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Colloids and Surfaces B: Biointerfaces 41 (2005) 299–304
COLLOIDS AND SURFACES B
www.elsevier.com/locate/colsurfb

The fabrication of a colloidal gold–carbon nanotubes composite film on a gold electrode and its application for the determination of cytochrome *c*

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Received 3 September 2004; accepted 1 December 2004

Abstract

Colloid Au (Au_{nano}) with a diameter of about 20 nm was prepared and used in combination with the multi-wall carbon nanotubes (MWNTs) to modify a gold electrode. Dihexadecylphosphate (DHP) dispersed in Au_{nano} aqueous solution was used to solubilize MWNTs. Deposition of Au_{nano} on MWNTs was realized as illustrated by TEM micrographs. The DHP formed a network that connected Au_{nano} and MWNTs to the gold electrode surface. The Au_{nano}–MWNTs–DHP composite film on the gold electrode surface was characterized by electrochemical impedance spectroscopy (EIS) and cyclic voltammmetry (CV). The composite film modified gold electrode was used to detect cytochrome *c* and a pair of well-defined redox waves was obtained. It was found that the composite film promoted the redox of horse heart cytochrome *c* and its effect was developed for the determination of cytochrome *c*.

Keywords: Cytochrome *c*; Colloidal gold; Carbon nanotubes; Dihexadecylphosphate

1. Introduction

Au colloids display unusual physical and chemical properties because of their unique size and shape. They are widely used as catalysts for many chemical reactions because of their high catalytic activities. Electrochemical behavior and applications of Au colloids in an electrochemical field have witnessed a significant growth in the past few years [1–7]. In particular, Au colloids possess biocompatibility and proteins bound to colloidal Au electrostatically can retain biological activity [8]. It has been demonstrated that several enzymes could maintain their enzymatic and electrochemical activity when immobilized on colloidal Au [9,10]. The immobilization of a redox protein on Au_{nano} can help the protein to keep a favored orientation or to make possible conducting channels between the prosthetic groups and the electrode surface, thus reducing the effective electron transfer distance and facilitating electron transfer between electrode and enzyme [11].

Carbon nanotubes, consisting of cylindrical graphite sheets with nanometer diameter, combine in unique properties of high electrical conductivity, high chemical stability and extremely high mechanical strength and modulus. Their subtle electronic properties suggest that carbon nanotubes have the ability to promote electron transfer reactions when used as electrode materials in electrochemical reactions. Multi-wall carbon nanotubes (MWNTs) were used first by Hill and co-workers [12] to realize the direct electrochemistry of proteins. Since that time, MWNTs have opened up new possibilities for studying the electrochemical processes of various proteins and enzymes [13–16].

The key idea of this paper is to combine Au_{nano} and MWNTs to modify gold electrodes in order to improve their electroactivity for cytochrome *c*. MWNTs offer a cubic network for Au_{nano} and the gelatinization of Au_{nano} in DHP ensures that the composite film remains stable on the surface of the gold electrode. It is demonstrated that Au_{nano} are in elec-

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doi:10.1016/j.colsurfb.2004.12.019

trical contact with the gold electrode through the MWNTs, enabling the composite film to be used as an electrode. The composite film on the gold electrode surface was characterized by electrochemical impedance spectroscopy and cyclic voltammetry (CV). It was found that the composite film promoted the direct electron transfer of horse heart cytochrome *c*. The optimum loading of MWNTs and Au_{nano} in the composite film was investigated and the optimized composite film was used to detect cytochrome *c* successfully.

2. Experimental details

2.1. Reagents

Horse heart cytochrome *c* was obtained from Sigma and used as received. Dihexadecylphosphate was purchased from Fluka. All the other chemicals were of analytical grade and were used without further purification. Doubly distilled water was used throughout and the supporting electrolyte was usually phosphate buffer containing 0.06 M Na_2HPO_4 and NaH_2PO_4.

The multi-wall carbon nanotubes, MWNTs (obtained from the Institute of Nanometer Materials, Central China Normal University, China), were synthesized by a catalytic pyrolysis method with a purity of 85%. Before being used, MWNTs were purified by treatment with concentrated HNO_3 as described by Tsang et al. [17]. Most of the MWNTs were, thereby, opened and their surface was oxidized with carboxylic, carbonyl and hydroxyl groups. The metals in the samples of carbon nanotubes were also removed by concentrated HNO_3.

