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Direct electrochemistry of cytochrome *c* at a novel gold nanoparticles-attached NH_2^+ ions implantation-modified indium tin oxide electrode

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ABSTRACT

A NH_2^+ ions implantation-modified indium tin oxide (NH_2/ITO) electrode was prepared and the existence of amino group on the electrode was verified by FT-IR spectra and X-ray photoelectron spectroscopy analysis (XPS). The gold nanoparticles (AuNPs) have been fabricated on the surface of NH_2/ITO electrode, resulting novel gold nanoparticles-attached NH_2^+ ions implantation-modified ITO (AuNPs/NH_2/ITO) electrode, which could provide a biocompatible surface for the adsorption of cytochrome *c* (Cyt *c*). The Ultraviolet–visible (UV–vis) spectra indicated that Cyt *c* adsorbed on the AuNPs interface retained the native structure. Electrochemical impedance spectra (EIS) and cyclic voltammetric techniques were employed to evaluate the electrochemical behaviors of Cyt *c*. The surface of the AuNPs/NH_2/ITO electrode was exhibited using the scanning electron microscopy (SEM) comparing with the surface of the gold nanoparticles-attached ITO electrode (AuNPs/ITO). We found that the functional surface of the NH_2/ITO electrode resulted in a high AuNPs loading. The obtained Cyt *c*/AuNPs/NH_2/ITO electrode gave an excellent electrocatalytic activity towards the reduction of H_2O_2, and the catalysis currents were proportional to the H_2O_2 concentration in the range of 2.0–300.0 μM with a detection limit of 0.5 μM ($S/N = 3$).

1. Introduction

Since the pioneer work of Hill and Eddowes in the 1970s [1], the direct electron transfer of redox proteins at solid matrices and related biosensing (the so-called "third generation biosensors") have received more and more attention. Understanding of these reactions fundamentally can provide insight into physiological electron transfer processes as well as a platform for fabricating biosensors, enzymatic biosensors and biomedical devices [2–5].

Due to the special biological function of Cyt *c* [6,7], many researches have been focused on the study of the direct electron transfer between Cyt *c* and electrode. Many electrode materials [8–10], such as glutathione [11], cardiolipin [12], TiO_2 phytate [13], and fullerene [14], have been used to prepare the modified electrode in order to improve the electrochemical properties of Cyt *c*, and some satisfactory results were obtained [15].

It is well known that, the alteration of electrode surface with the use of nano-structure material as a mediator is advantageous for achievement of direct electron transfer between the biomolecular and the electrode [16–18]. Nanomaterials are commensurate in size to proteins, and their multivalent functionalization on their surfaces holds great promise for controlling the bimolecular recognition [19,20]. In all kinds of nanomaterials, gold nanoparticles, due to their fascinating promises for optical, electronic, magnetic, catalytic and biomedical applications [21,22], are the subject of an exponentially increasing number of publications [23]. It has been also demonstrated that electrostatically bound AuNPs–protein conjugates typically retain biological activity [24].

On the other hand, the AuNPs are often immobilized on the composite film containing amino group through electrostatic interaction [25–28]. So a lot of peculiar binding molecules such as amines are used to assemble AuNPs on the electrode surfaces. However, the binding molecules often alter the conducting properties of the modified electrode [29–31]. To avoid these disadvantages, we utilize ion implantation to introduce amino group directly on the electrode in order to minimize the potential influence from the binding molecules.

Ion implantation, a kind of material surface modification technique, provides practical and excellent electrode material [32,33]. Our group have successfully fabricated the metal ion implanted ITO electrode and applied them to investigate the electrochemical behaviors of anti-cancer pharmaceuticals, DNA and proteins [34,35]. Recently, Fujishima's group have also successfully fabricated the Cu-, Ni-, and Pt-implanted BDD electrode and applied them for the application of the glucose and H_2O_2 sensor [36–39]. According to their research, in addition to its high catalytic activity, the ion implantation-modified electrode exhibited excellent electrochemical stability with low background current even after ultrasonic treatment [40].

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In this paper, a new NH_2/ITO electrode has been constructed by directly implanting NH_2^+ ions on ITO so AuNPs can be assembled on the NH_2/ITO electrode by the strong electrostatic interaction between AuNPs and the amino groups. Because Cyt *c* is positively charged in the case of solution pH below 10 (the isoelectric point) [41] and AuNPs possess uniformly negative charges on their surfaces [42], Cyt *c* can be immobilized on the AuNPs/NH_2/ITO electrode via electrostatic interaction. The electrochemical properties of the resulting Cyt *c*/AuNPs/NH_2/ITO electrode were studied by cyclic voltammetry (CV). By comparison, the immobilized Cyt *c* exhibited an enhanced electron transfer for its heme Fe(III)/Fe(II) redox couple at Cyt *c*/AuNPs/NH_2/ITO electrode. The UV–vis result shows that the immobilized Cyt *c* retains its original conformation. The electrocatalysis of immobilized Cyt *c* has also been investigated in detail.

