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Electrochemistry Communications 10 (2008) 355–358
electrochemistry communications
www.elsevier.com/locate/elecom

Synthesis, characterizations of silica-coated gold nanorods and its applications in electroanalysis of hemoglobin

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Received 28 November 2007; received in revised form 12 December 2007; accepted 12 December 2007
Available online 23 December 2007

Abstract

Gold nanorods (GNRs) were synthesized by a seed–mediated growth approach followed by TEOS polymerization leading to the formation of silica layer surrounding the gold nanorod core. TEM images showed that the silica-coated gold nanorods (GNRs@SiO_2) were dispersed with an average aspect ratio of 3.1 for the GNRs cores and a uniform thickness of the silica shell. The core/shell nanocomposites were further used as efficient supports for the immobilization of hemoglobin (Hb) to fabricate a novel biosensor. The immobilized Hb showed an enhanced electron transfer for its heme Fe(III) to Fe(II) redox couple. This biosensor showed an excellent bioelectrocatalytic activity towards H_2O_2 with a linear range from 8.0×10^{-7} to 6.1×10^{-5} M, and the detection limit was 6.0×10^{-8} M at 3σ. The apparent Michaelis–Menten constant of the immobilized hemoglobin was calculated to be 0.13 mM.

Keywords: Gold nanorods; Silica-coated gold nanorods; Hemoglobin; Biosensor

1. Introduction

Gold nanorods (GNRs) are important metal nanomaterial with distinctive shape-dependent optical properties. Especially, they possess two distinct plasmon bands, one associated with the transverse mode and the other with the longitudinal modes. These properties suggest several advantages of GNRs for the applications in biological sensing [1–4], imaging [5], and therapy [6]. However, there are three problems that limit the GNRs application in biosensing. One is that the large amount of cetyltrimethylammonium bromide (CTAB) in GNRs solution could interfere with biological processes and show high cytotoxicity [7]. The other is that the CTAB on the surface of GNRs is difficult to displace with biomolecules [8]. The last one is that the removal of CTAB may also result in uncontrollable aggregation of GNRs [9]. Great efforts have been made to reduce cytotoxicity of GNRs and stabilization in biocompatible conditions. Niidome and co-workers developed a technique to replace CTAB with phospholipids (PC) by extraction using a chloroform phase [10]. The resulting PC-modified GNRs showed acceptable stabilization and low cytotoxicity in comparison with twice-centrifuged GNRs. Sönnichsen and co-workers reported a general strategy to stabilize GNRs suspensions with mono- and bifunctional polyethylene glycol (PEG) and to attach a controlled number of nanoparticles or biomolecules [11]. Ma and co-workers presented an improved Stöber method for preparing silica-coated anisotropic gold nanorods [12,13]. These $Au_{rod}@SiO_2$ nanostructures have a pure-silica surface, which could be further used for the colorimetric biosensing [13]. However, to the best of our knowledge, no work has been reported on the studies of direct electron transfer of the immobilized proteins at GNRs@SiO_2 composite interfaces.

Herein, GNRs were synthesized by a seed–mediated growth approach followed by TEOS polymerization leading to the formation of silica layer surrounding the gold

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doi:10.1016/j.elecom.2007.12.017

nanorod core. The core/shell composites were further used as efficient supports to immobilize Hb. The immobilized Hb showed an enhanced electron transfer for its heme Fe(III) to Fe(II) redox couple. Moreover, on the GNRs@SiO_2 composites, Hb retained its bioactivity and displayed a high affinity to H_2O_2, producing a novel H_2O_2 sensor for a quick measurement of H_2O_2 down to 6.0×10^{-8} M.

2. Experimental

2.1. Reagents and apparatus

Hb and CTAB were purchased from Sigma Co. Hydrogen peroxide (30% (w/v)), $HAuCl_4 \cdot 4H_2O$ and tetraethyl orthosilicate (TEOS) were from Shanghai Chemical Reagent Co. (Shanghai, China). All other chemicals were of analytical grade and used without further purification.

Electrochemical measurements were performed using a CHI660a workstation (Shanghai Chenhua, China). All electrochemical experiments were performed with a conventional three-electrode system, using a platinum wire as the auxiliary, a saturated calomel electrode as the reference and the modified glass carbon electrode (GCE) as the working electrode. Electrolyte solutions were deoxygenated before and during measurements.

2.2. Synthesis of GNRs and GNRs@SiO_2 nanostructures

GNRs were prepared according to the seed growth method described by Pileni [14]. The synthesis of the GNRs@SiO_2 was as follows: First, the as-prepared GNRs (25 mL) were transferred into a 50 mL beaker followed by adjusting pH to ca. 10 after the addition of ca. 28 wt% ammonia. Subsequently, 5 mL of 10 mM TEOS ethanol solution was added into the beaker at a rate of 2 mL per hour under stirring, and the resulting solution was further allowed to react for 24 h. The GNRs@SiO_2 were collected by centrifugation at 5000 rpm for 15 min and further washed with water.

