



An amperometric biosensor fabricated from electro-co-deposition of sodium alginate and horseradish peroxidase

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ABSTRACT

A convenient and effective strategy for fabrication of hydrogen peroxide biosensor based on sodium alginate (SA) and polyvinyl butyral (PVB) as matrices was reported in this paper. The horseradish peroxidase (HRP) and SA were electro-co-deposited onto the surface of gold electrode, and the HRP-SA/Au electrode was further coated with PVB. The interaction between HRP and SA was characterized by UV–vis absorption spectroscopy, and the fabricating process of biosensor was characterized by electrochemical impedance spectroscopy (EIS). The electrochemical characteristics of the biosensor were studied by cyclic voltammetry and chronoamperometry. Experimental conditions were investigated which influence the performance of the biosensor, such as pH, and applied potential. The biosensor showed a linear response to H_2O_2 over a concentration range from 7.0×10^{-6} to 4.1×10^{-3} M with a detection limit of 1.8×10^{-6} M based on a signal-to-noise ratio of 3 under optimum conditions. The K_M^{app} value of HRP in the composite was evaluated to be 1.38 mM. The biosensor obtained from this study possesses high sensitivity, good reproducibility, and long-term stability.

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1. Introduction

Amperometric biosensors have attracted considerable interest in the field of clinical, biomedical analysis, chemical and pharmaceutical laboratories [1–5]. However, the exciting possibility of biosensors is usually hindered by their poor stability and short shelf life. To improve the stability and extend the shelf life of biosensor, various techniques such as cross-linking [6], sol–gel/hydrogel [7], self-assembly [8], covalent binding [9], and surfactant–enzyme complex [10,11] have been explored. Recently, nanomaterial such as carbon nanotubes [12,13], zirconium oxide [14], and nanometer-sized gold colloid particles [15] are also used for the fabrication of composite electrodes. The electro-co-deposition technology, as an effective immobilization of enzyme within a biocompatible material by using simple and controllable procedure, is of great significance and still being practiced today [16,17]. The advantages of the strategy come from the following two aspects. Firstly, electrochemical deposition process offers a simple and convenient technique for the fabrication of enzyme electrodes with minimum denaturation and strong adherence to electrode surface compared with other procedures [18,19]. Secondly, the biosensor fabrication is reproducible and the thickness of the resulting biocomposite film is controllable. However, the electro-enzymatic activity of

the immobilized enzyme depends on the chemical and physical properties of matrix employed in the immobilization process. And the leakage of enzyme from matrix in the testing surrounding is generally recognized. Consequently, more innovative approaches for enzyme immobilization have to be sought particularly for the applications in biomedical, biocatalytic, and biosensing fields [18,20].

Alginate is, being an anionic polymer with carboxyl groups, a natural and biocompatible polymer which can provide microenvironments to improve the enzyme stability or maintain its bioactivity [21–33]. Due to the possessing of carboxyl groups, alginate is a pH shift polymer. As a result, the solubility and net charge of alginate is pH-dependent. The deposition is performed based on the pH decrease at the anode owing to the decomposition of water. The electro-co-deposition film posed uniform coatings on substrates of complex shapes [34]. But no report on immobilization of biomolecules such as HRP by electro-co-deposition with alginate was found, to the best of our knowledge, in the literature.

It is worth emphasizing the two novel features in this study: First, natural biocompatible polymeric alginate was utilized as a matrix; second, the employment of electro-co-deposition technique during fabrication. In this paper, we attempt to disclose implementation of the above mentioned strategies for the successful development of an electrochemical biosensor which displays relatively high sensitivity, acceptable stability and good repeatability. The preparation method and main characteristic features were described and discussed in detail.

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2. Experimental

2.1. Reagent and materials

Horseradish peroxidase (HRP, EC 1.11.1.7, 250 U mg⁻¹) was obtained from Sigma. Sodium alginate (SA) was purchased from Shanghai Guoyao Chemical Co. Ltd., China. Polyvinyl butyral (PVB, 98%) was bought from Shanghai Chemical Reagent Co. Ltd., China. 0.1 M phosphate buffers solution (PBS) containing 0.1 M KCl with various pH values were prepared by mixing the stock solutions of K₂HPO₄ and KH₂PO₄, and adjusted by 0.1 M KOH and 0.1 M H₃PO₄ solution. Hydrogen peroxide (30%, w/v solution) was purchased from Chemical Reagent Company, Chongqing, China, and the solutions of the more diluted hydrogen peroxide were prepared freshly using a 30% hydrogen peroxide and determined by titration with potassium permanganate. All reagents were of analytical reagent grade and used as received. Double distilled water was used in this experiment throughout.

