ELSEVIER

Journal of Chromatography A, 787 (1997) 253–260

JOURNAL OF CHROMATOGRAPHY A

Determination of amino acids by high-performance capillary electrophoresis with on-line mode in-capillary derivatization

Shigeyuki Oguri*, Kenji Yokoi, Yuhko Motohase

Department of Home Economics, Aichi-Gakusen University, 28 Kamikawanari, Hegoshi-cho, Okazaki-city, Aichi 444, Japan

Received 13 March 1997; received in revised form 10 June 1997; accepted 11 June 1997

Abstract

Previously, we developed an on-line mode in-capillary derivatization method with 1-methoxycarbonylindolizine-3,5-dicarbaldehyde (IDA) reagent for the determination of some amino compounds using high-performance capillary electrophoresis (HPCE) [S. Oguri, T. Fujiyoshi and Y. Miki, Analyst, 121 (1996) 1683]. On-line mode in-capillary derivatization is a HPCE method where derivatization and separation are performed simultaneously in a capillary tube filled with a run buffer containing the derivatization reagent. Here, we report a further improvement in the sensitivity by using *o*-phthalaldehyde (OPA)/N-acetylcysteine (NAC) instead of IDA as a derivatization reagent. The HPCE separation of an amino acid mixture was much improved by adding β-cyclodextrin (β-CD) and phosphate–borate buffer (pH 10) to the run buffer. After optimization of the method, fifteen of the amino acids in a solution containing seventeen L-amino acids could be detected and separated using 15.4 mmol l^{-1} β-CD–2 mmol l^{-1} OPA/NAC–100 mmol l^{-1} phosphate–borate buffer (pH 10) as a run buffer and 22 kV as an applied voltage, respectively. Ammonia (NH_3) and cystine (Cys) did not give well defined peaks with this method. The precision ($n=5$) of this method is less than 3.0% (peak area) and 1.0% (migration time) of relative standard deviation (R.S.D.) at the 0.25 mmol l^{-1} level. Linearity was established over the concentration range 0.025 to 1.25 mmol l^{-1} of each derivative. The detection limits ($S/N=3$) range from 2.5 μmol l^{-1} for glycine to 10 μmol l^{-1} for threonine, glutamic acid and aspartic acid. In order to determine the accuracy of this method, the data obtained using a soybean protein hydrolysate were compared with data obtained using other HPLC methods. This method also enables D,L-amino acid enantiomers to be determined.

Keywords: Derivatization, electrophoresis; Soy bean; Amino acids; Phthalaldehyde; Acetylcysteine

1. Introduction

Over the past years, a number of papers have been presented on amino acid analysis. The determination of amino acids is generally accomplished by a combination of derivatization and separation of an amino acid using a pre- or post-column derivatization method with some chromophore or fluorophore, such as ninhydrin or *o*-phthalaldehyde (OPA)/thiol compound and high-performance liquid chromatography (HPLC) or high-performance capillary electrophoresis (HPCE), respectively. Although HPCE has become a useful and powerful separation technique, few practical methods have been available to date. One of the reasons is that the reproducibility of HPCE is inferior to that of HPLC, and another is that the derivatization method for HPCE is limited to a pre-column method that requires batchwise operations when an amino acid is being determined. In order to obtain reliable data, batchwise operation for

*Corresponding author.

