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Amperometric hydrogen peroxide biosensor based on the immobilization of HRP on DNA–silver nanohybrids and PDDA-protected gold nanoparticles

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ABSTRACT

A novel amperometric hydrogen peroxide biosensor based on the immobilization of horseradish peroxidase (HRP) on DNA–silver nanohybrids (DNA–Ag) and poly(diallyldimethylammonium chloride) (PDDA)-protected gold nanoparticles (PDDA–Au) was successfully fabricated by combining the self-assembly technique with an in situ electrochemical reduction of the DNA–Ag^+ complex. The preparation process of modified electrode was characterized with UV–vis absorption spectroscopy, transmission electron microscopy (TEM) and atomic force microscope (AFM). The electrochemical characteristics of the biosensor were studied by cyclic voltammetry and chronoamperometry. Experimental conditions influencing the biosensor performance such as pH, potential were optimized. The resulting biosensor (HRP/DNA–Ag/PDDA–Au/DNA–Ag/Au electrode) showed a linear response to H_2O_2 over a concentration range from 7.0 μM to 7.8 mM with a detection limit of 2.0 μM (S/N = 3) under optimized conditions. The apparent Michaelis–Menten constant (K_M^{app}) was evaluated to be 1.3 mM. The sensor exhibited high sensitivity, good accuracy and an acceptable stability.

1. Introduction

Highly sensitive and selective determination of H_2O_2 is of great importance in food, clinical, biological, environmental and many other fields. Many analytical methods have been developed for this purpose, such as titrimetry, photometry and electrochemistry. Among these methods, amperometric enzyme-based biosensors have received considerable attention due to its convenience, high sensitivity and selectivity [1,2]. However, a significant challenge to development of sensitive and stable sensors comes from the effective immobilization of enzyme to solid electrode surface [3]. Up to now, many materials have been used to immobilize enzyme on the surface of electrodes, such as quantum dots [4], polymers [5,6], mesoporous materials [7] and nanomaterials [8–10]. Among these materials, nanomaterials, especially functionalized nanocomposites have attracted great research interest in biosensor because of their versatility of the physical and chemical properties and other properties [11]. For example, SiO_2 functionalized by 3-aminopropyltrimethoxysilane was used in the glucose biosensor [12]. Zhang et al. immobilized tyrosinase in inorganic–organic hybrid titanium-oxo-polymers nanocomposite [13]. Ma et al. fabricated a polyaniline–TiO_2 composite film with in situ polymerization approach to detect trimethylamine at room temperature [14]. However, the conductivity is not very good because they are semiconductor nanocomposites, which resulted in the low sensitivity and the low accuracy in some degree, thereby they are limited in the biosensing application. Recently, more and more conducting metal nanoparticles modified by other materials were synthesized and applied widely in diverse areas. Tan et al. reported thiosalicylic acid-functionalized Ag nanoparticles which was synthesized in one-phase system [15]. Liu and Jiang used Nafion and PDDA–Pt nanoparticles synthesized by the alcoholic reduction of the Pt ions in ethanol/water system to construct polymer electrolyte fuel cells [16]. Wohltjen and Snow first reported the use of an octanethiol-coated Au nanoparticle material as a thin film on a chemiresistor device [17]. Hang and Chang proposed a novel colorimetric method for mercury (II) in aqueous solutions using mercaptopropionic acid-modified Au nanoparticles in presence of 2,6-pyridinedicarboxylic acid as a more stable complex compound with heavy metal ions than other metal ions [18]. To our best knowledge, although many applications of conducting metal nanoparticles modified by other materials have been reported, very few studies have been carried out for construction of hydrogen peroxide biosensor by taking advantage of the merits of composite materials which combine together the different properties of components and lead the way to a new, tunable behavior [19]. So, in this paper, we fabricated a hydrogen peroxide biosensor formed with DNA–Ag and PDDA–Au to entrap horseradish peroxidase (HRP).

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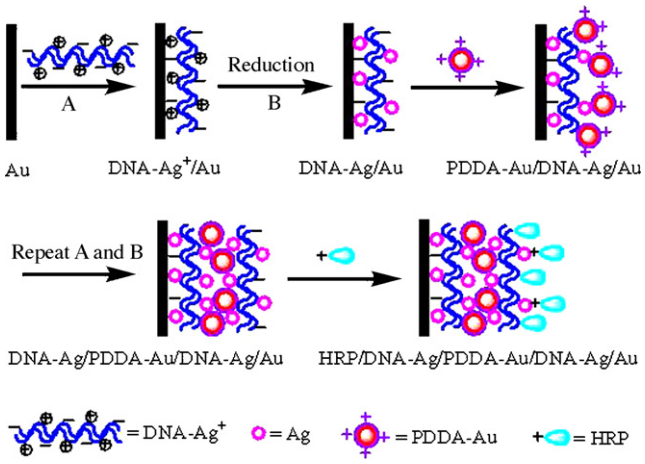

Scheme 1. Illustration of the preparation process of modified electrode.

