Determination of plant indoles by capillary electrophoresis with amperometric detection

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Abstract

A capillary electrophoresis (CE) method for separation and determination of the plant hormone indole-3-acetic acid (IAA) and one of its primary metabolites, indole-3-acetylaspartic acid (IAAsp), is described. Amperometric detection was employed. Detection limits were 5.2 amol for IAA and 110 amol for IAAsp. Homogenized plant tissue was extracted in phosphate buffer, filtered and desalted on a C_{18} solid-phase extraction column. The resulting methanol eluate was evaporated to dryness and redissolved in a water–methanol (90:10, v/v) mixture. This solution was then injected into the CE system. No further purification or derivatization steps were necessary. Xylem sap from one individual pea plant was injected directly without any pre-treatment and IAA was identified and determined semi-quantitatively.

Keywords: Plant hormones; Detection, electrophoresis; Indoleacetic acid; Indoleacetylaspartic acid

1. Introduction

Indole-3-acetic acid (IAA) is an ubiquitous plant hormone essential for normal growth and involved in almost every developmental process during the whole life cycle of the plant. Like plant hormones in general, IAA is present in the tissue at very low concentrations, ranging from 10–100 ng/g fresh mass, as compared to most plant secondary metabolites which are usually found at 1000-fold higher levels. Specific concentration levels of IAA are maintained by biosynthesis, degradation and conjugation with other small biomolecules [1]. Thus, a given tissue contains, together with IAA, a whole spectrum of IAA metabolites and conjugates at various concentrations [2]. The involvement of IAA in different growth processes may be reflected by an altered turnover rate of IAA and/or changes in the concentration of some of its metabolites, rather than in a change in the level of IAA itself [3]. Although IAA is the active hormone, it may thus be desirable also to determine simultaneously its metabolites. One important metabolite is indole-3-acetylaspartic acid (IAAsp).

Determination of IAA and associated compounds traditionally requires as much as 0.5–2 g plant material. The resulting extract is a multi-component mixture, where IAA and its metabolites are usually present at very low concentration levels, often considerably lower than the levels of other components with similar chemical structures. Consequently, a

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purification of the plant extract is required, involving steps of conventional liquid extraction and/or solid-phase extraction (SPE) and/or preparative liquid chromatography, in order to accomplish analyte enrichment and to reduce the concentration of the main matrix components [2,4]. Common for all sample pre-treatment procedures is that precautions must be taken to prevent oxidation of the analytes. For accurate quantitative measurements internal standards are used.

Gas chromatography (GC) is extensively employed in plant hormone analysis, due to its high efficiency, even though derivatization of the sample is required after purification [5]. Although several types of detectors, exhibiting various specificity, have been used, the most popular is indisputably mass spectrometry (MS), since structural information, selectivity and sensitivity can be provided. This technique also has the advantage of allowing simple and accurate determinations of isotopes, and thus isotopically labelled internal standards can be employed. Recently, a microscale GC method for determination of IAA in plants has been described, using a double-focusing HR MS detector [6]. By this method, the total amount of plant material could be reduced to about 1 mg and the sample pre-treatment steps were simplified. However, derivatization was still required. The relative standard deviation was found to be about 17%.

As an alternative separation and detection technique to GC–MS, HPLC–MS can be used [5]. Using the HPLC–MS technique, derivatization can be avoided, while still allowing positive identification of the various analytes. However, the demands on sample purity are very high.

Apart from the physicochemical techniques, immunoassay methods are used by some laboratories for plant hormone analysis [5]. Substantial sample purification is needed also for these methods, and therefore a preparative HPLC step is often included.

Extensive reviews dealing with currently used analysis methods for indoles have been presented [2,4,5].

Many important indolic compounds are lost, partly or completely, during the purification of the plant extract. An ultimate method for determination of IAA and related compounds in plant material would allow a 'direct' quantification of the analytes, thus eliminating all sample pre-treatment steps. Very few analytical techniques, if any, fulfil completely the requirements of separation efficiency and/or detector specificity. However, for this purpose, capillary electrophoresis (CE) is considered to be of interest to investigate further.

CE is a technique of increasing popularity, applicable to a wide area of analytical problems, ranging from determination of small inorganic ions to large, complex proteins. The real strength of CE is the small sample volumes required and the effective separation obtained, making it particularly useful in the fields of biotechnology and microanalysis of biological samples.

