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Short communication

Enantiomeric separations of primary amino compounds by non-aqueous capillary zone electrophoresis with a chiral crown ether

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Abstract

The enantiomeric separation by capillary electrophoresis (CE) in non-aqueous media was examined. The enantiomeric separations of eight primary amino compounds including aromatic amines, amino acids and amino alcohols were achieved by CE in formamide containing a chiral crown ether, (+)-18-crown-6 tetracarboxylic acid. The addition of tetra-*n*-butylammonium perchlorate as a supporting electrolyte to the electrophoretic solution improved the separation efficiency and gave the baseline enantiomeric separation of DL-1-phenylethylamine which has not been separated by any other separation mode of CE yet.

Keywords: Enantiomer separation; Chiral crown ethers; Host–guest complexation; 1-Naphthylethylamine; 1-Phenylethylamine; Phenylalanine; DOPA; Tryptophan; Norephedrine; Noradrenaline; 2-Amino-1,2-diphenylethanol

1. Introduction

Capillary electrophoresis (CE) is an attractive separation technique because of its advantages such as high efficiency, short analysis time and minimal sample volume requirement. The principle of CE separation is based mainly on the differences in electrophoretic mobilities between compounds in a capillary tube. For the electrophoretic solution, aqueous media such as buffers have generally been used in order to obtain effective conductivity. Sometimes a small amount of organic solvent is added to control the electroosmotic flow and to improve the selectivity. The use of aqueous media limits CE applications to substances that are stable and soluble in water. Also, some substances can not be separated because they have very similar electrophoretic mobilities in aqueous media. To overcome these limitations, researchers have begun to examine CE in non-aqueous media [1–4].

The recognition of molecular chirality is one of the most important themes in recent pharmacology and toxicology. CE has played an important role in the tremendous advances in enantiomer separation made in the last decade. Many of these separations have been carried out using chiral selectors such as cyclodextrins [5,6], the chiral crown ether (+)-18-crown-6 tetracarboxylic acid ($18C6H_4$, as shown in Fig. 1) [7–10], or chiral surfactants [11–14]. But all separations have been performed in aqueous media.

In this paper, we report the successful results of the enantiomer separation of primary amino compounds in non-aqueous media containing $18C6H_4$ using capillary zone electrophoresis (CZE). We used

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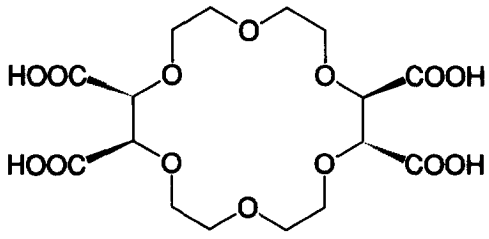

Fig. 1. Chemical structure of (+)-18-crown-6 tetracarboxylic acid.

formamide containing $18C6H_4$ as the electrophoretic solution and achieved the enantiomer separation of some primary amino compounds, including aromatic amines, amino acids and amino alcohols. We also found that the separation efficiency could be improved by adding tetra-*n*-butyl ammonium perchlorate (TBAP) as a supporting electrolyte.

2. Experimental

2.1. Apparatus

Electrophoresis was performed at room temperature (approximately 25°C) with a Jasco CE-800 system, which consisted of a ±30 kV high voltage power supply (890-CE) and a UV–Vis detector (870-CE) (Japan Spectroscopic, Tokyo, Japan). A Shimadzu C-R4AX (Kyoto, Japan) was used for data collection and manipulation. Capillary temperature was not controlled during the experiments. Untreated fused-silica capillary tubes (Otsuka Electronics, Osaka, Japan) with 75 μm I.D. (375 μm O.D.) were used as the separation columns. The total length of the capillary was 56 cm and detection was performed at 36 cm downstream. Each sample was dissolved in a solution with the same composition as the electrophoretic solution and introduced into the anodic end of the capillary by gravity, at a height of 10 cm for 5 s. A positive voltage of 20 or 30 kV was used for the electrophoretic separation. The detection wavelength was 260, 280 or 300 nm, depending on the absorbance spectrum of the each sample.

