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Evaluation of a reduced graphene oxide antimony nanocomposite horseradish peroxidase biosensor matrix for hydrogen peroxide

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Abstract

An exploration of reduced graphene oxide impregnated with antimony nanoparticles to form a nanocomposite as a platform for the immobilization of horseradish peroxidase for the amperometric detection of hydrogen peroxide is presented. Electrochemical measurements revealed that the heterogeneous electron transfer constant (ks) was evaluated to be 0.37 s^{-1}. The average surface concentration of electro-active HRP on the surface of the modified glassy carbon electrode was calculated to be 9.43×10^{-9} mol cm^{-2}. The voltammetric results revealed that the peak current of the modified electrode increased linearly with increasing concentration of hydrogen peroxide (from 1 nM to 4 nM) with a detection limit of 2.88 nM.

Keywords: Biosensor; Nanoparticles; Glassy carbon electrode; Horseradish peroxidase; Hydrogen peroxide

1. Introduction

Studies on the direct electrochemistry of redox enzymes are of great significance to bioelectrocatalytic synthesis and biosensor development [1]. Nanoparticles (NPs) have various unique features, which include biocompatibility, rapid and simple chemical synthesis, excellent electro-activity, and efficient coating by biomolecules. So if biosensors are contructed from NPs, it is proved to be of great benefit. A crucial step in the development of biosensors is the fast electron-transfer between the active site of the enzyme and the electrochemical transducer. Reduced graphene oxide (rGO) is rich in quinoid-like groups, which can assist electron transfer and activation of molecules in chemical reductions [2].Therefore, some quinoid structures might also facilitate enzyme-catalyzed redox processes. A number of methods have been used to immobilize the redox enzyme on the electrode surface and in the same time to preserve the enzymatic activity [3][4]. Among numerous enzymes, horseradish peroxidase (HRP), a heme-containing enzyme, is commonly chosen for amperometric biosensors due to its redox activity [5][6]. As an important heme-containing protein, HRP has been applied widely in biotransformation, organic synthesis and treatment of wastewaters [7]. However, due to the denaturalization of HRP adsorbed on the electrode surface, to study the HRP immobilized directly on a bare electrode is difficult [8]. In addition, the active site of HRP is deep inside the protein, and therefore, the ability of electrons to “escape” the confines of the enzyme to the electrode surface is restricted [9]. Hence, new materials or methodologies are required to modify electrode surfaces for HRP immobilization to obtain a more sensitive electrode. Various works have been done to improve the sensitivity of the electrode with HRP immobilized for instances, Zhang et al [10] reported a glassy carbon electrode (GCE) modified with horseradish peroxidase immobilized on partially reduced graphene oxide for detecting phenolic compounds. Nevertheless, no studies on the GCE modified with HRP that was immobilized within a reduced graphene oxide antimony nanocomposite (rGO-SbNPs) for the detection of H_2O_2 have been reported to date. In this study, it was found that the HRP/rGOSbNPs/GCE biosensor exhibits good electrocatalytic activity toward the reduction of H_2O_2, leading to a fast and low-cost method for hydrogen peroxide detection.

2. Experimental

The GCE surface was polished with alumina suspensions (1.0, 0.3, 0.05 µM), sonicated in water, ethanol and water successively, and then dried in air at room temperature. Horseradish peroxidase solution (2 mg ml^{-1}) was prepared by dissolving HRP (EC 1.11.1.7 type IV, Sigma) in phosphate buffer (0.1 M, pH 7.0). The composite of reduced graphene oxide impregnated with antimony nanoparticles was synthesized according to Silwana et al [11]. The HRP/rGOSbNPs enzyme-nanocomposite was prepared by incorporating the enzyme-sodium

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phosphate buffer (0.1 M, pH 7.0) for 1 h into the rGO-SbNPs nanocomposite through mixing and further dispersed in DMF-buffer solution. A 5 μL aliquot of this dispersion was dropped onto the glassy carbon electrode surface and left to dry overnight, then rinsed with copious amounts of phosphate buffer (pH 7.0) and ultrapure water. Voltammetric experiments were performed using an Epsilon analyser (BASi Instruments, West Lafayette, IN, USA) and performed at room temperature. All the voltammetric experiments were performed with Ag/AgCl (3 M NaCl) reference and platinum auxiliary electrodes in 0.1 M phosphate buffer (pH 7.0) solution.

