Gelatin-functionalized carbon nanotubes for the bioelectrochemistry of hemoglobin

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Abstract

In this paper, we compared the use of gelatin-functionalized carbon nanotubes (CNTs) as substrates for Hemoglobin (Hb) immobilization and as electrodes for electrochemical reduction of the absorbed Hb. The non-covalently gelatin-functionalized CNTs possessed an improved solubility in aqueous solution and may have an enhanced biocompatibility with Hb. The characteristics of Hb/gelatin-CNTs composite films were studied by using UV–vis spectroscopy, FTIR spectroscopy and electrochemical methods. The immobilized Hb showed a couple of quasi-reversible redox peaks with a formal potential of \(-0.35\) V (vs. SCE) in 0.1 M pH 7.0 phosphate buffer solution (PBS). The surface concentration of electroactive Hb immobilized on gelatin-CNT/GC electrode was about \(4.34 \times 10^{-10}\) mol cm\(^{-2}\).

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1. Introduction

In recent years, CNTs have attracted considerable research attention because of their unique electronic, structural, mechanical and electromechanical properties [1–3]. With synthesis technology and purification method of CNTs being gradually matured, more attention has been focused on the uses of CNTs to create the next generation electronic devices and networks. Promising nanotube-based devices include field emission transistors, memory elements, logic gates and tips in scanning probe microscopy [4,5]. With the subtle electronic properties, CNTs have been studied and can be used in electrochemical sensors, as reported in several recent reviews [6–9].

However, the surface of CNTs is strongly hydrophobic, which results in the aggregation of CNTs in most solvents and the difficulty of manipulating CNTs for practical applications and be integrated with biomacromolecules. Moreover, the strong interactions between proteins and CNTs substantially distort proteins, leading to the loss of their biocatalytic activities toward substrates. To improve the dissolution and biocompatibility of CNTs, it was proved to be an effective way to form supramolecular adducts with surfactants or polymers. In recent years, the present author has used the surfactants [10] and polymers [11] to disperse CNTs in aqueous solution, and to co-assemble some proteins onto CNTs successfully. An enhanced faradaic response was obtained by the direct electron transfer of the protein and enzyme. We attributed the enhanced response to the reconstruction of a three-dimension architecture which made the electron transfer of proteins away from electrode possible.

In this Letter, we used the gelatin, composed of eighteen kinds amino acid chains, to functionalize the side wall of the CNTs because it is a polymer of natural origin [12], biodegradable [13], biocompatible, and non-immunogenic [14,15]. These properties make the gelatin a suitable compound for biomedical applications [16]. Moreover, it is a
kind of natural surfactant. The non-polar amino acid chain of the gelatin could immobilize in the side wall of CNTs through hydrophobic–hydrophobic interaction, which inspires the motivation for using gelatin as the supported membrane in biosensors. To our best knowledge, no one has used gelatin to disperse CNTs for the direct electrochemistry of Hb. In this paper, we used gelatin to decorate CNTs for achieving the direct electron transfer of the Hb co-assembled onto CNTs. The prepared Hb/gelatin-CNT was found to possess good electron transfer properties which may pave a new way to CNT-based bioelectrochemical devices and the protein electrochemistry at functional interfaces.

2. Experimental

2.1. Materials and chemicals

Carbon nanotubes (CNTs, diameter 40–60 nm, approximately) were supplied by the Department of Physics, Hong Kong University of Science and Technology. The CNTs were purified by refluxing the as-received CNTs in 2.6 M HNO₃ for 10 h prior to use. Hemoglobin (Hb) was purchased from Sigma and was used without additional purification, whereas the hydrogen peroxide (30% (w/w)) and the gelatin were from Beijing Chemical Company (Beijing, China). The dilute solution of H₂O₂ was prepared daily. Other chemicals were of at least analytical grade and used as-received. Aqueous solutions were prepared with doubly distilled water. Potassium dihydrogen orthophosphate (0.10 M) was used to prepare the supporting electrolyte and its pH value was adjusted to 7.0 using KOH.

2.2. Preparation of the modified electrodes

Glassy carbon (GC, 3 mm-diameter), before use, was first polished with emery paper (# 2000), 0.3 and 0.05 μm alumina slurry on a woven cloth, and then cleaned under bath sonication for 10 min and finally thoroughly rinsed with distilled water.

For the preparation of Hb/gelatin-CNTs/GC electrodes, CNTs were dispersed into 1.0 mg ml⁻¹ aqueous solution of gelatin solution and the mixture was sonicated for 1 h to obtain a homogeneous and black dispersion containing 2 mg ml⁻¹ CNTs. A 4 μl of the dispersion was dip-coated onto the GC electrodes. A small glass bottle was fitted over the electrode, followed by a period of standing in air until it was dried. Then the gelatin-CNTs/GC electrodes were immersed in 0.10 M phosphate buffer containing 10 mg ml⁻¹ Hb for 8 h. The electrodes were rinsed with distilled water prior to electrochemical measurements. For comparison, the pristine CNTs without any functionalization were dispersed into DMF and 4 μl of the dispersion was coated on the GC electrode. The electrodes (CNTs/GC) were air-dried, rinsed with distilled water, and immersed in 10 mg ml⁻¹ Hb solution for 8 h. The resulting electrodes (Hb/CNTs/GC) were rinsed with the distilled water to remove the non-adsorbed Hb before the electrochemical measurements.