0021-9673/97/$17.00
PII S0021-9673(97)00664-X

tagging free amino acid is disadvantageous. Recently, methods that do not require batchwise operations have been developed. One such method is indirect chemiluminescence [1] or indirect absorbance detection [2] for HPCE. Lee and Lin [2] have developed a method for the HPCE determination of twenty common amino acids using indirect absorbance detection with a background electrolyte (BGE). Although these methods are useful and simple, indirect absorbance detection method may simultaneously detect some analytes that are not amino acids, such as organic or inorganic compounds, that interfere with their separation. Another method involves post-column derivatization. Zhu and Kok [3] developed and evaluated a post-column derivatization HPCE method [3]. In this system, the direct determination of a fourteen-component amino acid solution was achieved using OPA as the post-column derivatization reagent. It may be possible to fully automate this system, but the reactor needed is not commercially available at this time. In-capillary derivatization has been known as "sandwich-mode in-capillary derivatization" [4]. Sandwich-mode in-capillary derivatization refers to a method in which the front end of a HPCE separation capillary is used as a derivatization chamber, and sample buffer and derivatization reagent are introduced into the injection end of a capillary as a "sandwich", i.e., reagent buffer–sample solution–reagent buffer. Recently, we developed an "on-line mode in-capillary derivatization" method as a third derivatization method for HPCE [5]. The on-line mode in-capillary derivatization HPCE method involves simultaneous separation and derivatization in a capillary filled with a run buffer containing a new fluorescence derivatization reagent, 1-methoxycarbonylindolizine-3, 5-dicarbaldehyde (IDA) [6], which can be used for direct determinations without the need for batchwise operations. We have used this method for the determination of a seventeen-component amino acid mixture, a four-component polyamine mixture and a four-component aminocyclitol antibiotic solution. Although this reagent is useful for the highly sensitive determination of amino acids by HPLC [7] and for the determination of aminocyclitol antibiotics in human plasma by HPCE [8], the fluorescence intensity of IDA derivatives decreases for pH values in the neutral and alkaline ranges, and IDA reacts with analyte at pH 10. When IDA reagent is used with this method, fluorescence detection cannot be adapted. The on-line mode is limited as the separation has to be carried out at pH 10, so that the run buffer can combine with the derivatization buffer. On the other hand, Saito et al. [9] have reported on an on-column derivatization HPLC method that involves simultaneous separation and derivatization on an ODS column with a mobile phase of acetonitrile and borate buffer (pH 9.9) containing OPA and N-acetyl-L-cysteine (NAC) [9].

In this paper, we tested a labeling reagent, OPA/NAC, instead of IDA, for the determination of amino acids by an on-line mode in-capillary derivatization HPCE method linked with fluorescence detection. The migration behavior of each amino acid in a capillary was studied and the separation of amino acid using on-line mode in-capillary derivatization HPCE method was optimized. Comparisons of the results obtained using the present method for the determination of the composition of soybean protein hydrolysate and the determination of D- and L-amino acids are also described.

2. Experimental

2.1. Reagents and materials

β-Cyclodextrin (β-CD), OPA and NAC were of first, biochemical and special grade, respectively. These reagents were purchased from Wako, Tokyo (Japan), and used without further purification. The other reagents used were either of HPLC grade or the highest grade commercially available. All aqueous solutions were prepared using water purified with a Milli-Q purification system (Millipore, Milford, MA, USA). An individual D- or L-amino acid (Sigma, St. Louis, MO, USA or Wako) and ammonium chloride were dissolved in water (0.05 mol l^{-1} hydrochloric acid was used for cystine and tyrosine) to make 250 μmol l^{-1} solutions. The standard amino acids solution was prepared by diluting a 2.5 mmol l^{-1} seventeen-component L-amino acid solution (Type H; Wako, Osaka, Japan) ten times with water (to give a concentration of 250 μmol l^{-1} for each amino acid). The run buffers were prepared as follows. The pH of each phosphate–borate buffer (made by mix-

ing equimolar amounts of sodium dihydrogenphosphate and sodium tetraborate at concentrations of 10, 20, 40, 60, 80 and 100 mmol l^{-1}) was adjusted to pH 10 with 1 mol l^{-1} NaOH (for concentrations of 10–60 mmol l^{-1}) or 5 mol l^{-1} NaOH (for 80 and 100 mmol l^{-1} buffer), respectively. These buffer solutions were stored at room temperature prior to use. Then, 13.4 mg of OPA and 16.3 mg of NAC and 250, 500, 750, 875, 1000, 1125 or 1250 mg of β-CD were added to each 50 ml volume of these phosphate–borate buffers (pH 10) and they were dissolved by sonication for 5 min. A portion of each solution was filtered using a disposable syringe filter unit (DISMIC-13cp from Advantec, Tokyo, Japan). These run buffers were prepared just before use.

