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# Hydrogen bonding as a possible interaction for the chiral separation of DL-dopa and DL-3-*O*-methyl-dopa in a chiral teicoplanin column

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**Abstract**

The present study suggests that hydrogen bonding plays an important role in the mechanism of the chiral separation of DL-dopa and DL-3-*O*-methyl-dopa on a chiral teicoplanin column in high-performance liquid chromatography (HPLC). 

*Keywords:* Dopa; 3-*O*-Methyl-dopa; Chiral separation; HPLC

## 1. Introduction

The mechanism of chiral separation has been the objective of many studies [1–7]. The proposed mechanisms for the chiral separation include π–π complexation, dipole stacking, hydrogen bonding, hydrophobic interaction, steric interactions and cyclodextrin inclusion.

L-Dopa (L-3,4-dihydroxyphenyl-L-alanine) is one of the most effective drugs in the treatment of Parkinson's disease. L-3-*O*-Methyl-dopa is the metabolite of L-dopa and was found to compete with L-dopa for the neutral amino acid transport system in the blood–brain barrier [8,9]. D-Dopa was found to be able to elevate brain dopamine level in rats [10], thus the identification of DL-dopa and DL-3-*O*-methyl-dopa in humans is important for the further understanding of Parkinson's disease. Meanwhile, the chiral separation of DL-dopa and DL-3-*O*-methyl-dopa may provide some insight into the mechanism of chiral separation.

When using a commercially available high-performance liquid chromatographic column (Chirobiotic T™) to separate DL-dopa and DL-3-*O*-methyl-dopa, we noticed the reversed elution order compared with the non-chiral separation; thus, the hydrogen bonding is likely to be the main mechanism for the chiral separation of DL-dopa and DL-3-*O*-methyl-dopa.

## 2. Experimental

### 2.1. Chemicals and instrumentation

All chemicals are of analytical grade. DL-Dopa, DL-3-*O*-methyl-dopa and ammonium acetate were purchased from Sigma (St. Louis, MO, USA).

The high-performance liquid chromatography (HPLC) system consisted of a solvent delivery system (System Gold, programmable solvent module 116, Beckman Instruments Inc., Palo Alto, CA, USA), an analog interface (System Gold model 406, Beckman Instruments Inc.), an injection valve with a 50-μl sample loop, a 250 × 4.6 mm ID Chirobiotic T™ HPLC reversed-phase chiral column (StepBio, Bologna, Italy).

The mobile phase was prepared by mixing ethanol–water–methanol (60:40:5, v/v/v), and ammonium acetate to reach a final concentration of 10 mmol/l at pH 4.2. The mobile phase was degassed in an ultrasonic bath (Brason 2210 ultrasonic cleaner, Bransonic Ultrasonic Corporation, Darbury, CO, USA). The mobile phase flow rate was 1.0 ml/min and the back pressure was $< 2000$ psi through the column.

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The chromatographic stationary phase is packed by chemically bonding the macrocyclic antibiotic teicoplanin to a 5 μm silica gel. Teicoplanin is linked to silica gel through a spacer arm of optimum chain length which yields a non-hydrolytic stable ether link.

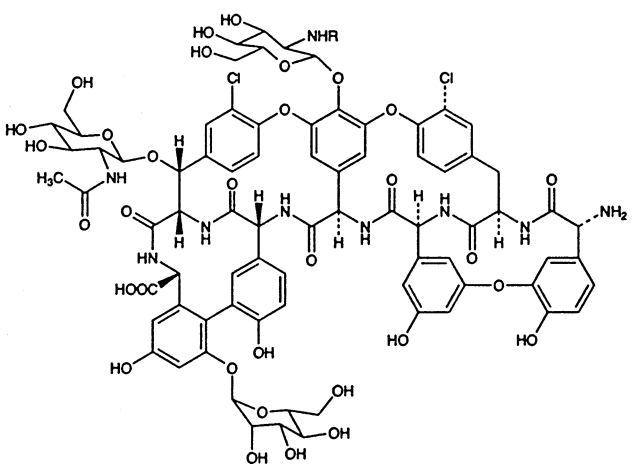
The electrochemical detection system was an ESA model 5200 Coulochem II (ESA, Bedford, MA, USA) with an ESA model 5010 analytical cell (ESA) with two working electrodes. The electrochemical detection system operated in DC mode. The potentials for the two working electrodes in the analytical cell were − 100 mV for the first ($E_1$) and 250 mV for the second ($E_2$), respectively.