2. Experimental

2.1. Apparatus

ITO was implanted using the BNU 400-keV implanter with ion dose of 1×10^{16} ions cm^{-2} at room temperature. Cyclic voltammetric experiments were performed on CHI660 electrochemical workstation (CH Instrument Inc, USA). The electrochemical measurements were carried out in a single-compartment three-electrode glass cell with a Cyt *c*/AuNPs/NH_2/ITO electrode as the working electrode, a Ag/AgCl (saturated KCl) as the reference electrode and a Pt-wire as the counter electrode. The pH measurements were performed using the pH-meter from Shanghai Leici instrument Ltd. The structure and morphology of the modified electrode were characterized by a scanning electron microscope (Hitachi X650, Japan). Transmission Fourier transform infrared (FT-IR) spectra were obtained by using the AVATRA 360 (Nicolet, USA) spectrophotometer. UV–vis absorption spectra were performed on a Cintra 10 UV–vis spectrophotometer (GBC, AUS). XPS measurement was performed on an Escalab MK II spectrometer (VG Company, UK).

2.2. Chemicals

Horse heart Cyt *c* was obtained from Sigma and used as received. All the chemicals were of analytical reagent grade and triple distilled water was used throughout. All measurements were performed at room temperature.

2.3. Electrode modification

The AuNPs solution was prepared according to Murphy's method [43]. Briefly, A 20 mL aqueous solution containing 2.5×10^{-4} M $HAuCl_4$ and 2.5×10^{-4} M tri-sodium citrate was prepared in a conical flask. Next, 0.6 mL of ice cold 0.1 M $NaBH_4$ solution was added to the solution all at once while stirring. The solution turned pink immediately after adding $NaBH_4$, indicating particle formation. Stirring was continued for 30 min and the AuNPs solution was kept in a brown bottle at 4 °C.

The NH_2^+ ions were produced from gaseous ammonia, and then they would be accelerated in an accelerating tube by an electric field at an energy level of 80 keV. A mass analyzer would be used to select the NH_2^+ ions. Finally the NH_2^+ ions were implanted on the ITO surface to form NH_2/ITO electrode.

Prior to the modification, the NH_2/ITO electrode was ultrasonicated in alcohol and washed in triple distilled water and dried in nitrogen. Then the electrode was immersed in the AuNPs solution at 4 °C for 4 h. Finally, the obtained AuNPs/NH_2/ITO electrode was immersed into 10.0 mM phosphate buffer (PBS) containing 2 mg mL^{-1} Cyt *c* for 24 h to from Cyt *c*/AuNPs/NH_2/ITO electrode. The electrodes were rinsed in distilled water prior to the electrochemical measurements. The modified electrode was stored at 4 °C in a refrigerator when it was not in use.

The Cyt *c*/AuNPs/ITO, Cyt *c*/NH_2/ITO and Cyt *c*/ITO electrodes were prepared with the same procedures as described above.

3. Results and discussion

3.1. Characterization of the Cyt *c*/AuNPs/NH_2/ITO electrode

3.1.1. FT-IR spectra of NH_2/ITO electrode

The ion implantation of amino group on the electrode can be verified by the FT-IR spectra. The peak centered at 3480 cm^{-1} is attributed to N–H stretching mode (curve *a* in Fig. 1). It is very clear that after the implantation process, this absorption peak increases modestly (curve *b* in Fig. 1), which shows that amino group is introduced on the ITO electrode.

3.1.2. XPS spectra of NH_2/ITO electrode

In order to confirm the existence of the amino groups on the surface of the NH_2/ITO electrode, XPS experiments were carried out. It can be seen that the N_{1S} spectrum of the bare ITO sample is low with the main peak at 400.0 eV representing N–H bond (curve *a* in Fig. 2). However, the N_{1S} peak reveals a remarkable increase after the ion implantation of NH_2^+ ions (curve *b* in Fig. 2). This corresponds to an increase in the nitrogen atom concentration from 0.63% to 0.98%. The XPS results thus show that NH_2^+ ions were successfully implanted into the ITO films and that the implanted NH_2^+ ions maintains the characteristics of the amino group.

3.1.3. Scanning electron microscopy

The surface image of AuNPs/NH_2/ITO, observed using a scanning electron microscopy, is shown in Fig. 3A. Compared with the surface of AuNPs/ITO (Fig. 3B), it was found that more AuNPs were dispersed on the electrode surface. XPS also show that the atom ratio of Au of AuNPs/NH_2/ITO is 1.7%, which is nine times larger than the atom ration of Au of AuNPs/ITO (0.19%). So we can conclude that the enhancement of the AuNPs loading might be the result of the interaction between the implanted amino groups and the AuNPs.

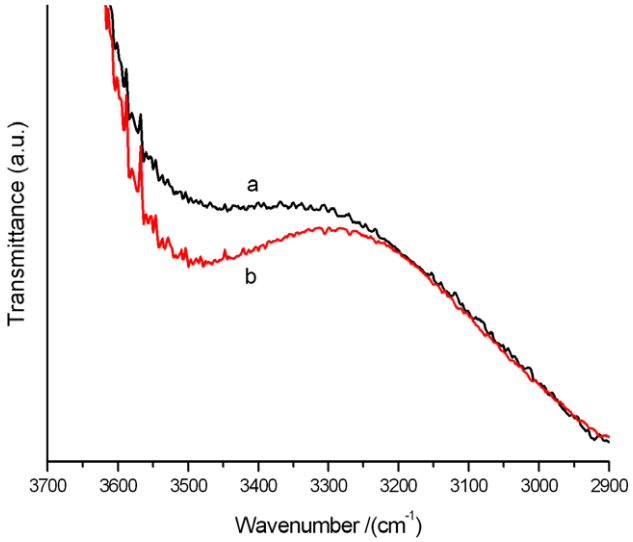

Fig. 1. FT-IR for (a) ITO electrode and (b) NH_2/ITO electrode.