Inclusion Complexes of Lipids with Branched Cyclodextrins[1)]

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The interactions of fatty acids, monoacylglycerols, diacylglycerols, and triacylglycerols with α-cyclodextrin (CD), β-CD, 6-*O*-α-D-glucosyl-α-CD (G-α-CD), and 6-*O*-α-D-glucosyl-β-CD (G-β-CD) were investigated by the solubility method and by differential scanning calorimetry. The complexation ability of G-α-CD for lipids was superior to that of G-β-CD. The reactivity of lipids with CDs was found to increase in the order of fatty acid ≥ monoacylglycerol ≫ diacylglycerol > triacylglycerol, and unsaturated lipids formed complexes more easily than corresponding saturated lipids. The complexation abilities of branched CDs and the parent CDs appeared to be almost the same, but the enhancement of lipid solubility by the branched CD, particularly by G-α-CD was much more marked than that by the parent CD.

Keywords branched cyclodextrin; glucosyl-α-cyclodextrin; glucosyl-β-cyclodextrin; lipid; fatty acid; acylglycerol; inclusion complex; solubility method; differential scanning calorimetry; solubilization

Branched cyclodextrins (CDs) are a kind of natural saccharides, and have one or more side chains consisting of an α-D-glucosyl unit or a (1→4)-α-D-glucan linking at C-6 of CDs. In recent years, branched CDs have been utilized for studies in the fields of biosynthesis,[2)] chemical synthesis,[3)] and analysis.[4)]

Previously, we have reported[5)] on the inclusion behavior, solubilities, specific rotations, and hemolytic activities of branched CDs, namely 6-*O*-α-D-glucosyl-α-CD (G-α-CD), 6-*O*-α-D-glucosyl-β-CD (G-β-CD), 6-*O*-α-D-glucosyl-γ-CD, 6-*O*-α-maltosyl-α-CD (G_2-α-CD), 6-*O*-α-maltosyl-β-CD (G_2-β-CD), 6-*O*-α-maltosyl-γ-CD, 6-*O*-α-maltotriosyl-α-CD, 6-*O*-α-maltotriosyl-β-CD, 6-*O*-α-maltotriosyl-γ-CD, and 6^A,6^D-di-*O*-α-D-glucosyl-β-CD. It has become apparent that these branched CDs have many advantages: higher solubilities in water and also organic solvents, excellent solubilizing ability for water-insoluble compounds by complex formation, and lower hemolytic activity compared with parent CDs.

This report deals with interaction of branched CDs (G-α-CD and G-β-CD) with various lipids which are useful as surface-active agents in the pharmaceutical field, and foods and cosmetics industries.

Experimental

Materials A branched CDs mixture which was prepared from maltose and CDs through the reverse action of pullulanase by Ensuiko Seito Co., Ltd. and is commercially available as Isoeleat, was a gift. α-CD and β-CD were used after recrystallization from water. Glucoamylase (GNL-3, *Aspergillus niger*) used for digestion of the branched CDs mixture was purchased from Amano Pharmaceutical Co., Ltd. The lipids, dodecanoic acid (lauric acid), tetradecanoic acid (myristic acid), hexadecanoic acid (palmitic acid), octadecanoic acid (stearic acid), *cis*-9-octadecenoic acid (oleic acid), 1-monododecanoyl-*rac*-glycerol (1-monolaurin), 1-monotetradecanoyl-*rac*-glycerol (1-monomyristin), 1-monohexadecanoyl-*rac*-glycerol (1-monopalmitin), 1-monooctadecanoyl-*rac*-glycerol (1-monostearin), 1-mono[(*cis*)-9-octadecenoyl]-*rac*-glycerol (1-monoolein), 1,2-dioctadecanoyl-*rac*-glycerol (1,2-distearin), 1,3-dioctadecanoylglycerol (1,3-distearin), 1,2-di[(*cis*)-9-octadecenoyl]-*rac*-glycerol (1,2-diolein), 1,3-di-[(*cis*)-9-octadecenoyl]glycerol (1,3-diolein), 1,2,3-trioctadecanoylglycerol (tristearin), 1,2,3-tri[(*cis*)-9-octadecenoyl]glycerol (triolein), were obtained from Sigma Chemical Co. All other materials were of analytical-reagent grade. Deionized and double-distilled water was used throughout this study. Reagent-grade organic solvents used for high-performance liquid chromatography (HPLC) were freshly distilled and filtered through a 0.45-μm membrane filter.

