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Comparative Study on Inclusion Complexation of Maltosyl-β-cyclodextrin, Heptakis(2,6-di-*O*-methyl)-β-cyclodextrin and β-Cyclodextrin with Fucosterol in Aqueous and Solid State

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Abstract—The complexation of fucosterol with three kinds of β-cyclodextrin (β-CyD) was investigated in aqueous solution and in the solid state. The solubility of fucosterol increased significantly on its complexation with maltosyl-β-CyD and heptakis(2,6-di-*O*-methyl)-β-CyD (DM-β-CyD), while no appreciable increase was observed when complexed with β-CyD. The stability constant of complexation with β-CyD estimated from solubility determinations was greater for a 1:2 complex than for a 1:1 complex. On the other hand, 1:1 complexation of fucosterol with maltosyl-β-CyD or DM-β-CyD was greater than 1:2 complexation. The solid complexes were obtained in molar ratios of 1:2 and 1:3 for β-CyD and maltosyl-β-CyD complexes, respectively. The inclusion behaviour of fucosterol with maltosyl-β-CyD was compared with β-CyD in the solid state using DSC, powder X-ray diffractometry and CP/MAS ^{13}C NMR. Maltosyl-β-CyD showed different inclusion behaviour compared with β-CyD, and produced an amorphous structure of fucosterol on complex formation. The dissolution rate of fucosterol-maltosyl-β-CyD complex was significantly faster than other complexes due to its high aqueous solubility and amorphous structure.

β-Cyclodextrin (β-CyD), a cyclic oligosaccharide consisting of seven glucose units, forms stable complexes with a number of drugs (Bender & Komiyama 1978; Szejtli 1982; Uekama & Otagiri 1987), but this form of CyD has low aqueous solubility which has restricted its application in the pharmaceutical field. Recently chemically modified CyDs and branched CyDs have received considerable attention because of their high aqueous solubility (Koizumi et al 1987; Okada et al 1988; Yamamoto et al 1989). The solubilization effects imparted by branched CyDs to poorly water-soluble vitamins are remarkable, and are related to the intrinsic high solubility as well as inclusion ability of branched CyDs (Okada et al 1989, 1990).

The aim of the present study was to compare the inclusion behaviour of branched β-CyD (maltosyl-β-CyD) and a chemically modified β-CyD (heptakis(2,6-di-*O*-methyl)-β-CyD, DM-β-CyD) with natural β-CyD in solution and in the solid state using fucosterol, a novel phytosterol, which is a very poorly water-soluble drug, as a model guest molecule. Although fucosterol increases the production of plasminogen activator (Hagiwara et al 1984, 1986), its low water-solubility is one of several reasons which has prevented further development of this compound as a drug.

Materials and Methods

Materials

Fucosterol was purified from marine brown algae. Maltosyl-β-, DM-β- and β-CyD were supplied from Ensuiko Sugar Refining Co. Ltd (Yokohama, Japan), Toshin Chemical Co. (Tokyo, Japan) and Nihon Shokuhin Kako Co. Ltd (Tokyo, Japan), respectively. All other materials and solvents were of analytical grade.

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Solubility studies

Phase-solubility studies were carried out according to the method of Higuchi & Connors (1965). Excess fucosterol was added to aqueous solutions containing various concentrations of CyDs and was shaken at a constant temperature (25°C) for 7 days. Each sample was centrifuged at 3000 rev min^{-1} for 10 min. After pipetting through a cotton filter, 0·5 mL of each sample was diluted with methanol (0·5 mL) and water (0·5 mL) and then extracted with 6 mL ethyl acetate by shaking for 10 min. Five millilitres of the organic phase was transferred to a new tube and then evaporated to dryness on a water bath under reduced pressure. The residue was dissolved in 0·5 mL of a mixture of hexane-isopropanol (15:1, v/v) and assayed by HPLC. The chromatograph (Hitachi 655A 11) was equipped with a UV detector (220 nm). For separation, a LiChrosorb Si 60 (7 μm, 4·6 mm i.d. × 25 cm) column was used. The mobile phase consisted of hexane-isopropanol (15:1, v/v). The flow rate was 1·0 mL min^{-1}. Peak height was used for quantitation.

