A new amperometric ß-ODAP biosensor

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Introduction

The *Lathyrus*/lathyrism challenge is broad-based by its nature and requires multi-disciplinary efforts of specialists in the field of epidemiology, neurology, biochemistry, chemistry, nutrition, and agronomy. The role of chemists is in systematic determination of minor natural products in *L. sativus* seeds so that the sole responsibility of ß-ODAP for neurolathyrism is ascertained [(1)]. Moreover, analytical chemists play a pivotal role in the development of a simple and reliable analytical method for ß-ODAP quantitation in seeds, food preparations, and biological samples taken from victims since lack of such a method hindered, in one way or another, research undertakings in the aforementioned disciplines. Our work addresses the latter role of targeting the development of an amperometric biosensor for ß-ODAP. This ß-ODAP biosensor is based on the pioneering work of Moges and Johannson that reported the activity of glutamate oxidase (GlOD) towards ß-ODAP [(2)]. One of the oxidation products, hydrogen peroxide, reduces the tetravalent manganese (modifier in the screen printed carbon electrode, SPCE) to lower oxidation states that reoxidize again electrochemically producing a current proportional to the concentration of ß-ODAP.

Material and Methods

MnO_2 bulk-modified SPCEs were produced in accordance to previous reports and the flow system and the electrochemical analyzer used were basically the same [(3)]. GlOD was immobilized by entrapment in neutralized Nafion® film as effected by drop coating the enzyme-polymer mixture onto the surface of the SPCE.

Results and Discussion

Operational parameters were assessed using the main substrate glutamate. An applied potential of 440 mV *vs.* Ag/AgCl, flow rate of 0.2 mL min^{-1}, and pH 7.75 of the carrier (0.1 mol L^{-1} phosphate buffer) were found to give the best signal as well as better sample throughput. These parameters were used for ß-ODAP biosensor except the flow rate. Flow rate of 0.1 mL min^{-1} was chosen in this case because of the slower reaction kinetics of the toxin towards the enzyme (equations 1 and 2) [(2, 4)].

$$\text{L-glutamate} + O_2 + H_2O \xrightarrow[\text{fast}]{\text{L-glutamate oxidase}} \alpha\text{–ketoglutarate} + NH_4^+ + H_2O_2 \qquad (1)$$

$$\text{ß-ODAP} + O_2 + H_2O \xrightarrow[\text{slow}]{\text{L-glutamate oxidase}} \alpha\text{–ketoacid} + NH_4^+ + H_2O_2 \qquad (2)$$

Linear relation between concentration of ß-ODAP and current response was observed in the range 50-500 mg L^{-1} (i [nA] = 0.25 c [mg L^{-1}] + 42.12, r^2 = 0.996). The detection limit (as 3σ values) from 6 injections of 100 µL standard ß-ODAP solution (50 mg L^{-1}) was found to be 29 mg L^{-1} and a relative standard deviation of 4.5% was recorded at this concentration of ß-ODAP. In comparison to previous reports [(5, 6)] the linear range between signal and concentration of ß-ODAP in this work was far better though the detection limit was a bit higher. The higher detection limit could be attributed to the diffusion barrier created by the Nafion-enzyme layer. As the thickness of layers increases the linear range extends but the detection limit becomes higher as reported elsewhere [(7-10)].

To destroy glutamate inherent in grass pea seed samples the enzyme glutamate decarboxylase (GlDC) was used. Incubation of glutamate solution with GlDC at 37 °C for 3 hours caused almost complete loss of the glutamate signal indicating the effectiveness and specificity of the enzyme in destroying glutamate (see reaction below).

L-glutamate $\xrightarrow{\text{GlDC}}$ γ-aminobutyric acid + CO_2

It was also observed that GlDC showed no activity at pH 7.75 (no effect on the glutamate concentration) but was effective at a pH between 4 and 5 (which is also the pH of distilled water) as recommended by the manufacturer. Wodajo *et al.* showed that extraction of ß-ODAP from grass pea seed powder could be made in distilled water as effectively as in phosphate buffer [(11)]. Thus, a solution of dihydrogen phosphate (pH 4.5) can be used for the extraction as well as for sample pre-treatment with GlDC; the solution can be adjusted to pH 7.75 using disodium phosphate solution before injecting it to the FI biosensor system.

The decarboxylase has no effect on ß-ODAP as checked by incubating 500 mg L^{-1} ß-ODAP solution overnight at 37 °C. There was no difference (relative error 2%) between the signals of ß-ODAP injection with and without GlDC treatment. Moreover, there was a significant difference (162%) in the response between grass pea extracts untreated and treated with GlDC.

