Inclusion Complexation of Prostaglandin $F_{2\alpha}$ with γ-Cyclodextrin in Solution and Solid Phases

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Abstract □ A solid complex of prostaglandin $F_{2\alpha}$ (dinoprost) with γ-cyclodextrin in a molar ratio of 1:1 was obtained on the basis of the B_S-type phase solubility diagram. The mode of interaction in the solid state was studied by powder X-ray diffractometry, thermal analysis, and carbon-13 cross polarization/magic angle spinning nuclear magnetic resonance (^{13}C-CP/MAS-NMR) spectrometry. The X-ray diffraction and NMR data suggested that prostaglandin $F_{2\alpha}$ is included in the cylindrical channels formed by coaxial alignment of γ-cyclodextrin molecules to give a channel type structure. Dissolution and thermal behaviors of the prostaglandin $F_{2\alpha}$–γ-cyclodextrin complex were examined and compared with the drug itself. The result indicated that the γ-cyclodextrin complex may have great utility as a rapidly dissolving form of prostaglandin $F_{2\alpha}$ with improved thermal stability.

Keyphrases □ Prostaglandin $F_{2\alpha}$—inclusion complex with γ-cyclodextrin, solution and solid phases, solubility, dissolution behavior □ γ-Cyclodextrin—inclusion complex with prostaglandin $F_{2\alpha}$, solution and solid phases, solubility, dissolution behavior □ Solubility—prostaglandin $F_{2\alpha}$–γ-cyclodextrin inclusion complex, dissolution behavior □ Dissolution—prostaglandin $F_{2\alpha}$–γ-cyclodextrin complex, solubility

Prostaglandins are essentially long-chain fatty acids containing a substituted cyclopentane ring system. The relative hydrophobic environment of the cyclodextrin cavity seems to be particularly favorable for inclusion of the highly hydrophobic prostaglandin molecules. The complexation of α- and β-cyclodextrins with some prostaglandin analogues including prostaglandin $F_{2\alpha}$ (dinoprost)[1] have been reported (1–3). Frank and Cho have recently reported the complexing behavior of dinoprostone (prostaglandin E_2) with β-cyclodextrin in water (4). Unfortunately, an attempt to isolate the solid complex from α- or β-cyclodextrin solution was unsuccessful because the prostaglandin molecule is too bulky to be completely included into the cavities of α- and β-cyclodextrins.

Continuing these investigations, we now report an inclusion complexation of prostaglandin $F_{2\alpha}$ with γ-cyclodextrin, which has a larger hydrophobic cavity than α- and β-cyclodextrins [internal diameters of α-, β-, and γ-cyclodextrins are 5.0, 6.2, and 7.9 Å, respectively (5)]. Solubility analysis, X-ray diffractometry, and thermal analysis were used in the present study, and in addition carbon-13 cross polarization/magic angle spinning nuclear magnetic resonance (^{13}C-CP/MAS-NMR) spectrometry was employed to gain insight into the mode of interaction in the solid phase. Furthermore, the dissolution and thermal behaviors of the complex were examined.

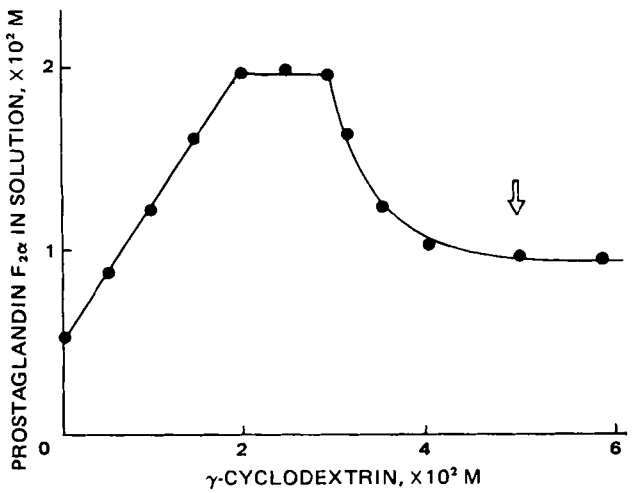

Figure 1—*Phase solubility diagram of the prostaglandin $F_{2\alpha}$–γ-cyclodextrin system in water at 25°C. The arrow shows the experimental condition of the preparation of the solid complex (see text).*

EXPERIMENTAL

Materials—Prostaglandin $F_{2\alpha}$[2] and γ-cyclodextrin[3] were used as supplied. All other materials and solvents were of analytical reagent grade. Deionized, double-distilled water was used.

