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Improvement of Dissolution Characteristics and Chemical Stability of Prostaglandin E_1 by γ-Cyclodextrin Complexation[1)]

KANETO UEKAMA,*,[a] AKIHIKO FUJISE,[a] FUMITOSHI HIRAYAMA,[a] MASAKI OTAGIRI,[a] and KOHJI INABA[b]

Faculty of Pharmaceutical Sciences, Kumamoto University,[a] Kumamoto 862, Japan and Central Research Institute, Ono Pharmaceutical Co., Ltd.,[b] Osaka 618, Japan

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Inclusion complexation of prostaglandin E_1 (PGE_1) with γ-cyclodextrin (γ-CyD) in aqueous solution and in the solid phase was assessed by the solubility method, X-ray diffractometry, and thermal analysis. A solid complex of PGE_1–γ-CyD in a 1:2 molar ratio was obtained, and its dissolution behavior and chemical stability were examined. The dissolution rate of the γ-CyD complex was extremely large compared with that of PGE_1. In addition, the dehydration of PGE_1 to PGA_1 was significantly retarded by inclusion complex formation. The data suggest that γ-CyD complex may have great utility as a fast-dissolving form of PGE_1 with good storage properties.

Keywords——prostaglandin E_1; γ-cyclodextrin; inclusion complexation; phase solubility diagram; dissolution profile; thermal stability; dehydration reaction

Prostaglandin E_1 (PGE_1) is essentially a long-chain unsaturated fatty acid containing a substituted cyclopentane ring system. The β-hydroxyketo moiety of PGE_1 is extremely susceptible to dehydration under acidic or alkaline conditions to give prostaglandin A_1 (PGA_1), which is isomerized consecutively to form prostaglandin B_1 (PGB_1) under alkaline conditions.[2,3)] The biological activity of PGE_1 decreases with the progress of these reactions.[4)] The chemical instability as well as the low aqueous solubility of PGE_1 have limited dosage form design and presented a substantial challenge to pharmaceutical scientists.[5,6)]

We have recently reported that some prostaglandin analogs including PGE_1 form inclusion complexes with α- and β-cyclodextrins (α- and β-CyDs) in aqueous solution.[7–10)] However, an attempt to isolate the PGE_1 complexes from α- and β-CyD solutions was unsuccessful because PGE_1 is too bulky to fit completely into the cavities of α- and β-CyDs (internal diameters of 5.7 and 7.8 Å for α- and β-CyDs, respectively). Recently, however, we have succeeded in isolating the solid complex of γ-CyD with PGE_1 from the saturated aqueous solution. Thus, we now report for the first time an inclusion complexation of PGE_1 with γ-CyD, which has larger hydrophobic cavity (internal diameter of 9.5 Å) and greater aqueous solubility (0.23 g/ml at 25 °C) than α- and β-CyDs. The dissolution behavior and chemical stability of the complex were examined.

Experimental

Materials——PGE_1 and PGA_1 were donated by Ono Pharmaceutical Co., Ltd. (Osaka, Japan). γ-CyD was supplied by Nihon Shokuhin Kako Co., Ltd. (Tokyo, Japan). All other materials and solvents were of analytical reagent grade. Deionized double-distilled water was used throughout.

Apparatus——The powder X-ray diffraction patterns were taken with a Rigaku Denki Geiger Flex 2012 (Tokyo, Japan) using Ni-filtered Cu-K_α radiation. Differential thermal analysis (DTA) was carried out at a scanning rate of 10 °C/min on a shimadzu DT-20B thermal analyzer (Kyoto, Japan). The sample weight was 2—10 mg. High performance liquid chromatography (HPLC) was run on a Hitachi 635 A machine (Tokyo, Japan) equipped with a

variable-wavelength ultraviolet (UV) monitor.

Solubility Studies——Solubility measurements and analytical methods for PGE_1 in the absence and presence of γ-CyD were essentially the same as those reported previously.[7] An apparent stability constant (K') was calculated from the initial linear portion of the phase solubility diagram according to the following equation.[11]

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

The solid complex was derived by mixing appropriate amounts of PGE_1 and γ-CyD in water. The amounts were calculated from the descending curvature of the phase solubility diagram (see Fig. 1). For example, 0.05 g of PGE_1 and 1.3 g of γ-CyD were added to 10 ml of water, then the mixture was sealed in a flask and stirred at 25 °C for 2 d. Under these experimental conditions, no appreciable decomposition of PGE_1 was observed. The complex, which precipitated as a micro-crystalline powder, was filtered and dried under a vacuum at 25 °C for 48 h. This powder corresponded to a 1 : 2 PGE_1–γ-CyD complex and had a molecular weight of 2949.

