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Journal of Chromatography A, 929 (2001) 13–21

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Liquid chromatographic determination of total and β-*N*-oxalyl-L-α,β-diaminopropionic acid in *Lathyrus sativus* seeds using both refractive index and bioelectrochemical detection

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Received 16 March 2001; received in revised form 11 July 2001; accepted 6 August 2001

Abstract

A further improved chromatographic method for the simultaneous determination of the total amount of ODAP, selectively the amount of its neurotoxic form, β-ODAP, and free L-glutamate in raw *Lathyrus sativus* (grass pea) seed samples is described using post-column refractive index in combination with bioelectrochemical detection. The biosensor is based on crosslinking horseradish peroxidase (HRP) and an Os-containing mediating polymer with poly(ethyleneglycol)(400) diglycidyl ether (PEGDGE), forming an inner hydrogel layer and then immobilising L-glutamate oxidase (GlOx) as an outer layer on top of a graphite electrode. Addition of polyethylenimine (PEI) to the hydrogel is believed to have sensitivity and stability enhancing effect on the biosensor. The double-layer approach in the biosensor construction avoided direct electrical wiring of GlOx and resulted in a higher sensitivity of 4.6 mA/*M* cm^2 with respect to β-ODAP and a wider linear range (1–250 μ*M*) for both L-glutamate and β-ODAP when compared with a single-layer approach where GlOx, HRP, and Os-polymer are crosslinked together. The limit of detection for the chromatographic–biosensor system was found to be 2 μ*M* with respect to β-ODAP and 0.7 μ*M* with respect to L-glutamate. The refractive index detection on-line with the biosensor enabled full control of the chromatographic system for the determination of the total amount of ODAP, selectively the amount of β-ODAP and L-glutamate. Ten grass pea samples have been collected from Lathyrism prone areas of Ethiopia to test the applicability of the presently developed analytical system for real sample analysis. The toxin levels of grass pea collections were determined in an aqueous extracts and ranged from 0.52 to 0.76%, dry mass basis. Comparison of results of an established spectrophotometric assay and that of the present system has shown an extraordinary degree of agreement as revealed by parallel ''*t*'' test (90% confidence limit). The present system has operational stability of more than 50 h. Analysis time per sample is 10 min after extraction for 90 min.

Keywords: *Lathyrus sativus*; Refractive index detection; Bioelectrochemical detection; β-*N*-Oxalyl-L-α,β-diaminopropionic acid; Glutamate oxidase; Horseradish peroxidase

1. Introduction

Grass pea (*Lathyrus sativus*) is the cheapest legume seed available in countries like Bangladesh, India, and Ethiopia, where it is a major protein

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0021-9673/01/$ – see front matter
PII: S0021-9673(01)01183-9

source for people in the lowest income groups [1,2]. The plant has a number of important agronomic and nutritional characteristics. Most notably it can thrive under adverse environmental conditions, and it is nutritious and tasty [3,4]. However, one negative factor in consumption of *Lathyrus sativus* is the presence of a unusual non-protein amino acid, β-*N*-oxalyl-L-α,β-diaminopropionic acid (β-ODAP), believed to be a neurotoxin and the causative agent for a neurodegenerative disease called Lathyrism, an irreversible paralysis of the lower limbs [5,6].

Under normal circumstances, the consumption of *Lathyrus sativus* seems to be under the threshold of toxicity. However, at times of persistent droughts or other calamities when most other crops fail and cause food scarcity, the more resistant crop of *Lathyrus sativus* becomes the only affordable survival food for the poorer sections of the starved population. This obviously underlies a much higher intake of the neurotoxin by the already malnourished people and a consequent outbreak of Lathyrism in epidemic proportions. The recent (1997) outbreak of Lathyrism in some pockets of the Wollo region of Ethiopia, which afflicted three up to four members of a family (ca. 6–8 persons), was preceded by drought and famine [7].

