A Study on the Chiral Recognition Mechanism of Enantioseparation of Adrenaline and Its Analogues Using Capillary Electrophoresis

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The purpose of this paper was to study the chiral recognition mechanism of enantioseparation of adrenaline and its analogues using capillary electrophoresis. The enantiomeric separation of adrenaline and its analogues has been developed using beta-cyclodextrins as the chiral selectors. All the tested compounds were separated under the same experimental conditions to study the chiral recognition mechanisms, using a low-pH buffer (50 mM Tris buffer at pH 2.5). By means of molecular docking the inclusion course between beta-cyclodextrins and enantiomers was investigated and thus the interaction energy was obtained by molecular mechanics calculations. The results suggest that the difference in interaction energy for the side chain part is most likely responsible for enantiomeric separation.

Key words chiral recognition mechanism; beta-cyclodextrin; docking; interaction energy; enantiomeric separation; capillary electrophoresis

Since the first report[1)] in 1985, capillary electrophoresis (CE) enantioseparation has become a very important area during the last two decades. In CE enantioseparation, the chiral selector can be directly added to the running buffer to provide chiral environment. Thus the two enantiomers can be separated owing to the difference in the interactions between chiral selectors and enantiomers. The most widely used chiral selectors are cyclodextrins (CDs) and various derivatives.[2—7)] Many papers on CE enantiomeric separation focused on optimization of experimental methods, for example, the type and concentration of CDs, buffer pH, ionic strength, *etc*.[8—14)] Vescina *et al.* reported the CE enantiomeric separation of 35 basic pharmaceutical compounds using a total of 26 different CD derivatives with different functional groups and degrees of substitution.[15)] The experimental conditions were chosen based on literature reports and their own experience and more than 1000 CE experiments were involved. Ren and Liu investigated the effects of pH, β-CD concentration, electrolyte concentration, and methanol concentration on the chiral separation of dioxypromethazine enantiomers using capillary electrophoresis.[16)] Wioleta reported the application of carboxymethyl-β-CD as a chiral selector in capillary electrophoresis for enantiomer separation of selected neurotransmitters.[17)] The experimental factors have been optimized, such as the type and concentration of chiral selector, concentration of borate buffer, content of methanol, pH of electrolyte, and method of sample introduction into the capillary.[17)] However, the literature studies on enantiomeric separation of pharmaceutical compounds were limited by the explanation of how the enantiomeric separation occurred. The current study used a molecular docking technique to gain insight into the selector-enantiomer interaction energy and provided useful supporting information for enantiomeric separations.

In this report, β-CDs were used as the chiral selectors and adrenaline and its analogues as the objects. The course of host-guest inclusion was determined by means of a molecular docking technique and thus the interaction energy was calculated by molecular mechanics calculations. Based on the results, the mechanism of chiral recognition is discussed.

Experimental

Chemicals Adrenaline, noradrenaline and isopropyladrenaline were purchased from Shanghai Hefeng Pharmaceuticals Co., Ltd. Terbutaline was obtained from Shanghai Institute for Drug Control. Tris reagent was obtained from Shanghai Shisheng Cell Biotechnique Co., Ltd. β-CD was purchased from Suzhou Flavorings Factory; 2,3,6-trimethyl-β-CD was synthesized in our laboratory based on references[18,19)] and the product was identified based on IR, NMR, and MS, which provided the physiochemical data such as the melting point of 156 to 157 °C and IR (cm^{-1}) of 2985, 2930, 2830, 1466, 1365, 1320, 1160, 1140, 1110, 1070. The uncoated fused-silica capillary was purchased from Hebei Yongnian Optical Fiber Factory.

Apparatus Separations were performed on an HP^{3D} capillary electrophoresis system (Agilent, U.S.A.) using an uncoated fused-silica capillary (58.5 cm×75 μm i.d., effective length 50 cm). The running buffer consisted of Tris buffer 50 mM (pH 2.5) with 1.0% β-CD or 2,3,6-trimethyl-β-CD. An online diode-array detector was used at the detection wavelength of 204 nm. Samples were injected at a pressure of 50 mbar for 3 s and separated at 20 °C using a constant voltage of 20 kV. The capillary was flushed with NaOH 0.1 M for 4 min and with the running buffer for 4 min before each run.

Preparation of Solutions Adrenaline, noradrenaline, isopropyladrenaline, and terbutaline were dissolved in double-distilled water to produce solutions containing about 40 μg per ml as sample solutions, respectively.

