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Cyclodextrin-modified micellar electrokinetic chromatography: separation of hydrophobic and enantiomeric compounds

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ABSTRACT

Cyclodextrin (CD)-modified micellar electrokinetic chromatography was applied to the separation of closely related aromatic compounds and enantiomeric separations using CD together with sodium dodecyl sulphate (SDS). Nine isomeric dimethylnaphthalenes, which were not resolved at all with an SDS solution, were successfully separated with y-CD and SDS. Sixteen polycyclic aromatic hydrocarbons were also almost completely separated. Dansylated (Dns)-DL-amino acids were optically resolved with combinations of SDS and β- or y-CD and sodium taurodeoxycholate and y-CD. Maltoheptaose was employed instead of CD, giving partial resolution of some Dns-Dr.-amino acids.

INTRODUCTION

Micellar electrokinetic chromatography (MEKC) is a mode of capillary electrophoresis based on the differential partitioning of an analyte between an ionic micelle and the surrounding aqueous phase [1–3]. Because the micelle and the aqueous phase migrate at different velocities, the analyte migrates at a velocity between two extremes, the electro-osmotic velocity and that of the micelle under neutral or alkaline conditions. That is, the range of the migration time is limited between the migration time of the bulk solution, t_0, and that of the micelle, t_{mc}. For high resolution, it is required that analytes do not have either too low or too high capacity factors [2]. The optimum capacity factor is calculated to be $(t_{mc}/t_0)^{1/2}$, provided that the plate number is constant irrespective of the capacity factor [4].

Hydrophobic compounds tend to be totally incorporated into the micelle and hence migrate at the same velocity as that of the micelle. Therefore, they cannot be resolved with a simple micellar solution. To solve the problem, several techniques have been developed: addition of an organic solvent to a micellar solution [5,6], the use of a bile salt surfactant instead of a long alkyl chain surfactant [7,8] and addition of cyclodextrin (CD) [9] or a high concentration of urea [10] to a micellar solution. Among these techniques, CD-modified MEKC (CD-MEKC) [9], which employs CD together with an ionic micellar solution, has been demonstrated to be useful for the separation of not only hydrophobic compounds [9,11] but also enantiomeric compounds [12,13].

In CD-MEKC, an analyte is distributed among three phases, the micelle, CD and the aqueous phase

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excluding the micelle and CD, although CD is not a true phase. As CD is electrically neutral, CD migrates at an identical velocity with the bulk solution. Therefore, the distribution of the analyte between the micelle and the non-micellar aqueous phase including CD directly affects the resolution. CD added to the micellar solution reduces the partitioning of the analyte to the micelle by increasing the fraction of the analyte in the non-micellar aqueous phase. If major fractions of the analytes are incorporated into the micelle or are they included by CD, the separation will be unsuccessful. Therefore, the distribution should not be close to either extreme.

CD is widely utilized in analytical separations, especially for enantiomeric separations [14]. Three CDs, a-, β- and y-CD, are the most popular but various derivatives have also been developed for modifying the selectivity or increasing the solubility in water. Although β-CD and its derivatives have cavity sizes suitable for inclusion of a wide range of analytes, y-CD has been found to be more effective than β-CD in CD-MEKC [9], probably owing to the co-inclusion of a monomeric surfactant molecule together with the analyte molecule.

In this paper, some examples of separations of highly hydrophobic compounds and enantiomers are presented. A combination of a bile salt surfactant and CD is also described for improved enantiomeric separation by taking advantage of chiral recognition by both the bile salt micelle and CD. A trial use of maltoheptaose, an open-chain form of β-CD, is briefly discussed.

EXPERIMENTAL

Apparatus

MEKC was performed with laboratory-built instruments or a Beckman (Palo Alto, CA, USA) P/ACE System 2000. The laboratory-built instruments consisted of a Jasco (Tokyo, Japan) Uvidec-100-IV or -V spectrophotometric detector and a high-voltage power supply, either a Matsusada Precision Devices (Kusatsu, Shiga, Japan) HCZE-30PN0.25-LDSW or a Bertan (Hicksville, NY, USA) 230-30R. A cell holder of the detector made of plastic (polyacetal) and an aperture (0.05 mm x 0.7 mm) made of stainless-steel were employed instead of the original cell holder for high-performance liquid chromatography (HPLC). The aperture was grounded to reduce electrical nois. A fused-silica capillary of 50 μm I.D. (Polymicro Technologies, Phoenix, AZ, USA) was glued to the aperture after removing the polyimide coating at the detector cell part. With the laboratory-built instruments the capillary was cooled with a small electric fan at ambient temperature. The detectors were operated at 210 nm.

