Solid-State $^{13}$C Nuclear Magnetic Resonance Spectroscopic Study on Amorphous Solid Complexes of Tolbutamide with 2-Hydroxypropyl-$\alpha$- and -$\beta$-Cyclodextrins

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Purpose. The objective of the study was to obtain structural information of inclusion complexes of tolbutamide with HP-$\alpha$- and -$\beta$-cyclodextrins in amorphous state. Method. The solid complexes of tolbutamide with HP-$\alpha$- and -$\beta$-CyDs in molar ratios of 1:1 and 1:2 (guest:host) were prepared by the spray-drying method, and their interactions were investigated by solid-state $^{13}$C nuclear magnetic resonance (NMR) spectroscopy. Results. The solid 1:1 and 1:2 tolbutamide/HP-CyD complexes showed halo pattern on the powder X-ray diffractogram and no thermal change in DSC curves, indicating they are in an amorphous state. $^{13}$C NMR signals of the butyl moiety were broader than those of the phenyl moiety in the HP-$\alpha$-CyD solid complex, whereas the phenyl moiety showed significantly broader signals than the butyl moiety in the HP-$\beta$-CyD solid complex. As temperature increased, signals of the phenyl carbons became markedly sharper, whereas the butyl carbons only sharpen slightly in the HP-d-CyD complex. In contrast, signals of the butyl carbons became significantly sharper whereas those of phenyl carbons only slightly changed in the HP-$\beta$-CyD complex. Conclusions. Solid state $^{13}$C NMR spectroscopic studies indicated that the butyl moiety of tolbutamide is predominantly included in the HP-$\alpha$-CyD cavity, whereas the phenyl moiety in the HP-$\beta$-CyD cavity in amorphous complexes.

KEY WORDS: tolbutamide; 2-hydroxypropyl cyclodextrins; inclusion complex; amorphous solid; solid-state $^{13}$C NMR spectroscopy.

INTRODUCTION

Cyclodextrins (CyDs) are cyclic oligosaccharides usually consisting of six to eight glucose units which form inclusion complexes with various drug molecules both in solution and solid states, and their host/guest interactions have been investigated using a number of chemical and physical techniques such as spectroscopies, potentiometric titration, kinetics, and solubility methods, etc. (1–3). Among these techniques, nuclear magnetic resonance (NMR) spectroscopy is particularly useful for structure determination of CyD inclusion complexes in solution, because it gives detailed information of molecular dimensions (4). Whereas single crystal X-ray analysis is the definitive method for structure determination of solid complexes (5,6), CyD complexes do not always give crystals with a size suitable for single crystal X-ray analysis, often producing microcrystals or even powder. In this latter case, powder X-ray diffractometry is used for structure elucidation using the peak fitting methods such as the Rietveld method (7–9), but even at present it is difficult to apply this technique to complicated compounds such as CyD complexes, particularly in an amorphous state. Therefore, it is difficult to thoroughly characterize amorphous solid CyD complexes from a structural viewpoint, as compared with the complexes in solution or in crystalline state. Recently, solid-state NMR spectroscopy has received attention as a complementary tool to X-ray analysis for structure determination of solid compounds, because it can apply to powder samples (10,11). For example, detection and characterization of polymorphic forms of drugs and their transformations such as polymorphic transitions, hydrations and dehydrations have been investigated using solid-state NMR spectroscopy.

2-Hydroxypropyl-$\beta$-CyD (HP-$\beta$-CyD) is a water-soluble CyD derivative that is used in two pharmaceutical preparations currently on the market, i.e., hydrocortisone mouthwash solution (Iceland) and itraconazole liquid preparation (USA and Belgium). Because HP-$\beta$-CyD is an amorphous compound, it can convert crystalline drugs to amorphous solids with higher aqueous solubility, through the inclusion complex formation (12,13). Although inclusion complex formations of HP-$\beta$-CyD with drugs in solution have been extensively studied, there are only a few reports on the structural aspect of amorphous HP-$\beta$-CyD complexes (14). Therefore, we conducted solid-state $^{13}$C NMR spectroscopic studies on the complex formation of HP-$\alpha$- and -$\beta$-CyD with an oral hypoglycemic agent, tolbutamide. This drug was chosen because of the presence of the alkyl and phenyl moieties that are of suitable size for the inclusion in the $\alpha$- and $\beta$-CyD cavities, respectively, in a molecule (15,16).