2.2. Apparatus

HPCE systems consisted of a Jasco Model CE-800 (Jasco, Tokyo, Japan) with a FP-920 fluorescence detector (Jasco) equipped with a capillary flow-cell unit for HPCE and a model 807-IT data processor (Jasco). A capillary tube of fused-silica (75 cm effective length×50 μm I.D.) was used throughout the work. The window (1.0 cm) for detection was made by removing the polyimide coating at the 25-cm position from the cathodic end. Sample solutions were introduced into the capillary tube from the anodic side by hydrostatic injection by raising the tube 10 cm higher than the level of the cathodic electrode for 10 s. Electropherograms were recorded by monitoring the fluorescence intensity at 450 nm at an excitation wavelength of 340 nm.

2.3. HPCE separation

Two reservoirs were set at an anodic site and a cathodic site in HPCE systems, respectively. The anodic and cathodic reservoirs each contained 1 ml of run buffer with 2 mmol l^{-1} OPA/NAC as a derivatization reagent and 10 ml of 100 mmol l^{-1} phosphate–borate buffer (pH 10), respectively. Before a sample solution was injected into a capillary at the cathodic site, the residue remaining in the capillary was removed with the run buffer and the capillary was subsequently filled with the same buffer by suction at the anodic site of the capillary. When the HPCE systems were not in use, the run buffer in the capillary and in both reservoirs was replaced with 20 mmol l^{-1} phosphate–borate buffer (pH 10) or water.

2.4. Preparation of soybean protein hydrolysate

A 10-mg amount of soybean protein (Soya-Farm, Nishinn Seiyu, Tokyo, Japan) was suspended in 10 ml of 6 mol l^{-1} hydrochloric acid in a 50-ml glass ampule. The ampule was heated at 110°C for 20 h after sealing it under a vacuum. Then, a 1-ml portion of the hydrolyzed solution was evaporated to dryness. The brownish solid was dissolved in 1 ml of 0.1 mol l^{-1} hydrochloric acid and diluted to 10 ml with water. The mixture was then filtered using a DISMIC-13cp syringe filter unit. The filtrate of the hydrolyzed solution of soybean protein was further diluted ten-fold with water.

3. Results and discussion

3.1. Optimization of HPCE separations and migration behavior

When determining amino acids using HPCE with on-line mode in-capillary derivatization, the pH of the run buffer is a dominant factor because the run buffer serves both as a separation buffer and as a derivatization buffer. OPA/NAC was used as the derivatization reagent in these studies and the pH of run buffer was fixed at 10, which was the optimum pH of this reagent [10]. The run buffer consisting of sodium dihydrogenphosphate and sodium tetraborate, was chosen as being the most suitable buffer based on peak performance on HPCE electropherograms. When solutions of sodium tetraborate, disodium hydrogenphosphate or sodium dihydrogenphosphate were adjusted at pH 10 with NaOH and used separately, the separations obtained were poor, as were peak responses. The concentrations of OPA and NAC were fixed at 2 mmol l^{-1}, but their migration behaviors were not influenced greatly by their concentrations. It was expected that the simultaneous determination of amino acids using a run buffer at pH 10 would cause difficulties due to the amino acids being zwitterionic. Some additives, such as

sodium dodecyl sulfate (SDS), ion-paired reagent, urea and organic solvent, were examined by adding them to run buffer at pH 10. As a result of having tested these additives, β-CD was found to be the most effective for use with the on-line mode in-capillary derivatization HPCE method. Optimization of the HPCE separation and migration behavior was performed using a seventeen-component L-amino acid standard solution. The migration parameters of analytes were expressed as migration time, not used as capacity factor. Aqueous phase times are necessary for calculation of the capacity factors of peaks. However, there were some cases when it was difficult to determine the migration time corresponding to the aqueous phase on electropherograms.

