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Note

Enantioseparation using cyclosophoraoses as a novel chiral additive in capillary electrophoresis

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Abstract

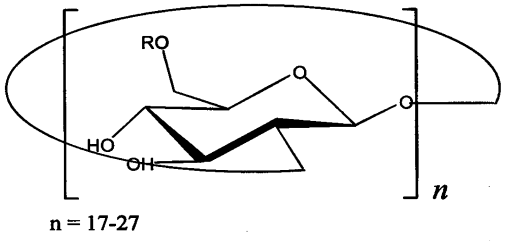
Cyclosophoraoses, cyclic β-(1→2)-D-glucans produced by *Rhizobium meliloti* 2011, were used as a novel chiral additive for the separation of terbutaline, amethopterin, thyroxine and *N*-acetylphenylalanine enantiomers in aqueous capillary electrophoresis (CE). Enantioseparation took place in the normal- or reversed-polarity mode when a high concentration of neutral (60 mM) or anionic (40 mM) cyclosophoraoses was added to the background electrolyte (BGE).

Keywords: Cyclosophoraoses; *R. meliloti* 2011; Enantiomeric separation; Capillary electrophoresis; Chiral additive

Chirality remains an important consideration for many compounds such as pharmaceuticals, food additives and agrochemicals. Enantiomeric separation by capillary electrophoresis (CE) has rapidly attracted attention as a promising technique because of its high efficiency, low cost, wide range of separation mechanisms, simplicity and flexibility, and very low consumption of buffer and samples. Recent capillary electrophoretic literature focuses on the utilization of chiral selectors in the background electrolyte (BGE) for the resolution of enantiomers.[1]

Cyclosophoraoses are a family of unbranched cyclic β-(1→2)-D-glucans produced as intra- or extraoligosaccharides by many strains of *Rhizobium* and *Agrobacterium* as a mixture of molecules with various sizes in a neutral or anionic form.[2] Recent reports have shown that cyclosophoraoses have potential as a complexation host for the solubility enhancement of poorly soluble guest molecules.[3b–d] Though the exact three-dimensional structure of cyclosophoraose is not known, recent nuclear magnetic resonance (NMR) studies[5] and molecular dynamics simulations[5,6,15,17] have provided molecular models with flexible glycosidic linkage backbones. The glucopyranose unit of neutral and anionic cyclosophoraoses composed of glycosidic β-(1→2) linkage is shown in Fig. 1. Recently, we reported that these macrocyclic molecules function as a chiral NMR solvating agent.[6] To the best of our knowledge, there have been no reports on the separation of enantiomers using cyclosophoraoses as a chiral additive in CE.

Herein, we describe for the first time that a family of cyclosophoraoses functions as a chiral selector in CE for enantiomeric separations of the antiasthmatic drug, terbutaline, the antineoplastic agent, amethopterin, thyroxine hormone, and an α-amino acid derivative, *N*-acetylphenylalanine.



n = 17-27

R = H; neutral cyclosophoraose
R = $PO_4CH_2CHOHCH_2OH$, or H; anionic cyclosophoraose

Fig. 1. The glucopyranose unit of neutral and anionic cyclosophoraoses.

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Table 1
Retention time (t_R), separation factor (α) and resolution (R_s) of enantiomers resolved by adding neutral [a] or anionic [b] cyclosophoraoses

Enantiomers	Migration time (min) t_R [c]	α	R_s
Terbutaline	5.06 [a]	1.07	3.9
Amethopterin	1.64 [a]	1.48	11.2
Thyroxine	9.91 [a]	1.02	13.6
Thyroxine	28.85 [b]	1.13	18.3
N-Acetylphenylalanine	5.95 [b]	1.14	8.4

[a] 60 mM neutral cyclosophoraoses were added to the BGE.
[b] 40 mM anionic cyclosophoraoses were added to the BGE.
[c] Migration time of the first enantiomers eluted from capillary column.

Structural analyses of the isolated neutral and anionic cyclosophoraoses were carried out as described previously.[3b–f] The ring sizes of the neutral[3b] or anionic[3e] cyclosophoraoses were confirmed through matrix-assisted laser desorption ionization-mass spectrometry (MALDI-MS) and electrospray ionization-mass spectrometry (ESI-MS) analyses, ranging from DP 17 to 27, respectively. Based on the MALDI-MS and ESI-MS analyses, the number-average molecular weight (M_n) of the neutral and anionic cyclosophoraoses was determined as 3568.6[3d] and 3940.2,[3e] respectively. Based on the previous results,[3e,18] we confirmed that the anionic cyclosophoraoses used in the present study contained the phosphoglycerol molecules as the substituent at C-6 of the glucose residue as shown in Fig. 1.