Preparation of solid complexes

The solid complexes were prepared by mixing the appropriate amounts of the CyDs and fucosterol in water. For example, 0·2 g fucosterol and 4·36 g maltosyl-β-CyD were added in 20 mL water and the mixture was stirred with a magnetic stirrer at 25°C for 7 days. The complex was filtered and dried under vacuum at room temperature (21°C) for 3 days. The solid complexes which were used in the dissolution studies were prepared by the kneading method (Tsuruoka et al 1981). Fucosterol was kneaded with maltosyl-β-CyD, β-CyD and DM-β-CyD in molar ratios of 1:3, 1:2 and 1:2,

respectively, with a small amount of water for 1 h. The paste thus obtained was dried under vacuum at room temperature for 3 days.

Instruments

Powder X-ray diffraction patterns were taken with a Rigaku Denki Geiger Flex 2012 diffractometer (Tokyo, Japan). The operation conditions were as follows: X-ray, Ni-filtered Cu-K_α radiation; voltage, 30 kV; current, 20 mA; time constant, 2 s; scanning rate, 1° min^{-1}.

The differential scanning calorimetric measurements were performed with the thermal analyser (model DT-20B, Shimadzu Co. Ltd, Kyoto, Japan) using a scanning rate of 10°C min^{-1}. Cross polarization/magic angle spinning ^{13}C nuclear magnetic resonance (CP/MAS ^{13}C NMR) spectra were recorded with an Otsuka Electronics CMX-300 spectrometer (Tokyo, Japan) operated at 75·3 MHz. The instrumental conditions were as follows: magic angle spinning rate, 2–4 kHz; contact time, 1 ms; spectral width, 30 kHz. ^{13}C-Chemical shifts were calibrated indirectly through the use of external benzene (128·5 ppm from tetramethylsilane).

Dissolution studies

The dissolution rate of the complex was determined according to the dispersion method (Nogami et al 1969). The drug (10 mg, < 100 mesh) or its equivalent amount of the solid complexes (< 100 mesh) was placed in the dissolution cell containing 25 mL water which was kept at 37°C and the dissolution medium was stirred at 91 rev min^{-1}. At appropriate intervals, 1·0 mL solution was withdrawn and filtered through a 0·45 μm membrane filter and then assayed by HPLC.

Results and Discussion

Complexation in aqueous solution

The complexation of fucosterol with three CyDs in aqueous solution was studied by the solubility method and the phase solubility diagrams are shown in Fig. 1. β- and Maltosyl-β-CyD systems showed solubility curves with solid complexes precipitating at high CyD concentrations. The low aqueous solubility of fucosterol (4×10^{-6} M) was markedly increased by maltosyl-β- and DM-β-CDs, whereas in the case of β-CyD, no appreciable increase in the solubility of fucosterol was observed. The highest solubility of fucosterol in β-CyD solution was 12×10^{-6} M, while the solubility of fucosterol increased to 7×10^{-3} M by the addition of DM-β- and maltosyl-β-CyDs. After isolation and chemical analysis of the solid complexes it was found that fucosterol formed solid complexes with β- and maltosyl-β-CyD in the molar ratios of 1:2 and 1:3, respectively.

The initial ascending parts of the solubility diagrams which are indicative of high order inclusion complexes were quantitatively analysed according to the optimization technique to estimate the stability constants of 1:1, 1:2 and 1:3 complexes. The results are given in Table 1. We could not calculate the $K_{1:3}$ stability constant for the fucosterol-maltosyl-β-CyD system, although the 1:3 solid complex was isolated, because a negative $K_{1:3}$ value was obtained when 1:3 complexation was assumed. Before isolation of the complex it was thought that fucosterol formed an inclusion complex with maltosyl-β-CyD in the molar ratio of 1:2 as with parent β-CyD which has the same cavity size. The differences in molar ratio of the complex in solution and solid state suggested that one maltosyl-β-CyD molecule in the solid complex might not directly participate in complexation with fucosterol. It was recently found by X-ray analysis that the glucopyranose moiety of glucosyl-α-CyD is included

Table 1. Stability constants of fucosterol-β-CyD systems.

