Use of selectively methylated β-cyclodextrin derivatives in chiral separation of dansylamino acids by capillary zone electrophoresis

Masanobu Yoshinaga[a], Minoru Tanaka[*,b]

[a]Kansai Technical Research Institute, Toppan Printing Co., Ltd., Ebie, Fukushima-ku, Osaka 553, Japan
[b]Department of Applied Chemistry, Faculty of Engineering, Osaka University, Yamada-oka, Suita, Osaka 565, Japan

Abstract

The chiral separation ability of heptakis(2-, 3- and 6-mono-O-methyl, 2,3-, 2,6- and 3,6-di-O-methyl and 2,3,6-tri-O-methyl)-β-cyclodextrins as chiral selectors in capillary zone electrophoresis was investigated using twelve dansylamino acids. Unmodified and 6-monomethylated β-cyclodextrins (β-CDs) exhibited similar high enantioselectivities. β-CD lost its high enantioselectivity after 2-methylation but still exhibited chiral separation ability after 3-methylation. In contrast to unmodified β-CD, methylation of the hydroxyl groups at the 6-position of 3-monomethylated β-CD resulted in the complete disappearance of the chiral separation ability but that of 2,3-dimethylated β-CD enhanced it. Moreover, the chemical modification of the secondary hydroxyl groups produced a reverse migration order of the enantiomers.

1. Introduction

Capillary zone electrophoresis (CZE) is attracting much attention as a method for separating a wide range of ionic and ionizable compounds owing to its rapid run times, extremely high separation efficiency, low sample requirements, etc. [1,2]. In particular, CZE is undergoing rapid development for chiral separations at the present time. In order to perform chiral separations, various chiral selectors are added to the CZE buffers. Unmodified and chemically modified cyclodextrins (CDs) have been successfully utilized for these purposes.

The chemical modification of CDs with various

functional groups has been extensively investigated in an attempt to improve the complexing and catalytic abilities of CDs. Various functional groups have been introduced on to their rims [3]. It is well known that the chemical modification of CDs brings about changes in the shape and size of their cavities, hydrogen-bonding ability and other physical properties.

Among chemically modified CD derivatives, methylated compounds have been widely used as chiral selectors in CZE [4–12]. In a previous paper [13], we reported the chiral separation of dansylamino acids by CZE using unmodified α-, β- and γ-CDs or 2,6-dimethylated and 2,3,6-trimethylated α- and β-CDs as chiral selectors. The chemical modifications of α- and β-CDs brought about remarkable changes in their enan-

* Corresponding author.

tioselectivities for dansylamino acids. To our knowledge, only 2,6-dimethylated and 2,3,6-trimethylated CDs have hitherto been applied as chiral selectors. Therefore, it was of interest to investigate the CZE enantioselectivity changes in CDs produced by selective methylation of the hydroxyl groups at their 2-, 3-, 6-, 2,3- or 3,6-positions. The results obtained may offer information concerning the evaluation of the chiral recognition of CDs.

In this paper, we describe the chiral separation of dansylamino acids by CZE in the presence of all the above-mentioned methylated β-CD derivatives.
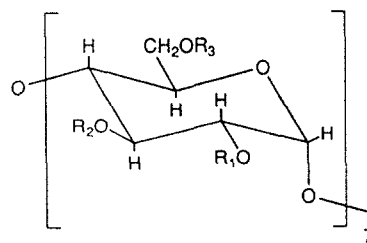
2. Experimental

2.1. Apparatus

An Applied Biosystems (Foster City, CA, USA) Model 270A fully automated CZE system was used with a 72 cm (50 cm from inlet to detector) × 50 μm I.D. fused-silica capillary. On-column UV detection was applied at 220 nm. The temperature and applied voltage were held constant at 30°C and 20 kV, respectively, unless specified otherwise. Sample solutions (0.2 mM) were injected by a vacuum technique (12.7 cmHg pressure difference for 1.0 s) after introducing methanol as a neutral marker to estimate the osmotic flow. Before each run, the capillary was rinsed successively with 0.1 M NaOH and the separation buffer. Electropherograms were recorded with a Hitachi (Tokyo, Japan) D-2500 Chromato-integrator. All experiments were run in duplicate to ensure reproducibility.

