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Some Pharmaceutical Properties of a New Branched Cyclodextrin, 6-*O*-α-(4-*O*-α-D-Glucuronyl)-D-glucosyl-β-cyclodextrin

SUMITRA TAVORNVIPAS[1], HIDETOSHI ARIMA[1], FUMITOSHI HIRAYAMA[1], KANETO UEKAMA[1,*], TOSHIHIRO ISHIGURO[2], MASAHIDE OKA[2], KENICHI HAMAYASU[3] and HITOSHI HASHIMOTO[3]

[1] *Faculty of Pharmaceutical Sciences, 5-1 Oe-honmachi, Kumamoto 862-0973, Japan;* [2] *Takeda Chemical Industries, Ltd., 2-17-85, Jusohonmachi, Yodogawa, Osaka 532-8686, Japan;* [3] *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 biological properties of a new branched cyclodextrin, 6-*O*-α-(4-*O*-α-D-glucuronyl)-D-glucosyl-β-cyclodextrin GUG-β-CyD) were investigated. Further, the interaction of GUG-β-CyD with several drugs was studied by the solubility and spectroscopic 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 the 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.

Introduction

Branched cyclodextrins, in which mono- or di-saccharides are introduced onto one or two primary hydroxyl groups of cyclodextrin through the α-1,6 glycosidic bond, have advantages over the parent cyclodextrins such as higher solubility, lower hemolytic activity and higher bioadaptability. Recently, glucuronylglucosyl-β-cyclodextrin (GUG-β-CyD, Figure 1) has been prepared by the oxidation of G_2-β-CyD with *Pseudogluconobacter saccharoketogenes* [1]. Since GUG-β-CyD contains a negatively charged carboxylate group in a molecule, the inclusion ability of GUG-β-CyD may be different from that of non-ionized CyDs such as parent CyD (β-CyD) and branched CyD (G_2-β-CyD). The present paper deals with comparison of the inclusion complexation of GUG-β-CyD, G_2-β-CyD and β-CyD with neutral (*p*-hydroxybenzoic acid esters: parabens), anionic (flurbiprofen) and cationic (chlorpromazine) drugs. Further, some cellular effects of GUG-β-CyD were compared with those of β-CyD and G_2-β-CyD.

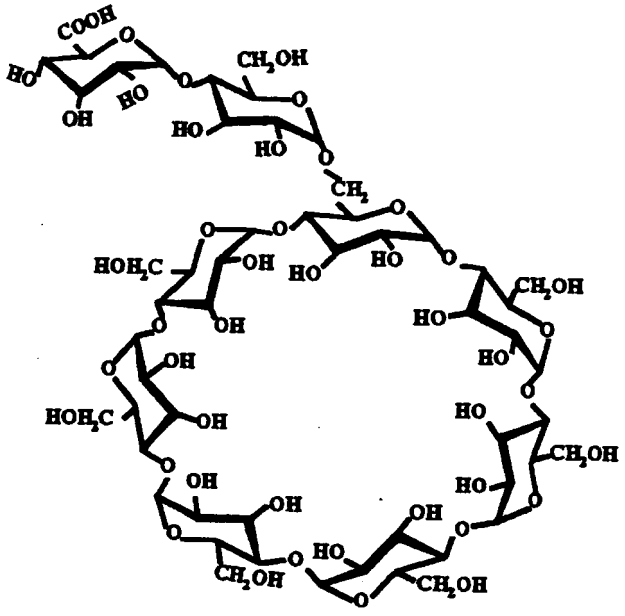

Figure 1. Chemical Structure of GUG-β-CyD.

* Author for correspondence. E-mail: uekama@gpo.kumamoto-u.ac.jp

Experimental

Materials

β-CyD was supplied by Nihon Shokuhin Kako Co. (Tokyo, Japan). G_2-β-CyD and GUG-β-CyD were gifted from Bio Research Corporation of Yokohama (Yokohama, Japan). All other materials and solvents were of analytical reagent grade. Deionized and double-distilled water was used throughout the study.