3. Results and discussion

3.1. Characteristics of the nanocomposite containing HRP

The integrity of the immobilized HRP construction and its ability to exchange electrons with the nanocomposite rGOSbNPs/GCE surfaces were assessed by voltammetry. The cyclic voltammograms (CVs) of a HRP/rGOSbNPs/GCE biosensor in 0.1 M PBS (pH 7.0) containing 0.01 mM H_2O_2 at various scan rates were investigated. The redox process of the HRP/rGOSbNPs/GCE was a typical quasi-reversible electrochemical process relating a redox active compound attached to the electrode. A linear dependence of cathodic and anodic peak current versus scan rate was observed. The amount of electroactive enzyme on the electrode surface was estimated by the following equation [12]:

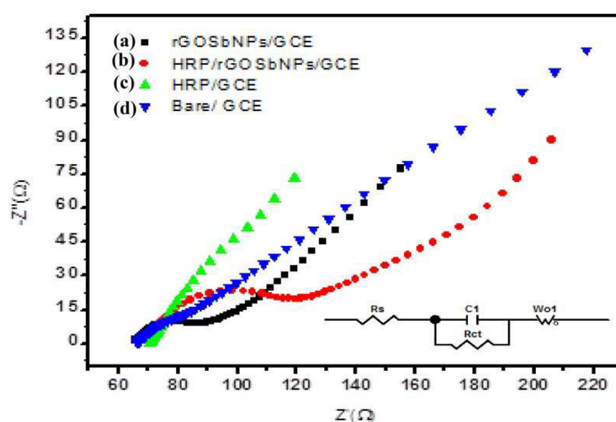
$$Ip = \frac{n^2F^2vA\Gamma *}{4RT} \qquad Eqn\ 1$$

Comparing the calculated value of the surface concentration Γ with the previous reported values, the surface concentration of the HRP/rGOSbNPs/GCE biosensor has been determined as 9.43×10^{-9} mol cm^{-2} which was higher than the HRP/GA/PANI biosensor at 7.136×10^{-9} mol cm^{-2} [13] and the HRP-PANI-ClO_4/ITO biosensor at 5.81×10^{-9} mol cm^{-2} [14]. We further studied the relationship between redox peak potentials and scan rate, and investigated the electron transfer kinetics of HRP using the Laviron model [15]. The charge-transfer coefficient (α) was estimated as 0.62. The heterogeneous electron transfer rate constant (ks) was estimated according to the following equation:

$$[logks = \propto \log(1-\propto) + (1-\propto)log \propto -log\frac{RT}{nFv} - \frac{\propto (1-\propto)nF\Delta Ep}{2.3RT} \qquad Eqn\ 2$$

The heterogeneous electron transfer rate constant (ks) of HRP in this study was calculated to be 0.37 s^{-1}, which was found to compare very well to previous studies [13][14].

The step-wise fabrication process of the biosensor was further characterized by electrochemical impedance spectroscopy (EIS) in order to investigate the electrochemical properties at the electrode surface as can be seen in Fig 1. The electron-transfer kinetics and diffusion characteristics can be determined from the shape of the impedance spectrum. The diameter of the semicircle is equal to the Rct value which is indicative of the electron transfer kinetics of the redox probe at the electrode/electrolyte interface. The electron transfer characteristics were interpreted by using the Randle's equivalent circuit [8]. The results are tabulated in Table 1.



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