The effects of storage on the crystallization, dissolution and absorption of tolbutamide from amorphous tolbutamide–2-hydroxypropyl-β-cyclodextrin (HP-β-CyD) complex were investigated, in comparison with those of polyvinylpyrrolidone (PVP) solid dispersion. The amorphous solid complex of tolbutamide with HP-β-CyD and the solid dispersion of tolbutamide with PVP were prepared by a spray-drying method. During storage, a stable form of tolbutamide (form I) was crystallized from the amorphous PVP dispersion, whereas a metastable form of tolbutamide (form II) was crystallized from the HP-β-CyD complex. The dissolution rate of tolbutamide from both HP-β-CyD complex and PVP dispersion was significantly faster than that of tolbutamide alone. However, the dissolution rate from the PVP dispersion markedly decreased with storage, because of the formation of slow dissolving form I crystals. On the other hand, the dissolution rate from the HP-β-CyD complex was only slightly decreased due to the formation of fast dissolving form II crystals. These in vitro dissolution characteristics were clearly reflected in the in vivo absorption of tolbutamide and the glucose plasma level after oral administration in dogs. The results suggested that HP-β-CyD is useful not only for converting crystalline tolbutamide to an amorphous substance, but also for maintaining the fast dissolution rate of the drug over a long period. Furthermore, the crystallization of drugs from CyD complexes, with storage, seemed to be different from that involving polymer excipients such as PVP.

Key words tolbutamide; 2-hydroxypropyl-β-cyclodextrin; amorphous complex; crystallization; dissolution; absorption

Crystal modifications significantly affect various pharmaceutical properties such as the solubility, dissolution rate, stability and bioavailability of drugs.1–3) As a consequence, the rational control of crystal growth, habit and polymorphic transition, using pharmaceutical additives, becomes an attractive and intriguing area of drug research and development. Cyclodextrins (CyDs), cyclic oligosaccharides consisting of 6–8 glucose units linked through α-1,4 glycosidic bonds, form inclusion complexes with various drug molecules in solution and in solid state and have been utilized successfully to improve certain properties such as the solubility, stability and bioavailability of drugs.4–6) Many reports have shown that crystalline drugs can be converted to an amorphous form by complexation with amorphous CyDs such as 2-hydroxypropyl-β-CyD (HP-β-CyD), and their aqueous solubility and dissolution rates thus markedly increased.7–9) However, the effects of storage on pharmaceutical properties of amorphous CyD complexes, such as crystallization, dissolution and absorption behavior, etc., have not fully been elucidated.10) In the present study, we studied the effects of aging on the crystallization, dissolution and absorption of the HP-β-CyD complex with an oral hypoglycemic agent, tolbutamide (Fig. 1), in comparison with those of the solid dispersion of tolbutamide with polyvinylpyrrolidone, because HP-β-CyD can convert crystalline drugs such as nifedipine to amorphous solids.11,12) Tolbutamide was chosen because it has several polymorphic forms, such as a stable form (form I) and metastable forms (forms II, III, IV).13–16)

Fig. 1. Structure of Tolbutamide

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plex in a molar ratio of 1:1 (weight ratio of 1:5.2) and the solid tolbutamide–PVP dispersion in a weight ratio of 1:5.2 were obtained by dissolving the components in a mixed solvent of ethanol–dichloromethane, followed by spray-drying under the above condition.

**Aging Studies** The test powder (3–5 g < 100 mesh) was placed in glass containers in desiccators at a constant humidity of 75% R.H. (solution saturated with sodium chloride),20–21 then stored in incubators at constant temperatures (40–70 °C). At appropriate time intervals, samples were withdrawn, dried under reduced pressure for about 1 d at room temperature, and subjected to powder X-ray diffractometry and DSC analysis. The content of tolbutamide polymorphs (form I and form II) in matrices was calculated from areas of the diffraction peaks at 2θ = 19.9° and 10.3° characteristic of the former and the latter crystals, respectively. The standard calibration curve was made by mixing the polymorphs and HP-β-CyD or PVP (<100 mesh) in appropriate ratios and measuring the areas of above diffraction peaks (minimum content for the detection of forms I and II was about 3.0%).

**Dissolution Studies** The dissolution rate was measured according to the dispersed amount method.19) A fixed amount (tolbutamide 100 mg < 100 mesh) of test powders was put into 25 ml of Japanese Pharmacopoeia XIII (JP XIII) second fluid (pH 6.8) and stirred at 91 rpm at 37 °C. At appropriate intervals, an aliquot (1.0 ml) was withdrawn with a cotton-plugged pipette and spectrophotometrically analyzed for tolbutamide at 230 nm. A constant volume of dissolution medium was maintained by adding an equal volume of the original medium.

