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Direct chiral separation of amino acids derivatized with 2-(9-anthryl)ethyl chloroformate by capillary electrophoresis using cyclodextrins as chiral selectors
Effect of organic modifiers on resolution and enantiomeric elution order

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Abstract

Direct chiral separation of amino acids derivatized by 2-(9-anthryl)ethyl chloroformate (AEOC) has been performed by capillary electrophoresis in capillary zone electrophoretic (CZE) mode and micellar electrokinetic (MEKC) mode using cyclodextrins (CDs) as chiral selectors. The concentrations of chiral selector, surfactant and organic modifier were optimized. Application of β- and γ-CD as chiral selectors in MEKC, resulted in D/L separation of 12 and 13 amino acids, respectively. In CZE, chiral separation was obtained only with γ-CD, 8 amino acids were chirally separated in this mode. Separation in the MEKC mode resulted in higher resolutions of the enantiomers than in the CZE mode. Addition of 2-propanol to the buffer caused reversal of the enantiomeric elution order for some amino acids in MEKC mode.

Keywords: Chiral selectors; Enantiomer separation; Amino acids; Cyclodextrins; 2-(9-Anthryl)ethyl chloroformate

1. Introduction

Amino acids are an important group of substances and much effort has been devoted to their separation. Most amino acids do not absorb in the UV region and a large number of derivatization methods that can be used with UV or fluorescence detection has been proposed [1–4]. These methods were originally developed for HPLC but are now applied to CE. Besides improved detection, the derivatization leads to a strong improvement of chiral separation capabilities. It became evident that the aromatic moieties of the derivatization reagents are beneficial for the complexation with cyclodextrins. Further, derivatization, in general, takes place on an amino group and the pK_a of the amino acid is thus influenced; this may facilitate the simultaneous separation of basic and acidic amino acids. The drawbacks of the derivatization approach have been discussed [5]. A general drawback is the low yield obtained when

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derivatizing analytes occurring in very low concentrations and matrix effects could also be disadvantageous. However, there is a great number of applications for which these drawbacks are irrelevant.

A number of methods for the separation of amino acids, derivatized or underivatized, by capillary zone electrophoresis (CZE) or micellar electrokinetic chromatography (MEKC) has been described and these were recently summarized [5–8]. In a recent paper [9], two approaches for chiral separation of amino acids by means of CE were compared. These were direct chiral separation and indirect separation after the formation of diastereomers. For this purpose, two chemically similar derivatization reagents were used, 9-fluorenylmethyl chloroformate (FMOC) and 1-(9-fluorenyl)ethyl chloroformate (FLEC). It was found that the indirect method was easier to optimize and gave more rapid separations. Further, the number of amino acids that could be separated in one run was larger than when applying the direct approach. However, the indirect method is afflicted with an inherent problem, this being the enantiomeric purity of the derivatization reagent. This is evident in the determination of trace enantiomeric impurities. In such cases, a small impurity in the chiral reagent may result in a relatively large error in the enantiomeric excess determination if the impurity is not taken into account. For this reason, it is of interest to investigate the direct chiral resolution that can be obtained with the application of different chiral selectors and derivatization reagents.

In the present work, investigation was made of the enantioselectivity possible for derivatized amino acids in CE, using cyclodextrins as chiral selectors, when applying an alternative chloroformate reagent, 2-(9-anthryl)ethyl chloroformate (AEOC) for the derivatization. This reagent was first presented by Faulkner et al. [10] and it was recently used in connection with non-chiral separation of amino acids in capillary electrophoresis [11]. The main advantage of AEOC, as compared to FMOC, is the high molar absorption, which was reported to be 190 000 at 256 nm [10].

The aim of the present work was to evaluate the performance of amino acids derivatized with AEOC regarding direct chiral separation. Enantiomeric separation of amino acids derivatized by AEOC using β- or γ-cyclodextrin as chiral selector has thus been optimized.