	Stability constant (M^{-1})	
System	$K_{1:1}$	$K_{1:2}$
β-CyD	60	170
DM-β-CyD	1610	13
Maltosyl-β-CyD	680	50

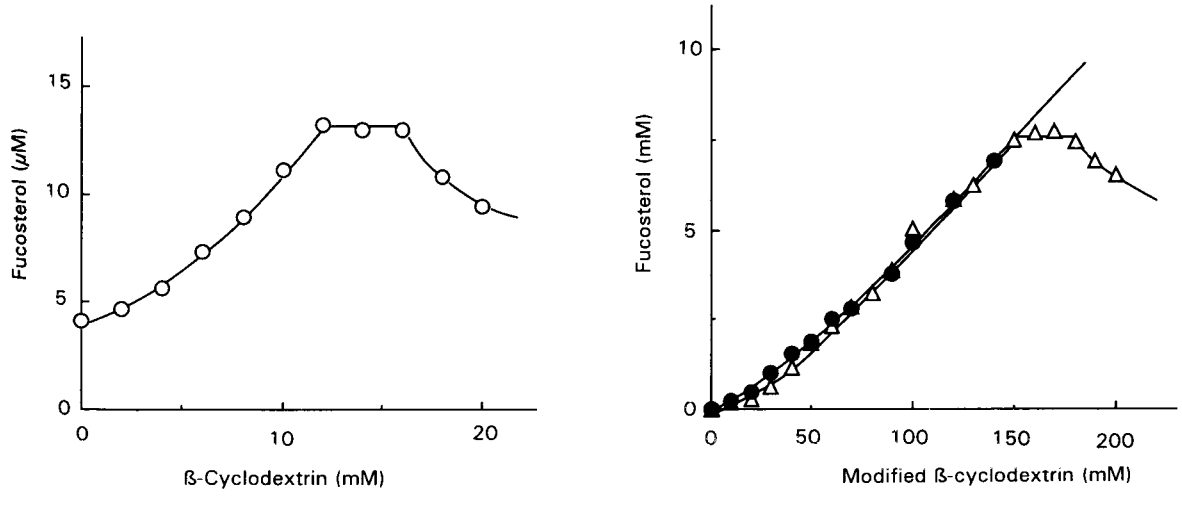

FIG. 1. Solubility diagrams of fucosterol with β-, DM-β- and maltosyl-β-CyD in water at 25°C. ○ β-CyD system, ● DM-β-CyD system, △ maltosyl-β-CyD system.

deeply into the cavity of another glucosyl-α-CyD in the crystal (Fujiwara et al 1989). Furthermore, the steroid compounds are generally liable to form the 1:2 complex with β-CyD (Liu et al 1990; Djedaini & Perly 1991). Therefore, probably two molecules of maltosyl-β-CyD included one fucosterol molecule, and the maltosyl moiety of maltosyl-β-CyD in the complex might be included in the cavity of another maltosyl-β-CyD in the high concentration of maltosyl-β-CyD, and thus a 1:3 solid complex was formed. Also it was assumed that the complex of fucosterol with DM-β-CyD was in the molar ratio of 1:2 in order to obtain a positive value of the stability constant. The stability constants of fucosterol with modified CyDs were greater than that of β-CyD. It is apparent from the magnitudes of $K_{1:1}$ and $K_{1:2}$ that the fucosterol molecule first forms an inclusion complex with one molecule of maltosyl-β- or DM-β-CyD, which apparently is a more favoured process over the binding of a 1:1 complex with the second CyD molecule. In the case of β-CyD, the association of the second β-CyD molecule with the 1:1 complex is a favoured process over the first association. Such anomalous behaviour could be due to the formation of intermolecular hydrogen bonds between β-CyDs. β-CyD forms a head-to-head type complex with a number of guest molecules, through which an intermolecular hydrogen bond is formed between secondary hydroxyl groups of two β-CyDs (Uekama et al 1983; Saenger 1984). The 1:1 complex of fucosterol with β-CyD might also be stabilized by the association of the second β-CyD molecule. However, maltosyl-β-CyD and DM-β-CyD could not form the head-to-head structure due to their steric hindrance (Harata 1984).