Reagents

Sodium dodecyl sulphate (SDS) of protein research grade and β-CD were purchased from Nacalai Tesque (Kyoto, Japan), α- and y-CDs, maltoheptaose, dansylated (Dns) amino acids and dimethylnaphthalenes from Wako (Osaka, Japan), sodium taurodeoxycholate from Sigma (St. Louis, MO, USA) and polycyclic aromatic hydrocarbons (PAHs) from Accustandard (New Haven, CT, USA). Other reagents were of analytical-reagent grade. All the chemicals were used as received. Deionized water was purified with a Milli-Q system (Nippon Millipore, Tokyo, Japan).

Procedure

A micellar solution containing CD and urea was prepared by dissolving a weighed amount of a surfactant, CD and urea in a measuring flask with a pertinent buffer solution and filtered through a membrane filter of 0.45-μm pore size. The analytes were mostly dissolved in methanol at an appropriate concentration (usually 0.1–0.5 mg ml^{-1}). When the analytes were poorly soluble in methanol, tetrahydrofuran was added to methanol.

RESULTS AND DISCUSSION

Separation of dimethylnaphthalenes

Nine isomers of dimethylnaphthalenes, which were commercially available, were not separated by MEKC with 100 m*M* SDS solution (pH 8.45) (Fig. 1A), but they were successfully resolved by CD-MEKC using 70 m*M* γ-CD in 100 m*M* SDS solution (pH 8.45) containing 2 *M* urea (Fig. 1B). In the absence of y-CD, the migration times of dimethylnaphthalenes were identical with that of the SDS micelle measured with Sudan IV, suggesting that dimethylnaphthalenes were totally incorporated into the micelle. The addition of 2 *M* urea was not necessary for the separation shown in Fig. 1B

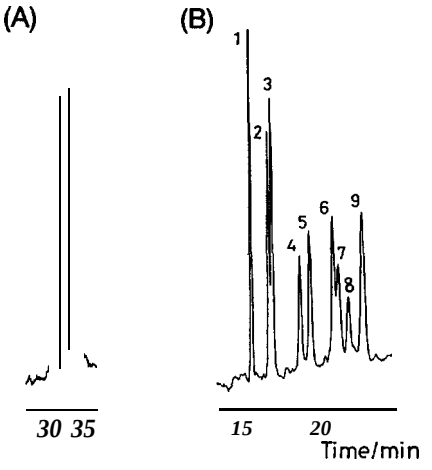

Fig. 1. Separation of dimethylnaphthalene isomers by MEKC (A) without and (B) with y-CD. 1 = 1,4-; 2 = 2,3-; 3 = 1,6-; 4 = 1,2-; 5 = 1,8-; 6 = 2,6-; 7 = 1,3-; 8 = 2,7-; 9 = 1 ,5-dimethylnaphthalene. Conditions: capillary, 70 cm × 50 μm I.D. (50 cm to the detector); separation solution, (A) 100 m*M* SDS in 100 m*M* borate buffer (pH 8.45), (B) 70 m*M* y-CD and 2 *M* urea in the same SDS solution as used in (A); applied voltage, 18 kV.

but urea was added to prevent dimethylnaphthalene-CD complexes from precipitating. These isomers were not resolved with addition of methanol or 2 Murea to the SDS solution in the absence of y-CD.

