1-Methoxycarbonylindolizine-3,5-dicarbaldehyde as a Derivatization Reagent for Amino Compounds in High-performance Capillary Electrophoresis

Shigeyuki Oguri, Chikako Uchida and Yasuko Miyake

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

Yasuyoshi Miki and Kazuaki Kakehi

Faculty of Pharmaceutical Sciences, Kinki University, 3-4-1 Kowakae, Higashi-Osaka, 577, Japan

The indolizine derivative 1-methoxycarbonylindolizine-3,5-dicarbaldehyde (IDA) was synthesized from 2-(1,3-dioxolan-2-yl)pyridine in four steps. The reactivity of the reagent towards primary amines was investigated by using alanine (Ala) as a model compound. The reagent easily reacted with Ala in 20 mmol l^{-1} phosphate–borate buffer at pH 10 containing 25–50% v/v of ethanol in the dark at room temperature, and the reaction was completed within 15 min. The IDA derivative of alanine (IDA-Ala) showed a strong absorption at 280 nm ($\varepsilon = 3.31 \times 10^4$ l mol^{-1} cm^{-1}) and 409 nm ($\varepsilon = 2.18 \times 10^4$ l mol^{-1} cm^{-1}). The derivative also showed fluorescence at 482 nm when irradiated at 282 or 414 nm. These wavelengths did not overlap with those of IDA. IDA-Ala showed a 1.5 times stronger absorption than that produced by the ninhydrin method, and the fluorescence intensity of IDA-Ala was about three times that produced by *o*-phthalaldehyde-Ala. On analysis of IDA-Ala by high-performance capillary electrophoresis (HPCE) in the capillary zone electrophoresis (CZE) mode with detection at 280 nm, the calibration graph showed good linearity over the range 0.0172–21.5 μmol ml^{-1} of alanine. The detection limit for alanine was about 5 nmol ml^{-1}. The relative standard deviations in the determination of IDA-Ala at 10.8 and 21.5 μmol ml^{-1} were less than 2% ($n = 5$). Application to the analysis of a mixture of amino acids by HPCE using the micellar electrokinetic chromatography mode is also described.

Keywords: *1-Methoxycarbonylindolizine-3,5-dicarbaldehyde; amino acids; high-performance capillary electrophoresis; micellar electrokinetic chromatography capillary zone electrophoresis*

Introduction

Amino compounds having primary amino groups such as amino acids or aminocyclitol antibiotics lack chromopores or fluorophores in their molecules. Derivatization is required for their detection with high sensitivity using high-performance liquid chromatography (HPLC) or high-performance capillary electrophoresis (HPCE) with photometric and fluorimetric detections.[1–6]

Recently, HPCE has become an attractive separation method owing to its high resolution. In spite of a wide variety of detection techniques developed for HPCE,[7–10] UV detection is still one of the most popular methods for commercially available HPCE systems.

We have studied the synthesis of indolizine analogues, which have shown UV absorption and fluorescence.[11] Some of the intermediate compounds in their syntheses possibly react with various biological compounds having amino or aldehyde groups. In addition, the indolizine derivatives contain hetero-aromatic N-containing rings in their molecules, and seem to be advantageous for separation by HPCE owing to their electric charge.

In this paper, we report the synthesis of the indolizine derivative 1-methoxycarbonylindolizine-3,5-dicarbaldehyde (IDA), from 2-(1,3-dioxolan-2-yl)pyridine in four steps, and an investigation of its reactivity towards primary amines using amino acids as model compounds.

Experimental

Chemicals

Standard samples of amino acids were purchased from Takara Kohsan (Tokyo, Japan). A standard solution of an amino acid mixture (Type H) for amino acid analysers was obtained from Wako (Dosho-machi, Osaka, Japan). Other reagents were of the highest or HPLC grade commercially available. All aqueous solutions were prepared by using water purified with a Milli-Q purification system (Millipore, Milford, MA, USA).

Reagents for derivatization of amino acids

For ease of handling, the dried reagent (IDA) in a tube was prepared and used in this study. The derivatization procedure was easily started by placing the reaction buffer and the sample solution in a tube. IDA was then dissolved in ethyl acetate by sonication to give a 1.00 mg ml^{-1} solution. A portion (100 μg per 100 μl, 0.43 μmol) was transferred into a 0.6 ml polypropylene microcentrifuge tube. The solvent was carefully evaporated to dryness by placing the tubes in a desiccator under reduced pressure. The reagent tubes were then tightly capped and stored in a refrigerator in the dark until use.