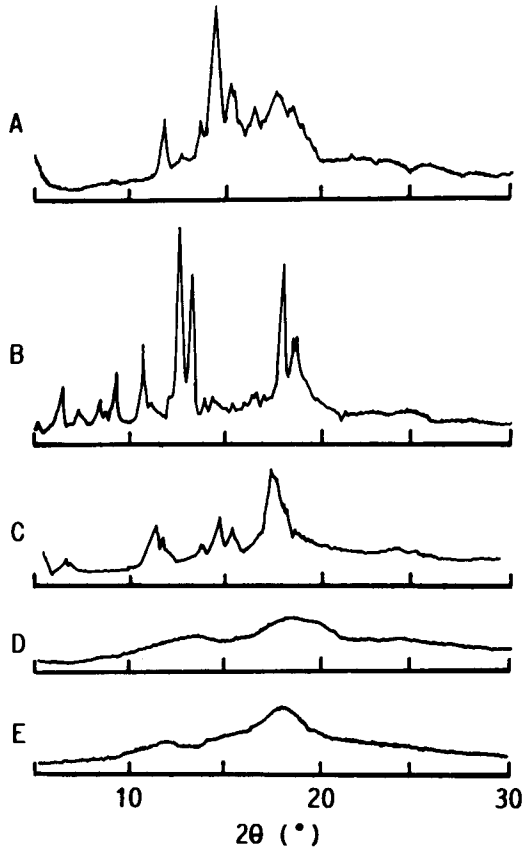

FIG. 2. Powder X-ray diffraction pattern of fucosterol-cyclodextrin systems. A Fucosterol, B physical mixture of fucosterol and β-CyD, C fucosterol-β-CyD complex, D physical mixture of fucosterol and maltosyl-β-CyD, E fucosterol-maltosyl-β-CyD complex.

Complexation in the solid state

Inclusion complex formation of fucosterol with β- and maltosyl-β-CyDs in the solid phase was compared by X-ray powder diffractometry, differential scanning calorimetry (DSC) and CP/MAS ^{13}C NMR measurements. As shown in Fig. 2, the diffraction patterns of the β-CyD complex were clearly different, indicating a new solid phase. On the other hand, the fucosterol-maltosyl-β-CyD complex showed an amorphous structure. In general, a CyD complex which precipitates in an aqueous solution is liable to crystallize. Although the amorphous complexation by maltosyl-β-CyD is difficult to explain, it is possible that maltosyl-β-CyD cannot crystallize in water.

Fig. 3 shows the DSC thermograms of intact fucosterol, fucosterol-CyD complexes and the physical mixtures of fucosterol and CyDs. Intact fucosterol and its physical mixtures showed an endothermic peak at around 121°C. However, the endothermic peak disappeared with the formation of the complex. More material was needed to complete