2.2. Reagents

Unmodified β-CD was purchased from Ensuiko Seito (Yokohama, Japan) and heptakis(2,6-di-O-methyl)- and heptakis(2,3,6-tri-O-methyl)-β-CDs were prepared by well-known methods [14,15]. Heptakis(2-, 3- and 6-mono-O-methyl and 2,3- and 3,6-di-O-methyl)-β-CDs were synthesized by modifying the literature method [16]. The mono-, di- and trimethylated β-CDs obtained are denoted by

R₁	R₂	R₃	Abbreviation
CH₃	H	H	2-MM-β-CD
H	CH₃	H	3-MM-β-CD
H	H	CH₃	6-MM-β-CD
CH₃	CH₃	H	2,3-DM-β-CD
CH₃	H	CH₃	2,6-DM-β-CD
H	CH₃	CH₃	3,6-DM-β-CD
CH₃	CH₃	CH₃	2,3,6-TM-β-CD

Fig. 1. Structures of methylated β-CD derivatives used as chiral selectors.

prefixing the unmodified β-CD with MM-, DM- and TM-, respectively (Fig. 1). Dansylamino acids were obtained from Sigma (St. Louis, MO, USA) and other compounds from Wako (Osaka, Japan).

Separation buffers were prepared by dissolving each methylated β-CD at 10 mM except for 6-MM-β-CD (2 mM) in 0.1 M sodium borate–0.05 M sodium phosphate buffer (pH 9.0). They were filtered through a membrane filter after ultrasonication for 10 min prior to use.

3. Results and discussion

3.1. Characterization of methylated β-CD derivatives

After isolation, crude methylated β-CD derivatives were fractionated by silica gel column

chromatography, using chloroform–methanol as eluents [16]. The methylated β-CD derivatives thus obtained were characterized by ^1H and ^{13}C NMR spectrometry and fast atom bombardment mass spectrometry (FAB-MS). Each fractionated β-CD derivative was a mixture of heptakis(methyl)-β-CD and its under- and/or over-methylated products. Ion peaks corresponding to the methyl–oxygen bond scission products [i.e., loss of methyl group(s)] were not observed in FAB tandem mass spectra. Therefore, the composition of each methylated β-CD derivative could be estimated from the relative intensities of the $[M + K]^+$ ions (KI was adulterated with the samples).

Table 1 gives the composition of the methylated β-CD derivatives used here for CZE. It has been reported that several commercial 2,6-DM-β-CD derivatives were mixtures with broad and roughly symmetrical distributions of the degree of substitution and that the content of heptakis(2,6-di-O-methyl)-β-CD was 41% at most [17]. A higher purity of 52.6% was achieved with our 2,6-DM-β-CD (Table 1). Compared with this, the other methylated β-CDs were much more enriched in the desired heptakis(methyl)-β-CD derivatives.

3.2. Chiral separation of dansylamino acids

For a clear separation, the extent of separation of the two peaks of a racemate is represented by

$R' = 100(H - H')/H$, where H and H' are the height of the first peak and that of the valley between the two peaks, respectively. In this definition, the greater the R' value the better is the resolution, and $R' = 100$ represents a baseline separation of the two peaks.

The CZE conditions optimized for separating the dansylamino acid enantiomers were given under Experimental. The pH of the separation buffer solutions was fixed at 9.0 in order to run the solutes in the fully ionized forms. Under these CZE conditions, the β-CD derivatives having no charge are transported towards the negative electrode by electroosmotic flow (V_{eo}). In the absence of the β-CD derivatives, each negatively charged dansylamino acid migrates toward the negative electrode with the difference between V_{eo} and its electrophoretic velocity (V_{ep}) due to $V_{eo} > V_{ep}$. When included in a β-CD cavity, the solute is transported towards the negative electrode faster, because of the decrease in V_{ep}. This, therefore, indicates that a faster migrating enantiomer interacts more strongly with the β-CD cavity than the other.