Reaction buffer. The reaction buffer was prepared by mixing an equal volume of ethanol and 20 mmol l^{-1} phosphate–borate buffer (Equal volumes of 20 mmol l^{-1} sodium dihydrogenphosphate and 20 mmol l^{-1} sodium tetraborate were mixed and the pH was adjusted to 10 by adding 1 mol l^{-1} sodium hydroxide).

IDA solution. A 40 μl volume of the reaction buffer was added to the reagent tube. The solution was prepared just before use.

Aqueous solution of alanine as standard sample. An aqueous solution of alanine (21.5 μmol ml^{-1}) was prepared by dissolution of alanine (19.2 mg) in 10.0 ml of water. A standard solution of alanine containing 3-nitrophenol

(internal standard; MNP) was also prepared in the same manner except for the use of a 2.5 mg ml^{-1} aqueous solution of MNP as the solvent. These solutions were stored in a refrigerator.

Apparatus

Proton nuclear magnetic resonance (NMR) spectra were recorded on a JEOL FX-200 spectrometer at 200 MHz using tetramethylsilane as the internal standard. Infrared (IR) spectra were recorded with a Hitachi EPI-G2 spectrophotometer. UV/VIS absorption spectra were obtained with a Hitachi 220A spectrophotometer using a 1 cm quartz cell. For measurement of the excitation and emission spectra, a Hitachi F-3010 spectrofluorimeter with a 1 cm quartz cell was employed.

HPCE was performed on a JASCO CE-800 system with a JASCO 807-IT data processor. A capillary tube of fused silica (50 μm i.d., 50 cm effective length) was used throughout. The window (0.7 mm) for detection was made by removing the polyimide coating at the 10 cm position from the cathodic end. The applied voltage with 20 kV throughout. Sample solutions were introduced into the capillary tube from the anodic side by hydrostatic injection by raising the tube 15 cm higher than the level of the cathodic electrode for 5 s. The electropherograms were recorded by monitoring the UV absorption at 280 nm. The carrier electrolyte was 20 mmol l^{-1} phosphate–borate buffer (pH 10) for the capillary zone electrophoresis (CZE) mode, and 20 mmol l^{-1} sodium dodecyl sulfate (SDS)–40 mmol l^{-1} phosphate–borate buffer (pH 7) containing methanol at a concentration of 3% v/v for the micellar electrokinetic chromatography (MEKC) mode.

Synthesis of 1-methoxycarbonylindolizine-3,5-dicarbaldehyde (IDA)

1-(tert-Butoxycarbonylmethyl)-2-(1,3-dioxolan-2-yl)pyridinium bromide (2)

A solution of 2-(1,3-dioxolan-2-yl)pyridine[12] (**1**; 15.1 g, 0.1 mol) and *tert*-butyl bromoacetate (19.5 g, 0.1 mol) in dry acetonitrile (100 ml) was refluxed for 7 h and the reaction mixture was kept overnight at room temperature. The precipitated crystalline material was collected and washed with diethyl ether to give 26.8 g (78%) of **2**: m.p. 110–112 °C (acetonitrile); IR (Nujol, cm^{-1}), 1740; NMR ($CDCl_3$), δ 1.51 (m, 9H), 4.0–4.2 (m, 4H), 5.97 (s, 2H), 6.43 (s, 1H), 8.16 (ddd, *J* 8, 6, 2 Hz, 1H, H-5), 8.24 (dd, *J* 8, 2 Hz, H-3), 8.63 (br t, *J* 8 Hz, 1H, H-4), 9.77 (dd, *J* 6, 1 Hz, 1H, H-6). Analysis: calculated for $C_{14}H_{20}NO_4Br$: C, 48.57; H, 5.82; N, 4.05; found: C, 48.58; H, 6.15; N, 4.85%.

