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Short communication

Reversal of enantiomer elution order in capillary electrophoresis using charged and neutral cyclodextrins

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Abstract

Three different techniques for the reversal of the enantiomer elution order in capillary electrophoresis (CE) are reported in this paper. Intrinsic chiral recognition of the chiral selector remains the same in all cases and the reversal of enantiomer elution order is achieved by manipulation of the mobilities of the chiral selector and selectand. The mechanism of the reversal of the enantiomer elution order is described in detail for each particular case. In the first case a reversal of the enantiomer elution order is achieved by modification of the self-mobility of a chiral selector by introduction of an anionic substituent. In the second case the same effect was achieved by changing the pH of the separation buffer and in the third one by combination of the pH change and the reversal of polarity of the high-voltage supply.

Keywords: Enantiomer separation; Binaphthyldiyl hydrogenphosphate; Cyclodextrins

1. Introduction

In the chiral analysis of nonracemic mixtures of enantiomers it is always desirable to detect the minor enantiomer in front of the major one. Reversal of the enantiomer elution order is extensively studied in high-performance liquid chromatography, whereas only few papers are published concerning this phenomenon in capillary electrophoresis (CE) [1–9]. The most trivial way of a reversal of the enantiomer elution order universal for all separation techniques (GC, SFC, HPLC, CE) is the use of a chiral selector with the opposite configuration. This is not possible for cyclodextrins (CDs) which are natural macrocycles available in one enantiomeric form only. Therefore, search for alternative techniques is especially important when CDs are used as chiral selectors. Reversal of the enantiomer elution order of several dansylated amino acids was described by Tanaka and co-workers depending on the number and position of methyl substituents in CDs [1–3]. Several possibilities of reversal of elution order dependent on the type of capillary (coated or uncoated), the direction of the electroosmotic flow (EOF), the type and the concentration of the chiral selector and the pH of the separation buffer in combination with reversed polarity of the electrodes have been summarized [4–6]. Recently, a reversal of the enantiomer elution order of chiral 3,4-dihydro-2H-1-benzopyran derivative was also described [8]. This compound contains both phenolic hydroxy and tertiary amino groups and

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exists either in anionic or in cationic forms depending on the pH of the separation buffer and consequently possesses self-mobility to the direction of anode or cathode.

Three different types of reversal of the enantiomer elution order are described in this paper. The nature of the reversal of the enantiomer elution order is explained in detail in each case and rationales are proposed to cover a wide variety of chiral selectors and chiral compounds.

2. Experimental

2.1. Instrumentation

2.1.1. Capillary electrophoresis

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 an untreated fused-silica capillary (Grom) of 60 cm total length× 50 μm I.D. The sample was introduced hydrostatically (10 cm) during 5 s. Detection was carried out at 210 nm.

The selectivity of the enantioseparation was characterized with α_{rel} which is the ratio of the effective mobilities of the enantiomers as an average of two measurements.

2.1.2. NMR

^{1}H NMR, ^{13}C NMR, homonuclear correlated spectroscopy (HOMCOR), heteronuclear chemical shift correlation (HETCOR), attached proton test (APT) and distortionless enhancement by polarization transfer (DEPT) spectral analysis were carried out with a Varian Gemini 200 NMR-spectrometer at 200 MHz (^{1}H) and 50 MHz (^{13}C). $^{2}H_2O$+10% $C^{2}H_3O^{2}H$ was used as a solvent and a solution of tetramethylsilan (TMS) in tetrachloromethane served as external standard. Peak assignments of (±)-1,1′-binaphthyl-2,2′-diyl-hydrogen phosphate (±)-I in ^{1}H and ^{13}C NMR were performed using HOMCOR, HETCOR, APT and DEPT spectra.

2.2. Chemicals and reagents

Racemic (±)-1,1′-binaphthyl-2,2′-diyl-hydrogen phosphate (I) and optically pure enantiomers of I were purchased from Aldrich (Steinheim, Germany). For studying enantiomer elution order the racemic I was spiked with *S*-(+)-I to give a ratio of *S*-(+):*R*-(−)=2:1.