Table 2 gives the migration times and the R' values for the twelve pairs of dansylamino acid enantiomers in the presence of unmodified and monomethylated β-CDs. Unmodified β-CD and 6-monomethylated β-CD (6-MM-β-CD), whose secondary hydroxyl groups at the 2- and 3-positions are not methylated, exhibited good and comparable enantioselectivities. The D-enantiomers invariably migrated faster than the corresponding L-enantiomers in the presence of β-CD or 6-MM-β-CD, indicating their stronger interaction with the D-enantiomers. These results strongly suggest the major participation of the secondary hydroxyl groups in the chiral recognition process and the minor or scarce participation of the primary hydroxyl groups.

It was of great interest to investigate which secondary hydroxyl groups play an important role in the chiral recognition process. Therefore, 2- and 3-monomethylated β-CDs (2-MM-β-CD and 3-MM-β-CD, respectively) were synthesized as chiral selectors. Methylation of the 2-hydroxyl groups (2-monomethylation) in β-CD resulted in the complete disappearance of enantioselectivity except for dansyl-DL-α-aminobutyric acid ($R' =$

Table 1
Composition of methylated β-CD derivatives

β-CD derivative	Composition (%)[a]			
	$-CH_3$	0	$+CH_3$	$+2CH_3$
2-MM-β-CD	15.4	76.9	7.7	0
3-MM-β-CD	14.3	76.2	9.5	0
6-MM-β-CD	2.9	97.1	0	0
2,3-DM-β-CD	6.5	93.5	0	0
2,6-DM-β-CD	0	52.6	36.8	10.6
3,6-DM-β-CD	0	90.6	9.4	0
2,3,6-TM-β-CD	5.6	88.8	5.6	0

[a] 0 = Desired heptakis(methyl)-β-CD; − = under-methylated β-CD derivative; + = over-methylated β-CD derivative.

Table 2
Chiral separation of dansylamino acids in the presence of unmodified and monomethylated β-CDs

Dansylamino acid	β-CD		2-MM-β-CD		3-MM-β-CD		6-MM-β-CD	
	Migration time (min)	R'	Migration time (min)	R'	Migration time (min)	R'	Migration time (min)	R'
α-Amino-n-butyric acid	D 9.20 L 9.24	12.7	D 10.20 L 10.28	83.6	10.26		D 11.44 L 11.57	62.1
Aspartic acid	D 12.84 L 13.18	100	20.30		19.01		D 23.02 L 23.70	100
Glutamic acid	D 12.72 L 12.93	100	19.09		17.78		D 21.86 L 22.25	93.4
Leucine	D 8.96 L 9.08	52.5	9.77		L 9.56 D 9.64	90.1	D 11.54 L 11.57	90.3
Methionine	D 9.28 L 9.33	31.6	10.33		L 10.06 D 10.10	14.2	D 11.70 L 11.76	11.7
Norleucine[a]	9.16 9.22	35.9	10.01		9.70 9.76	55.0	11.45 11.54	18.3
Norvaline	D 9.14 L 9.20	44.1	10.32		L 9.89 D 9.93	18.5	D 11.36 L 11.49	64.7
Phenylalanine	8.84		9.57		L 9.42 D 9.49	69.7	11.56	
Serine	D 9.48 L 9.53	38.8	11.05		10.62		12.37	
Threonine	D 9.13 L 9.22	94.0	10.77		10.49		D 12.05 L 12.17	63.8
Tryptophan	8.89		9.50		L 9.50 D 9.57	68.3	11.40	
Valine	D 9.00 L 9.08	76.4	10.40		10.06		D 11.56 L 11.76	96.5

[a] Neither enantiomer was obtained.