Methyl 3-tert-butoxycarbonyl-5-(1,3-dioxolan-2-yl)indolizine-1-carboxylate (3)

To a suspension of **2** (51.9 g, 0.15 mol) in tetrahydrofuran (1500 ml) were added potassium carbonate (62.1 g, 0.45 mol) and methyl propiolate (15.12 g, 0.18 mol) and the mixture was stirred for 9 d at room temperature. The insoluble material was removed by filtration and the filtrate solution was concentrated *in vacuo*. The residue was applied on a column of silica gel and eluted with hexane–ethyl acetate (20 + 1–10 + 1). The fractions containing **3** were collected and evaporated to dryness to afford 35.0 g (67%) of **3**: m.p. 138–139 °C (hexane); IR (Nujol, cm^{-1}), 1690; NMR ($CDCl_3$), δ 1.63 (s, 9H), 3.7–4.05 (m, 4H), 3.91 (s, 3H), 6.85 (s, 1H), 7.2–7.3 (m, 2H, H-6 and H-7), 7.78 (s, 1H, H-2), 8.3–8.4 (m, 1H, H-8). Analysis: calculated for $C_{18}H_{21}NO_6$: C, 62.24; H, 6.10; N, 4.03; found: C, 62.19; H, 6.11; N, 4.21%.

1-Methoxycarbonylindolizine-5-carbaldehyde (4)

A solution of **3** (20.82 g, 0.06 mol) in a mixture of 10% hydrochloric acid (60 ml) and tetrahydrofuran (600 ml) was refluxed for 6 h and the mixture was concentrated to about one quarter of the original volume. Water was added to the mixture, which was then extracted with chloroform. The chloroform layer was washed with water and dried over sodium sulfate. The solution was evaporated and purified on a column of silica gel with hexane–ethyl acetate (10 + 1) as the eluent to afford 11.58 g (95%) of **4**: m.p. 135–137 °C (methanol); IR (Nujol, cm^{-1}), 1675, 1695; NMR ($CDCl_3$), δ 3.92 (s, 3H), 7.18 (dd, *J* 9.7 Hz, 1H, H-7), 7.38 (d, *J* 3 Hz, 1H, H-2), 7.41 (dd, *J* 7.2 Hz, 1H, H-6), 8.57 (br d, *J* 9 Hz, 1H, H-8), 8.84 (d, *J* 3 Hz, 1H, H-3), 9.86 (s, 1H, CH). Analysis: calculated for $C_{11}H_9NO_3$: C, 65.02; H, 4.46; N, 6.89; found: C, 65.07; H, 4.55; N, 7.03%.

1-Methoxycarbonylindolizine-3,5-dicarbaldehyde (IDA)

To a solution of **4** (12.18 g, 0.06 mol) in dry dimethylformamide (116 ml, 1.5 mol) was added phosphorus oxytrichloride (17 ml, 0.18 mol) at 0 °C under an argon atmosphere, and the mixture was stirred for 1 h at room temperature. The reaction mixture was poured into water and made alkaline (pH 9.0) with 5% potassium carbonate solution. The solution was extracted with chloroform and the chloroform layer was washed with water and dried over sodium sulfate. The solution was concentrated to form a precipitate, which was collected by filtration to yield 7.41 g (53%) of IDA. The mother liquid was further concentrated to afford a residue, which was purified on a column of silica gel with hexane–ethyl acetate (5 + 1) as the eluent to recover 1.06 g (8%) of IDA: m.p. 164–165 °C (methyl acetate); IR (Nujol, cm^{-1}), 1640, 1700; NMR ($CDCl_3$), δ 3.97 (s, 1H), 7.5–7.65 (m, 2H, H-6 and H-7), 8.09 (s, 1H, H-2), 8.55–8.65 (m, 1H, H-8), 9.67 (s, 1H), 10.11 (s, 1H). Analysis: calculated for $C_{12}H_9NO_4$: C, 62.34; H, 3.92; N, 6.06; found: C, 62.40; H, 4.04; N, 6.06%.

Measurement of Absorption and Fluorescence Spectra of the Derivatized Product of Alanine with Ninhydrin, o-Phthalaldehyde (OAP) and IDA

The derivatization reactions of alanine with ninhydrin and *o*-phthaldehyde were performed according to the literature.[13,14]

Ninhydrin method

Ninhydrin (200 mg) was dissolved in a mixture of 4 mol l^{-1} sodium acetate buffer (pH 5.5, 2.5 ml) and 2-methoxyethanol (7.5 ml). The solution (40 μl) was mixed with a standard solution of alanine (40 μl) in a 0.6 ml polypropylene microcentrifuge tube. The mixture was kept for 15 min at 90 °C in a water-bath and cooled to room temperature.