Solubility Studies—Solubility measurements were carried out according to Higuchi and Lach (6). Excess amounts of prostaglandin $F_{2\alpha}$ were added to aqueous solutions containing various concentrations of γ-cyclodextrin and were shaken at 25 ± 0.5°C. After equilibration was attained (~2 d), an aliquot was centrifuged and pipetted through a cotton filter. A 0.5-mL aliquot of the sample solution was extracted with 5 mL of ether from acidified media, and 3 mL of the ether phase was evaporated to dryness under reduced pressure. The concentration of prostaglandin

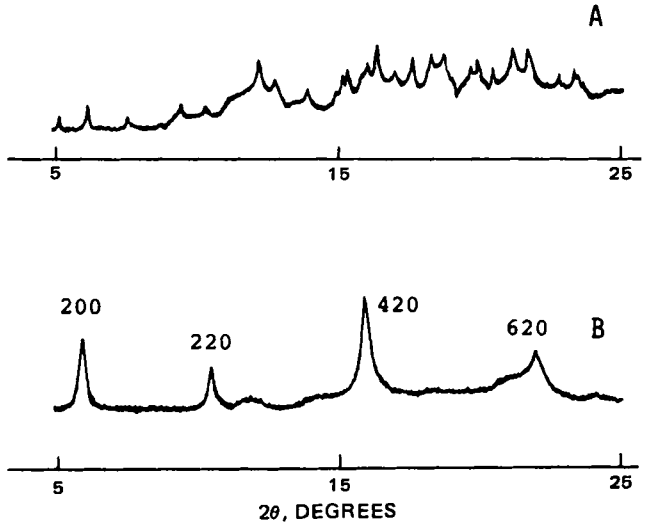

Figure 2—*Powder X-ray diffraction patterns of the prostaglandin $F_{2\alpha}$–γ-cyclodextrin system. Key: (A) γ-cyclodextrin; (B) prostaglandin $F_{2\alpha}$–γ-cyclodextrin complex. The reflection peaks of the complex were indexed according to Ref. 9.*

[1] (5Z,9α,11α,13E,15S)-9,11,15-Trihydroxyprosta-5,13-dien-1-oic acid.

[2] Ono Pharmaceutical Co. Ltd., Osaka, Japan.

[3] Nippon Shokuhin Kako, Tokyo, Japan.

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Table I—Diffraction Data for Prostaglandin $F_{2\alpha}$–γ-Cyclodextrin Complex

hkl[a]	d, Å Observed	d, Å Calculated
200	14.68	14.68
220	8.47	8.48
420	5.54	5.55
620	4.07	4.07

[a] Hexagonal indices.

$F_{2\alpha}$ was determined by GC according to the method of Roseman and Yalkowsky (7). The chromatographic conditions were the same as those reported previously (1). The apparent 1:1 stability constant (K') was calculated from the slope and intercept of the initial straight-line portion of the phase solubility diagram according to the following (8):

$$K' = \frac{\text{slope}}{\text{intercept}\,(1 - \text{slope})}$$

Preparation of the Solid Complex—The solid complex was derived by mixing appropriate amounts of prostaglandin $F_{2\alpha}$ and γ-cyclodextrin in water. The amounts were calculated from the descending curvature of the phase solubility diagram. For example, 100 mg of prostaglandin $F_{2\alpha}$ and 650 mg of γ-cyclodextrin were added to 10 mL of water, sealed in a flask, and stirred at 25°C for 2 d. The complex, which precipitated as a microcrystalline powder, was filtered and dried under vacuum at room temperature for 2 d. This powder corresponded to a complex of prostaglandin $F_{2\alpha}$–γ-cyclodextrin, in a molar ratio of 1:1 which had a molecular weight of 1652 (±5%).

X-Ray Diffractometry—The powder X-ray diffractometer[4] was operated under the following conditions: X-ray, Ni-filtered Cu-K_α radiation; voltage, 30 kV; current, 20 mA; time constant, 2 s; scanning speed, 1°/min.

