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Characterization of itraconazole/2-hydroxypropyl-β-cyclodextrin inclusion complex in aqueous propylene glycol solution

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Abstract

The interaction of itraconazole, a triazole antifungal agent, with 2-hydroxypropyl-β-cyclodextrin (HP-β-CyD) in water and 10% v/v propylene glycol/water solution at pH 2.0 was investigated by the solubility method and ultraviolet and ^{1}H-nuclear magnetic resonance (NMR) spectroscopies. The solubility of itraconazole in water significantly increased as the concentrations of HP-β-CyD were augmented, showing an A_P type phase solubility diagram. The upward curvature closely corresponded to the simulation curve which was calculated on the basis of the 1:2 (guest:host) complexation model. The 1:2 complex was formed even in the presence of 10% v/v propylene glycol, although the co-solvent system made the interaction with HP-β-CyD weaker due to the competitive inclusion. The ultraviolet spectroscopic studies also supported the 1:2 complex formation of itraconazole with HP-β-CyD in 10% v/v propylene glycol/water solution at pH 2.0. The ^{1}H-NMR spectroscopic studies suggested that the triazole and triazolone moieties of itraconazole are involved in the 1:2 inclusion complexation.

Keywords: Itraconazole; 2-Hydroxypropyl-β-cyclodextrin; Inclusion complexation; Solubility; Stability constants; Stoichiometry

1. Introduction

Itraconazole is an orally active triazole antifungal agent to inhibit most human fungal pathogens (Heykants et al., 1989; Van Cutsem, 1989; Ne-

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groni and Arechavala, 1993). This drug is practically insoluble in water at physiological pH conditions and soluble only under extremely acidic media, leading to a poor oral bioavailability with large individual variations (Van Peer et al., 1989). Therefore, an oral liquid preparation of itraconazole has been developed recently using 2-hydroxypropyl-β-cyclodextrin (HP-β-CyD) and propylene glycol as the solubilizing agents in concentrations of 40% w/v and 10% v/v, respectively (Cartledge et al., 1997). This solution may have superior oral bioavailability characteristics compared with the oral capsules already on the market and enables a more patient adjusted dose (Hostetler et al., 1992; Van de Velde et al., 1996). The use of propylene glycol to increase the solubilizing and stabilizing effects of CyDs has been reported for other drugs (Aboutaleb et al., 1986, 1988; Vikmon and Szejtli, 1990; Lee et al., 1997). However, propylene glycol employed as a solubilizing agent may work as a competing agent and thus hinder the inclusion complex formation of the drug with HP-β-CyD in aqueous solution. Therefore, the present study deals with the inclusion complexation of itraconazole with HP-β-CyD mainly in 10% v/v propylene glycol/water solution, in order to confirm the complexation in the co-solvent system and to gain insight into the mode of inclusion and the solubilization mechanism of the drug.

2. Materials and methods

2.1. Materials

Itraconazole and HP-β-CyD (degree of substitution: 4.41) were supplied from Janssen-Kyowa (Tokyo, Japan). Propylene glycol (analytical grade) was purchased from Nacalai tesque (Kyoto, Japan). Other chemicals and solvents were of analytical reagent grade, and deionized double-distilled water was used throughout the study.

2.2. Solubility studies

The solubility method was employed according to the method of Higuchi and Connors (1965).

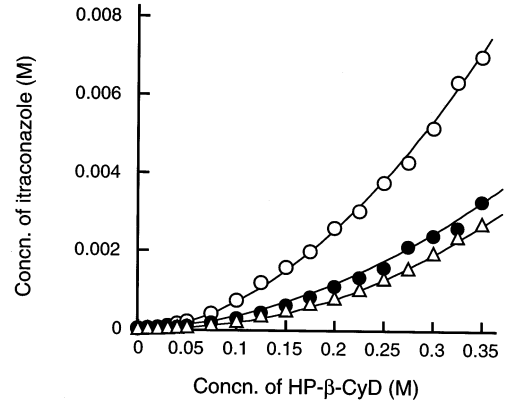

Fig. 1. Phase solubility diagrams of itraconazole/HP-β-CyD systems in acidic solution (pH 2.0) at 25°C. ○, without additives; ●, with 10% v/v propylene glycol; △, with all additives.