2.3. Preparation of Hb–GNRs@SiO_2 bioconjugates

For the fabrication of Hb–GNRs@SiO_2 bioconjugates, 20 mg of GNRs@SiO_2 composites were dispersed in 2 mL of pH 7.0 phosphate buffer solution (PBS) containing 4 mg of Hb and shaken at room temperature overnight, followed by centrifugation and washing.

2.4. Construction of the biosensors

The GCE with 3 mm in diameter was polished with 1.0, 0.3 and 0.05 μm α-alumina powder, and sonicated in acetone and water successively. The Hb–GNRs@SiO_2 bioconjugates obtained above were resuspended in 0.5 mL of water, and 5 μL of this suspension was dropped onto the electrode and dried in a silica gel desiccator. After 30 min, this coating process was repeated. Finally, the electrode was left to dry at 4 °C overnight. The GNRs-modified GCE, GNRs@SiO_2-modified GCE and Hb–GNRs-modified GCE were prepared in the same way except that GNRs, GNRs@SiO_2 or Hb–GNRs was used instead of the bioconjugates. The modified electrode was stored under the same conditions when not used.

3. Results and discussion

3.1. Characterization of GNRs@SiO_2 and Hb–GNRs@SiO_2 bioconjugates

Amorphous silica nanoparticles have been proven to be compatible with the immobilization of biomolecules such as proteins via physical adsorption or electrostatic interaction without preventing their biological activity [15]. Here, well-defined GNRs@SiO_2 was synthesized by using an improved Stöber method as described in experimental Section 2.2. Fig. 1A is the TEM image of the GNRs@SiO_2 composite. It can be seen that the GNRs cores have an average aspect ratio of 3.1 with length of ca. 30.7 nm and width of ca. 9.8 nm, and the amorphous silica shell have a thickness of ca. 6.8 nm.

UV–vis spectroscopy was used to monitor the assembly process. As shown in Fig. 1B, the as-prepared GNRs (curve c) showed a weak transverse plasmon band at 508 nm and a strong longitudinal plasmon band at 744 nm. After coating of silica, the adsorption peaks (curve b) changed to 512 nm and 746 nm, respectively. This slightly red shift may be due to the formation of the silica shell, which increases the local refractive index of the medium surrounding the GNRs. When further immobilization of Hb, the bioconjugate of Hb–GNRs@SiO_2 (curve a) showed a characteristic Soret absorption band at 407 nm, same as that of native Hb (curve d). This indicated that Hb retained its native structure after adsorption on the GNRs@SiO_2 composite. It should be noted that the Hb–GNRs@SiO_2 also showed two peaks at 768 nm and 517 nm corresponding to the longitudinal and transverse plasmons, respectively. The shift as well as broadening of the SPR bands may be attributed to the aggregation of the GNRs@SiO_2 composites after bioconjugation.

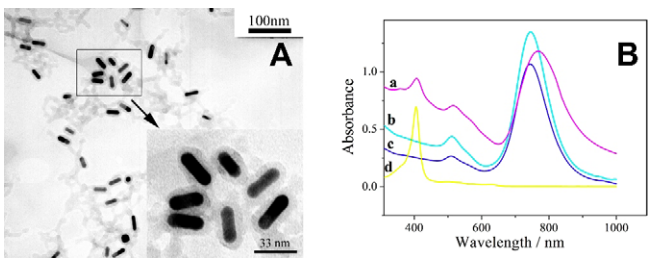

Fig. 1. (A) TEM image of GNRs@SiO_2 composites. (B) UV–visible spectra of (a) Hb–GNRs@SiO_2, (b) GNRs@SiO_2, (c) GNRs, and (d) Hb in 0.1 M pH 7.0 PBS.

3.2. Direct electrochemistry of Hb–GNRs@SiO_2 -modified electrode

The electrochemical behavior of the immobilized Hb was studied with cyclic voltammetry. Fig. 2A showed typical cyclic votammograms of different modified electrodes. A pair of stable and well-defined redox peaks of Hb for the Hb(Fe(III))/Hb(Fe(II)) redox couple transformation, which could be ascribed to the electron transfer between the hemoglobin and the underlying electrode, were observed at the Hb–GNRs@SiO_2-modified GCE (a). The anodic and cathodic peak potentials were located at −0.201 and −0.308 V (vs. SCE), respectively. In contrast, the redox peaks observed at the Hb–GNRs-modified GCE (b) were much smaller. Moreover, no redox peak was observed for the CVs of GNRs-modified GCE (c) and GNRs@SiO_2-modified GCE (d), indicating the electro-inactiveness of GNRs and GNRs@SiO_2 composite. The direct electron transfer of Hb could be achieved through immobilization on the GNRs@SiO_2 composite. It revealed that the GNRs@SiO_2 composite provided a biocompatible microenvironment for the protein to retain its native structure and the GNRs played an important role in facilitating the direct electron transfer of Hb.

