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Electrochemical investigations of lipase enzyme activity inhibition by methyl parathion pesticide: Voltammetric studies

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ABSTRACT

Candida Rugosa Lipase mobilized sensor method was developed for the detection of organophosphorus pesticides like methyl parathion. Here *p*-Nitrophenyl Acetate was used as a substrate which releases *p*-Nitrophenol by the enzymatic hydrolysis and gives an anodic oxidation on electrochemical oxidation peak potential at 0.05 V versus SCE. Methyl parathion is a toxic substance and releases *p*-Nitrophenol when it is hydrolyzed and it also has the property of inhibiting the enzymatic activity and this is taken as a basis for the inhibition studies. The current response under the influence of pH, substrate concentration, enzyme concentration and time variation effects on the hydrolysis process was studied for the reaction between enzyme and substrate which releases *p*-Nitrophenol. A linear calibration for the methyl parathion was obtained in the concentration range of 10–70 ppb with a correlation coefficient of 0.948 under the optimized conditions by following the incubation time of 25 min. The detection limit was found as 26.32 ppb of methyl parathion with optimal conditions of 750 μM substrate concentration, 125 U of enzyme, pH 7.0, 25 min of hydrolysis time and incubation time of 25 min and the proposed method has a quantification limit of 87.72 ppb.

1. Introduction

Organophosphorus (OP) pesticides (herbicides, fungicides, insecticides) are the most toxic compounds and are widely used as pest controllers in agriculture, medicine and industry. Most of the aquatic eco-systems are contaminated by these pesticide residues due to the heavy runoff rain water. The increasing concentrations of organophosphorus pesticide residues are found to be present in many sampled soils, aquatic ecosystems and waste-water streams. It has generated the need to understand and evaluate the biological effects of pollutants on aquatic ecosystems. In this sense, a large number of studies have used biomarkers as functional tools to evaluate the toxicity of such compounds for natural populations [1]. Methyl parathion is an organophosphorus insecticide (Table 1) and it is widely used in the agriculture for the control of most of the pest verities. The mistreatment of these pesticides results in contamination of fields, crops, aquatic environment and air. Methyl parathion is relatively insoluble in water, and readily soluble in most of the organic solvents. The residues of these pesticides in air, aquatic environment, soil and organisms in the environment will influence several chemical and biological factors. Methyl parathion is readily absorbed via all routes of exposure (oral, dermal, inhalation) and is rapidly distributed to the tissues of the living organisms. Most of the traditional analytical methods like High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC), Liquid Chromatography (LC) coupled to sensitive and specific detectors such as nitrogen–phosphorous detectors (NPD) [2–4], flame ionization detectors (FID), ultraviolet detectors (UV) or diode array detector (DAD) [5–7] and mass spectrometry (MS) [8,9] are used for the quantification of environmental pollutants, pesticide residues in water, soil and other sources. These sophisticated techniques are time-consuming processes; require highly trained personnel, tedious extraction and clean-up procedures prior to instrumental analysis and therefore are not very suitable for routine analysis.

An electrochemical method of sol–gel immobilization acetylcholine esterase enzymatic biosensor method is developed for the determination of pesticide residues in environmental matrices due to their good selectivity, sensitivity and rapid response towards pesticides due to their eco-friendly nature [10]. An immobilizing potentiometric biosensor [11] was developed by using Candida Rugosa Lipase enzyme which shows an intrinsic capability to catalyze carboxylic ester bonds to the corresponding alcohol and acid and the same enzyme was used for the surface acoustic wave impedance sensor for the determination of organophosphorus pesticides [12]. *p*-Nitrophenol is used as a starting substrate for the synthesis of many fungicides, pesticides and pharmaceutical products. In particular *p*-Nitrophenol is a toxic derivative of methyl parathion insecticide [13].

Based on electrochemical response of phenolic compounds [14] towards carbon paste electrode, a simple voltammetric mobilized

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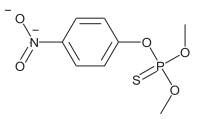
Table 1
The structure and molecular formulae of methyl parathion.

Sl. no	Selected organophosphorus pesticide	Molecular formula	Chemical structure
01	Methyl parathion	$C_8H_{10}O_5NPS$	

mono-enzyme sensor for the indirect, sensitive and selective determination of methyl parathion is developed. In this work a differential pulse voltammetric lipase enzyme mobilized method is also developed which can be applied for the determination of methyl parathion in the environmental samples.

2. Experimental section

2.1. Reagents and chemicals

All chemicals were obtained from commercial sources and used without further purification. Lipase from Candida Rugosa (EC 3.1.1.3, type VII, ≥700/mg) *p*-Nitrophenyl Acetate, methyl parathion was purchased from Sigma-Aldrich Chemicals. The pesticide stock solutions were prepared by dissolving in acetone (GR grade solution). The fine graphite powder was obtained from Merck chemicals. Silicon oil, acetone (GR grade) was procured from Himedia chemicals. Phosphate Buffer [0.1 M] was prepared by using 0.1 M disodium hydrogen phosphate and 0.1 M sodium dihydrogen phosphate. All chemicals were of analytical grade and aqueous solutions were prepared with double distilled water. The enzyme stock solution was stored at −5 °C and all stock and working solutions were stored at 5 °C.

