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Fabrication of a novel hydrogen peroxide biosensor based on the AuNPs–C@SiO_2 composite

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ABSTRACT

The composite of C@SiO_2 with gold nanoparticles (AuNPs–C@SiO_2) was fabricated by layer-by-layer assembly technique and was used to fabricate a novel hydrogen peroxide (H_2O_2) biosensor. The composite was composed of a colloidal carbon sphere core with an average diameter of 200 nm and a porous silica shell with a uniform thickness of about 50 nm, and decorated with gold nanoparticles on the surface. The porous silica shell could promote the composite to show high bio-compatibility and chemical stability. The AuNPs–C@SiO_2 composite combined with hemoglobin (Hb) was used to construct a novel biosensor for the determination of H_2O_2, displaying a wide linear range from 5.0 to 80 μM with a detection limit of 0.08 μM at 3σ. The K_m^{app} value for the biosensor was determined to be 71.49 μM.

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1. Introduction

Along with the development of nanoscience, more and more new bio-compatible and electro-conductive materials have been synthesized and used in electrochemical bioassay [1]. Carbon materials are widely used due to their high conductivity [2–4]. Monodisperse silica as the most attractive colloids played a prominent role in research [5], especially in bioanalysis due to its excellent bio-compatibility [6]. However, compared with other conductive materials, the low electro-conductivity of silica limits its applications. Coating silica on carbon sphere could combine the advantages both good electro-conductivity and good bio-compatibility together and the surface area could be enlarged as well. The existence of the SiO_2 shell could keep carbon spheres monodisperse and control the interparticle spacing and interactions [7]. Furthermore, the special structure of C@SiO_2 was also helpful to increase the bio-compatible of carbon sphere dramatically, while keeping the advantages of its original characteristics, such as high conductivity and chemical stability. Besides, the porous silica shell enlarged the functional surface, which could make them suitable to immobilize more proteins. After the C@SiO_2 was assembled with gold nanoparticles, the electro-conductivity and good bio-compatible could be further increased. However, to the best of our knowledge, no work has been reported on the synthesis of the AuNPs–C@SiO_2 composite and the direct electron transfer of immobilized proteins on its interfaces.

Herein, an AuNPs–C@SiO_2 composite was fabricated by layer-by-layer method and was further used to fabricate a novel hydrogen peroxide biosensor. The composite could exhibit improved physical and chemical properties over their single-component counterparts, making them attractive in both scientific and technological viewpoints. The biosensor combined AuNPs–C@SiO_2 and hemoglobin (Hb) was fabricated and could be used to determine H_2O_2 (Scheme 1). The direct electrochemistry of Hb also was investigated. It displayed good performance for the detection of hydrogen peroxide with a wide linear range and a low detection limit. Moreover, the resulting biosensor exhibited fast amperometric response, good stability and reproducibility.

2. Experimental

2.1. Reagent and apparatus

Bovine heart Hb and Poly (diallyldimethylammonium chloride) (PDDA, 20%) were purchased from Sigma Co. Hydrogen peroxide (30% (w/v)), and tetraethyl orthosilicate (TEOS) were obtained from Shanghai Chemical Reagent Co. (Shanghai, China). Five milligram per milliliter Hb was prepared in pH 5.0, 0.1 M phosphate-buffered solution (PBS). All other chemicals were of analytical grade and used as received.

Electrochemical experiments were performed in a conventional three-electrode system in the CHI660a workstation (Shanghai Chenhua, China), using a platinum wire as the auxiliary electrode, a saturated calomel electrode (SCE) as the reference and the modified glass carbon electrode (GCE, Φ = 3 mm) as the working