General Methods Lyophilization was carried out with an FD-1 freeze-dryer (Tokyo Rika, Tokyo, Japan). HPLC analyses of lipids were performed using a Familic-300S HPLC pump, a model VL-614 injector, and a UVIDEC-100V variable-wavelength ultraviolet detector (all from JASCO, Tokyo, Japan). The columns used were a YMC-Pack A-802 C_4 (150 × 4.6 mm i.d.), a YMC-Pack A-212 C_8 (150 × 6 mm i.d.) (both from Yamamura Chemical, Kyoto, Japan), a μBondasphere 5 μ C_4-100 Å (150 × 3.9 mm i.d.) (Waters Assoc., Milford, MA, U.S.A.) and a TSK gel Octadecyl-4PW (150 × 4.6 mm i.d.) (Tosoh, Tokyo, Japan). Preparative HPLC was carried out using a Twincle pump and a VL-611 injector (both from JASCO) with an SE-11 refractive index (RI) monitor (Showa Denko, Tokyo, Japan). An HPLC system consisting of an 880-PU Intelligent HPLC pump (JASCO), a model 7125 injector (Rheodyne, Cotati, CA, U.S.A.) and an SE-61 RI detector (Showa Denko) was used to monitor the purity of G-α-CD and G-β-CD isolated.

Preparation and Isolation of G-α-CD and G-β-CD Fifty grams of the branched CDs mixture and 0.1 ml of glucoamylase GNL-3 were dissolved in 500 ml of water, and then the solution was incubated at 50 °C for 24 h. After complete conversion from G_2-CDs to G-CDs was confirmed by HPLC, the reaction mixture was heated at 100 °C for 5 min and then the denatured enzyme was removed by filtration through a 0.45-μm membrane filter. The filtrate, including mainly G-α-CD, G-β-CD, diglucosyl-α-CD, diglucosyl-β-CD, α-CD, β-CD, and glucose, was applied to a column packed with LiChroprep RP-18 (5—20 μm, 300 × 20 mm i.d.) (Yamazen, Osaka, Japan) using methanol–water (10 : 90) as an eluent, and G-α-CD and G-β-CD were isolated. The purity of G-α-CD and G-β-CD was checked by HPLC, on a Hibar LiChrosorb NH_2 column (250 × 4 mm i.d.) (Merck, Darmstadt, F. R. G.) with acetonitrile–water (60 : 40) and on a YMC-Pack A-302 ODS column (150 × 4.6 mm i.d.) (Yamamura Chemical) with methanol–water (6 : 94).

Solubility Studies Estimation of the complex-forming ability of CDs by the solubility method[6)] was conducted according to the procedure described previously.[5a)] Chromatographic conditions for determination of the lipids are summarized in Table I.

Preparation of Solid Complexes and Thermal Analysis The solid complexes were prepared in the same manner as described before.[5b)] The contents of guest and host in the solid complexes were determined by HPLC. Lipid/G-α-CD molar ratios in the solid complexes obtained were 1/2 in lauric acid– and myristic acid–G-α-CD systems, 2/5 in 1-monolaurin– and 1-monomyristin–G-α-CD systems, 1/3 in palmitic acid– and 1-monopalmitin–G-α-CD systems, 2/7 in the 1-monoolein–G-α-CD system, 1/4 in the oleic acid–G-α-CD system, and 1/5 in stearic acid– and 1-monostearin–G-α-CD systems. These numerical values are thought to be smaller than the actual composition ratios of guest and host in complexes, since the solid complexes obtained are assumed to contain some free G-α-CD, but not free lipids, which are practically water-insoluble. These values were determined for reference, for the preparation of physical mixtures used for thermal analyses. Thermal analysis was done using a Thermo Flex DSC-8230 B (Rigaku, Tokyo, Japan) at a scanning speed of 5 °C/min and scanning range of −30—100 °C.