3.1.1. Effect of β-CD concentrations

The effect of β-CD concentration on the migration time of each amino acid was investigated over a concentration range from 4.4 to 22.0 mmol l^{-1}, using a buffer concentration of 100 mmol l^{-1} (pH 10) containing 2 mmol l^{-1} OPA/NAC. The applied voltage used for these studies was 25 kV. The results are shown in Fig. 1. Although about ten peaks were detected on an electropherogram when β-CD was not added (data were not shown), fifteen peaks were observed when β-CD was added at concentrations of between 15.4 and 22.0 mmol l^{-1}. Two components of the seventeen-component L-amino acid solution, ammonia (NH_3) and cystine (Cys), did not give rise to definite peaks in this system. It is not clear why separations are improved by adding β-CD to the run buffer. However, it is generally well known that β-CDs are toroid-shaped cyclic oligosaccharides that are made up of seven α-1,4-linked D-glucopyranose units, with the inside of the cavity being hydrophobic. The molecule probably has the ability to form specific inclusion complexes with amino acid–OPA/NAC derivatives that depend on the size and polarity of the host molecule. In fact, the migration times of aspartic acid and glutamic acid, both of which have a carboxymethyl group (CH_2-COOH), decrease more with increasing β-CD concentration than those of the other amino acids.

3.1.2. Effect of buffer concentration

The effect of the concentration of phosphate–borate run buffer at pH 10 on these separations was examined at concentrations of 10, 20, 40, 60, 80 and 100 mmol l^{-1}, with 15.4 mmol l^{-1} β-CD and 2 mmol l^{-1} OPA/NAC. An applied voltage of 25 kV was used in all cases. As shown in Fig. 2, the

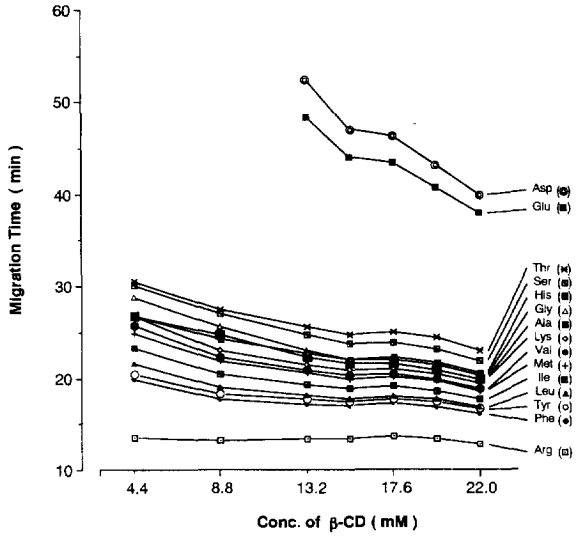

Fig. 1. Effect of β-CD concentration on migration time. The run buffer and applied voltage used were 2 mmol l^{-1} OPA/NAC–100 mmol l^{-1} phosphate–borate buffer (pH 10) and 25 kV, respectively. Other HPCE conditions were as described in Section 2.

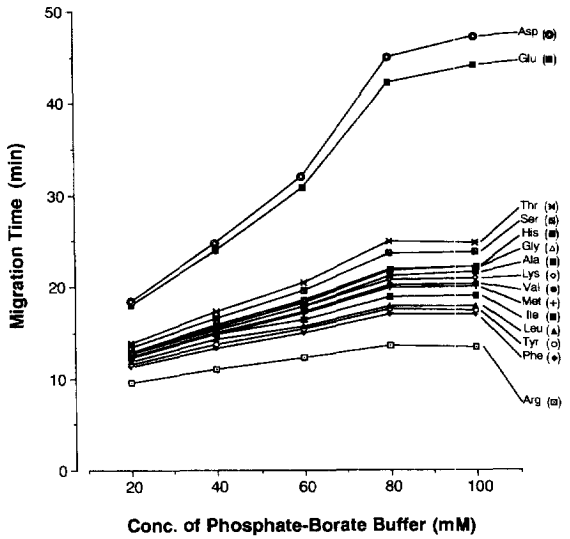

Fig. 2. Effect of phosphate–borate buffer (pH 10) concentration on migration time. The concentration of β-CD in the run buffer was 15.4 mmol l^{-1}. Other HPCE conditions as in Fig. 1.

migration order of each amino acid is changed slightly, and the separation is much improved on increasing the concentration of buffer. As a result of this studies, a phosphate–borate buffer concentration of 100 mmol l^{-1} was taken to be optimal.