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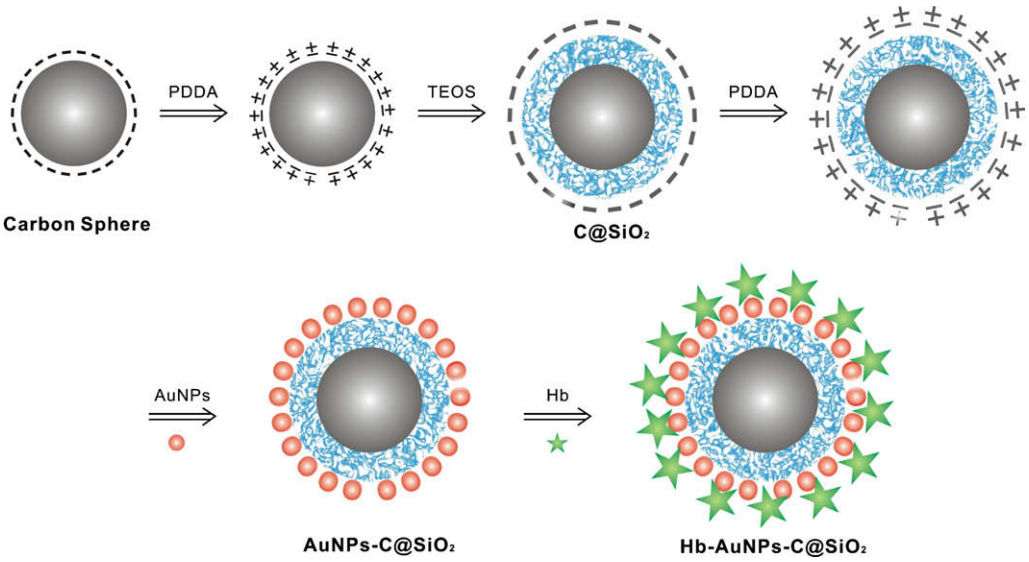

Scheme 1. Procedure for preparation of Hb–AuNPs–C@SiO_2.

electrode. All solutions were deoxygenated by highly pure nitrogen before and during the measurements.

2.2. Synthesis of AuNPs–C@SiO_2 composite

Colloidal carbon spheres and Au colloid were prepared as described in our previous work [8]. The synthesis of C@SiO_2 was as follows: first, colloidal carbon spheres were assembled with PDDA, then were dispersed in 25 ml water followed by adjusting pH to ca. 11 after adding ammonia (ca. 28 wt%). Subsequently, 5 ml of 40 mM TEOS ethanol solution was injected at a rate of 2 ml per hour under stirring and the resulting solution was further allowed to react for 24 h. The C@SiO_2 spheres were positive charged by the same way, and then spreaded in 30 ml Au colloid solution and stirred for 20 min. After centrifugation, the dark purple AuNPs–C@SiO_2 composite was obtained and the supernatant liquor was colorless.