TABLE I. Conditions of Lipid Determination by HPLC

Lipid	Column[a)]	Eluent	Column temperature (°C)	Retention time (min)
Lauric acid	II	CH_3CN : 0.01% H_3PO_4 (70 : 30)	40	10.5
Myristic acid	II	CH_3CN : 0.01% H_3PO_4 (77 : 23)	40	11.0
Palmitic acid	II	CH_3CN : 0.01% H_3PO_4 (85 : 15)	40	9.5
Stearic acid	II	CH_3CN : 0.01% H_3PO_4 (90 : 10)	40	10.0
Oleic acid	II	CH_3CN : 0.01% H_3PO_4 (90 : 10)	40	8.0
1-Monolaurin	I	CH_3OH : H_2O (68 : 32)	40	9.5
1-Monomyristin	I	CH_3OH : H_2O (72 : 28)	40	10.5
1-Monopalmitin	I	CH_3OH : H_2O (77 : 23)	40	10.0
1-Monostearin	I	CH_3OH : H_2O (80 : 20)	40	10.5
1-Monoolein	I	CH_3OH : H_2O (78 : 22)	40	10.0
1,2-Distearin	II	C_2H_5OH : H_2O (89 : 11)	45	11.5
1,3-Distearin	II	C_2H_5OH : H_2O (89 : 11)	45	11.5
1,2-Diolein	I	CH_3OH : H_2O (92 : 8)	40	9.0
1,3-Diolein	I	CH_3OH : H_2O (92 : 8)	40	9.0
Tristearin	IV	C_2H_5OH : H_2O : THF (90 : 10 : 5)	55	13.0
Triolein	III	CH_3OH : H_2O (95 : 5)	45	10.0

a) Column: I = YMC-Pack A-802 C_4 (150 × 4.6 mm i.d.), II = YMC-Pack A-212 C_8 (150 × 6 mm i.d.), III = μBondasphere 5 μ C_4-100 Å (150 × 3.9 mm i.d.), IV = TSK gel Octadecyl-4PW (150 × 3.9 mm i.d.). Flow rate was 1.0 ml/min. Determined at 210 nm.

Results and Discussion

Preparation and Isolation of G-α-CD and G-β-CD from the Branched CDs Mixture The branched CDs mixture containing α-CD, G_2-α-CD, β-CD, G_2-β-CD, dimaltosyl-α-CD ($(G_2)_2$-α-CD), $(G_2)_2$-β-CD, linear glucans, and other minor components is useful as a commercially available source of branched CDs (Fig. 1(a)).

The solubility of G_2-α-CD, which is the main component of the branched CDs mixture, is significantly lower than those of other branched CDs.[5b)] Moreover, steric hindrance between the longer branch and the guest molecule and distortion of the CD ring with the larger branch seemed to lower the stability of the complex, especially at higher concentrations of branched CD solution.[5)] Therefore, G-α-CD and G-β-CD were selected as the host compounds in this study. In order to convert G_2-CDs to G-CDs, the branched CDs mixture was treated with glucoamylase, whereupon most of the contaminating linear glucans were degraded to glucose (Fig. 1 (b)). From the digest of the branched CDs mixture, G-α-CD and G-β-CD were isolated by HPLC on an ODS-column eluted with methanol–water system.

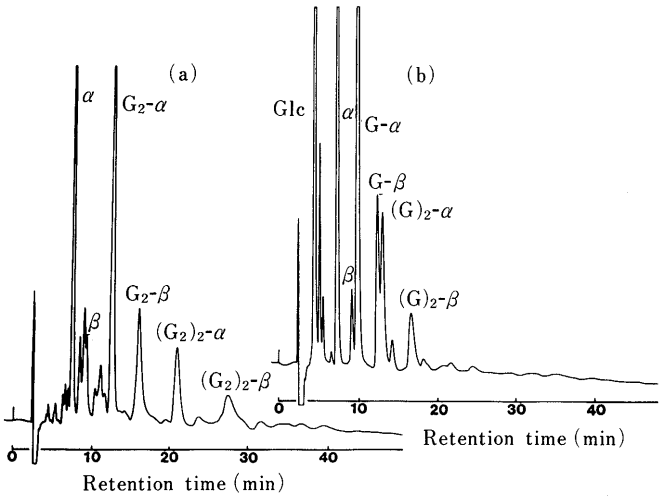

Fig. 1. Chromatograms of the Branched CDs Mixture (a) and the Branched CDs Mixture Treated with Glucoamylase (b)