The capacity factor, k', in MEKC is defined as

$$k' = \frac{n_{mc}}{n_{aq}} = K \cdot \frac{V_{mc}}{V_{aq}} \quad (1)$$

where n_{mc} and n_{aq} are the numbers of moles of the solute incorporated into the micelle and in the aqueous phase, respectively, K is the distribution coefficient, V_{mc}/V_{aq} is the phase ratio and V_{mc} and V_{aq} are the volumes of the micelle and the remaining aqueous phase, respectively. In CD-MEKC, the apparent capacity factor, k'_{app}, is described similarly as

$$k'_{app} = \frac{n_{mc}}{n_{non\text{-}mc}} = \frac{n_{mc}}{n_{aq} + n_{CD}} \quad (2)$$

where $n_{non\text{-}mc}$ is the number of moles of the solute in the non-micellar phase, which is equal to $n_{aq} + n_{CD}$, where n_{CD} is the number of moles of the solute included by CD. In order to clarify the difference between eqns. 1 and 2, the different term k'_{app} is employed in eqn. 2. However, it should be noted that k'_{app} in eqn. 2 can be equally used as k' in eqn. 1 for the calculation of various chromatographic parameters. When the solute is insoluble in water, as with dimethylnaphthalene, eqn. 2 can be rewritten as

$$k'_{app} = \frac{n_{mc}}{n_{CD}} = K_{app} \cdot \frac{V_{mc}}{V_{CD}} \quad (3)$$

where K_{app} is the apparent distribution coefficient between CD and the micelle. Here, K_{app} is introduced again to indicate the distribution between the micelle and CD instead of K between the micelle and the aqueous phase. It is apparent that K_{app} holds the same relationship as K only when the solute is not soluble in water.

Eqn. 3 can be rewritten again as

$$k'_{app} = \frac{K_{app}}{\bar{v}_{CD} C_{CD}} (C_{srf} - CMC)\, \bar{v}_{mc} \quad (4)$$

where C_{srf} and CMC are the concentration of the surfactant and the critical micelle concentration, respectively, C_{CD} is the concentration of CD and $\bar{v}_{mc}$ and $\bar{v}_{CD}$ are the partial specific volumes of the micelle and CD, respectively. Eqn. 4 indicates that the apparent capacity factor is linearly proportional to the surfactant concentration. Eqn. 3 can be further modified as

$$\frac{1}{k'_{app}} = \frac{\bar{v}_{CD}}{K_{app} \bar{v}_{mc} (C_{SDS} - CMC)} \cdot C_{CD} \quad (5)$$

Eqn. 5 suggests the reciprocal of the apparent capacity factor is directly proportional to the concentration of CD.

The capacity factor is obtained experimentally according to

$$k' = \frac{t_R - t_0}{t_0 (1 - t_R/t_{mc})} \quad (6)$$

where t_0, t_R and t_{mc} are the migration times of the bulk solution or the solute which is free from the micelle, the sample solute and the micelle, respectively [2]. The migration times t_0 and t_{mc} can be obtained with tracers such as methanol for t_0 and Sudan III or IV for t_{mc}. The apparent capacity factor can be obtained according to eqn. 6. However, t_{mc} in the presence of CD is difficult to measure, because most tracers of the micelle are partially included by CD also.

In order to test the validity of eqn. 4, the dependence of the apparent capacity factor on the

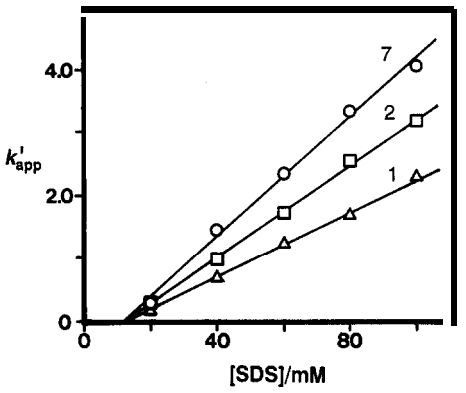

Fig. 2. Dependence of the apparent capacity factor (k'_{app}) on the concentration of SDS. 1 = 1,4-; 2 = 2,3-; 7 = 1,3-dimethylnaphthalene. Conditions: capillary, 57 cm x 75 μm I.D. (50 cm to the detector); separation solution, 20 mM y-CD and 2 M urea in SDS solutions in 100 mM borate buffer (pH 8.45); applied voltage, 15 kV; temperature, 20°C.

concentration of SDS at a constant CD concentration (20 mM) was examined in the presence of 2 A4 urea (Fig. 2). A similar linear dependence of k'_{app} on SDS concentration was observed also for 1,8- and 1,5-dimethylnaphthalene (not shown in Fig. 2). The migration time of the micelle required to calculate apparent capacity factors was obtained by assuming that the electrophoretic mobility of the micelle is equal to that in the absence of y-CD. The slope of the plots of k'_{app} against SDS concentration is equal to