Dissolution Studies——The dissolution properties of PGE_1 and its γ-CyD complex in water were measured according to the dispersed amount method.[12] A sample powder (100 mesh, 44 mg as PGE_1) was weighed and put in a dissolution cell. The dissolution medium (25 ml) was maintained at 25 °C and stirred at 91 rpm. At appropriate intervals, 0.5 ml of solution was sampled by the use of a pipette with a cotton filter. The assay procedure for PGE_1 was the same as that in the solubility study.

Stability Studies——Stability tests for PGE_1 and its γ-CyD complex were conducted at 90 °C. Citric acid (30% w/w) was added to the test samples to prevent PGB_1 formation. The disappearance of PGE_1 and the appearance of PGA_1 were simultaneously determined by HPLC according to the method of Fitzpatrick.[13] The chromatograph was operated at a flow rate of 0.6 ml/min, and the eluent was monitored spectrophotometrically at 254 nm. The separation was achieved on a column of LiChrosorb RP-18 (5 μm in 4φ × 250 mm, Merck), with water–methanol–acetonitrile (5 : 12 : 8) as the mobile phase. Components were quantitated by measuring peak heights and comparing them with those of known amounts of internal standard, hexyl 4-hydroxybenzoate.

Results and Discussion

Inclusion Complex Formation of PGE_1 with γ-CyD

The complexation of PGE_1 with γ-CyD was studied by the solubility method, X-ray diffractometry and thermal analysis. The phase solubility diagram obtained for PGE_1 with γ-CyD in water is shown in Fig. 1. The plot shows a typical B_s-type solubility curve,[11] where the initial rising portion is followed by a plateau region, and then a decrease in total PGE_1 concentration with precipitation of the micro-crystalline complex. As a tentative measure of inclusion complexation, the apparent stability constant (K') was estimated from Eq. (1) based on the assumption that a 1 : 1 complex is initially formed. The K' value was calculated to be 530 M^{-1} from the initial rising portion of the solubility diagram. The K' value of γ-CyD complex was found to be the smallest among the three CyD complexes (α-CyD, 1430 M^{-1}; β-CyD, 1700 M^{-1} reported previously[6]). The 1 : 2 stoichiometry of the γ-CyD complex in the solid state was ascertained on the basis of the data in the plateau region of the solubility diagram. To gain further insight into the stoichiometry of the complex, the solid phase that precipitated beyond the plateau region was analyzed.[14] The analysis at several points beyond the plateau region in Fig. 1 gave the following results for 10^2 L_t (the total concentration of γ-CyD) and X_s (the mole fraction of PGE_1 in the solid phase): 8.0, 2.05; 10.0, 2.07; 16.0, 2.10; 20.0, 2.02, respectively. These data indicate that 1 : 2 complex formation of PGE_1 with γ-CyD is predominant at higher γ-CyD concentration. Inspection of a space-filling molecular model[15] also showed that two molecules of γ-CyD are available for the complete inclusion of PGE_1, where PGE_1 fits suitably into the interior space of the γ-CyD channels. Thus, the 1 : 2 solid complex corresponding to the region of descending curvature of the B_s-type solubility diagram was used for further study.[16]

Figure 2 shows the powder X-ray diffraction patterns of the PGE_1–γ-CyD system, in comparison with the corresponding physical mixture at the same molar ratio. The diffraction pattern of the physical mixture was simply the sum of those of the two components, while that of the complex was apparently different from the pattern of either constituent, as shown in

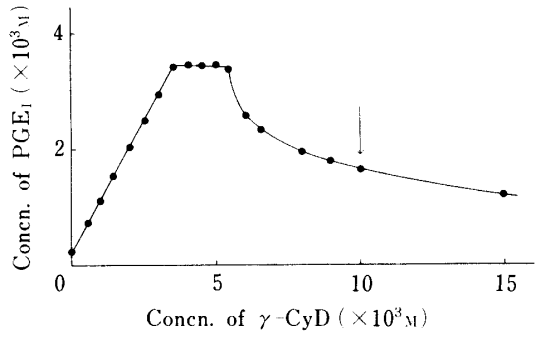

Fig. 1. Phase Solubility Diagram of the PGE_1–γ-CyD System in Water at 25 °C

The arrow shows the experimental conditions used for the preparation of the solid complex (see the text).