Apparatus

Optical rotations were determined with a DIP-100 digital polarimeter (Jasco, Tokyo, Japan). Melting points were measured with a micro melting point apparatus (Yanaco, Japan). Ultraviolet (UV), fluorescence and circular dichroism (CD) spectra were measured at 25 °C using Hitachi U-2000 UV and F-4500 fluorescence spectrometers (Tokyo, Japan) and a Jasco J-720 polarimeter (Tokyo, Japan), respectively.

Solubility studies

The solubility methods were carried out according to the method of Higuchi and Connors [2]. Excess amounts of drugs were added to an aqueous solution containing various concentrations of CyDs, and the solutions were shaken at 25 °C. After equilibrium was attained, the solution was centrifuged, pipetted through a cotton plug and analyzed for drugs by UV (flurbiprofen) or HPLC (parabens) methods. The HPLC conditions were as follow: a Jasco PU-1580 pump and a UV-970 UV detector. (Tokyo, Japan), a YMC AM-312 column (6.0 × 150 mm), a flow rate of 1.0 mL/min. The stability constants ($K_{1:1}$) of 1 : 1 complexes were calculated from the slope and intercept of straight line of the phase solubility diagram according to the equation of Higuchi and Connors [2]. The A_P type diagrams were analyzed according to the method of Kristiansen [3] to obtain the 1 : 1 and 1 : 2 ($K_{1:2}$, guest:host) stability constants.

Hemolysis assays

From freshly drawn rabbit blood, erythrocytes were separated by centrifugation at 1000 × g for 5 min, washed three times with isotonic phosphate buffer solution (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 CyD solution at various concentrations. The mixture was incubated for 30 min at 37 °C and centrifuged at 1000xg 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 which was obtained when water was used instead of the buffer solution.

Cytotoxicity

The intracellular enzyme activity was assayed by using a Cell Counting Kit (WST-1 method) from Wako Pure Chemical Industries (Osaka, Japan). Caco-2 cells were seeded at 5 × 10^5 cells onto 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 three times with HBSS (pH 7.4) and then incubated for 1 h with 100 μL of HBSS containing β-CyDs or Tween 20 at various concentrations in a humidified atmosphere. After washing three times with HBSS to remove β-CyDs and Tween 20 again, 100 μL of fresh HBSS and 10 μL of WST-1 reagent 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, NY, U.S.A.).

Spectroscopic studies

The spectroscopic changes (UV, CD and fluorescence) of drugs in the presence of β-CyDs were analyzed at 25 °C in 0.1 M phosphate buffer. The stability constants of the complexes (K_c) were obtained from the Scott equation [4].

$$a.b/d = 1/K_c.\varepsilon_c + b/\varepsilon_c$$

where a is the total concentration of drugs, b is the total concentration of β-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 β-CyDs. The stoichiometry of the complexes in isotonic phosphate buffer (pH 7.4) at 25 °C was determined by the continuous variation method [5].

Results and discussion

Physico- and biochemical properties

Table 1 lists some physicochemical and biological properties of branched β-CyDs. The solubility of GUG-β-CyD (> 2000 mg/ml) and G_2-β-CyD (> 1500 mg/ml) was about 100 times and 80 times, respectively, greater than that of β-CyD in water at 25 °C. Values of surface tension of branched β-CyDs were about the same as that of water (71 mN/m), suggesting negligible surface activity. Figure 2 showed the hemolytic effects of branched β-CyDs on rabbit erythrocytes in isotonic solution. The hemolytic activity of branched CyDs was lower than that of each parent CyD and decreased in the order of β-CyD > G_2-β-CyD > GUG-β-CyD. Okada et al. investigated the relationship between aqueous solubility and hemolytic activity of doubly-branched CyDs, 6^1,6^n-di-*o*-α-D-glucopyranosyl-β-cyclodextrins (1, n-$(Glc)_2$-β-CyDs; n = 2–4) [6], and reported that the 1,4-isomer with low aqueous solubility has the stronger hemolytic activity than 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 aqueous solubility. Further, we found that the inclusion ability of GUG-β-CyD to drugs having steroid skeletons is slightly 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.