2. Experimental

2.1. Apparatus

The CE separations were performed on a Prince Autosampler (Lauerlabs, Emmen, Netherlands) with a high-voltage supply (0–±30 kV). A CV^4 UV detector (ISCO, Lincoln, NE, USA) was employed for on-column detection. The separation capillaries (360 μm O.D. and 25 μm I.D.) were from Polymicro Technologies (Phoenix, AZ, USA). Micellar electrokinetic chromatograms and electropherograms were recorded with an ELDS 900 laboratory data system (Chromatography Data Systems, Kungshög, Sweden).

2.2. Reagents

The amino acids tested in this study, and α-, β- and γ-cyclodextrin were from Sigma (St. Louis, MO, USA). The 2-(9-anthryl)ethyl chloroformate (AEOC) reagent was from Eka Nobel (Surte, Sweden). Sodium dodecyl sulfate (SDS) was from USB (United States Biochemical, Cleveland, OH, USA), and urea was from Merck (Darmstadt, Germany). Other chemicals, methanol, acetonitrile, 2-propanol, etc., used in this work were of analytical grade.

2.3. Derivatization procedure

Derivatization was made by adding 200 μl of 10 m*M* AEOC to 200 μl of 2 m*M* amino acid in 0.2 *M* borate buffer (pH 9.0). This mixture was kept for 2 min, then extracted with 0.5 ml pentane to remove excess of reagent. After dilution, ten times with water, the sample was ready for introduction.

2.4. Separation conditions

The length of the capillaries was 67 cm (46 cm to detector) for MEKC and 61 cm (45 cm to detector) for CZE. New separation capillaries were rinsed with 0.2 *M* sodium hydroxide for 2 h. Prior to each

sample injection, the separation capillaries were first flushed for 10 min with 0.2 *M* sodium hydroxide to which 10% methanol had been added, then with water for 5 min and finally the capillaries were equilibrated with the running buffer for 10 min. All buffers were prepared in water from an Elgastat UHQ II (Elga, High Wycombe, UK) water purification system and degassed prior to use. The buffer concentration, SDS concentration, urea concentration and pH are given as they were before the addition of organic modifiers. Sample injections were made by pressure (50 mbar×0.25 min). The temperature of the capillaries was controlled at 25°C. The detection was performed at 256 nm.

2.5. Optimization and calculations

Optimization design and response charts were obtained by Codex programme (AP Scientific Service, Sollentuna, Sweden). The calculation of selectivity, 'α-value', was made without consideration of micellar elution time [9]. Resolution, R_s, was calculated according to conventional methods.

3. Results and discussion

Direct separation of enantiomers in CE may be influenced by many factors such as type of chiral selector and its concentration, pH, buffer ionic strength, field strength, and the presence of organic modifiers [6,12]. As has been shown in an earlier paper for the separation of FMOC-derivatized amino acids, the organic modifiers may, in some cases, play an important role in chiral recognition [9]. The proper choice of key separation parameters may result in favourable resolutions.

3.1. Capillary zone electrophoresis

The resolution, R_s, in electrophoresis is given by [13]:

$$R_s = \frac{N^{0.5}}{4} \times \left(\frac{\Delta\mu}{\mu_{av} + \mu_{eo}} \right) \quad (1)$$

where N is the number of theoretical plates, $\Delta\mu$ and μ_{av} are the difference in electrophoretic mobilities between two analytes and their average mobilities and μ_{eo} is the electroosmotic mobility. According to Wren and Rowe [14,15] $\Delta\mu$ can be expressed as:

$$\Delta\mu = \frac{[C](\mu_f - \mu_c)(K_2 - K_1)}{1 + [C](K_1 + K_2) + K_1K_2[C]^2} \quad (2)$$

where [C] is the concentration of chiral selector, K_1 and K_2 are inclusion complex formation constants of two enantiomers, μ_f and μ_c are the mobilities of the free enantiomer and the enantiomer in complexed form, respectively.

A combination of Eq. 1 and Eq. 2 yields:

$$R_s = \frac{N^{0.5}}{4(\mu_{av} + \mu_{eo})} \cdot \frac{[C](\mu_f - \mu_c)(K_2 - K_1)}{1 + [C](K_1 + K_2) + K_1K_2[C]^2} \quad (3)$$

It can be seen from Eq. 3 that a large difference between μ_f and μ_c results in increased R_s, which suggests that chiral selectors having a charge sign opposite to that of the analytes will be useful. The existence of an optimal CD concentration for each pair of amino acids is predicted by Eq. 3.