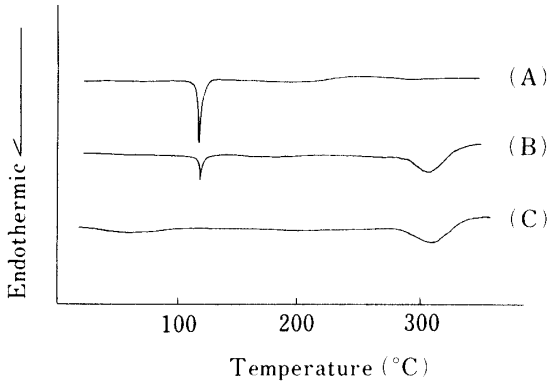

Fig. 3. DTA Thermograms of PGE_1–γ-CyD System

(A): PGE_1.
(B): physical mixture of PGE_1 with γ-CyD.
(C): PGE_1–γ-CyD complex.

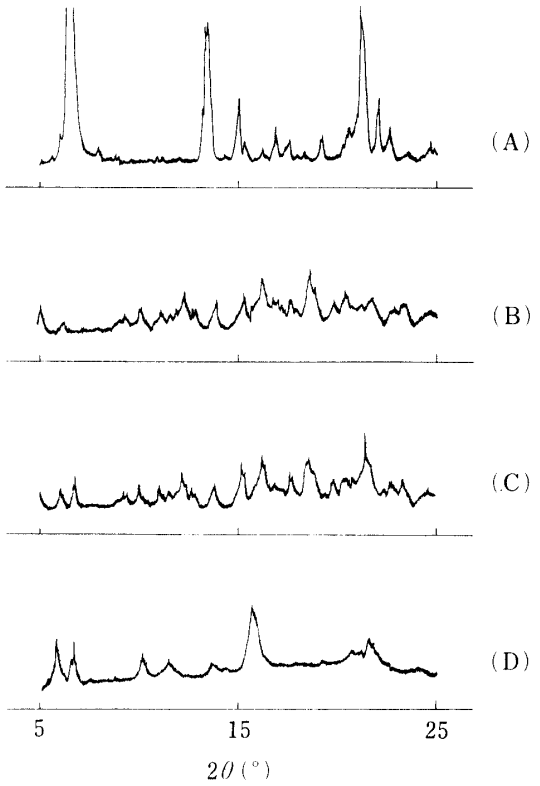

Fig. 2. Powder X-Ray Diffraction Patterns of PGE_1–γ-CyD System

(A): PGE_1.
(B): γ-CyD.
(C): physical mixture of PGE_1 with γ-CyD.
(D): PGE_1–γ-CyD complex.

Fig. 2. It was also found that the complex gave a somewhat diffuse diffraction pattern with decreased intensities, suggesting that it is less crystalline than the physical mixture.

Figure 3 shows DTA thermograms of the PGE_1–γ-CyD system. In the cases of PGE_1 and the physical mixture of PGE_1 and γ-CyD, an endothermic peak due to the melting of PGE_1[17] was observed around 117 °C. In contrast, the complex showed no appreciable endothermic peak. These results also indicate that the complexed form of PGE_1 is less crystalline than PGE_1 itself, as expected from Fig. 2.

Dissolution Behavior and Chemical Stability

The dissolution profiles of PGE_1 and its γ-CyD complex in water are shown in Fig. 4. It is evident that the complexed form of PGE_1 dissolved much more rapidly (about 10-fold) than PGE_1 itself. The observed increase in rate may be due to the increase in solubility and the decrease in crystallinity of PGE_1 by inclusion complexation, as expected from Fig. 1 and Fig. 2, respectively. It is interesting to note that the dissolution profile of the complex showed a negative curvature with the passage of time. This may be due to the dissociation of the complex after the start of dissolution, resulting in an increase in the fraction of free PGE_1 in the dissolution medium. Similar dissolution behavior has been observed recently for prostaglandin $F_{2\alpha}$–γ-CyD complex.[18]

The thermal stabilities of PGE_1 and its γ-CyD complex were examined at 90 °C. Figure 5