OPA method

A solution of OPA was prepared by dissolution of OPA (28.8 mg) in methanol (1 ml) and 0.1 mol l^{-1} sodium hydroxide (9 ml). A solution of 2-mercaptoethanol (2-ME) was prepared by dilution of 2-Me (18.7 μl) with 0.1 mol l^{-1} sodium hydroxide solution (10 ml). Volumes of 20 μl of each of OPA, 2-ME and the standard solution of alanine was mixed and kept for 10 min at room temperature.

IDA method

A mixture of IDA solution (40 μl) and the standard solution of alanine (20 μl) was kept for 15 min at room temperature in the dark.

For measurement of absorption spectra, 10 μl of the reaction solution obtained by the ninhydrin method and the IDA method were diluted with water (3 ml) and used for the observation of absorption spectra. A portion of the mixture (10 μl) obtained from the OPA and the IDA method was also diluted with water (50 ml) and used for measurement of fluorescence spectra.

Optimization of Derivatization

For studies of the effect of pH on the course of the reaction of IDA and alanine, buffer solutions of pH 6–12 were used. Buffers of pH 6–9 were prepared by mixing 20 mmol l^{-1} sodium dihydrogenphosphate and 20 mmol l^{-1} sodium tetraborate. Buffers of pH 10–12 were prepared by mixing 500 ml of 20 mmol l^{-1} sodium dihydrogenphosphate and 500 ml of 20 mmol l^{-1} sodium tetraborate and adjusting the pH with 1 mol l^{-1} sodium hydroxide solution. The buffer solutions were mixed with an equal volume of ethanol and used as the reaction buffer. IDA (100 μg, 0.43 μmol) in a tube was dissolved in the reaction buffer (40 μl). After addition of the standard solution of alanine containing MNP (20 μl), the mixture was kept at room temperature in the dark. An aliquot of the mixture was subjected to the HPCE in the CZE mode.

Comparison of the Stabilities of the Derivatized Products Between the Present Method and the OPA method

A mixture of IDA solution (40 μl) and a standard solution of alanine containing MNP (20 μl) was kept at room temperature and an aliquot was introduced into the HPCE capillary at the intervals specified in Table 1. The reagent solution in water (20 μl) was also kept in the dark, and an aliquot was introduced into the capillary to confirm the stability. The reaction between OPA and alanine was performed in the same manner as described above. An aliquot was introduced into the capillary without dilution at the same intervals as described for the present method. With OPA-Ala, a detection wavelength of 340 nm was employed for the HPCE systems.

Results and Discussion

Synthesis of IDA

1-Methoxycarbonylindone-3,5-dicarbaldehyde (IDA) was synthesized from 2-(1,3-dioxolan-2-yl)pyridine (**1**) as the starting material as shown in Scheme 1.

Pyridine derivative **1** was treated with *tert*-butyl bromoacetate to give a pyridinium salt (**2**). This pyridinium salt was then condensed with methyl propiolate in the presence of potassium carbonate to afford methyl 3-*tert*-butoxycarbonyl-5-(1,3-dioxolan-2-yl)indolizine-1-carboxylate (**3**). Deprotonation and decarboxylation of the indolizine derivative (**4**) followed by formylation with dimethylformamide and phosphorus oxychloride gave IDA in the total yield of 30% from **1**. From the proton NMR investigation of IDA, disappearance of the proton signals at 7.38 and 8.84 ppm in **4** showed subsitution of the 3-position, which is more reactive than the 4-position. The two signals observed at 9.67 and 10.11 ppm showed clearly the presence of two aldehyde groups. Thus, IDA was easily confirmed to have the structure shown in Scheme 1.