The most important factors for chiral separations would be: control of μ_{eo}, use of optimal concentration of chiral selectors and high separation efficiency.

However, for the separation of AEOC-derivatized amino acids, a low pH could prevent dissociation of the amino acids. As a result, the selectivity could be lost. Therefore, in the present work, a pH range of 4.5–6.5 was selected for the scouting experiments. The other parameters were the concentration of β-CD (5–20 m*M*) and γ-CD (5–30 m*M*), respectively, and three different organic modifiers, methanol, acetonitrile and 2-propanol (5–15%, v/v). The AEOC derivatives of Leu, Met, Phe and Val were used as test analytes. The scouting results showed that selectivity in CZE mode for these amino acids was observed only with γ-CD. Optimal conditions for separation of the mixture were: 15 m*M* γ-CD, pH 5.3, 25 m*M* borate– 25 m*M* phosphate. In addition to the four test analytes, also Glu, Ser, Trp and Tyr were chirally separated under these conditions. How-

ever, for the separation of one pair of amino acids at a time, optimal conditions were different for the different pairs. For example, optimal conditions for AEOC-Met were found at a concentration of γ-CD below 5 m*M*, but for AEOC-Phe optimal conditions were at values higher than 30 m*M* γ-CD.

The separations were not improved when adding any of the organic modifiers tested. This was completely different from the results obtained with FMOC-amino acids using β-CD as the chiral selector, where an organic modifier, 2-propanol, was a prerequisite for chiral recognition [9]. High concentration (30 and 40 m*M*) of β-CD in buffers containing 1 and 2 *M* urea, respectively, were also tested at pH 5.0 with and without organic modifiers, but no selectivity for AEOC-amino acids, except for Ile, was observed. Ile differs from the other amino acids tested in having two chiral centres.

3.2. Chiral separation with β-CD – enhancement of resolution with SDS

Interestingly, chiral separation of AEOC-amino acids with β-CD was observed at pH 7.5 (at a relatively large μ_{eo} value) when the buffer contained 2-propanol and SDS. It was found that an increase in SDS concentration resulted in increased resolution for the four examined amino acids. It was also observed that an increase of SDS concentration resulted in higher efficiency. In addition, it should be noted that the chiral separation of these AEOC-amino acids was achieved only in the presence of 2-propanol. Chiral recognition was thus not obtained in the presence of methanol or acetonitrile. As mentioned earlier, the presence of 2-propanol was a prerequisite for the chiral separation of FMOC-amino acids with β-CD [9]. The special benefits of 2-propanol as a modifier in chiral separation by means of CE was recently pointed out by Armstrong et al. [16].

In order to obtain optimal conditions in MEKC, a factorial design of two parameters, 2-propanol (8–22%, v/v) and β-CD (25–54 m*M*), was performed at pH 7.5. Considering that the migration times would become impractically long when using a high SDS concentration, a constant concentration of 40 m*M* SDS was used. Further, urea (1 *M*) was added to the buffer in order to increase the solubility of β-CD. A mixture of four AEOC-amino acids, Leu, Met, Phe and Val was used for the evaluation. The results obtained from the optimization showed that these four amino acids had similar optimal separation conditions: β-CD (42–47 m*M*) and 2-propanol (14–17%, v/v). Response surfaces for the chiral resolution of the tested amino acids are shown in Fig. 1. No interaction effects of β-CD and 2-propanol on the resolution of the system were observed. However, the main effects were found to significantly affect the resolution as shown in Fig. 1. All four models have a relatively flat maximum close to 45 m*M* β-CD and 15% 2-propanol; these conditions could thus be suitable for separation of AEOC-amino acids. Under these conditions, 12 of the 19 examined amino acids were separated (Table 1). In Fig. 2 the separation of a mixture of 10 AEOC-amino acids is shown.