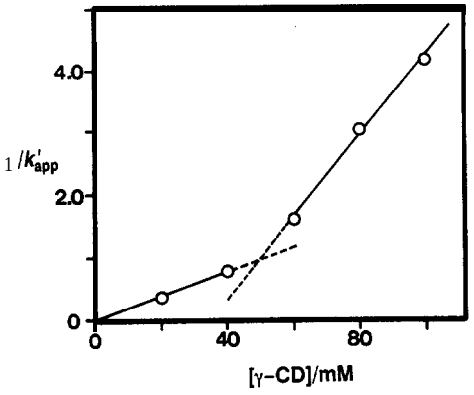

Fig. 3. Dependence of the reciprocal of the apparent capacity factor of 1,4-dimethylnaphthalene on the concentration of γ-CD. The concentration of SDS was 100 mM. Other conditions as in Fig. 2, except for the concentration y-CD.

$K_{app}\bar{v}_{mc}/(\bar{v}_{CD}C_{CD})$, as seen from eqn. 4. If we assume that the partial specific volume of y-CD is equal to that of a-CD, 0.671 ml g^{-1} [15], and the partial specific volume of the SDS micelle is 0.856 ml g^{-1} [16], a K_{app} value of 1.8 1 is obtained for 1 ,4-dimethylnaphthalene from Fig. 2. This apparent distribution coefficient value indicates that 1,4-dimethylnaphthalene, which has the smallest k'_{app} among the nine isomers, tends to be incorporated into the SDS micelle slightly more than into the cavity of y-CD in the presence of monomeric SDS.

The reciprocal of k'_{app} was plotted against the concentration of y-CD at constant concentrations of SDS (100 mM) and urea (2 M), according to eqn. 5 (Fig. 3). A similar dependence of $(k'_{app})^{-1}$ on the concentration of y-CD was also observed for 2,3- and 1,3-dimethylnaphthalenes. The plot suggests that the partition mechanism was altered at a y-CD concentration of cu. 50 mM. At concentrations of y-CD below *ca.* 50 mM, K_{app} was 1.82, which was consistent with the value obtained from Fig. 2, and above ***ca.*** 50 mM, K_{app} was 0.56. These values were calculated by assuming the CMC of SDS to be 3 mM [16]. Above cu. 50 mM y-CD, K_{app} decreased by a factor of ca. 3 or the dimethylnaphthalene was more easily included by y-CD in comparison with y-CD concentrations below ca. 50 mM. The reason for the alteration of K_{app} at high concentrations of y-CD is not clear. A possible explanation is the change in the stoichiometric ratio of the inclusion complex between CD and the solute from 1: 1 to 2: 1. However, it does not seem probable that the distribution coefficient for the 2: 1 complex is significantly larger than that for the 1:1 complex.

Separation of polycyclic aromatic hydrocarbons

In a previous paper [9], the separation of eight PAHs was shown. Fig. 4 shows the separation of sixteen PAHs using 20 mM y-CD and 100 mM SDS. The addition of a high concentration of urea was helpful to prevent the adsorption of PAHs on the capillary wall, although the addition of urea was not essential for dissolving y-CD in the SDS solution. Unless urea was added, the capillary had to be rinsed after each run, still giving poor reproducibility. Two pairs of PAHs, fluoranthene (8 in Fig. 5) and benz[*a*]anthracene (**10**), and benzo[*k*]fluoaranthene (12) and dibenz[a,h]anthracene (**14**), were not resolved. It is interesting that two unresolved pairs (8 and 10,