3.1.3. Effect of applied voltage

The effect of the applied voltage on the separation of amino acids was examined by varying the applied voltage from 17 to 25 kV. The concentrations of β-CD and buffer were fixed at 15.4 and 100 mmol l^{-1}, respectively. Fig. 3 shows the profile of migration time versus applied voltage. The migration time of each amino acid decreases gradually with increasing applied voltage, and the migration order is also changed slightly. In these studies, 22 kV was chosen as the optimum applied voltage. Fig. 4 shows a typical electropherogram of the seventeen-component L-amino acid standard solution; fifteen of the L-amino acids were well separated from each other, the exceptions being NH_3 (* on Fig. 4) and Cys (** on Fig. 4), which did not form well-defined peaks. It is well known that Cys–OPA–2-mercaptoethanol (2ME) derivatives have only about 5% of the fluorescence of other natural amino acids. However, Benson and Hare [11] overcame this shortcoming by increasing the concentration of 2-ME ten-fold. In our system, resolution might be improved by increasing the concentrations of NAC in the run buffer.

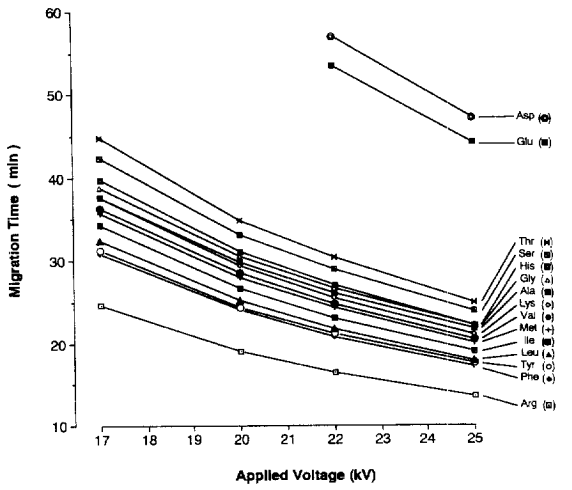

Fig. 3. Effect of applied voltage on migration time. The run buffer used was 15.4 mmol l^{-1} β-CD–2 mmol l^{-1} OPA/NAC–100 mmol l^{-1} phosphate–borate buffer (pH 10). Other HPCE conditions as in Fig. 1.

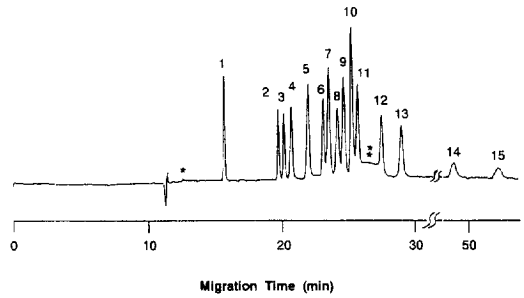

Fig. 4. Typical HPCE pherogram of a seventeen-component L-amino acid solution. The run buffer and the applied voltage were 15.4 mmol l^{-1} β-CD–2 mmol l^{-1} OPA/NAC–100 mmol l^{-1} phosphate–borate buffer (pH 10) and 22 kV, respectively. Other HPCE conditions as in Fig. 1. Peaks: 1 = L-Arginine (Arg); 2 = L-phenylalanine (Phe); 3 = L-tyrosine (Tyr); 4 = L-leucine (Leu); 5 = L-isoleucine (Ile); 6 = L-methionine (Met); 7 = L-valine (Val); 8 = L-lysine (Lys); 9 = L-alanine (Ala); 10 = glycine (Gly); 11 = L-histidine (His), 12 = L-serine (Ser); 13 = L-threonine (Thr); 14 = L-glutamic acid (Glu) and 15 = L-aspartic acid (Asp). The symbols * and ** represent ammonia (NH_3) and cystine (Cys), respectively.