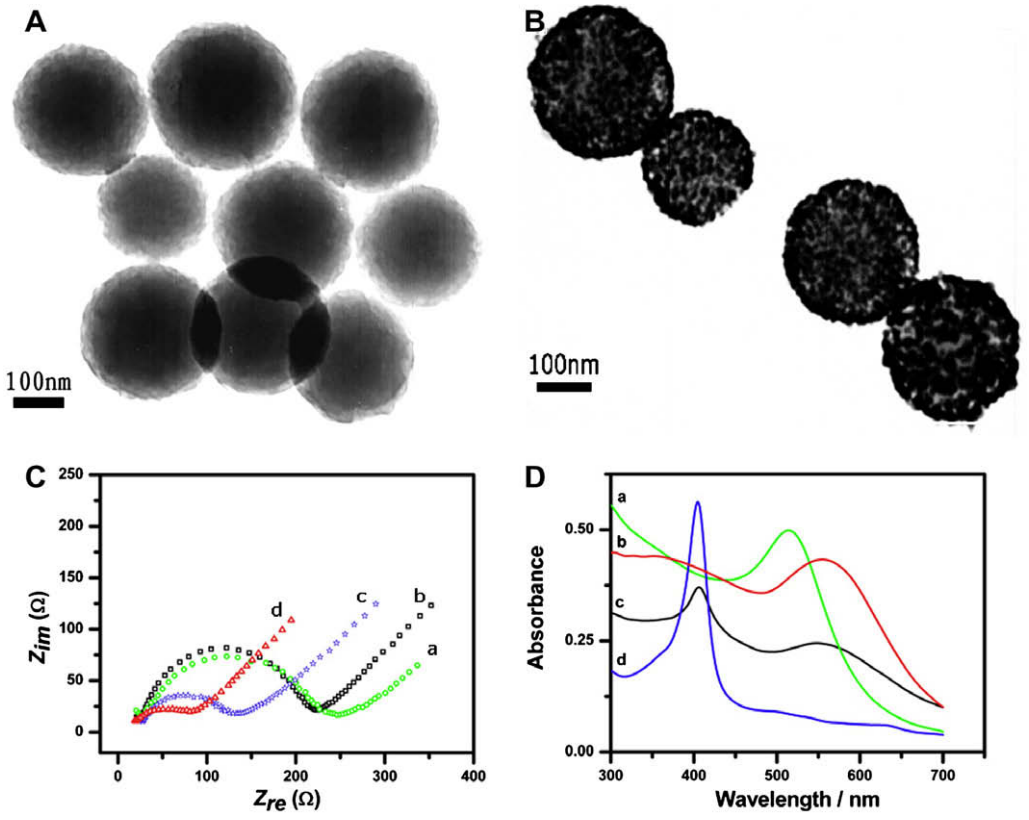

Fig. 1. (A) TEM images of C@SiO_2, (B) AuNPs–C@SiO_2 microsphere, (C) Nyquist plots of modified GCE recorded in solution containing 0.1 M KCl and 2 mM $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$: (a) C/GCE, (b) C@$SiO_2$/GCE, (c) AuNPs–C/GCE, (d) AuNPs–C@SiO_2/GCE, (D) UV–Vis spectra of (a) Au, and (b) AuNPs–C@SiO_2, (c) Hb–AuNPs–C@SiO_2, (d) Hb in 0.1 M pH 5.0 PBS.

2.3. Hb immobilization and construction biosensors

For the combination of Hb, 2 ml Hb was mixed with 2 ml AuNPs–C@SiO_2 (≈1.4 wt%). Then the mixture was equilibrated for 24 h at room temperature and centrifuged. The obtained Hb–AuNPs–C@SiO_2 composite was used for further test.

GCE was polished with 1.0, 0.3, and 0.05 μm alumina powder successively, followed by successive sonication in acetone and water and dried at room temperature. The Hb–AuNPs–C@SiO_2 composite above was resuspended in 1 ml water, and 10 μl of the suspension was dropped onto the electrode surface and then dried in desiccator. Finally, the electrode was stored at 4 °C.

3. Results and discussion

3.1. Characteristics of C@SiO_2, AuNPs–C@SiO_2 and Hb–AuNPs–C@SiO_2

Fig. 1A is the TEM image of C@SiO_2 microspheres. The surface of C@SiO_2 microsphere has a porous shell compared with original carbon sphere, and the average diameter of the microsphere is increased from 200 to 250 nm, both of which indicate that the SiO_2 is coated outside the carbon sphere. After adsorbing the PDDA, the surface of C@SiO_2 was positive charged, which ensured the efficient adsorption of negatively charged AuNPs by means of electrostatic interaction. Fig. 1B shows the rough structure of AuNPs–C@SiO_2 composite. It can be seen that the numerous individual dark nanodots spread around the grey nanosphere.

Fig. 1C shows the electrochemical impedance spectroscopy (EIS) spectra observed upon the changes of surface-modified process. Generally, the semicircle portion at higher frequencies corresponds to the electron transfer limited process, and the semicircle diameter equals to the electron transfer resistance, R_{et} [9]. Compared with that of original carbon sphere modified GCE (curve a), the R_{et} of C@SiO_2-modified GCE (curve b) is almost unchanged because the porous structure outside does not inhibit the electronic transfer. Compared with curve b and c, the R_{et} (curve d) decreased dramatically, showing that much more gold nanoparticles were adsorbed successfully owing to the enlarged surface area of porous silica layer.

UV–Vis absorption was also used to monitor the modification process. In Fig. 1D, the AuNPs (curve a) shows a strong transverse plasmon band at 513 nm. After the AuNPs was adsorbed by C@SiO_2, the absorption peak (curve b) changes to 532 nm and became wider than pure AuNPs. This red shift as well as the broadening of the bands may be due to the aggregation of AuNPs on the surface of C@SiO_2. Upon further immobilization of Hb, it has a characteristic Soret absorption [10] at 405 nm (curve c), as same as that of native Hb (curve d). It indicates Hb entrapped in the AuNPs–C@SiO_2 composite has a similar structure to the native Hb.