MATERIALS AND METHODS

Materials

HP-$\alpha$-CyD (degree of substitution (D.S.) 4.1) and HP-$\beta$-CyD (D.S. 4.8) were supplied by Japan Maize Co. (Tokyo, Japan). Tolbutamide was donated by Hoechst-Marion-Roussel Ltd. (Tokyo, Japan). Other chemicals and solvents were of analytical reagent grade, and deionized double-distilled water was used throughout the study.

Solubility Measurements

Solubility studies were carried out according to the method of Higuchi and Connors (17). The screw capped vials containing tolbutamide (50 mg) in an excess amount in aqueous HP-CyD solutions (3.0 ml) at various concentrations were shaken at 25°C. After equilibrium was attained (about 14 days), the solution was centrifuged at 800 g force for 5 min and the supernatant was filtered through a membrane filter (ADVANTEC DISMIC 13P, TOYO-Roshi, Tokyo, Japan) and the filtrate was analyzed for tolbutamide by high-performance liquid chromatography under the following conditions: a Hitachi L-6000 pump and a 635-A UV detector (Tokyo, Japan), a Yamamura YMC AQ-312 ODS column (5 μm, 6 mm x 150 mm i.d., Kyoto, Japan), a mobile phase of acetonitrile/0.05 M NaH2PO4 solution (45:55 v/v), and a flow rate of 1.6 ml/min, and detection of 230 nm.

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Preparation of Solid HP-CyD Complexes

Solid complexes of tolbutamide with HP-α- and β-CyD were prepared by the spray-drying method, using a Yamato Pulvis GA32 spray dryer (Tokyo, Japan). The calculated quantities of tolbutamide and HP-CyDs corresponding to 1:1 and 1:2 (guest:host molar ratio) were dissolved in the mixed solvent of ethanol/dichloromethane (1:2.1 v/v) and then spray-dried under the following conditions: an air flow rate of 0.4 m³/min, an air pressure of 1.0 kgf/cm², and inlet and outlet temperatures of 85°C and 55°C, respectively.

Powder X-ray Diffractometry and Differential Scanning Calorimetry (DSC)

Powder X-ray diffraction patterns were measured with a Rigaku Rint-2500 diffractometer (Tokyo, Japan) under the following conditions: Ni-filtered Cu-Kα radiation (1.542 Å), voltage of 40 kV, a current of 40 mA, a divergent slit of 1.74 mm (1º), a scattering slit of 0.94 mm (1º), a receiving slit of 0.15 mm, and a goniometer angular increment of 1º/min. DSC analyses were carried out using a Perkin-Elmer DSC-7 thermal analyzer (Norwalk, CT) with a data analysis system (DEC station 325C computer, USA), operated with a sample weight of 5 mg and a scanning rate of 10°C/min.