As pointed out by Wren and Rowe,[7] weakly interacting analytes with a chiral additive typically require higher concentrations of the chiral additive than strongly interacting ones. However, the longer migration time accompanying higher concentrations of a chiral additive may also limit the utility of the chiral additive. To overcome this problem, negatively charged chiral addtives have been predominantly employed to the effective separations in reversed polarity mode.[8–10] As the representative chiral additives, various cyclomaltooligosaccharides (cyclodextrins, CDs), which contain α-(1→4)-glycosidically linked glucopyranose units, and their derivatives have been employed.[12]

Herein, we performed effective separations of the enantiomers with cyclosophoraoses used as a novel chiral additive in the normal/reversed polarity mode (Table 1 and Fig. 2). Intrinsic high solubility[3a] of cyclosophoraoses in aqueous solution gave benefit to the CE experiment.

In the case of racemic terbutaline, a resolution (R_s) value of 3.9 was obtained with 60 mM neutral cyclosophoraoses added to the acidic (pH 3.0) running buffer (separation factor (α) = 1.07, Table 1) in the normal polarity mode. It has been reported that the chiral additives such as CD derivatives containing ionizable sulfate groups,[10] nonionizable dimethyl[19] and hydroxypropyl groups[11] as well as native β-CD[19] provide moderate enantiomeric resolutions for racemic terbutaline in CE. In the present study, the racemic terbutalines were separated into each enantiomer by adding neutral cyclosophoraoses to the BGE.

Racemic amethopterin was known to be moderately resolved by macrocyclic antibiotics as chiral additives in CE.[12,13] In the present study, chiral separation occurred with much higher enantioselectivity ($\alpha = 1.48$, $R_s = 11.2$, Fig. 2A) in the normal polarity mode when the 60 mM neutral cyclosophoraoses were added as a chiral additive to the BGE. The (+)-enantiomer of amethopterin migrated before the (−)-enantiomer.

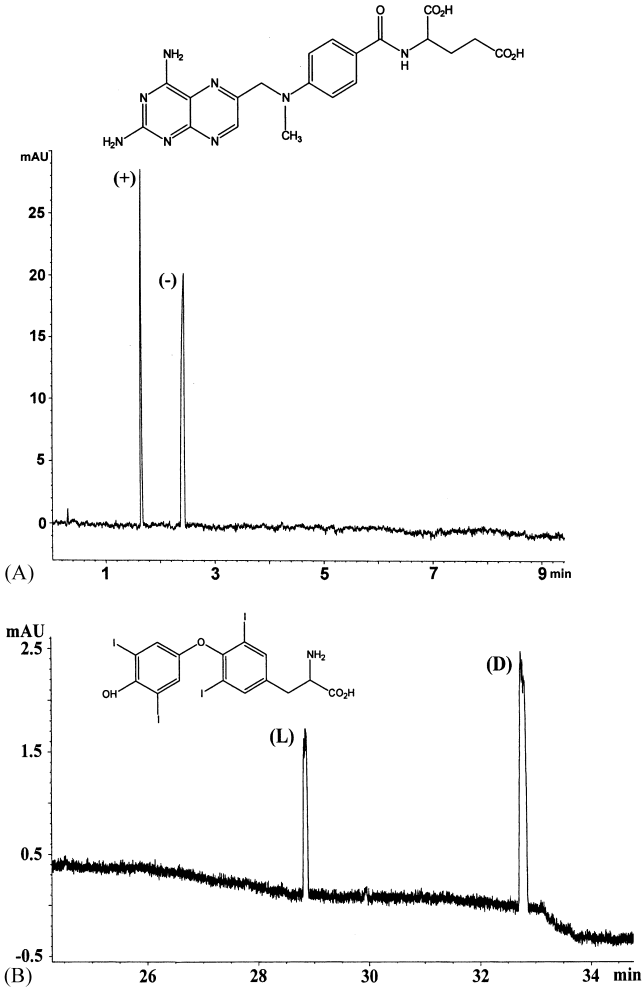

Fig. 2. Electropherograms showing enantiomeric separations induced by neutral or anionic cyclosophoraoses used as a novel chiral additive in CE. (A) Enantiomeric separation of a nonracemic mixture of amethopterin ((+):(−) = 3:2)) induced by 60 mM neutral cyclosophoraoses in the normal polarity mode at 10 kV. (B) Enantiomeric separation of nonracemic mixture of thyroxine (D:L = 1:2) induced by 40 mM anionic cyclosophoraoses in the reversed polarity mode, pH 3.0 at −10 kV.