Connection of the CE separation system to an amperometric detector results in one of the most sensitive analytical tools available today. This is mainly due to the fact that no reduction in sensitivity occurs on miniaturization of such a detector [7,8]. In addition, the amperometric detector provides selectivity towards electroactive compounds. This is an advantage when dealing with complex matrices.

Ewing and co-workers were the first to suggest a system for CE with amperometric detection [9]. Detection was performed in the off-column mode, but later also an end-column detection mode was employed [10], and it was shown that this latter mode was applicable for capillaries with an internal diameter of 25 μm or less [11].

Using CE with UV detection, Yeo et al. optimized the separation of nine synthetic plant growth regulators, four of which may also be found in plants [12]. However, no real samples were analysed. Recently, Chan et al. separated tryptophan and related indoles with micellar electrokinetic chromatography [13]. Indole profiles of directly injected human urine and serum were obtained. The detection principle was KrF laser-induced fluorescence, and the detection limits were in the range of 5.5 to 13.7 nM.

In the present work, a CE system with amperometric detection has been utilized to separate a laboratory prepared mixture of naturally-occurring indolic compounds, related to IAA. Identification and quantitation of IAA and IAAsp in plant extract and in xylem were also performed. The plant extract was purified to varying degrees, or just desalted, while xylem sap was injected directly in the CE system.

2. Experimental

2.1. Capillary electrophoresis system

Untreated fused-silica capillaries, 60 cm×20 μm I.D.×150 μm O.D., were obtained from Polymicro Technologies, (Phoenix, AZ, USA). Before use, the capillaries were washed with 0.1 M NaOH for 30 min, distilled water for 20 min, and operating electrolyte for 20 min.

The input of the high-voltage power supply, ±0–30 kV, (Brandenburg, Thornton Heath, UK), together with the injection end of the capillary, were placed in a plexiglass box with an interlock on the access door for protection. Rinsing of the capillary was accomplished by applying pressure on the injection buffer. Injections of samples were made hydrodynamically by raising the injection end of the capillary 3 cm for 280 s.

Amperometric detection was performed in the end-column mode using a three electrode configuration. The auxillary electrode was used for grounding in the detector cell. The potentiostat was designed according to the circuit scheme presented by Cassidy et al. [14]. Two car batteries, 12 V, were used as power supply. Control of the detector potential and data acquisition was accomplished with a PC equipped with a PCL-812PG lab card (Advantech, Taiwan), and an in-house developed QBASIC computer program.

A schematic diagram of the detector cell is shown in Fig. 1a. The working electrode consisted of a 30 μm carbon fibre disk electrode cast into a plastic stopper using epoxy glue. The stopper was fitted into a glass tube, forming the detection cell, which was placed vertically onto an X–Y–Z micro-positioner (Photon Control, Cambridge, UK). The capillary, together with the auxiliary and the reference electrodes, were attached to an aluminium plate by the aid of which they were inserted into the detection cell from above. As reference electrode was used a Ag/AgCl electrode in 3 M KCl (BAS, West Lafayette, IN, USA) and the auxiliary electrode was a platinum wire with a diameter of 2 mm (Goodfellow, Cambridge, UK). Each day, the working electrode was washed with bichromate–sulphuric acid for a few min, and then with deionized water. It was positioned directly in front of the capillary outlet at a
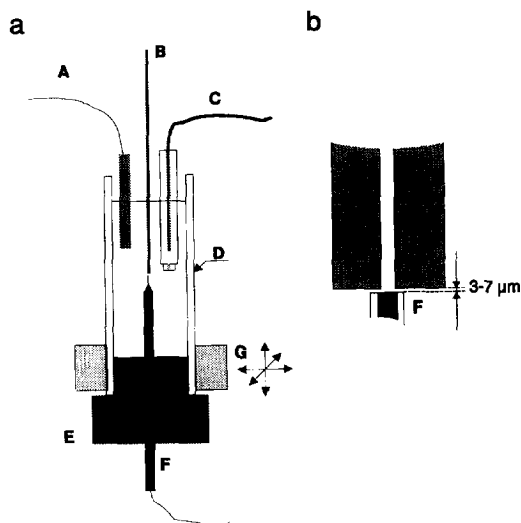

Fig. 1. (a) Sideview of amperometric detection cell. A=auxiliary electrode (Pt); B=capillary; C=reference electrode (Ag/AgCl); D=glass tube; E=plastic stopper; F=working electrode (carbon fibre); G=micromanipulator. (b) Detailed view of working electrode, capillary alignment.

distance of approximately 5 μm, see Fig. 1b. This was accomplished by adjusting the working electrode with the micropositioner under the view of a microscope. The detector and the injection arrangements were accommodated in Faraday cages.