3.3. Chiral separation with γ-CD in MEKC

When γ-CD (20 m*M*) was first employed to test six AEOC-amino acids, Asp, Ile, Leu, Nval, Thr and Val in MEKC, four of these were separated (Table 2). However, after adding 15% 2-propanol (v/v) to the buffer, an improvement in resolution was observed for most of these AEOC-amino acids, although the resolution for Nval was lost with the addition of 2-propanol. The effect of γ-CD concentration on the resolution of Thr, Val, Leu and Ile, was further investigated with buffers containing 15% 2-propanol (v/v). An optimal concentration of 10 m*M* γ-CD was observed for these four AEOC-amino acids (Fig. 3a). However, the efficiency was increased and the migration times were decreased with the increase in γ-CD concentration (Fig. 3b). With the concentration of 10 m*M* γ-CD, nine amino acids were baseline separated (Table 2). The separation of a mixture of seven AEOC-amino acids is shown in Fig. 4a. As mentioned above, the organic modifier was effective for separation of all examined AEOC-amino acids when using β-CD as chiral selector, but when using γ-CD, the presence of 2-propanol resulted in deteriorated separation for some AEOC-amino acids. The same buffers, in the absence of 2-propanol, were thus examined for the separation of

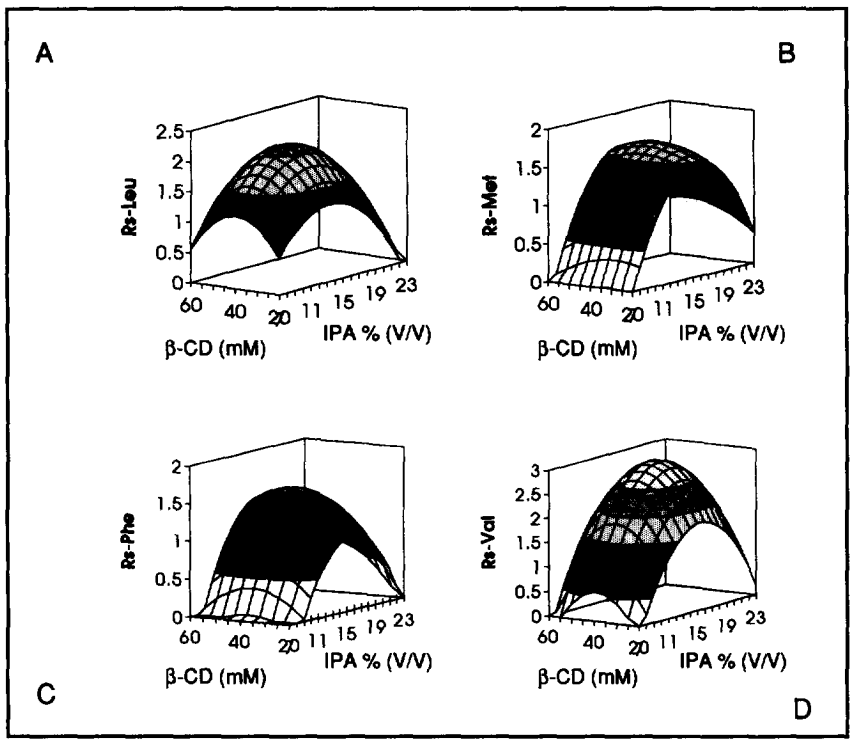

Fig. 1. Three-dimensional surfaces of optimization for four AEOC-amino acids. A=Leu; B=Met; C=Phe; D=Val. Column: 67 cm (46 cm to detector)×25 μm I.D. Conditions: buffer, 50 m*M* phosphate (pH 7.5), 40 m*M* SDS; β-CD, 20–55 m*M*; 2-propanol, 8–22% (v/v); 1 *M* urea; 30 kV; temperature 25°C; UV detection at 256 nm.