**Absorption Studies** In vivo absorption studies were carried out using male beagle dogs (9–11 kg) that were fasted for 24 h before drug administration. The sample (equivalent to 100 mg tolbutamide/body) was wafer wrapped and administered orally with water (50 ml), using a catheter. Blood samples (1 ml) were withdrawn from the cephalic vein using a heparinized injection syringe and centrifuged at 1000 × g for 10 min. The plasma (0.2 ml) was added to the acetonitrile solution of an internal standard, chlorpropane (1.0 mg/ml, 0.5 ml), and extracted with ethyl ether (4.0 ml). The organic phase (3.0 ml) was evaporated, the residue was dissolved in acetonitrile–0.05 m NaH₂PO₄ solution (45:55 v/v), a flow rate of 1.6 ml/min, and detection at 230 nm. Plasma levels of glucose were determined using a glucose measuring kit (Glucose CII Test Wako, Wako Pure Chemical, Ltd., Osaka, Japan), i.e., the plasma (0.02 ml) was mixed with the kit solution (3.0 ml) and incubated at 37 °C for 5 min and the UV absorption was measured at 595 nm.

**Results and Discussion**

**Crystallization of Tolbutamide during Storage** Tolbutamide is known to form a 1:2 complex with β-CyDs in aqueous solution. However, our previous study using nuclear magnetic resonance spectroscopy indicated that tolbutamide predominantly forms a 1:1 inclusion complex with HP-β-CyD in aqueous solution and in the solid state, and the tolue ne moiety is preferentially included in the cavity (stability constants of the 1:1 and 1:2 complexes: 225 and 25 M⁻¹, respectively). Therefore, the complex was prepared by spray-drying the drug and the host in a molar ratio of 1:1 (weight ratio of 1:5.2). The solid dispersion of tolbutamide with PVP was prepared in a weight ratio of 1:5.2. Figure 2 shows changes in the powder X-ray diffraction pattern of tolbutamide–HP-β-CyD complex and tolbutamide–PVP solid dispersion during storage at 60 °C, 75% R.H. The complex and the PVP dispersion gave a halo-pattern in the powder X-ray diffractogram, indicating that they are in an amorphous state. On the storage of the complex and the PVP dispersion, X-ray diffraction peaks appeared due to tolbutamide crystals formed in HP-β-CyD and PVP amorphous matrices. The HP-β-CyD and PVP matrices spray-dried without tolbutamide showed no diffraction peaks under the present storage conditions. The PVP dispersion gave diffraction peaks characteristic of a stable crystal form (form I) of tolbutamide at 2θ = 8.7°, 12.1°, 12.9°, 14.1°, 15.3°, 17.1°, and 19.9°. On the other hand, the HP-β-CyD complex gave diffraction peaks at 10.3°, 11.3°, 15.5°, 19.6° and 21.3°, characteristic of form II, and no form I peaks were observed for at least one month. On further storage of the HP-β-CyD complex, the form II diffraction peaks disappeared and the diffraction pattern changed to that of form I. The crystallization rate of tolbutamide from the complex and the PVP dispersion was measured by monitoring the change in peak areas at 19.9° and 10.3°, characteristic of form I and form II, respectively, and the results are shown in Fig. 3. In the PVP matrix, tolbutamide crystallized exclusively to form I, and the crystallization was completed within about 2 d under the experimental conditions. In sharp contrast, the HP-β-CyD complex gave predominantly form II crystals which were stable in the matrix and began to convert to form I crystals from about 2 months after the storage. The crystallization of tolbutamide to form II in the HP-β-CyD matrix was about 75% of the original amount of the drug, and the remaining tolbutamide (about 25%) may exist as an amorphous complex. The crystallization-time profiles of tolbutamide in these matrices were analyzed according to the Hancock and Sharp equation.

![Fig. 2. Changes in Powder X-Ray Diffraction Patterns of HP-β-CyD Complex (A) and PVP Dispersion (B) during Storage at 60 °C and 75% R.H. and Powder X-Ray Diffraction Patterns of Forms I and II of Tolbutamide (C)](image-url)
rate-determining step for the crystallization of form II in the using data of total sample crystallized to tolbutamide, and Storage at 60 °C and 75% R.H. On the other hand, the PVP system gave an and 6.64 (6) were linear, and crystallization rate constants (k) were about 7.1 cp and 1.3 cp, respectively. Therefore, the crystallization of tolbutamide from the HP-β-CyD matrix, (B) to Form I in PVP matrix. (©), 40 °C; (●), 45 °C; (△), 50 °C; (▲), 55 °C; (◇), 60 °C; (▼), 70 °C. Each point represents the mean±S.E. of 2—3 experiments.