Compounds: Glc, glucose; α, α-cyclodextrin (CD); β, β-CD; G-α, 6-*O*-α-D-glucosyl-α-CD; G_2-α, 6-*O*-α-maltosyl-α-CD; $(G)_2$-α, di-*O*-α-D-glucosyl-α-CD; $(G_2)_2$-α, di-*O*-α-maltosyl-α-CD; G-β, 6-*O*-α-D-glucosyl-β-CD; G_2-β, 6-*O*-α-maltosyl-β-CD; $(G_2)_2$-β, di-*O*-α-maltosyl-β-CD.

Chromatographic conditions; column, Hibar LiChrosorb NH_2 (250 × 4 mm i.d.); eluent, acetonitrile–water (60 : 40); flow rate, 1.0 ml/min; detector, Shodex RI SE-61 at 2×10^{-5} RI units full scale; temperature, ambient.

Solubility Study The complexing behavior of sixteen lipids (fatty acids, monoacylglycerols, diacylglycerols, triacylglycerols) with G-CDs in water was investigated by the solubility method.[6)]

Figures 2—4 show the phase solubility diagrams obtained for each lipid with G-α-CD and G-β-CD in water at 30 °C. For all lipids examined except 1-monolaurin, enhancement of the water-solubilities by complexation with G-α-CD was greatly superior to that with G-β-CD. Corey–Pauling–Kaltum models show that carbon chains of lipids fit favorably in the G-α-CD cavity, whereas the G-β-CD cavity is too wide.[7)] Moreover, solubilities of lipid–G-β-CD complexes in water were generally much lower than those of lipid–G-α-CD complexes. For comparison, the complexation behavior of the parent CDs was also examined. In many cases, complexes with α-CD and β-CD precipitated in lower concentrations (< 0.01 M) and therefore, lipids could not be solubilized.

In Fig. 2, solubility curves of fatty acids other than stearic acid in G-α-CD solutions can be classified as type A,[6)] while those of stearic acid in G-α-CD solutions and of all fatty acids reacted in G-β-CD solution are type Bs.[6)] As calculations of apparent stability constants (K'), using the equation $K' = \text{slope}/\text{intercept}\ (1-\text{slope})$,[6)] could not be carried out because of the intercept ≒ 0, the reactivity of lipids with G-CDs was evaluated for convenience in terms of the slope of the initial straight line portion of each solubility diagram. The reactivity of lauric, myristics, palmitic, oleic, and stearic acid with G-CDs decreased in that order. However, the interaction of G-β-CD with oleic acid started at a lower concentration than that with palmitic acid. These findings suggest that G-CDs preferably form inclusion complexes with smaller fatty acids and the unsaturated fatty acid is more favorable than the corresponding saturated one.

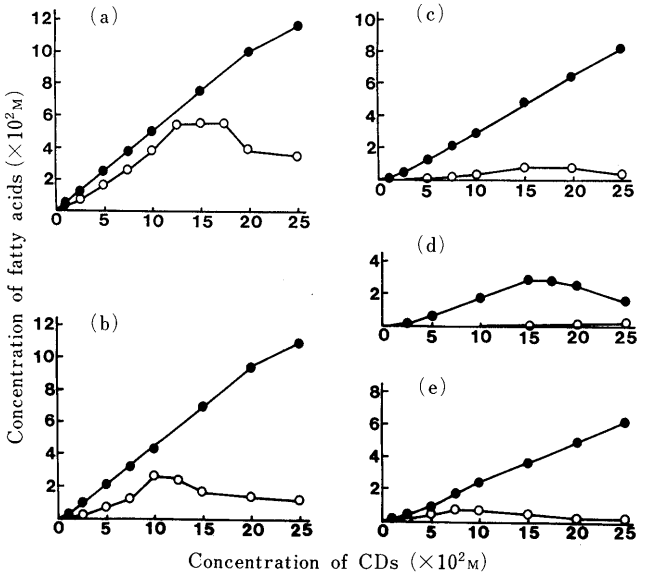

Fig. 2. Phase Solubility Diagrams of Fatty Acids–Branched CDs Systems in Water at 30 °C

●, G-α-CD; ○, G-β-CD. (a), lauric acid; (b), myristic acid; (c), palmitic acid; (d), stearic acid; (e), oleic acid.