It is already documented in the literature that β-ODAP in solution undergoes a transformation to its non-toxic form, α-ODAP, until an equilibrium mixture of β-ODAP to α-ODAP (3:2 ratio) is reached at an elevated temperature of about 55°C [8]. The α- and β-isomers differ in the position of the oxalyl moiety, i.e., α when it is linked to the α amino group and β to the β amino group of the diaminopropionic acid parent chain, see Fig. 1. The equilibration process between α- and β-forms takes a few hours in mildly acidic and alkaline aqueous solution at elevated temperature. Longer time is required at neutral pH [8,9]. The process should be considered as positive since it is observed that the α-isomer is not acutely toxic to 1-day-old chicks, to neonatal mice [10,11] and to rat spinal cord in vivo [12]. These studies suggest that different processing and/or cooking methods could be used for partial detoxification of grass pea.

A number of analytical methods have been developed in the past to assist various efforts of detoxification of the crop in the field or post-harvest [13–18]. The most widely used method for determining the neurotoxin is a spectrophotometric method (denoted the Rao method) that involves alkaline hydrolysis of the toxin to L-α,β-diaminopropionic acid (DAP), followed by reaction with orthophthalaldehyde (OPA), which, in the presence of ethanethiol gives a coloured adduct which can be measured at 476 nm [13]. The method, however, does not discriminate between the non-toxic α- and the toxic β-isomeric forms as both of them hydrolyse to DAP. Obviously this method is limited and the search for a more convenient method is demanded that can monitor selectively β-ODAP. An immobilized glutamate oxidase reactor system in a flow injection analysis mode [17] was developed for the selective determination of β-ODAP. However, a decrease in the response of the reactor after several injections, and interference from L-glutamate, were some limitations of this method. Later, a liquid-chromatographic separation of β-ODAP from interfering amino acids, coupled with an amperometric enzyme electrode (graphite rod modified with an $Os^{2+/3+}$ redox polymer cross-linked with glutamate oxidase and horseradish peroxidase) was developed [18]. This, however, lacked linear calibration characteristics for β-ODAP.

In the present investigation, a chromatographic system coupled on line with both refractive index detection and an improved amperometric biosensor based on a previous biosensor prototype [18] that

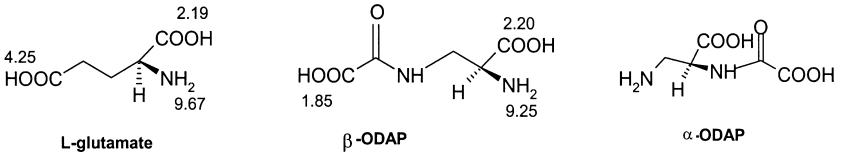

Fig. 1. Structures of L-glutamate, β-ODAP (toxic isomer), and α-ODAP (non toxic isomer), with pK_a values of functional groups in L-glutamate and β-ODAP.

lacked linear calibrations characteristics for β-ODAP, is described for the simultaneous determination of the total amount of ODAP, selectively the amount of β-ODAP, and also the free L-glutamate in raw grass pea samples. The biosensor is based on L-glutamate oxidase (GlOx) that has activity not only for L-glutamate but also for β-ODAP (and to some extent also for aspartate), however, it has no activity for α-ODAP. Analysis results by the presently described method were favourably compared with that of the Rao method.

2. Experimental

2.1. Chemicals and reagents

The enzyme electrode was prepared using spectroscopic graphite (SGL Carbon, Werke Ringsdorff, Bonn, Germany, type RW001, 3.05 mm diameter). Tris(hydroxymethyl)aminomethane (Tris) and potassium chloride were from Merck (Darmstadt, Germany). Sodium salt of DAP was obtained from the Organic Research Laboratory, Department of Chemistry (Addis Ababa University, Addis Ababa, Ethiopia), L-glutamate oxidase (GlOx), *Streptomyces* Sp X119-6 was obtained from Yamasa (Tokyo, Japan). Horseradish peroxidase (HRP Type VI, EC 1.11.1.7, catalog No. P-8375), polyethylenimine (PEI), L-glutamic acid, β-ODAP and OPA were purchased from Sigma (St. Louis, MO, USA). Poly(1-vinylimidazole) {osmium(4,4′ - dimethyl-bpy)$_2$Cl}$^{2+/+}$ was a gift from TheraScience (Alameda, CA, USA). Poly(ethylene glycol)(400) diglycidyl ether (PEGDGE) was purchased from Polysciences (Warrington, PA, USA, catalog No. 08210). All solutions were prepared with water purified with a Milli-Q (Millipore, Bedford, MA, USA) system.