2.2. Chemicals and reagents

All chemicals were of analytical grade and solutions were prepared from deionized water. The background electrolytes used for both separation and detection were degassed and filtered before use. These had the following compositions:

Electrolyte 1: Acetate buffer, 30 mM, adjusted to pH 4.5.

Electrolyte 2: As electrolyte 1, but with addition of $CuSO_4$ (0.01–1.0 mM).

Electrolyte 3: As electrolyte 1, but with addition of 30 mM sodium dodecyl sulphate (SDS).

Standard stock solutions (100 μM in methanol) were prepared with the following indoles obtained from SIGMA (St. Louis, MO, USA): indole-3-carboxylic acid (ICA), indole-3-acetic acid (IAA), indole-3-propionic acid (IPrA), indole-3-butyric acid

(IBA), indole-3-acetyl-L-aspartic acid (IAAsp), indole-3-aldehyde (IA), indole-3-acetaldehyde (IAAld), 3-indole-methanol (IM), 3-(2-hydroxy-ethyl)-indole (IE) and indole-β-D-glucose (IAGlu).

Working standard solutions were prepared daily by mixing and diluting the stock solutions with a water–methanol (90:10, v/v) mixture.

2.3. Preparation procedure of plant material

The applied method described has previously been used for quantitative determination of IAA by HPLC and GC–MS [3,15].

2.3.1. Extraction and filtering

Seeds of pea (Pisum sativum L. cv. Marma) were grown in trays with vermiculite in a growth chamber. After 10 days, pieces from the shoot apex or from the stem were collected and homogenized in liquid nitrogen. The resulting powder, about 0.75 g, was extracted in 10 ml 0.005 M phosphate buffer, pH 7.0, during 1 h at 4°C. This extract was filtered through a glass fibre filter, GF/C 1.2 μm (Whatman, Clifton, NJ, USA), and then washed with 5 ml of the extraction buffer. After adjusting the volume of the filtrate to 20 ml by addition of buffer, three 6 ml aliquots were taken (sample 1–3) which were cleaned to different degrees of purity, as described below.

2.3.2. Purification, sample 1 (desalting only)

A 0.5-g Bond Elute C_{18} column (Analytichem International, USA), was conditioned with 2×2 ml ethanol, flushed with 2 ml distilled water, and pH adjusted with 2 ml 0.005 M phosphate buffer (pH 2.5). Sample 1 was pH-adjusted to 2.5 using phosphoric acid and was then allowed to run through the column at a flow-rate of 1 to 5 ml/h; the column was then washed with 4 bed volumes (2 ml) distilled water. At this pH, the uncharged analyte species will be retained in the column, while some of the interfering, charged species will pass through (desalting).

Elution of the analyte species from the column was made with 1 ml 100% methanol. After evaporation of the eluate to dryness, the solid analytes were redissolved in 0.1 ml water–methanol (90:10, v/v),

mixture. The resulting solution being then introduced into the CE system.

2.3.3. Purification, sample 2

Sample 2 was run through a C_{18} column pre-treated as described above but with the exception that the final rinsing was made with a pH 6.5 phosphate buffer. At this pH, the analyte species will pass through the column while many interfering species will be retained. The resulting eluate, thus containing the analyte species, was collected and acidified to pH 2.5 by addition of phosphoric acid, and subsequently desalted according to the procedure described in Section 2.3.2.

2.3.4. Purification, sample 3

For sample 3, the most extensively purified sample, the procedure used was identical with that for the sample 2 but with one exception: in the last wash step, the desalting step, the column was washed with 2 ml distilled water, 2 ml ethanol–acetic acid–water (20:2:78, v/v), and a final portion of 2 ml distilled water.

2.4. Direct sampling of xylem

Xylem is that part of the vascular system which is responsible for the transport of water, containing dissolved substances, from the root system and upwards to the different parts of the shoot. Limited information is available concerning the contents of IAA and other indole derivatives in the xylem sap.