2.2. Chemicals

$18C6H_4$ was obtained from Merck (Darmstadt, Germany). Formamide and TBAP were purchased from Nakarai Tesque (Kyoto, Japan). The primary amino compounds tested for the enantiomeric separation and other organic solvents were obtained from various suppliers.

3. Results and discussion

Crown ethers having macrocyclic polyether ring systems are known to form stable inclusion complexes (host–guest complexes) with alkaline metals, alkaline earth metals, ammonium ion or protonated amines [15,16]. On the basis of this principle between host and guest, Kuhn et al. [7–9] succeeded in the enantiomeric separation of various primary amines in aqueous media containing $18C6H_4$ by CZE mode. The proposed mechanisms of the chiral recognition are as follows [7]: (1) the four optically active carboxylic acid groups act as chiral barriers to the guest molecules and (2) lateral electrostatic interactions occur between host and guest.

To establish which solvents would be the best for the enantiomeric separation by non-aqueous CE, we tried using ten organic solvents with various physicochemical properties as electrophoretic solutions to which 10 m*M* $18C6H_4$ was added. The enantiomeric separation of the test compound DL-1-naphthylethylamine was achieved only in formamide or in dimethylsulfoxide. The better separation of this compound with a suitable analysis time was achieved in formamide (separation factor [8], $\alpha=1.12$). Furthermore, the baseline separation of (1*S*/2*R*), (1*R*/2*S*)-2-amino-1,2-diphenylethanol was achieved under the same conditions, as shown in Fig. 2 (resolution [8], $R_s=2.31$; $\alpha=1.10$). Using the same condition, we were successful with the enantiomeric separation of eight primary amino compounds such as aromatic amines, amino acids and amino alcohols (Fig. 3, the range of α was 1.01–1.12).

In order to improve the separation efficiency, some electrophoretic conditions were examined. The enantiomeric separations of the examined compounds except for DL-norephedrine were improved with varying the concentration of $18C6H_4$ and/or adding a supporting electrolyte. The some electrophoretic data are given in Table 1. The addition of TBAP as the supporting electrolyte to the electrophoretic solution improved the separation efficiency

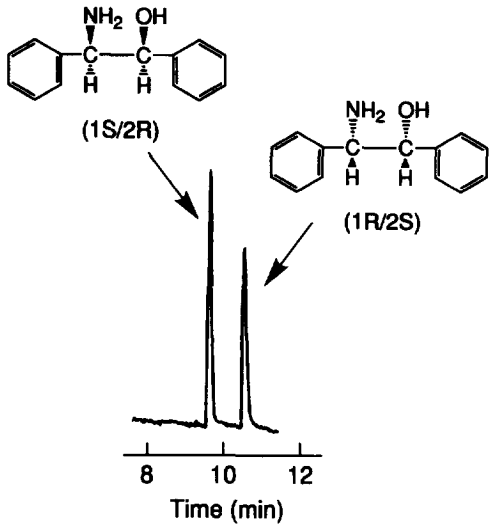

Fig. 2. Enantiomeric separation of (1*S*/2*R*), (1*R*/2*S*)-2-amino-1,2-diphenylethanol. Conditions: electrophoretic solution, formamide containing 10 m*M* 18C6H$_4$; detection wavelength, 300 nm; applied voltage (current), +20 kV (2 μA).