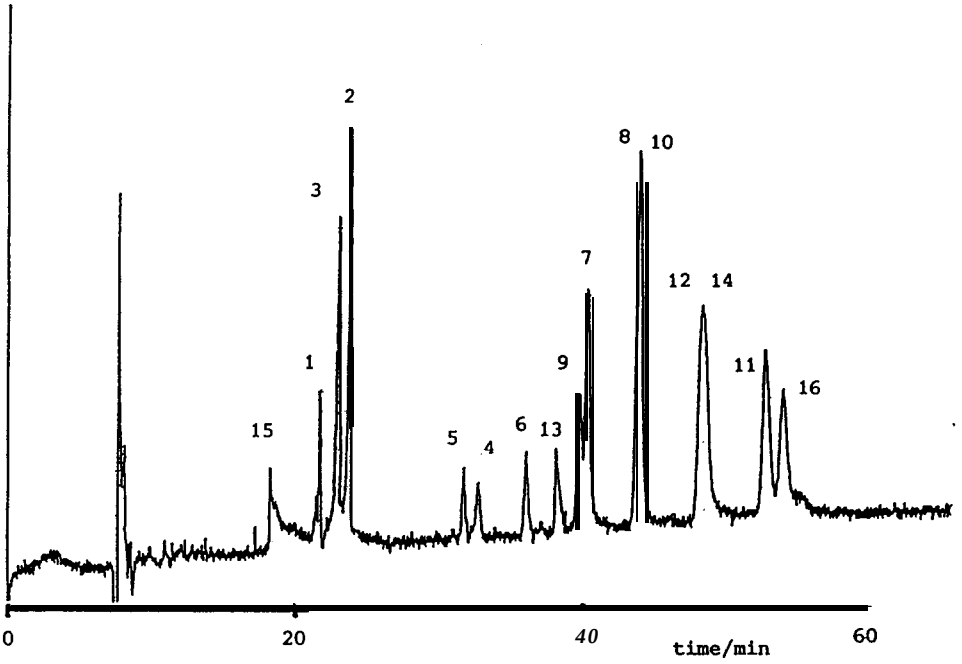

Fig. 4. Separation of sixteen PAHs by CD-MEKC. 1 = Naphthalene; 2 = acenaphthene; 3 = acenaphthylene; 4 = fluorene; 5 = anthracene; 6 = phenanthrene; 7 = pyrene; 8 = fluoranthene; 9 = chrysene; 10 = benz[*a*]anthracene; 1 1 = benzo[*b*]fluoranthene; 12 = benzo[*k*]fluoranthene; 13 = benzo[*a*]pyrene; 14 = dibenz[*a,h*]anthracene; 15 = benzo[*ghi*]perylene; 16 = indeno[1,2,3-*cd*]pyrene. Conditions: capillary, 80 cm × 50 μm I.D. (60 cm to the detector); separation solution, 20 m*M* γ-CD, 5 *M* urea and 100 m*M* SDS in 100 m*M* borate buffer (pH 9.0); applied voltage, 25 kV.

and 12 and 14) have similar molecular structures, as shown in Fig. 5.

The use of 75 m*M* β-CD instead of y-CD together with 100 m*M* SDS gave a different selectivity, as shown in Fig. 6. The results suggest that the cavity of β-CD can accommodate all the tricyclic PAHs and two of the tetracyclic PAHs among the sixteen PAHs. Two unresolved pairs in Fig. 4 were completely separated in Fig. 6. However, β-CD showed poor resolution as a whole, especially for penta- and hexacyclic PAHs. The use of a mixed solution of β- and y-CD was explored but the complete separation of the sixteen PAHs was not successful.

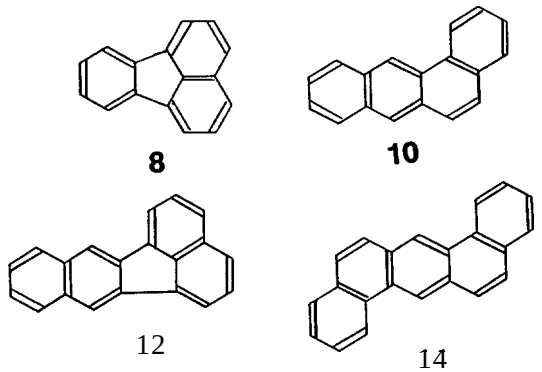

Fig. 5. Molecular structures of (8) fluoranthene, (10) benz[*a*]-anthracene, (12) benzo[*k*]fluoranthene and (14) dibenz[*a,h*]anthracene.