2.2. Methods

2.2.1. Spectrophotometry

The content of ODAP in 80.0 mg of grass pea powder sample was extracted with 8.0 ml of distilled water in an ultrasonic bath (40–45°C) for 1 h [19]. Prior vortexing for 30 s facilitated the leaching of ODAP. The extract was filtered through a Whatman filter paper (No. 41, 9 cm) and centrifuged at 4000 rpm. A 0.1-ml aliquot of the extract (supernatant) was pipetted into a 10-ml test tube and hydrolysed to DAP using 0.2 ml of 3 *M* KOH. The hydrolysis was done in a boiling water bath for about 30 min. Following a neutralisation step with 0.2 ml of 3 *M* HCl and making up the volume to 1.0 ml with distilled water, a coloured adduct was formed by addition of 2.0 ml of OPA reagent [19]. The colour intensity reached maximum within 30 min. The absorbency of the coloured adduct was measured at a λ_{max} of 476 nm (ϵ in the order of 10^5) against a reagent blank using a Milton Roy Spectronic 1001 PLUS spectrophotometer (USA).

2.2.2. Liquid chromatography

The chromatographic system consisted of a pump (2150, LKB Bromma, Sweden), a Carbopac anion-exchange column (PA1, 250×4 mm I.D. Model No. P/N 35359, Dionex, Sunnyvale, CA, USA), and a guard column of the same sort (PA1 Guard, 10-32, P/N 43096). The effluent from the column was directed to a refractive index detector (ERC-7512, Erma, Tokyo, Japan) and then to the electrochemical flow through cell of the wall-jet type [20]. The cell was equipped with three electrodes, the enzyme modified working electrode poised at −50 mV, an Ag|AgCl (0.1 *M* KCl) reference electrode, and a Pt wire counter electrode. All connections between the different parts except between the refractive index detector and electrochemical cell were made of polyether ether ketone (PEEK) tubing (0.76 mm I.D.). In order to reduce the peak broadening effect of the refractive index detector as much as possible before the eluents reach the electrochemical detector, the inner diameter of the tubing was kept as low as possible (0.178 mm) with a length of 15 cm. The electrochemical cell was connected to a potentiostat (Zäta Electronik, Lund, Sweden). The responses of the electrode and refractive index detector were registered using a double pen recorder (Kipp & Zonen, BD 112, The Netherlands). Samples were injected with an injector (Rheodyne, type 7125 LabPRO, CA, USA) supplied with an injection loop of 30 μl. The flow carrier solution Tris–Cl$^-$ buffer (10/10 m*M*, pH 7.5) was filtered through a 0.45-μm membrane (Millipore) and degassed before use to

prevent bubble formation at the surface of the working electrode.

2.3. Sample preparation

Dehusked and split grass pea samples were ground to fine powder with an analytical mill (IKA A10 Maschinen, Germany). A 50-mg amount of powdered grass pea sample was suspended in 10 ml of buffer (Tris–KCl, 10/10 m*M*, pH 7.5) and constantly stirred with a magnetic stirrer for 90 min at room temperature. The extract was then filtered through a Millex-GS filter (0.2 μm, Molsheing, France). Protein and other macromolecules co-extracted with the analytes of interest were removed by centrifugation using centrifugal filter devices (Centricon, molecular cut-off 3000, Bedford, MA, USA) at ca. 4000 rpm for 2 h. The final aliquots (30 μl) of the extract were loaded onto the separation column using an electronically operated injector valve (Rheodyne, type 7125) for isocratic chromatographic separation and post-column refractive index and electrochemical detection.

2.4. Fermented samples

Solid-state fungal fermentation using the strains of *Aspergillus oryzae* CCUG 33812 and *Rhizopus oligosporus* sp T3 was carried out with some grass pea samples following the procedure in Ref. [21]. After fermentation the samples were freeze–dried and the rest of sample preparation was as for raw grass pea samples.