A section of about 5 cm of the upper part of the pea seedlings was removed. The xylem sap then accumulated as a droplet on the cut surface of the remaining root-bearing part. This droplet, about 0.3 μl, was collected with a microcap and transferred to a small cavity (2 μl) of a home-made sample plate of PTFE, serving as an injection vial. Spiking of the xylem sap samples was performed by transferring a portion of a methanol solution of IAA to the cavity in the PTFE plate and there evaporating to dryness; this portion corresponded to 0.1 pmol IAA. The xylem sample was then transferred to the cavity and after 'mixing', the spiked sample was introduced in the CE system.
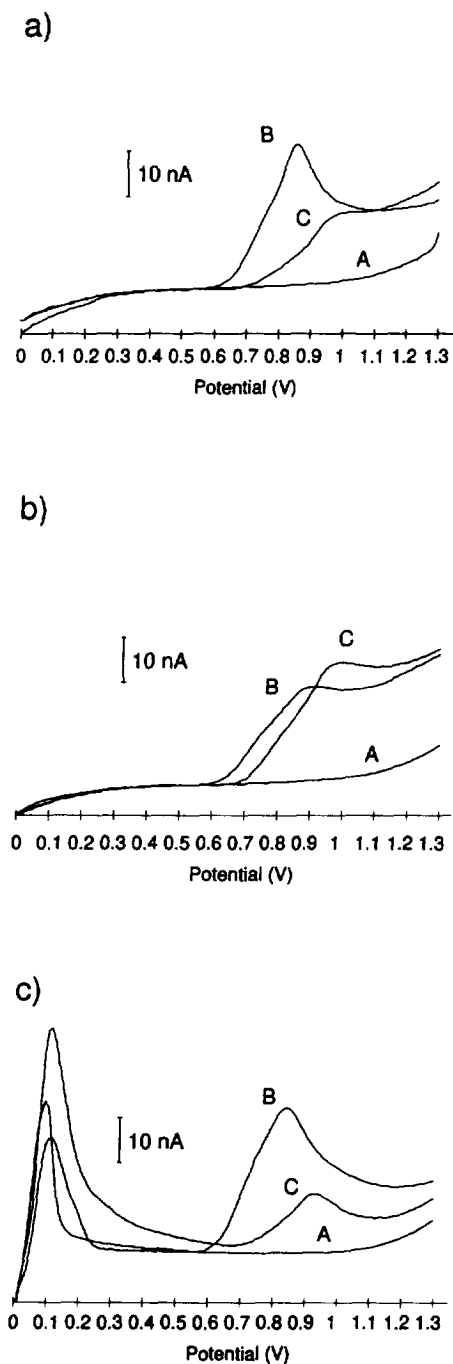
a)

b)

c)

Fig. 2. Positive scans from cyclic voltammograms at a 30 μm carbon fibre disk electrode, with an effective scan rate 0.08 V/s of background electrolyte (A) 1.0 mM IAA, (B) 1.0 mM IAAsp, (C) obtained in (a) 30 mM acetate pH 4.5; (b) 30 mM acetate pH 4.5, 30 mM SDS; (c) 30 mM acetate pH 4.5, 1.0 mM CuSO₄.

3. Results and discussion

3.1. Electrochemical conditions for the CE system

The electrochemical properties of the indoles IAA and IAAsp dissolved in different electrolytes were investigated by staircase voltammetry, using the described CE set up but with constant pressure flow only. All experiments were performed in one, uninterrupted run using the same electrode alignment, to ensure constant flow conditions. Staircase voltammetry sequences were created from a home-made QBASIC program. Electrolytes no 1 (pH 4.5), 2 and 3 were used.

In Fig. 2a–c are depicted the resulting positive scan voltammograms. Addition of Cu^{2+} to the buffer systems did not markedly change the shape of the voltammograms, see Fig. 2a, c. Addition of SDS, on the other hand, resulted in an increased response value for IAAsp, see Fig. 2b.

The optimal detection potential for CE with regard to signal-to-noise ratio was also established. For IAA about 0.8 V vs. Ag/AgCl was required, whereas IAAsp required a somewhat higher potential, namely 1.0 V. Consequently, a potential of 1.0 V was chosen and used throughout.

3.2. Separation of indoles in standard indole mixtures

Shown in Fig. 3 is an electropherogram recorded of a laboratory-prepared mixture containing nine different indoles. A potential of 1.0 V vs. Ag/AgCl was applied. The electrolyte, 30 mM acetate pH 4.5 with 30 mM SDS, was selected in order to receive good separation of all indoles. No attempt was made to optimize the separation. It should be noted that this CE method can be used to separate neutral indoles when SDS is present in the electrolyte. However, in the following investigations, attention was focused on separation of the negatively charged indoles IAA and IAAsp because their presence in plant material is well documented [3].