Table 1 Stabilities of IDA, IDA-Ala and OPA-Ala. Each value represents the peak-area ratio of IDA, IDA-Ala or OPA-Ala to MNP obtained by the HPCE method in the CZE mode. The detection wavelength for IDA and IDA-Ala was 280 nm and that for OPA-Ala was 340 nm

	Time/h				
Sample	0.5	1.0	4.0	6.0	24
IDA	0.51	0.51	0.52	0.51	0.49
IDA-Ala	1.71	1.73	1.70	1.65	1.68
OPA-Ala	4.89	4.84	4.28	3.99	2.33

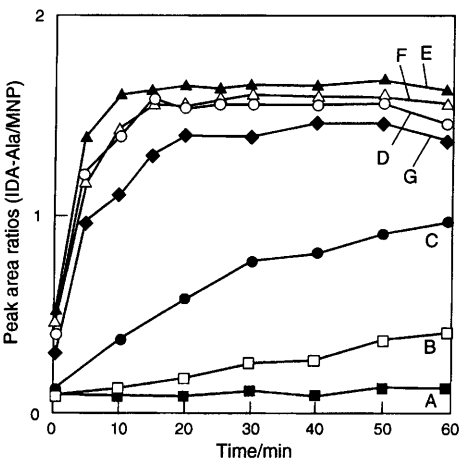
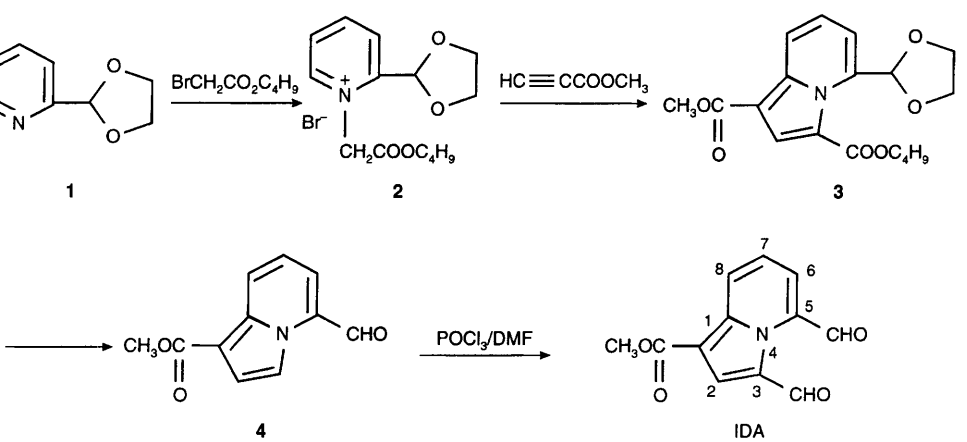


Fig. 1 Optimization of the reaction conditions. The reaction course was traced using HPCE. Analytical conditions for HPCE: carrier electrolyte, 20 mmol l^{-1} phosphate–borate buffer (pH 10); capillary tube, 50 cm × 50 μm (i.d.) fused silica; applied voltage, 20 kV; detection wavelength, 290 nm; hydrostatic injection (15 cm, 10 s). pH: A, 6; B, 7; C, 8; D, 9; E, 10; F, 11; G, 12.



Scheme 1

Optimization of Reaction Conditions

Effect of pH and reaction time

Alanine was used as the standard primary amino compound for the optimization study of the derivatization reaction. The effect of pH on the derivatization efficiency is shown in Fig. 1. The time course of the derivatization is also shown in Fig. 1.

At around neutral pH (6–8), the reaction proceeded slowly. On the other hand, the reaction was completed within 10 min under weakly basic conditions (pH 9 and 10). However, a decrease in the yield was observed at higher pH, probably owing to degradation of the product. At pH 10, the yield became the highest and constant after 10 min of reaction.

Effect of ethanol as an organic additive

For complete dissolution of the reagent and the products, an organic solvent should be added to the reaction buffer. Ethanol was chosen for this. The effect of the ethanol concentration on the reaction efficiency is shown in Fig. 2. By using buffer solutions containing ethanol at a concentration of 25–50%, the yields were almost constant.

Spectral Characteristics of IDA-Ala

The absorption spectra of the reagent (IDA) and the reaction solution of the IDA derivative of Ala (IDA-Ala) are shown in Fig. 3. IDA-Ala showed strong absorption at 280 nm ($\varepsilon = 3.31 \times 10^4$ l mol^{-1} cm^{-1}) and 409 nm ($\varepsilon = 2.18 \times 10^4$ l mol^{-1} cm^{-1}). On the other hand, the reagent did not show absorption maxima at these wavelengths. The reagent showed little absorption at 409 nm where IDA-Ala showed the strongest absorption.

