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Short communication

Enantiomeric resolution of anionic *R*/*S*-1,1′-binaphthyl-2,2′-diyl hydrogen phosphate by capillary electrophoresis using anionic cyclodextrin derivatives as chiral selectors

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Abstract

The enantioseparation of racemic 1,1′-bi-2-naphthol (1), 1,1′-binaphthyl-2,2′-diyl hydrogen phosphate (2) and 1,1′-binaphthyl-2,2′-diamine (3) using native β-cyclodextrin (β-CD) and its anionic derivatives such as carboxymethyl-β-CD (CM-β-CD), sulfoethyl ether of β-cyclodextrin (SEE-β-CD) and sulfobutyl ether of β-cyclodextrin (SBE-β-CD) has been studied. The successful resolution of 2 in the anionic form using negatively charged cyclodextrin derivatives shows that the role of the Coulombic interactions is not critical for the chiral guest–host recognition in capillary electrophoresis (CE). The application of SEE-β-CD as a chiral selector in CE has been demonstrated for the first time. Comparison of the enantioseparation efficiencies of SEE-β-CD and SBE-β-CD shows that the spacer length and the substitution pattern are factors with rather low importance and that the resolution efficiency is mainly determined by the counter-current mobility of the chiral selector.

1. Introduction

Cyclodextrins (CDs) belong to the most commonly used chiral selectors in analytical chemistry, particularly for gas chromatographic (GC), high-performance liquid chromatographic (HPLC), supercritical fluid chromatographic (SFC) and capillary electrophoretic (CE) enantioseparations. The role of electrostatic forces has been recognized to be very important for the chiral selector–selectand interactions with charged cyclodextrins in CE [1–3,5]. Therefore neutral chiral selectors or chiral selectors carrying a charge opposite to that of the racemic solutes are commonly used for CE enantioseparations. In our previous study the CE enantioseparation of the neutral thalidomide molecule was achieved using very low concentrations of the anionic SBE-β-CD as a chiral selector [4]. No measurable electrostatic interactions exist between the neutral racemic guest and the ionic

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chiral host. This result prompted us to investigate the possibility of chiral recognition in pairs where the racemic solute and the chiral selector both are negatively charged.

2. Experimental

2.1. Equipment

A Grom capillary electrophoresis system 100 (Herrenberg, Germany), equipped with a Linear Instruments (Reno, NV, USA) UVIS 200 detector and a HP 3396 A integrator (Hewlett-Packard, Avondale, PA, USA) was used with (a) an untreated fused-silica capillary (Grom) 41 cm effective length × 50 μm I.D and (b) a polyacrylamide coated capillary with 41 cm effective length × 50 μm I.D. Electric field strength was 400 V/cm, temperature 21 ± 1°C. The racemic samples were introduced hydrostatically (10 cm) for 5 s at the anodic end. Detection of the solutes was carried out at 210 nm. The anode and cathode buffers had the same pH and molarity as the run buffer but contained no chiral selector.

2.2. Chemicals and reagents

The racemic compounds 1,1′-bi-2-naphthol (1), 1,1′-binaphthyl-2,2′-diyl hydrogen phosphate (2) and 1,1′-binaphthyl-2,2′-diamine (3) were purchased from Aldrich (Aldrich-Chemie, Steinheim, Germany). Tris(hydroxymethyl)-aminomethane (Trizma base) was from Sigma (Sigma, St. Louis, MO, USA). Methacrylic acid-3-trimethoxysilylpropylester, Tris-borate-EDTA-buffer, acrylamide, ammoniumpersulfate and N,N,N′,N′-tetramethylethylendiamine used for preparation of polyacrylamide coated capillaries following the procedure described in Ref. [6] were all from Fluka (Fluka Chemie, Buchs, Switzerland). SBE-β-CD (substitution degree approx. 3.14, $M_r = 1684$) was a gift from Prof. J.F. Stobaugh and Prof. V.J. Stella (Center for Drug Delivery Research, The University of Kansas, Lawrence, KS, USA), SEE-β-CD (substitution degree approx. 0.4) and CM-β-CD (substitution degree approx. 0.3) were gifts from Wacker Chemie (Munich, Germany). Analytical grade KH_2PO_4, Na_2HPO_4, H_3PO_4 and NaOH were purchased from Merck (Darmstadt, Germany).