Differential Thermal Analysis (DTA)—The thermometer[5] was operated at a scanning rate of 10°C/min over the temperature range of 10–350°C. The sample weight was 2–10 mg.

^{13}C-NMR Spectrometry—Single-contact ^{13}C-CP/MAS measurements[6] were obtained at 75.46 MHz with a spectrometer equipped with a CP/MAS accessory. The sample (~300 mg) was placed in a Andrew–Beams-type rotor machined from perdeuterated poly(methyl methacrylate) and spun as fast as 3–4 kHz. The contact time was chosen as 1 ms (not optimized, but chosen to avoid buildup of strong signals from residual signals from the rotor and probe assembly), and repetition time was 2 s. The spectral width and data points were 30 kHz and 4K, respectively. Carbon-13 chemical shifts were calibrated indirectly through external benzene (128.5 ppm from tetramethylsilane).

Dissolution Studies—The dissolution behaviors of prostaglandin $F_{2\alpha}$ and its γ-cyclodextrin complex in water were compared at the same concentration of prostaglandin $F_{2\alpha}$ according to the dispersed-amount method (9). An 850-mg sample of the complex as a 100-mesh powder was weighed and put in a dissolution cell. Because prostaglandin $F_{2\alpha}$ is easy to melt at room temperature (the melting temperature is ~30°C), an ether solution containing 182 mg of the drug was placed in the dissolution cell and evaporated to dryness. The dissolution medium (25 mL) was maintained at 25°C and stirred at 91 rpm. At an appropriate interval, 0.5 mL of solution was sampled by a pipet with a cotton filter. The assay procedure for prostaglandin $F_{2\alpha}$ was the same as that used in the solubility studies.

RESULTS AND DISCUSSION

Figure 1 shows the equilibrium phase solubility diagram obtained for prostaglandin $F_{2\alpha}$ with γ-cyclodextrin in water. The plot shows a typical B_S-type solubility curve (8). The initial rising portion is followed by a plateau region and then decreases in total concentration of prostaglandin $F_{2\alpha}$ with precipitation of a microcrystalline complex at high γ-cyclodextrin concentration. In sharp contrast, the phase solubility diagrams of prostaglandin $F_{2\alpha}$ with α- and β-cyclodextrins showed an A_L-type curve, and no precipitation of the complexes was observed (1). The apparent 1:1 stability constant (K') of the γ-cyclodextrin complex was calculated to be 480 M^{-1} from the initial straight-line portion of the solubility diagram in Fig. 1. The K' value of the γ-cyclodextrin complex was found to be intermediate between the α-cyclodextrin complex (254 M^{-1}) and the β-cyclodextrin complex (1240 M^{-1}) values reported previously (1). The 1:1 stoichiometry of the γ-cyclodextrin complex in the solid phase was ascertained on the basis of data in the plateau region of the solubility diagram. The result was in good agreement with that obtained by isolation and analysis of the solid complex. Thus, the 1:1 solid complex isolated from the descending curvature of the B_S-type solubility diagram was used for further study.

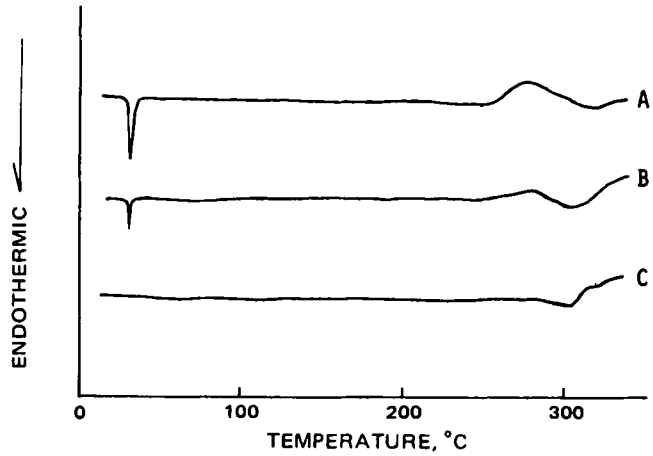

Figure 3—*DTA thermograms of prostaglandin $F_{2\alpha}$–γ-cyclodextrin system. Key: (A) prostaglandin $F_{2\alpha}$; (B) physical mixture of prostaglandin $F_{2\alpha}$ and γ-cyclodextrin in a molar ratio of 1:1; (C) prostaglandin $F_{2\alpha}$–γ-cyclodextrin complex.*