3.2. Analytical reproducibility, linearity, peak response factor and detection limit

Analytical reproducibility was investigated by performing five identical analyses on fifteen amino acids of the seventeen-component L-amino acid standard solution. Table 1 shows the results in terms of reproducibility (relative standard deviation, R.S.D.) corresponding to peak area response and migration time, respectively. The R.S.D. values (%) for peak area and migration time were less than 3.0 and 1.0%, respectively, for each amino acid. R.S.D. values of less than 2.0% (peak area) and 1.1% (retention time), respectively, were obtained previously [12] using a pre-column HPLC method with 9-fluorenylmethyl chloroformate and an automated amino acid analyzer. Therefore, the reproducibility of our method is relatively good.

The linearity of each peak area, corresponding to its amino acid response at concentrations of between 0.025 and 1.25 mmol l^{-1}, was calculated by the least squares regression method for $y = A + Bx$, where y, x, A and B are peak area counts, concentration of amino acid (mmol l^{-1}), intercept at y axis and slope,

Table 1
Linearity for peak area response, detection limit, peak area response factor and reproducibility

Amino acid	Linearity ($\times 10^5$)[a]			Detection limit (μmol l^{-1}) ($S/N=3$)	Peak response factor[b]	Reproducibility (R.S.D., %, $n=5$)	
	A	B	r			Area	Time
Arginine (Arg)	−0.020	1.245	0.996	5	1.000	1.3	0.4
Phenylalanine (Phe)	−0.013	0.735	0.999	5	0.513	1.1	0.5
Tyrosine (Tyr)	−0.002	0.638	0.999	5	0.545	1.7	0.5
Leucine (Leu)	−0.035	1.233	0.995	5	0.859	1.8	0.5
Isoleucine (Ile)	−0.046	1.826	0.996	5	1.341	1.8	0.5
Methionine (Met)	−0.016	0.901	0.996	5	0.687	1.4	0.6
Valine (Val)	−0.034	1.892	0.998	5	1.394	1.6	0.5
Lysine (Lys)	0.011	1.000	0.999	5	1.099	1.4	0.5
Alanine (Ala)	−0.050	1.971	0.995	5	1.441	1.1	0.4
Glycine (Gly)	−0.064	2.229	0.997	2.5	1.744	2.0	0.4
Histidine (His)	−0.063	1.445	0.999	5	0.812	1.5	0.5
Serine (Ser)	−0.003	1.515	0.999	5	0.958	2.6	0.5
Threonine (Thr)	−0.016	0.740	0.996	10	0.394	2.1	0.6
Glutamic acid (Glu)	−0.031	0.381	0.991	10	0.202	3.0	0.7
Aspartic acid (Asp)	−0.011	0.235	0.998	10	0.186	2.9	1.0

[a] y (peak area count) $=A+Bx$ (amino acid concentration, 0.025–1.25 mmol l^{-1}), r = correlation factor.
[b] Normalized to arginine at a concentration of 0.25 mmol l^{-1}.

respectively. The results obtained were good, with correlation factors (r) ranging between 0.991 and 0.999. The peak area response was normalized to arginine at the 0.25 mmol $l-^{1}$ level and detection limits were obtained using a signal to noise ratio (S/N) of three. These parameters are also listed in Table 1. Based on these data, the detection limits range from 2.5 μmol l^{-1} for glycine to 10 μmol l^{-1} for threonine, glutamic acid and aspartic acid. The detection limits are approximately the same as those reported previously [3] using a post-column labeling HPCE method.

3.3. Comparison of the present method with other HPCE methods

Aliquots of soybean protein hydrolysate were analyzed by the present method, and a typical electropherogram is shown in Fig. 5. To determine the accuracy of the present method, these data were compared with HPLC data that were cited in a previous paper [7] using the IDA HPLC method and a classical amino acid analyzer, respectively. The results of the data are listed in Table 2. Although, the data obtained using the present method (I in Table 2) vary by about 5 to 10% from the corresponding data obtained using the IDA HPLC method (II in Table 2) or the classical amino acid analyzer method (II in Table 2), the present method is relatively good, considering the native abilities of HPCE.