The fluorescence spectra of IDA and IDA-Ala are shown in Fig. 4. The excitation maxima (292 and 414 nm) observed from IDA-Ala were different from those observed for IDA (312 and 344 nm), as shown in Fig. 4(*a*). The emission spectra of IDA-Ala showed maxima at 482 nm [irradiated at 292 and 414 nm, Fig. 4(*a*) and (*b*), respectively]. When IDA-Ala was irradiated at 414 nm, the fluorescence observed at 482 nm had little interference from the reagent.

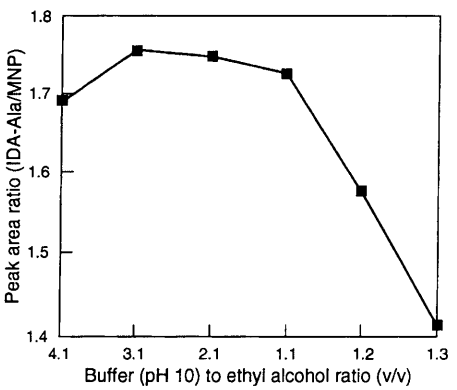


Fig. 2 Effect of the concentration of ethanol in the reaction buffer. Other conditions as in Fig. 1.

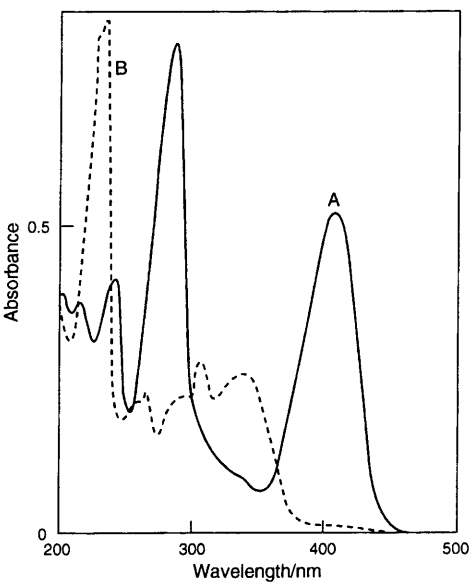


Fig. 3 Absorption spectra of A, IDA–Ala and B, IDA in water.

Stabilities of the Reagent and the Derivatized Product

The stabilities of the reagent and the product (IDA-Ala) were investigated by HPCE. As a reference method OPA-Ala was also investigated in the same manner. Both the reagent (IDA)

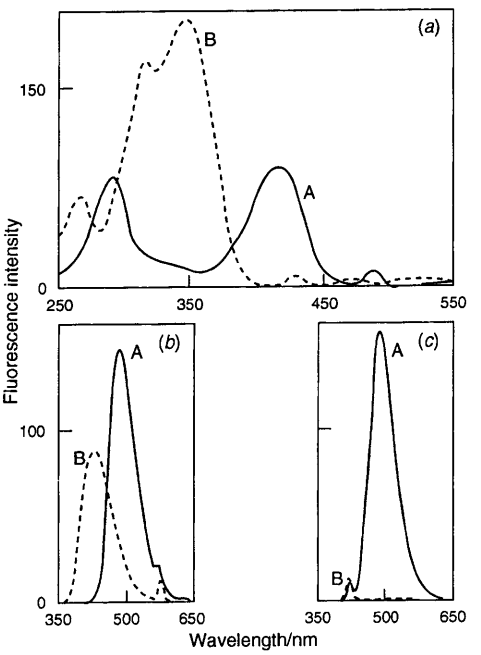


Fig. 4 Fluorescence spectra of A, IDA–Ala and B, IDA in water. (*a*) Excitation spectra (emission at 482 nm); (*b*) emission spectra (excitation at 292 nm); and (*c*) emission spectra (excitation at 414 nm).

Table 2 Peak-area response of IDA–Ala with changing concentration of alanine

Alanine concentration/ μmol ml^{-1} (x)*	Peak-area ratios of IDA–Ala to MNP† (y)
7.16	1.70
3.58	0.880
0.72	0.183
0.14	0.0384
0.029	0.00882
0.0057	0.00214
0.0014	Not detected‡

* $y = 0.0792x + 0.00707$ ($r = 0.9999$).
† MNP = *m*-nitrophenol (internal standard).
‡ IDA–Ala peak cannot be detected at an attenuation of 0.005 (a.u.f.s.).

and the product (IDA-Ala) were stable for at least 24 h, as shown in Table 1. On the other hand, the peak intensity of OPA-Ala rapidly decreased and became one third of the original value after 24 h.