The dependence of the peak currents (i_p) on the scan rate (v) was investigated. As shown in Fig. 2B, both cathodic and anodic peak currents increased linearly with scan rates from 20 to 300 mV s^{-1}, which is characteristic of thin-layer electrochemistry [16]. According to Faraday's law, $Q = nFA\Gamma^*$ where F is Faraday constant, Q can be obtained by integrating the reduction peak of Hb, n and A stand for the number of electron transferred and the area of the electrode surface, respectively, the surface concentration of electroactive Hb (Γ^*) at Hb–GNRs@SiO_2-modified GCE was estimated to be 4.8×10^{-11} mol cm^{-2}, which is larger than the theoretical monolayer coverage of Hb (ca. 1.89×10^{-11} mol cm^{-2}). The value obtained in experiments showed that a multilayer of proteins participated in the electron-transfer process in the three-dimensional composite.

An estimation of the rate constant (Ks) has been made from the peak potential separation value using the relation given for thin-layer voltammetry. A value of 0.83 s^{-1} has been obtained, which was much larger than Hb/Au colloid/cysteamine-modified gold electrode [17]. This fast electron transfer rate indicated that GNRs@SiO_2 composite could make a suitable microenvironment for Hb to undergo facile electron transfer reaction.

3.3. Electrocatalysis of Hb–GNRs@SiO_2-modified electrode to reduction of H_2O_2

The electrocatalytic reactivity of Hb–GNRs@SiO_2-modified electrode toward H_2O_2 was investigated by CV. Fig. 3A shows the cyclic voltammograms obtained for the H_2O_2 biosensor in PBS (pH 7.0) containing varied concentration of H_2O_2. The cathodic peak (∼−0.35 V) was greatly enhanced in the presence of H_2O_2, while the corresponding anodic peak decreased, suggesting that an electrocatalytic reduction of H_2O_2 occurred. Furthermore, the reduction peak current increased with the increase of H_2O_2 concentration. This phenomenon was not observed at a GNRs-modified electrode or a GNRs@SiO_2-modified electrode; therefore, the catalytic reduction of H_2O_2 was due to the presence of Hb.

The electrocatalytic curve (i_{cat}) of the Hb–GNRs@SiO_2-modified electrode to H_2O_2 concentration obtained with cyclic voltammetry was shown in Fig. 3B. Here the i_{cat} value is defined as the difference between i_{pc} in the presence of H_2O_2 and i_{pc} in the absence of H_2O_2 for the enzyme electrode. The i_{cat} values are linear with increasing concentration of H_2O_2 in the range of 0.8–61.0 μM (Fig. 3B (inset A)). The linear regression equation was $y = 0.018x + 0.037$ μA, with a correlation coefficient of 0.998. From the slope of 0.018 μA μM^{-1}, the detection limit was estimated to be 6.0×10^{-8} M at 3σ.

The apparent Michaelis–Menten constant (K_{Mapp}) provides an indication of the enzyme-substrate kinetics and a way to compare this H_2O_2 sensor with others. It can be calculated from the electrochemical version of the

