Chiral Discrimination of Aromatic Amino Acids by Capillary Electrophoresis in (+)- and (−)-(18-Crown-6)-2,3,11,12-tetracarboxylic Acid Selector Modes

Wonjae Lee,† Sookie La, Youngmie Choi, and Kyoung-Rae Kim*

College of Pharmacy, Sungkyunkwan University, Suwon 440-746, Korea
†College of Pharmacy, Chosun University, Gwangju 501-759, Korea
Received May 26, 2003

Key Words : Aromatic amino acids, Chiral capillary electrophoresis, 18-Crown-6-tetracarboxylic acids

Optically active (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid (18C6H4) (Figure 1) has been successfully employed as a chiral selector in enantioseparations of diverse amino acids and chiral primary amines in capillary electrophoresis (CE).[1-7] The host-guest complexation mechanisms for the chiral recognition of crown ethers toward chiral amines have been well established.[2,6] The accurate chiral discrimination of each separated analyte has become an important task for their optical purity control and stereoselective pharmacokinetic studies in chiral drug development. The migration order of separated enantiomers is mainly determined by co-electrophoresis with enantiomerically pure standards and matching its migration time or relative migration time (RMT) with that of the reference. However, crosschecking two RMT sets measured with two chiral selectors of different enantioselectivities could enhance confidence in the identification of CE peaks and the chiral discrimination as well. Based on this concept, simultaneous chiral discrimination of 15 racemic aromatic amino acids was achieved with chiral CE in neutral and charged cyclodextrin modes in our previous report.[8] The surest way of the accurate chiral discrimination is the reversal of enantiomer migration order (EMO).[9-16] It is especially desirable when peak tailing, fronting or overlapping is observed. Among the diverse ways of EMO reversal developed, suppressing or reversing the direction of electroosmotic flow has been most widely used.[9,12-16] In our recent report, simultaneous EMO reversal of nine chiral profens was achieved in the normal polarity and reversed polarity modes.[16] The most certain and easy method of EMO reversal might be, however, the use of two chiral selectors with the opposite chiral recognition ability, like (+)-18C6H4 and (−)-18C6H4 when employing crown ether-modified CE systems. However, no attempt to exploit the crosschecking each EMO measured in these two selector modes has been made in chiral CE of amino acids to date. The present study was undertaken to investigate EMO reversal by crown ether modified-CE system in the two selector modes with (+)-18C6H4 and (−)-18C6H4 for the chiral discrimination of nine aromatic amino acids.

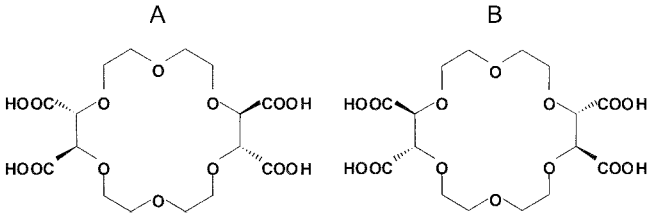

Figure 1. The chemical structures of (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid (A) and (−)-(18-crown-6)-2,3,11,12-tetracarboxylic acid (B) used as chiral selectors in this study.

When (+)-18C6H4 was added at 5 mM to 20 mM Tris-citric acid buffer (pH 2.50), each enantiomeric pairs of nine aromatic amino acids studied were baseline-resolved with similar enantioselectivity and resolution factor except for 3-hydroxyphenylglycine (Table 1). 3-Hydroxyphenylglycine showed much stronger interaction with the selector than other analytes, thereby yielding a very large separation factor (1.684) and resolution factor (21.99). In good agreement with the previous reports.[1,2,4] each D-enantiomer migrated slower than the corresponding L-isomer, indicating that the D-isomers were bound more strongly with (+)-18C6H4 in CE. The (*R*)-baclofen migrated ahead of the (*S*)-enantiomer. When (+)-18C6H4 was replaced in the same buffer condition with its antipode, (−)-18C6H4 (Figure 1), the EMO of each analyte was correctly reversed (Table 1). The L-enantiomers migrated slower than corresponding D-forms, indicating that the L-forms were bound more strongly with (−)-18C6H4 in CE. Unexpectedly, all separation factors and resolution factors were, however, lowered with decrease in their migration times compared to those in the (+)-18C6H4 selector mode (Table 1). The optical purity of (−)-18C6H4 might be responsible for these observations since enantioselectivity of a selector decreases as its optical purity lowers. The optical rotation of (−)-18C6H4 used for this study was slightly smaller than that of (+)-18C6H4, suggesting its lower optical purity than that of (+)-18C6H4.[17] The typical dual-electropherograms of three amino acids measured in the two selector modes demonstrated that they were complements each of the other in the accurate chiral discrimination (Figure 2). To our knowledge, crosschecking each EMO measured in the two selector modes of (+)-18C6H4 and (−)-18C6H4 was first exploited in the present study. Further optimization of the present crown ether-modified CE systems is under way to include other chiral amino acids so