Table 1
Data of AEOC-amino acids separated with β-CD by MEKC

Amino acid	*t* (min)[a]	Elution order[b]	Selectivity α>	$\Delta\mu\times(10^{-10})$ ($m^2 V^{-1} s^{-1}$)	Resolution (R_s)	N/m ($\times 10^5$)
Ala	21.19	L	1.038	6.72	3.07	7.24
Glu	18.82	L	1.031	5.27	2.13	7.46
Ile	32.05	L	1.067	8.64	5.98	5.74
Leu	34.87	L	1.030	3.89	2.75	6.48
Met	20.52	L	1.015	2.48	1.35	8.87
Nleu	40.44	L	1.031	3.40	2.83	5.20
Nval	27.04	L	1.028	4.18	2.60	7.13
Phe	45.43	L	1.018	1.99	1.51	3.85
Ser	18.51	L	1.022	3.20	1.82	8.24
Thr	20.22	L	1.041	6.66	3.27	7.65
Trp	58.65	L	1.020	1.73	1.42	2.72
Val	25.16	L	1.022	3.50	1.89	5.48

Conditions: buffer, 50 m*M* phosphate, pH 7.50, 40 m*M* SDS, 1 *M* urea, 15% (v/v) 2-propanol (IPA), 45 m*M* β-CD; separation column, 67 cm (46 cm to detector)×25 μm I.D.; applied voltage, 30 kV; current, 11 μA; temperature, 25°C.

[a] Migration times for the first eluted enantiomer.

[b] Configuration for the first eluted enantiomer.

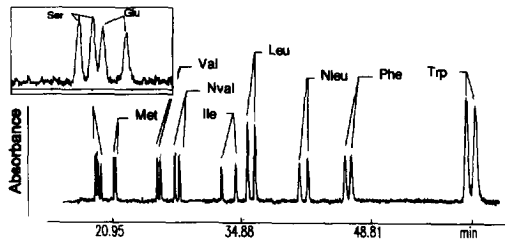

Fig. 2. Micellar electrokinetic chromatogram showing D/L separation of ten AEOC-amino acids. Conditions: buffer, 50 m*M* phosphate (pH 7.5), 40 m*M* SDS, 45 m*M* β-CD, 2-propanol, 15% (v/v); 1 *M* urea; other conditions as in Fig. 1. The insert shows an enlarged version of the first peaks in the chromatogram.

these AEOC-amino acids. As a result, four of these were well separated with an optimal resolution at 25 m*M* γ-CD. However, the maximum efficiency was found at 20 m*M* γ-CD. In Fig. 4b is shown the separation of three AEOC-amino acids with 20 m*M* γ-CD in the absence of organic modifier.

Comparing the results obtained with γ-CD and β-CD as chiral selectors, the longer migration times obtained with β-CD were probably due to the increase in viscosity resulting from the high β-CD concentration and the use of urea in the buffer. The presence of 2-propanol, however, not only resulted in improved separations for most AEOC-amino acids, but for Ala, Leu and Val, it also led to reversed enantiomeric elution order when γ-CD was used as chiral selector (Fig. 5). To our knowledge, such alteration of elution orders has not previously been demonstrated for CE. Reversal of the enantiomeric elution order, initiated by organic mobile phase additives, has, however, been observed on protein-bonded stationary phases in column liquid chromatography [17].

In the present work, reversal of enantiomeric elution order was achieved only for amino acids having a non-polar side chain. Also Ser, Thr and Ile could be separated both with or without the presence of 2-propanol, there was no change in elution order however (Table 2). The amino acids Ser and Thr have polar side chains. Further, Ile and Thr differ by having two chiral centres; D/L separation of these two compounds was, however, obtained. Another

