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Development of enzyme biosensor based on pH-sensitive field-effect transistors for detection of phenolic compounds

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Abstract

This article describes a biosensor based on pH-sensitive field-effect transistors (pH-FETs) as transducer, and immobilised enzyme tyrosinase as biorecognition element, which was used for the determination of phenolic compounds in water solutions. The biologically active membrane was formed by cross-linking of tyrosinase with bovine serum albumin (BSA) in saturated glutaraldehyde (GA) vapours on the sensitive transducer surface. The main analytical characteristics were studied under different conditions as well as the possibility to optimise these working parameters. Different factors such as the pH of immobilisation, the enzyme loading, the time of exposition to glutaraldehyde vapours were investigated in regards to the influence on sensitivity, limit of detection, dynamic range, and operational and storage stability.

Keywords: Biosensor; pH-FET; Tyrosinase; 4-Chlorophenol

1. Introduction

Chlorophenols are a major group of chemicals, which are widely used in the manufacture of various industrial products such as pesticides, disinfectants, antioxidants, dyes, etc. [1]. Organophosphorous and chlorinated phenoxyacids also yield chloro and nitrophenols as major degradation products [2].

The conventional methods for phenols determination are chromatography and spectrometry [3]. However, these techniques do not easily allow continuous on-site monitoring, are expensive, time-consuming, need skilled operators, and sometimes require preconcentration steps. The application of biosensors is favourable due to some generally claimed advantages such as the selectivity, the relatively low cost of realisation, sometimes the good storage stability, the potential for miniaturisation and the easy automation, and for the construction of simple portable device for fast screening purposes and in-field/on-site monitoring. In particular, several biosensors based on tyrosinase were elaborated for the determination of phenols. They include the various kinds of electrochemical detection, such as the amperometric of dioxygen consumption [4], the direct reduction of generated *o*-quinone [5] and the mediated reduction of *o*-quinone by hexacyanoferrate(II) [6]. This article describes the biosensor based on pH-sensitive field-effect transistors (pH-FETs) as transducer, and immobilised enzyme tyrosinase as biorecognition element, which was used for the determination of phenolic compounds in water solutions.

2. Experimental

Tyrosinase (EC 1.14.18.1, 6680 U/mg) from mushroom, bovine serum albumin (BSA) and aqueous solutions (25% w/v) of glutaraldehyde (GA) were purchased from Sigma. All other chemicals were of analytical grade.

The biologically active membrane was formed by cross-linking of tyrosinase with BSA in saturated GA vapour on

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the sensitive transducer surface [7]. The 10% solutions of tyrosinase and BSA were prepared in 20 mM phosphate buffer. Prior to the deposition on a sensitive area of a transducer, these solutions were mixed, and glycerol was added to the final concentration of 10%. As a differential experimental set-up was used, one drop of the enzyme-containing mixture was deposited on the working ISFET, while on the reference ISFET, only a mixture containing 10% BSA and 10% glycerol was deposited. Then the sensor chip was placed in a saturated glutaraldehyde vapour for a certain time called immobilisation time. After exposure in GA, the membranes were dried at room temperature for 15 min.

The potentiometric sensor chip contains two identical pH-FETs, the design and operation mode are given elsewhere [8]. Measurements were conducted in daylight at room temperature (25 °C) in a glass cell (5-ml volume) filled with phosphate buffer. The biosensors were immersed in a vigorously stirred sample solution. After the initial drift of the output signal, 4-chlorophenol was added to the vessel. The differential output signal between the measuring and reference pH-FET was registered using laboratory ISFET-meter from the Institute of Microtechnology (Neuchatel, Switzerland) and the steady-state response of the biosensor was plotted as a function of 4-chlorophenol concentration.

3. Results and discussion

Tyrosinase (phenol oxidase) contains copper, which forms part of the prosthetic group in its active site, and acts as a built-in carrier undergoing reversible oxidation and reduction of the enzyme. The conversion of phenols by tyrosinase proceeds in two consecutive steps involving molecular oxygen. The first step is referred to as the enzyme's hydroxylase activity (also cresolase activity) where phenol is hydroxylated by the aid of molecular oxygen to produce catechol (*o*-hydroquinone). In the second step, known as enzyme's catecholase activity, catechol is oxidized to an *o*-quinone, and simultaneously, tyrosinase is oxidized by oxygen to its original form with the production of water. The potentiometric detection of phenols can be used, since the subsequent local decreasing of concentration of protons inside the membrane results in a change of the measuring FET gate voltage.

