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Enantioselective inclusion between terbutaline enantiomers and hydroxypropyl-β-cyclodextrin

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Abstract

Solid inclusion complexes between terbutaline (racemate, *S*-(+)-enantiomer, and *R*-(−)-enantiomer, respectively) and hydroxypropyl-β-cyclodextrin (HP-β-CD) were prepared by the lyophilization method. Characterization of each complex was achieved with differential scanning calorimetry (DSC). Stoichiometry between terbutaline and HP-β-CD was studied by UV spectroscopy, and stability constants between terbutaline (racemic, *S*-(+)-enantiomer and *R*-(−)-enantiomer) and HP-β-CD was also determined by UV absorption and chromatographic retention method. Structural study to confirm exact location of chiral discrimination between terbutaline enantiomers and HP-β-CD was performed by ^{1}H NMR spectroscopy. From this experiment, enantioselective binding of terbutaline enantiomers to HP-β-CD was clearly demonstrated.

Keywords: Enantiomer; Enantioselective binding; HP-β-CD; ^{1}H NMR spectroscopy; Stability constants; Terbutaline

1. Introduction

Cyclodextrins (CDs) are oligoglycosides of six (α-CD), seven (β-CD) or eight (γ-CD) glucose units forming a ring. The most stable three dimensional configuration of CDs takes the form of a toroid with the upper (larger) and lower (smaller) opening of toroid presenting secondary and primary hydroxyl groups, respectively to the solvent environment. CDs are known for their ability to bind organic molecules in aqueous medium by non-covalent interactions and the complexation driving forces have been attributed to hydrophobic interactions, van der Waals forces, and hydrogen bonds. For these reasons, CDs have been used in order to enhance the drug's poor aqueous solubility, protect them in their micro-environment, create and maintain stable homogeneous distributions, provide more convenient physical

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forms (suspension to solution, oil to solid) and alter their physical properties (smell, taste, photodegradation) (Duchene and Wouessidjewe, 1990; Bekers et al., 1991; Veyron and Tachon, 1996)

CDs are optically active and offer the potential discrimination of enantiomeric substances (Dodziuk et al., 1992; Wenzel et al., 1994; Chen et al., 1996). Most often, the discrimination involves the formation of hydrogen bonds between the CDs and substances (guest molecules). The proximity of hydrogen bonding moieties can vary markedly for a pair of enantiomer, with concomitant difference in association constants (K_c). These characteristics of CDs permit them to be employed as stationary phase in gas and liquid chromatography for separation of drug enantiomers. CDs are also used as chiral selectors in the form of mobile phase additives in liquid chromatography, and have been developed as chiral selectors in capillary electrophoresis (CE) for separation of drug enantiomers (Benzhan et al., 1996; Salvatore, 1996). Alternatively, the complexes formed between CDs and a pair of enantiomers are diastereomer (stereoisomer not positioned in mirror image). In this case, even with comparable association constants, enantiomeric resolution caused by chemical shift nonequivalence between enantiomers may be observed in NMR spectra (Park and Park, 1996). NMR studies represent very useful tools in obtaining, respectively direct evidence of the complexation and a better description of supramolecular assemblies in solution (Moyano et al., 1997). These NMR techniques give structural information on chiral discrimination of enantiomers by CDs and further could be utilized for elucidation of chiral discrimination mechanisms by CDs.