3.3. Effect of Cu addition

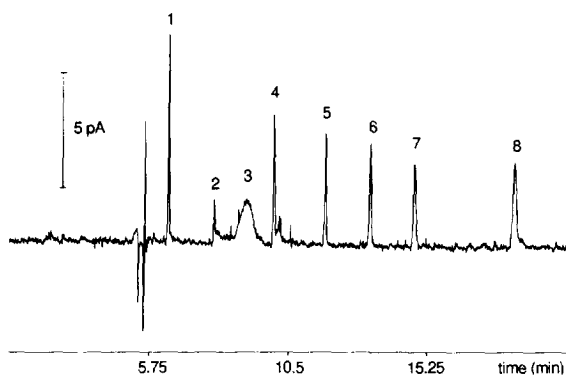Addition of 1 mM $CuSO_4$ to the acetate electrolyte was found to enhance the signal-to-noise ratio

Fig. 3. Electropherogram of a standard mixture of indoles 0.2 mg/l each. Amperometric detection at 8 μm carbon fibre disk electrode, 1.0 V vs. Ag/AgCl; buffer: 30 mM acetate, 30 mM SDS, pH 4.5; separation capillary: 60 cm×25 μm I.D.; separation potential: 30 kV; hydrodynamic injection: 0.4 nl; peaks: 1=IAGlu, 2=IM, 3=IAAld, 4=IE, 5=IAA, 6=IPrA, 7=IAAsp, 8=IBA.

3 times. Higher concentrations caused a significant decrease in the signal-to-noise ratio. In addition to the enhanced detection limits, presence of Cu(II) had the effect of decreasing the mobility of the indoles, due to complex formation, see Fig. 4. The effect was
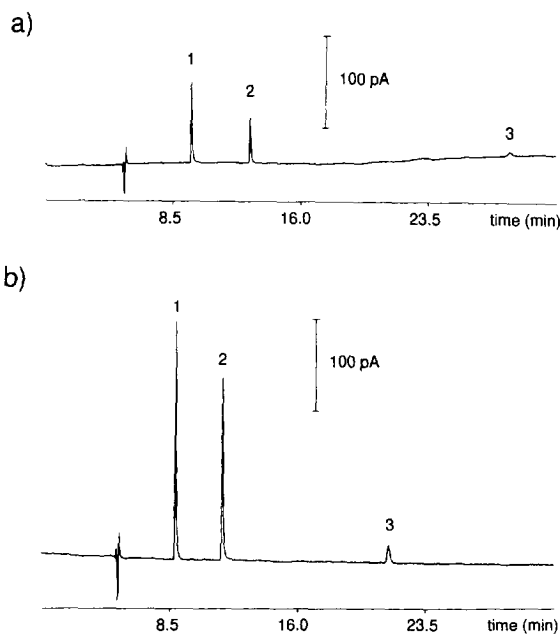

Fig. 4. Effect of presence of Cu²⁺ on migration times and responses for a standard mixture of 1 mg/l; peaks: 1=IBA, 2=IAA and 3=IAAsp in buffers containing (a) 30 mM acetate pH 4.5; (b) 30 mM acetate pH 4.5 and 1 mM CuSO₄.

greater with IAAsp than with the other indoles, since IAAsp has two carboxylic acid groups. On addition of Cu(II), the electroosmotic flow decreased only slightly, 4%. This change was small compared with the mobility change of IAAsp, 29%. Dynamic ranges for IAA and IAAsp were examined for an injection volume estimated to 0.4 nl, see Table 1.

Lunte et al. used copper complexation for determination of peptides by CE with electrochemical detection [16]. They reported a gradual noise increase with time, which they concluded was due to accumulation of Cu(II) in a Nafion joint, isolating the detector from the electric field in the capillary. This accumulation obstructed the ion-exchange function. In this work, where no such joint was utilized, no noise was observed.

3.4. Separation of indoles in real samples

Electropherograms of spiked and unspiked desalted extracts from pea plants (sample 1) are shown in Fig. 5. As can be seen, well-separated peaks were obtained and the IAA and IAAsp peaks are easily identified among all the other electrochemically active compounds in the sample. The concentrations of IAA and IAAsp were determined by standard addition to 22 and 27 ng/g fresh mass, respectively. This is in accordance with earlier results [3]. No fouling of the electrode surface was observed but plant extracts only subjected to filtration were found to contaminate the electrode to such an extent that it had to be removed and cleaned. For samples subjected to purification steps in addition to desalting (samples 2 and 3), no improvement for the peaks of interest was observed with respect to resolution and detection properties. Thus, this developed CE method can be applied to plant extract samples that are less purified than what generally is required when employing alternative methods.

