Enzyme and Microbial Technology 52 (2013) 377–385

Contents lists available at SciVerse ScienceDirect

Enzyme and Microbial Technology

journal homepage: www.elsevier.com/locate/emt

A novel electrochemical biosensor based on horseradish peroxidase immobilized on Ag-nanoparticles/poly(L-arginine) modified carbon paste electrode toward the determination of pyrogallol/hydroquinone

P. Raghu [a], T. Madhusudana Reddy [a,*], K. Reddaiah [a], L.R. Jaidev [b], G. Narasimha [b]

[a] Electrochemical Research Laboratory, Department of Chemistry, S.V.U. College of Sciences, Sri Venkateswara University, Tirupati 517502, Andhra Pradesh, India
[b] Applied Microbiology Laboratory, Department of Virology, Sri Venkateswara University, Tirupati 517502, Andhra Pradesh, India

ARTICLE INFO

Article history:
Received 28 January 2013
Received in revised form 21 February 2013
Accepted 24 February 2013

Keywords:
Horseradish peroxidase
Silica sol–gel
Pyrogallol
Hydroquinone
Carbon paste electrode
Silver nanoparticle

ABSTRACT

A novel electrochemical biosensor for the determination of pyrogallol (PG) and hydroquinone (HQ) has been constructed based on the poly L-arginine (poly(L-Arg))/carbon paste electrode (CPE) immobilized with horseradish peroxidase (HRP) and silver nanoparticles (AgNPs) through the silica sol–gel (SiSG) entrapment. The electrochemical properties of the biosensor were characterized by employing the electrochemical techniques. The proposed biosensor showed a high sensitivity and fast response toward the determination of PG and HQ around 0.18 V. Under the optimized conditions, the anodic peak current of PG and HQ was linear with the concentration range of 8 μM to 30×10^{-5} M and 1–150 μM. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 6.2 μM, 20 μM for PG and 0.57 μM, 1.92 μM for HQ respectively. The electrochemical impedance spectroscopy (EIS) studies have confirmed that the occurrence of electron transfer at HRP-SiSG/AgNPs/poly(L-Arg)/CPE was faster. Moreover the stability, reproducibility and repeatability of the biosensor were also studied. The proposed biosensor was successfully applied for the determination of PG and HQ in real samples and the results were found to be satisfactory.

1. Introduction

Pyrogallol (PG) and hydroquinone (HQ) are derivatives of phenolic compounds which are important contaminants in medical food and environmental matrices. Reliable analytical procedures are required for the determination of PG and HQ in various matrices with high sensitivity. So far, many methods have been developed for their determination, including liquid chromatography [1,2], synchronous fluorescence [3], chemiluminescence [4,5], spectrophotometry [6], gas chromatography/mass spectrometry [7], pH based-flow injection analysis [8], electrochemical methods [9,10], etc., However, most of the above methods have some disadvantages, such as time consuming, high cost, low sensitivity and complicate pretreatment. In recent past, more attention on the development of biosensor was made due to its advantages such as easy preparation, fast detection, low consume of time and high sensitivity [11].

Horseradish peroxidase (HRP) is an important enzyme and is always used as an electron acceptor. Among peroxidases, HRP has been one of the most widely studied enzymes in the development of enzyme based biosensor. Because of the deep embedding of the HRP-active site, which is in unfavorable orientation [12], it is a challenging task to obtain the direct electrochemistry of HRP. According to Marcus theory, the electron transfer (ET) distance is a decisive factor for the direct electrochemistry of redox enzyme, which depends on the overall distance between the redox site within the enzyme and the electrode surface, and the orientation of the enzyme on the electrode [13]. In order to prepare good biosensors, many materials such as nanoparticles, redox dyes, conducting polymers, biomolecules and ionic liquids were employed to improve the microenvironment around the enzyme to provide suitable orientation and to accelerate the electron transfer between the enzyme and the surface of the electrode [11].