Table 2
Data of AEOC-amino acids separated with γ-CD by MEKC

Amino acid	t (min)[a,b]	Elution order[c]		Selectivity[b] $\alpha >$	$\Delta\mu\times(10^{-10})$[b] ($m^2\ V^{-1}\ s^{-1}$)	Resolution[b] (R_s)	N/m[b]
		0% IPA	15% IPA				($\times 10^5$)
Ala	16.41	D	L	1.022	4.86	1.96	8.0
Asp	14.19	–[d]	L	1.031	6.72	2.37	7.82
Glu	14.23	–	L	1.030	6.78	2.42	8.37
Ile	23.70	L	L	1.053	9.54	5.28	7.00
Leu	26.07	D	L	1.031	5.10	3.07	6.61
Met[e]	9.58	D	–	1.073	25.9	5.49	8.93
Nleu[e]	7.72	D	–	1.067	26.7	5.87	10.9
Nval[e]	9.84	D	–	1.034	10.8	3.06	7.78
Phe	31.04	–	L	1.048	7.26	4.84	5.93
Ser	15.51	L	L	1.030	6.60	2.61	8.52
Thr	16.16	L	L	1.039	8.46	3.50	8.48
Trp[e]	12.21	D	–	1.027	7.20	2.09	6.15
Val	19.90	D	L	1.020	4.24	1.91	7.56

Conditions: buffer, 50 m*M* phosphate, pH 7.50, 40 m*M* SDS, 15% (v/v) 2-propanol (IPA), 10 m*M* γ-CD; separation column, 67 cm (46 cm to detector)×25 μm I.D.; applied voltage, 30 kV; current, 12 μA; temperature, 25°C.

[a] Migration times for the first eluted enantiomer.

[b] Data from 15% (v/v) 2-propanol (IPA).

[c] Configuration for the first eluted enantiomer.

[d] –: No separation.

[e] 25 m*M* γ-CD without 2-propanol; current, 20 μA.

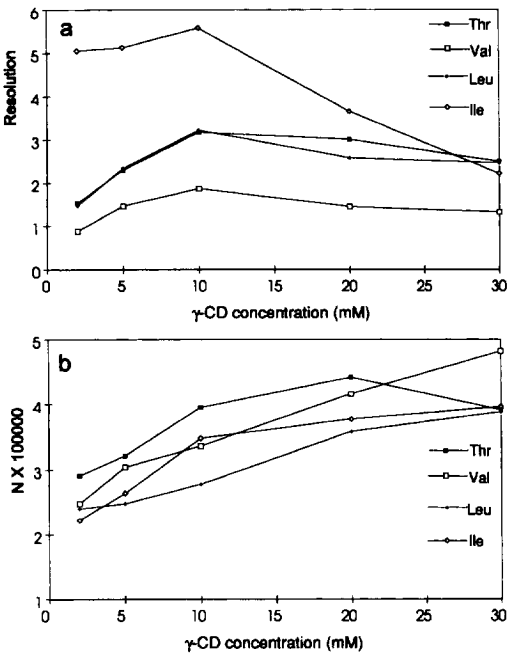

Fig. 3. Effect of γ-CD concentration on (a) the resolution and (b) efficiency of four AEOC-amino acids. Conditions: buffer, 50 m*M* phosphate (pH 7.5), 40 m*M* SDS; 2-propanol, 15% (v/v); γ-CD varied from 2 to 30 m*M*; other conditions as in Fig. 1.

group of amino acids, Met, Nleu, Nval and Trp, could only be separated in buffers without 2-propanol (Table 2). These amino acids belong to the group having longer or straight non-polar side groups. Finally, three amino acids could only be separated in the presence of 2-propanol, these being Asp, Glu and Phe. The amino acids Asp and Glu have two negative charges and Phe differs by having an extended retention time. In the buffer system used here, long retention time is an indication of strong affinity to the micelles and/or weak association with the CD. The stronger the interaction with CD, the shorter the retention time will be [18]. The outside surface of the CD is hydrophilic, thus CD will not interact with the micelle, and the CD behaves electrophoretically as the aqueous phase [19].

3.4. Chiral separation parameters

The separation mechanisms, active in the chiral separation of derivatized amino acids when using cyclodextrins as chiral selectors, were discussed by Fujimura et al. [20] and by Copper et al. [21]. Following their approach, the three points of interaction required for enantioselectivity in the present system would be inclusion of the anthrylic moiety or part of this in the CD cavity and interaction of the amino acid –NH and –COO– groups with the CD hydroxyls at the rim of the CD. Tanaka and coworkers [22,23] have demonstrated that monomethylation in the 3-position, dimethylation in the 2,3-positions and trimethylation in the 2,3,6-positions of β-CD resulted in reversed enantiomeric elution order for some dansylated amino acids having hydrophobic side chains. Obviously, such methylation affects the steric and/or hydrogen bonding interactions between CD and analyte [24,25].