The excess amount (20 mg) of itraconazole was added in the screw capped vials containing HP-β-CyD solutions (1.0 ml) at various concentrations (1.0×10^{-2}–3.5×10^{-1} M) and 10% v/v propylene glycol. pH of the solutions was adjusted to 1.5, 2.0 and 2.5 by adding concentrated hydrochloric acid. The vials were shaken at 15, 25 and 37°C. After equilibrium was attained (about 14 days), the solution was filtered through a membrane filter (cellulose acetate membrane filter, ADVANTEC DISMIC 3CP045 (Toyo-Roshi), Tokyo, Japan) and analyzed for itraconazole us-

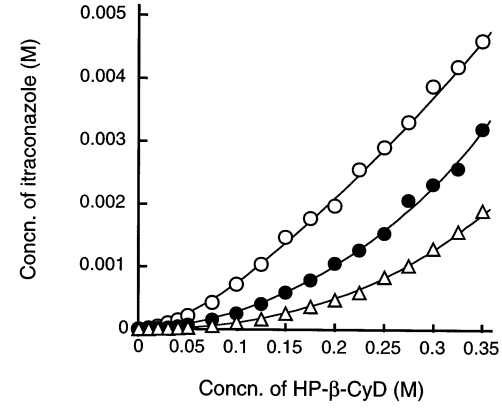

Fig. 2. Phase solubility diagrams of itraconazole/HP-β-CyD systems in acidic solutions containing 10% v/v propylene glycol at 25°C. ○, pH 1.5; ●, pH 2.0; △, pH 2.5.

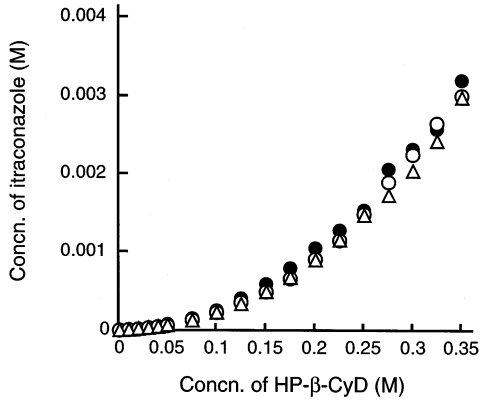

Fig. 3. Phase solubility diagrams of itraconazole/HP-β-CyD systems in acidic solution (pH 2.0) containing 10% v/v propylene glycol at various temperatures. ○, at 15°C; ●, 25°C; △, 37°C.

ing high-performance liquid chromatography (HPLC) under the following conditions: a Hitachi L6000 pump and a L-4000 UV detector at 254 nm; a GL-Science Hypersil ODS-5 column (4.6 mm i.d. × 150 mm, Kyoto, Japan); a mobile phase of acetonitrile/water (56:44 v/v) containing 0.02% diethylamine; a flow rate of 1.0 ml/min. The pH change before and after the equilibrium was within 0.1.

2.3. Spectroscopic studies

Ultraviolet (UV) spectra were recorded with a Hitachi U-2000A spectrophotometer at 25°C. The concentration of itraconazole was 1.4×10^{-5} M and that of HP-β-CyD was changed from 1.0×10^{-3} to 2.0×10^{-1} M in the acidic solution (pH 2.0) containing 10% v/v propylene glycol. The pH of the solution was adjusted using concentrated hydrochloric acid. ^{1}H-nuclear magnetic resonance (NMR) spectra were obtained with a JEOL JNM-α 500 instrument (Tokyo, Japan) with a 5 mm inverse broad band probe, operating at 500 MHz and a sweep width of 10 000 Hz, at 25°C. Chemical shifts are given as parts per million (ppm) downfield from that of an external standard, tetramethylsilane, with an accuracy of 0.005 ppm. The two-dimensional nuclear Overhauser effect (NOESY) spectra were measured under the following conditions: sweep width, 5000 Hz; carrier frequency, 2.95 ppm; spin-lock field, 4 kHz; mixing time, 200 ms; 32 scans for each t1 point with a pulse delay of 1.5 see; data matrix, 2 × 256 × 1K. Itraconazole (5.0×10^{-3} or 1.0×10^{-2} M) and HP-β-CyD (1.0×10^{-2}, 2.0×10^{-2} or 5.0×10^{-2} M) were dissolved in deuterated dimethyl sulfoxide (DMSO-d_6), because of the extremely low solubility in water (9.94×10^{-7} M in 10% v/v propylene glycol/water).