83.6). On the other hand, after 3-mono-methylation of β-CD, six pairs of dansylamino acid enantiomers could still be resolved. This suggests a positive contribution of the 2-hydroxyl groups to the chiral recognition. In contrast to the results for β-CD and 6-MM-β-CD, the L-enantiomers migrated faster in the presence of 3-MM-β-CD. Moreover, 3-MM-β-CD could not resolve dansyl-DL-aspartic and -glutamic acid, -serine and -threonine bearing a hydrophilic OH or a COOH group in their side-chains on asymmetric carbons, although β-CD could. However, dansyl-DL-phenylalanine and -tryptophan bearing aromatic groups in their side-chains could be resolved in the presence of 3-MM-β-CD but not in the presence of β-CD. Dansyl-DL-leucine and -norleucine bearing relatively long hydrophobic isobutyl and n-butyl

side-chains, respectively, could also be resolved well in the presence of 3-MM-β-CD, although dansyl-DL-α-aminobutyric acid and -valine bearing shorter ethyl and isopropyl side-chains, respectively, could not.

The reason for these remarkable changes in the chiral recognition after 3-monomethylation of β-CD may be speculated as follows. In an inclusion model where a dansyl group is included in the CD cavity and an amide and/or a COO⁻ group closely contact the secondary hydrophilic rim (2- and 3-hydroxyl groups, located inside and outside the cavity, respectively) of unmodified β-CD, the above-mentioned side-chains of the L- and D-enantiomers are located in the proximity of and far from the secondary rim of β-CD, respectively [18]. Thus the bulky side-chain is far from the hydrophilic rim in the case of the

D-enantiomer but is in the proximity of the hydrophilic rim in the case of the L-enantiomer. The approach of the side-chain to the hydrophilic rim is considered to be less favourable for the inclusion. Therefore, the D-enantiomer is included more stably than the L-enantiomer; thus, the D-enantiomer migrates faster, as mentioned previously. After 3-monomethylation of β-CD, all the 3-hydroxyl groups are replaced by OCH_3 groups. This produces a rim with both a hydrophilic and a hydrophobic ring composed of the 2-hydroxyl and 3-methoxy groups, respectively, in place of the original rim with two hydrophilic rings in unmodified β-CD. As described above, provided that a dansyl group is included in the cavity and an amide and/or a COO^- group closely contacts the 2-hydroxyl groups of 3-MM-β-CD, the approach of the hydrophobic side-chain to the hydrophobic ring is more favourable for the inclusion complex formation. Consequently, the complex of 3-MM-

β-CD with the L-enantiomer is stabilized more than that with the D-enantiomer. This brings about the reversal of the migration order observed in the presence of 3-MM-β-CD. Considering the high resolution of dansyl-DL-leucine ($R' = 90.1$) and the lack of resolution of dansyl-DL-valine ($R' = 0$) in the presence of 3-MM-β-CD, the longer isobutyl side-chain may closely contacts the 3-methoxy groups but the shorter isopropyl side-chain may not. This trend is also true for the comparison of dansyl-DL-norleucine ($R' = 55.0$) with dansyl-DL-norvaline ($R' = 18.5$). Another drastic enantioselectivity change after 3-monomethylation of β-CD can be seen in the appearance of chiral resolution for dansyl-DL-phenylalanine ($R' = 69.7$) and -tryptophan ($R' = 68.3$) bearing the hydrophobic aromatic chains.

Similar data in the presence of dimethylated and trimethylated β-CDs are listed in Table 3. Di- or trimethylation of unmodified β-CD resulted in both a significant decrease in the chiral

Table 3
Chiral separation of dansylamino acids in the presence of di- and trimethylated β-CDs

| Dansylamine acid	2,3-DM-β-CD		2,6-DM-β-CD		3,6-DM-β-CD		2,3,6-TM-β-CD	
	Migration time (min)	R'	Migration time (min)	R'	Migration time (min)	R'	Migration time (min)	R'
α-Amino-n--butyric acid	10.57		8.72		12.29		11.46	
Aspartic acid	21.10		L 13.68	31.8	22.13		22.90	
			D 13.77					
Glutamic acid	19.73		13.06		20.37		21.33	
Leucine	L 10.10	99.2	L 8.13	41.9	12.12		L 10.85	100
	D 10.28		D 8.17				D 11.04	
Methionine	10.57		8.45		12.56		11.40	
Norleucine[a]	10.02	26.4	8.28		12.29		10.76	92.7
	10.09						10.90	
Norvaline	10.34		8.42		12.24		L 11.22	48.0
							D 11.32	
Phenylalanine	9.97		8.04		12.08		L 10.50	92.1
							D 10.69	
Serine	11.08		8.82		12.57		11.88	
Threonine	10.86		8.77		12.60		11.62	
Tryptophan	L 10.38	21.0	8.01		12.50		L 10.90	76.6
	D 10.48						D 11.02	
Valine	L 10.37	25.6	8.49		12.12		L 11.06	38.7
	D 10.44						D 11.14	

