complexes. These branched β-CyDs gave no precipitation of solid complexes with *p*-hydroxybenzoic acid esters under the experimental conditions. On the other hand, parent β-CyD gave B_S type diagrams with the ethyl to hexyl esters, precipitating solid complexes at higher CyD concentrations, except for the methyl ester giving a A_L type diagram.

The stability constant of the complexes increased as the alkyl chain length increases, and those of the complexes with the methyl to butyl esters were in the order of β-CyD > G_2-β-CyD > GUG-β-CyD. The stability constant ($K_{1:1}$) of the 1:1 complexes of the hexyl ester was also G_2-β-CyD > GUG-β-CD, although the comparison with parent β-CyD was difficult because of the different stoichiometry. The weaker interaction of the branched β-CyDs, compared with that of parent β-CyD, may be due to a steric hindrance of the branched sugar units (maltose and glucuronylglucose) appended to the β-CyD rim. Further, the negatively charged carboxylate moiety of GUG-β-CyD seemed to affect disadvantageously the inclusion of neutral guest molecules.

Table 3
Apparent stability constants (K_c, M^{-1})[a] and types of phase solubility diagrams for inclusion complexation of *p*-hydroxybenzoic acid esters with β-CyDs in 0.1 M phosphate buffer (pH 7.4,$I = 0.2$) at 25 °C

Ester	β-CyD		G_2-β-CyD		GUG-β-CyD	
	K_c	Type	K_c	Type	K_c	Type
Methy1	640±50	A_L	260±10	A_L	170±20	A_L
Ethyl	740±20	B_S[b]	460±30	A_L	410±30	A_L
Propyl	1160±90	B_S[b]	1000±50	A_L	530±40	A_L
Butyl	1930±40	B_S[b]	1670±100	A_L	1630±80	A_L
Hexyl	2770±70	B_S[b]	8420[c]±120	A_P	7260[d]±140	A_P

Each value represents the mean±S.E. of 3–4 experiments.
[a] K_c = Slope/so(1-slope).
[b] Molar ratio of host:guest = 1:1.
[c] $K_{1:1} = 6270\ M^{-1}$ and $K_{1:2} = 20\ M^{-1}$.
[d] $K_{1:1} = 5970\ M^{-1}$ and $K_{1:2} = 30\ M^{-1}$.

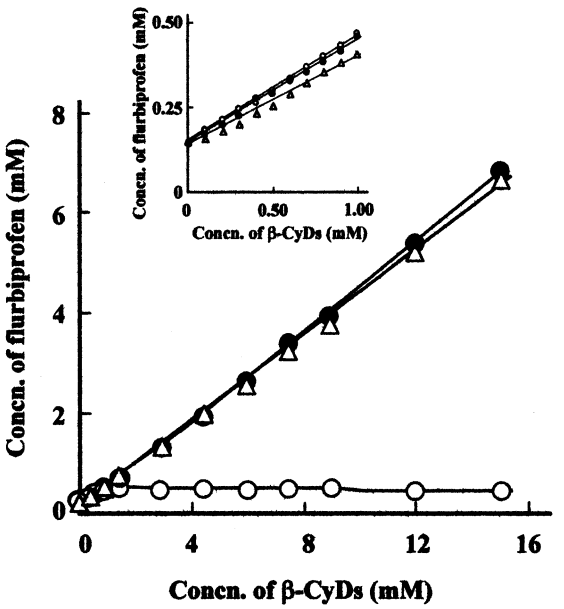

Fig. 4. Phase solubility diagrams of flurbiprofen/β-CyD systems in water at 25 °C. The inset shows those in low CyD concentrations (0–1 mM). (○) β-CyD; (●) G_2-β-CyD; (△) GUG-β-CyD.

3.3.2. Acidic drugs

The interaction of the three β-CyDs with an acidic drug, flurbiprofen, was investigated in water and pH 7.4 phosphate buffer by the solubility and spectroscopic methods, respectively. Fig. 4 shows the phase solubility diagrams of flurbiprofen/β-CyD systems in water at 25 °C. As described before, the GUG-β-CyD and G_2-β-CyD systems showed A_L type diagrams, whereas the parent β-CyD system showed a B_S type diagram, precipitating the solid complex in a molar ratio of 1:1 (guest:host) at higher β-CyD concentrations. The 1:1 stoichiometry was determined by analysis of length of the plateau region of the B_S diagram as well as by chemical analysis of the solid complex isolated. The stability constants were 4000 ± 130, 3800 ± 70 and 3100 ± 100 M^{-1} for the β-CyD, G_2-β-CyD and GUG-β-CyD complexes, respectively. The stability constant of the GUG-β-CyD complex was smaller than those of the parent β-CyD and G_2-β-CyD complexes.