First, we electrochemically reduced the DNA–Ag^+ complex to obtain negatively charged immobilization matrix (DNA–Ag) to immobilize PDDA–Au. Here, immobilization of PDDA–Au was attributed to the two forces. The one is electrostatic force between positively charged PDDA–Au and the negatively charged DNA. The other is the adsorption of the nano-Ag to the PDDA–Au. Then, the second layer of DNA–Ag was assembled onto the modified electrode based on the electrostatic force and excellent film-forming ability of DNA. Finally, the positively charged HRP was adsorbed tightly onto the DNA–Ag layer just as the adsorption of PDDA–Au to obtain hydrogen peroxide biosensor without losing their biological activity (see Scheme 1).

This strategy has following advantages. First, Au and Ag nanoparticles not only possess larger specific surface area, good biocompatibility, but also possess good conductivity. They can make possible conducting channels to facilitate charge transfer between the prosthetic groups and the electrode surface [20]. Secondly, PDDA can act as the reducing and stabilizing agents to fabricate the PDDA-protected Au nanoparticles simultaneously [21]. Furthermore, PDDA is a positively charged ionic polymer, and the PDDA-protected Au nanoparticles can be effectively self-assembled onto the negatively charged DNA–Ag membrane by electrostatic interaction. DNA, a robust negatively charged biopolymer with excellent film-forming and adhesion ability, biocompatibility and the efficient electroconductivity [22], is widely recognized as an ideal candidate for enzyme immobilization. Finally, compared with the other methods, our proposed strategy in this paper is not necessary to introduce any other noxious material or energy to the system, so it is very good to avoid the contamination and deactivation of the enzyme [20] and obtain a hydrogen peroxide biosensor which showed high sensitivity, an acceptable stability and good repeatability.

2. Experimental

2.1. Reagents and apparatus

HRP, gold chloride ($HAuCl_4$), poly(diallyldimethylammonium chloride) (PDDA, MW 200,000–350,000) and double-stranded calf thymus DNA were all obtained from Sigma Chemical Co. Silver nitrate ($AgNO_3$, AR) and potassium nitrate (KNO_3, AR) were purchased from Beijing Chemical Co. (China). Hydrogen peroxide (H_2O_2, 30%, w/v solution) was purchased from Chemical Reagent Co. (Chongqing, China). The concentration of the more diluted hydrogen peroxide solutions prepared from 30% hydrogen peroxide was determined by titration with potassium permanganate. All other chemicals employed were of analytical grade and were used without further purification. All solutions were made up with doubly distilled water.

Electrochemical measurements were performed on CHI660A electrochemical workstation (CH Instruments, Chenhua Co., Shanghai, China). A conventional three-electrode system was employed with a modified Au disk electrode (φ = 4.0 mm) as a working electrode, a platinum wire as an auxiliary electrode, and a saturated calomel electrode (SCE) as a reference electrode. All the potentials given in this paper were referred to the SCE. The size of PDDA–Au was estimated from transmission electron microscopy (TEM, TECNAI 10, Philips Fei Co., Holland). UV–vis absorption spectra were recorded in the range of 250–700 nm using a Lambda 17 UV–vis 8500 spectrometer (PE Co., USA) with a quartz cell (path length 1 cm) at room temperature. The morphologies of the bare Au and the substrate modified with different materials were investigated with atomic force microscope (AFM, Vecco, USA).

2.2. Preparation of PDDA–Au nanoparticles

PDDA–Au nanoparticles were synthesized according to Ref. [21] with a little change. Briefly, 250 μL PDDA solution of certain concentration, 40 mL water, 200 μL 0.5 M NaOH and 100 μL $HAuCl_4$ (1%) were added into a beaker. After thoroughly mixed for a few minute, the mixed solution continued heating until the color of the solution changed to red and no further color change occurred. The UV–vis spectrum and TEM were used to confirm and characterize the production. Fig. 1 shows UV–vis spectra of PDDA solution (curve a) and PDDA–Au solution (curve b). When the solution just only contained PDDA, no obvious absorption peak can be observed. But when the solution contained the production, a maximum adsorption in UV–vis spectrum was observed at 524 nm, which is a typical plasmon absorbance of spherical Au nanoparticles [21–23]. It is proved that PDDA–Au nanoparticles have been successfully synthesized. In order to further confirm and characterize the size and size distribution of PDDA–Au nanoparticles, Fig. 2 shows typical TEM image of the PDDA–Au nanoparticles. As shown in Fig. 2, all of the PDDA–Au nanoparticles are almost spherical, evenly distributed without obvious aggregation and the average diameter is about 15 nm. This is ascribed to the surrounding PDDA polymers, which could limit particle aggregation and thus yielded uniform, isolated, small particles.

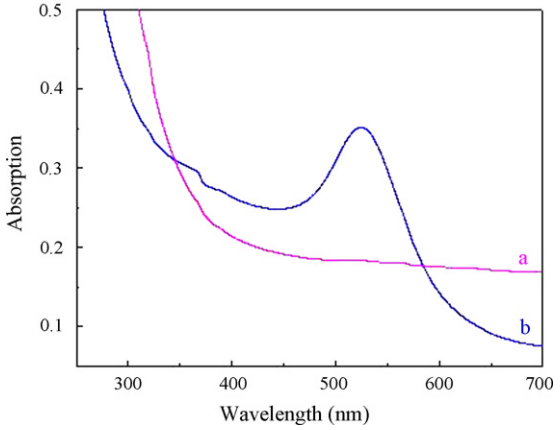

Fig. 1. UV–vis absorption spectrum of PDDA solution (a) and PDDA–Au solution (b).