2.3. Apparatus and procedures

Electrochemical measurements were carried out with a computer-controlled electrochemical analyzer (CHI 660 A, CHI, Austin, TX) in a two-compartment and three-electrode cell. The modified GC electrodes were used as working electrodes, a platinum spiral wire as counter electrode, and a saturated calomel electrode (SCE) as reference electrode. A 0.10 M phosphate buffer (pH 7.0) was used as the supporting electrolyte. For the experiments conducted under anaerobic conditions, the electrolyte was bubbled with pure N₂ gas for more than 30 min and N₂ gas was kept flowing over the solution during the electrochemical measurements. All electrochemical measurements were performed at ambient temperature (18 ± 2 °C).

UV–visible (UV–vis) absorption spectroscopy was carried out by using a 2550 spectrophotometer (Shimadzu, Japan). Sample films for measurements were prepared by casting Hb aqueous solution and dispersion of CNTs onto ITO glass slides and drying them up in air.

FT–IR spectra were obtained on a Perkin–Elmer Spectromate 100 spectrometer (Perkin–Elmer Company, USA) at room temperature. Sample films for FT–IR spectroscopy were prepared by casting Hb/gelatin-CNTs, gelatin-CNTs or Hb solutions onto glass slides and allowed to dry up in air. The films were then stripped off and tableted with KBr powders for measurements.

Transmission electron micrographs were recorded on a transmission electron microscope (TEM, Philips Tecnai 10) using an accelerating voltage of 200 kV.

3. Results and discussion

3.1. Solubility of the CNTs with gelatin

The morphologies and the structures of CNT are characterized using the transmission electron microscopy (TEM). As shown in Fig. 1, the nanotubes are multi-walled carbon nanotubes with a diameter of 40–60 nm, the inner of which is composed by the continuously connecting taper structure tubes with a length of 20–40 nm.

Due to the strong attractive interactions between the hydrophobic side walls of the CNTs, they tend to aggregate and precipitate in water (Fig. 2b), indicating a poor solubility. However, the CNTs modified with gelatin can form homogeneous and black dispersion in aqueous solution (Fig. 2a) and such homogeneous dispersion is found to be essentially stable for at least two weeks. These indicate that there are strong interactions between the CNTs and the gelatin, leading to that the CNTs are hydrated. It has been known that the CNTs can be wrapped with the hydrophobic chains of surfactants, which makes the CNTs modified with the hydrophilic groups of the surfactants and
thus, can be dispersed into the aqueous solution [17,18]. Just like surfactants, the gelatin has a zwitterionic structure, forasmuch there should be hydrophobic interactions between the hydrophobic amino acid chain of the gelatin and the hydrophobic side walls of the CNTs, which make the CNTs modified with the hydrophilic groups and thus, can be dispersed into the aqueous solution.

3.1.1. Spectroscopic characterization of Hb/gelatin-CNTs composite film

Structural variations of Hb are firstly considered after the proteins are immobilized into CNTs composite film. FT–IR spectroscopy is an effective means to probe into the secondary structure of proteins. The characteristic amide I and II bands of proteins provide detailed information on the secondary structure of polypeptide chain [19]. The amide I band (1700–1600 cm\(^{-1}\)) is caused by the combination of N–H bending and C–N stretching of the peptide groups. Fig. 3 shows the FT–IR spectra of the Hb, the gelatin-CNT and the Hb/gelatin-CNT composite film. As shown in Fig. 3a and b, the spectra of amide I and II bands of the Hb/gelatin-CNT composite film (1651 and 1538 cm\(^{-1}\)) are nearly the same as those obtained for the native Hb (1653 and 1544 cm\(^{-1}\)). The similarities of the two spectra suggest that the Hb retains the essential features of its native secondary structure in the Hb/gelatin-CNT composite film. Thus, the gelatin-CNT composite film may provide a promising matrix for the enzyme immobilization and the biosensor fabrication because of its satisfying biocompatibility. It should be noted that the gelatin-CNT films also show the FT–IR absorption bands (1640 cm\(^{-1}\)) in the Hb amide I regions, as shown in Fig. 3c, which is attributed to the C=O stretching mode of the oxygen-containing groups [20–22]. However, the absorption bands (1640 cm\(^{-1}\)) are not consistent with that of the Hb, and the spectra of amide II band of the Hb (1540 cm\(^{-1}\)) are not evident in Fig. 3c.