β-CD, hydroxypropyl-β-CD [HP-β-CD; average substitution degree (s.d.) approx. 4.2], permethyl-β-CD (PM-β-CD; s.d. approx. 1.8) and carboxymethyl ether of β-CD (CM-β-CD; s.d. approx. 2.1) were donated by Wacker Chemie (Munich, Germany). Sulfobutyl ether of β-CD (SBE-β-CD; s.d. approx. 4.0) was a gift from Prof. J.F. Stobaugh and Prof. V.J. Stella (Center for Drug Delivery Research, The University of Kansas, Lawrence, KA, USA), heptakis-2,3,6-trimethyl-β-CD (TM-β-CD) was purchased from Sigma (Sigma–Aldrich, Deisenhofen, Germany). Trimethylammonium salt of β-CD (TMA-β-CD) and 6-amino-β-CD (6-Am-β-CD; s.d. 1.0) were prepared as described [10,11]. Analytical grade KH_2PO_4, H_3PO_4, NaOH, $^{2}H_2O$ and $C^{2}H_3O^{2}H$ were obtained from Merck (Darmstadt, Germany).

2.3. Buffer and sample preparation

A stock solution of 50 m*M* KH_2PO_4 was prepared in double distilled, deionized water. The pH was adjusted to the desired value with 0.3 *M* H_3PO_4 or 0.3 *M* NaOH. The run buffers consisting of 90% phosphate buffer and 10% methanol were prepared in the same way after the addition of the appropriate amount of the chiral selectors. All solutions were filtered and degassed by sonication before use.

3. Results and discussion

3.1. Enantioseparation of I with CDs containing various substituents and the reversal of enantiomer elution order

The enantioseparation of chiral organic acids in CE is usually performed at neutral or basic pH, where they are negatively charged and possess an electrophoretic mobility towards the anode. Under these conditions in uncoated silica capillaries the

EOF directed towards the cathode is usually higher than the electrophoretic mobility of the chiral organic acid towards the anode. This enables the injection of an acidic solute on the anodic end and the detection on the cathodic end of the capillary. For example, the enantioseparation of I has been reported at pH 6.0 using sulfoethyl ether of β-CD (SEE-β-CD), SBE-β-CD and CM-β-CD as chiral selectors [12].

In contrast to pH 6.0, at pH 3.7 the EOF is very slow. At this pH compound I has to be injected on the cathodic end and to be detected on the anodic end of the capillary. As Table 1 shows, all CDs in this study resolved enantiomers of I at pH 3.7. The elution order of the enantiomers of I has been *R*-(−) before *S*-(+) for all CDs excluding of SBE-β-CD. However, as ^{1}H NMR studies show SBE-β-CD binds *S*-(+)-I stronger than *R*-(−)-I, similar to other CDs in this study (Fig. 1). The higher mobility of the multiple-negatively-charged chiral selector than the mobility of the anionic chiral substance in the same direction can be another reason of the observed reversal of an enantiomer elution order.

To confirm the higher self-mobility of SBE-β-CD in comparison to I at pH 3.7 the following experiments were performed. (a) The separation capillary was filled with a 2.5 mg/ml solution of SBE-β-CD and the selector free buffer was added in the cathodic vial. No enantioseparation of I was observed in this case (Fig. 2a). (b) Both the separation capillary and the cathodic vial were filled with a 2.5 mg/ml solution of SBE-β-CD in the buffer solution. The enantioseparation of I was observed in this experiment with the elution order *S*-(+)-I before *R*-(−)-I (Fig. 2b). (c) The separation capillary was filled with chiral selector free buffer and a 2.5 mg/ml solution of SBE-β-CD in the buffer was added only in the cathodic vial. The enantioseparation observed in this

