Improvement of Dissolution Characteristics and Chemical Stability of Prostaglandin E₁ by γ-Cyclodextrin Complexation

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(Received June 6, 1983)

Inclusion complexation of prostaglandin E₁ (PGE₁) 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 PGE₁–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 PGE₁. In addition, the dehydration of PGE₁ to PGA₁ was significantly retarded by inclusion complex formation. The data suggest that γ-CyD complex may have great utility as a fast-dissolving form of PGE₁ with good storage properties.

Keywords—prostaglandin E₁; γ-cyclodextrin; inclusion complexation; phase solubility diagram; dissolution profile; thermal stability; dehydration reaction

Prostaglandin E₁ (PGE₁) is essentially a long-chain unsaturated fatty acid containing a substituted cyclopentane ring system. The β-hydroxyketo moiety of PGE₁ is extremely susceptible to dehydration under acidic or alkaline conditions to give prostaglandin A₁ (PGA₁), which is isomerized consecutively to form prostaglandin B₁ (PGB₁) under alkaline conditions.²,³ The biological activity of PGE₁ decreases with the progress of these reactions.⁴ The chemical instability as well as the low aqueous solubility of PGE₁ have limited dosage form design and presented a substantial challenge to pharmaceutical scientists.⁵,⁶

We have recently reported that some prostaglandin analogs including PGE₁ form inclusion complexes with α- and β-cyclodextrins (α- and β-CyDs) in aqueous solution.⁷–¹⁰ However, an attempt to isolate the PGE₁ complexes from α- and β-CyD solutions was unsuccessful because PGE₁ 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 PGE₁ from the saturated aqueous solution. Thus, we now report for the first time an inclusion complexation of PGE₁ 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—PGE₁ and PGA₁ 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-Kα 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 PGE₄ in the absence and presence of γ-CyD were essentially the same as those reported previously. An apparent stability constant ($K'$) was calculated from the initial linear portion of the phase solubility diagram according to the following equation:

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

(1)

The solid complex was derived by mixing appropriate amounts of PGE₄ 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 PGE₄ 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 PGE₄ 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 PGE₄–γ-CyD complex and had a molecular weight of 2949.

**Dissolution Studies** — The dissolution properties of PGE₄ and its γ-CyD complex in water were measured according to the dispersed amount method. A sample powder (100 mesh, 44 mg as PGE₄) 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 PGE₄ was the same as that in the solubility study.

**Stability Studies** — Stability tests for PGE₄ and its γ-CyD complex were conducted at 90 °C. Citric acid (30 w/w) was added to the test samples to prevent PGE₄ from precipitation and the appearance of PGA₄ was simultaneously determined by HPLC according to the method of Fitzpatrick. The chromatography 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 4.0 x 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 PGE₄ with γ-CyD**

The complexation of PGE₄ with γ-CyD was studied by the solubility method, X-ray diffractometry and thermal analysis. The phase solubility diagram obtained for PGE₄ with γ-CyD in water is shown in Fig. 1. The plot shows a typical B₁-type solubility curve, where the initial rising portion is followed by a plateau region, and then a decrease in total PGE₄ 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). 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. The analysis at several points beyond the plateau region in Fig. 1 gave the following results for $L_i$ (the total concentration of γ-CyD) and $X_i$ (the mole fraction of PGE₄ 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 PGE₄ with γ-CyD is predominant at higher γ-CyD concentration. Inspection of a space-filling molecular model also showed that two molecules of γ-CyD are available for the complete inclusion of PGE₄, where PGE₄ 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 B₁-type solubility diagram was used for further study.

Figure 2 shows the powder X-ray diffraction patterns of the PGE₄–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 PGE₁-\(\gamma\)-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 PGE₁-\(\gamma\)-CyD System

(A): PGE₁
(B): physical mixture of PGE₁ with \(\gamma\)-CyD.
(C): PGE₁-\(\gamma\)-CyD complex.

Fig. 2. Powder X-Ray Diffraction Patterns of PGE₁-\(\gamma\)-CyD System

(A): PGE₁,
(B): \(\gamma\)-CyD,
(C): physical mixture of PGE₁ with \(\gamma\)-CyD,
(D): PGE₁-\(\gamma\)-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 PGE₁-\(\gamma\)-CyD system. In the cases of PGE₁ and the physical mixture of PGE₁ and \(\gamma\)-CyD, an endothermic peak due to the melting of PGE₁\(^{17)}\) was observed around 117 °C. In contrast, the complex showed no appreciable endothermic peak. These results also indicate that the complexed form of PGE₁ is less crystalline than PGE₁ itself, as expected from Fig. 2.

**Dissolution Behavior and Chemical Stability**

The dissolution profiles of PGE₁ and its \(\gamma\)-CyD complex in water are shown in Fig. 4. It is evident that the complexed form of PGE₁ dissolved much more rapidly (about 10-fold) than PGE₁ itself. The observed increase in rate may be due to the increase in solubility and the decrease in crystallinity of PGE₁ 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 PGE₁ in the dissolution medium. Similar dissolution behavior has been observed recently for prostaglandin F₂₆-\(\gamma\)-CyD complex.\(^{18}\)

The thermal stabilities of PGE₁ and its \(\gamma\)-CyD complex were examined at 90 °C. Figure 5
shows the degradation curves of PGE₁ and the complex, where the appearance of PGA₁ was simultaneously determined in the course of stability tests. No decomposition product other than PGA₁ was detected under these experimental conditions. The rate of degradation was found to be very slow for both PGE₁ and its complex during 12h. However, after 12h the amount of PGE₁ decreased rapidly and that of PGA₁ rose concomitantly. In contrast to PGE₁ alone, the degradation of the complex was significantly slow even after 12h. The difference in thermal stabilities observed for PGE₁ and the complex can be explained on the basis of the extent of formation of unstable intermediates such as the PGE₁-PGA₁ complex. In the case of PGE₁ 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 PGE₁ molecule can be included within the cavity of γ-CyD to prevent the interaction with PGA₁. In fact, the dehydration reaction of PGE₁ was
significantly accelerated by the addition of PGA₁, 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 PGE₁–γ-CyD complex may have great utility in the development of fast-dissolving dosage forms of PGE₁ with good storage properties.

Acknowledgement  This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

References and Notes

1) A part of this work was presented at the 103rd Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April 1983.
15) Corey–Pauling–Koltun molecular model.
16) An X-ray crystallographic analysis of PGE₁–γ-CyD complex is under way.
17) The melting point of PGE₁ was determined to be 118–120 °C (uncorrected value), using a micro melting point apparatus (Yanagimoto Co., Ltd.).