2.5. Preparation of electrode

A two-layer bienzyme electrode was used involving a mediator, an Os-containing polycation, poly(1-vinylimidazole) {osmium(4,4′ - dimethylbpy)$_2$-Cl}$^{2+/+}$}, denoted PVI$_{12}$-dmeOs [22], a cross linking polymer PEGDGE, and a highly positively charged polymer, PEI with the enzymes GlOx and HRP. For the construction of the redox hydrogel on the top of the graphite electrode, first the graphite electrode was polished on a wet emery paper (P1200) to obtain a smooth surface. The electrode was carefully rinsed with MilliQ water and allowed to dry at room temperature. A premixed solution composed of 1 μl of PVI$_{12}$-dmeOs (10 mg/ml in water), 1.5 μl of HRP (10 mg/ml in 10/10 m*M* Tris–Cl^{-1} buffer, pH 7.5), 0.5 μl of a freshly prepared PEGDGE solution (5 mg/ml in water) and 1.5 μl of PEI (0.1%, w/w, in water) was placed on top of the polished end of the electrode and spread evenly using the micro syringe tip. After 10 min waiting time, 3 μl of GlOx (20 mg/ml in 10/10 m*M* Tris–Cl^{-1} buffer, pH 7.5) was evenly spread on top of the first layer of the enzyme electrode and allowed to stand a couple of hours at room temperature before mounting the electrode into the electrochemical cell. Electrode preparations for later use (within a few hours) were kept in a desiccator at room temperature.

2.6. Dry substance determination

About 1 g of powdered grass pea sample was put into a pre-weighed crucible and was kept in an oven at 110°C for more than 24 h. Crucibles with dry matter were re-weighed after cooling to room temperature in a desiccator. Percent of dry matter in each sample was determined by mass differences.

3. Results and discussion

3.1. Detection system

The biosensor employed as a post-column electrochemical detector is based on a previously reported biosensor prototype [18]. Further optimisation and improvements that were made recently to improve some of the analytical characteristics of the biosensor are fully described separately [23]. It contains two enzymes GlOx and HRP. GlOx has a high activity primarily for L-glutamate but also for β-ODAP and to some extent also for L-aspartate. However, GlOx is non-responsive to the α-form of the neurotoxin and based on this characteristic, a number of successful β-selective enzymatic methods have been reported in the literature [17,18,24–26]. Thermal isomerisation studies conducted using immobilised GlOx reactors in flow injection analysis mode [26] show results quite comparable with the results obtained using nuclear magnetic resonance (NMR) techniques [8] and a high-performance liquid chro-

matography (HPLC) method [16]. This finding convincingly proved the β-selectivity of methods based on the enzyme GlOx. Injection of pure α-ODAP in the present system could have been an additional proof for the β-selectivity of the method used, however, this was not possible due to the absence of any commercial preparation of this compound.

In the catalytic reaction sequence of the biosensor, the substrate is oxidised and the enzyme cofactor reduced. The natural reoxidising agent is molecular oxygen, which in the reoxidation reaction of the enzyme is reduced to hydrogen peroxide. To be able to follow the reaction electrochemically the hydrogen peroxide formed can be directly electrochemically oxidised. However, this reaction only occurs at high potentials (+600 mV vs. Ag|AgCl), which would open up the sensing system for interfering reaction to occur with bias response signals as a result.

Another way would be to exchange molecular oxygen for an artificial electron acceptor, which in turn can be reoxidised at the electrode at a potential lower than that for direct oxidation of hydrogen peroxide. $Os^{2+/3+}$-complexes have proven to function as electron acceptors for GlOx [18], however, with moderate reaction rates. Various $Os^{2+/3+}$ containing polymers have been constructed for biosensor work [27] with $E^{\circ\prime}$-values of the $Os^{2+/3+}$ redox couple ranging between +80 and 200 mV vs. Ag|AgCl. The necessary applied potential for such a redox polymer used in its oxidative mode would require a working potential of around +200 mV, which still remains too high to minimise interfering reactions.

An alternative way is to couple the oxidation reaction yielding hydrogen peroxide with a second reaction catalysed by HRP. Here the hydrogen peroxide produced by GlOx is reduced to water and the oxidised HRP can very efficiently be re-reduced with a high reaction rate with the $Os^{2+/3+}$-based mediator and a cathodic response current can be registered at potentials more negative than the $E^{\circ\prime}$-value of the mediator. With the polymeric mediator used, PVI_{12}-dmeOs, maximum response currents are found at around 0 mV [23]. The redox cycle of the electrode and the structure of the redox polymer mediating the electron transfer are shown in Fig. 2.

