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Enantiomer separation of basic drugs by capillary electrophoresis using ionic and neutral polysaccharides as chiral selectors

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Abstract

The enantiomer separation of basic drugs by capillary electrophoresis was investigated employing two types of polysaccharides as chiral selectors. One type consists of electrically neutral polysaccharides such as dextran and dextrin, in which the separation mode is capillary zone electrophoresis (CZE), and ionic drugs are suitable for this mode. The other consists of ionic polysaccharides such as chondroitin sulfate C. Chondroitin sulfates are known as mucopolysaccarides and natural components, being charged, linear, sulfated polysaccharides of high mass. Therefore, the latter approach can be classified as affinity electrokinetic chromatography (AEKC). Both ionic and neutral drugs can be separated by AEKC owing to its separation principle. The separation of enantiomers of some basic drugs such as diltiazem and trimetoquinol was investigated with both CZE and AEKC employing polysaccharides as mentioned above and the enantioselectivity was compared. A brief mechanism of enantiorecognition by polysaccharides is also described.

Keywords: Enantiomer separation; Drugs, basic; Polysaccharides

1. Introduction

Capillary electrophoresis (CE) is a powerful and versatile separation technique because of its fast separation and high resolution [1,2]. Various separation modes such as capillary gel electrophoresis (CGE) [3,4] and micellar electrokinetic chromatography (MEKC) [5,6] have been developed within CE techniques. Among them, electrokinetic chromatography (EKC), including MEKC, and capillary zone electrophoresis (CZE) have been used for the separation of a wide variety of drugs, which are typically small aromatic compounds. Especially the separation of enantiomers has been successful by CZE or EKC through the addition of chiral selectors to the running buffer solutions [7–9].

The advantages of CZE and EKC for the separation of enantiomers are the ultra-high separation efficiency, easy changes of separation media and extremely small volumes of the sample and media, etc., in comparison with HPLC. In the development of a chiral separation method, one can easily alter the running buffer solution to find the optimum separation medium and can also use an expensive chiral selector because of the small volume requirement. Nowadays most of the direct CZE enantiomer separations have been performed with cyclodextrins (CDs) as chiral selectors [10–12]. In EKC, chiral

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surfactants such as bile acids [13,14] and sodium N-dodecanoyl-L-valinate [15,16] have been found to be effective for enantiomer separations. CDs were also effective in EKC modes known as cyclodextrin-mediated MEKC (CD-MEKC) [17,18] and EKC with charged CDs (CD-EKC) [19,20].

Recently, polysaccharides, which have already been found to be useful as chiral stationary phases for HPLC, were applied to CE chiral separations in two modes. One in CZE with an electrically neutral maltodextrin [21–23] and the other is EKC with an ionic polysaccharide [24–26]. In the latter method, we consider that the mode can be included in affinity EKC (AEKC), which is used for CE chiral separations using proteins as chiral selectors [27–29]. In AEKC, biological components are employed as an ionic pseudo-stationary phase and the mode can be applicable to the electrically neutral solutes as in MEKC.

In this work, chondroitin sulfate C (sodium salt) and chondroitin sulfate A (sodium salt), which are both known as mucopolysaccharides and natural components, were employed as chiral selectors in AEKC for the separation of the enantiomers of some basic drugs. CZE enantiomer separation by using dextran and dextrin was also investigated for the same analytes. A brief separation mechanism or enantiorecognition of polysaccharides is described from the comparison of the results obtained by AEKC and CZE.

2. Experimental

2.1. Apparatus

The instrument used was a P/ACE system 5510 equipped with a photodiode-array detector (Beckman, Fullerton, CA, USA). An untreated fused-silica capillary tube of 47 cm total length (effective length 40 cm) × 75 μm I.D. was used for the separation. The capillary was thermostated at 20°C with a liquid coolant. The applied voltage was held constant at 20–30 kV. The detection wavelength was adjusted to 220 or 240 nm. The instrument control and data collection were performed with a personal computer (COMPAQ ProLinea 4/33).

2.2. Materials

Chondroitin sulfate A (sodium salt) and chondroitin sulfate C (sodium salt) were of reagent grade and purchased form Nacalai Tesque (Kyoto, Japan), and were used without further purification. Dextran 70 (molecular mass ca. 70 000) was obtained from Tokyo Kasei Kogyo (Tokyo, Japan). Dextrin of Japanese Pharmacopoeia (JP) grade was purchased from Nichiden Kagaku Kogyo (Tokyo, Japan). From the investigation of the iodine colour reaction and gel permeation chromatographic (GPC) analysis, the molecular mass of JP-grade dextrin seems to be around 3700, which is classified as achrodextrin [30]. The unit structures of these polysaccharides are shown in Fig. 1.