3. Results and discussion

3.1. Solubility studies

The solubility method is useful for investigating inclusion complexation of poorly water-soluble drugs with CyDs in water, because it gives not only the solubilizing ability of host molecules but also the stability constant of the complexes by analyzing the solubility curve (Higuchi and Kristiansen, 1970). Fig. 1 shows the phase solubility diagrams of itraconazole with HP-β-CyD in the absence and presence of 10% v/v propylene glycol in pH 2.0 solution at 25°C. Furthermore, the solubility study of the itraconazole/HP-β-CyD system was conducted in the presence of various ingredients such as sodium saccharin (0.6 mg), sorbitol (70% solution, 0.19 ml), cherry flavors 1 and 2 (0.25 and 0.5 mg, respectively) and caramel (0.2 mg), which are formulated in the itraconazole oral solution (1.0 ml, Okamoto et al., 1998). The pH of 2.0 was chosen because itraconazole is more soluble in acidic solutions (pK_a of the nitrogen atom of the triazolone moiety is 4.0). The solubility of itraconazole increased with a rise in the HP-β-CyD concentrations, showing a positive deviation from linearity. The solubility enhancement by the complexation with HP-β-CyD was decreased by the addition of 10% v/v propylene glycol, and the solubility curve was shifted to higher CyD concentrations. This may be ascribed to the decrease in the effective CyD concentration necessary for the complexation with itraconazole, due to the competitive inclusion of propylene glycol. The addition of the ingredients had insignificant effect on the solubilization. These solubility curves can be classified as type A_P, as

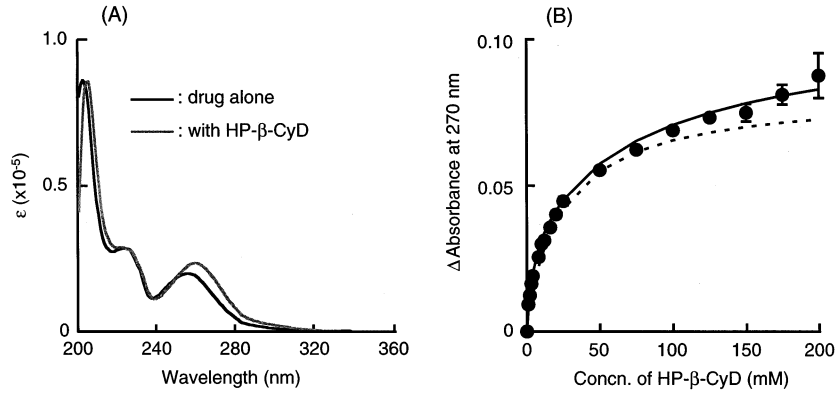

Fig. 4. (A) Effect of HP-β-CyD (0.2 M) on UV spectrum of itraconazole (1.4×10^{-5} M) in acidic solution (pH 2.0) containing 10% v/v propylene glycol at 25°C. (B) Change in UV absorption at 270 nm of itraconazole (1.4×10^{-5} M) as a function of HP-β-CyD concentration. The dotted and solid lines are the theoretical curves calculated according to the 1:1 and 1:2 complexation models, respectively.

defined by Higuchi and Connors (1965), suggesting the formation of higher-order complexes Therefore, the upward curvatures were quantitatively analyzed according to the optimization technique to obtain the stability constants of higher order complexes ($K_{1:n}$) (Higuchi and Kristiansen, 1970).