The present method is not sensitive enough to determine the secondary amino acids, proline and

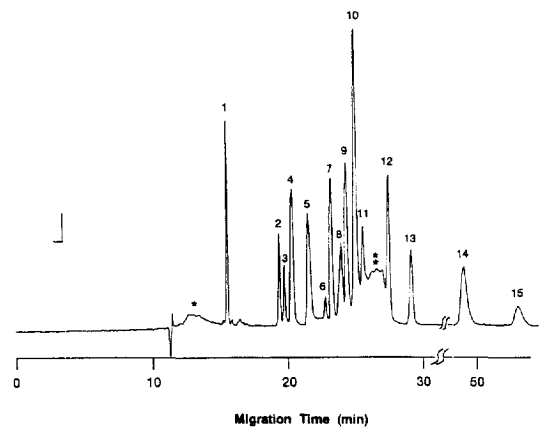

Fig. 5. Typical HPCE pherogram of soybean protein hydrolysate. The procedures used for the hydrolyzation of protein have been described in ref. [6]. Other HPCE conditions as in Fig. 4.

Table 2
Compositional data from hydrolyzed soybean protein

Amino acid	HPCE	HPLC	
	I[a]	II[b]	III[c]
Arginine (Arg)	832	801.5	804.8
Phenylalanine (Phe)	614	627.4	629.1
Tyrosine (Tyr)	347	–	336.0
Leucine (Leu)	1268	1252.6	1258.3
Isoleucine (Ile)	768	701.5	703.1
Methionine (Met)	138	151.2	150.5
Valine (Val)	850	827.1	840.4
Lysine (Lys)	698	724.6	737.7
Alanine (Ala)	1072	965.4	973.1
Glycine (Gly)	1116	1203.4	1218.4
Histidine (His)	326	347.2	333.8
Cystine (Cys)	–	13.6	12.8
Serine (Ser)	964	1151.1	1157.2
Threonine (Thr)	636	695.3	689.2
Glutamic acid (Glu)	3038	3331.8	3284.4
Asparagine (Asp)	1662	1819.5	1827.9

Data are presented at the nmol/ml of hydrolyzed solution level. I[a], II[b] and III[c] are data obtained using the present method, the IDA HPLC method and the amino acid analyzer, respectively. Both II and III are taken from Ref. [7].

oxyproline. However, if oxidation (e.g. with sodium hypochlorite) was performed to open the proline ring, these amino acids would be detectable.

3.4. Application to the chiral separation of D,L-amino acids

OPA/NAC reagent reacts with primary amino acids to form diastereomers. A few applications as to a chiral separation of D,L-amino acid by using this diastereomer have been published [13]. The present method can also be applied to the separation of enantiomers of D,L-amino acids. Fig. 6 shows a typical electropherogram obtained for a six-component D,L-amino acid solution (Arg, Ile, His, Ala, Glu and Asp) at a concentration of 0.5 mmol l^{-1}. A list of their migration times and resolutions (R_s) are given in Table 3. R_s values were calculated using the following equation

$$R_s = 1.18\Delta t/(\omega_{(0.5)1} + \omega_{(0.5)2})$$

where Δt is the difference in migration times and $\omega_{(0.5)1}$ and $\omega_{(0.5)2}$ are the peak widths at half the peak height corresponding to each enantiomer peak, respectively. Although further trials were carried out by changing the contents of the run buffer (while keeping the pH at 10), D,L-serine (Ser) could not be separated.