Terbutaline, (5-[2-[(1,1-dimethylethyl) amino]-1-hydroxyethyl]-1,3-benzenediol), which was used as guest molecule in this report, has been widely used as β_2-adrenergic bronchodilator and administered as racemic mixture, and *R*-(−)-terbutaline avoided the side effects associated with the administration of the racemic terbutaline (Morley, 1992). Recently, enantioselective synthesis of *R*-(−)-terbutaline was attempted (Donohue and Jackson, 1995). Many reports have been presented for separation of terbutaline enantiomers using β-CD and hydroxypropyl-β-cyclodextrin (HP-β-CD) as chiral selectors in high performance liquid chromatography (HPLC) and CE (Sheppard et al., 1995; Sarah et al., 1997). ^{1}H NMR spectroscopy of inclusion complex between racemic terbutaline and β-CD was performed by Sarah et al. (1997), and chemical shift changes between free and complexed state terbutaline was also studied in respect to structure of the inclusion complex. However, because of difficulties in preparation of optically pure single enantiomers of terbutaline, NMR and other structural studies such as enantioselective inclusion of terbutaline with HP-β-CD have not been studied and therefore, exact location of chiral discriminating site between terbutaline and HP-β-CD also have not been discussed. In this report, optically pure single enantiomer of terbutaline (*S*-(+)- and *R*-(−)-) was prepared with semi-preparative chiral HPLC system equipped with chiral column, and then complexed with HP-β-CD (*S*-complex, *R*-complex and *RS*-complex, respectively). Difference of stability constants between each inclusion complex was observed with spectroscopic absorption and chromatographic retention methods, respectively, and structural studies for elucidation of exact location of chiral discriminating site were also performed with ^{1}H NMR spectroscopy. Preparation of solid inclusion complex was characterized with differential scanning calorimetry (DSC), and stoichiometry between terbutaline and HP-β-CD was also discussed with UV spectroscopy. The present report could be used for understanding chiral discrimination mechanisms of CDs, and utilized for the purpose of pharmaceutical formulation with CDs.

2. Experimental

2.1. Materials

Racemic terbutaline sulfate and hydroxypropyl-β-cyclodextrin (MS = 0.8, M_w = 1500) were purchased from Aldrich (MW, USA). All other materials were HPLC or analytical grade and used without further purification.

2.2. Preparation of terbutaline enantiomers from racemate

Single enantiomer of terbutaline was prepared by semi-preparative chiral HPLC system composed of Shimadzu LC-9A pump (Shimadzu, Japan), UV/VIS spectrophotometric detector (Shimadzu) and C-R4AD data recorder (Shimadzu). Sumichiral OA-4700 column (5 μm, 10 × 250 mm, GL Science, Japan) was used as chiral stationary phase for preparation of terbutaline enantiomer. The mobile phase consisted of a mixture of n-hexane, 1,2-dichloroethane, methanol and trifluoroacetic acid (240:140:20:1, v/v). Detection was set at UV 278 nm and flow rate was 4.0 ml/min. Optical purity of each enantiomer was confirmed with chiral HPLC system composed of HPLC instruments described above and Sumichiral OA-4700 column (5 μm, 4 × 250 mm, GL Science) as chiral stationary phase. The mobile phase was composed of a mixture of n-hexane, 1,2-dichloroethane, methanol and triflluoroacetic acid (240:140:27:1, v/v). Detection was set at UV 278 nm and flow rate was 0.8 ml/min.

2.3. Determination of stoichiometry between terbutaline and HP-β-CD

Various molar ratios of mixture of racemic terbutaline and HP-β-CD, from 1:9 to 9:1 relating terbutaline to HP-β-CD concentration, were dissolved with distilled-deionized water and stirred to the equilibrium for 24 h at 278 K. UV absorbance changes between free and complexed terbutaline was observed with Shimadzu UV 1601PC (Shimadzu) at UV 278 nm. Continuous variation plots was described to determination of stoichiometry.

2.4. Determination of stability constants between enantiomer of terbutaline and HP-β-CD

The stability constants (K_c) between each enantiomer of terbutaline and HP-β-CD in aqueous surroundings was determined by UV absorption and chromatographic retention method, respectively. In UV absorption method, UV absorption changes of terbutaline (constantly 0.3 mM) in the presence of HP-β-CD (various concentration of 0.3, 0.9, 1.5, 3.0, 6.0, and 12.0 mM) were measured at UV 278 nm. All experiments were performed in triplicate and the stability constant (K_c) was calculated by the conventional Scott's equation as follows (Scott, 1956).

$$\frac{[D][CD]}{d} = \frac{1}{K_c \cdot \varepsilon} + \frac{[CD]}{\varepsilon}$$

Where [D] and [CD] are the total concentration of terbutaline and HP-β-CD, respectively; d is the change in UV absorbance between free and complexed terbutaline; K_c is the stability constant; and ε is the difference of molar absorptivity.