A separation buffer of acetate at pH 4.5 was chosen. It is advantageous to select a pH close to the pK_a values of the analytes in order to obtain a large separation power [17], and this was confirmed also for this application, since buffers with higher pH values resulted in unsatisfactory separation. For three repetitive injections of the same sample the R.S.D. values for times and areas were: 2.8% and 8.2% for IAA and 3.6% and 8.5% for IAAsp.

Table 1
Calibration data

Analyte	Migration time		R.S.D.$_{area}$ [a] (%)	Detector response (pA/fmol)	Concentration range (μM)	LOD[b] (amol)	r[c]
	min	R.S.D.$_{time}$ [a] (%)					
IBA	10.64	1.1	6.7	87	0.1–10	5.6	0.998
IAA	13.93	1.3	6.5	89	0.1–10	5.2	0.999
IAAsp	34.7	2.4	6.8	4.3	1.0–100	108	0.997

[a] Relative standard deviation calculated from eight injections of 1 μM standard solution.
[b] $S/N = 3$.
[c] Based on 6 values.

Even with the selectivity provided by the amperometric detector, there is no guarantee that the peaks corresponding to IAA and IAAsp are completely separated from other constituents in the sample. However, consistent results were obtained when the separation conditions were changed in the CE system, e.g., by addition of copper to the electrolyte buffer.
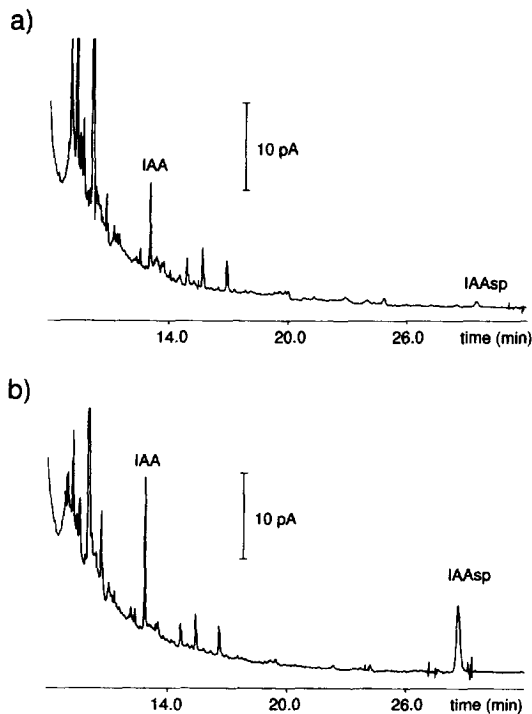
3.5. Identification of indoles in xylem

A peak corresponding to IAA was found in a sap of xylem, directly injected by hydrodynamic injection into the CE-system, see Fig. 6a. No peak
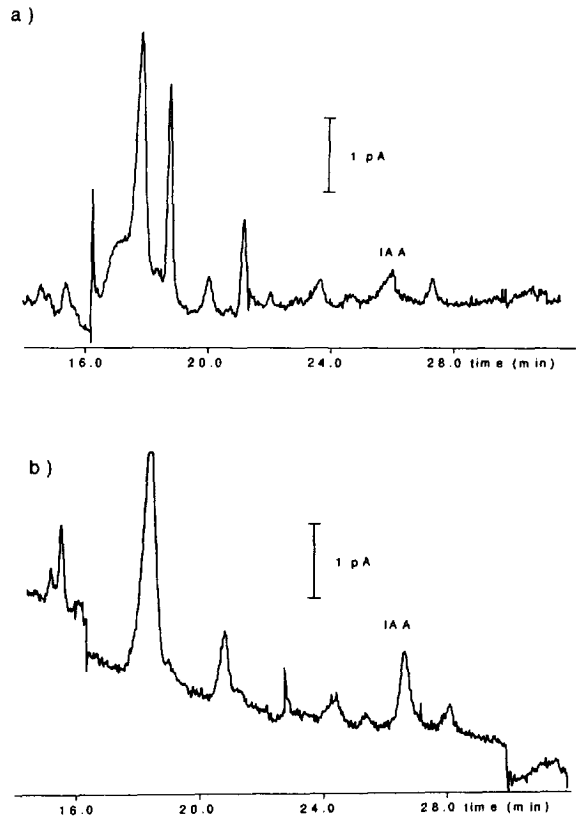

Fig. 5. Electropherogram of plant extract; (a) unspiked; (b) spiked with 0.25 μM IAA and 2.5 μM IAAsp. Amperometric detection at 30 μm carbon fibre disk electrode, 1.0 V vs. Ag/AgCl; buffer: 30 mM acetate, 1.0 mM CuSO$_4$, pH 4.5; separation capillary: 60 cm×20 μm I.D.; separation potential: 30 kV; hydrodynamic injection: 0.4 nl.