2.3. Buffer and sample preparation

Stock solutions of 50 m*M* KH_2PO_4 and 30 m*M* benzoic acid were prepared in double distilled, deionized water. The pH was adjusted with 0.5 *M* H_3PO_4 or 0.5 *M* NaOH for phosphate buffer and 0.1 m*M* Trizma base for the benzoic acid buffer. The run buffers for the chiral separations were prepared accordingly after the addition of appropriate amounts of the chiral selectors. All solutions were filtered and degassed by sonification before use. Stock solutions of 1 mg/ml of the racemic compounds 1, 2 and 3 were stored at 4°C and diluted to 60 μg/ml before use. CD derivatives were injected into the capillary as 10 mg/ml solutions in the 30 m*M* benzoic acid buffer. The composition and the electrophoretic profile of the chiral anionic selectors were characterized using a polyacrylamide coated capillary. The latter was used to exclude the contribution of electroendoosmotic flow (EOF) in the total mobility of the cyclodextrin derivatives. The 30 m*M* benzoic acid buffer at pH 6.0 was used to detect the CD derivatives at 254 nm (indirect detection mode, due to the shift of the UV absorption of the benzoic acid in the cyclodextrin complexes).

3. Results and discussion

All anionic CD derivatives used are heterogenous mixtures of components with different degrees of substitution and as a consequence with different anionic mobilities at pH 6.0 characterized by CE in a polyacrylamide coated capillary (Fig. 1).

The enantioseparations were carried out using all four chiral selectors at pH 3.0 and 6.0 to ensure that both 2 and 3 are resolved as anionic and cationic species respectively. The existence of 2 in an anionic form under the experimental

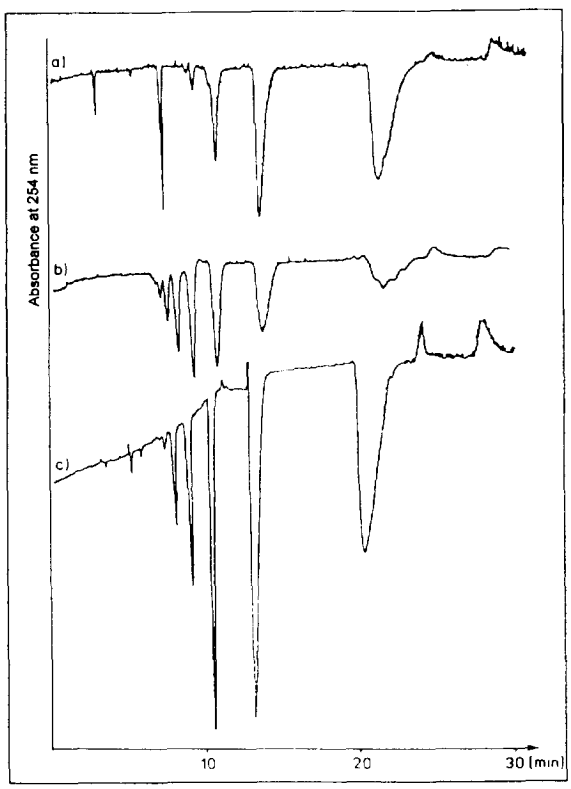

Fig. 1. Electropherograms of CM-β-CD (a), SBE-β-CD (b) and SEE-β-CD (c) used as chiral selectors. Polyacrylamide coated fused-silica capillary with 41 cm effective length was filled with 30 m*M* benzoic acid buffer at pH 6.0. Solutions (5 m*M*) of the CD derivatives were injected hydrostatically on the cathodic end and detected using indirect detection technique at 254 nm. The field strength was maintained at −345 V/cm.

conditions used, but without cyclodextrins, has been additionally confirmed by comparison of the mobility with the EOF, which has been measured as the migration time of the neutral marker mesityl oxide (MSO). The migration times of MSO and compound 2 were 6.2 and 21.3 min, respectively.