$$[S]_t = S_o + K_{1:1} \cdot S_0 \cdot [CyD] + K_{1:1} \cdot K_{1:2} \cdot S_0 \cdot [CyD]^2 + K_{1:1} \cdot K_{1:2} \cdot K_{1:3} \cdot S_0 \cdot [CyD]^3 + \cdots + K_{1:1} \cdot K_{1:2} \cdot K_{1:3} \cdots K_{1:(n-1)} \cdot K_{1:n} \cdot S_0 \cdot [CyD]^n \quad (1)$$

$$[CyD]_t = [CyD] + K_{1:1} \cdot S_0 \cdot [CyD] + 2 \cdot K_{1:1} \cdot K_{1:2} \cdot S_0 \cdot [CyD]^2 + 3 \cdot K_{1:1} \cdot K_{1:2} \cdot K_{1:3} \cdot S_0 \cdot [CyD]^3 + \cdots + n \cdot K_{1:1} \cdot K_{1:2} \cdot K_{1:3} \cdots K_{1:(n-1)} \cdot K_{1:n} \cdot S_0 \cdot [CyD]^n \quad (2)$$

where $K_{1:1}$, $K_{1:2}$, $K_{1:3}$,…$K_{1:n}$ are the stability constants of complexes with the stoichiometry of 1:1, 1:2, 1:3, …, 1:n (guest:host), respectively. S_0 and $[S]_t$ represent the solubility and the total concentration of the drug, respectively. [CyD] and $[CyD]_t$ stand for the free and total concentrations of HP-β-CyD, respectively. By setting $[CyD] = [CyD]_t$ as a first approximation, Eq. (1) was analyzed by a nonlinear least-squares method to obtain each apparent stability constant. [CyD] values were then calculated from Eq. (2) using the apparent stability constants. This procedure was repeated until each stability constant converged on a constant value. The fitness between the observed and calculated solubility profiles was judged from Akaike's information criterion (Yamaoka et al., 1981). This analysis of the solubility curves (Fig. 1) was carried out in every case of 1:2, 1:3 and 1:4 guest/host molar ratios to gain insight into the stoichiometry of the itraconazole/HP-β-CyD complex, and gave almost the same Akaike's indices after the optimization: −120, −122 and −121 for the 1:2, 1:3 and 1:4 stoichiometries, respectively, without propylene glycol and −157, −159 and −158 for those with propylene glycol. Therefore, it was difficult to determine the stoichiometry by a simple comparison of the Akaike's index. However, the analysis of the 1:3 and 1:4 inclusion models gave negative values of the 1:3 and 1:4 stability constants. These results suggested that itraconazole forms preferentially the 1:1 and 1:2 complexes with HP-β-CyD under experimental conditions. Therefore, the solubility curves were analyzed according to the 1:2 model ($n = 2$ in Eqs. (1) and (2)) and the interaction parameters obtained were as follows: $S_0 = 4.24 \times 10^{-7}$ M, $K_{1:1} = 2050$ (± 270) M^{-1} and $K_{1:2} = 60$ (± 10) M^{-1} for the system without propylene glycol and any additives, $S_0 = 9.94 \times 10^{-7}$ M, $K_{1:1} = 120$ (± 30) M^{-1} and $K_{1:2} = 240$ (± 70) M^{-1} for the system with 10% v/v propy-