The racemic drugs tested were diltiazem hydrochloride (calcium channel blocker), clentiazem maleate (8-chlorodiltiazem; calcium channel blocker), trimetoquinol hydrochloride (bronchodilator), trimetoquinol isomer, sulconazole nitrate (antifungal), verapamil hydrochloride (calcium channel blocker), propranolol hydrochloride (β-blocker) and primaquine (antimalarial). The structures are shown in Fig. 2, where optically active drugs are described as their active forms. These drugs were purchased from

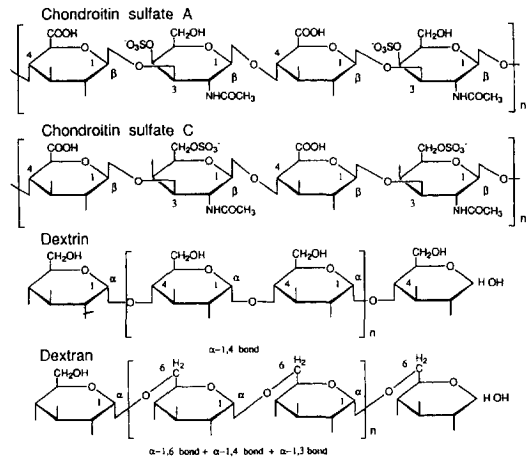

Fig. 1. Unit structure of ionic and neutral polysaccharides.

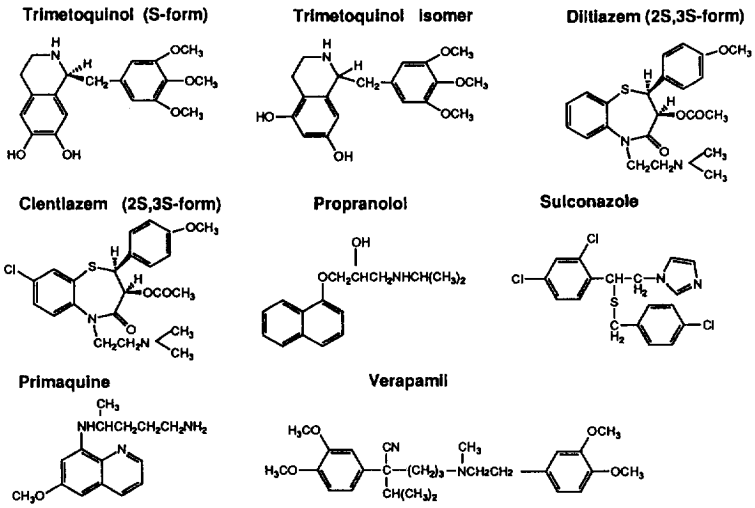

Fig. 2. Structures of the tested solutes.

Aldrich (Milwaukee, WI, USA), Wako (Osaka, Japan) or Nacalai Tesque, except diltiazem, clentiazem, trimetoquinol, its isomer and sulconazole, which were obtained from Tanabe Seiyaku. HPLC-grade methanol was purchased from Katayama Kagaku Kogyo (Osaka, Japan). All other reagents were of analytical-reagent grade from Katayama Kagaku Kogyo. Water was purified with a Milli-RO 60 water system (Millipore Japan, Tokyo, Japan).

2.3. Procedure

Running buffer solutions were prepared by dissolving each ionic or neutral polysaccharide in 20 m*M* phosphate buffer of pH 2.5 or 2.9. The concentration ranges investigated were 1–3% for chondroitin sulfates, 1–15% for dextran and 1–9% for dextrin. These buffer solutions were filtered through a membrane filter of 0.45-μm pore size (Gelmann Science Japan, Tokyo, Japan) and degassed by sonication with a Branson Model B-2200 ultrasonic cleaner (Yamato, Tokyo, Japan) prior to use. For AEKC with chondroitin sulfates and CZE with dextran, the capillary was rinsed with the running buffer solution for 1 min before every run, and washed with 0.1 *M* NaOH solution followed by water daily, usually at the end of an experiment. However, in CZE with dextrin, a delay of the migration times, i.e., the change of electroosmotic flow (EOF), was observed when capillary conditioning with the running buffer solution was employed. Washing with 0.5 *M* NaOH was effective for the recovery of the initial EOF velocity in the dextrin system [30]. Injection was performed by the pressure method [0.5 p.s.i. (1 p.s.i. = 6894.76 Pa), 2–10 s].