The results of the enantioseparations are shown in Fig. 2 and Table 1. These results illustrate that contrary to the expectations the anionic chiral selector SBE-β-CD more effectively separates the enantiomers of the negatively charged compound 2 than those of the corresponding neutral analyte 1 at pH 6.0. This result was confirmed with the other negatively

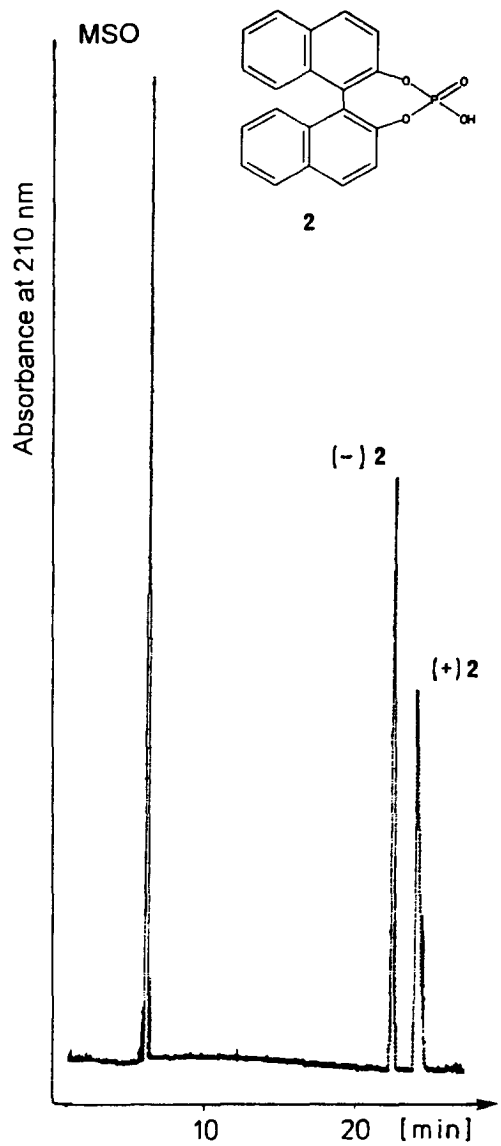

Fig. 2. Electropherogram of 1,1′-binaphthyl-2,2′-diyl hydrogen phosphate 2. Uncoated fused-silica capillary with 43 cm effective length was filled with 0.5 m*M* SBE-β-CD/50 m*M* phosphate buffer at pH 6.0. The field strength was maintained at 400 V/cm. The sample was injected hydrostatically at the anode. The EOF was measured as the migration time of mesityl oxide (MSO).

charged CD derivatives SEE-β-CD and CM-β-CD. Amine 3 exists at pH 6.0 as a neutral substance and is resolved as such. However the enantioseparation is more efficient at pH 3.0.

Table 1
Enantioseparation parameters

Solute	pH	α_{rel}			
		SBE-β-CD	SEE-β-CD	CM-β-CD	β-CD
1	6.0	poor chiral recognition			no separation
2	6.0	1.03	1.02	1.06	no separation
3	3.0	1.05	1.04	1.14	1.09

The latter result can not necessarily be ascribed to a more stereospecific binding of the protonated form of 3 to SBE-β-CD in comparison with the neutral form. Another explanation is the strongly inhibited EOF at low pH while the EOF does not favor the enantioseparation [4,5]. The results given in Table 1 confirm that negatively charged CD-derivatives resolve the enantiomers not only of neutral and cationic, but also of anionic racemates.

In conclusion, the anionic cyclodextrin derivatives such as CM-β-CD, SEE-β-CD and SBE-β-CD exhibit a chiral recognition ability not only to positively charged and neutral analytes but also for negatively charged analytes. On the basis of this finding the role of the Coulombic interactions in the host–guest complexation using CD-hosts seems not to be critical. Additionally, SEE-β-CD has been used as a chiral selector in CE for the first time and it exhibited a chiral recognition ability comparable to that of SBE-β-CD (Table 1). These two derivatives have a different spacer length and degree of substitution but surprisingly they are characterized by a quite similar electrophoretic profile (Fig. 1). Also the CM-β-CD proved to be a mixture of several compounds. This result leads to the conclusion that the role of the spacer length and the substitution pattern seem to be factors of rather low importance and that the efficiency of the enantioseparation is determined mainly by the counter-current mobility of the chiral selectors as already emphasized in previous studies by us [4,7] and others [5,8].

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