of most of compounds. For example, the α value for DL-1-naphthylethylamine increased from 1.12 to 1.24 with the addition of 100 m*M* TBAP to formamide containing 10 m*M* 18C6H$_4$ and that for (1*S*/2*R*), (1*R*/2*S*)-2-amino-1,2-diphenylethanol increased from 1.10 to 1.21 with the addition of 40 m*M* TBAP to formamide containing 10 m*M* 18C6H$_4$. We propose the mechanisms of these improvements as follows: (1) the hydrophobic interaction may occur between the *n*-butyl group of TBAP and the substituent(s) of the primary amino compounds and (2) the chiral recognition of 18C6H$_4$ may be enhanced with the substituent which partially behaves like a bulkier substituent than itself owing to this interaction. Furthermore, the addition of 100 m*M* TBAP to formamide containing 10 m*M* 18C6H$_4$ gave the baseline separation of DL-1-phenylethylamine (R_s= 2.80, α=1.10), as shown in Fig. 4. This compound has not been separated by CE in aqueous media containing 18C6H$_4$ [7,8] nor by any other enantiomeric separation mode of CE yet. Our additional proposals for the mechanisms of this successful enantiomeric separation are as follows: (1) the stability of the host–guest complex in the solvent which has a higher dielectric constant is lower than that in the solvent which has a lower dielectric constant because of the enthalpic effect [16]; (2) the stability of the 1-phenylethylamine-18C6H$_4$ complex in formamide (the dielectric constant, ε=111) may be lower than that in water (ε=78); (3) this lower stability may make the steric interaction between the optically active carboxylic acid groups of 18C6H$_4$ and the substituents of 1-phenylethylamine more effective for the enhancement of the difference in the stability constants between the enantiomers and (4) the *n*-butyl group of TBAP may help to improve the chiral recognition as described above. This result demonstrates one of the advantages of CE in non-aqueous media, that is, solvent effects on the enhancement of chiral recognition of chiral selectors could be expected.

Migration time reproducibility was examined for two selected compounds at +30 kV in formamide containing 10 m*M* 18C6H$_4$ and 2.5 m*M* TBAP. The relative standard deviations of the migration times for DL-1-naphthylethylamine and (1*S*/2*R*), (1*R*/2*S*)-2-amino-1,2-diphenylethanol were 3.5 and 1.5%, respectively, over the course of six injections each. The migration time reproducibility would be improved by using a more sophisticated CE instrument with a capillary thermostating system.

4. Conclusion

We achieved the enantiomeric separations of eight primary amino compounds by CZE using formamide containing 18C6H$_4$. Varying the concentration of 18C6H$_4$ and/or adding TBAP improved the separation efficiency of most of the examined compounds. Among these compounds, DL-1-phenylethylamine, which has not been separated by CE in aqueous media nor by any other enantiomeric separation mode of CE yet, was separated when TBAP was added as a supporting electrolyte to the electrophoretic solution. This result demonstrates one of the advantages of CE in non-aqueous media.

The chiral selector used in this paper, 18C6H$_4$, is soluble in both aqueous and non-aqueous media. Since non-aqueous media can dissolve various additives that are insoluble or much less soluble in aqueous media, CE in non-aqueous media with such additives is a way to improve selectivity. In addition, the enantiomeric separation by non-aqueous CE