FIG. 3. Time Courses for Crystallization of Tolbutamide Polymorphs (Form I (©) and Form II (▼)) in HP-β-CyD (A) and PVP (B) Matrices during Storage at 60 °C and 75% R.H.

Each point represents the mean±S.E. of 2—3 experiments.

\[
\text{ln}[-\ln(1-\alpha)] = m \ln t + \ln B
\]  

(1)

where \( m \) is the intrinsic value for various theoretical equations of solid-state decomposition, \( \alpha \) is the fraction of the total sample crystallized to tolbutamide, \( t \) is the storage time, and \( B \) is a constant. The plot of \( \text{ln}[-\ln(1-\alpha)] \) versus \( \text{ln} t \) using data of \( m=0.99 \pm 0.03 \) (mean±standard error (S.E.), \( n=3 \), correlation coefficient \( r=0.99 \)) for the crystallization to form II in the HP-β-CyD matrix at 60 °C and 75% R.H. The \( m \) value of 0.99 indicates that a random nucleation on each particle is a rate-determining step for the crystallization of form II in the HP-β-CyD matrix (a criterion for this conversion is \( m=1.0 \)),\(^{21}\) and the rate obeys the equation \( -\ln(1-\alpha)=kt \). On the other hand, the PVP system gave an \( m \) value of 0.63±0.02 (\( n=3 \), \( r=0.99 \)). The \( m \) value of 0.63 for the PVP system indicates a one-dimensional diffusion-controlled crystallization (a criterion for this conversion is 0.62),\(^{21}\) and its rate obeys \( \alpha^t=kt \). Therefore, the crystallization rates of tolbutamide from the HP-β-CyD complex and the PVP dispersion were analyzed, respectively, according to the aforementioned equations, and the plots of results at various temperatures are shown in Fig. 4. The plots of \( -\ln(1-\alpha) \) or \( \alpha^t \) versus \( t \) were linear, and crystallization rate constants (\( k \)) were obtained from the slopes: crystallization of form II from the HP-β-CyD complex, \( k=1.28 \pm 0.06 \times 10^{-2} \text{h}^{-1} \) (40 °C), 1.81 \((\pm 0.04)\times 10^{-2} \text{h}^{-1} \) (45 °C), 2.55 \((\pm 0.03)\times 10^{-2} \text{h}^{-1} \) (50 °C), 3.30 \((\pm 0.04)\times 10^{-2} \text{h}^{-1} \) (55 °C), 6.62 \((\pm 0.01)\times 10^{-2} \text{h}^{-1} \) (60 °C), and 1.19 \((\pm 0.02)\times 10^{-1} \text{h}^{-1} \) (70 °C), and crystallization of form I from the PVP dispersion, \( k=5.14 \pm 0.02 \times 10^{-3} \text{h}^{-1} \) (40 °C), 6.32 \((\pm 0.02)\times 10^{-3} \text{h}^{-1} \) (45 °C), 8.09 \((\pm 0.02)\times 10^{-3} \text{h}^{-1} \) (50 °C), 1.32 \((\pm 0.03)\times 10^{-2} \text{h}^{-1} \) (55 °C), 2.49 \((\pm 0.03)\times 10^{-2} \text{h}^{-1} \) (60 °C), and 6.64 \((\pm 0.05)\times 10^{-2} \text{h}^{-1} \) (70 °C). The Arrhenius plots of these rate constants gave straight lines (\( r=0.999 \)), from which the activation parameters of 68 \((\pm 1)\) and 78 \((\pm 2)\) kJ/mol were obtained for the crystallization of form II from the HP-β-CyD complex and form I from the PVP dispersion, respectively. The diffusion-controlled crystallization of tolbutamide in the PVP matrix may be attributable at least partly to the high water absorption property of the excipient, because its water content and viscosity were much higher than those of HP-β-CyD, i.e., the water content of PVP and HP-β-CyD at equilibrium (stored at 60 °C, 75% R.H for 1 d) was about 15.2% and 3.4%, respectively, and the viscosities of the former and latter excipients (15% solution, for example) were about 7.1 cp and 1.3 cp, respectively. Therefore, stable form I of tolbutamide may crystallize from viscous PVP solution after dissolution in adsorbed water. Similar diffusion-controlled crystallization in a PVP matrix was observed for nifedipine, as reported previously.\(^{12}\) On the other hand, the crystallization of tolbutamide form II from the HP-β-CyD complex cannot be ascribed merely to such environmental factors. Although further studies should be done in order to elucidate its crystallizing mechanism, the inclusion effect of the host molecule seems to be operative in the form II crystallization, because the same phenomenon was also observed in the crystallization from aqueous HP-β-CyD solutions (data not shown here). The detailed crystallizing mechanism of tolbutamide from CyD complexes will be reported elsewhere.