Changes in UV, CD and fluorescence spectra of flurbiprofen by the addition of GUG-β-CyD were investigated in pH 7.4 phosphate buffer where the carboxylic acids of the guest (p*K*a 4.2, Anderson and Conradi, 1985) and host (p*K*a 3.5, Ishiguro et al., 2001) molecules are dissociated into anion forms. The UV absorption intensity of flurbiprofen ($\lambda_{max} = 247$ nm) decreased with a concomitant shift to longer wavelength by the addition of β-CyDs, magnitude of the change being β-CyD > G_2-β-CyD ≈ GUG-β-CyD. A new CD band of flurbiprofen was induced at 244 nm by the addition of β-CyDs, where the cotton effect was β-CyD > G_2-β-CyD > GUG-β-CyD, in qualitative agreement with magnitude of the stability constants. The fluorescence intensity of flurbiprofen ($\lambda = 312$ nm) increased in the order of G_2-β-CyD > β-CyD ≈ GUG-β-CyD. These spectral changes may be the reflection of not only the binding affinity but also the hydrophobicity of the cavity or surrounding environment of the branched β-CyDs. Fig. 5A shows continuous variation plots of flurbiprofen/β-CyD systems, where the maximum fluorescence change was observed at the 1:1 composition, indicating that the guest forms the inclusion complexes with β-CyDs in 1:1 stoichiometry. The stability constants of the complexes were determined by analyzing the UV change according to the Scott equation (Fig. 5B), and were 6000 ± 50, 5400 ± 220 and 2900 ± 280 M^{-1} for the β-CyD, G_2-β-CyD and GUG-β-CyD complexes, respectively. Again, the stability constant of the GUG-β-CyD complex was significantly smaller than those of the β-CyD and G_2-β-CyD complexes. These results suggest that the negatively charged carboxylate anion of GUG-β-CyD affects disadvantageously the inclusion of anionic guest molecules, probably because of an electrostatic repulsion.

3.3.3. Basic drugs

The interaction of the three β-CyDs with basic drugs was investigated, using chlorpromazine (p*K*a 9.2, Chrzanowski et al., 1985) and cinnarizine (pKa_1 1.9 and pKa_2 7.5, Järiven et al., 1995) as model drugs, in pH 7.4 phosphate buffer. UV and CD spectroscopic studies on the interaction with chlorpromazine were conducted, because of its soluble property in water. Chlorpromazine gave a UV absorption maximum at 255 nm and a

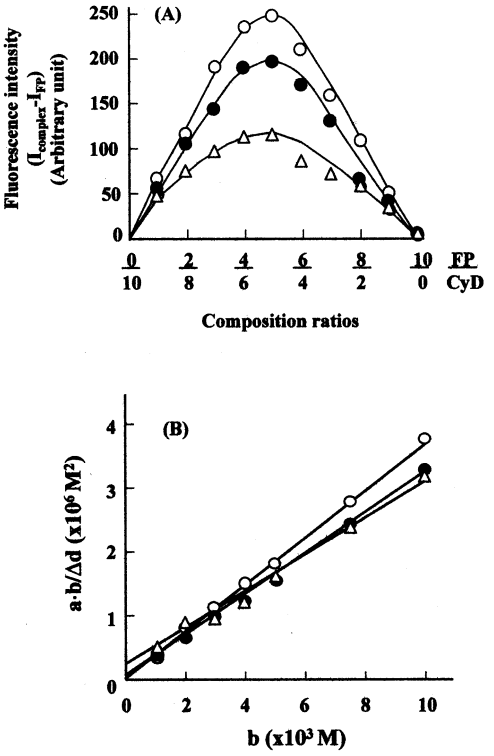

Fig. 5. (A) Continuous variation plots for flurbiprofen/β-CyD systems in 0.1 M phosphate buffer (pH 7.4, $I = 0.2$) at 25 °C. The total concentration of the drug and β-CyDs was 0.1 mM. (B) Scott plots for changes in UV absorption intensity (247 nm) of flurbiprofen (0.04 mM) by the addition of β-CyDs in 0.1 M phosphate buffer (pH 7.4, $I = 0.2$) at 25 °C. (○) β-CyD; (●) G_2-β-CyD; (△) GUG-β-CyD.