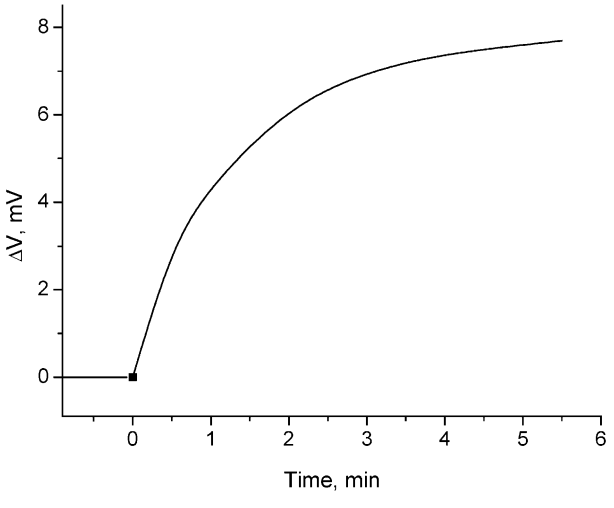

Fig. 1. A typical response curve of tyrosinase biosensor for 4 mM 4-chlorophenol. Measurements were conducted in 5 mM phosphate buffer, pH 6.0.

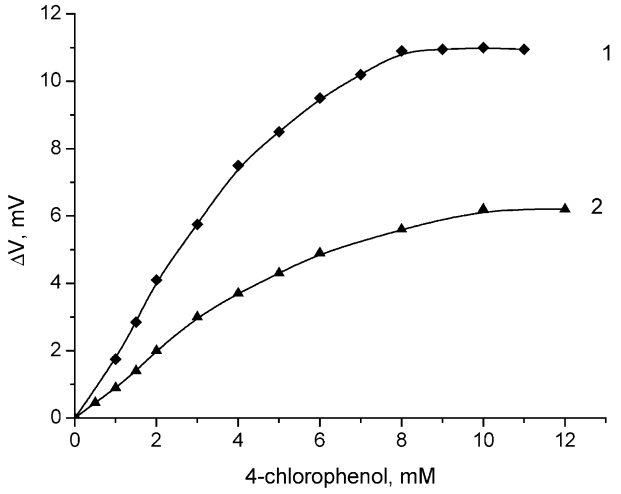

Fig. 2. Calibration curves for 4-chlorophenol determination for pH of membrane immobilisation mixture of 6.0 (1) and 7.4 (2). Measurements were conducted in 5 mM phosphate buffer, pH 6.0. Time of exposition to glutaraldehyde vapours—30 min.

A typical dependence of the potentiometric tyrosinase biosensor response on the time after 4-chlorophenol additions to a vessel is shown in Fig. 1. After the biosensor reached a stable baseline in blank phosphate buffer solution, injection of 4-chlorophenol stock solutions into blank solution caused significant sensor response, which resulted from subsequent local decreasing of concentration of protons inside the membrane from the enzymatic oxidation of phenol. As it can be seen, the biosensor steady-state response time, i.e. time necessary to reach 90% of the steady-state amplitude was about 5 min.

The main analytical characteristics of tyrosinase biosensor based on pH-sensitive field-effect transistors for the detection of phenolic compounds were studied under different conditions such as the pH of sample solution and immobilisation mixture, the enzyme loading in immobilisation mixture, time of exposition to glutaraldehyde vapours.

An optimum for the sensor response was observed at solution pH 6.0. This result is in good agreement with results obtained for tyrosinase by other authors [9,10]. The pH in the immobilisation mixture may also influence the analytical characteristics of enzyme biosensors. Fig. 2 presents the calibration curves for 4-chlorophenol determinations for different pH of membrane immobilisation mixture. Here, it is clearly seen that the sensitivity of biosensor

is higher in case of immobilisation at pH 6.0, i.e. the pH range where the tyrosinase activity is optimal. This pH of immobilisation mixture was selected for all further experiments.

The effect of the enzyme loading in immobilisation mixture on tyrosinase biosensor response to 4-chlorophenol was investigated. Different ratios tyrosinase/(BSA + tyrosinase) were tested to optimise the amount of loaded tyrosinase with the sensor response. It was shown that the best ratio for enzyme loading was about 0.4 with general containing of protein 10%. For this ratio, the best combination of operational stability and response value was obtained.

Concerning time of incubation in glutaraldehyde vapours, the best result was obtained for 30 min. For longer incubation time, a dramatic decrease of response values was occurred. This phenomenon can be connected with the formation of a great number of covalent bonds between the glutaraldehyde and the enzyme molecules, which block enzyme active centres.

The response of the tyrosinase biosensor based on pH-sensitive field-effect transistor is reproducible; the relative standard deviation of intra-sensor responses was about 3% for each concentration. The relative standard deviation of inter-sensor responses was about 7%.

These biosensors could be used also for the detection of diuron-type herbicides (atrazines, simazine, diuron, isoproturon, etc.), because these substances can inhibit the activity of tyrosinase [11].

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