Fig. 6. Electropherograms of untreated xylem sample; (a) unspiked; (b) from the same location in an other plant, spiked with 1 μM IAA. Amperometric detection at 30 μm carbon fibre disk electrode, 1.0 V vs. Ag/AgCl; buffer: 30 mM acetate, 1.0 mM CuSO$_4$, pH 4.5; separation capillary: 20 μm I.D., 60 cm; separation potential: 30 kV; injection: hydrodynamic, 0.4 nl.

corresponding to IAAsp was detected. Repeatable injections of the same xylem sample could not be performed due to significant evaporation of the small volume sample. However, several xylem sap samples were collected from plants which had been treated the same way, and the peak areas found for IAA were all very similar. The concentration of IAA was estimated by running a separate, spiked xylem sample, see Fig. 6b, and it was found to be about 40 nM.

3.6. Specific properties of the developed CE method

Current methods for determination of indoles in plant tissues are tedious, since they require clean up and/or derivatization as well as relatively large amounts of plant material. Furthermore, the sample handling is crucial, since indoles are so readily oxidized. This study shows that it is possible to reduce the number of sample preparation steps when CE is applied as a separation technique and when the amperometric detection principle is used. The recoveries of IAA and IAAsp after the desalting step (sample 1) used were found to be 95 and 82%, respectively. However, in the extensive cleanup (sample 3, Section 2.3.4) which is required for HPLC and GC–MS, as much as 80–90% of the IAAsp is lost [3,15]. Thus, with only the desalting step, it is possible also to determine indoles of higher polarity such as IAAsp simultaneously with IAA.

The amount of extract injected into the CE system corresponds to approximately 6 μg of plant tissue. If the sample preparation method is transferred to microscale, it should be possible to reduce the plant material required for one analysis.

When the desalting step alone is performed, as suggested in this work, all indoles which are neutral at pH 2.5 will be retained on the separation column, while the charged species will be eluted. These indoles, which are recovered from the column through elution with methanol, represent the majority of all plant indoles of interest. Selective discrimination of some of the indoles can be made by altering the pH during the desalting step.

One advantage of the CE system is the ease by which the separation pattern can be altered. This is achieved by changing the pH and the composition of the separation electrolyte. When dealing with samples containing a large variety of impurities in large amounts this possibility is of a great value. By manipulation, resolution between the analyte species and the interfering species can be enhanced, and the separation order can be changed.

3.7. Different detection techniques for indoles in CE

The amperometric detection principle was compared with UV detection using a 75 μm capillary, see Table 2. Amperometry is about 1000 times more sensitive for IAA. But, above all, amperometric detection is more selective than UV detection, see Fig. 7. The lack of selectivity by the UV detector is, for this application, a great disadvantage and would require rigorous sample pre-treatment to be practically useful.

In HPLC analysis of indoles also fluorescence detection has been employed [5]. Fluorescence detection is both selective and sensitive. However, currently available lamp fluorescence (non-laser) detectors for CE do not provide the sensitivity needed for this application, because of the small cell length. Lasers with an excitation in the low UV region are extremely expensive; an optimum excitation wavelength would be 288 nm for indole determination. A pulsed KrF laser with an excitation

Table 2

Limits of detection for indoles with amperometric and UV detection

Analytes	Amperometric detection (μM)	UV detection (μM)
IBA	0.014	8.8
IPrA	0.013	11
IAA	0.013	15
IAAAsp	0.270	8.8