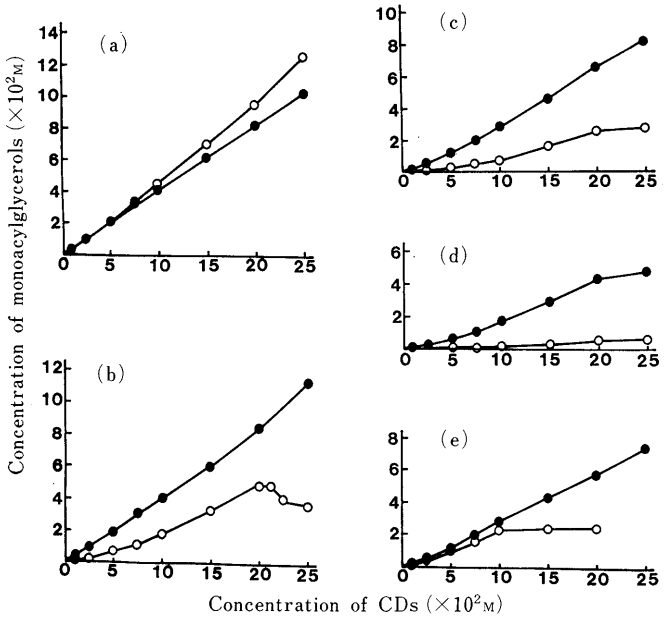

Fig. 3. Phase Solubility Diagrams of 1-Monoacylglycerols–Branched CDs Systems in Water at 30 °C

See Fig. 2 for symbols. (a), laurin; (b), myristin; (c), palmitin; (d), stearin; (e), olein.

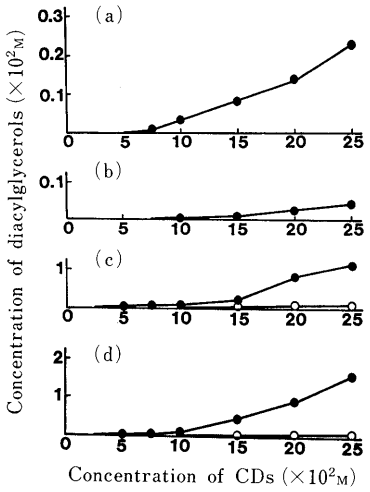

Fig. 4. Phase Solubility Diagrams of Diacylglycerols–Branched CDs Systems in Water at 30 °C

See Fig. 2 for symbols. (a), 1,2-distearin; (b), 1,3-distearin; (c), 1,2-diolein; (d), 1,3-diolein.

Complex-forming ability of G-β-CD with monoacylglycerols (1-monolaurin, 1-monomyristin, 1-monopalmitin, 1-monostearin, and 1-monoolein) was greater than that with the corresponding fatty acids (Fig. 3). In particular, the water-solubility of 1-monolaurin was exceptionally higher in G-β-CD solution than in G-α-CD solution. The order of reactivity of monoacylglycerols with G-CDs was 1-monolaurin > 1-monomyristin > 1-monopalmitin ≥ 1-monoolein > 1-monostearin, as in the case of fatty acids.

Diacylglycerols interacted with G-CDs much more weakly than fatty acids and monoacylglycerols (Figs. 2—4). At high concentrations G-α-CD slightly solubilized 1,2- and 1,3-distearin, and 1,2- and 1,3-diolein, whereas G-β-CD interacted little with dioleins and not at all with distearins.

Bulky and very hydrophobic triacylglycerols (tristearin and triolein) had great difficulty in forming inclusion complexes with G-CDs and CDs: the solubility of triolein increased slightly only at high concentrations (>0.2 M) of G-α-CD.