Noble metal nanoparticles have been extensively used in the designing and in construction of enzyme biosensors due to their unique characteristics, such as high surface energy and surface to volume ratio, ability to decrease proteins–metal particle distance, good mechanical, thermal and chemical stability [14,15]. So far, the direct electron transfer of HRP has been reported at nanomaterial surfaces such as gold nanoparticles (AuNPs) [16], gold nanowire array electrodes [17], cadmium sulphide [CdS] nanorods [18], and carbon nanotubes (CNTs) [19,20]. The stability and sensitivity of a biosensor could be improved by choosing suitable

* Corresponding author. Tel.: +91 877 2289303.
E-mail addresses: tmsreddysvu@gmail.com, tmsreddy_svu@yahoo.co.in (T. Madhusudana Reddy).

0141-0229/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.enzmictec.2013.02.010

enzyme immobilization matrix and by adapting better enzyme immobilization strategies. Recently several valuable immobilization strategies have been employed including absorption [21], cross-linking [22], layer-by-layer assembly [23], sol–gel entrapment [24], electropolymerization [25,26], etc., Among those silica sol–gel (SiSG) entrapment technology has attracted much attention in the field of immobilization of a variety of biomolecules, because of its special features such as chemical inertness, physical rigidity, high-thermal stability, biodegradation and optical transparency [27,28]. However, these HRP-based biosensors require mediators to transfer electrons between the electrode and HRP.

In our previous work, we have developed a biosensor based on the CPE immobilized with HRP, through SiSG entrapment for the determination of hydroquinone (HQ). The electrochemical properties of biosensor were characterized by employing the electrochemical methods like cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The electrocatalytic response of HQ was detected in methanol, ethanol, 2-propoanol, 1-butanol and acetone. The good results were obtained in ethanol as a solvent and acetate buffer solution (ABS) as supporting electrolyte, the experiments were carried out in combination of these two media. The electrochemical impedance spectroscopy (EIS) result confirmed the occurrence of rapid electron transfer at HRP-SiSG/CPE. The proposed sensor was successfully applied for the determination of HQ in real samples and the result were found to be commensurate [29].

In this present work, we have concentrated on embedding HRP into the network of SiSG/AgNPs/poly(L-Arg)/CPE. This technology exhibited a remarkable advantage of AgNPs/poly(L-Arg) and SiSG network. HRP was effectively embedded into the network of AgNPs/poly(L-Arg) which in turn can promote the direct electron transfer of the enzyme immobilized on the electrode surface. The electrocatalytic behavior of this biosensor has also been investigated in detail. The resulting biosensor exhibited high sensitivity and good stability.

2. Experimental

2.1. Reagents

All chemicals were obtained from commercial sources and used without further purification. Horseradish peroxidase (E.C. 1.11.1.7 type-VI-A-S/5 mg, Amoracia rusticana source, 1840 U/mg), pyrogallol and hydroquinone were purchased from Sigma–Aldrich chemicals Co., USA. Tetraethyl orthosilicate (TEOS), cetyltrimethyl ammonium bromide (CTAB), Triton-X-100 were obtained from Sigma–Aldrich chemicals Co., USA. The graphite fine powder was procured from Lobo Chemie and silicon oil from Himedia. Silver nanoparticles (AgNPs) used in the present study were synthesized from fungal culture *Aspergillis niger* and their size (1–3 nm) and shape (spherical) were characterized according to Jaidev and Narasimha [30]. Acetate buffer solution (ABS) was prepared by mixing 0.1 M sodium acetate and 0.1 M acetic acid. All the aqueous solutions were prepared with double distilled water. The enzyme stock solution and working solutions of chemicals were stored in cool place.

2.2. Apparatus

The electrochemical measurements were conducted in a three electrodes cell at a room temperature 25 ± 2 °C. The working electrode was a enzyme immobilized carbon paste electrode (HRP-SiSG/AgNPs/poly(L-Arg)/CPE). The reference electrode was a saturated calomel electrode system and glassy carbon rod electrode was used as an auxiliary electrode. Electrochemical measurements were carried out using CH-Electrochemical Analyzer (Model CHI-660D, CH Instruments, USA).