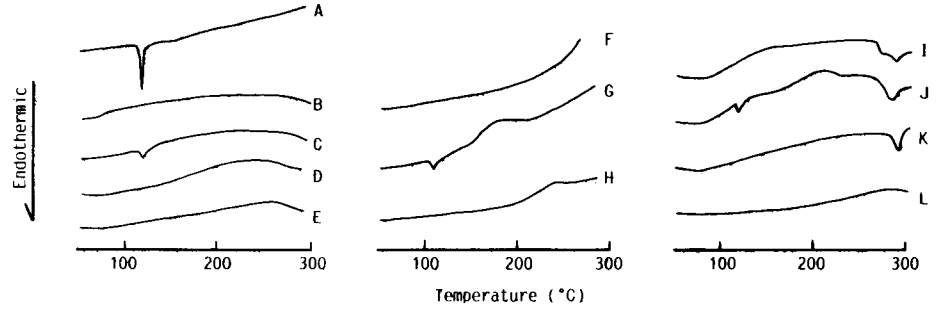

FIG. 3. DSC thermograms of fucosterol-CyD systems. A Fucosterol, B β-CyD, C physical mixture of fucosterol and β-CyD, D fucosterol-β-CyD complex, E kneaded mixture of fucosterol and β-CyD, F DM-β-CyD, G physical mixture of fucosterol and DM-β-CyD, H kneaded mixture of fucosterol and DM-β-CyD, I maltosyl-β-CyD, J physical mixture of fucosterol and maltosyl-β-CyD, K fucosterol-maltosyl-β-CyD complex, L kneaded mixture of fucosterol and maltosyl-β-CyD.

the dissolution studies, so kneading mixtures of fucosterol with β-, DM-β- and maltosyl-β-CyD in molar ratios of 1:2, 1:2 and 1:3, respectively were prepared and the interaction in the solid state was checked by DSC measurements. As shown in Fig. 3, the fucosterol endothermic peak disappeared on kneading, suggesting that fucosterol interacted with three kinds of β-CyD in the kneading mixture as well as in the solid inclusion complex isolated from aqueous solution.

Fig. 4 shows the CP/MAS ^{13}C NMR spectra of β-CyD and maltosyl-β-CyD and their inclusion complexes with fucosterol. The ^{13}C-signals from guest molecules in the fucosterol-maltosyl-β-CyD complex were obscured because the amount of fucosterol was low compared with that of the glucose residues, whereas fucosterol resonances appeared in the spectra of the 1:2 β-CyD complex. It is suggested that the molecular motion of fucosterol was not extensively affected on complexation with β-CyD, and that the fucosterol molecule was loosely included in the β-CyD cavity, consistent with a small stability constant of the β-CyD complex. On the other hand, complex formation resulted in a change in the shape of the glucose signals in β-CyD, especially at C-1, C-4 and C-6. The chemical shifts of C-1 and C-4 reflect the rotation state about the glycosidic linkage which is specified by the dihedral angles (Saito et al 1982; Kuan et al 1986). Therefore, the splitting of the signals in β-CyD can be explained in terms of superposition of displaced signals from glucose residues whose dihedral angles are distributed to some extent because of the distorted macrocyclic conformation. The β-CyD macrocycle is markedly perturbed by fucosterol, and the smaller distribution of dihedral angle might be induced by complexation. Although the ^{13}C-resonances of maltosyl-β-CyD did not show this splitting, the line-width of each peak was reduced by 1·2- to 1·5-fold through complexation with fucosterol. The reduction of the peak-width can be explained in the following manner. The glucose units of maltosyl-β-CyD were not magnetically equivalent to each other due to introduction of the maltosyl group, even though maltosyl-β-CyD had a round macrocyclic conformation. The macrocyclic conformation of maltosyl-β-CyD was distorted, so that the magnetic and conformational distribution of glucose residues and dihedral angles were observed as a broadening of peak-width. Further, the amorphous structure of maltosyl-β-CyD might also be responsible for broadening of the peak as reported by Gidley & Bociek (1988), who have shown that the amorphous structure of a polysaccharide induces the broadening of peak. The line-width of each ^{13}C-resonance was reduced by the decrease in the extent of glucose conformation and dihedral angle caused by complexation.