*Correspondence. Tel: +82-31-290-7703; Fax: +82-31-292-8800; E-mail: krkim@skku.edu

Table 1. Enantioseparations of nine aromatic amino acids by CE in (+)-18C6H$_4$ and (−)-18C6H$_4$ selector modes

Analytes	(+)-18C6H$_4$ selector			(−)-18C6H$_4$ selector		
	MT[a]	α[b]	R_s[c]	MT	α	R_s
L-Tryptophan	12.67			12.50		
D-Tryptophan	12.95	1.022	1.60	12.25	1.020	1.46
(*R*)-Baclofen	12.93			10.53		
(*S*)-Baclofen	13.85	1.071	3.12	10.15	1.037	1.91
L-5-Hydroxytryptophan	12.97			13.12		
D-5-Hydroxytryptophan	13.28	1.024	1.77	12.83	1.023	1.47
L-Phenylalanine	13.70			13.47		
D-Phenylalanine	13.98	1.020	2.02	13.22	1.019	1.11
L-Tyrosine	13.90			13.67		
D-Tyrosine	14.20	1.022	1.43	13.47	1.015	0.87
L-Homophenylalanine	14.22			14.48		
D-Homophenylalanine	14.88	1.046	4.24	13.97	1.037	2.40
L-3-Methoxytyrosine	14.63			14.85		
D-3-Methoxytyrosine	15.07	1.030	1.77	14.48	1.026	1.65
L-3,4-Dihydroxyphenylalanine	14.93			14.65		
D-3,4-Dihydroxyphenylalanine	15.43	1.033	2.46	14.37	1.019	1.36
L-3-Hydroxyphenylglycine	27.23			30.98		
D-3-Hydroxyphenylglycine	45.85	1.684	21.99	23.00	1.347	11.68

CE conditions: uncoated fused silica capillary (470 mm × 50 μm ID; 394 mm to detector window); 20 mM Tris-citric acid (pH 2.50) run buffer containing 5 mM (+)-18C6H$_4$ or (−)-18C6H$_4$; 23.5 kV; UV 200 and 210 nm; temperature 20 °C. [a]Migration time (min). [b]Separation factor. [c]Resolution factor.

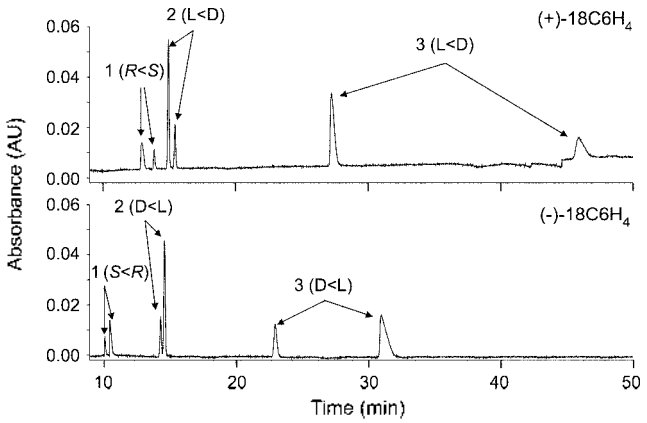

Figure 2. Enantioseparations of three enantiomerically enriched (3 : 1) amino acids in (+)-18C6H$_4$ (top) and (−)-18C6H$_4$ (bottom) selector modes. Peaks: 1 = baclofen; 2 = 3,4-dihydroxyphenylalanine; 3 = 3-hydroxyphenylglycine. See Table 1 for experimental conditions.

that they can be used as a chiral discrimination tool for optical purity tests of amino acid drugs.