In aqueous solutions, the CD-cavity may accommodate water molecules [26], but in buffers containing an organic modifier, the water in the cavities may have been replaced by the modifier. A presupposition is, however, that the modifier size and shape are suitable for the CD type in question. For example, it has been demonstrated that, for α-CD, the hydroxy-group of propanol interacts with the 6-hydroxyls of the CD [26]. It was proposed that, in this case, the propanol enters the cavity from the geometrically more open O(2),O(3) side of the CD-torus. In addition, it has been suggested that an organic modifier may aid the desorption of analytes from the CD-cavity [27].

In the CD-MEKC system applied here, the analytes are distributed between the CD, the micelles and the aqueous phase. The partitioning of the analytes between these three phases is strongly influenced by the presence of an organic modifier. Since we are aiming at chiral separations, the interactions with the CD are the main issue here. It seems that 2-propanol can influence interactions either by means of a dynamic modification of the CD-hydroxyls and/or by inclusion in the cavity, thus modifying the cavity size. It should be noted that such cavity size modification can be achieved also by inclusion of SDS monomers [28].

For the separation of relatively large molecules, such as AEOC-amino acids (see Fig. 6) with a dimension of ca. 8.8 Å, a tight geometrical fit between the anthryl group and the hydrophobic cavity of CDs could occur [23]. It has been demon-

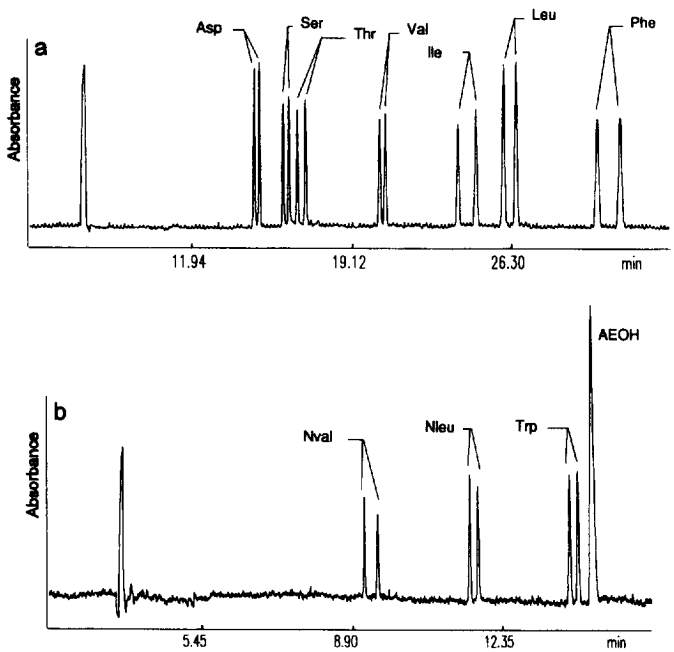

Fig. 4. Micellar electrokinetic chromatograms showing D/L separation of AEOC-amino acids. Conditions: (a) buffer, 50 m*M* phosphate (pH 7.5), 40 m*M* SDS; 10 m*M* γ-CD; 2-propanol, 15% (v/v); current, 12 μA; other conditions as in Fig. 1. (b) 40 m*M* SDS, 20 m*M* γ-CD; IPA, 0% (v/v); current, 20 μA; other conditions as in Fig. 1. AEOH=AEOC hydrolysis product.

strated that the way of fitting of an amino acid derivative, alanine β-naphthylamine, into the cavity of cyclodextrins may be strongly dependent of CD-type and modification [24]. It seems likely, that such differences in the mode of CD cavity penetration of the aromatic part of the derivative, as a function of cavity size and modification, have resulted in the reversal of elution orders observed here.

