A SCREEN-PRINTED ENZYMATIC ELECTRODE FOR THE DETERMINATION OF ORGANO-PHOSPHOROUS PESTICIDES

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Evolution of a previously reported bienzymatic biosensor for the determination of organo-phosphorous pesticides are reported. This class of pesticides are detected thanks to their high reversible inhibition power towards acid phosphatase (AcP). The catalytic activity of AcP was detected by means of a screen-printed electrode, in presence of the substrate ascorbic acid 2-phosphate (A2P). The current change due to the electrochemical oxidation of the ascorbic acid as reaction product was monitored. Particularly we focused our attention to the determination of the widely diffused pesticides, i.e. Malathion. Results obtained show a high sensitivity, with a lower detection limit of about 5 ppb.

1. Introduction

Most of the biosensors, either electrochemical, optical or piezoelectric, which are used for pesticides determinations, are based on the detection of the catalytic activity of several enzymes in the presence of pesticides [1-8]. The enzymes which have been most extensively employed for the realization of pesticide-sensitive biosensors are the cholinesterases, especially acetylcholinesterases (primarily among them butyrylcholinesterase and acetyl-cholinesterase) owing to the strong and mostly irreversible inhibition of their catalytic activity operated by different classes of pesticides [9-12]. The major drawback of those
biosensors is due to the fact that the irreversible inhibition of the enzyme activity leads to a rapid decrease in the sensitivity and performance of most of the cholinesterase based biosensors, so that each biocatalytic membrane can be used only for few assays. To overcome this limitation of cholinesterase-based biosensors, and on the basis of the same approach that has already been followed in the past for the realization of an organophosphorous pesticide biosensor, we are proposing an improvement of this pesticide-selective sensor, based on the reversible inhibition of acid phosphatase (AcP) [1] coupling with the screen printing technology [13-18]. The method here proposed is based on the AcP catalyzed reaction which produces ascorbic acid amperometrically detected. The determination of Malathion was carried out by measurements of its inhibition values of the catalytic activity of AcP by means of the A2P-selective sensor.

2. Experimental

2.1. Materials

L-Ascorbic Acid 2-phosphate, Acid Phosphatase (Orthophosphoric-monoester phosphohydrolase; EC 3.1.3.2 from potato), Malathion were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The reference solution was obtained from BAS (Bioanalytical Systems Inc. IN, USA). Carbon paste, silver conductive inks and dielectric polymer ink were purchased from Acheson.

2.2. Methods

Screen-printing was performed with a manual printing machine (Fleischle). Screen-printed graphite (SPG) electrodes were designed for use in batch and flow cell measuring systems. Electrodes were supported on a PVC layer. Silver ink acting as conductive medium was printed and cured at 70°C for 15 min. Carbon paste ink was printed and cured at the same temperature. An insulator layer was finally applied to cover the body of the electrode as reported in the figure 1. Electrodes were cut from the printed sheet.
and placed in 10 mL of 0.1M NaOH. Voltammetric cycles were carried out between -1500 and +1500 mV (vs. Ag/AgCl) at a scan rate of 100 mV/s for 40 minutes. Amperometric measurements were carried out by connecting the previously described electrodes to a potentiostat at a constant potential of +400mV vs. Ag/AgCl. Experiments were carried out in a glass cell, by ensuring an uniformity of solution by a constant magnetic stirring, in 2.5 mL of citrate buffer 0.1 M, pH=5.5 and KCl 0.01M. The electrode was employed both in the absence of the enzyme, to the direct determination of ascorbic acid concentration, and in the presence of AcP in the buffer solution, for the determination of A2P concentration. All the assays were performed under the same experimental conditions as described for the calibration experiments. The measurements were performed by dipping the sensor in citrate buffer 0.1 M, pH=5.5 and KCl 0.01M containing AcP and A2P at concentrations of respectively 0.132 U/mL and 26 M. After stabilization of the current signal, increasing quantities of the pesticide solution were added under constant stirring to calibrate the system. The current decrease, proportional to the lower ascorbic acid production due to the AcP inhibition, was recorded for 30 minutes. It represents the best compromise between the optimal response of the electrode and short time of analysis. In this way the calibration curve gives the decrease of current as a function of the concentration of added inhibitor in an easy and reliable way.

3. Results

The calibration graph, obtained with the standard solution of ascorbic acid without AcP, is reported in figure 2 together with calibration curve performed in the presence of AcP and carried out on standard solution of A2P. Figure 3 shows measurements carried out in the presence of several concentrations of pesticide.

Table 1 summarizes the main electroanalytical features of the screen-printed electrode referred to the inhibition assays. Figure 4 shows the amperometric a recording in a typical inhibition experiment performed by means of the enzyme sensor here described. The first arrow refers to the addition of A2P, to a final concentration of 26 µM, the following arrow to the addition of a malathion solution to a final concentration of 50 ppb

4. Conclusions and future studies

The performance of the AcP based screen printed electrode proves to be a close correlation with previously obtained analogous bienzymatic biosensor, with the advantage of the requirement of only a single enzyme. Another advantage of
the AcP based sensor with respect to the cholinesterase based sensor is that the inhibition of AcP by organophosphorous pesticides is almost completely reversible, so that no reactivating treatment is required. The reversibility of the inhibition of AcP, in the case of the immobilized enzyme biosensor, could be also responsible for the relatively long shelf life of the sensor, leading to a drastic reduction of the overall costs of operation.

Additional experiments carried out to study the appropriate enzyme immobilization procedure for the optimization of the electroanalytical features of the screen-printed biosensor, are currently in progress.

<table>
<thead>
<tr>
<th>pH:</th>
<th>5.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer:</td>
<td>Citrate 0.1M</td>
</tr>
<tr>
<td>Response time:</td>
<td>30 min</td>
</tr>
<tr>
<td>Equation of calibration curve: $Y = \Delta I \ (\text{nA}); X = \text{Malathion (ppb)}$</td>
<td>$Y = 0.69 + 0.057 \times X$</td>
</tr>
<tr>
<td>Linearity range (ppb):</td>
<td>10-100</td>
</tr>
<tr>
<td>Correlation coefficient:</td>
<td>0.9908</td>
</tr>
<tr>
<td>Lower detection limit (ppb):</td>
<td>5</td>
</tr>
<tr>
<td>Reproducibility of measurements:</td>
<td>2.5 %</td>
</tr>
</tbody>
</table>

(as “pooled standard deviation” in the linearity range)
Figure 4. Example of the measurement procedure (see text)

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References