Dissolution behaviour of complexes

Fig. 5 shows the dissolution behaviour of fucosterol and its CyD complexes in water. Intact fucosterol dissolved extremely slowly in water. The dissolution rate from fucosterol-β-CyD increased slightly as compared with fucosterol. On the other hand, the two other complexes, particularly fucosterol-maltosyl-β-CyD complex, significantly improved the dissolution rate of fucosterol. Although the solubility of complexes was the same for maltosyl-β-CyD and DM-β-CyD (see Fig. 1), fucosterol-maltosyl-β-CyD, which has an amorphous structure, dissolved more quickly than fucosterol-DM-β-CyD. The enhanced dissolution rate of fucosterol thus might be due to an increase of solubility of this compound and the formation of an amorphous structure through complexation with maltosyl-β-CyD. The enhanced solubility of fucosterol may be useful for the formulation of various dosage forms, and the enhanced dissolution rate may be helpful for its absorption. The toxicity of maltosylated CyD has been reported to be lower than native CyD, but that of dimethylated CyD is higher than that of native CyD (Okada et al 1988; Uekama et al 1991). Therefore, maltosylated CyD which makes an amorphous structure through

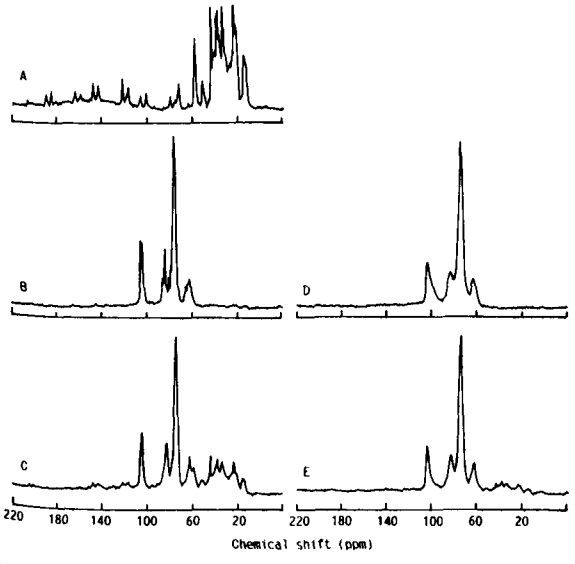

FIG. 4. CP/MAS ^{13}C NMR spectra of fucosterol-CyD systems. A Fucosterol, B β-CyD, C fucosterol-β-CyD complex, D maltosyl-β-CyD, E fucosterol-maltosyl-β-CyD complex.

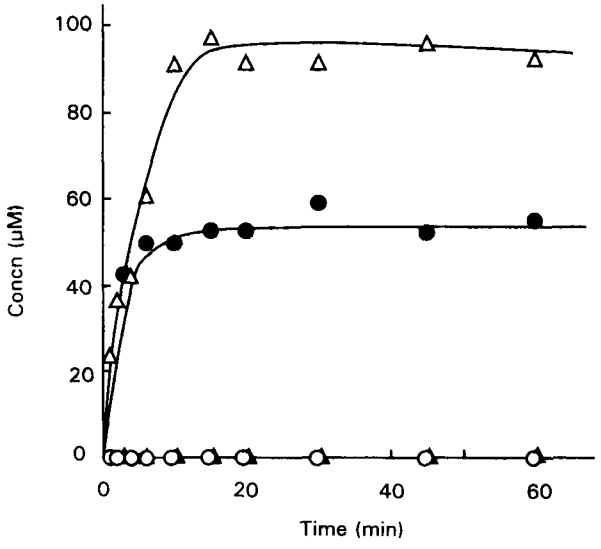

FIG. 5. Dissolution behaviours of fucosterol and its CyD complexes in water at 37°C. ▲ Fucosterol alone, ○ fucosterol-β-CyD complex, ● fucosterol-DM-β-CyD complex, △ fucosterol-maltosyl-β-CyD complex.

complexation with fucosterol, might be useful to improve the dissolution rate of poorly soluble steroid compounds.

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