Stock solutions of each racemic sample were prepared in methanol at concentrations of ca. 1.0 mg/ml. The sample solutions for enantioseparation were prepared at concentrations of ca. 0.1 mg/ml by mixing several stock solutions and diluting with water.

3. Results and discussion

Enantiomer separation by AEKC with chondroitin sulfate C, heparin and dextran sulfate has been investigated previously for about 20 racemic drugs [26]. Among these chiral selectors, chondroitin sulfate C was the most effective for enantiorecognition. This was interpreted by its low ionic character, which permitted its application under acidic conditions [26]. Basic drugs did not migrate towards the negative end within 40 min under acidic conditions with a 50-cm capil-

Table 1
Migration times (min) in CE enantiomer separations with polysaccharides

Analyte	Chondroitin sulfate C (3%, 20 kV)		Chondroitin sulfate A (3%, 20 kV)		Dextran 70 (6%, 25 kV)		Dextrin JP (3%, 25 kV)	
Diltiazem	15.81	17.08	14.05	15.00	8.01	–	7.60	8.01
Clentiazem	18.04	20.18	15.79	17.29	7.93	–	7.80	8.35
Trimetoquinol	18.93	19.91	17.19	17.43	8.95	8.98	6.79	–
Trimetoquinol isomer	20.87	21.25	16.36	16.52	8.01	8.08	6.90	–
Verapamil	17.38	17.62	15.62	15.83	8.48	–	7.39	–
Sulconazole	18.68	18.98	13.46	13.67	7.70	–	7.25	7.50
Propranolol	18.40	18.70	13.36	–	7.07	–	7.29	–
Primaquine	16.88	17.53	12.38	12.64	4.33	–	4.05	4.22

lary at 15 kV when heparin and dextran sulfate were employed as chiral selectors in AEKC. This time, we employed another mucopolysaccharide, chondroitin sulfate A, in AEKC under acidic conditions for enantiomer separations.

Comparison of the results for the tested analytes with those obtained by CZE with dextran and dextrin is summarized in Tables 1 and 2. The concentration of chiral selectors for AEKC (chondroitin sulfates) and CZE with dextrin was 3%. Enantiorecognition for the tested analytes was not observed in CZE with 3% dextran. Therefore, the results in 6% and 15% dextran are given in the tables. Partial separation was observed in 6% dextran and baseline separation was obtained when 15% dextran was used. The separation factor, α, was calculated from the ratio of the electrophoretic mobilities of the enantiomers. Typical enantiomer separations obtained by AEKC with chondroitin sulfates are shown in Figs. 3 and 4, where 3% of each chondroitin sulfate was added to the 20 m*M* phosphate buffer solution of pH 2.9. Enantiomer separation of propranolol by AEKC with chondroitin sulfate A was unsuccessful and a reversal of the migration order was observed for some drugs. Overall, the enantioselectivity (i.e., number of enantioseparated analytes and α values) of ionic polysaccharides was better than that of neutral polysaccharides for the tested analytes at

Table 2
Separation factors (α) in CE enantiomer separations with polysaccharides

Analyte	Chondroitin sulfate C (3%, 20 kV)	Chondroitin sulfate A (3%, 20 kV)	Dextran 70 (15%, 30 kV)	Dextrin JP (3%, 25 kV)
Diltiazem	1.08	1.07	NS[a]	1.06
Clentiazem	1.12	1.10	NS	1.07
Trimetoquinol	1.05	1.01	1.02	NS
Trimetoquinol isomer	1.02	1.01	1.02	NS
Verapamil	1.01	1.01	NS	1.02[b]
Sulconazole	1.02	1.02	NS	1.03
Propranolol	1.02	NS	NS	NS
Primaquine	1.04	1.02	NS	1.04

[a] NS: not enantioseparated.
[b] 15%, 30 kV.