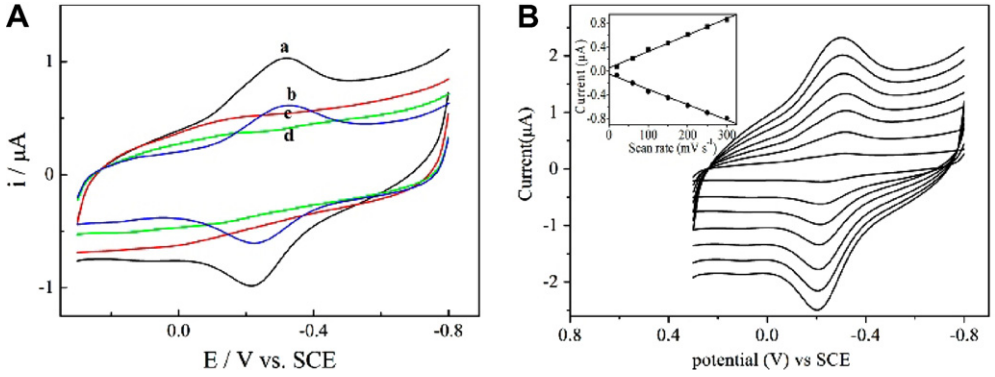

Fig. 2. (A) Cyclic voltammograms of (a) Hb–GNRs@SiO_2/GCE, (b) Hb–GNRs/GCE, (c) GNRs/GCE, and (d) GNRs@SiO_2/GCE in 0.1 M pH 7.0 PBS at 0.1 V s^{-1}. (B) Cyclic voltammograms of Hb–GNRs@SiO_2-modified GCE at scan rate of 20, 60, 100, 150, 200, 250, and 300 mV s^{-1} in 0.1 M pH 7.0 PBS, respectively and (inset) plots of peak currents vs. scan rates.

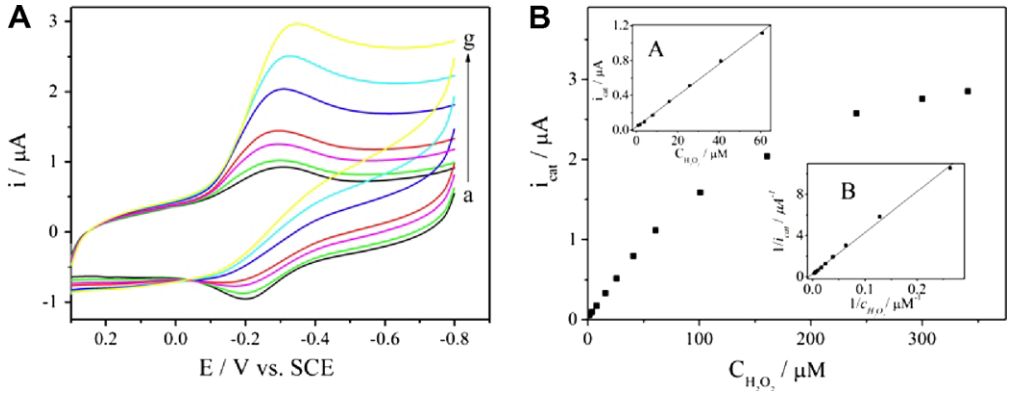

Fig. 3. (A) Cyclic voltammograms of the Hb–GNRs@SiO_2/GCE at scan rate of 0.1 V s^{-1} in 0.1 M pH 7.0 PBS solution with (a) 0, (b) 3.8, (c) 15.8, (d) 25.8, (e) 60.8, (f) 100.8 and (g) 160.8 μM H_2O_2. (B) Plots of the electrocatalytic current (I_{cat}) vs. H_2O_2 concentration. Inset: Linear plots of I_{cat} vs. H_2O_2 concentration (A) and the Lineweaver–Burk plot (B).

Lineweaver–Burk equation. The K_M value for the Hb–GNRs@SiO_2-modified electrode was determined to be 0.13 mM, which was smaller than that of 2.87 mM for Hb encapsulated in mesoporous silicas [18], 1.9 mM for Hb incorporated SP Sephadex [19] and 0.898 mM for a Hb/sol–gel film modified carbon paste electrode [20]. Therefore, the present study clearly shows that GNRs@SiO_2 composite is an excellent matrix for Hb to exhibit efficient direct electron transfer and higher catalytic activity to H_2O_2.

The relative standard deviation (R.S.D.) is 3.7% for six successive measurements at 20 μM hydrogen peroxide, showing the proposed biosensor possesses a good reproducibility. The stability of the Hb–GNRs@SiO_2-modified electrode was investigated by CVs in the presence of 20 μM H_2O_2. The electrode is tested every month. When not in use, it was stored in the refrigerator at 4 °C. The biosensor retained 94% of its original response after one month storage. This is comparable or better than the performance of some other Hb based H_2O_2 biosensors. The good long-term stability can be attributed to the efficient biocompatibility and stability of the hybrid material.

4. Conclusions

In this paper, GNRs@SiO_2 nanostructures with a pure and clean silica surface were successfully synthesized using an improved Stöber method. The solubility and biocompatibility of GNRs@SiO_2 were further used for Hb immobilization and biosensor fabrication. This biosensor showed a fast direct electron transfer of Hb. Additionally, the immobilized Hb displayed a high stability and excellent catalytic activity to hydrogen peroxide. Thus, this hybrid material provides a novel and promising platform for the study of electron transfer of proteins and the development of biosensors.

Acknowledgments

The work was supported by the National Natural Science Foundation of China for Distinguished Young Scholars (20325516), the Key Program (20635020), Creative Research Group (20521503), General Program (20605011, 20575026) and Jiangsu Scientific Foundation (BK2006114).

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