[a] Neither enantiomer was obtained.
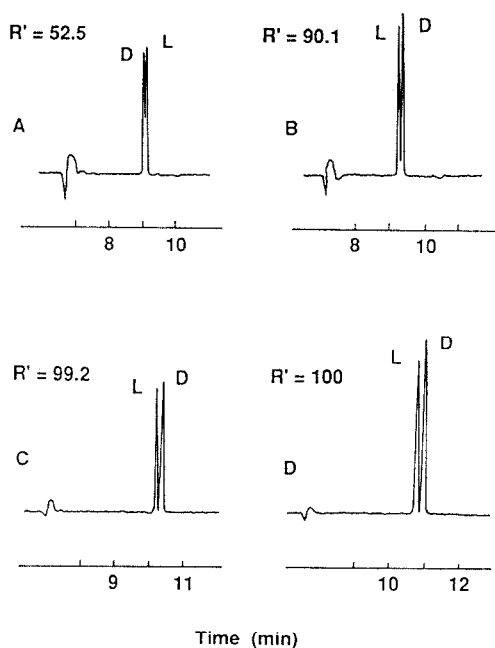
Fig. 2. Chiral separations of dansyl-DL-leucine with (A) β-CD, (B) 3-MM-β-CD, (C) 2,3-DM-β-CD and (D) 2,3,6-TM-β-CD.

recognition ability and a reversal of the migration order of the enantiomers. Fig. 2 shows typical electropherograms for dansyl-DL-leucine in the presence of 2,3-DM- and 2,3,6-TM-β-CDs, together with those in the presence of unmodified and 3-MM-β-CDs. Near and complete baseline separations of the enantiomers were obtained. The chiral separation of dansyl-DL-leucine or -norleucine is also superior to that of dansyl-DL-valine or -norvaline, respectively, with 2,3-DM- or 2,3,6-TM-β-CD as well as 3-MM-β-CD. This is probably due to the closer contact of the hydrophobic rims of methylated β-CDs with the longer n- and isobutyl side-chains than with the shorter n-and isopropyl side-chains, which is supported by the CPK molecular models.

None of the dansylamino acid enantiomers could be resolved in the presence of 3,6-DM-β-CD. After methylation of the hydroxyl groups at the 6-positions of 3-MM-β-CD, its high chiral recognition completely disappeared. On the other hand, similar 6-methylation for 2,3-DM-β-

CD enhanced the chiral recognition (2,3,6-TM-β-CDE). As already described, 6-mono-methylation of unmodified β-CD scarcely produced the enantioselectivity changes. Hence methylation of the hydroxyl groups at the 6-positions of the β-CD derivatives (unmodified, 3-MM- and 2,3-DM-β-CDs) produced different changes in the enantioselectivity. The reason for this variety is not clear at present. The penetration depth of the dansylamino acids into the cavities may be sensitively affected by 6-methylation.