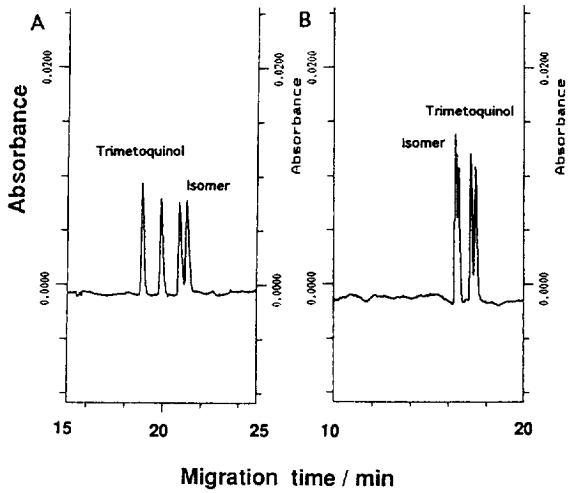

Fig. 3. Separation of enantiomers of trimetoquinol and its isomer by AEKC with (A) chondroitin sulfate C and (B) chondroitin sulfate A. Conditions: 3% chondroitin sulfate in 20 m*M* phosphate buffer (pH 2.9); separation tube, 47 cm (effective length 40 cm) × 75 μm I.D.; applied voltage, 20 kV; detection wavelength, 240 nm; temperature, 20°C; injection times of the sample solutions, 5 s.

the same concentration (3%). Of two ionic polysaccharides, chondroitin sulfate C gave better enantioseparation than chondroitin sulfate A.

The enantioselectivity of four polysaccharides

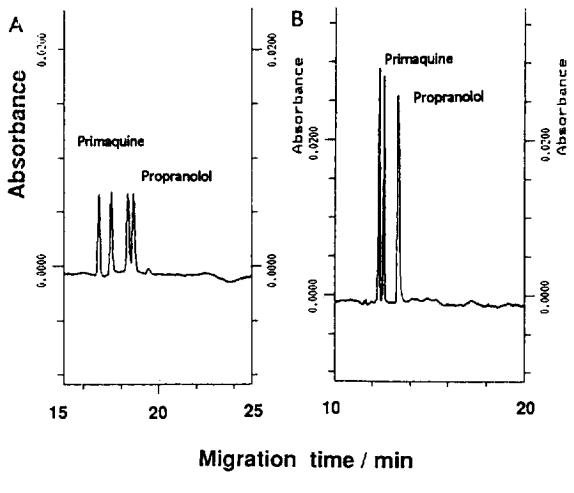

Fig. 4. Separation of enantiomers of primaquine and propranolol by AEKC with (A) chondroitin sulfate C and (B) chondroitin sulfate A. Conditions as in Fig. 3.

may be interpreted from the migration times. The migration times of the analytes in the neutral polysaccharide system (dextran and dextrin) were all shorter than those obtained in the anionic polysaccharide system, chondroitin sulfate. This can be interpreted by the contribution of the ionic interaction between the analyte and chondroitin sulfates, even if this is smaller than that in heparin and dextran sulfate. For two chondroitin sulfates, the migration times of the analytes in chondroitin sulfate A were all shorter than those obtained in chondroitin sulfate C. This smaller ionic interaction of chondroitin sulfate A may be due to the position of the sulfate groups in the glucose unit. The structural difference between these two is in the position of sulfate groups, as shown in Fig. 1. Chondroitin sulfate C is a 6-sulfate and chondroitin sulfate A is a 4-sulfate. Hence there may be some steric hindrance in the 4-sulfate compared with the 6-sulfate. Basic analytes must interact more strongly with the 6-sulfate.

Electrically neutral polysaccharides such as dextrin and dextran have been found to have the capability of enantiorecognition, even though there is no ionic interaction. Five of the tested analytes were successfully enantioseparated by CZE with dextrin. A typical separation of enantiomers of sulconazole by CZE with 3% dextrin, in which other analytes except primaquine migrated with almost the same velocity as sulconazole, as summarized in Table 1, is shown in Fig. 5A. On the other hand, the concentration of dextran required for the baseline separation of trimetoquinol and its isomer (see Fig. 5B and C), which were not enantioseparated by the dextrin system, was much higher (15%) than the typical concentration employed for the dextrin system. The interaction between the analytes and dextran was small and it required a higher concentration to give different migrations for the enantiomeric pairs. The better enantioselectivity of dextrin may be ascribed to its helical structure [23].