**Dissolution and Absorption Behavior** The different crystallization of tolbutamide from the HP-β-CyD complex and the PVP dispersion on storage will affect the dissolution of tolbutamide. Figure 5 shows dissolution profiles of tolbutamide–HP-β-CyD complex and tolbutamide–PVP solid dispersion immediately after the preparation, in addition to profiles of samples stored for one week at 60 °C, 75% R.H. The
dissolution rates of these amorphous complexes and PVP dispersion were fast, and there was insignificant difference in the dissolution rate between them. With storage, however, the dissolution rate from the PVP dispersion significantly decreased, whereas that of the HP-β-CyD complex only slightly decreased. Under the above storage conditions, tolbutamide had crystallized to form I in PVP matrix and to form II in the HP-β-CyD matrix. The dissolution rate of tolbutamide polymorphs was in the order of form IV > form II > form III > form I, as reported previously. Therefore, the large deceleration of the PVP system as a result of storage is attributable to the formation of form I crystals with a slow dissolution characteristic, whereas the slight deceleration is attributable to the formation of form II crystals with a fast dissolution characteristic. The insignificant decrease in dissolution rate of the tolbutamide–HP-β-CyD complex was reflected in the in vivo absorption behavior of the drug. Figure 6 shows plasma levels of tolbutamide after oral administration in dogs of a tolbutamide–HP-β-CyD complex, tolbutamide–PVP dispersion immediately after the preparation, or those after storage for one week at 60°C, 75% R.H. Each point represents the mean ± S.E. of 3—4 experiments.

Table 1. Bioavailability Parameters of Tolbutamide after Oral Administration of Tolbutamide–HP-β-CyD Complex or Tolbutamide–PVP Dispersion (Equivalent to 100 mg Drug/Body) in Dogs

<table>
<thead>
<tr>
<th>System</th>
<th>$t_{\text{max}}$ (h)$^b$</th>
<th>$C_{\text{max}}$ (μg/ml)$^c$</th>
<th>$AUC$ (h μg/ml)$^d$</th>
<th>MRT (h)$^e$</th>
<th>$F$ (%)$^f$</th>
</tr>
</thead>
<tbody>
<tr>
<td>HP-β-CyD complex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before storage</td>
<td>3.3±0.4</td>
<td>105.8±11.4</td>
<td>1043.8±23.7</td>
<td>7.5±0.3</td>
<td>88.8±2.6</td>
</tr>
<tr>
<td>After storage</td>
<td>2.8±0.3</td>
<td>95.1±3.1</td>
<td>811.0±15.9</td>
<td>7.1±0.2*</td>
<td>69.0±1.4</td>
</tr>
<tr>
<td>PVP dispersion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before storage</td>
<td>2.9±0.4</td>
<td>107.4±9.0</td>
<td>989.2±16.7</td>
<td>7.7±0.4</td>
<td>84.2±2.1</td>
</tr>
<tr>
<td>After storage</td>
<td>3.1±0.3</td>
<td>52.4±4.2**</td>
<td>259.0±11.3***</td>
<td>6.9±0.3*</td>
<td>22.0±1.3**</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E. of 3—4 dogs; significant differences from HP-β-CyD complex or PVP dispersion before storage are noted as follows: *$p<0.05$; **$p<0.01$; ***$p<0.001$. $^b$ The time required to reach the maximum plasma level. $^c$ The maximum plasma level. $^d$ The area under the plasma level versus time curve up to 24 h post-administration. $^e$ The mean residence time in plasma. $^f$ The extent of bioavailability compared with the $AUC$ value of intravenously administered tolbutamide.
excipients such as PVP, and attention should be given to the crystallization of drugs from CyD complexes, particularly drugs with many polymorphs.

References