Enantiomeric separation of dansylated amino acids

The enantiomeric separation of nine dansylated (Dns) DL-amino acids using 60 m*M* y-CD and 100 m*M* SDS was described previously [17]. Table I summarizes enantiomeric separations of Dns-DL-amino acids with 60 m*M* y-CD or 60 m*M* β-CD together with 100 m*M* SDS. The effect of the addition of 20% of methanol to 60 m*M* β-CD in 100 m*M* SDS is also included in Table I. In general, y-CD gave a better resolution than β-CD. However, all the Dns-DL-amino acids except Dns-DL-Ser were

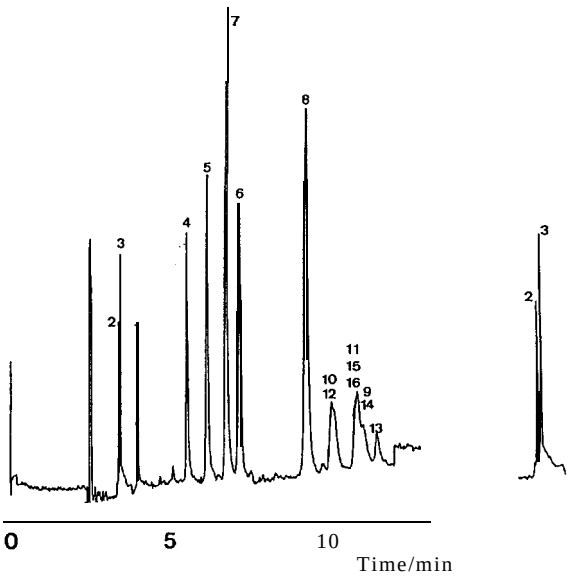

Fig. 6. Separation of sixteen PAHs by CD-MEKC using β-CD. Peak identification numbers in Fig. 4. The inset shows the separation of peaks 2 and 3 on an expanded time scale. Conditions: capillary, 47 cm x 50 μm I.D. (30 cm to the detector); separation solution, 75 mM β-CD, 5 M urea and 100 mM SDS in 100 mM borate buffer (pH 9.0); applied voltage, 22.5 kV.

at least partially resolved with either β-CD or y-CD. The addition of 20% of methanol substantially improved the chiral discrimination of β-CD but some of the Dns-DL-amino acids were only partially resolved.

The results in Table I suggest that the use of a mixture of β- and y-CD may provide better results than those obtained with the use of a single CD. Table II gives the results obtained using a mixture of 50 mM β-CD and 10 mM y-CD in 100 mM SDS solution. Except for Dns-DL-Ser, all the Dns-DL-amino acids were almost completely resolved. The addition of 10% of methanol to the mixed solution of β- and γ-CD did not improve the resolution, as shown in Table II, although the migration times were slightly increased. A further increase in methanol content decreased the resolution, in spite of considerable increases in migration times. Fig. 7 shows examples of the enantiomeric separation of six Dns-DL-amino acids using a mixed solution of β- and γ-CD in the absence or presence of 10% of methanol. Glucosyl-P-CD was also used for the separation of Dns-DL-amino acids in CD-MEKC but the results were almost identical with those obtained with β-CD.

TABLE I

ENANTIOMERIC SEPARATION OF Dns-DL-AMINO ACIDS BY CD-MEKC WITH β-CD OR y-CD

Conditions: capillary, 70 cm x 50 μm I.D. (50 cm to the detector); separation solution, 100 mM SDS in 0.1 M borate buffer (pH 8.6); applied voltage, 20 kV.