However, as $Os^{2+/3+}$ has reactivity for both GlOx (in its Os^{3+}-form) and for HRP (in its Os^{2+}-form), it

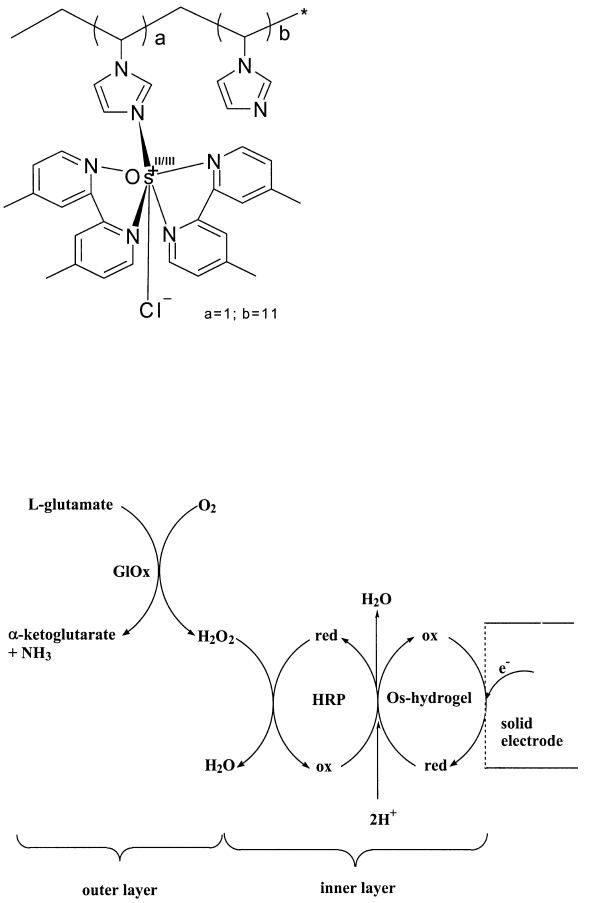

Fig. 2. Structure of the mediating redox polymer, PVI_{12}-dmeOs (upper) and the reaction sequence for the double-layer bienzyme modified electrode (lower). The electrode has an electrically wired HRP inner layer and a non-wired GlOx outer layer. H_2O_2 diffuses between the layers.

is necessary to prevent contact between GlOx and the Os-mediator to prevent short circuiting of the sensing system. When the redox centres of the GlOx are electrically connected to the mediating polymeric network, the substrate reduced redox centres of the enzyme are also oxidised by the redox centres of the mediator. This will have the effect of reducing the measured cathodic current by the system [22]. In our previous work a single-layer approach was utilised, where all the components of the sensing layer were mixed prior to deposition onto the electrode surface [18]. That electrode showed good linear calibration characteristics only for L-glutamate but not for β-

ODAP. The double-layer electrode construction therefore alleviates most of this problem and gave improvement in the sensitivity of the biosensor. Moreover, three equally prepared biosensors were shown to be excellently reproducible (Fig. 3).

With the refractive index detection system, the total amount of the neurotoxin (α- and β-forms) in the eluent is registered and upon reaching the electrochemical cell, the biosensor responded only to the β-form of the toxin.

3.2. Chromatographic separation

As mentioned above GlOx has a high activity mainly for L-glutamate but also to β-ODAP, both of which are extractable from grass pea seeds. Chromatographic separation of these components is therefore a necessary requirement for an accurate analysis of the neurotoxin, β-ODAP and free glutamate in grass pea. Following the procedure of chromatographic separation described elsewhere [18] it was impossible to obtain sufficient baseline separation of L-glutamate and β-ODAP. One of the reasons for this could be the difference in batches of the column preparation though the same sort of column was used. Therefore, chromatographic separation was studied and optimised for best separation and compatibility with the post-column biosensor. As a general trend, L-glutamate eluted first followed by ODAP. Phosphate buffer (100 m*M*, pH 9.2) gave good separation, however, long-term stability of the enzyme-modified electrode was doubtful at this high pH. Appearance of an unspecific peak over which the peak for β-ODAP appears in mixed analytes was also another factor to discard this buffer system. Lowering the concentration of phosphate in the buffer to 10 m*M* and/or to pH 7.8 did not bring the desired effect either in the separation or in the biosensor performance. Tris-buffer at 10 m*M* with different pH adjustments was also investigated. This was also not sufficient to bring the desired chromatographic separation. Next a combination of Tris and KCl, with different pH adjustment was tried and Tris–KCl (10/10 m*M*, pH 7.5) gave the best performance for both the chromatographic and biosensor system. The chromatograms of this run buffer were symmetrical with complete baseline separation and minimum band broadening (Fig. 4). This was therefore the run buffer chosen throughout the subsequent work.