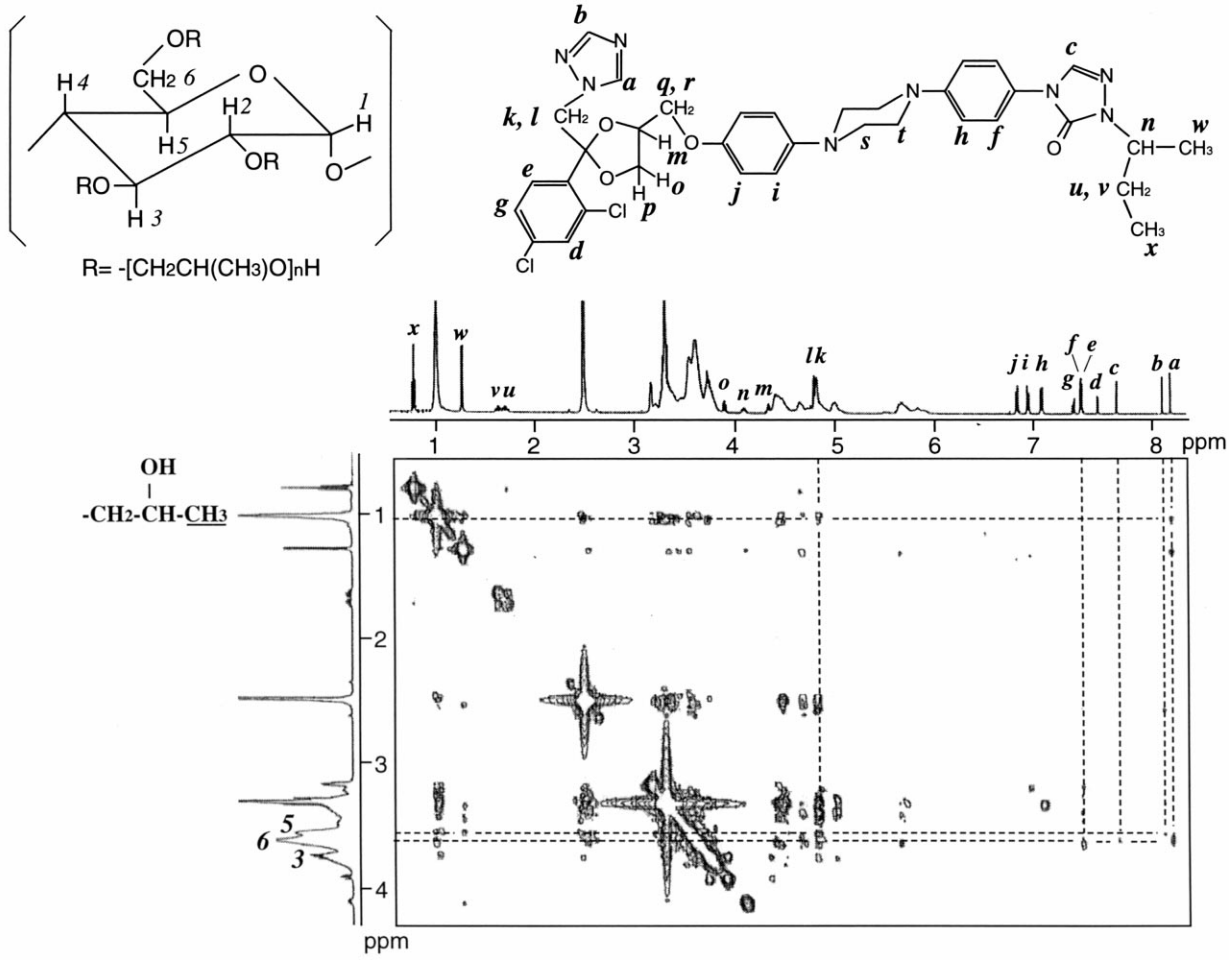

Fig. 5. Partial contour plot of NOESY spectrum of itraconazole (1.0×10^{-2} M) in the presence of HP-β-CyD (1.0×10^{-2} M) in DMSO-d_6 at 25°C.

lene glycol, and $S_0 = 1.81 \times 10^{-6}$ M, $K_{1:1} = 180$ (± 10) M^{-1} and $K_{1:2} = 60$ (± 20) M^{-1} for the system with 10% v/v propylene glycol and the ingredients described above. The addition of 10% v/v propylene glycol increased about 2.3-fold the intrinsic solubility (4.24×10^{-7} M at pH 2.0 and 25°C) of itraconazole and the ingredients increased further 2-fold the solubility, probably because of the decrease in polarity of the solvent. The $K_{1:1}$ value (2050 M^{-1}) in the absence of propylene glycol was larger than the $K_{1:2}$ value (60 M^{-1}), indicating that the 1:1 complex is more stable than the 1:2 complex. By the addition of propylene glycol, the former constant was significantly decreased, whereas the latter constant was increased, although propylene glycol made the apparent total binding ($K_{1:1} \times K_{1:2}$) weaker (1.23×10^5 M^{-2} without the solvent to 2.88×10^4 M^{-2} with the solvent). These results suggest that propylene glycol facilitates the access of the second host molecule to the guest molecule, due to alteration of hydration around the second binding site. Fig. 2 shows the phase solubility diagrams of itraconazole/HP-β-CyD system at pH 1.5, 2.0 and 2.5. The interaction parameters obtained were as follows: $S_0 = 3.46 \times 10^{-6}$ M, $K_{1:1} = 670$ (± 30) M^{-1} and $K_{1:2} = 20$ (± 4) M^{-1} at pH 1.5, $S_0 = 9.94 \times 10^{-7}$ M, $K_{1:1} = 120$ (± 30) M^{-1} and