Methods of Molecular Construction, Optimization, and Docking Molecular constructions were carried out on the MODIFY/SKETCH module of SYBYL6.2 software. A molecular mechanics Powell method[20)] was applied for structure optimization. All molecular mechanics calculations and quantum chemistry calculations were carried out on a Silicon Graphics Indigo II Station.

The three-dimensional structure of β-CD was constructed using the crystal structures of β-CD and its enzymatic complex substance obtained from the Protein Crystal Database (www.rcsb.org/pdb). It was then optimized using the molecular mechanics Powell method. The structure of 2,3,6-trimethyl-β-CD was derived from the above three-dimensional structure of β-CD.

Molecular docking serves as a method to simulate the interactions of two molecules (such as ligand and receptor) and to predict their binding mode and affinity. DOCK 4.0.1 software[21,22)] was applied for molecular docking and the docking software has been validated for simulating the interactions of two molecules. The Connolly molecular surface was calculated and then all possible ligand fields were generated using Sphgen software. The optimized enantiomers were then inserted into the hydrophobic cavity of β-CDs and the interaction energy of docking was obtained by calculating the steric

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energy and electrostatic energy in the docking region.

Results and Discussion

Enantiomeric Separation of Adrenaline and Its Analogues on β-CDs As shown in Fig. 1, all the test compounds have an aromatic ring and the same chiral center, but they contain different structures in the side chains. Generally, the CE experimental conditions affect the enantiomeric separation. To study the chiral recognition mechanism of β-CDs and ensure the results can reflect the inclusion statement, the same conditions were used, such as the same pH, ionic strength, temperature, no addition of organic solvent, *etc*. In this study, Tris buffer 50 mmol/l at pH 2.5 containing 1.0% chiral selector (native β-CD or 2,3,6-trimethyl-β-CD) was applied for all the tested compounds. Figure 2 shows the separation of the enantiomers. Using native β-CD as the chiral selector, terbutaline enantiomers can be easily separated and isopropyladrenaline enantiomers can be slightly separated. Better separation of terbutaline enantiomers was achieved using 2,3,6-trimethyl-β-CD as the chiral selector. The elution orders of terbutaline and isopropyladrenaline enantiomers were identified by analysis of individual enantiomers. However, adrenaline and noradrenaline enantiomers could not be separated under the same conditions.

Calculation of Interaction Energies between the Enantiomers and β-CDs The structures of enantiomers and β-CDs were constructed on the computer according to the above described method. Docking between each enantiomer and β-CDs was carried out. Interaction energy parameters were calculated. Table 1 lists the data of interaction energies between four pairs of enantiomers and β-CDs. From the table, it can be observed that the absolute value of the total interaction energy (*I.E.*) for each enantiomer was greater than 15. This was probably because all the test compounds contained an aromatic ring, which had higher hydrophobility and tended to be included in the hydrophobic cavity of β-CDs. It was generally suggested that the key point for enantiomeric separation is in the difference of interaction energy (expressed in *ΔI.E.*) between two enantiomers included in β-CDs.[23] As seen in Table 1, the *ΔI.E.* value of terbutaline was 4.576, which was much greater than that of the other compounds. This was consistent with the experimental result that terbutaline enantiomers could be easily separated. The *ΔI.E.* values of both adrenaline and noradrenaline were also greater than that of isopropyladrenaline, but interestingly, the enantiomers of both adrenaline and noradrenaline could not be separated while isopropyladrenaline enantiomers could be slightly separated. There is a slight difference in the side chains of these compounds. Therefore the interaction energies for the aromatic ring part and side chain part of enantiomers combined with β-CD were calculated.

The difference in interaction energy for the side chain part ($\Delta I.E._{(s)}$) was thus obtained. The related data are summarized in Table 2. It was obvious that the interaction energy for the aromatic ring part was greater than that for the side chain part. The $\Delta I.E._{(s)}$ values of isopropyladrenaline and terbutaline (2.681, 5.821) were greater than those of adrenaline and noradrenaline. The results were consistent with the practical separation and thus indicated that the side chain part of enantiomers played a more important role in chiral recognition on β-CDs, while the existence of an aromatic ring only