Due to the relatively smaller cavity of β-CD, a tighter fit between β-CD and the anthryl moiety can be expected. The observed high optimal concentration of β-CD indicates a relatively weak affinity of β-CD to AEOC-amino acids. Application of β-CD resulted in lower efficiencies than the application

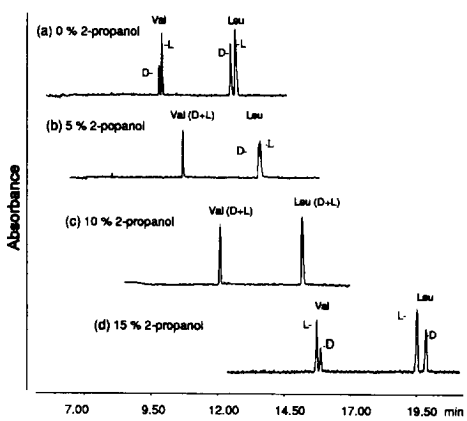

Fig. 5. Micellar electrokinetic chromatograms showing reversal of enantiomeric elution order. Conditions: buffer, 50 m*M* phosphate (pH 7.5), 40 m*M* SDS, 20 m*M* γ-CD; 2-propanol varied from 0 to 15% (v/v); other conditions as in Fig. 1.

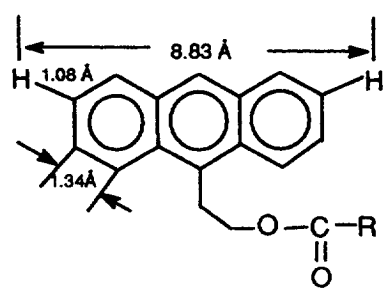

Fig. 6. Structure of AEOC-amino acids.

of γ-CD. This result suggests that a slower dynamic exchange occurred between β-CD and AEOC-amino acids. Most likely, this is due to the tighter structural fit in the β-CD cavity. On the other hand, the enhancement of efficiency in the presence of SDS, as discussed above, could be due to the speed of dynamic exchange between AEOC-amino acids, β-CD and the micelles. This hypothesis is further supported by the experimentally observed decrease in efficiency with the increase in SDS concentration when chiral selectors were absent. This is because the increase in SDS concentration, in the absence of β-CD, led to slower mass transfer, and thereby decreased efficiency. In the application of γ-CD as chiral selector, separation efficiency was, however, decreased with increasing concentration of SDS.

In contrast, γ-CD with a relatively large cavity, on the average, gives improved selectivity and efficiency because of more rapid dynamic exchange and stronger affinity to AEOC-amino acids. Thus, the optimal concentrations of γ-CD were lower than that for β-CD.

It should be noted that α-CD failed to give chiral separation for any of the AEOC-amino acids tested. Obviously, the large anthryl group could not enter the cavity of the α-CD, which thus affected the steric interaction between AEOC-amino acids and α-CD. This further demonstrates the important role of the anthryl moiety in chiral discrimination of AEOC-amino acids with cyclodextrins.

4. Conclusions

Direct chiral separation of amino acids derivatized with 2-(9-anthryl)ethyl chloroformate has been achieved with β- and γ-cyclodextrins in MEKC after optimization.

Comparing the results obtained with β-CD and γ-CD, the latter offered higher efficiency as well as selectivity for the separation of AEOC-derivatized amino acids. This could be due to the fact that γ-CD possesses a larger hydrophobic cavity and thus stronger affinity towards the anthryl group of AEOC-amino acids. Further, the optimal concentrations of γ-CD were lower than those of β-CD.

The presence of 2-propanol was found to be necessary for the separation of all AEOC-amino acids with β-CD as chiral selector. The other organic modifiers investigated were not effective for separation of AEOC-amino acids. When γ-CD was employed as chiral selector, seven of the AEOC-amino acids were separated in the absence of organic modifier and in this case the D-form was first eluted. The presence of 15% 2-propanol in the buffer deteriorated the resolution of four of these AEOC-amino acids, but improved the resolution for most of the AEOC-amino acids. Moreover, for three of the amino acids tested, the enantiomeric elution order was reversed when 2-propanol was added, the L-form now being eluted first.

Acknowledgments

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