Calibration and Reproducibility

The calibration graph for IDA-Ala showed good linearity over the range 0.0172–21.5 μmol ml^{-1} with a satisfactory correlation (r = 0.999), as shown in Table 2. The detection limit of alanine was 5 nmol ml^{-1} at a signal-to-noise ratio of 3.

Repeated analyses (n = 5) of IDA-Ala at 10.8 and 21.5 μmol ml^{-1} gave excellent reproducibility with relative standard deviations of 1.75% and 1.33%, respectively, as shown in Table 3.

Comparison of the Present Method with Ninhydrin and o-Phthalaldehyde (OPA) Method

The absorption spectrum of IDA-Ala is shown in Fig. 5 together with that of Ruhemann's Purple chromphore (abbreviated as 'ninhydrin-Ala' in this paper) obtained from the ninhydrin method. The absorbance of IDA-Ala at 280 nm showed almost a 2.5 times stronger absorption than that of ninhydrin-Ala at 570 nm. The absorbance at 414 nm was 1.5 times higher.

The fluorescence spectra of IDA-Ala are shown in Fig. 6 together with that of OPA-Ala. The fluorescence intensity of IDA-Ala at 482 nm (irradiated at 414 nm) was about three times stronger than that of OPA-Ala at 461 nm (irradiated at 342 nm).

Table 3 Precision of derivatization procedure using HPCE in the CZE mode. Each value represents the peak-area ratio of IDA–Ala to MNP

	Alanine concentration/μmol ml^{-1}	
Sample	10.8	21.5
1	0.848	1.70
2	0.868	1.71
3	0.888	1.75
4	0.878	1.72
5	0.879	1.75
Mean*	0.872 (1.75%)	1.70 (1.33%)

* The values in parentheses are relative standard deviations (n = 5).

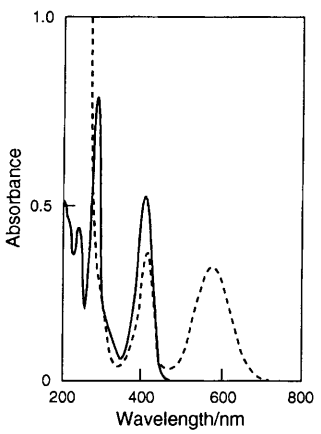


Fig. 5 Comparison of the sensitivity between the ninhydrin method and the present method. A, IDA–Ala; and B, ninhydrin–Ala.

Separation of the Labelled Amino Acids by Micellar Electrokinetic Chromatography

After derivatization of a mixture of standard amino acids by the present method, the reaction mixture was analysed by HPCE. Proline and hydroxyproline were not derivatized by the present method; separation of the other amino acids is shown in Fig. 7.

Tryptophan and threonine did not give clear peaks. This was probably due to oxidative degradation under the alkaline conditions, as occasionally observed for the alkaline degradation of glycoproteins. Separation by simple zone electrophoresis did not afford a satisfactory separation especially for the group Phe, Lys and Arg (data not shown). Addition of SDS and a small volume of methanol enhanced the resolution of these amino acids, although the separation among Glu, Asp and Gly became slightly worse.

The two aldehyde groups in the IDA molecule are not equivalent. Only one of the aldehyde groups (probably that at the 5-position owing to its higher reactivity) seems to react with an amine to form a Schiff base. The Schiff base produced becomes stable by formation of a conjugated diene with the other aldehyde group at the 3-position in solution. Further, the conjugated dienes produced may show strong bathochromic shifts in their spectra. Although the Schiff base seems stable in solution, the attempt to isolate the reaction product was not successful owing to its instability in the isolated state.

