Short communication

Electrochemical biosensor for catechol using agarose–guar gum entrapped tyrosinase

Sanket Tembe a, Shaukat Inamdar a, Santosh Haram a, Meena Karve a,*, S.F. D’Souza b,**

a Department of Chemistry, University of Pune, Ganeshkhind, Pune 411 007, India
b Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Mumbai 400 085, India

Received 21 June 2006; received in revised form 13 September 2006; accepted 15 September 2006

Abstract

An electrochemical biosensor using tyrosinase was constructed for the determination of catechol. The enzyme was extracted from a plant source Amorphophallus companulatus and entrapped in agarose–guar gum composite biopolymer matrix. Catechol was determined by direct reduction of biocatalytically liberated quinone species at $-0.1 \text{ V}$ versus Ag/AgCl (3 M KCl). The response was found to be linear and concentration dependent in the range of $6 \times 10^{-5}$ to $8 \times 10^{-4}$ M with a lower detection limit of $6 \mu\text{M}$. It has reusability up to 20 cycles and a shelf life of more than 2 months when stored at $4 ^\circ \text{C}$.

Keywords: Tyrosinase; Catechol; Biopolymers; Entrapment; Electrochemical sensor

1. Introduction

In view of major concern regarding toxicity, considerable attention has been given to the reliable quantification of phenols in complex environmental matrices. Among many analytical methods for measuring phenolic compounds, electrochemical biosensors based on immobilized tyrosinase have received the major share of attention (Chen et al., 2001; Cosnier and Popescu, 1996; Wang and Chen, 1995; Xue and Shen, 2002; Yu et al., 2003).

Tyrosinase (or polyphenol oxidase) is a copper-containing enzyme (EC 1.14.18.1) that catalyses conversion of phenolic substrate to the corresponding quinone species that can be electrochemically reduced to allow convenient low-potential detection of phenolic analyte (Kazandjian and Klibanov, 1985; Rivas and Solis, 1991). A variety of methods for the immobilization of tyrosinase with an electrochemical transducer

* Corresponding author. Tel.: +91 20 25601225; fax: +91 20 25691728.
** Corresponding author. Tel.: +91 22 25593632; fax: +91 22 25505151.
E-mail addresses: mskarve@chem.unipune.ernet.in (M. Karve), sfdsouza@apsara.barc.ernet.in (S.F. D’Souza).
have been reported such as adsorption (Yaropolov et al., 1995) or cross-linking (Tillyer and Gobin, 1991) on the surface of electrodes, incorporation within a carbon paste matrix (Wang et al., 1994a,b) or graphite-epoxy composite electrodes (Onnerfjord et al., 1995; Wang et al., 1994a,b), entrapment in polymer films and hydrogels (Deng et al., 1996; Shan et al., 2002; Wang et al., 2002). Electropolymerization (Rajesh et al., 2004; Vedrine et al., 2003), self-assembled monolayers (Campuzano et al., 2003; Tatsuma and Sato, 2004), silica sol–gel (Li et al., 1998; Wang et al., 2000), alumina sol–gel (Liu et al., 2000a,b) and nanoparticles (Li et al., 2006) have also been applied for immobilization of tyrosinase for the detection of phenolic compounds.

Recently we reported the development of electrochemical biosensor for L-dopa and dopamine using a composite biopolymer matrix, agarose and guar gum (Tembe et al., 2006). Agarose and guar gum are naturally occurring biopolymers having high permeability towards water. The composite material provides natural microenvironment to the enzyme and also gives sufficient accessibility to electrons to shuttle between the enzyme and the electrode. Its good film forming and adhesion ability, together with its nontoxicity and biocompatibility, has developed growing interest in using it for tyrosinase entrapment and subsequent sensor fabrication. In the present article, the potential application of this tyrosinase-polysaccharide bioelectrode in environmental pollution monitoring is described using catechol as a representative of phenols. The probing of catechol is important owing to its applicability in a broad range of chemical manufacturing processes and its inherent toxicity. Here we developed electrochemical biosensor for catechol using enzyme tyrosinase entrapped in agarose–guar gum composite biopolymer matrix and analytical performance of the biosensor was studied.

2. Materials and methods

2.1. Materials

Tyrosinase derived from a plant source Amorphophallus complanatus (Paranjpe et al., 2003) was used. Catechol and agarose were from Sigma Chemical Co., St. Louis, USA. Guar gum was procured from Sisco Research Laboratory (SRL, India). All other chemicals were of analytical grade and all solutions were prepared with water from Millipore Milli-Q system.

2.2. Apparatus

Cyclic voltammetric and differential pulse voltammetric experiments were performed with Autolab 100 potentiostat. All experiments were carried out in an electrochemical cell with a working volume of 1 ml with a conventional three-electrode system. The working electrode was glassy carbon covered with an enzyme layer, and a platinum wire as the counter electrode. All the potentials quoted here were relative to an Ag/AgCl (3 M KCl) reference electrode.

2.3. Preparation of immobilized tyrosinase film

Tyrosinase was entrapped in agarose–guar gum composite as described earlier (Tembe et al., 2006). Briefly, aqueous solutions of agarose (3%) and guar gum (1%), 1 ml each, were mixed and the appropriate amount of tyrosinase (2550 U) was then added. The mixture was spread uniformly on glass plate over the surface area 9 cm² and kept overnight in a dust free hood for drying. The thin membrane thus obtained was stored at 4 °C under dry conditions.

