Prominent Inhibitory Effect of 2-Hydroxybutyl-β-cyclodextrin on Solution-mediated Polymorphic Transition of Chlorpropamide

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The effects of cyclodextrins on crystallization of chlorpropamide from aqueous solutions were investigated. Parent α-, β-, and γ-cyclodextrins and 2-hydroxypropyl-α-, and -β-cyclodextrins and glucose did not change the crystallization of the drug, providing the stable Form A crystal. On the other hand, 2-hydroxybutyl-β-cyclodextrin inhibited the solution-mediated polymorphic transition of the drug, providing the metastable Form II at higher cyclodextrin concentrations and Form III at lower cyclodextrin concentrations. The results suggested that 2-hydoxybutyl-β-cyclodextrin is useful for preparing metastable crystals during crystallization according to the Ostwald rule.

Polymorphism is a phenomenon that a compound has more than one crystalline arrangement in the solid. Polymorphism plays an important role in pharmaceutical industries, because different crystalline polymorphs exhibit different physicochemical properties such as solubility, dissolution rate, bioavailability, and chemical and physical stabilities.[1] Therefore, it is of great importance to discover, produce, and isolate crystalline polymorphs of a given solid drug and to control their polymorphic transformations. Further, for the development of high-quality drugs, it is important to select a proper polymorph and to be able to control its polymorphic transformations, and diverse crystal forms provide valuable intellectual property protection in drug development.

Cyclodextrins (CyDs), cyclic oligosaccharides consisting of usually 6 to 8 D-glucose units, form inclusion complexes with various molecules in aqueous solution and in solid states, and are successfully utilized for improvement of pharmaceutical properties of drugs.[2] In previous papers, we reported that 2,6-di-*O*-methyl-β-cyclodextrin (DM-β-CyD) is useful for selective preparation of a metastable polymorph of tolbutamide.[3] In this study, we investigated crystallization and polymorphic transition behavior of an oral hypoglycemic drug, chlorpropamide (CPM), in the absence and presence of cyclodextrins, and report that 2-hydroxybutyl-β-cyclodextrin (HB-β-CyD) significantly inhibits solution-mediated polymorphic transition of CPM in aqueous solutions, giving metastable polymorphs.

The crystallization of CPM in the absence and presence of CyDs in aqueous solution was conducted as follows: CPM was dissolved at 10 mM concentration in the absence and presence of CyDs in pH 8.0 sodium phosphate buffer (20 mL) at room temperature. The solution was slowly titrated with aqueous 0.5 M HCl (about 1 mL) to about pH 5 where CPM did not yet precipitate. The solution was paper filtered, and the filtrate was put in a refrigerator (about 4 °C) for 1 day. The precipitated CPM crystals were collected by filtration, and the content of different polymorphs was determined by powder X-ray diffraction, using the peak areas at $2\theta = 6.6$, 18.5, and 15.3 degrees for Forms A, II, and III, respectively.

Figure 1 shows powder X-ray diffraction patterns of crystals precipitated from aqueous CPM solutions in the absence and presence of CyDs, together with those of CPM polymorphs, Forms II, III, and A where Form A crystal (or Form IV) is the most stable form and Forms II and III are metastable forms. It is apparent that in the absence of CyDs, CPM crystallized into stable Form A, giving diffraction peaks typical for CPM Form A crystals at $2\theta = 6.6$, 11.7, and 19.5°. Stable Form A crystals of CPM were also obtained from solutions containing 5 mM α-, β-, and γ-CyDs and 2-hydoxypropyl-α- and -β-CyDs (HP-α- and -β-CyDs) and 35 mM glucose. In sharp contrast, a solution containing 5 mM HB-β-CyD yielded metastable Form II crystals, giving diffraction peaks typical for CPM Form II crystals at $2\theta = 9.2$, 11.0, 12.1, and 18.5°. In the case of lower concentration of HB-β-CyD (0.5 mM), Form III crystals of CPM were obtained, giving diffraction peaks at $2\theta = 7.4$, 12.3, and 15.3°. On the other hand, in the presence of 5 mM 2,6-di-*O*-methyl-β-CyD (DM-β-CyD) a mixture of stable Form A and metastable Form III crystals precipitated, giving a superimposed diffraction pattern of these metastable crystals.

To gain insight into the crystallization mechanism of the metastable form, the effect of HB-β-CyD concentration on the crystallization was investigated. Figure 2 shows polymorph contents in the precipitates obtained after 1 day-storage of CPM solutions containing various HB-β-CyD concentrations.

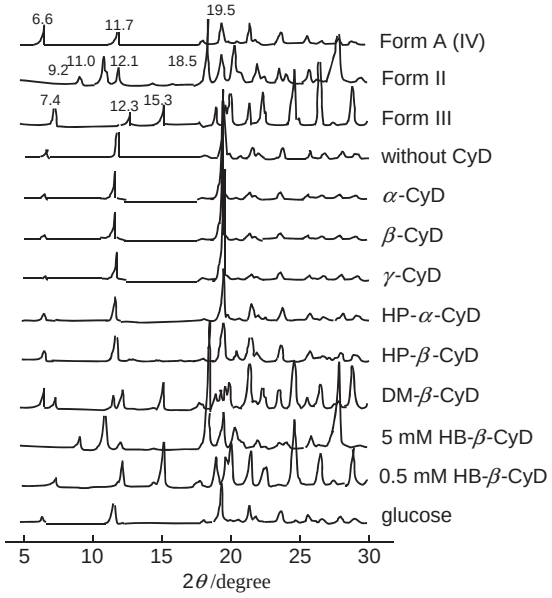


Figure 1. Powder X-ray diffraction patterns of crystals obtained from 10 mM CPM in phosphate buffer in the absence and presence of 5 mM CyDs (0.5 and 5 mM in the case of HB-β-CyD) or 35 mM glucose, stored at 4 °C for 1 day.