2.3. Preparation of poly(L-arginine) modified carbon paste electrode (poly(L-Arg)/CPE)

The carbon past electrode was prepared by hand mixing 85% graphite powder and 15% silicon oil in an agate mortar. The carbon paste was then packed into the cavity of a homemade carbon paste electrode with a diameter of 2 mm and smoothed on a weighing paper [31,32]. The 0.038 M of aqueous solution of L-arginine was placed in the electrochemical cell and dipped with CPE and was scanned for eight multiple cycles between the potential ranges from −0.6 V to +1.6 V at 100 mV/s. After polymerization, the poly(L-Arg) film was rinsed sufficiently with double distilled water.

2.4. Fabrication of HRP-SiSG/AgNPs/poly(L-Arg)/CPE

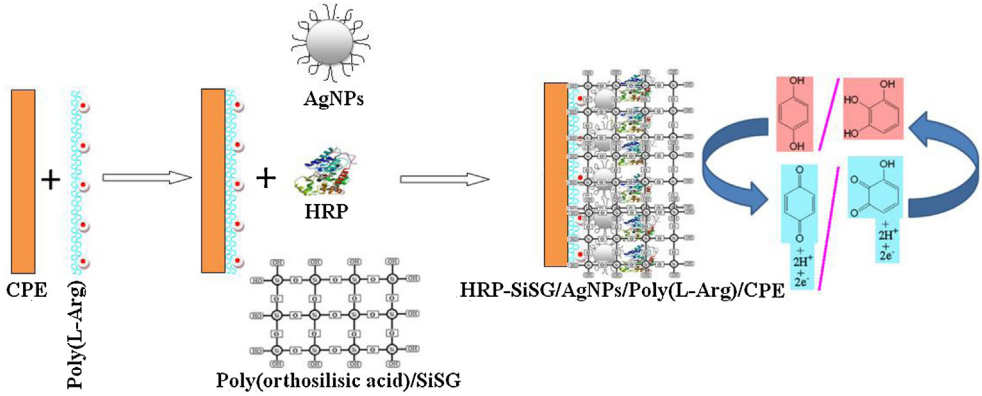
A homogenous TEOS silica sol–gel was prepared by mixing 2 ml of TEOS, 1 ml of H_2O, 50 μl of 0.1 M KCl, 25 μl of 10% Triton-X-100. The mixture was stirred for 1 h for obtaining clear sol. The sol can be stored for about one month when it was kept in refrigerator.

The 5 μl of 5 mg/ml enzyme stock solution was added to the mixture of 5 μl of stock SiSG solution, 40 μl of ABS and 10 μl of AgNPs. A drop of this dispersion with a volume of 5 μl was cast onto the surface of the poly(L-Arg)/CPE, then it was allowed to polymerize at room temperature for 3–5 min. The electrode was gently washed with ABS and was used for further experimental procedure [33]. The 2.5 U of enzyme was immobilized on the electrode surface. The fabrication procedure of the biosensor is illustrated in Scheme 1.

3. Results and discussion

3.1. Electrochemical polymerization of L-arginine on carbon paste electrode

L-Arginine is an amino acid and its electrochemical polymerization potential was between +1.6 V to −0.6 V. The potential window scan lies in the positive direction and this was the most important factor in preparing the poly(L-Arg) film. If the potential window was less than 1.6 V or greater than −0.6 V, it was observed that the formation of poly film on the CPE was not stable. On the other hand, a stable poly film was obtained by the electropolymerization between the potential windows of −0.6 V to +1.6 V and with a maximum of eight cycles on CPE. Fig. 1A shows the growth of polymer film of 0.038 M aqueous L-Arginine solution on the surface of carbon



Scheme 1. A schematic diagram showing the steps involved in the fabrication of HRP-SiSG/AgNPs/poly(L-Arg)/CPE with reaction mechanism.