The retention times of L-glutamate and β-ODAP as they are detected coming out from the chromato-

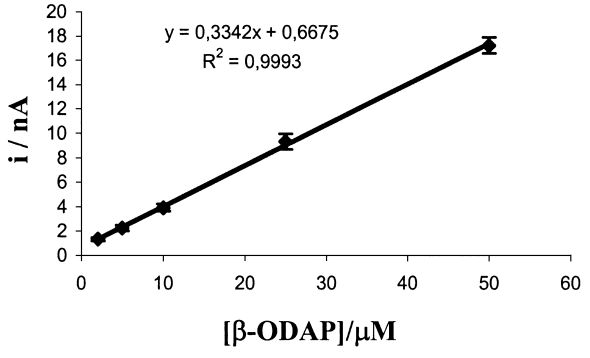

Fig. 3. Reproducibility of response to β-ODAP of three equally prepared biosensors.

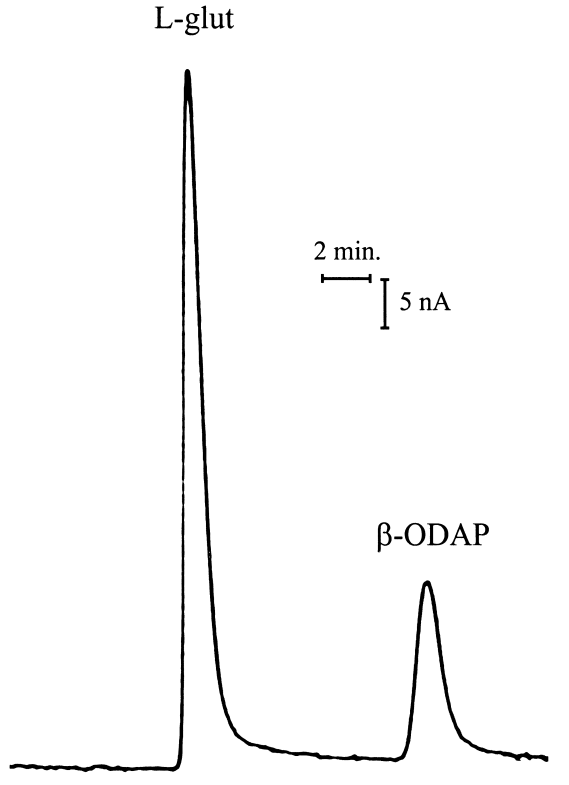

Fig. 4. Chromatograms revealing the status of separation of L-glutamate (40 μ*M*) and β-ODAP (20 μ*M*) using a mobile phase consisting of Tris–Cl^- 10/10 m*M*, pH 7.5.

graphic column by the refractive index detector were 4 and 9 min, respectively. The chromatographic results show that separation of L-glutamate and β-ODAP increases at higher pH of the buffer. Under the buffer conditions used and the pK_a values of the different groups in L-glutamate and β-ODAP (Fig. 1), both molecules seem to attain a net negative charge of one, since the two carboxylic groups are deprotonated and the amine group protonated in each one of them. However, since the pK_a value of the carboxyl group further from the amine group in L-glutamate is higher (4.25) compared to the carboxyl group of the oxalyl moiety of β-ODAP (1.85), the ease of ionisation is difficult in L-glutamate and hence accounting its first elution in the chromatographic separation. Differences in the carbon chain length in the two molecules might have a synergic effect in the separation.

The flow-rate of the mobile phase was optimised to yield both good chromatographic resolution and electrode responses. Lower flow-rates (0.2, 0.4, and 0.6 ml/min) were accompanied by peak broadening. A flow-rate of 0.8 ml/min gave quite acceptable results with minimum peak broadening and better peak symmetry. Running the system at a flow-rate of 0.9 ml/min did not show further improvement. Flow rates higher than 0.9 ml/min were not tried to avoid high backpressure of the chromatographic system. Therefore a flow-rate of 0.8 ml/min was selected.

