An electrochemical biosensor constructed by nanosized silver particles doped sol–gel film

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Abstract

The nanosized silver particles are used to dope into the sol–gel film to prepare a biosensor. The horseradish peroxidase (HRP), mediator methylene blue (MB), nanosized silver particles and sol–gel solution are mixed and coated on the surface of glass carbon (GC) electrode to get the biosensor. The silver nanoparticles in the sol–gel film can adsorb the enzyme molecules and improve the sol–gel film conductivity. The biosensor has a high sensitivity, quickly response to H2O2 and good stability. The biosensor responds to H2O2 in the linear range from 1 μM to 1 mM. The detection limit was down to 0.4 μM when the signal to noise ratio is 3. The apparent Michaelis-Menten constant of the biosensor to H2O2 was estimated to be 1.2 mM, showing a high affinity.

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1. Introduction

The sol–gel process, a method for the production of ceramic materials that was first reported some 150 years ago, is gaining renewed interest because it provides a convenient way to embed active proteins to prepare the biosensor [1–3]. Some new methods and new composite sol–gel were studied to improve the character. The sol–gel film modified with gold nanoparticles has been proved that it has new properties [4,5].

Metal nanoparticles have good interaction with the protein. Specific antibody–antigen coupling has also been used to organize gold and silver nanoparticles into extended three-dimensional networks [6]. The protein can be used to protect the Ag nanoparticle surface from rapid expansion of supercritical solution into aqueous solution [7]. The determination of hydrogen peroxide is of great relevance, ascribable to both the facts that it is the product of the reactions catalyzed by a large number of oxidase enzymes and that it is essential in food, pharmaceutical, and environmental analysis. The electrochemical analysis offers improved sensitivity, extended dynamic range and rapid response time. The direct electrochemical detection of H2O2 often requires relatively high overpotentials. However, hydrogen peroxide can be detected enzymatically at low applied potentials by employing peroxidase as bioelectrocatalysts for its electrochemical reduction. Because the redox center of the peroxidase is inner, the electron transfer is difficult between the peroxidase and the electrode surface. The different mediators were often chosen to facilitate electron transfer [8].

In this paper, the silver nanoparticles is doped into the sol–gel network to prepare composite material, then HRP, MB and the composite material are commixed and dropped on the surface of glassy carbon electrode to prepare the biosensor. The biosensor responds to the H2O2 sensitively and stability.

2. Experimental

2.1. Reagents

Horseradish peroxidase (HRP) was obtained from Sigma. H2O2 (30% w/v solution) were purchased from Shanghai...
Chemical Reagent Company. The concentration of the more diluted hydrogen peroxide solutions prepared was determined by titration with cerium (IV) to a ferroin endpoint. Silver nanoparticles were prepared according to the reference [9]. All other chemicals were of analytical grade. All the solutions were prepared with doubly distilled water.

2.2. Apparatus

CHI660 Electrochemistry workstation (CHI) was used for electrochemical measurements. A three-electrode system incorporating this H$_2$O$_2$ biosensor as the working electrode, a saturated calomel reference electrode (SCE) and a platinum wire counter electrode were used for the measurements. A magnetic stirrer and a stirring bar provided the convective transport for the amperometric experiments.

UV spectra were obtained in the range of 300—600 nm on a type BRAIC 1200 UV instrument (Beijing, China) with quartz cuvette (path length 1 cm) at room temperature. The images for transmission electron micrographs (TEM) were obtained by using a JEOL-JEM 200CX electron microscope.

2.3. Preparation of the biosensor

The GC electrode with a diameter 4 mm was polished in turn with 1.0, 0.3, and 0.05 µm aluminium oxide, rinsed thoroughly with deionized water, sonicated in deionized water and ethanol, and dried in air. The sol solution is prepared according to the reference [10]. The sol solution, the Ag colloid, 0.1 mM MB, and 2 mg/ml HRP solution were mixed in proportion and dropped on the electrode surface. In order to get a thin and uniform sol–gel film, the spin coating technique was used to prepare the biosensor.

3. Result and discussion

3.1. The TEM characterization

The Ag nanoparticles is prepared as the reference and the size and shape are studied by the TEM. The size of materials is about 40 nm and the shapes are particle or square according to the Fig. 1.