2.2. Apparatus

Cyclic voltammetric experiments were performed with a CHI model 660c electrochemical work station with a connection to a personal computer that was used for electrochemical measurement and treating of data. A conventional three electrode cell was employed throughout the experiments, with a bare carbon paste electrode (homemade cavity of 3.0 mm diameter) as a working electrode, saturated calomel electrode (SCE) as a reference electrode and a platinum wire as a counter electrode. All the experiments were carried out at room temperature 25 ± 2 °C.

2.3. Preparation of bare carbon paste electrode

The bare carbon paste electrode was prepared by hand mixing of 70% graphite powder and 30% silicon oil in an agate mortar to produce a homogenous carbon paste. The paste was packed into the cavity of homemade PVC (3 mm in diameter) and then smoothed on a weighing paper. The electrical contact was provided by copper wire connected to the paste at the end of the tube [15].

3. Results and discussion

3.1. Cyclic voltammetric studies

The electrochemical behavior of in situ generated *p*-Nitrophenol from the hydrolysis of *p*-Nitrophenyl Acetate substrate in the presence of Candida Rugosa Lipase enzymatic hydrolysis was examined. Fig. 1 shows a cyclic voltammogram of the *p*-Nitrophenol in the absence and presence of 125 units of Lipase enzyme with 750 μM substrate in 0.1 M phosphate buffer (pH 7.0) at a scan rate of 30 mV/s. In the absence of enzyme and substrate (blank) the electrode gave no response and only a small background current was observed (peak a). In the presence of only 750 μM substrate a little back ground peak current was observed (peak b). When enzyme was added to 750 μM of substrate after 25 min a relatively large anodic peak current at a potential of 0.05 V (peak c) was obtained. The *p*-Nitrophenol molecule readily undergoes electrooxidation. Primarily, it is oxidized to nitro-phenoxy radical as shown in Eq. (1) according to literature report [14].

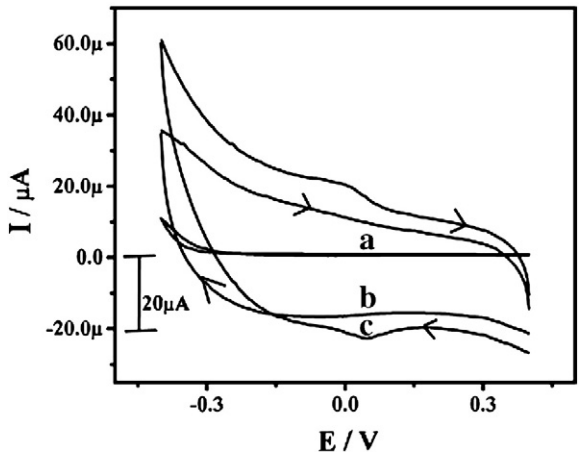


Fig. 1. Cyclic voltammograms of in situ generated *p*-Nitrophenol in 0.1 M phosphate buffer, pH 7.0 a) blank; b) 750 μM substrate alone; and c) substrate in the presence of 125 U of enzyme.

$$C_6H_4NO_2OH \longrightarrow C_6H_4NO_2O^{\bullet} + H^+ + e^- \quad (1)$$

This radical intermediate subsequently undergoes polymerization leading to the formation of a non-sticky thin film on the electrode surface. For further studies the non-sticky polymeric film electrode surface was removed by physically smoothing against a tissue paper [14]. When the concentration of enzyme was gradually increased from 25 U to 250 U there was a gradual increase in the peak current response and this response was finally saturated at 125 U of enzyme concentration where the current values observed were almost constant (Fig. 2) and the optimized enzyme concentration is presented (Table 2).

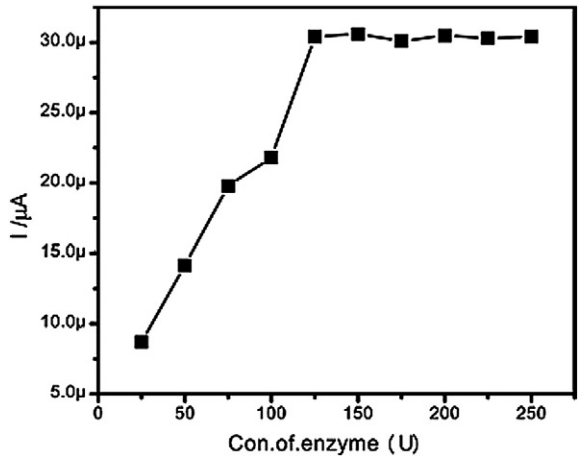


Fig. 2. Response of increasing concentrations of Lipase enzyme in 0.1 M phosphate buffer pH 7.0.