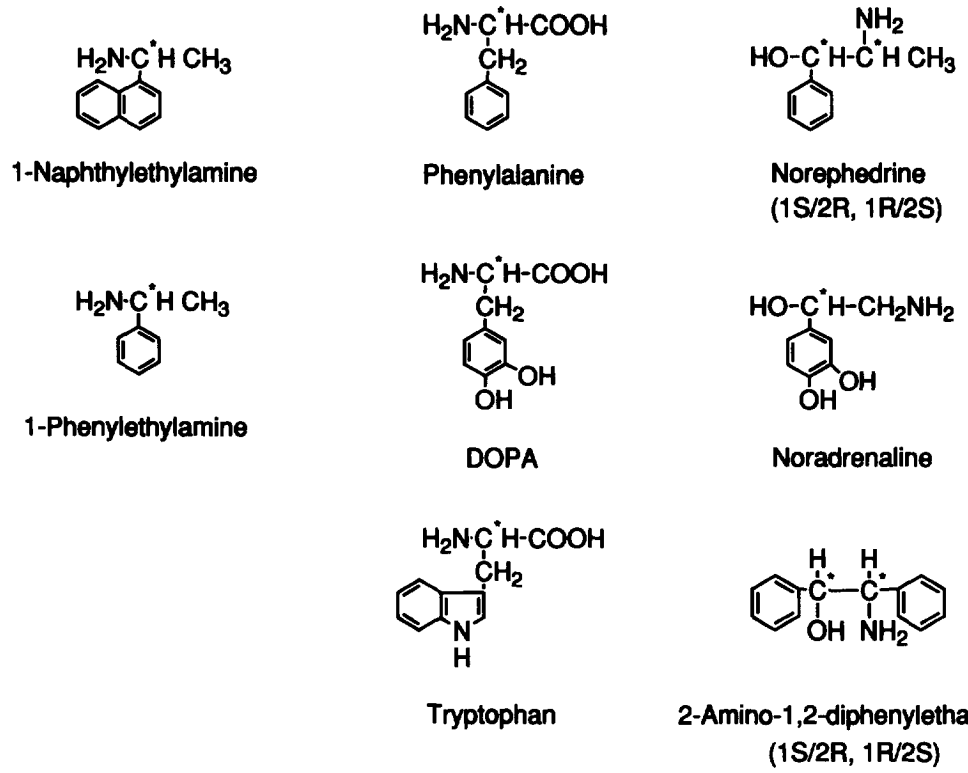

Fig. 3. Chemical structures of the optically active primary amino compounds separated by non-aqueous CZE mode using $18C6H_4$ as a chiral selector.

Table 1
Electrophoretic data for the analytes separated by non-aqueous CZE mode using $18C6H_4$ as a chiral selector

Compound	Condition[a]		Migration time (min)		α[b]
	$18C6H_4$ (mM)	TBAP (mM)	t_{M1}	t_{M2}	
Aromatic amines					
1-Naphthylethylamine	10	100	49.49	61.48	1.24
1-Phenylethylamine	10	100	25.95	28.42	1.10
Amino acids					
Phenylalanine	25	2.5	22.80	23.37	1.03
DOPA	25	2.5	23.53	24.18	1.03
Tryptophan	50	2.5	22.47	23.29	1.04
Amino alcohol					
Norephedrine	10	–	15.07	16.56	1.10
Noradrenaline	2.5	2.5	11.69	12.13	1.04
2-Amino-1,2-diphenylethanol	10	40	21.73	26.23	1.21

[a] Other conditions: capillary, fused-silica capillary (75 μm I.D.; effective length, 36 cm; total length, 56 cm); solvent, formamide; applied voltage, +20 kV.

[b] $\alpha = t_{M2}/t_{M1}$: where t_{M1} is the migration time of the first eluted enantiomer and t_{M2} is the migration time of the antipode.

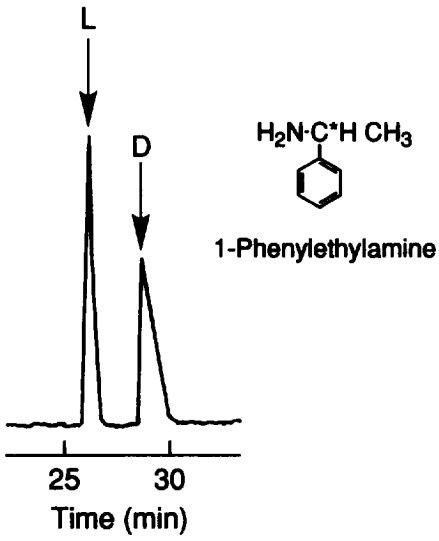

Fig. 4. Enantiomeric separation of DL-1-phenylethylamine. Conditions: electrophoretic solution, formamide containing 10 m*M* $18C6H_4$ and 100 m*M* TBAP; detection wavelength, 260 nm; applied voltage (current), +20 kV (32 μA).

combined with a mass spectrometric analysis system should be useful for the determination and the structural analysis of optically active drugs and their metabolites in biological fluids, as well as unstable or insoluble substances in aqueous media.

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