Figure 2 shows the powder X-ray diffraction pattern of the prostaglandin $F_{2\alpha}$–γ-cyclodextrin complex, comparing with that of γ-cyclodextrin itself. It was difficult to obtain the diffraction pattern of prostaglandin $F_{2\alpha}$ under these experimental conditions because of the low melting temperature. The diffraction pattern of the complex was apparently different from that of γ-cyclodextrin, indicating the constitution of a new solid phase. It was noted that the diffraction diagram of the complex in Fig. 2 showed a hexagonal nature of pattern similar to that observed for the *n*-propyl alcohol–γ-cyclodextrin complex (10). The *n*-propyl alcohol–γ-cyclodextrin complex is known to have a channel structure (10, 11) with a hexagonal close packing of the cylinders of γ-cyclodextrin molecules. Therefore, the diffraction pattern of the complex was indexed on the basis of a two-dimensional hexagonal unit cell having $a = b = 33.90$ Å, where *d*-spacing for the 200 reflection peak on the diagram was used to calculate the unit cell dimensions (10). As shown in Table I, the calculated *d*-spacing was in excellent agreement with that observed. This suggests that prostaglandin $F_{2\alpha}$ is included

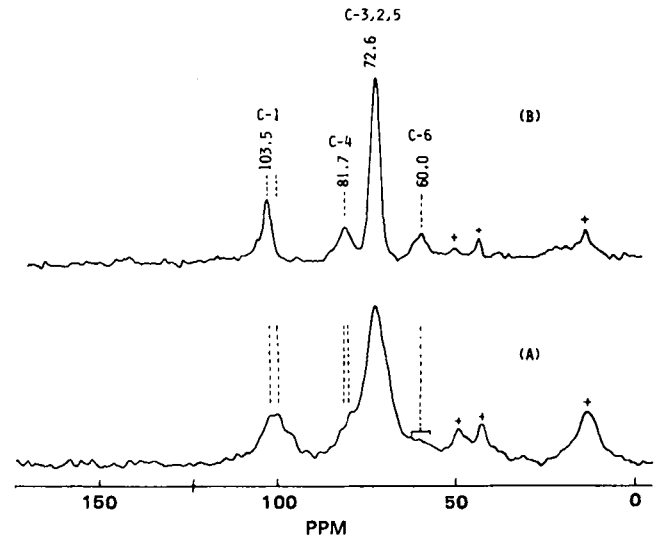

Figure 4—*^{13}C-CP/MAS-NMR spectra of prostaglandin $F_{2\alpha}$–γ-cyclodextrin system in solid state. Key: (A) γ-cyclodextrin; (B) prostaglandin $F_{2\alpha}$–γ-cyclodextrin complex.*

[4] Geiger Flex-2012 diffractometer; Rigaku Denki, Tokyo, Japan.
[5] Model DT-20B thermal analyzer; Shimadzu, Kyoto, Japan.
[6] CXP-300 spectrometer; Bruker, Silber-Streifen, West Germany.

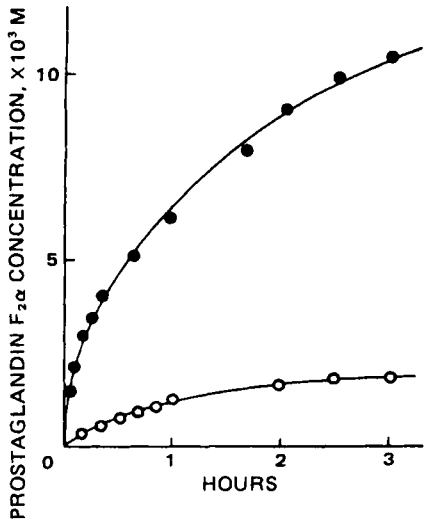

Figure 5—*Dissolution profiles of prostaglandin $F_{2\alpha}$ and its γ-cyclodextrin complex in water at 25°C. Key: (○) prostaglandin $F_{2\alpha}$; (●) prostaglandin $F_{2\alpha}$–γ-cyclodextrin complex.*

within the cylindrical channels formed by coaxial alignment of γ-cyclodextrin molecules to give a channel type structure.