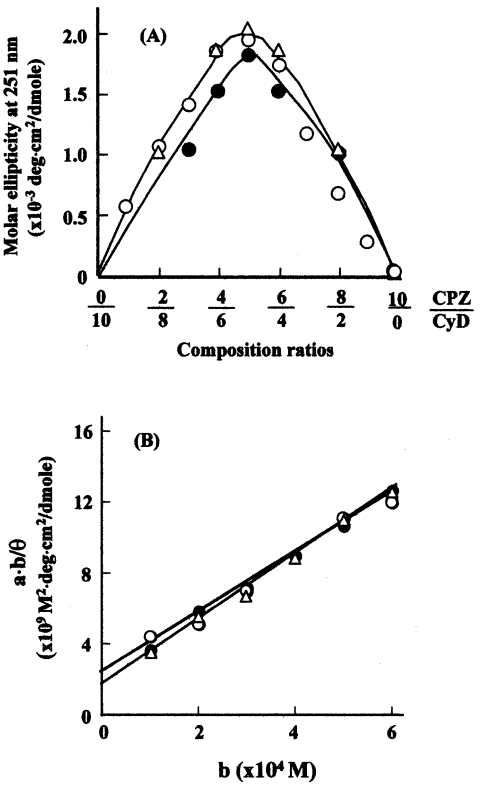

Fig. 6. (A) Continuous variation plots for chlorpromazine/β-CyD systems in 0.1 M phosphate buffer (pH 7.4, $I = 0.2$) at 25 °C. The total concentration of the drug and β-CyDs was 0.05 mM. (B) Scott plots for changes in CD molar ellipticities (251 nm) of chlorpromazine (0.025 mM) by the addition of β-CyDs in 0.1 M phosphate buffer (pH 7.4, $I = 0.2$) at 25 °C. (○) β-CyD; (●) G_2-β-CyD; (△) GUG-β-CyD.

second small peak at 307 nm. The intensity of these UV bands was decreased with concomitant shifts to longer wavelength by the addition of β-CyDs, where the spectral changes were comparable to parent β-CyD, G_2-β-CyD and GUG-β-CyD. In the CD spectra, new induced-cotton effects were observed at 230 nm with negative sign and at 251 nm with positive sign, together with alternate small positive and negative bands in 280–320 nm, when β-CyDs were added to the chlorpromazine solution. The positive CD intensity at 251 nm increased in the order of G_2-β-CyD ≈ GUG-β-CyD > β-CyD. Fig. 6A shows continuous variation plots of chlorpromazine/β-CyD systems, where the maximum CD change was observed at the 1:1 composition, indicating the 1:1 complexation. The stability constants of the complexes were determined by analyzing the CD change according to the Scott equation (Fig. 6B), and were 8340 ± 100, 8000 ± 140 and 9000 ± 150 M^{-1} for the β-CyD, G_2-β-CyD and GUG-β-CyD complexes, respectively. In contrast to the case of the neutral and acidic drugs studied above, chlorpromazine interacted more strongly with GUG-β-CyD than neutral β-CyD and G_2-β-CyD. Since chlorpromazine and GUG-β-CyD are in cationic and anionic forms, respectively, at pH 7.4, this enhanced affinity of GUG-β-CyD to the drug can be ascribed to an electrostatic interaction between the positive and negative charges of the guest and host molecules, in addition to the inclusion. ^{13}C-

NMR spectroscopic studies were conducted to gain insight into the inclusion mode of the chlorpromazine/GUG-β-CyD complex. The ^{13}C-chemical shift changes ($\Delta\delta = \delta_{\text{with GUG-β-CyD}} - \delta_{\text{drug alone}}$) of chlorpromazine (10.0 mM) by the addition of GUG-β-CyD (25.0 mM) in deuterium oxide were as follows (Fig. 7 for carbon numbering of chlorpromazine); C1 ($\Delta\delta = +0.15$ ppm), C2 (+0.11), C3 (+1.39), C4 (+0.39), C5 (+0.80), C6 (−0.12), C7 (+0.96), C8 (+2.19), C9 (+0.17), C10 (+0.60), C11 (−0.23), C12 (+1.89). Large shift changes (>0.2 ppm) were observed for C3 and C4 carbons of the chlorobenzene ring, C5, C7 and C8 carbons of the benzene ring, and C10, C11 and C12 carbons of the alkyl side chain of chlorpromazine, where the C3, C8 and C12 carbons showed the markedly large displacement. These results suggested that GUG-β-CyD either includes the chlorobenzene ring or the benzene ring of the drug, the mode similar to the parent β-CyD complex reported by Otagiri et al. (1975). In addition, the carboxylate anion of GUG-β-CyD may interact electrostatically with the tertiary ammonium cation of chlorpromazine, enhancing the guest/host interaction, as shown in Fig. 7.