Conditions with UV detection (230 nm): buffer 30 mM acetate pH 4.5, capillary 75 cm×50 μm I.D., 30 kV, injection volume 1.0 nl. Conditions with amperometric detection: working electrode potential (1.0 V), buffer 30 mM acetate pH 4.5, capillary 60 cm×20 μm I.D., separation voltage 30 kV, injection 0.4 nl.
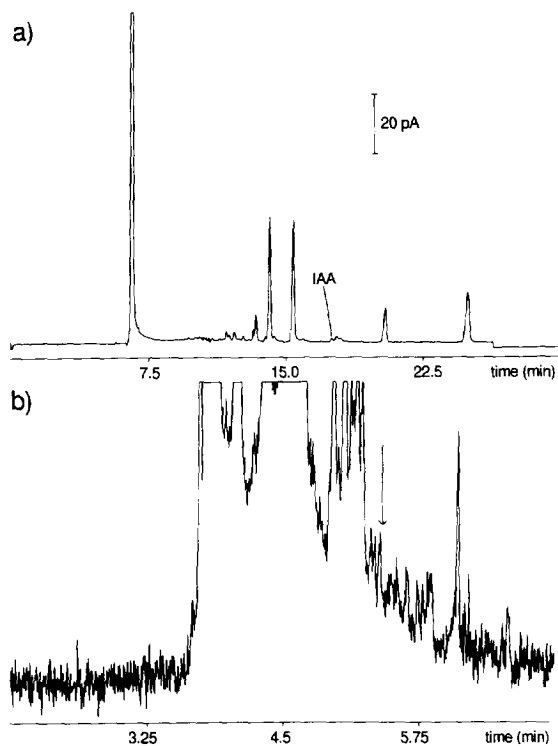
a)

b)

Fig. 7. Electropherogram of a plant extract with (a) amperometric detection; (b) UV detection; detection conditions as in Table 2. The arrow indicates the migration time for IAA.

wavelength of 248 nm has been used for tryptophan and indole determination in urine and serum samples [14] and would, consequently, be of interest to apply also to plant extract.

For the future, CE–MS should be a technique with a great potential for this type of application, since a highly effective separation system then is combined with a detector capable of giving structural information. Unfortunately, the sensitivity for the MS detector is too low when it is combined with CE.

4. Conclusions

It has been shown that CE with amperometric detection is a suitable technique for determination of plant indoles since:

1. The number of pre-treatment steps can be substantially reduced to one simple desalting step of the crude plant extract; no derivatization is required.
2. The sample amount needed is much smaller in comparison with the amounts needed for any of the currently applied methods.
3. The detector is selective for IAA and its metabolites.
4. The technique can be used for screening samples subjected to no or just a single pre-treatment step. Hence, this method can be regarded as an important supplement to currently used physico-chemical quantitative methods.
5. The problem with interference peaks overlapping analyte peaks can be dealt with by altering the composition of the electrolyte buffer so that the elution order is changed.

5. Acknowledgement

References

[1] R.S. Bandurski, J.D. Cohen, J. Slovin and D.M. Reinecke, in P.J. Davis (Editor), Plant Hormones, Kluwer Academic Publ., Dordrecht, Netherlands, 1995, p. 39.

[2] G. Sandberg, A. Crozier and A. Ernstsen (Editors), The Principles and Practice of Plant Hormone Analysis, Academic Press, London, 1987, Ch.4.

[3] A-C. Nordström and L. Eliasson, Physiol. Plant, 82 (1991) 599.

[4] P. Hedden, Plant Physiol. Plant Mol. Biol., 44 (1993) 107.

[5] R. Horgan, in P.J. Davis (Editor), Plant Hormones, Kluwer Academic Publ., Dordrecht, Netherlands, 1995, p. 415.

[6] A. Edlund, S. Eklöf, B. Sundberg, T. Moritz and G. Sandberg, Plant Physiol., 108 (1995) 1043.

[7] R.L. III. St. Claire and J.W. Jorgenson, J. Chromatogr. Sci., 23 (1985) 186.

[8] L.A. Knecht, E.J. Guthrie and J.W. Jorgenson, Anal. Chem., 56 (1984) 479.

[9] R.A. Ross and A.G. Ewing, Anal. Chem., 59 (1987) 1762.

[10] X. Huang, R.N. Zare, S. Sloss and A.G. Ewing, Anal. Chem., 63 (1991) 189.

[11] L. Wenzhe and R.M. Cassidy, Anal. Chem., 66 (1994) 200.

[12] S.K. Yeo, C.P. Ong and S.F.Y. Lee, Anal. Chem., 63 (1991) 2222.

[13] K.C. Chan, G.M. Muschik and H.J. Issaq, J. Chromatogr. A, 718 (1995) 203.

[14] W. Lu, R.M. Cassidy and A.S. Baranski, J. Chromatogr., 366 (1993) 433.

[15] A-C. Nordström and L. Eliasson, Plant Growth Reg., 12 (1993) 83.

[16] M. Deacon, T.J. O'Shea and S.M. Lunte, J. Chromatogr. A, 652 (1993) 377.

[17] F. Foret, L. Krivankova and P. Bocek, Capillary Zone Electrophoresis, VCH, Weinheim (1993) 219.