3.2. Direct electrochemistry of Hb–AuNPs–C@SiO_2

The electrochemical behavior of the Hb–AuNPs–C@SiO_2-modified electrode was studied in 0.1 M PBS (pH 5.0) at 100 mV s^{-1}. Fig. 2A shows the cyclic voltammograms obtained from different modified electrodes. No current peak is observed at AuNPs–C@SiO_2-modified electrode (curve a), indicating the nonelectroactive property of AuNPs–C@SiO_2. After combining with Hb (curve b), a pair of small but stable and well redox peaks appears which shows the electron transfer between Hb and the underlying electrode. The anodic and cathodic peak potentials are located at −0.220 and −0.294 V (vs. SCE) respectively. In Fig. 2A (curve c), with the addition of H_2O_2, the reduction peak current is greatly enhanced which also indicates that Hb is successfully immobilized and the heme groups in Hb molecules still retain their structure and electrochemical activity.

The dependence of the peak currents on the scan rate is illustrated in Fig. 2B. The electrode modified with the Hb–AuNPs–C@SiO_2 composite showed a pair of well-defined redox peaks, and peak currents (i_p) increased with the increase of scan rate. As shown in Fig. 2B (inset), the cathodic and anodic peak currents increase linearly with the scan rate from 20 to 300 mV s^{-1}. Thus, the Hb adsorbed on the surface undergoes a reversible and surface-controlled electron transfer. According to Faraday's law ($Q = nFA\Gamma$), where Q is the total amount of charge, n the number of electron transferred, F Faraday's constant, and A the electron area, the average Γ values of electroactive Hb can be estimated to be 8.8×10^{-11} mol cm^{-2}, which is larger than the theoretical monolayer coverage of Hb (≈1.89×10^{-11} mol cm^{-2}). This indicates that a multilayer of proteins participated in the electron transfer process in the composite.

3.3. The determination of hydrogen peroxide

Fig. 3A depicts the cyclic voltammograms obtained at a GCE modified with Hb–AuNPs–C@SiO_2 in PBS (pH 5.0) containing varied concentrations of H_2O_2. The reduction peak at approximately −0.30 V is greatly enhanced, while the anodic peak decreases, suggesting an electro catalytic reduction of H_2O_2 occurred. Moreover, the reduction current increases dramatically with the increasing concentration of H_2O_2, and the anodic peak leads to the gradual disappearance simultaneously. However, this phenomenon is not observed at AuNPs–C@SiO_2-modified GCE; therefore, the catalytic

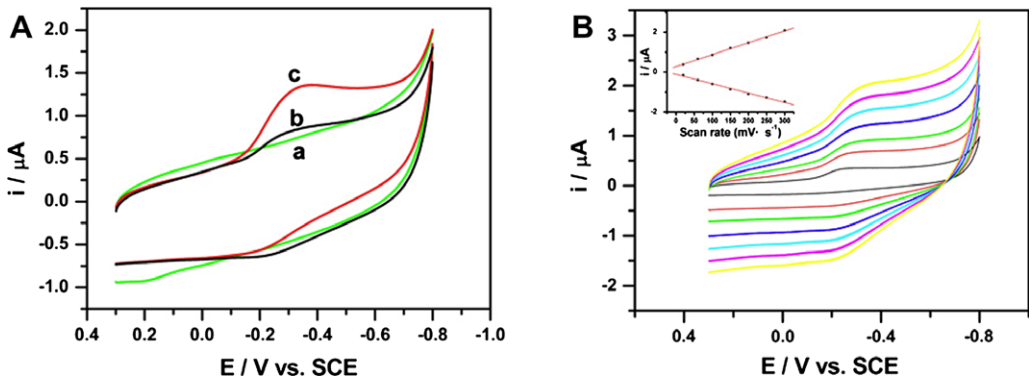

Fig. 2. (A) Cyclic voltammograms of AuNPs–C@SiO_2/GCE (a), Hb–AuNPs–C@SiO_2/GCE (b), Hb–AuNPs–C@SiO_2 with 10 μM H_2O_2 (c) in 0.1 M pH 5.0 PBS solution. (B) Cyclic voltammograms of Hb–AuNPs–C@SiO_2-modified GCE in PBS (0.1 M, pH 5.0) at different scan rates (from inner to outer curve: 20, 60, 100, 150, 200, 250, 300 mV s^{-1}), and (inset) plots of cathodic and anodic peak currents vs. scan rates.