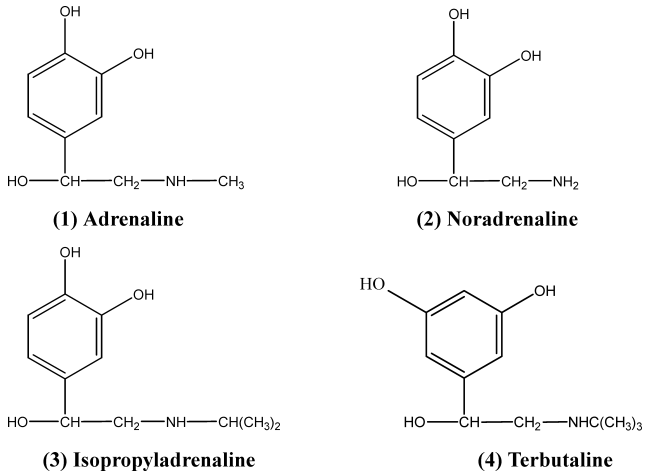

Fig. 1. Chemical Structures of All Tested Compounds

Table 1. Data on the Difference in Total Interaction Energy (β-CD as Chiral Selector)

Enantiomer		Total *I.E*[a)]	*ΔI.E.*
Adrenaline	*R*-	−18.795	
	S-	−21.216	2.421
Noradrenaline	*R*-	−20.871	
	S-	−17.412	3.459
Isopropyladrenaline	*R*-	−19.898	
	S-	−20.354	0.456
Terbutaline	*R*-	−20.875	
	S-	−25.451	4.576

a) *I.E.*, interaction energy; *ΔI.E.*, difference in total interaction energy.

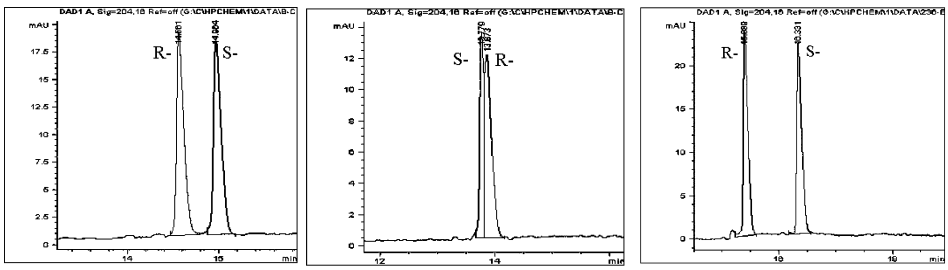

Fig. 2. Electropherograms for Separation of Enantiomers

(A) Terbutaline; (B) isopropyladrenaline, both in the presence of Tris buffer 50 mmol/l containing 1.0% β-CD; (C) terbutaline, in the presence of Tris buffer 50 mmol/l containing 1.0% 2,3,6-trimethyl-β-CD; voltage, 20.0 kV; temperature, 20 °C.

Table 2. Data on the Difference in Interaction Energy for the Side Chain Part (β-CD as Chiral Selector)

Enantiomer		Total $I.E.$[a)]	$I.E._{(a)}$	$I.E._{(s)}$	$\Delta I.E._{(s)}$
Adrenaline	*R*-	−18.795	−13.971	−4.824	
	S-	−21.216	−13.832	−7.384	2.560
Noradrenaline	*R*-	−20.871	−13.294	−7.577	
	S-	−17.412	−9.911	−7.501	0.076
Isopropyladrenaline	*R*-	−19.898	−9.465	−10.433	
	S-	−20.354	−12.602	−7.752	2.681
Terbutaline	*R*-	−20.875	−14.004	−6.870	
	S-	−25.451	−12.760	−12.691	5.821

a) $I.E.$, interaction energy; $I.E._{(a)}$, interaction energy for the aromatic ring part; $I.E._{(s)}$, interaction energy for the side chain part; $\Delta I.E._{(s)}$, difference in interaction energy for the side chain part.

Table 3. Data on the Difference in Interaction Energy for Side Chain Part (2,3,6-Trimethyl-β-CD as Chiral Selector)

Enantiomer		Total $I.E.$[a)]	$I.E._{(a)}$	$I.E._{(s)}$	$\Delta I.E._{(s)}$
Adrenaline	*R*-	−14.335	−8.775	−5.560	
	S-	−16.783	−9.490	−7.293	1.733
Noradrenaline	*R*-	−14.346	−11.058	−3.288	
	S-	−12.377	−7.154	−5.223	1.935
Isopropyladrenaline	*R*-	−17.872	−10.699	−7.173	
	S-	−13.681	−9.073	−4.608	2.565
Terbutaline	*R*-	−20.503	−11.556	−8.947	
	S-	−18.024	−11.931	−6.093	2.854

a) Meanings of symbols are the same as in Table 2.

increased the chiral discrimination.