2.2. Electrode modification procedure

A bulk gold disk electrode ($d = 4$ mm) was carefully polished with 1.0, 0.3 and 0.05 μ m alumina slurry, and ultrasonically cleaned in ethanol and double distilled water before modification. The gold electrode was then chemically cleaned by repeatedly scanning, at the potential range of -0.3 to 1.5 V, in a 0.5 M H₂SO₄ solution until the voltammograms were reproducible. HRP–SA/Au electrode was achieved by cycling at the potential between -1.0 and 1.0 V for 25 consecutive cycles in 5 ml sodium alginate (2%, w/v) doped with 2 mg/ml HRP (PBS, pH 6.0). Then, the HRP–SA/Au electrode was dipped into PVB ethanol solution (2%, w/v) to a depth of 10 mm for 10 min.

2.3. Apparatus

Cyclic voltammetry (CV) and chronoamperometry (CA) were carried out on AUTOLAB system with PGSTAT12 (Eco Chemie B.V., Utrecht, Netherlands). The electrochemical cell consists of a three-electrode system, bare or modified gold electrodes were employed as working electrode, platinum wire as auxiliary electrode and saturated calomel as reference electrode (SCE). Electrochemical impedance spectroscopy (EIS) was performed with a Model IM6e (ZAHNER Elektrik, Germany). UV–vis absorption spectra were recorded in the range of 250–800 nm using a Lambda 17 UV–vis 8500 spectrometer (PE Co, USA) with a quartz cell (path length 1 cm). All potentials were measured and reported versus the SCE and all experiments were carried out at room temperature.

3. Results and discussion

3.1. Fabrication of the H₂O₂ biosensor

The possibility of deposition of alginate and composite coatings for surface modification of materials opens new opportunities in the fabrication of advanced biomedical implants [34]. In the work, an electrochemical sensing platform was developed based on the integration of natural polysaccharide alginate and enzyme (HRP). Alginate is an anionic polymer with carboxyl groups and ideally suited for electrodeposition since its net charge and solubility are pH dependent. Electro-co-deposition was used for one-step construction of H₂O₂ biosensors by local formation of SA–HRP bio-composite film on the surface of electrode (Fig. 1). The results of deposition of alginate doped HRP suggested that the alginate composite film with HRP were successfully fabricated on the surface of gold electrode. The proposed mechanism of gel formation is

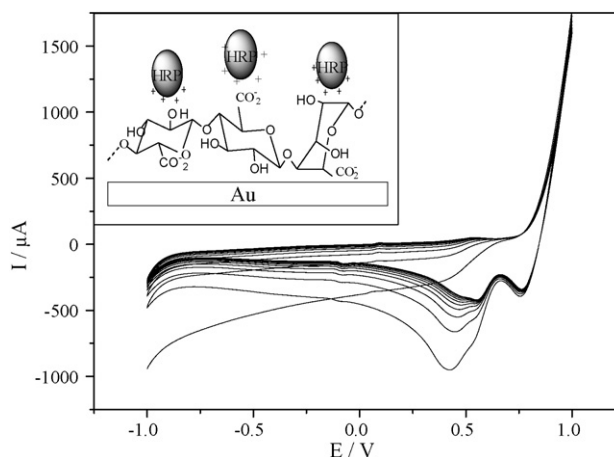


Fig. 1. The cyclic voltammograms of HRP–SA composites progressive course on bare Au electrode between -1.0 and 1.0 V (vs. SCE) at a scan rate of 100 mV/s for 25 consecutive scans. Insert: illustration of the electro-co-deposition process of modified electrode.

based on the pH decrease at the anode owing to the electrochemical decomposition of water [35].



The dissociation of sodium alginate (Na–SA) results in the formation of anionic SA⁻ species.



It is suggested that SA⁻ species formed alginic acid (H–SA) gel in the low pH region around the electrode.



Obtained bulk gel film incorporated with HRP is locally electro-co-deposited on the anode surface (gold electrode). Then, the coating of PVB was to prevent the leakage of HRP out of HRP–SA composite. After storing for about 24 h at 4 °C, the PVB/HRP–SA/Au electrode was ready for testing.

3.2. UV studies of the interaction between HRP and SA

UV–vis absorbance spectroscopy was employed to characterize the conformational change of protein and the interaction between protein and other compositions [36]. UV–vis spectra of SA, HRP and HRP–SA composites were presented in Fig. 2. The adsorption peak of HRP is 403 nm (Fig. 2b), while no shift is observed when HRP is immobilized in the HRP–SA composites (Fig. 2c). After stored in a refrigerator at 4 °C for 7 days, the absorption peak of HRP–SA composites still remain at 403 nm (Fig. 2c2), but shifted to 405 nm (Fig. 2c3) 1 month later. This indicates that the interaction between HRP and SA does not destroy the conformational structure of HRP. Instead, SA may provide a microenvironment for HRP to maintain its native structure and bioactivity as reported by others [31,32].

3.3. Electrochemical impedance spectroscopy (EIS) of the modifying process

EIS is one of the most effective electro-analytical techniques used in studying the interfacial properties of modified electrodes. EIS is particularly useful for understanding the chemical transformations and processes associated with the conductive supports [37,38]. The typical impedance spectrum (presented in the form of the Nyquist plot) includes a semicircle portion at higher frequencies corresponding to the electron-transfer-limited process and a linear

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