Dns-DL-Amino acid	*60* mM β-CD				*60* mM β-CD + *20%* methanol				*60* mM y-CD			
	t_D (min)	t_L (min)	α^a	R_s	t_D (min)	t_L (min)	α^b	R_s	t_D (min)	t_L (min)	a'	R_s
Thr	12.46		1	_	15.53	15.78	1.04	1.22	10.23	10.37	1.05	> 1.5
Ser	12.71		1	_	16.04	16.17	1.02	0.71	10.58		1	—
Aba	13.48	13.55	1.02	0.79	16.87	17.03	1.02	0.71	10.07	10.29	1.08	> 1.5
Val	13.79	13.91	1.03	1.18	17.38	17.61	1.03	1.40	10.08	10.36	1.05	> 1.5
Nva	14.77	14.85	1.02	0.90	17.17	17.32	1.02	0.98	10.24	10.63	1.14	> 1.5
Met	14.80		1	_	17.78	17.92	1.02	0.87	11.09	11.38	1.31	> 1.5
Leu	16.84	17.01	1.03	1.20	18.81	18.24	1.03	1.44	10.76	11.63	1.29	> 1.5
Glu	17.18	17.31	1.02	0.81	27.18	27.70	1.03	> 1.5	13.83	14.08	1.06	> 1.5
Asp	17.21	17.36	1.03	0.98	29.89	30.68	1.05	> 1.5	14.53		1	_
Trp	17.77	17.99	1.04	> 1.5	18.02	18.13	1.01	0.68	10.84		1	_
Phe	18.09	18.36	1.04	> 1.5	18.72	19.01	1.03	> 1.5	9.22	9.65	1.21	> 1.5
Nle	18.06	18.18	1.02	0.81	19.05	19.23	1.02	1.04	11.10	11.63	1.16	> 1.5

[a] The migration time of the micelle was assumed to be equal to that of timepidium bromide.

[b] The migration time of the micelle was assumed to be equal to the maximum run time until when the peak oftimepidium bromide was not observed.

TABLE II

ENANTIOMERIC SEPARATION OF Dns-DL-AMINO ACIDS BY CD-MEKC WITH A MIXTURE OF β-CD AND γ-CD

Conditions as shown in Table I except for applied voltages.

Dns-DL-Amino acid	50 m*M* β-CD + 10 m*M* γ-CD[a]				50 m*M* β-CD + 10 m*M* γ-CD + 10% methanol*			
	t_D (min)	t_L (min)	α^c	R_s	t_D (min)	t_L (min)	α^d	R_s
Thr	11.30	11.42	1.04	1.28	12.50	12.70	1.04	1.01
Ser	11.52		1	–	13.08		1	–
Aba	11.42	11.60	1.05	>1.5	12.57	12.84	1.06	>1.5
Val	11.56	11.82	1.07	>1.5	12.68	13.01	1.07	>1.5
Nva	12.21	12.52	1.08	>1.5	13.32	13.63	1.06	>1.5
Met	12.76	12.98	1.05	>1.5	13.62	13.84	1.04	>1.5
Leu	12.84	13.57	1.18	>1.5	14.06	14.77	1.12	>1.5
Glu	14.88	15.16	1.04	>1.5	19.54	19.88	1.03	>1.5
Asp	15.38	15.53	1.03	1.35	13.76	13.87	1.02	1.08
Phe	11.06	11.87	1.26	>1.5	11.78	12.58	1.20	>1.5
Nle	13.54	14.06	1.12	>1.5	14.56	15.05	1.08	>1.5

[a] Applied voltage 20 kV.
[b] Applied voltage 25 kV.
[c] See the footnote a in Table 1.
[d] See the footnote *b* in Table I.

Enantiomeric separations by MEKC using bile salts have been reported [18–21]. The enantiomeric separation of Dns-DL-amino acids using sodium taurodeoxycholate (STDC) was not very successful [18]. Other bile salts were less effective for the separation Dns-DL-amino acids. Whereas STDC incorporates Dns-L-amino acids more strongly [18], CD includes D-isomers more than L-isomers. Therefore, the use of CD in combination with STDC seems promising for the enantiomeric separation of