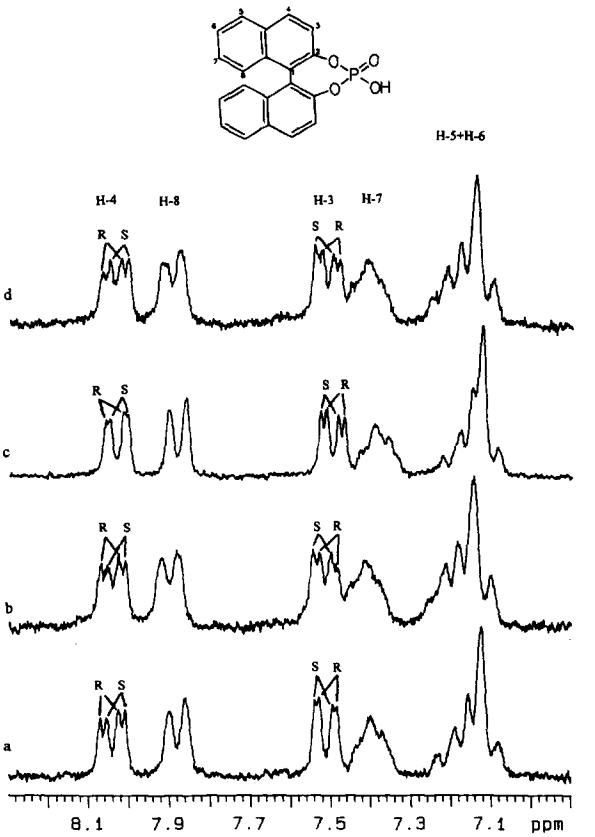

Fig. 1. ^{1}H NMR spectra of (±)-I in the presence of ca. 2 molar excess of β-CD (a,c) and SBE-β-CD (b,d) at pH 3.3 (a,b) and pH 6.5 (c,d). Only the aromatic region is depicted. Conditions as in text.

Table 1
Enantioseparation of (±)-1,1′-binaphthyl-2,2′-diyl hydrogen phosphate (I) using various CDs

Selector	Concentration (mg/ml)	t_1 (min)	t_2 (min)	α_{rel}	First eluted enantiomer
None	–	11.39	–	–	–
β-CD	0.5	11.95	12.19	1.02	*R*-(−)
HP-β-CD	0.5	11.97	12.36	1.03	*R*-(−)
TM-β-CD	0.5	12.61	12.77	1.01	*R*-(−)
TMA-β-CD	0.5	10.89	11.17	1.02	*R*-(−)
6-Am-β-CD	0.5	10.32	10.59	1.03	*R*-(−)
CM-β-CD	2.5	13.17	14.41	1.02	*R*-(−)
SBE-β-CD	2.5	8.95	9.19	1.03	*S*-(+)

pH 3.7. Applied voltage, −400 V/cm.

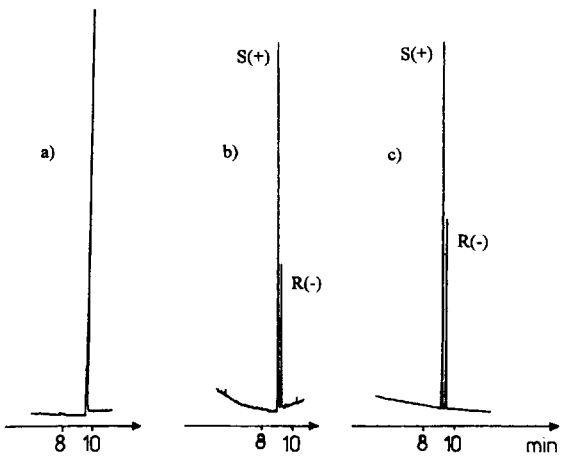

Fig. 2. Enantioseparation of I [nonracemic mixture of *S*-(+)/*R*-(−), 2:1] using SBE-β-CD at pH 3.7. Applied voltage, −400 V/cm. (a) The separation capillary was filled with 2.5 mg/ml SBE-β-CD in buffer and selector free buffer was added in cathodic vial. (b) The separation capillary and the cathodic vial were filled with 2.5 mg/ml SBE-β-CD in buffer. (c) Only the cathodic vial was filled with 2.5 mg/ml SBE-β-CD in buffer.

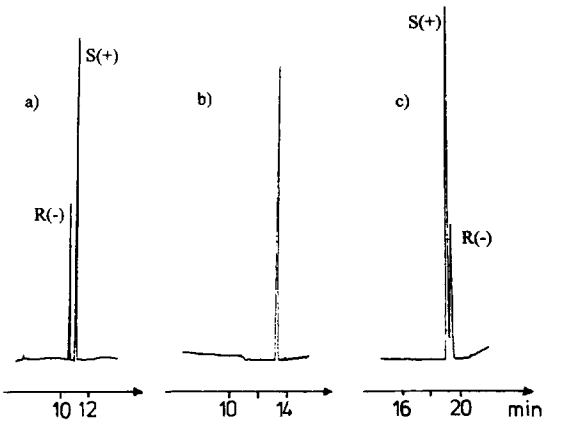

Fig. 3. pH dependent reversal of the enantiomer elution order of I [nonracemic mixture of *S*-(+)/*R*-(−), 2:1] using 5.0 mg/ml CM-β-CD at various pH. Applied voltage, −400 V/cm. (a) pH 3.0, (b) pH 4.3, (c) pH 5.0.

case (Fig. 2c) is absolutely the same as in case (b). This experiment confirms undoubtedly that the higher self-mobility of SBE-β-CD in comparison to I in the same direction is responsible for the observed reversal of the enantiomer elution order.