In the chromatographic retention method, determination of stability constants was performed with reversed-phase HPLC using HP-β-CD as mobile phase additives. The HPLC system was composed of PU 610 pump (GL Science, Japan), UV 620 UV/VIS spectrophotometer (GL Science), EZChrom chromatography data system (GL Science) and Inertsil ODS-3 column (5 μm, 4 × 150 mm, GL Science). The mobile phase was composed of various concentrations of HP-β-CD (0, 1, 2, 4, 6 and 8 mM) in the mixed solution of ammonium acetate (0.05 M, pH = 6.0 adjusted with acetic acid) and methanol (95:5, v/v). Detection was set at UV 278 nm with 0.005AUFS and the flow rate was 1.2 ml/min. A sample of 10 μl of racemic terbutaline (0.1 mg/ml) was injected into the HPLC system with various mobile phases, and the stability constant was calculated concerning HP-β-CD concentration to $1/k'$ by the conventional Scott's equation as follows.

$$\frac{1}{k'} = \frac{K_c}{k_D}[CD] + \frac{1}{k_D}$$

Where k' is the capacity factor of the peaks; [CD] is the concentration of HP-β-CD in the mobile phase; k_D is a phase constant; and K_c is the stability constant.

2.5. Preparation, characterization and ^1H NMR studies of solid inclusion complexes

Solid inclusion complex was prepared by lyophilization method adding equimolar ratios of terbutaline (racemate and enantiomers) and HP-

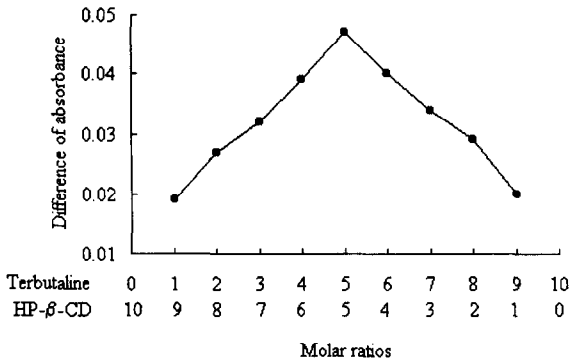

Fig. 1. Continuous variation plot for the terbutaline-HP-β-CD system.

β-CD in distilled-deionized water, and then characterized with DSC (Perkin Elmer DSC-7, Perkin Elmer, USA). ^{1}H NMR spectroscopy was performed with a Brucker DPX-400 NMR spectrometer (400.132MHz, Brucker, Germany) for racemic terbutaline, HP-β-CD and each inclusion complex, and chemical shift change of terbutaline protons were compared to acquire structural information on each complex. All samples were saturated with D_2O (Merck, Germany) and sodium 2,2-diethyl-2-silapentane-5-sulfonate (DSS, Merck) was used as internal standard.

3. Results and discussion

Optically pure terbutaline enantiomers could be acquired with semi-preparative HPLC, quantitatively (data not shown). Fig. 1 shows the continuous variation plots of UV absorbance changes between free and complexed state racemic terbutaline, and it was found that changes in absorbance intensity have a maximum at the terbutaline-HP-β-CD molar fraction in solution 5:5. This means that the complex of 1:1 stoichiometry is predominant in the solution.

Table 1
Stability constants (K_c) of the terbutaline-HP-β-CD system

Complexed with	Stability constants[a] (K_c, M^{-1})	
	UV absorption method	Chromatographic retention method
Racemic terbutaline	185	—
S-(+)-terbutaline	332	183
R-(−)-terbutaline	236	127

Fig. 2A shows Scott's plot for the determination of stability constants between terbutaline (racemic, *S*-(+)- and *R*-(−)-enantiomer) and HP-β-CD by the UV absorption method. The effects of HP-β-CD on the UV absorption of terbutaline was observed by increasing the concentration of HP-β-CD, indicating that intrinsic Cotton effects of terbutaline were affected by the complexation with HP-β-CD. Stability constants of each complex were summarized in Table 1, showing that *S*-(+)-terbutaline forms more stable complex than *R*-(−)-terbutaline, and single enantiomer of terbutaline forms a more stable