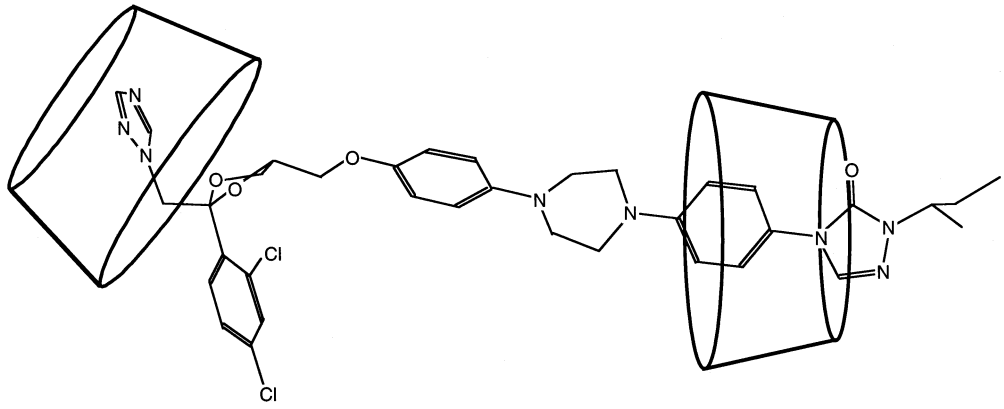

Fig. 6. Proposed inclusion mode of itraconazole/HP-β-CyD complex in 1:2 molar ratio.

$K_{1:2} = 240$ (± 70) M^{-1} at pH 2.0, $S_0 = 2.48 \times 10^{-7}$ M, $K_{1:1} = 140$ (± 30) M^{-1} and $K_{1:2} = 330$ (± 90) M^{-1} at pH 2.5. The intrinsic solubility of itraconazole decreased with a rise in pH of the solution, owing to the decrease in concentration of the ionized moiety of the drug (pK_a 4.0). The apparent total binding constant increased with a rise in pH of the solution, because the unionized moiety is preferably included in the CyD cavity. With a rise in pH, the $K_{1:1}$ value decreased while the $K_{1:2}$ value increased, suggesting that the second host molecule can readily access after the deprotonation of the cationized moiety (the triazolone) of itraconazole. Fig. 3 shows the phase solubility diagrams of itraconazole/HP-β-CyD system at temperatures of 15, 25 and 37°C. The interaction parameters obtained were as follows: $S_0 = 5.36 \times 10^{-7}$ M, $K_{1:1} = 140$ (± 20) M^{-1} and $K_{1:2} = 320$ (± 40) M^{-1} at 15°C, $S_0 = 9.94 \times 10^{-7}$ M, $K_{1:1} = 120$ (± 30) M^{-1} and $K_{1:2} = 240$ (± 70) M^{-1} at 25°C, and $S_0 = 1.13 \times 10^{-6}$ M, $K_{1:1} = 110$ (± 10) M^{-1} and $K_{1:2} = 190$ (± 20) M^{-1} at 37°C. With a rise of temperature, the intrinsic solubility of the drug increased while the stability constants decreased, as is usual with the dissolution and CyD complexation of organic guest molecules. The effect of temperature on the solubilization seemed to be negligible under the experimental conditions. Unfortunately, the thermodynamic parameters for the complexation could not be accurately determined, because of the relatively large standard deviations of the stability constants.