Figure 3 shows DTA thermograms of the prostaglandin $F_{2\alpha}$–γ-cyclodextrin system. In the cases of prostaglandin $F_{2\alpha}$ and its physical mixture with γ-cyclodextrin (1:1 molar ratio), an endothermic peak due to the melting was observed around 30°C. In sharp contrast, the complex showed no appreciable endothermic peak within the degradation temperature of γ-cyclodextrin (~290°C) (12), suggesting that the complexed form of prostaglandin $F_{2\alpha}$ is considerably stable compared with the drug itself.

^{13}C-CP/MAS-NMR techniques were employed to gain further insight into the mode of interaction between prostaglandin $F_{2\alpha}$ and γ-cyclodextrin in the solid state. Figure 4 shows the ^{13}C-CP/MAS-NMR spectra of γ-cyclodextrin and its inclusion complex with prostaglandin $F_{2\alpha}$. The signals are readily assigned to the individual carbons of the glucose residues, as indicated in Fig. 4 [signals marked by the + are ascribed to the residual signals from the rotor of magic angle spinning machined from perdeuterated poly(methyl methacrylate)]. In the case of the complex, no appreciable signal from the guest molecule was observed under the experimental conditions used. Carbon-13 signals from guest molecules are known to be, in many instances, lost or obscured because the amount of the guest molecules is usually low compared with that of the glucose residues; also, buildup of the ^{13}C-magnetization might be strongly influenced by plausible molecular motions within the cavity (13, 14). Nevertheless, it is clearly seen that the complex formation resulted in the substantial reduction of the line widths of the glucose residues, especially in the C-1, C-4, and C-6 region, as well as the significant displacement of these signals. The distinct spectral change on complex formation might arise from a macrocyclic conformational change associated with the inclusion of the guest molecule.

In this connection, it is emphasized that the C-1 and C-X carbon-13 shifts at the glycosidic linkages for a number of polysaccharides (15, 16) are substantially displaced depending on their conformations. In particular, such displacements of the C-1 and C-4 shifts of α-D-(1,4)-linked glucans are well related to the dihedral angles ϕ and ψ for the C(1)—O_{gly} and O_{gly}—C(4) rotations (13, 14), respectively, which characterize the conformation of the individual glucose residues, as manifested from the previous ^{13}C-CP/MAS-NMR studies on polymorphs of amylose and various types of cyclodextrin complexes (13, 14). Therefore, broad carbon-13 signals in the γ-cyclodextrin are explained in terms of superposition of slightly displaced signals from glucose residues whose dihedral angles are distributed to some extent because of distorted macrocyclic conformation (15). It should be also noted that the ^{13}C-chemical shift changes of this complex are identical to those of γ-cyclodextrin–*n*-propyl alcohol complex within the experimental error (±1 ppm) (13, 14). Obviously, this finding is consistent with the X-ray diffraction study described above and also with the work by Lindner and Saenger (11).

The dissolution behavior of the γ-cyclodextrin complex was examined in anticipation of improved dissolution characteristics of prostaglandin $F_{2\alpha}$. It would be interesting to compare the effect of complexation on the dissolution rate for the three cyclodextrins, but an attempt to isolate the solid complexes of prostaglandin $F_{2\alpha}$ with α- and β-cyclodextrins was unsuccessful.

Figure 5 shows the dissolution profiles of prostaglandin $F_{2\alpha}$ and its γ-cyclodextrin complex in water. It was found that the complex dissolved much more rapidly (>10-fold) than prostaglandin $F_{2\alpha}$ itself. The observed increase in the rate may be due to the increase in solubility, as expected from Fig. 1, although other factors such as diffusion coefficient, wettability, and the dissociation of the complex in the dissolution medium (17) should be considered in the rate enhancement.

Thus, the increased dissolution rate suggests that prostaglandin $F_{2\alpha}$–γ-cyclodextrin complex may have great utility in the development of fast-dissolving dosage forms with improved bioavailability. Furthermore, the increased melting temperature and/or thermal stability of prostaglandin $F_{2\alpha}$ by γ-cyclodextrin complexation may facilitate the ease of handling the pharmaceutical preparations.

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