UV–vis spectroscopy is a useful tool for the conformational study of the heme proteins. The Soret absorption band of the heme prosthetic group may provide information on the tertiary structure of the heme proteins, especially the conformational change around the heme region [23]. As can be seen from Fig. 4, Hb/gelatin-CNT composite film on ITO glass slides shows the soret band at 408 nm, as same as that of the native Hb film (curve a and curve b in Fig. 4). It indicates that the Hb entrapped in the composite film has a similar structure to the native of the Hb and retains its near–native conformations. In the UV–vis absorption spectra of the gelatin-CNT, no characteristic peak is observed, as shown in Fig. 4 (curve c).

3.2. Electrochemistry of hemoglobin in gelatin-CNT/GC electrode

Fig. 5 depicts the typical cyclic voltammogram (CV) of the gelatin-CNT/GC, Hb/gelatin-CNT/GC and Hb/
CNT/GC electrodes in 0.10 M phosphate buffer solution of pH 7.0 at 100 mV s$^{-1}$, respectively. As shown in CV (Fig. 4), a pair of redox peaks are observed at the gelatin-CNT/GC electrode (dashed curve, Fig. 4) at -0.10 V, of which the redox peaks at -0.10 V is attributed to the redox process of the oxygen-containing groups produced [24]. However, the disappearance of these peaks after immobilization of Hb is indicative of the adsorption of Hb at the CNTs (solid and dotted curves, Fig. 5). Moreover, a pair of well-defined redox peaks is observed at the Hb/gelatin-CNT/GC electrode (solid curve, Fig. 5). The formal potential $E^0$ (defined as the average of anodic peak potential, $E_{pa}$ and cathodic peak potential, $E_{pc}$) is -0.35 V (vs. SCE) with a peak-to-peak separation of ca. 70 mV, which is consistent with the reported values for the Fe(III)/Fe(II) redox center of the heme group in the Hb [25,26], suggesting that the Hb molecules entrapped in the gelatin-CNT composite film retain their native structure. In comparison, we find that the peak currents obtained at the Hb/gelatin-CNT/GC electrode (solid curve, Fig. 5) are remarkably larger than that obtained at the Hb/CNT/GC electrode (dotted curve, Fig. 5), suggesting that it is difficult to realize the direct electron transfer of the Hb at the pristine CNTs, which coincides with the previous reports [27,28]. The above experiments show that gelatin-CNT compound could facilitate the direct electron transfer of the Hb and enhance the electrochemical signal. The reason is that CNTs have a high surface area and good electrical conductivity, furthermore, the gelatin-dispersed CNTs film electrode possesses a three-dimensional architecture. Moreover, the gelatin allows the Hb maintain its suitable conformation and activity, which make the direct electron transfer between the Hb and the electrode easy. Hence, it can be concluded that the gelatin is useful for solubilization, thereby avoids the aggregation of CNTs, and the gelatin-dispersed CNTs are useful for facilitating electron transfer of the proteins.

The dependence of the peak currents and the peak potentials on the scan rate is shown in Fig. 6. It can be observed that the cathodic and anodic peak currents ($I_p$) of the Hb increase with the increase of the scan rate, at the same time, the cathodic and anodic peak potentials exhibited a small shift and the peak-to-peak separation also increased with the scan rate. This indicates that the electron transfer process is diffusion-controlled.
increases. As shown in inset of Fig. 6, the cathodic and anodic peak currents increase linearly with the scan rate from 100 to 900 mV s\(^{-1}\). Thus, it is clear that the Hb adsorbed onto the surface undergoes a reversible and surface-confined electron transfer. The surface concentration of the Hb (\(\Gamma^*\)) can be deduced according to the Faraday’s law, \(Q = nF\Gamma^*\). Here \(F\) is Faraday’s constant, \(Q\) can be obtained by integrating the cathodic 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 at the Hb/gelatin-CNT/GC electrode is estimated to be 4.34 \(\times\) 10\(^{-10}\) mol cm\(^{-2}\), which is much larger than the theoretical monolayer coverage of the Hb. These results indicate that the Hb undergoes a saturated adsorption onto the three-dimensional gelatin-CNT composite films with multilayer coverage which participated in the electron transfer process.

4. Conclusions

In this study, the gelatin-CNT modified glassy carbon electrode was prepared, and the electrochemical behavior of the Hb on the modified electrode was studied. The use of gelatin to functionalize CNTs essentially increased the solubility of CNTs into water and leads to a good separation of the aggregated CNTs, thus substantially facilitated the bioelectrochemical applications of CNTs. The Hb underwent a saturated adsorption onto the three-dimensional gelatin-CNT composite films with multilayer coverage. The direct electron transfer of the Hb was largely facilitated onto the functionalized CNTs, compared with the CNTs modified GC electrode. The Hb can retain its original conformation in the gelatin-CNT composite films.

References