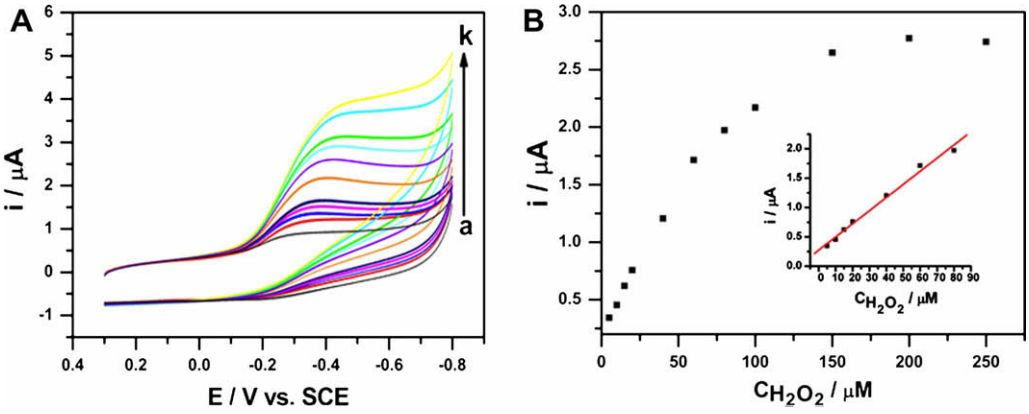

Fig. 3. (A) Cyclic voltammograms of the Hb–AuNPs–C@SiO_2/GCE at scan rate of 0.1 Vs^{-1} in 0.1 M pH 5.0 PBS solution with (a) 0, (b) 5.0, (c) 10.0, (d) 15.0, (e) 20.0, (f) 40.0, (g) 60.0, (h) 80.0, (i) 100.0, (j)150.0, and (k) 200.0 μM H_2O_2. (B) Plots of the electrocatalytic current (*i*) vs. H_2O_2 concentration. Inset: linear plots of *i* vs. H_2O_2 concentration.

reduction of H_2O_2 is only due to the presence of Hb. The mechanisms can be expressed as the following [11]:

$$Hb(Fe^{3+}) + H_2O_2 \rightarrow Compound\,I(Fe^{4+}{=}O) + H_2O$$

$$Compound\,I(Fe^{4+}{=}O) + e + H^+ \rightarrow Compound\,II$$

$$Compound\,II + e + H^+ \rightarrow Hb(Fe^{3+}) + H_2O$$

The reductive i_p has a linear relationship with H_2O_2 concentration from 5 to 80 μM (Fig. 3B (inset)), while at the concentration of 200 μM, the CV response tends to saturate (Fig. 3B). The linear regression equation is $y = 0.02242x + 0.27336$ μA, with a correlation coefficient of 0.99492. From the slope of 0.024 μA μM^{-1}, the detection limit is estimated to be 0.08 μM at 3σ [12,13].

When the concentration of H_2O_2 is greater than 200 μM, a response plateau is observed, showing a typical Michaelis–Menten kinetic mechanism. The apparent Michaelis–Menten constant (K_m^{app}) is calculated by Lineweaver–Burk equation [14] to evaluate the catalytic activity of intercalated protein and compare this sensor with others:

$$1/I_{SS} = 1/I_{max} + K_m^{app}/I_{max}C$$

Here I_{ss} is the steady-state current, C the concentration of substrate, K_m^{app} the apparent Michaelis–Menten constant, and I_{max} the maximum current measured under the saturated substrate condition. The K_m^{app} value for the Hb–AuNPs–C@SiO_2-modified electrode is determined to be 71.49 μM, which is much smaller than those reported previously [15,16] , suggesting a higher affinity to H_2O_2 and a higher enzymatic activity to H_2O_2 reduction for the intercalation of Hb into AuNPs–C@SiO_2.

Additional experiments were carried out to test the stability. The modified electrode was stored in phosphate buffer solution at pH 5.0 in the refrigerator at 4 °C for a week and no obvious change was found. The biosensor retained 96% of its original response after a month when it was investigated by CVs in the presence of 20 μM H_2O_2.

4. Conclusion

The synthesized AuNPs–C@SiO_2 composite was used to fabricate a novel biosensor for the determination of H_2O_2. The electrochemical results showed a fast direct electron transfer of Hb. Additionally, the immobilized Hb retained its native conformation and showed the good electrocatalytic effect to the reduction of H_2O_2. Therefore it could provide a novel and promising platform for the study of the construction of biosensor.

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