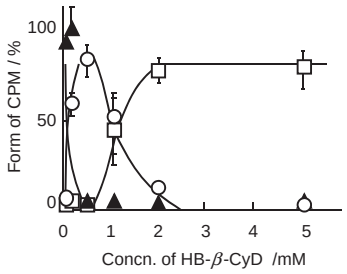
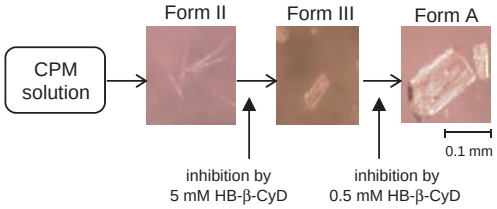


Figure 2. Effects of CyD concentrations on crystallization of CPM (10 mM) in the presence of HB-β-CyD in phosphate buffer, stored at 4 °C for 1 day. ▲: Form A, □: Form II, ○: Form III. Each point represents the mean ± SE of 3–4 experiments.



Scheme 1. Possible mechanism for the inhibition of the solution-mediated polymorphic transition of CPM in the presence of HB-β-CyD.

It is apparent that CPM precipitated in the stable Form A in the absence of HB-β-CyD. At lower concentrations of HB-β-CyD (0.5–1 mM), CPM precipitated in metastable Form III crystal (open circle in Figure 2), whereas in metastable Form II crystal (open square in Figure 2) at higher HB-β-CyD concentrations (>2 mM). In general, the solution-mediated polymorphic transition or crystallization proceeds according to "Ostwald's rule of stages[4]" i.e. the least stable crystal with the highest solubility[1c] appears first from solution, but this form is quickly transformed to a second crystal that is more stable than the former, because the solubility of the first crystal is higher than that of the second and the solution is supersaturated with respect to that of the second crystal. Therefore, the first metastable crystal with the least stability is consecutively transformed to the most stable crystal via several metastable intermediates. Our present results suggest that CPM is crystallized from solutions through metastable Forms II and III to the stable Form A, as shown in Scheme 1. The addition of higher HB-β-CyD concentrations (>2 mM) inhibits the transition of the least stable Form II crystal to Form III crystal, while the addition of lower HB-β-CyD concentrations (0.5–1 mM) can not inhibit the transition of the least stable form but inhibit the transition of the second metastable Form III to the stable Form A crystal. The content of Form II in the precipitates obtained in the presence of HB-β-CyD (>2 mM) was about 80–90%, and the remaining CPM may be in amorphous state. In the case of DM-β-CyD, both transitions may be appropriately inhibited, giving Form A and III crystals.

To gain insight into the transition mechanism, time courses of appearance and disappearance of Form II, III, and A crystals of CPM in the presence of 2 mM HB-β-CyD were investigated.

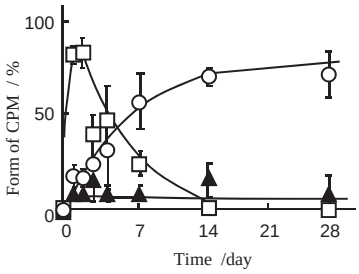


Figure 3. Time courses for appearance and disappearance of CPM polymorphs in the presence of HB-β-CyD (2 mM) in phosphate buffer at 4 °C. ▲: Form A, □: Form II, ○: Form III. Each point represents the mean ± SE of 3–8 experiments.

This CyD concentration was chosen because both Forms II and III appeared in the precipitates under the experimental conditions. As shown in Figure 3, CPM crystallized in Form II after 1 day storage, after which the content of Form II decreased with concomitantly increasing Form III. Form A crystals co-existed in the precipitates, although the content was only slight. These results indicate that CPM crystallizes to the stable Form A crystal consecutively through metastable Forms II and III, and HB-β-CyD inhibits the transition of Form II to Form III at higher concentrations and that of Form III to Form A at lower concentrations.

The inhibitory effect of HB-β-CyD may be ascribed to not only inclusion complex formation (stability constant, *K*c, of the complexes determined by the solubility method:[5] 163 ± 11 M^{-1} for β-CyD, 120 ± 7 M^{-1} for HP-β-CyD, 389 ± 15 M^{-1} for DM-β-CyD, and 182 ± 6 M^{-1} for HB-β-CyD), but also other factors such as interactions of CyDs with crystal surfaces, because the *K*c value of HB-β-CyD complex was lower than that of the DM-β-CyD complex.

In conclusion, HB-β-CyD may be useful as a tool for detection of metastable intermediates that occur during crystallization according to "Ostwald's rule of stages," and for selective isolation of metastable solid forms. Further investigation is now under way to elucidate the inhibition mechanism of HB-β-CyD.

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