3.2. Spectroscopic studies

Fig. 4A shows the UV spectra of itraconazole in the absence and presence of HP-β-CyD in the acidic solution (pH 2.0) containing 10% v/v propylene glycol. By the addition of HP-β-CyD, the absorption maximum at 260 nm was shifted to a longer wavelength with a concomitant increase in the molar absorption coefficient. Similar spectral changes were observed when the drug was dissolved in less polar solvents such as ethanol, suggesting that the drug chromophore is incorporated in the hydrophobic environment of the HP-β-CyD cavity (Bender and Komiyama, 1978). Fig. 4B shows the change of the absorbance of itraconazole at 270 nm as a function of HP-β-CyD concentration under the above condition. These spectral changes were analyzed according to Eqs. (3) and (4) for the 1:1 and 1:2 complexation models, respectively, using a nonlinear least-squares method.

$$\mathrm{Abs} = \frac{(\varepsilon_0 + \varepsilon_1 \cdot K_{1:1} \cdot [\mathrm{CyD}]) \cdot [\mathrm{ITZ}]_t}{1 + K_{1:1} \cdot [\mathrm{CyD}]} \tag{3}$$

$$\mathrm{Abs} = \frac{(\varepsilon_0 + \varepsilon_1 \cdot K_{1:1} \cdot [\mathrm{CyD}] + \varepsilon_2 \cdot K_{1:1} \cdot K_{1:2} \cdot [\mathrm{CyD}]^2) \cdot [\mathrm{ITZ}]_t}{\times 1 + K_{1:1} \cdot [\mathrm{CyD}] + K_{1:1} \cdot K_{1:2} \cdot [\mathrm{CyD}]^2} \tag{4}$$

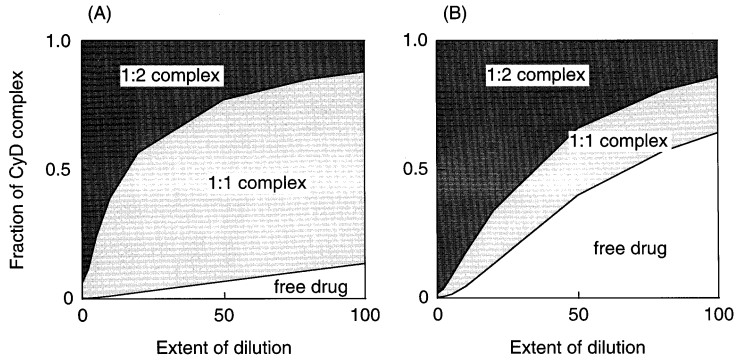

Fig. 7. Changes in fractions of free itraconazole and its 1:1 and 1:2 complexes by dilution of itraconazole:HP-β-CyD solution without (A) and with (B) 10% v/v propylene glycol. The initial concentrations of the drug and HP-β-CyD were assumed to be 1.42×10^{-2} and 2.88×10^{-1} M, respectively, and the fraction of each species was calculated using the stability constants (2050 and 60 M^{-1} of the 1:1 and 1:2 complexes for without 10% v/v propylene glycol system and 120 and 240 M^{-1} of the 1:1 and 1:2 complexes for with 10% v/v propylene glycol system, respectively) obtained by the solubility method at pH 2.0 and 25°C.

where Abs symbolizes the absorbance of the drug at 270 nm in the presence of HP-β-CyD, ε_0, ε_1 and ε_2 are molar extinction coefficients of the drug alone and the drug included in the 1:1 and 1:2 complexes, respectively, and $[ITZ]_t$ stands for the total concentration of itraconazole. The spectral changes fitted in the 1:2 model rather than the 1:1 model (Akaike's index: -98 and -114 for the 1:1 and 1:2 models, respectively), indicating once again that itraconazole forms the 1:2 inclusion complex with HP-β-CyD under experimental conditions. The interaction parameters obtained were as follows: $\varepsilon_0 = 12\,270$ (± 70) cm^{-1} M^{-1}, $\varepsilon_1 = 14\,700$ (± 640) cm^{-1} M^{-1} and $\varepsilon_2 = 19\,580$ (± 820) cm^{-1} M^{-1}, $K_{1:1} = 245$ (± 80) M^{-1} and $K_{1:2} = 10$ (± 5) M^{-1}. The $K_{1:1}$ value was almost comparable whereas the $K_{1:2}$ value was smaller than those determined by the solubility method. This discrepancy of the $K_{1:2}$ value may be due to the difference in the experimental conditions such as concentration ranges of the host and guest molecules.