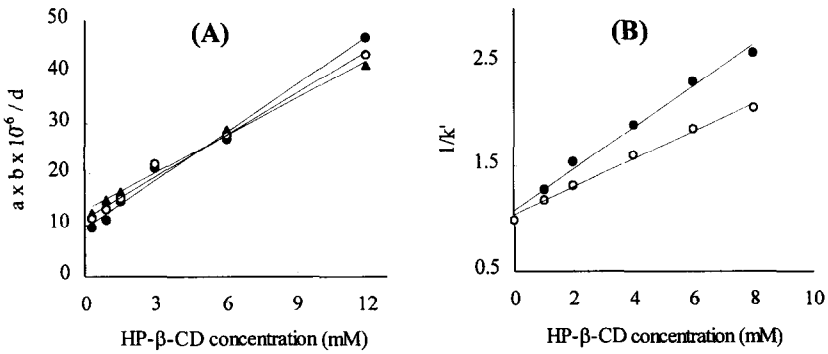

Fig. 2. Scott's plot for interaction between racemic (▲), *S*-(+)- (●) or *R*-(−)-terbutaline (○) and HP-β-CD, by (A) UV absorption method and (B) chromatographic retention method.

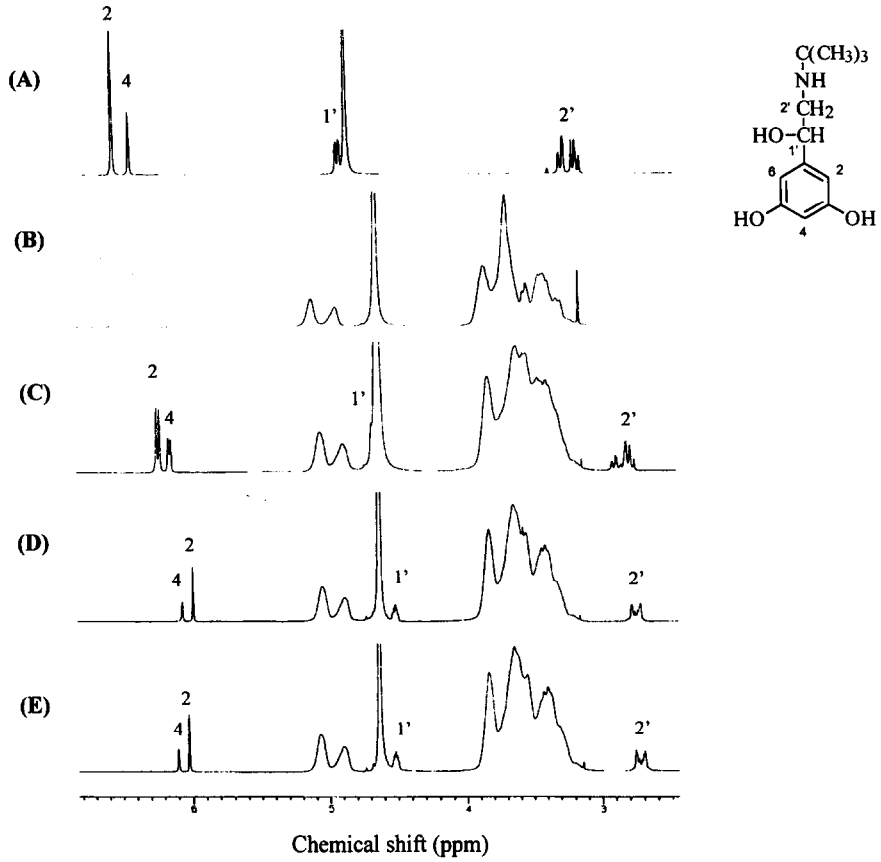

Fig. 3. Comparison of ^{1}H NMR spectra of (A) terbutaline base (racemate), (B) HP-β-CD, (C) *RS*-complex, (D) *S*-complex and (E) *R*-complex (400 MHz, D_2O, DSS as internal standard).

complex than racemic terbutaline. Ratios of stability constants, relating *S*-complex to *R*-complex, were 1.41:1, and these ratios were similar to that of the chromatographic retention method described below.

In the chromatographic retention method, the retention time of terbutaline enantiomers was diminished in HP-β-CD containing solution compared with HP-β-CD-free solution. The stability constants of each complex, which is calculated with the resulting retention time, permit the deduction of perceptible enantioselective bindings of each enantiomer (Fig. 2B). Stability constants of each complex were different in comparison with values calculated by UV absorption method, and these differences may result from nonequivalence of experimental conditions such as temperature, HP-β-CD concentration, and other various parameters. The results were summarized in Table 1, indicating a similar tendency of stability constants between enantiomers and the previous report (Benzhan et al., 1996).