Experimental Section

CE analyses were performed with a Beckman P/ACE^{TM} 5500 CE system (Beckman-Coulter, Fullerton, CA, USA), interfaced to a Gold version 8.12 data station and equipped with a photo diode array detector, an automatic injector and an uncoated fused silica capillary (Polymicro Technologies, AZ, USA; 470 mm × 50 μm ID; 394 mm to detector window) installed inside a fluid-cooled column cartridge. Samples (*ca.* 3 nL) were introduced in pressure injection mode for 3 sec at 3.4 kPa to the capillary maintained at 20 °C. The run buffer was 20 mM Tris-citric acid (pH 2.50) containing 5.0 mM of (+)-18C6H$_4$ or (−)-18C6H$_4$. The applied voltage along the capillary was 23.5 kV. The UV absorbance was measured at 200 and 210 nm, and the absorption spectra from 190 to 390 nm. The capillary was rinsed between runs sequentially with water, 0.1 M NaOH and water for 3 min, and finally with buffer solution for 2 min. All analytes used in this study were available from prior studies.[8] (+)-and (−)-18C6H$_4$ were purchased from Fluka and Aldrich, respectively. A racemic mixture of baclofen, 3,4-dihydroxyphenylalanine and 3-hydroxyphenylglycine was enriched with enantiomerically pure (*R*)-baclofen, L-forms of 3,4-dihydroxyphenylalanine and 3-hydroxyphenylglycine in excess (3 : 1).

Acknowledgement. This study was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (02-PJ2-PG3-21503-0006).

References

1. Kuhn, R.; Stoecklin, F.; Erni, F. *Chromatographia* **1992**, *33*, 32.
2. Kuhn, R.; Erni, F.; Bereuter, T.; Hausler, J. *Anal. Chem.* **1992**, *64*, 2815.
3. Kuhn, R.; Wagner, J.; Walbroehl, Y.; Bereuter, T. *Electrophoresis* **1994**, *15*, 828.
4. Nishi, H.; Nakamura, K.; Nakai, H.; Sato, T. *J. Chromatogr. A* **1997**, *757*, 225.
5. Verleysen, K.; Vandijck, J.; Schelfaut, M.; Sandra, P. *J. High Resol. Chromatogr.* **1998**, *21*, 323
6. Kuhn, R. *Electrophoresis* **1999**, *20*, 2605.
7. Cho, S. I.; Jung, H.; Chung, D. S. *Electrophoresis* **2000**, *21*,

3618.
8. La, S.; Ahn, S.; Kim, J.-H.; Goto, J.; Choi, O.-K.; Kim, K.-R. *Electrophoresis* **2002**, *23*, 4123.
9. Chankvetadze, B.; Schulte, G.; Blaschke, G. *J. Chromatogr. A* **1996**, *732*, 183.
10. Katayama, H.; Ishihama, Y.; Asakawa, N. *J. Chromatogr. A* **1997**, *764*, 151.
11. Chankvetadze, B.; Schulte, G.; Blaschke, G. *J. Chromatogr. A* **1997**, *771*, 259.
12. Chankvetadze, B. G. *J. Chromatogr. A* **1997**, *792*, 269.
13. Katayama, H.; Ishihama, Y.; Asakawa, N. *J. Chromatogr. A* **2000**, *875*, 315.
14. Chankvetadze, B. *Electrophoresis* **2002**, *23*, 4022.
15. Abusgoffa, A.; Fillet, M.; Servais, A.-C.; Hubert, P.; Crommen, J. *Electrophoresis* **2003**, *24*, 343.
16. La, S.; Kim, J.; Kim, J.-H.; Goto, J.; Kim, K.-R. *Electrophoresis* **2003**, in press.
17. The optical rotation of (+)-18C6H$_4$ purchased from Fluka and (−)-18C6H$_4$ purchased from Sigma-Aldrich was +70° and −65°, respectively.