The solute complexed by SBE-β-CD migrates faster; hence the mobility of the preferentially bonded enantiomer is higher than the mobility of the less bonded one. The opposite is true for all other CDs in Table 1 which migrate at low pH slower than the solute. Therefore, the enantiomer elution order in case of SBE-β-CD is reversed to all other CDs in this study (Table 1).

3.2. The pH dependent reversal of enantiomer elution order

The study of the pH dependence of the mobilities of CM-β-CD [13] and I leads to another type of the reversal of enantiomer elution order. At pH 3.0 the electrophoretic mobility of CM-β-CD is lower than that of I. With increasing pH the mobility of CM-β-CD increases gradually due to the higher degree of dissociation and above pH 4.3 it exceeds the electrophoretic mobility of I. The reversal of the enantiomer elution order should be observed at this pH. Indeed, as Fig. 3 shows, peak coalescence of the enantiomers of I occurs at pH 4.3 where the effective mobilities of the chiral solute and selector become equal. Reversal of the elution order appears at above pH 4.3. Neither the reversal of the polarity of the high voltage supply nor the elimination of the EOF is required to observe this effect.

3.3. Reversal of enantiomer elution order using neutral and charged CDs

At pH 3.3 the EOF is very slow. Therefore, the anionic solute is injected at the cathodic end of the capillary and detected at the anodic end. With increasing pH the EOF increases in a silica capillary due to the increasing dissociation of the silanol groups. As mentioned before, at the pH where the EOF is higher than the self-mobility of the chiral solute a reversal of the polarity is required in order to achieve the detection of the solute. If a chiral selector is able to discriminate enantiomers of a given chiral compound before (pH 3.3, Table 2) and after the reversal of the polarity (pH 6.5, Table 2), then the reversal of enantiomer elution order should be observed by the reversal of polarity. The precondition for achieving this effect is that the relative affinity of both enantiomers to the CD selector remains the same.

As Fig. 1 shows all CD derivatives in this study

Table 2
Reversal of the enantiomer elution order of (±)-1,1′-binaphthyl-2,2′-diyl hydrogen phosphate (I) using neutral, positively and negatively charged CDs in an uncoated silica capillary

Selector (2.5 mg/ml)	pH 3.3, −400 V/cm t_1/t_2 (min)	pH 6.5, +400 V/cm t_1/t_2 (min)
β-CD	11.56/12.38 (*R/S*)	10.65/11.32 (*S/R*)
HP-β-CD	11.66/12.82 (*R/S*)	16.04/18.27 (*S/R*)
PM-β-CD	11.56/11.75 (*R/S*)	16.16/16.55 (*S/R*)
TMA-β-CD	11.69/12.52 (*R/S*)	10.25/10.88 (*S/R*)
CM-β-CD	11.05/11.41 (*R/S*)	21.51/22.75[a] (*R/S*)
SBE-β-CD	9.91/10.22[b] (*S/R*)	31.04/34.15[a] (*R/S*)

[a] 5.0 mg/ml selector, pH 6.0.
[b] pH 3.7.

bind *S*-(+)-I preferably than *R*-(−)-I in the pH range of 3.0–6.5. Consequently, a reversal of the enantiomer elution order of I has been observed after the reversal of the polarity of the high voltage supply for all CD derivatives except CM-β-CD independent whether they are neutral, positively or negatively charged (Fig. 4, Table 2). The reason for this apparent exception is the above-mentioned pH dependent reversal which is unique for CM-β-CD.

The reversal of the enantiomer elution order described in the present work is observed in contrast to Refs. [5,6,8] also with neutral CDs and in uncoated silica capillaries (Fig. 4, Table 2). Thus, the number of chiral selectors and racemic solutes useful for this technique is expanded substantially. The last seems to be the most universal and well predictable technique for the reversal of the enantiomer elution order applicable for a wide variety of chiral solutes and chiral selectors.

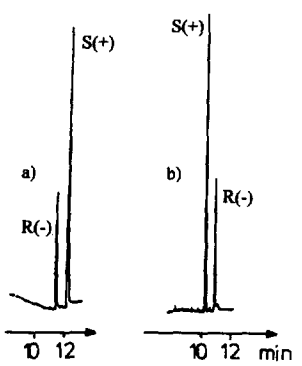

Fig. 4. Reversal of the enantiomer elution order of I [nonracemic mixture of *S*-(+)/*R*-(−), 2:1] in uncoated silica capillary using 2.5 mg/ml β-CD. (a) pH 3.3, −400 V/cm; (b) pH 6.5, +400 V/cm.

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