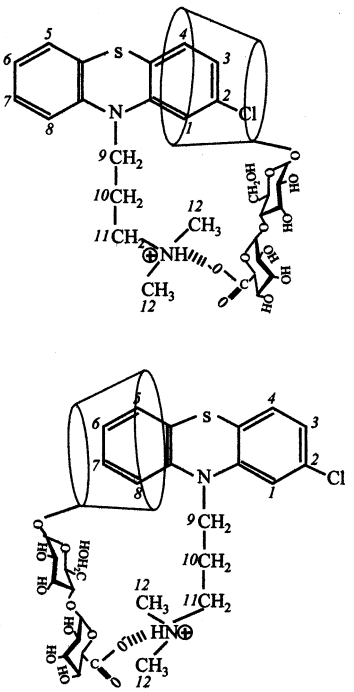

Fig. 7. Proposed inclusion mode of chlorpromazine/GUG-β-CyD complex. The figures in italics are for carbon numbering.

The interaction of a basic drug, cinnarizine, with GUG-β-CyD was studied by the solubility method, in comparison with and G_2-β-CyD. However, a quantitative comparison was difficult, because the phase solubility diagrams showed A_L or A_P types, depending on the hosts employed (GUG-β-CyD and G_2-β-CyD) and pH of solutions. Therefore, the solubility-enhancement of cinnarizine by β-CyDs at various pHs of solution was investigated in detail. Table 4 lists the solubility of cinnarizine at various pHs (1.8, 4.4, 5.9, 8.5 and 10.3) and the solubility-enhancement (S_c/S_o) in the presence of GUG-β-CyD and G_2-β-CyD (0.6, 3.0 and 6.0 mM), where S_o and S_c stand for the solubility of the drug in the absence and presence of β-CyDs, respectively. At pH 1.8 where cinnarizine is in cationic form while GUG-β-CyD and G_2-β-CyD are in neutral form, the solubility-enhancement was almost the same between the hosts. On the other hand, at pH 4.4 and 5.9 where the drug is in cationic form while GUG-β-CyD is in anionic form but G_2-β-CyD is in neutral form, the solubility-enhancement of GUG-β-CyD was much higher than that of G_2-β-CyD, indicating a favorable interaction of the cationic cinnarizine with negatively charged GUG-β-CyD over that of neutral G_2-β-CyD. In the case of pHs 8.5 and 10.3 where cinnarizine is in neutral form while GUG-β-CyD is in anion form but G_2-β-CyD is in neutral from, the enhancement was significantly higher for G_2-β-CyD than for GUG-β-CyD, indicating that the carboxylate anion of GUG-β-CyD works disadvantageously in the interaction with the neutral cinnarizine. Unfortunately, the solubility difference in 0.6 mM could not be detected because of the low solubility of cinnarizine at pHs 8.5 and 10.3. Again, these results suggest that an electrostatic interaction plays an important role in the complexation of GUG-β-CyD.

4. Conclusion

Some physico- and bio-chemical properties of a newly developed branched β-CyD, GUG-β-CyD, were investigated, in comparison with parent β-

Table 4
Enhancement of solubility of cinnarizine by complexation with G2-β-CyD and GUG-β-CyD at various pHs of solutions

PH	S_o (M)	Enhancement ratio (S_c/S_o)					
		0.6 mM		3.0 mM		6.0 mM	
		G2-β-CyD	GUG-β-CyD	G2-β-CyD	GUG-β-CyD	G2-β-CyD	GUG-β-CyD
1.8	3.46×10^{-4}	1.35	≈ 1.30	3.56	≈ 3.55	6.78	≈ 6.50
4.4	1.96×10^{-4}	1.54	< 1.81	3.25	< 3.68	4.76	< 5.05
5.9	2.43×10^{-6}	2.05	< 3.43	12.8	< 18.0	32.0	< 54.7
8.5	1.43×10^{-6}	1.98[a]	1.97[a]	9.91	> 7.97	21.1	> 14.6
10.3	7.75×10^{-7}	1.64[a]	2.03[a]	17.2	> 10.3	34.2	> 24.5

Average of the values for 2–4 experiments, which coincided with each other within ±2%.
[a] Accuracy of ±5% due to the low solubility.

CyD and G_2-β-CyD. The hemolytic activity of GUG-β-CyD was lower than those of β-CyD and G_2-β-CyD, and the cytotoxicity of the host was negligible. The ability of GUG-β-CyD to include cationic drugs was stronger than β-CyD and G_2-β-CyD due to an additional electrostatic interaction, although that to neutral and acidic drugs was slightly smaller. These results indicate that GUG-β-CyD has potential as a safe solubilizer for poorly water-soluble drugs, particularly for basic drugs.

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