3.3. Real sample analysis and extraction efficiency

After calibrating the present analytical system with standards of L-glutamate and β-ODAP, the applicability of the method to real samples was tried. The regression lines for the calibration curves (Fig. 5a) have R^2 values of 0.9955 and 0.9978 for L-glutamate (upper) and β-ODAP (lower), respectively. For real sample analysis 10 grass pea seed samples at 10 different localities were collected. Fig. 6 shows chromatograms obtained for a raw and a fermented grass pea sample. The extraction procedure employed was fast and efficient. Previously it has been shown that different extraction procedures such as using 60–70% ethanol (with tumbling for 6–12 h), pure water (40–45°C with sonication for 1 h), and phosphate buffer (pH about 7, with stirring) have practically the same performances [19,24]. The

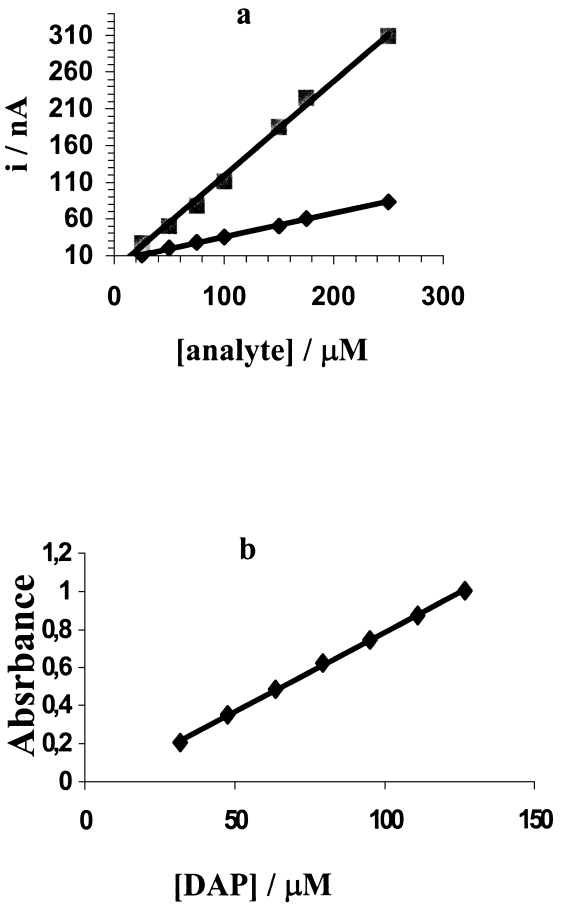

Fig. 5. Calibration curves (a) for L-glutamate (■) and β-ODAP (♦) for the chromatographic/biosensor system and (b) for DAP using the RAO method.

degree of recovery of extraction of ODAP using phosphate buffer was found to be 98% [18]. A similar degree of recovery (99%) was obtained with ethanol extraction [16]. In view of possible isomerisation, a fast and yet efficient isolation is essential for an accurate evaluation of the toxin level. The built in β-selectivity of the biosensor with relatively high turn over rate of the catalytic reaction could be well suited to the analysis of real samples not only in grass pea but also in a variety of other specimens that may involve considerable isomerisation.

3.4. Method validation

The traditional Rao method as described in Ref.

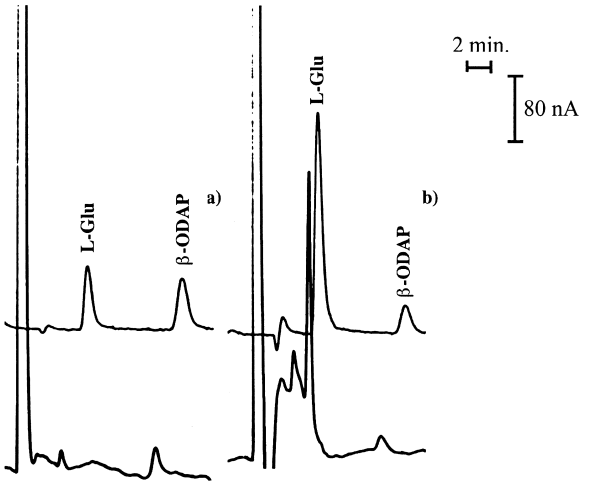

Fig. 6. Chromatograms of real sample analysis: electrochemical detection (upper) and refractive index detection (lower) demonstrating baseline separation of β-ODAP and L-glutamate (''a'' is raw grass pea sample and ''b'' is fermented grass pea sample). Conditions: carrier buffer – Tris–Cl^- (10/10 m*M*, pH 7.5), flow-rate: 0.8 ml/min, injection volume: 30 μl.