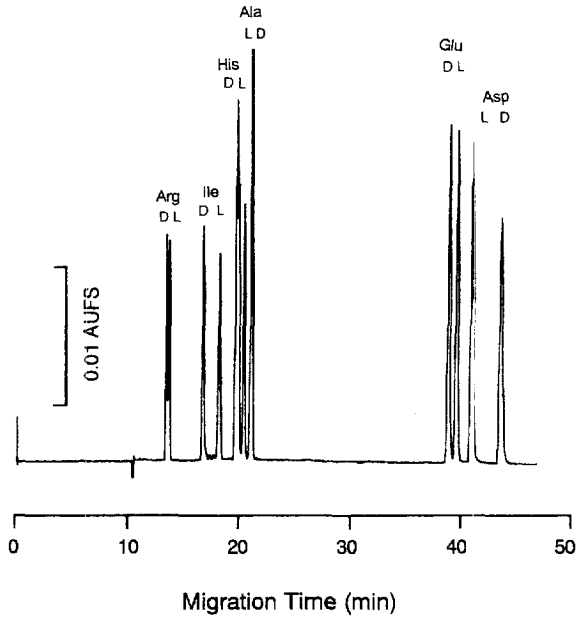

Fig. 6. Typical HPCE pherogram of a six-component D,L-amino acid solution. The run buffer and applied voltage used were 8.8 mmol l^{-1} β-CD–2 mmol l^{-1} OPA/NAC–60 mmol l^{-1} phosphate–borate buffer (pH 10) and 25 kV, respectively. Other HPCE conditions as in Fig. 1.

4. Conclusion

In a previous paper, we developed an on-line mode in-capillary derivatization method using HPCE with IDA reagent. Here, IDA was replaced by an OPA/NAC reagent system and fluorescence detection was carried out linking a fluorescence detector linked to the capillary flow-cell unit. Although the present method is not useful for cystine, secondary amines and ammonia, its sensitivity and reproducibility can be used for the direct determination of amino acids without the need for a clean-up step or batchwise operations prior to HPCE analysis. Furthermore, this system may also be used for the determination of the enantiomers of D,L-amino acids.

Table 3
Migration time and resolution of individual D,L-amino acids

Amino acid	Migration time (min)		Resolution
Arginine (Arg)	D	11.47	0.767
	L	11.65	
Phenylalanine (Phe)	L	15.53	2.819
	D	16.38	
Isoleucine (Ile)	D	15.49	5.962
	L	17.40	
Leucine (Leu)	D	16.26	1.573
	L	16.71	
Valine (Val)	D	16.47	5.282
	L	18.33	
Methionine (Met)	D	17.73	1.991
	L	18.28	
Histidine (His)	D	18.48	0.593
	L	18.78	
Lysine (Lys)	D	18.89	0.952
	L	19.46	
Threonine (Thr)	D	19.64	2.995
	L	20.95	
Alanine (Ala)	L	20.58	2.103
	D	21.38	
Serine (Ser)	D	21.33	0
	L	21.33	
Glutamic acid (Glu)	D	38.96	2.068
	L	39.76	
Aspartic acid (Asp)	L	40.96	4.430
	D	43.78	

Data were obtained using 8.8 mmol l^{-1} β-CD–2 mmol l^{-1} OPA/NAC–60 mmol l^{-1} phosphate–borate buffer (pH 10) and 25 kV (applied voltage). Other HPCE conditions were as described in Section 2.

References

[1] S.-Y. Liao, C.-W. Whang, J. Chromatogr. A 736 (1996) 247.
[2] Y.-H. Lee, T.-I. Lin, J. Chromatogr. A 680 (1994) 287.
[3] R. Zhu, W.T. Kok, J. Chromatogr. A 716 (1995) 123.
[4] S. Honda and S. Terabe (Editors), Capillary Electrophoresis, Kohdanshya Scientific, Tokyo, Japan, 1995, Ch. 2.
[5] S. Oguri, T. Fujiyoshi, Y. Miki, Analyst 121 (1996) 1683.
[6] S. Oguri, C. Uchida, Y. Miyake, Y. Miki, K. Kakehi, Analyst 120 (1995) 63.
[7] S. Oguri, C. Uchida, M. Mishina, Y. Miki, K. Kakehi, J. Chromatogr. A 724 (1996) 169.
[8] S. Oguri, Y. Miki, J. Chromatogr. B 686 (1996) 205.
[9] K. Saito, M. Horie, N. Nose, K. Nakagomi, H. Nakazawa, J. Chromatogr. 595 (1992) 163.
[10] M. Roth, A. Hampai, J. Chromatogr. 83 (1973) 353.
[11] J.R. Benson, P.E. Hare, Proc. Natl. Acad. Sci. U.S.A. 72 (1975) 619.
[12] P.A. Haynes, D. Sheumack, J. Chromatogr. 588 (1991) 107.
[13] N. Nimura, T. Kinoshita, J. Chromatogr., 352 (1986) 169.