^{1}H-NMR spectroscopic studies were carried out to gain insight into the inclusion mode of itraconazole/HP-β-CyD complex. Since the solubility of itraconazole was too low to take high-quality NMR spectra even in acidic solutions containing propylene glycol, the drug and HP-β-CyD were dissolved in DMSO-d_6. The essential feature of the interaction of itraconazole with HP-β-CyD in DMSO may be similar to that in water, because the relative magnitude of dielectric constants of the solvents ($\varepsilon = 46.8$ and 80, respectively) against the hydrophobic guest molecule is not significantly different between DMSO and water (Jyothirmayi et al., 1991; Matsui et al., 1994; Gafni and Cohen, 1997). Upon addition of HP-β-CyD, the proton signals of itraconazole shifted to upfield, among which the shift of the b proton (see Fig. 5) of the triazole ring and the f and h protons of the benzene ring was larger than that of other protons (8.31, 7.49 and 7.10 ppm for the b, f and h protons) (data not shown). Fig. 5 shows a two-dimensional NOESY spectrum of itraconazole/HP-β-CyD system. The cross-peaks were observed between the a, b, l and k protons of the drug and the methyl proton of 2-hydroxypropyl group of HP-β-CyD and between the a, b, c, and f protons of the drug and the protons of the glucose skeleton of HP-β-CyD. These cross-peaks were not observed for the drug alone. These results, together with those of the solubility and UV spectroscopic studies, indicate that the triazole and triazolone rings of the drug should be involved in the complexation, which is initiated in the inclusion of the triazole ring into HP-β-CyD cavity preferentially and the second HP-β-CyD may include the triazolone ring and its neighboring benzene, as shown in Fig. 6.

The aforementioned results apparently indicate that itraconazole forms the inclusion complex with HP-β-CyD in a 1:2 stoichiometry both in water and 10% v/v propylene glycol/water solution. The addition of propylene glycol made the inclusion strength of HP-β-CyD weaker, because it works as a competing agent.

After oral administration, the primary driving force for dissociation of the complex is supposed to be simple dilution, although other factors such as competitive displacement of the drug by biological components and pH of gastrointestinal tracts may contribute (Uekama et al., 1994; Stella and Rajewski, 1997). Then, the effect of dilution on the fraction of drug bound to HP-β-CyD was analyzed in the absence and presence of propylene glycol. Fig. 7 shows changes in the fraction of free itraconazole and its HP-β-CyD complexes by dilution, which was theoretically calculated using the $K_{1:1}$ and $K_{1:2}$ values determined by the solubility method under the initial concentrations of the drug and HP-β-CyD of 1.42×10^{-2} M and 2.88×10^{-1} M, respectively. In the absence of any competing agents, the 1:2 complex exists exclusively at the initial stage, whereas after the 100-fold dilution the 1:1 complex is the main species followed by the 1:2 complex and a minute fraction of the free form. In the presence of 10% v/v propylene glycol, a 100-fold dilution provided more than 50% of free itraconazole, followed by the 1:1 complex and the 1:2 complex. These simulations apparently suggest that after dilution the co-solvent system gives a larger free fraction of drug which is favorable for intestinal absorption. However, such dilution may cause the drug to precipitate, due to the dissociation of the CyD complex. Therefore, attention should be given not only to the change in the fraction of free drug but also to the precipitation in gastrointestinal fluids, in the formulation of the drug whose intrinsic solubility is lower than the dilution concentration line at a given HP-β-CyD concentration (Fig. 7). Knowledge of this kind will be particularly useful for design and evaluation of the CyD-based pharmaceutical formulations.

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