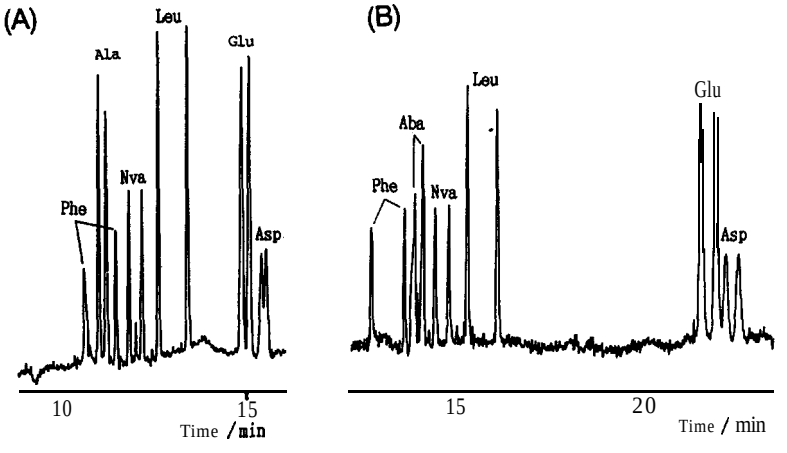

Fig. 7. Separation of Dns-DL-amino acids by CD-MEKC using a mixture of β-CD and y-CD. Peaks are identified by names of amino acids. Conditions: separation solution, (A) 50 m*M* β-CD, 10 m*M* γ-CD and 100 m*M* SDS in 100 m*M* borate buffer (pH 8.6); (B) the same solution but containing 10% of methanol. Other conditions as in Table I.

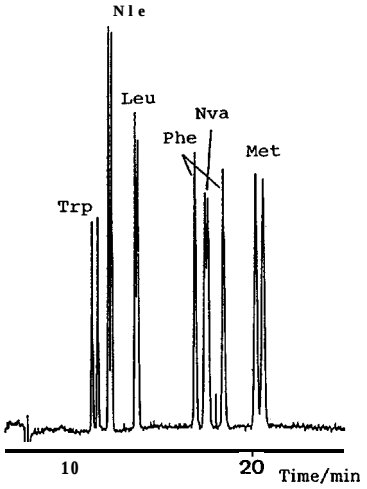

Fig. 8. Separation of Dns-DL-amino acids by CD-MEKC using y-CD with sodium taurodeoxycholate. Peaks are identified by names of amino acids. Conditions as in Table III.

Dns-DL-amino acids, because the chiral discrimination by both STDC micelles and CD should mutually enhance the resolution. Fig. 8 shows the separation of Dns-DL-amino acids using 10 m*M* γ-CD and 50 m*M* STDC at pH 3.0.

Although the addition of γ-CD significantly improved the resolution and reduced the migration times, as shown in Table III, in comparison with the results obtained with STDC alone [18], enantiomeric separations of Dns-DL-amino acids were more successful when CD were used with the achiral SDS micelle under alkaline conditions. The chiral discrimination of γ-CD may be different under acidic and alkaline conditions for Dns-DL-amino acids.

Maltoheptaose has a molecular structure corresponding to an open-ring form of β-CD. The addition of 20 m*M* maltoheptaose to 60 m*M* SDS solution (pH 7.0) instead of β-CD also resulted in the enantiomeric separation of Dns-DL-Leu, -Phe and -Nle. A decrease in the capacity factors was observed for all the Dns-DL-amino acids even though optical resolution was unsuccessful, suggesting an interaction of maltoheptaose with Dns-amino acids.

TABLE III

ENANTIOMERIC SEPARATION OF Dns-DL-AMINO ACIDS BY CD-MEKC WITH SODIUM TAURODEOXYCHOLATE AND y-CD

Conditions: capillary, 37 cm × 50 μm I.D. (30 cm to the detector); separation solution, 10 m*M* y-CD in 50 m*M* sodium taurodeoxycholate solution in 50 m*M* phosphate buffer (pH 3.0); applied voltage, 12.5 kV. Other conditions as given in Table I.

Dns-DL-Amino acid	t_L (min)		t_D (min)	t_D/t_L
Trp	11.28		11.58	1.027
Nle	12.16		12.32	1.013
Leu	13.61		13.75	1.010
Val		13.8		1
Phe	16.86		18.39	1.091
Aba		17.03		1
Nva	17.40		18.39	1.057
Met	20.16		20.56	1.020
Thr	28.44		29.01	1.020
Glu		26.78		1
Asp		34.25		1
Ser[a]	–		–	–

[a] The peak was not observed owing to the long migration time.

CONCLUSIONS

CD-MEKC has two advantages over simple MEKC: the separation of hydrophobic compounds, which are mostly incorporated into the micelle, and enantiomeric separation based on the chiral recognition of CDs. In CD-MEKC the capacity factor or migration time was manipulated by changing either the surfactant concentration or the concentration of CD. In enantiomeric separations, the choice of CD is important and a mixture of different CDs may be advantageous. Addition of methanol also affects not only the width of the migration time window and the capacity factors but also the selectivity.

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