NMR Spectroscopies

¹H NMR spectra were obtained with a JEOL JNM-α-500 instrument (Tokyo, Japan) with a 5 mm inverse broadband probe, operating at 500 MHz and a sweep width of 10000 Hz, at 25°C. The concentration of tolbutamide in 0.1 M sodium borate buffer (pH 9.3) in deuterium oxide (D₂O) was 5.0 × 10⁻³ M, and that of HP-CyDs varied from 0 to 2.0 × 10⁻² M. In the case of the continuous variation plot, the total concentration of the guest and the host was kept constant (1.0 × 10⁻² M). Chemical shifts are given as part per million (ppm) downfield from that of tetramethylsilane with an accuracy of 0.005 ppm. Solid-state ¹³C NMR spectra were taken on a JEOL JNM EX-270 spectrometer with a cross polarization/magic angle spinning (CP/MAS) accessory (Tokyo, Japan), operating at 270 MHz (¹H). The CP radio frequency field strength was about 56 kHz, the contact time was 5 ms, the repetition time of accumulation was 4 s, and the MAS was 6.2–6.4 kHz. The ¹H decoupling frequency was chosen to be 3 ppm downfield from tetramethylsilane. The ¹³C chemical shifts were measured in ppm with respect to the methine carbon of adamantane (29.7 ppm downfield from the resonance of tetramethylsilane) as an external reference. Variable-temperature measurements were accomplished using a JEOL MVT temperature controller (Tokyo, Japan). Signal accumulation was started 20 min after the desired temperature was achieved (25–80°C). The chemical shifts of tolbutamide were assigned according to the report of Ueda et al. (16).

RESULTS AND DISCUSSION

Figure 1 shows the phase solubility diagrams of tolbutamide with HP-α- and β-CyDs in water, where the solubility of the guest molecule increased as a function of CyD concentration. The solubility curve of the HP-β-CyD system deviated positively from a straight line and that of the HP-α-CyD system deviated slightly positively. These curves were classified as A₂ type (17), indicating a formation of higher order complexes. Therefore, the 1:1 and 1:2 stability constants (K₁,1 and K₁,2) of the tolbutamide/CyD complexes were calculated by analyzing the solubility curves according to the iteration method of Kristiansen (18), and were 73 (±3) and 9 (±1) M⁻¹ for the 1:1 and 1:2 HP-α-CyD complexes and 194 (±8) M⁻¹ and 17 (±1) M⁻¹ for the 1:1 and 1:2 HP-β-CyD complexes, respectively. The fact that the 1:1 stability constant was much larger than the 1:2 stability constant, suggests that tolbutamide forms predominantly the 1:1 complex in aqueous solution and at higher CyD concentrations it forms the high order complex.

In order to gain insight into the inclusion mode of tolbutamide/HP-CyD complexes in aqueous solution, ¹H NMR spectroscopic studies were carried out. Because the solubility of tolbutamide in water (Fig. 1) was too low to measure accurately NMR spectra, the drug was dissolved in 0.1 M sodium borate/D₂O buffer (pH 9.3) where it is in ionized form (pKₐ 5.34). Tolbutamide gave ¹H signals of H₁, H₃ and H₄ of tolbutamide moiety and H₉, H₁₀, H₁₁ and H₁₂ of the butyl moiety at 2.31 (singlet), 7.63 (doublet), 7.29 (doublet), 2.91 (triplet), 1.28 (quintet), 1.15 (sextet), and 0.75 (triplet) ppm, respectively, in the borate buffer. Figure 2 shows changes in chemical shifts of these protons with the addition of CyDs. In the case of HP-α-CyD, ¹H signals of the butyl protons (H₉-H₁₂) markedly shifted downfield and the shift became greater at higher CyD concentrations, whereas those (H₁, H₃ and H₄) of the tolbutamide moiety shifted only slightly. In sharp contrast, the addition of HP-β-CyD brought about a large shift of the tolbutamide protons, whereas those of the butyl protons shifted only slightly. These results suggested that HP-α-CyD having the smaller cavity interacts preferably with the butyl moiety of tolbutamide, whereas HP-β-CyD having the larger cavity interacts with the tolbutamide moiety in aqueous solution. Maincent et al. (19) reported that the tolbutamide/parent β-CyD system gives the B₃ type phase solubility diagram, where the solubility of tolbutamide increased at low CyD concentration, followed by a plateau region and then decreased with increase in CyD concentration, and that the stoichiometry of the complex is 1:2 (molar ratio of guest:host). Therefore, the stoichiometry of tolbutamide/HP-CyD complexes was estimated by the continuous variation.