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[a] (mg/mL)	Surface tension[a] (dyne/cm)	Hemolytic activity[b] (mM)
β-CyD	7	1135	158	19	75	5.7
G_2-β-CyD	9	1159	166	>1500	72	8.4
GUG-β-CyD	9	1473	155	>2000	73	11.6

[a] At 25 °C in water.
[b] The concentration of CyDs to induce 50% hemolysis of rabbit erythrocytes.

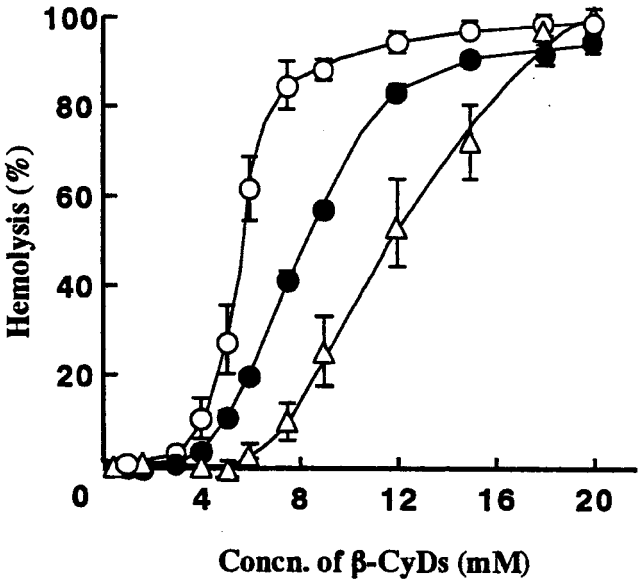

Figure 2. Hemolytic effects of β-CyDs on rabbit erythrocytes in isotonic phosphate buffer (pH 7.4) at 37 °C. ○: β-CyD, ●: G_2-β-CyD, △: GUG-β-CyD. Each point represents the mean ± S.E. of 6–8 experiments.

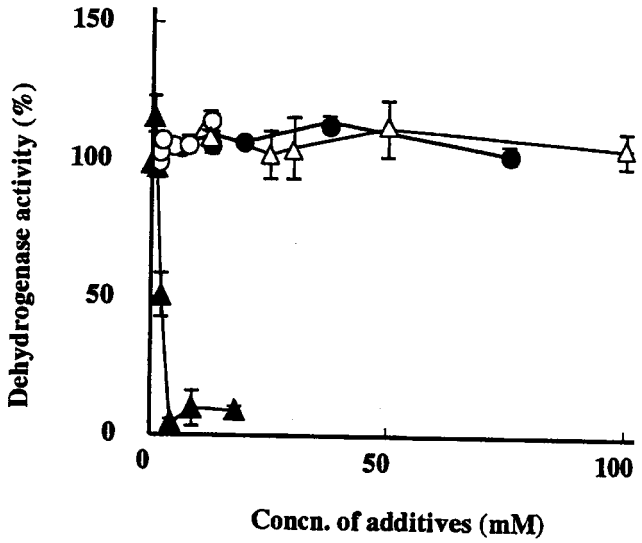

Figure 3. Effects of additives on intracellular dehydrogenase activity of Caco-2 cells at apical side after incubation for 1 h in HBSS (pH 7.4) at 37 °C. ○: β-CyD, ●: G_2-β-CyD, △: GUG-β-CyD, ▲: Tween 20. Each point represents the mean ± S.E. of 6 experiments.

The cytotoxicity of β-CyDs toward Caco-2 cells was studied by measuring intracellular dehydrogenase activity (WST-1 method), and the results were shown in Figure 3. Tween 20, a non-ionic surfactant, was used as a positive control. GUG-β-CyD and G_2-β-CyD showed negligible cytotoxicity on Caco-2 cells up to at least 0.1 M, and that of β-CyD was also negligible up to its solubility limit (15 mM).