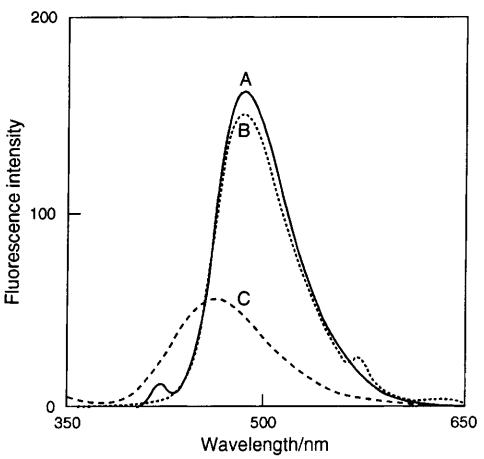


Fig. 6 Comparison of the sensitivity in spectral characteristics between OPA method and the present method. A, IDA–Ala excited at 414 nm; B, IDA–Ala excited at 292 nm; and C, OPA–Ala excited at 342 nm.

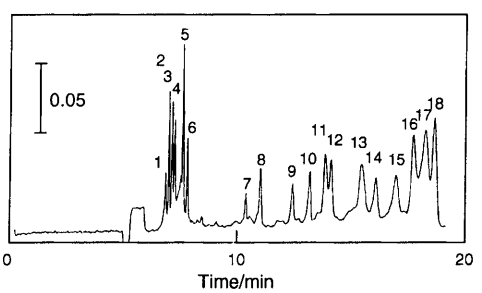


Fig. 7 Separation of a mixture of 15 amino acids by MEKC. Conditions: carrier electolyte, 20 mmol l^{-1} SDS–40 mmol l^{-1} borate–phosphate buffer (pH 7.0) containing methanol at a concentration of 3% v/v; applied voltage, 20 kV; other conditions as in Fig. 1. 1, Ser; 2, Cys; 3, Glu; 4, Asp; 5, Gly; 6, Ala; 7, Val; 9, His; 10, Met; 11, Ile; 12, Leu; 13, NH_3; 14, Phe; 15, Lys; 16, Arg, 17, Tyr. Peaks 8 and 18 are due to the reagent. Each amino acid concentration used was 1.43 μmol ml^{-1}.

Conclusion

The indolizine dialdehyde derivative IDA was prepared and used for labelling of amino acids. The derivative has strong UV absorption at 280 and 409 nm and also fluorescence at 482 nm (with irradiation at 282 or 414 nm). The reagent did not show interferences in the determination of the amines. Using the present derivatization method, a mixture of amino acids was derivatized and separated by HPCE in the MEKC mode. The analysis is performed within 40 min including derivatization and separation.

References

1 Barth, H. G., Barber, W. E., Lochmuller, C. H., Majors, R. E., and Regnier, F. E., *Anal. Chem.*, 1988, **60**, 387R.
2 Roth, M., *Anal. Chem.*, 1971, **43**, 880.
3 Benson, J. R., and Hare, P. E., *Proc. Natl. Acad. Sci. USA*, 1975, **72**, 619.
4 Lindroth, P., and Mopper, K., *Anal. Chem.*, 1979, **51**, 1667.
5 Wilkinson, J. M., *J. Chromatogr. Sci.*, 1978, **16**, 547.
6 Carpino, L. A., and Han, G. Y., *J. Org. Chem.*, 1972, **37**, 3404.
7 Chervet, J. P., Ursem, M., Salzmann, J. P., and Vannoort, R. W., *J. High. Resolut. Chromatogr.*, 1989, **12**, 278.
8 Heiger, D. N., in *High Performance Capillary Electrophoresis*, report, Hewlett-Packard, Waldbronn, 1992, p. 100.
9 Wang, T., Aiken, J. H., Huie, C. W., and Hartwick, R. A., *Anal. Chem.*, 1991, **63**, 1372.
10 Tsuda, T., Sweedler, J. V., and Zare, R. N., *Anal. Chem.*, 1990, **62**, 2149.
11 Miki, Y., Hachiken, H., Yoshikawa, M., Takemura, S., and Ikeda, M., *Heterocycles*, 1991, **32**, 655.
12 Bradsher, C. K., and Parham, J. P., *J. Org. Chem.*, 1963, **28**, 83.
13 Moore, S., and Stein, W. H., *J. Biol. Chem.*, 1954, **211**, 907.
14 Morineau, G., Azoulay, M., and Frappier, F., *J. Chromatogr.*, **467**, 209.

Paper 4/02577F
Received May 3, 1994
Accepted September 9, 1994