Solid inclusion complex of terbutaline (racemate, enantiomers) was successfully characterized with DSC (data not shown) and ^{1}H NMR spectroscopy. ^{1}H NMR studies of complexes permit the deduction of structure of inclusion complexes. Fig. 3 shows a comparison of ^{1}H NMR spectra between free and complexed terbutaline with HP-β-CD. Generally, up-field chemical shifts were observed in complexed state terbutaline (racemate, *S*-(+)- and *R*-(−)-terbutaline) compared with free state terbutaline. These results imply that protons of terbutaline were surrounded by

electron density of HP-β-CD after the complexation with HP-β-CD. Among the hydrogen of terbutaline, aromatic Ar-2,6H have shown the largest chemical shift changes than any other proton, especially when complexed with terbutaline enantiomers. In detail, up-field chemical shift changes of aromatic 2,6H of racemic terbutaline, S-(+)-terbutaline and R-(−)-terbutaline were 0.371, 0.540 and 0.534 ppm, respectively, while other protons such as methyl and methine has shown $0.160 \sim 0.385$ ppm of chemical shift changes. Methylene protons of terbutaline also show significant up-field chemical shift changes ranging from 0.333 to 0.475 ppm. From the above results, it is supposed that the aromatic moiety of terbutaline is included deeply into the inner cavity of HP-β-CD, and $\alpha(1 \rightarrow 4)$ linkage oxygen of HP-β-CD carries electron densities to aromatic moiety of terbutaline. Up-field chemical shift changes of methylene protons of terbutaline were supposed to be affected by upper torus of HP-β-CD. Methyl and methine protons are also affected by the complexation with HP-β-CD, but not as much as aromatic or methylene protons. From the above results, it could be characterized that the aromatic moiety of terbutaline is embedded into the HP-β-CD cavity and the methylene protons are located near the cavity mouth of cyclodextrin. The methyl group was supposed to be located at the outside of the cavity, and therefore show little chemical shift changes compared with other protons. The supposed embedded view of terbutaline into the HP-β-CD was as suggested in Fig. 4.

Chemical shift non-equivalence between the complexes of terbutaline enantiomer, a focus in this report, was observed in ^{1}H NMR spectra. After complexation with HP-β-CD, aromatic protons of racemic terbutaline were split into two peaks, while non-complexed racemic terbutaline or complexes of each enantiomer have shown single peaks. It may be a result of enantioselective binding of each enantiomer with HP-β-CD. These phenomena were more obvious between the complexes of each enantiomer. The S-complex was shown to have larger chemical shift changes than that of the R-complex. Methylene and methine protons of terbutaline were presented to largest chemical shift non-equivalence between complexes of each enantiomer. In detail, differences of chemical shift changes between S- and R-complexes were 0.010, 0.012 ppm for methine and methylene protons, respectively while other protons showed below 0.060 ppm. From these results, it is supposed that methine and methylene protons play significant roles in chiral discrimination of the terbutaline-HP-β-CD system. However, the exact location of chiral discrimination was not determined due to insufficient assignment of the HP-β-CD peaks of ^{1}H NMR spectra which was caused by uncertainty of the substitution site of HP-β-CD, and therefore further research base on structure of cyclodextrin was required such as NOE of NMR and computer-aided molecular modelling.

Based on above results, HP-β-CDs could be used as probes for elucidation of optical purity of terbutaline by NMR spectroscopy. Furthermore, complexation of terbutaline with HP-β-CD could be applied to pharmaceutical formulations for the purpose of enhancement of stability in aqueous surroundings and development of controlled release terbutaline, especially for side effect-free R-(−)-terbutaline. The present study also reveals that methylene and methine protons play significant roles in chiral discrimination of terbutaline-HP-β-CD systems, and these facts could be applied to understand chiral discrimination mechanisms of cyclodextrins in various compounds.

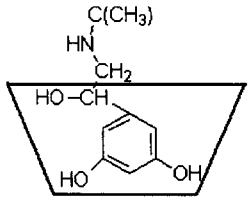

Fig. 4. Embedded view of terbutaline into HP-β-CD.

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