Inclusion ability of GUG-β-CyD

Neutral drugs

The phase solubility diagrams of GUG-β-CyD and G_2-β-CyD with parabens (methyl to butyl esters) in pH 7.4 phosphate buffer showed A_L types, where the solubility of drugs increased linearly with CyD concentrations. On the other hand, parent β-CyD gave Bs type diagrams in that crystalline complexes with a limited solubility precipitated at higher CyD concentrations (> 0.01 M). The stability constants of three β-CyDs increased as the alkyl chain length of parabens increases, and were generally in the order of β-CyD > G_2-β-CyD > GUG-β-CyD. The stability constant of 1 : 1 β-CyD, G_2-β-CyD and GUG-β-CyD complexes and the type of the phase solubility diagrams (in parenthesis) are as follows: K_c = 641 (A_L), 255 (A_L) and 167 (A_L) M^{-1} for methyl paraben, K_c = 740 (B_S), 456 (A_L) and 414 (A_L) M^{-1} for ethyl paraben, K_c = 1160 (B_S), 1000 (A_L) and 533 (A_L) M^{-1} for propyl paraben, K_c = 1930 (B_S), 1670 (A_L) and 1630 (A_L) M^{-1} for butyl paraben and K_c = 2770 (B_S), 8420 (A_P) and 7260 (A_P) M^{-1} for hexyl paraben, respectively. 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 β-CyD molecule.

Acidic drugs

The phase solubility diagram obtained for flurbiprofen (FP) with β-CyDs in water at 25 °C showed Bs type solubility curve. On the other hand, G_2-β-CyD and GUG-β-CyD showed A_L type solubility curve. The stability constants of inclusion complexes determined by the solubility and UV method were in good agreement with each other, and the magnitude of stability constants was in the order of β-CyD ≈ G_2-β-CyD > GUG-β-CyD (solubility method: K_c = 3960, 3820 and 3080 M^{-1}, UV method: K_c = 5990, 5380 and 2920 M^{-1}, for the β-CyD, G_2-β-CyD and GUG-β-CyD complexes, respectively). The stoichiometry of the

complexes was determined to be 1 : 1 (molar ratio of host and guest), by the continuous variation method [5].

The UV absorbance of FP was decreased by the addition of β-CyDs. In contrast to UV changes, the fluorescence intensity of FP with β-CyDs was increased. In the CD spectra, the optical activity was induced positively by the addition of three β-CyDs and was in the order of β-CyD > G_2-β-CyD > GUG-β-CyD. The weaker interaction of the branched β-CyDs may be ascribed to either a steric hindrance of branched sugar units in G2- and GUG-β-CyDs and/or an electrostatic repulsion between the drug and GUG-β-CyD in pH 7.4 phosphate buffer where both components are in anionic forms (pKa = 4.2 and 3.5 for flurbiprofen and GUG-β-CyD, respectively).

Basic drugs

Chlorpromazine (CPZ) formed the inclusion complexes with three β-CyDs in a molar ratio of 1 : 1 in pH 7.4 phosphate buffer. CPZ gave a UV absorption maximum at 255 nm, and by the addition of β-CyDs the intensity was decreased with concomitant shifts to longer wavelength. In the CD spectra, when β-CyDs were added to the CPZ solution, an induced positive cotton effect was observed at 251 nm and was in the order of G_2-β-CyD > GUG-β-CyD > β-CyD. The stability constant of CPZ/GUG-β-CyD complex was greater than those of β-CyD and G_2-β-CyD complexes (K_c = 8340, 8000 and 9000 M^{-1} for the complexes with β-CyD, G_2-β-CyD and GUG-β-CyD, respectively). The higher affinity of CPZ to GUG-β-CyD, than that to β-CyD, may be ascribed to an electrostatic attraction between CPZ and GUG-β-CyD in pH 7.4 phosphate buffer where the drug and the host exist as cationic and anionic species (pKa = 9.2 and 3.5, respectively).

Conclusion

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 no appreciable cytotoxicity on Caco-2 cells up to at least 0.1 M. In the interaction studies, GUG-β-CyD had greater affinity for the basic guest molecules, compared with β-CyD and G_2-β-CyD. Thus GUG-β-CyD may be useful as a safe solubilizing agent particularly for basic drugs.

Acknowledgements

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