2.4. Fabrication of enzyme electrode

The working electrode was a tyrosinase modified glassy carbon electrode prepared by immobilizing the tyrosinase in agarose–guar gum composite. Prior to casting of gel, glassy carbon electrode was polished with alumina powder, rinsed thoroughly with distilled water. Composite gel (30 µl) containing 271 U of enzyme extract [29.7 µg as determined by Lowry method (Lowry et al., 1951)] was directly cast on the active surface of glassy carbon electrode. The electrode was then allowed to dry in a dust-free hood. When not in use, electrode was stored at 4 °C.

2.5. Electrochemical measurements

Determination of catechol was carried out electrochemically by measuring the intensity of current,
which corresponds to the electrochemical reduction of the enzymatically generated quinone. This was done by immersing the working electrode in a 0.1 M phosphate buffer at pH 6.0 and applying a polarization voltage of +100 mV to the platinum electrode against an Ag/AgCl (sat. KCl). When the background current had stabilized, an appropriate amount of catechol was introduced in an electrochemical cell. All the measurements were carried out at room temperature under continuous stirring. Low concentrations of catechol were analyzed by differential pulse voltammetry (DPV) at modified GCE. A sensitive cathodic reduction peak was used for quantitative determination. A good linear relationship was observed between cathodic peak currents and catechol concentration.

3. Results and discussion

3.1. Cyclic voltammetric behavior of tyrosinase modified electrode

Cyclic voltammograms of tyrosinase electrode in sodium phosphate buffer (pH 6.0, 0.1 M) without catechol and with increasing concentrations of catechol are shown in Fig. 1. It was observed that the reduction peak increased after catechol was added to phosphate buffer on enzyme immobilized electrode. Such an increase in reduction peak is due to the reduction of quinone species liberated from the enzymatic reaction catalyzed by tyrosinase on enzyme electrode.

DPVs of thus-prepared composite film-covered electrode in the presence of catechol are shown in Fig. 1. Cyclic voltammograms obtained at agarose–guar gum entrapped tyrosinase electrode for solutions of increasing catechol concentration from 0 μM (a), 200 μM (b), 400 μM (c), 600 μM (d), 800 μM (e) and 1000 μM (f) and dashed line: agarose–guar gum membrane without tyrosinase for 400 μM catechol. Scan rate: 100 mV/s. Electrolyte: phosphate buffer (0.1 M, pH 6.0).
Fig. 2. Differential pulse voltammograms obtained at agarose–guar gum entrapped tyrosinase electrode for solutions of increasing catechol concentration from 0 μM (a), 60 μM (b), 80 μM (c), 100 μM (d), 200 μM (e), 400 μM (f), 600 μM (g) and 800 μM (h). The inset shows linear response for catechol.

Fig. 3. (A) Calibration curve for catechol and (B) corresponding Lineweaver–Burk plot. Electrode: agarose–guar gum entrapped tyrosinase electrode.
Fig. 2. Reduction current measured at $-0.1 \text{ V}$ was indicative of enzymatic activity of electrode.

3.2. Electrode response characteristics

The initial experiments were conducted to establish the optimum pH. Phosphate buffers at different pH values were used as carrier solution. The maximum response for catechol was obtained at pH 6.0. This pH value was used throughout the work.

A linear response for catechol obtained in phosphate buffer (0.1 M, pH 6.0) is shown in Fig. 2 inset. Catechol sensor gave a linear plot for the range $6 \times 10^{-5}$ to $8 \times 10^{-4}$ M with a linear regression equation $y = 0.001x + 0.5402$, $r^2 = 0.9981$ where $y$ represents the current ($\mu$A) and $x$, the substrate concentration. A detection limit of $6 \times 10^{-6}$ M is achieved with a signal-to-noise ratio <1. A calibration curve for catechol is shown in Fig. 3A. Corresponding Lineweaver–Burk plot for catechol (based on data of Fig. 2) is given in Fig. 3B. An apparent $K_m$ value of 22 $\mu$M was obtained for catechol.

With low concentrations of catechol, no decrease in response was observed for at least 20 cycles in continuous testing. A decrease in response of enzyme electrode was observed for a high concentration (7 mM) of catechol, attributed to slow surface fouling by the reaction product (Koile and Johnson, 1979). To determine the storage stability, the performance of enzyme electrode was monitored over a period of 4 months. When stored at 4 $^\circ$C under dry conditions, only a marginal loss of enzyme activity was observed after 2 months.

3.3. Selectivity of the sensor

Polyphenol oxidase or tyrosinase catalyzes the oxidation of phenol group to $\alpha$-quinone, thus allowing a variety of phenolic compounds to be used as substrates of this enzyme. It is well known that this enzyme presents broad substrate specificity.

In order to determine the selectivity of the sensor, the voltammetric response of this sensor to other phenolic compounds including dopamine hydrochloride, caffeic acid and hydrocaffeic acid was checked. These phenolic compounds actually show no current at $-0.1 \text{ V}$, suggesting that there is no interference in the presence of these substrates. Therefore, it is reasonable that this sensor may be used to selectively detect catechol without interference.

4. Conclusion

In the present work, an electrochemical biosensor for catechol was developed using enzyme tyrosinase entrapped in agarose–guar gum composite matrix. The analytical characteristics of this sensor, including linear range, lower detection limit and kinetic constants are described. The biosensor exhibited good performance in terms of reusability, operational stability, fabrication simplicity and shelf life. This simple, easy-to-construct, reagentless electrode is suitable for micromolar quantification of catechol.

Acknowledgement

Research fellowship to S.T. from University of Pune-Bhabha Atomic Research Centre collaborative research programme is acknowledged.

References