The successful enantiorecognition by CZE with neutral polysaccharides means that hydrophobic interaction and hydrogen bonding through the glucose unit are two of the effective

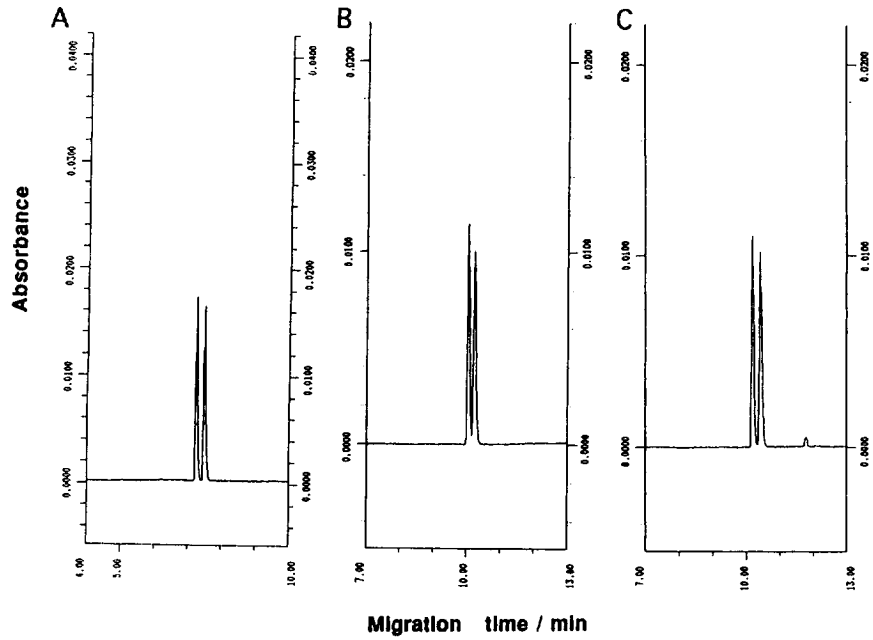

Fig. 5. Separation of enantiomers of (A) sulconazole, (B) trimetoquinol and (C) trimetoquinol isomer by CZE with dextrin or dextran. Buffers, (A) 3% dextrin and (B, C) 15% dextran in 20 m*M* phosphate buffer (pH 2.5); applied voltage, (A) 25 kV and (B, C) 30 kV; temperature, 20°C; detection wavelength, 220 nm.

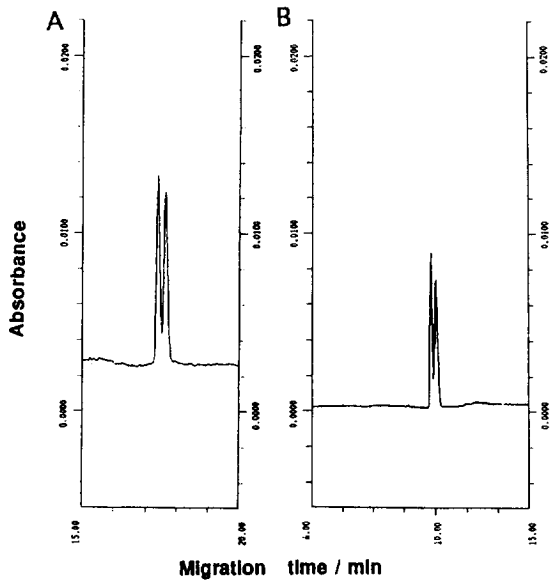

Fig. 6. Separation of enantiomers of verapamil by (A) AEKC with chondroitin sulfate C and (B) CZE with dextrin. Buffers, (A) 3% chondroitin sulfate C and (B) 15% dextrin in 20 m*M* phosphate buffer [(A) pH 2.9; (B) pH 2.5]; applied voltage, (A) 20 kV and (B) 30 kV; temperature, 20°C; detection wavelength, (A) 240 nm and (B) 220 nm.

parameters for the enantiorecognition. In ionic polysaccharides such as chondroitin sulfate C, the enantioselectivity must be much improved by combining these parameters and ionic interaction. As a typical example, the separation of enantiomers of verapamil by these two modes is shown in Fig. 6. The concentration of chondroitin sulfate C required for the separation (3%) was much lower than that of dextrin (15%), as mentioned above for trimetoquinol.

4. Conclusion

Chiral selectors having moderate ionic interactions (not as strong as heparin or dextran sulfate), together with other interactions such as hydrogen bonding and hydrophobic interactions, will probably show enantioselectivity for a wide variety of compounds in CE enantiomer separations. Recently, macrocyclic antibiotics such as vancomycin [31] and ristocetin A [32] were found to be effective for the enantioseparation of a

wide variety of compounds. In their case, also multiple interactions such as hydrogen bonding and ionic interactions (we may call these affinity interactions), seem to be essential for their wide enantioselectivity. The same considerations must be applicable to proteins, which have been successfully used in AEKC as chiral selectors [27–29].

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