Spiking glutamate (50 mg L^{-1}) to ß-ODAP solution (100 mg L^{-1}) and treating the mixture with GlDC did not make any difference in the ß-ODAP signal. Thus, determination of ß-ODAP in grass pea seed was done in accordance to this finding. Recovery test by spiking 50 mg L^{-1} standard ß-ODAP to one of the samples gave 98.6 ± 3.2 %.

Moreover, the biosensor exhibited extraordinary stability retaining 50% of the original response even after 65 days on-line in the FI system as monitored by injection of standard glutamate solution regularly. It also showed sufficient activity for glutamate when stored in the working buffer for more than 2 months.

To our knowledge, this is the first ß-ODAP biosensor produced using SPCEs. Interferences from glutamate present in grass pea seed extracts have been eliminated using the enzyme GlDC. GlDC has no effect on ß-ODAP (which is also reported for the first time) but completely destroys glutamate in the sample after 3 hours incubation. Extraction of ß-ODAP and elimination of glutamate has been effected in dihydrogen phosphate solution (0.1 mol L^{-1}). The off-line sample pre-treatment is a bit time consuming. However, it can further be improved by adding sodium chloride that is known to activate GlDC [(12, 13)]. It should be noted that the same amount of sodium chloride should be added in the carrier solution to avoid a change in the ionic strength that may otherwise affect the current response. Addition of chloride solution to the carrier can also have the additional advantage of maintaining the stability of the reference electrode, which is chloride concentration dependent. Once, sodium chloride is introduced the enzymatic decarboxylation of glutamate can be faster than observed in this work and the sample pre-treatment can be done on-line by using dual channel flow system as shown in Fig. 1.

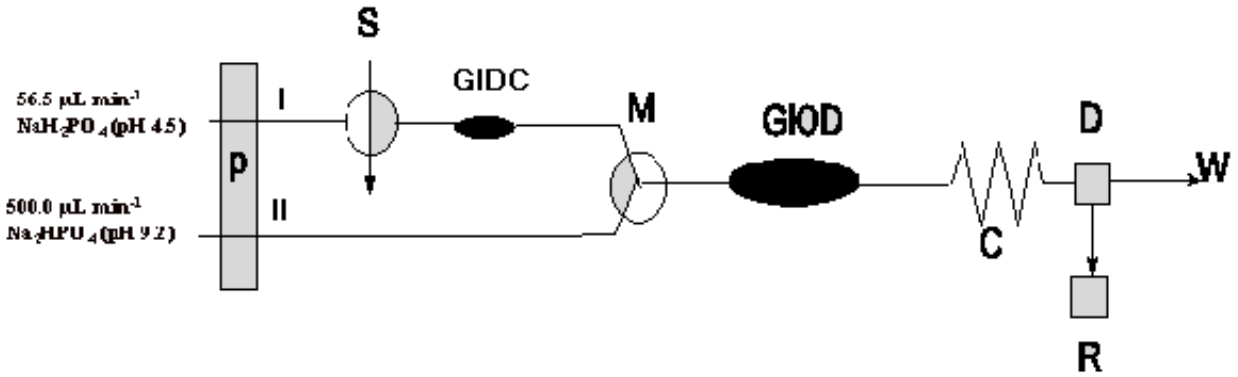

Fig. 1. Proposed dual flow system for improvement of ß-ODAP biosensor.

The first flow channel (I) is to propel the dihydrogen phosphate solution (pH 4.5) at a very low flow rate (Fig. 1) and the second channel (II) to propel disodium phosphate solution (pH 9.2) at higher flow rate. The injection port can be placed somewhere in channel 1 before the GlDC reactor (column). The two channels combine in the mixing tee (M) adjusting the pH to 7.75 and then pass to the GlOD electrode for the main analytical reaction. One further advantage of using GlDC is that it is cheaper and can be produced easily from green pepper [(14)]. Further research could investigate simple sensors with multi-enzymatic layer configurations where the upper most layer contains GlDC to destroy glutamate and the bottom layer contains GlOD with enough activity to oxidize ß-ODAP.

In conclusion, this work demonstrated that immobilization of GlOD in a Nafion® film on MnO_2 bulk-modified carbon electrodes (screen printed) can be used for constructing biosensors for the determination of ß-ODAP. The biosensor exhibited a wider linear range than biosensors of previous studies as well as extraordinary stability. Furthermore, this work showed the effectiveness of GlDC in removing any interference from inherent glutamate that may be present in grass pea seeds.

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