3.3. Interaction of dansylamino acids with β-CD derivatives

As already described, a shorter migration time means a stronger interaction of a solute with the β-CD cavity. Strictly, all the CZE conditions for the migration time data in Tables 1 and 2 are not always the same, because their measurements required many days. Therefore, the data cannot be directly used to estimate the interaction of one dansylamino acid with the β-CD derivatives. Fig. 3 shows the migration times of dansyl-DL-leucine as a model solute in the presence of each β-CD derivative, which were measured successively under the same CZE conditions as carefully as possible. Considering the migration time values, the interaction of the solute increases in the order 2,3-DM-β-CD < 2,3,6-TM-β-CD < 6-MM-β-CD < β-CD < 2-MM-β-CD < 3,6-DM-β-
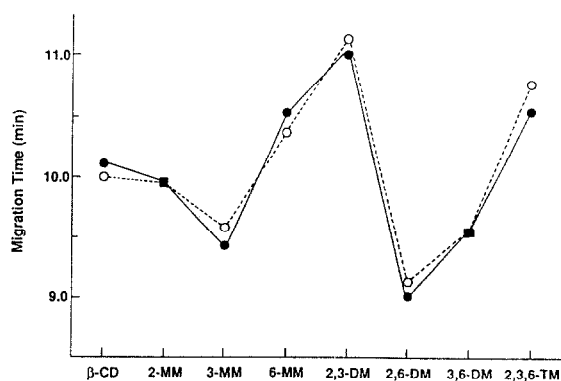

Fig. 3. Migration times of dansyl-DL-leucine in the presence of each β-CD derivative. Solutes: O = D-enantiomer; ● = L-enantiomer; ■ = DL-enantiomers.

CD $\approx$ 3-MM-β-CD < 2,6-DM-β-CD. On the other hand, 2,3,6-TM- and 2,3-DM-β-CDs exhibited complete and almost complete baseline separations of dansyl-DL-leucine and interacted with it more weakly than any other β-CD derivatives. 3-MM- and 6-MM-β-CD also exhibited high resolution abilities. 2,6-DM-β-CD, although it interacted most strongly, could produce only a partial resolution. Consequently, in this case, it is apparent that the chiral separation of the solute has not necessarily been correlated with its interaction strength with the CD.

In conclusion, methylation of the hydroxyl groups on the rim of each β-CD cavity produced large enantioselectivity changes for dansylamino acid enantiomers, as already described. Further work on interactions between guests and CD derivatives bearing groups different in bulkiness and/or polarity may provide more information for the chiral recognition process.

Acknowledgements

References

[1] S.F.Y. Li, Capillary Electrophoresis, Elsevier, Amsterdam, 1992.

[2] P.D. Grossman and J.C. Colburn, Capillary Electrophoresis, Academic Press, San Diego, 1992.

[3] A.P. Croft and R.A. Bartsch, Tetrahedron, 39 (1983) 1417–1474.

[4] S. Fanali, J. Chromatogr., 474 (1989) 441–446.

[5] J. Snopek, H. Soini, M. Novotny, E. Smolkova-Keulemansova and I. Jelinek, J. Chromatogr., 559 (1991) 215–222.

[6] T.E. Peterson and D. Trowbridge, J. Chromatogr., 603 (1992) 298–301.

[7] S. Fanali, M. Flieger, N. Steinerova and A. Nardi, Electrophoresis, 13 (1992) 39–43.

[8] P. Gareil, J.P. Gramond and F. Guyon, J. Chromatogr., 615 (1993) 317–325.

[9] T.E. Peterson, J. Chromatogr., 630 (1993) 353–361.

[10] M.W.F. Nielen, Anal. Chem., 65 (1993) 885–893.

[11] S.A.C. Wren and R.C. Rowe, J. Chromatogr., 635 (1993) 113–118.

[12] M. Heuermann and G. Blaschke, J. Chromatogr., 648 (1993) 267–274.

[13] M. Tanaka, M. Yoshinaga, S. Asano, Y. Yamashoji and Y. Kawaguchi, Fresenius' J. Anal. Chem., 343 (1992) 896–900.

[14] J. Boger, R.J. Corcoran and J.-M. Lehn, Helv. Chim. Acta, 61 (1978) 2190–2218.

[15] J. Szejtli, A. Lipták, I. Jodá, P. Fügedi, P. Nanási and A. Neszmélyi, Starch/Stärke, 32 (1980) 165–169.

[16] K. Takeo, H. Mitoh and K. Uemura, Carbohydr. Res., 187 (1989) 203–221.

[17] T. Irie, K. Fukunaga, J. Pitha, K. Uekama, H.M. Fales and E.A. Sokolowski, Carbohydr. Res., 192 (1989) 167–172.

[18] K. Fujimura, S. Suzuki, K. Hayashi and S. Masuda, Anal. Chem., 62 (1990) 2198–2205.