3.2. UV studies of the interaction between the Ag and HRP

Because the surface properties of the metal nanoparticle are always represented the plasma absorption in the UV–Vis range, the UV method can be used to study the interaction between the HRP and Ag nanoparticles. The results are showed in Fig. 2. The absorption peak of the Ag nanoparticles is about 411 nm. The absorption band is broader and shifts to 406 nm when the HRP molecules are added in the Ag nanoparticles solution. The surface charge is negative on the surface on the Ag nanoparticle. The HRP molecule is positive according to the reference. The interaction between the Ag nanoparticle and HRP molecule changes the surface properties and the plasma absorption changes from 411 to 406 nm.

3.3. Electrochemical characterization of the biosensor

Typical cyclic voltammograms of the biosensor in 0.1 M phosphate buffer solution (pH 7.0) at different rates are shown in Fig. 3. The $E_{1/2}$ shifted negatively for 25 mV in comparison with that of the redox process of free MB in the buffer solution, and the peak currents of the MB-HRP/NME are proportional to the scan rates up to 200 mV/s, indicating the surface redox reaction of the MB-HRP/NME. Furthermore, no essential decrease of the peak current was found, after the potential swept cyclically from0.6 to 0.1 V at a scan rate of 10 mV/s for 100 cycles. It is indicated that the MB molecules are stable in the sol–gel structure.

3.3.1. The electrochemical response to H$_2$O$_2$

The cyclic voltammograms of resulting electrode in the absence and presence of H$_2$O$_2$ are shown in Fig. 4. A significant increase of the cathodic peak currents was generated in 1 mM H$_2$O$_2$ solution. A little peak-potential
shift toward negative direction with the increase of H\textsubscript{2}O\textsubscript{2} concentration was observed. The reaction mechanism of the biosensor is same with the reference. The MB in the sol–gel film can be recycled at the electrode as the mediator leading to an increase of its reduction current.

3.4. Optimization of H\textsubscript{2}O\textsubscript{2} monitoring

In order to determine the optimal working potential for the H\textsubscript{2}O\textsubscript{2} sensing, we research the electrochemical response of the H\textsubscript{2}O\textsubscript{2} in different potentials. The result is showed in Fig. 5. The steady-state current increased with an increase of cathodic potential, and when the potential reached $-0.3$ V, the electroreduction of H\textsubscript{2}O\textsubscript{2} hardly changed. So the $-0.3$ V was chosen as working potential.

3.5. Detection of H\textsubscript{2}O\textsubscript{2}

Fig. 6 shows the dynamic response of the biosensor at a working potential of $-0.3$ V with successive injections of H\textsubscript{2}O\textsubscript{2}. The current curve is typical Michaelis-Menten response according with the enzyme-substrate kinetics. The curve clearly demonstrates the fast response and high sensitivity of the sensor to H\textsubscript{2}O\textsubscript{2}. The linear relation between the current and concentration is showed in Fig. 7. The biosensor responds to H\textsubscript{2}O\textsubscript{2} in the linear range from 1 μM to 1 mM. The detection limit was down to 0.4 μM when the signal to noise ratio is 3. The relative standard deviation...
is 4.2% for six repetitive measurements of 0.1 mM H₂O₂ solution. The sensitivity of the biosensor is higher than that reported in reference without Ag nanoparticle [11,12]. The current response decreased about 10% in 1 week, and 18% in 2 weeks. It shows that the biosensor has a good stability. The sol–gel film is made of hydrolysis SiO₂, the SiO₂ semiconductor on the electrode can block the electron transfer. The Ag nanoparticles in the sol–gel film not only improve the conductivity, but also adsorb the enzyme molecules to keep the stability of the biosensor. So the biosensor has higher sensitivity than the traditional sol–gel biosensor.

The apparent Michaelis-Menten constant \( K_{M}^{\text{app}} \), which gives an indication of the enzyme-substrate kinetics, can be calculated from the Lineweaver-Burk equation [13]. The value of the biosensor was found to be 0.15 mM. The presence of the Ag nanoparticles resulted in a higher affinity to H₂O₂.

4. Conclusion

In this paper we have introduced a novel biosensor based on the combination of sol–gel and Ag nanoparticle properties. The Ag nanoparticle in the sol–gel film have two advantages to improve the character of the biosensor. One is that the metallic properties of the Ag nanoparticle can enhance the electron conductivity of sol–gel film, the other can adsorb the protein by the interaction between the enzyme and Ag nanoparticle. The biosensor exhibited higher sensitivity than the traditional sol–gel biosensor.

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