The enantiomers of thyroxine were known to be resolved by using anionic CDs as chiral additives in CE.[14] Likewise, thyroxine enantiomers were separated with a very high resolution ($\alpha = 1.13$, $R_s = 18.3$) by adding 40 mM anionic cyclosophoraoses to the BGE in the reversed polarity mode (Table 1 and Fig. 2B). This enantiomers were also fully resolved ($\alpha = 1.02$, $R_s = 13.6$) in the normal polarity mode with 60 mM neutral cyclosophoraoses added as a chiral additive (Table 1). The L enantiomer of thyroxine migrated before the D enantiomer by adding either neutral or anionic cyclosophoraoses.

In the previous study, we showed that the *N*-acetylphenylalanine enantiomers were discriminated throughout the NMR analysis by neutral cyclosophoraoses in aqueous solution.[4] A nonracemic mixture (D:L = 2:3) of this α-amino acid derivative was also resolved in a CE experiment at the reversed polarity mode ($\alpha = 1.14$, $R_s = 8.4$, Table 1).The *N*-acetyl-D-phenylalanine enantiomer migrated before the L enantiomer. The migration order of each enantiomer in CE could explain the extent of the interaction of cyclosophoraoses with each enantiomer as shown in the previous NMR data.[4]

Throughout the present investigation, we showed that a family of neutral or anionic cyclosophoraoses was successfully utilized as novel chiral additives for enantioseparation in CE. Cyclosophoraoses seemed to provide the capacity for the required difference in both the binding of the enantiomers and the appropriate mobility. They shortened the analysis time as well as greatly increased the efficiency of enantioseparation of the analytes in the normal or reversed polarity mode. Although the exact molecular mechanism of the chiral separation by cyclosophoraoses remains to be elucidated, we postulate that the chiral recognitions are likely to be induced by the portions associated with the β-glycosidic linkages of cyclosophoraoses as we described previously.[6]

1. Experimental

1.1. Chemicals and reagents

All chemicals, including enantiomers or racemates of terbutaline, amethopterin, thyroxine and *N*-acetylphenylalanine used in this study were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

1.2. Preparation of neutral and anionic cyclosophoraoses

R. meliloti 2011 was cultured in a 5-L jar fermenter containing GMS medium as previous report.[16] Isolation and purification of neutral and anionic cyclosophoraoses were achieved as our previous reports.[3b–f]

1.3. Structural analyses of neutral and anionic cyclosophoraoses

The structure and molecular weight of neutral and anionic cyclosophoraoses was confirmed through NMR spectroscopy,[3c–e] ESI-MS[3e] and MALDI-MS[3b] analyses as our previous reports.

1.4. Capillary electrophoretic conditions

All CE experiments were performed using a Agilent 3D CE System (Wilmington, DE, USA) equipped with a diode array detector. Separations were carried out with uncoated fused-silica capillary (50 μm i.d., 33 cm total length, effective length 24.5 cm). The polyimide coating of the capillary was stripped to create a 0.5-cm detection window. The BGE consisted of an aqueous solution of 50 mM KH_2PO_4, adjusted to pH 3.0 with phosphoric acid. The sample solutions were prepared in the run buffer to give final concentrations of 0.02 μg mL^{-1} for terbutaline, 1.36 μg mL^{-1} for amethopterin, 2.33 μg mL^{-1} for thyroxine and 1.0 μg mL^{-1} for *N*-acetylphenylalanine, respectively. The analytes were monitored at 211 nm for terbutaline, at 201 nm for amethopterin, at 220 nm for thyroxine, and at 205 nm for *N*-acetylphenylalanine, respectively. The neutral or anionic cyclosophoraoses were used as chiral additives for enantioseparation of terbutaline, amethopterin, thyroxine and *N*-acetylphenylalanine in the normal/reversed polarity mode at ± 10 kV. The samples were injected by a pressure of 5 kPa for 1–3 s. Before each analysis, the capillary was rinsed for 2 min with water and 3 min with the BGE solution. The capillary was thermostated at 20 °C.

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