In the same way, the difference in interaction energy for the side chain part was determined using 2,3,6-trimethyl-β-CD as the chiral selector. Table 3 lists the calculation results. The values also confirmed the above separation results. Most of values were less than those in Table 2, which was probably because stronger effects of space hindrance occurred in 2,3,6-trimethyl-β-CD than in native β-CD. Thus it can be concluded that the interaction between the side chain part of enantiomers and β-CDs was the key point in chiral recognition and the difference in the total interaction energy ($\Delta I.E.$) could not completely reflect the chiral discrimination. Therefore it is recommended that the difference in the interaction energies for the side chain part be used in predicting the enantiomeric separation, especially when the interaction energy for the aromatic ring part is much greater.

Molecular Docking between *R*/*S*-Terbutaline and β-CD From the molecular modeling, it was found that the aromatic ring of both terbutaline enantiomers were included into the cavity of β-CD and the side chains located near the rim of β-CD, as illustrated in Fig. 3. This indicates that the chiral recognition mechanisms between terbutaline and β-CD are involved in the formation of inclusion complexes. However, as seen in Fig. 3, there were some differences in the interaction between the side chain and β-CD. *S*-Terbutaline deeply penetrated into the cavity, and thus the interaction between the side chain and the rim of β-CD was stronger than that of *R*-terbutaline. Both enantiomers can be separated mainly due to the difference in the interaction energy for the side chain.

Conclusion

In this paper, enantioseparation of adrenaline and its analogues by CE using β-CDs as the chiral selectors was investigated. The molecular docking technique was applied for the first time to determine the course of host-guest inclusion and the selector-enantiomer interaction energy was calculated by molecular mechanics calculations.

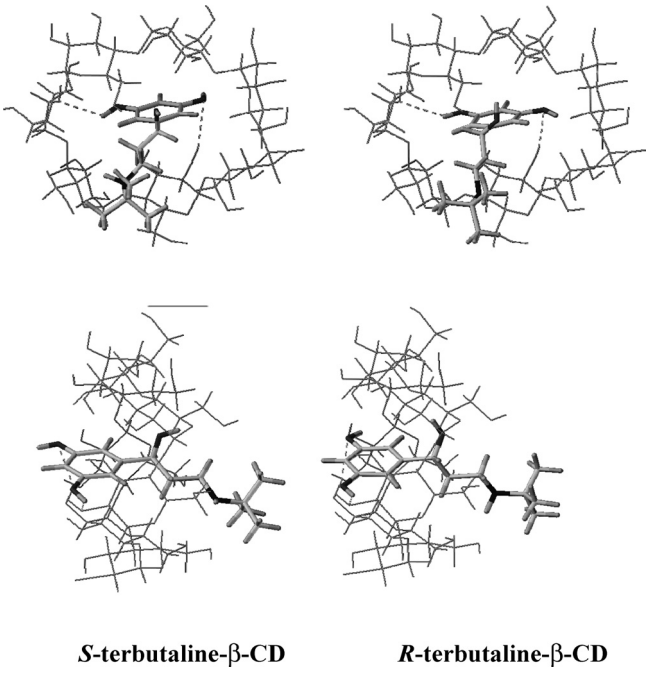

Fig. 3. Molecular Docking between *R*/*S*-Terbutaline and β-CD
The graphs were viewed from the *x*- and *z*-axes, respectively.

Enantiomers and β-CDs formed reversible inclusion complexes with different structures and properties by inclusion within the cavity and complexation outside β-CDs during the course of chiral recognition. The inclusion can occurs at multiple points in the cavity, while complexation occurs at one point or multiple points. There must be a difference in the interaction at one complexation point outside the cavity to achieve chiral separation. This complexation point can be the entire side chain or groups attached directly to the chiral carbon. Based on the results of molecular docking and enantioseparation, the chiral recognition mechanism of enantioseparation of adrenaline and its analogues by CE using β-CDs was proposed, which involved a combination at multiple points and determination by one point. The mechanism was distinguished from the three-point-interaction rule.[24] Molecular mechanics calculations suggested the difference in interaction energy for the side chain part was most likely responsible for enantiomeric separation.

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