[19] was used for the analysis of ten grass pea collections as part of method validation. Sodium salt of DAP was used as a standard for calibration (Fig. 5b). Results of the spectrophotometric method and that of the presently described method agree very well (90% confidence limit parallel ''*t*'' test) after correction of the results of spectrophotometric analysis to 95% β-ODAP level (Table 1). As pointed out above the spectrophotometric method does not distinguish between the toxic β-form and the non-toxic α-form as both of them give rise to the same hydrolysis product, DAP, whereas the biosensor detection system is responsive only to the β-form. The occurrence of β-ODAP in grass pea seed extracts has been reported by different researchers [16,26] to be 95% of the total ODAP content. This level might well depend on the speed of extraction and analysis and it is even reported to be an artifact of isolation rather than an in vivo composition [28]. This explains the constant level of occurrence of the toxic form for at least similar extraction conditions. The strongest evidence for the 95% correction factor comes from studies of thermal isomerisation of β-ODAP by enzymatic flow injection analysis method that used the same extraction procedure as in the present investigation [26]. In this work results after being corrected were quite comparable to those obtained by a completely different method of analysis. In the present study as well, the consistent agreement of the spectrophotometric results (after correcting to 95% β-ODAP) for all the 10 samples with the results of the chromatographic method (all with small standard deviations, Table 1) is also another indication to the constant level of occurrence of β-ODAP in the extracts. Although currently there is a lack of information as to what mechanism keeps 100% β-ODAP in the plant biosynthesis, all the observations in fact convince us to believe that the occurrence of the α-form of the toxin in the seed extracts is generated during the separation process.

Table 1
β-ODAP and free L-glutamate levels of raw grass pea

Sample code	% β-ODAP		% L-Glutamate[c]
	OPA[a]	Chrom/biosensor[b]	
F 1	0.77	0.76	0.11
F 2	0.73	0.73	0.11
F 3	0.64	0.65	0.13
F 4	0.74	0.77	0.12
F 5	0.71	0.66	0.12
F 6	0.67	0.70	0.14
B 1	0.62	0.69	0.16
B 2	0.77	0.83	0.15
B 3	0.58	0.56	0.17
B 4	0.53	0.52	0.16

[a] Dry mass basis, mean value of three determinations ± 0.017 (mean SD).
[b] Dry mass basis, mean value of three determinations ± 0.014 (mean SD).
[c] Dry mass basis, mean value of three determinations ± 0.021 (mean SD).

4. Conclusion

It is claimed that a fast, convenient and reliable β-selective method has been developed for the toxin in grass pea and food preparations thereof. Assay of the toxin on a grass pea collection in particular areas was done in an attempt to relate toxicity with environmental factors. In fact one of the two main unsolved problems in *Lathyrus* research is the question why some pockets of high incidence of Lathyrism occur when the entire district has the same

socio–economic problems and the whole population is consuming grass pea. The other is the individual susceptability for the neurotoxin, β-ODAP. It is also believed that the β-selective electrochemical detection system after chromatographic separation could find application in body fluid specimens in an effort to explain the fate of the toxin after ingestion. In general the inherent β-selectivity of the enzymatic system is well suited to samples wherein considerable amount of isomerisation might have taken place. The long-term operational stability of the biosensor makes it an economical method of analysis.

Acknowledgements

We thank the Swedish International Development Cooperation Agency/Swedish Agency for Research Collaboration with Developing Countries (SIDA/SAREC) and the Swedish Natural Science Research Council (NFR) for financial support. The authors also thank Carolyn A, TheraSense, CA, USA for donating the redox polymer. Department of Chemistry, Addis Ababa University, Addis Ababa, Ethiopia is acknowledged for allowing the facility for the spectrophotometric analysis of the neurotoxin ODAP. Y.Y. is grateful to Professors Jan Åke Jönsson and Lennart Mathiasson, Department of Analytical Chemistry, Lund University for their helpful discussion of the chromatographic separation.

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