The reactivities of lipids with G-CDs can be summarized as follows: the complexation ability of G-α-CD was superior to that of G-β-CD; the suitability of lipids as the guest compound was in the order of fatty acid ≥ monoacylglycerol ≫ diacylglycerol > triacylglycerol; regarding the lipid carbon chain, the reactivities of dodecanoyl (laurin, C12:0), tetradecanoyl (myristin, C14:0), hexadecanoyl (palmitin, C16:0), (*cis*)-9-octadecenoyl (olein, C18:1) and octadecanoyl (stearin, C18:0) decreased in that order; the solubilities of fatty acids and monoacylglycerols were greatly enhanced in G-α-CD solution, *e.g.*, the concentrations of myristic acids and 1-monomyristin reached 0.11 M in 0.25 M G-α-CD solution.

Lagendijk and Pennings[8] investigated the complex-forming capacity of amylose and amylopectin for monoacylglycerols. They reported that the reactivity of monoacylglycerols with amylose increased in the order of 1-monopalmitin > 1-monostearin > 1-monomyristin > 1-monolaurin, the complex-forming ability of amylopectin was considerably smaller than that of amylose and the reactivity of monoacylglycerols with amylopectin increased with increasing chain length of the monoacylglycerol. These results are different from findings in reactions of monoacylglycerols with branched CDs.

It is generally recognized that the minimum requirement for inclusion complex formation is a size compatibility between host and guest molecules.[9] In addition, inclusion complexes are formed readily in water solution, and hydrophobic molecules are included much more easily than hydrophilic molecules, because the cavity of CD is more hydrophobic than water.[10,11] Heredia *et al.*[12] and Cox *et al.*[13] claimed that the polarity of the internal cavity of β-CD is similar to that of ethanol. It can be assumed that the polarity of branched CDs cavities may be almost the same as that of the parent CDs cavities, and guest molecules

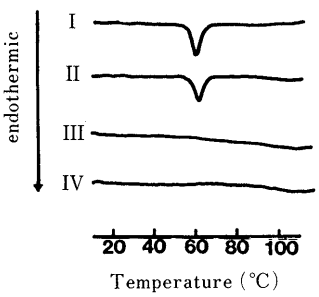

Fig. 5. DSC Thermograms of Palmitic Acid–G-α-CD Systems

I, palmitic acid alone; II, physical mixture of palmitic acid and G-α-CD in 1 : 3 molar ratio; III, 1 : 3 complex of palmitic acid with G-α-CD; IV, G-α-CD alone.

having very different polarity would not readily be included in CDs, because of unfavorable interaction of guest and host molecules. Although the polarity of lipids is much lower than that of ethanol and they are insoluble or poorly soluble in water, the solubilities of a series of homologous lipids increase as the number of carbon atoms in the chain decreases and with the introduction of an unsaturated bond and/or a hydrophilic group.[14] These facts may explain in part the results obtained in this work.

Figure 5 shows the differential scanning calorimetry (DSC) thermograms of palmitic acid and G-α-CD systems. The physical mixtures showed an endothermic peak around 65 °C corresponding to the melting point of palmitic acid. However, the endothermic peak disappeared with formation of the complex. In lauric acid–, myristic acid–, stearic acid–, oleic acid–, 1-monolaurin–, 1-monomyristin–, 1-monopalmitin–, 1-monostearin–, and 1-monoolein–G-α-CD systems, similar phenomena were observed. The results indicate that these lipids interact with G-α-CD in the solid state.

Monoacylglycerols are used as surface-active agents in the manufacture of foods throughout the world. For example, they are used in bakery products (breads, biscuits, cookies, sponge-cakes, *etc.*), fats (margarins, shortenings, *etc.*), milk products (ice creams, whip creams, *etc.*), and sweets (candies, caramels, *etc.*), as an anti-staling agent, a forming agent, an emulsifier, a plasticizer, an anti-moisture agent and so on. However, as monoacylglycerols are poorly soluble in water, they can not be effective unless dispersed by heating in the process of mixing. If their solubilities can be increased to allow them to be dispersed into aqueous solution at room temperature, their uses could be further extended. In this regard, monoacylglycerol–G-α-CD complex may be useful in food industries.

Acylglycerols and fatty acids are secreted from various glands, in analogy with phospholipid, glycolipid and bile acid, and function as surface-active agents *in vivo*. Therefore, we are considering a study of the effect of administration of branched CDs on *in vivo* lipid behavior.

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