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Direct electrochemistry of horseradish peroxidase immobilized on the layered calcium carbonate–gold nanoparticles inorganic hybrid composite

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ABSTRACT

A mediator-free hydrogen peroxide (H_2O_2) biosensor was fabricated based on immobilization of horseradish peroxidase (HRP) on layered calcium carbonate–gold nanoparticles ($CaCO_3$–AuNPs) inorganic hybrid composite. The proposed biosensor showed a strong electrocatalytic activity toward the reduction of H_2O_2, which could be attributed to the favored orientation of HRP in the well-confined surface as well as the high electrical conductivity of the resulting $CaCO_3$–AuNPs inorganic hybrid composite. The hybrid composite was obtained by the adsorption of AuNPs onto the surfaces of layered $CaCO_3$ through electrostatic interaction. The key analytical parameters relative to the biosensor performance such as pH and applied potential were optimized. The developed biosensor also exhibited a fast amperometric response (3 s), a good linear response toward H_2O_2 over a wide range of concentration from 5.0×10^{-7} to 5.2×10^{-3} M, and a low detection limit of 1.0×10^{-7} M. The facile, inexpensive and reliable sensing platform based on layered $CaCO_3$–AuNPs inorganic hybrid composite should hold a huge potential for the fabrication of more other biosensors.

1. Introduction

Direct electron transfer between the redox enzymes and the electrode surface is a goal for which researchers have been pursuing in recent years (Topoglidis et al., 2003; Gooding et al., 2003; Liu et al., 2005a,b) because it can establish a desirable and ideal model for the fundamental study of the redox behavior of the enzymes in biological systems, which may help to elucidate the relationship between their structures and biological functions (Feng et al., 2005). To achieve this propose, much effort has been devoted to designing biocompatible matrix to retain the native structure of redox enzymes (Feng et al., 2005), synthesizing new layered or mesopore structured materials to increase the amount of immobilized redox enzymes (Liu et al., 2005a,b; Cai et al., 2006), or using different nanoparticles to serve as tiny conduction centers to facilitate electron transfer, such as gold nanoparticles (AuNPs) (Cai et al., 2006), titanate nanotubes (Gooding et al., 2003), carbon nanotubes (Liu et al., 2005a,b, 2008), and silica nanoparticles (Luo et al., 2007).

Recently, AuNPs have received much attention for the immobilization of enzymes owing to their good biocompatibility and nano-scaled dimension effect (Daniel and Astruc, 2004). AuNPs can also provide a suitable microenvironment similar to that of enzymes in native systems and allow a favorable orientation of immobilized enzymes for the effective electron transfer, which are essentially important for the achievement of direct electrochemistry of redox enzymes (Feng et al., 2005). AuNPs are often combined with other biocompatible materials such as polymer matrix, sol–gel matrix, and inorganic micro/nanomaterials for enzyme loading. A distinct synergic effect in the electrochemistry of enzyme is often expected compared with a single one. Among them, calcium carbonate ($CaCO_3$) could be deemed as such a good candidate due to its porous surface structure, good biocompatibility, and large surface area (Rosu et al., 1998). Usually, spherical $CaCO_3$ is used in the fabrication of different biosensors (Cai et al., 2006; Wang et al., 2007; Sun et al., 2007), however, layered $CaCO_3$ is rarely reported (Watabe, 1965; Nakahara et al., 1982; Kato et al., 2002). Layered $CaCO_3$ has a high mechanical strength and large surface area, also can provide a friendly environment for enzymes to retain their activity. Natural layered $CaCO_3$, such as nacre of abalone, could be formed via a biomineralization process in the presence of living organisms (Kato et al., 2002), however, the biomineralization process is complex and hard to control. In contrast, in the present paper, a simple method was adopted to prepare layered $CaCO_3$. Furthermore, a novel inorganic hybrid composite material was obtained with the adsorption of AuNPs onto the surface of layered $CaCO_3$ via electrostatic interaction.

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In order to explore the distinct advantages of current special inorganic hybrid composite in enzyme adsorption and bioactivity, horseradish peroxidase (HRP) was chosen as a model and the direct electron transfer ability of HRP to the electrode surface was investigated. A mediator-free HRP based H_2O_2 biosensor was further constructed by immobilizing HRP on the layered $CaCO_3$–AuNPs inorganic hybrid composite modified Au electrode. Owing to the synergic effect of layered $CaCO_3$ and AuNPs, the prepared biosensor toward H_2O_2 shows good stability, a very wide linear calibration, and a low detection limit. The current fabricated biosensor with the use of layered $CaCO_3$–AuNPs inorganic hybrid composite could be easily extended for more applications in other electrochemical biosensors.

2. Experimental

2.1. Reagents

HRP (EC 1.11.1.7, RZ > 3.0, 250 U mg^{-1}) was obtained from Boao Biotechnology Co. Ltd., China. Hydrogen peroxide (H_2O_2) solutions were prepared freshly using a 30% H_2O_2 solution (Nanjing Chemical Reagent Factory, China), and the concentration of H_2O_2 solutions was determined by titration with $KMnO_4$. Calcium chloride ($CaCl_2$) and sodium carbonate (Na_2CO_3) were purchased from Tianjin Bodi Chemical Co. Ltd., China. $HAuCl_4 \cdot 4H_2O$ was purchased from Sigma. Tween-80 was purchased from Tianjin Kermel Chemical Reagent Co. Ltd. The 0.1 M phosphate buffer solutions (PBS) containing 0.1 M KCl at various pH values were prepared by mixing the stock solutions of NaH_2PO_4 and Na_2HPO_4, and then adjusting the pH with 0.10 M H_3PO_4 or NaOH. Doubly distilled water (DDW) was used throughout this work.