Signals were collected using System Gold™ Personal™ Chromatography Software (version 6.01, Beckman Instruments Inc.).

## 3. Results and discussion

Teicoplanin has three sugar moieties surrounding four cavities and a methyl dodecyl side chain (Fig. 1) [11]; therefore, all the defined mechanisms for chiral recognition are included in this structure.

Fig. 2 shows the simultaneous separation of DL-dopa and DL-3-*O*-methyl-dopa. As observed previously [12], the retention time of D-isomers is longer than their corresponding L-isomers. However, the retention time of D-dopa is longer than that of D-3-*O*-methyl-dopa, while retention time of L-dopa is shorter than that of L-3-*O*-methyl-dopa.



| Teicoplanin | R | |
|---|---|---|
| | $A_2$-1 | (Z)-4-decenoic acid |
| | $A_2$-2 | 8-methylnonanoic acid |
| | $A_2$-3 | n-decanoic acid |
| | $A_2$-4 | 8-methyldecanoic acid |
| | $A_2$-5 | 9-methyldecanoic acid |

Fig. 1. The structure of teicoplanin.

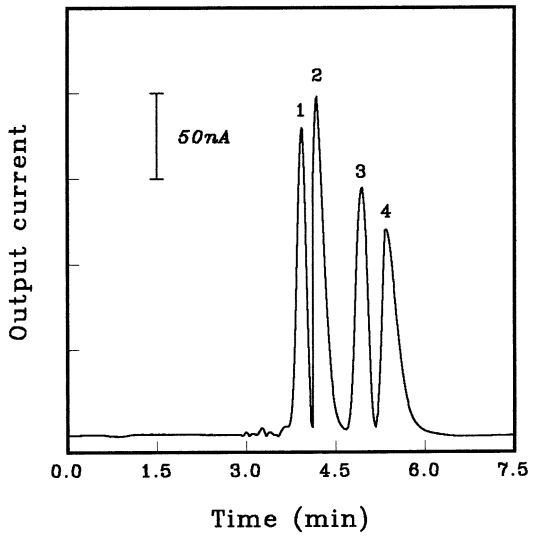



Fig. 2. Separation of DL-dopa and DL-3-*O*-methyl-dopa using a teicoplanin chiral column with mobile phase of ethanol–water–methanol (60:40:5, v/v/v) and 10 mmol/l ammonium acetate at 1.0 ml/min flow and less than 2000 psi back pressure at a detector sensitivity of 200 nA full scale. (1) L-Dopa; (2) L-3-*O*-methyl-dopa; (3) D-3-*O*-methyl-dopa; (4) D-dopa.

Using the current technique of simultaneous detection of dopa and 3-*O*-methyl-dopa with non-chiral columns, the retention time of dopa is shorter than that of 3-*O*-methyl-dopa [13–15]. Therefore, our results suggest that the chiral column changes the retention times of D-dopa and D-3-*O*-methyl-dopa to the different magnitudes leading to a reversal in their elution positions compared with non-chiral columns. The chiral column is more selective to DL-dopa than DL-3-*O*-methyl-dopa, because the resolution between D-dopa and L-dopa (3.6) is larger than that between D-3-*O*-methyl-dopa and L-3-*O*-methyl-dopa (2.1).

When performing chiral separation, several other amino acids also show the similar elution order as our results, i.e. D-first amino acid, D-second amino acid, L-second amino acid and L-first amino acid, such as alanine versus arginine, and valine versus methionine [16]. Other substances, such as α-antiaspartame also show different elution orders during the chiral separation [17]. All these substances contain an analogous alanine part in their structures, perhaps the chiral separation mechanism of DL-dopa and DL-3-*O*-methyl-dopa is likely to be similar to the separation of these substances.

When the hydrogen is substituted by the methyl group in case of dopa and 3-*O*-methyl-dopa, the resolution of DL-dopa is larger than that of DL-3-*O*-methyl-dopa. Therefore, the separation mechanism in the separation of DL-dopa and DL-3-*O*-methyl-dopa is likely to be the hydrogen bonding in the chiral column.

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