2.2. Surface preparation and modification

Colloidal Au was prepared as described by Hillier and co-workers [18]. In brief, $HAuCl_4$ aqueous solution (4 mL of 0.4 mg mL^{-1}) was boiled, and then 1 mL sodium citrate (1% aqueous) was added to the boiled solution. The resulting solution was maintained at 100 °C for several minutes until the color of the solution did not change.

A 5 mM DHP was dispersed in 4 mL Au_{nano} aqueous solution or distilled water by ultrasonicating for 16 h until it was homogeneous and a Au_{nano}–DHP or DHP aqueous dispersion was obtained. Then, MWNTs were dispersed uniformly in the above Au_{nano}–DHP dispersion or DHP aqueous dispersion for 5 min aided by ultrasonication and a Au_{nano}–MWNTs–DHP or MWNTs–DHP dispersion was obtained. Prior to modification, a gold electrode with a diameter 1.5 mm was polished with 0.3 and 0.05 μm alumina slurry (CH instrument, Inc.) in sequence, and then sonicated in ethanol and doubly-distilled water, respectively. Finally, the gold electrodes were coated with 4 μL of Au_{nano}–MWNTs–DHP dispersions and dried in air at room temperature. The Au_{nano}–DHP modified gold electrode or MWNTs–DHP modified gold electrode was prepared by the same procedure as explained above. The active surface area of the modified electrodes was determined by cyclic voltammetry in a solution of 5 mM $K_4[Fe(CN)_6]$ with 0.1 M potassium chloride as the supporting electrolyte.

2.3. Apparatus and procedures

TEM images were obtained using a TEM–100 CXII (Japan) microscope. To obtain TEM images, a drop of the Au_{nano}–DHP, MWNTs–DHP or Au_{nano}–MWNTs–DHP dispersion was placed on a standard TEM sample holder containing holey carbon film and allowed to dry in air at room temperature.

All the CV experiments were performed with a computer controlled Model CHI 830A electrochemical analyzer (ChengHua Instrument Co., Shanghai, China). A conventional three-electrode cell was employed with a modified gold electrode as a working electrode, a platinum wire as counter electrode and a saturated calomel electrode (SCE) as a reference electrode. All experiments were done at room temperature in a conventional electrochemical cell. All experimental solutions were deaerated by bubbling nitrogen for 15 min and a nitrogen atmosphere was kept over the solution during measurements.

For EIS experiments, a Model RDE 5 Bi-potentiostat (USA) and a Model 5210 Lock-in Amplifier interfaced to an EG&G 273 A Potentiostat/Galvanostat (USA) were used. The impedance measurements were performed in the presence of equimolar (5 mM) $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ as a redox probe. The supporting electrolyte was 0.1 M KCl. The EIS experiments were done at the formal potential (+0.20 V) at an amplitude of 5.0 mV (rms) with a wide frequency range of 100 kHz to 0.1 Hz.

3. Results and discussion

3.1. Assembly of Au_{nano} on MWNTs

Pristine carbon nanotubes are very hydrophobic and most metals do not adhere to the carbon nanotubes [19]. The oxidation of the carbon nanotube surface is required to create functional groups and facilitate metal deposition on the carbon nanotubes [20]. Our method for the deposition of Au_{nano} on MWNTs is simple and effective. First, dihexadecylphosphate, a negatively charged surfactant, was dispersed in colloidal gold and then MWNTs were solubilized in the dispersion. Au_{nano} were assembled on the MWNTs by interaction with carboxylic, carbonyl and hydroxyl groups on the oxidized carbon nanotube surface [21]. As shown in the TEM micrograph in Fig. 1a, a high and uniform dispersion of Au_{nano} in DHP was obtained. The Au nanoparticles in DHP were spherical with a mean diameter of about 20 ± 10 nm. The size distribution based on a count of 1023 particles was quite symmetrical. No change in the particle size was noticed after 2 months when the Au_{nano} in DHP was stored in the dark at