2.2. Apparatus and instrumentations

Amperometric and cyclic voltammetric (CV) experiments were performed with a CHI 832B electrochemical analyzer (Shanghai CH Instrument Company, China). Electrochemical impedance spectroscopy (EIS) was performed on a CHI660C electrochemical analyzer (Shanghai CH Instrument Company, China). A conventional three-electrode system was used with bare gold (Au) electrode or modified Au electrode as the working electrode, Ag/AgCl electrode (sat. KCl) as the reference electrode and platinum wire electrode as auxiliary electrode, respectively. Scanning electron microscopy (SEM) images were obtained at 5.0 kV on a SIRION (FEI, USA) field emission scanning electron microscope.

2.3. Preparation of AuNPs and the layered $CaCO_3$ microparticles

All glassware used in the following procedures was cleaned in a bath of freshly prepared solution of HNO_3–HCl (3:1, v/v), rinsed thoroughly in DDW and dried in air. AuNPs were prepared by the conventional citrate reduction of $HAuCl_4$ in aqueous solution according to the literature (Doron et al., 1995). In brief, sodium citrate solution was added to a boiling $HAuCl_4$ solution with a molar ratio of $HAuCl_4$ to sodium citrate of 0.26.

10 mL Tween-80 was dissolved in 20 mL 0.05 M $CaCl_2$ solution under stirring to achieve a homogeneous solution, into which an equal volume of 0.05 M Na_2CO_3 solution was rapidly poured at room temperature under ultrasonication (Li and Li, 2007). 10 s later, the precipitated layered $CaCO_3$ was collected by centrifugation and washed three times with DDW.

2.4. Preparation of the layered $CaCO_3$–AuNPs inorganic hybrid composite

0.5 g layered $CaCO_3$ was dispersed in 50 mL Au colloid solution and sonicated for 2 min. The AuNPs–$CaCO_3$ inorganic hybrid composite was obtained with a color of light purple after centrifugation. The hybrid composite was further washed with DDW and dried (Cai et al., 2006).

2.5. Preparation of the enzyme modified electrode

Au electrode was used as the base electrode for the fabrication of the enzyme biosensor. Before modification, bare Au electrode was immersed in freshly prepared piranha solution (H_2SO_4/H_2O_2, 7:3, v/v) for 30 min. Then, it was carefully polished with 1.0, 0.3, and 0.05 μm α-Al_2O_3 slurry, respectively. The obtained electrode was rinsed with DDW, followed by sonicating in ethanol and DDW alternatively. Finally, the electrode was left to dry at room temperature.

The assembly of 4-aminothiophenol (ATP) monolayer was performed by direct immersion of the clean bare Au electrode into ethanol solution containing 0.5 mM ATP for 8 h. After that, the obtained electrode was washed carefully with 0.1 M PBS (pH 7.0), and then 10 μL of layered $CaCO_3$–AuNPs hybrid composite suspension was deposited on the surface of ATP modified Au electrode. 4 h later, the modified electrode was washed thoroughly with PBS (pH 7.0). Subsequently, the resulting electrode was incubated in 3 mg/mL HRP for 8 h to allow HRP molecules loading onto the surface of the layered AuNPs–$CaCO_3$ modified electrode, and the enzyme electrode was denoted as HRP/$CaCO_3$–AuNPs/ATP/Au. All the resulted electrodes were washed with PBS (pH 7.0) and stored at 4 °C when not in use.

2.6. Characterization of the developed biosensor

CV experiments were carried out in quiescent solutions at a scan rate of 50 mV s^{-1}. Amperometric experiments were carried out in a stirred system by applying a potential of −0.2 V to the working electrode. Typical steady-state response of the biosensor to successive injection of H_2O_2 was recorded after a steady-state current had been achieved. EIS experiments were performed in 1.0 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ (1:1) mixture containing 0.1 M KCl with the frequency ranging from 10^4 to 10^{-1} Hz.

3. Results and discussion

3.1. Immobilization of HRP on layered $CaCO_3$–AuNPs inorganic hybrid composite

In present investigation, a mediator-free H_2O_2 biosensor was fabricated based on immobilization of HRP on layered $CaCO_3$–AuNPs inorganic hybrid composite modified Au electrode. Schematic illustration of the fabrication process is given in Fig. 1. With the isoelectric point (IEP) at pH 8.5 (Volodkin et al., 2004), the layered $CaCO_3$ were positively charged at pH 7.0, while AuNPs were negatively charged at the same pH. As a result, AuNPs were adsorbed on the surface of layered $CaCO_3$ through electrostatic interaction (Cai et al., 2006).

Self-assembling technique has been proved as a promising method for the construction of interface with specific functionality and controllability (Song et al., 2008). The ATP was firstly self-assembled on the bare Au electrode surface via sulfur–Au interaction (Jiao et al., 2005; Zhang et al., 2003). The exterior amine group on the monolayer could be further used to attach layered $CaCO_3$–AuNPs composite on the electrode surface through Au–NH_2 interaction (Ferapontova, 2004). Then, the HRP, its IEP of 8.8, could