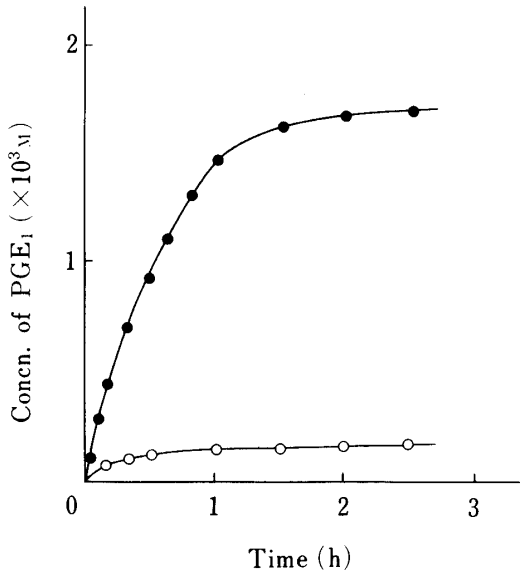

Fig. 4. Dissolution Profiles of PGE_1 and Its γ-CyD Complex in Water at 25 °C, Measured by the Dispersed Amount Method

○; PGE_1: ●; PGE_1–γ-CyD complex.

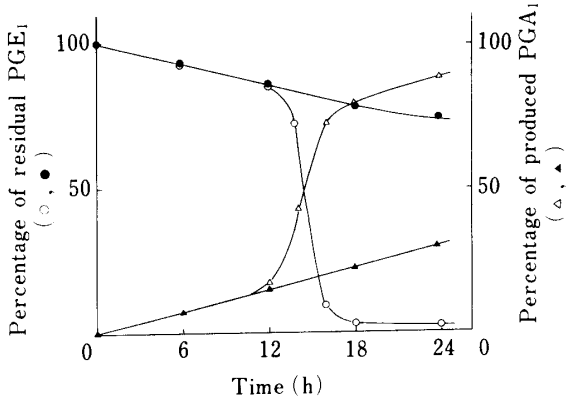

Fig. 5. Time Courses of Degradation of PGE_1 and Production of PGA_1 from PGE_1 and Its γ-CyD Complex at 90 °C

○, △; PGE_1: ●, ▲; γ-CyD complex.

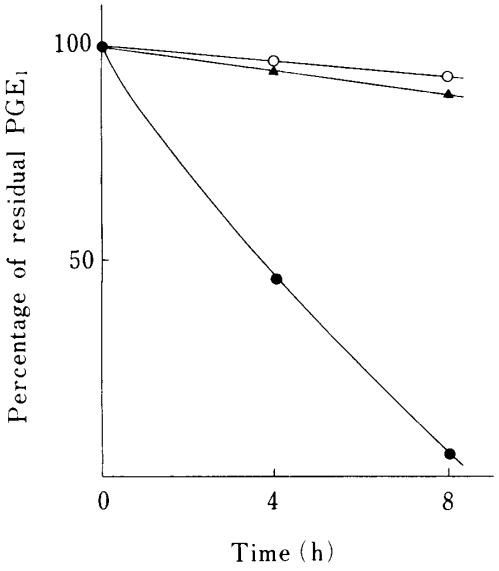

Fig. 6. Catalytic Effect of PGA_1 on the Dehydration Reaction of PGE_1 and Its γ-CyD Complex at 90 °C

○; PGE_1 alone (4 mg).
●; PGE_1 (4 mg) + PGA_1 (1 mg).
▲; γ-CyD complex (4 mg as PGE_1) + PGA_1 (1 mg).

shows the degradation curves of PGE_1 and the complex, where the appearance of PGA_1 was simultaneously determined in the course of stability tests. No decomposition product other than PGA_1 was detected under these experimental conditions. The rate of degradation was found to be very slow for both PGE_1 and its complex during 12 h. However, after 12 h the amount of PGE_1 decreased rapidly and that of PGA_1 rose concomitantly. In contrast to PGE_1 alone, the degradation of the complex was significantly slow even after 12 h. The difference in thermal stabilities observed for PGE_1 and the complex can be explained on the basis of the extent of formation of unstable intermediates such as the PGE_1–PGA_1 complex. In the case of PGE_1 alone, the amount of the activated complex may increase progressively with the passage of time. In the case of the γ-CyD complex, however, it may be difficult for the intermediate to accumulate because the PGE_1 molecule can be included within the cavity of γ-CyD to prevent the interaction with PGA_1. In fact, the dehydration reaction of PGE_1 was

significantly accelerated by the addition of PGA_1, compared with that of the γ-CyD complex alone, as shown in Fig. 6.

The increased dissolution rate together with improved chemical stability suggest that the PGE_1–γ-CyD complex may have great utility in the development of fast-dissolving dosage forms of PGE_1 with good storage properties.

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References and Notes

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