Direct Electrochemistry of Cytochrome c at a Glassy Carbon Electrode Modified with Single-Wall Carbon Nanotubes

Jianxiu Wang, Meixian Li, Zujin Shi, Nanqiang Li,* and Zhennan Gu

College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, People's Republic of China

The electrode reaction of cytochrome c was studied by cyclic voltammetry at a glassy carbon electrode modified with single-wall carbon nanotubes (SWNTs). A pair of well-defined redox waves was obtained in cytochrome c aqueous solution at an activated SWNT film-modified electrode. The optimal conditions for activating the SWNT film-modified electrode have been determined. The electrode reaction of cytochrome c is a diffusion-controlled process. The peak current increases linearly with the concentration of cytochrome c in the range from 3.0 × 10⁻⁵ to 7.0 × 10⁻⁴ M. The detection limit is 1.0 × 10⁻⁵ M. The activated SWNT film was characterized by scanning electron microscopy. Furthermore, interaction of cytochrome c with adenine was characterized by electrochemical and spectral methods.

Carbon nanotubes, consisting of cylindrical graphene sheets with nanometer diameter, combine in a unique way with high electrical conductivity, high chemical stability, and extremely high mechanical strength and modulus. These special properties of both single-wall and multiwall carbon nanotubes (SWNTs and MWNTs, respectively) have attracted increasing attention. Numerous novel applications of carbon nanotubes have been investigated, including their use as field emitters, nano electronic devices, nanotube actuators, batteries, probe tips for scanning probe microscopy, and nanotube-reinforced materials.

Carbon nanotubes behave electrically either as metals or as semiconductors, depending on their atomic structure. The subtle electronic properties suggest that carbon nanotubes have the ability to promote electron-transfer reactions when used as an electrode material in electrochemical reactions, representing a new application of carbon nanotubes. The better performance of the carbon nanotube electrodes in comparison with other forms of carbon electrode has been found due to the carbon nanotube dimensions (of the tubes, the channels that are inherently present in the tubes), the electronic structure, and the topological defects present on the tube surface. Furthermore, it has been proved that a carbon nanotube has better conductivity than graphite.

The MWNTs were first used to fabricate carbon nanotube electrodes by using a binder, which were successfully used in the oxidation of dopamine, electrochemistry of protein, and electrocatalysis of oxygen. Their performance has been found to be superior to other carbon electrodes in terms of reaction rate and reversibility.

Since the discovery of SWNTs in 1993, they have been the focus of intense interest. SWNTs exhibit unique properties and potential applications in nanoscale science and technology. However, the electrochemistry of SWNTs is less studied. To date, only Liu et al. have reported the electrochemistry of cast films of SWNTs on Pt and Au electrodes, but the films do not show well-resolved voltammograms. After the SWNTs were treated with nitric acid during the purification process, the carboxylic acid groups were introduced on the SWNT surface. They have also been found to be superior to other carbon electrodes in terms of reaction rate and reversibility.

Published on Web 03/30/2002

10.1021/ac0010978u CCC: $22.00 © 2002 American Chemical Society

Analytical Chemistry, Vol. 74, No. 9, May 1, 2002 1993
electrode to form a carbon nanotube film. The film shows very
stable electrochemical behavior, and it can be used to catalyze
the electrochemical reaction of some biomolecules such as
dopamine, epinephrine, and ascorbic acid.24,25

Cytochrome c is a water-soluble heme protein that exists in
the cytosol between the inner and outer membranes of mitochon-
dria. It plays an important role in the biological respiratory chain,
whose function is to receive electrons from cytochrome c reduc-
tase and deliver them to cytochrome c oxidase. So the electro-
chemical study of cytochrome c is very important. Whereas the
voltammetric response of cytochrome c is quite poor at bare metal
electrodes, most likely due to protein denaturation at the metal
electrode surface leading to extremely slow electron-transfer
kinetics. Although direct electrochemistry of cytochrome c at
M WNT electrodes has been reported, the M WNT electrodes are
time-consuming to prepare and the electrochemical response is
weak.18

In this paper, the SWNTs were oxidized in air during the
purification process.26 A dispersion of the SWNTs in N,N-
dimethylformamide (DMF) was cast on a GC electrode to form a
carbon nanotube film. A pair of well-defined redox waves of
cytochrome c was obtained at an activated SWNT film-modified
electrode. As much more work had been done on the interaction
of macromolecules such as cytochrome c with small molecules
in recent years,27–32 the interaction of cytochrome c with adenine
was characterized by electrochemistry, FT-IR, and UV in our
studies.

EXPERIMENTAL SECTION

Instruments and Chemicals. Cyclic voltammetry (CV) mea-
surement was carried out by using a PAR (Princeton Applied
Research) 273 potentiostat/galvanostat with model 270 electro-
chemical software. Electrochemical measurements were
performed by using a conventional cell, with a glassy carbon electrode
as the working electrode, a platinum electrode as the counter
electrode, and a saturated calomel electrode (SCE) as the
reference electrode. Water was triply distilled with a quartz
apparatus. Horse heart cytochrome c was obtained from Merck.

Adenine hemisulfate salt was purchased from Sigma. A 0.1 M
phosphate buffer solution (KH2PO4 + Na2HPO4, pH 6.24) was used
as the supporting electrolyte. All electrochemical experiments
were conducted under nitrogen atmosphere at the ambient
temperature 25 ± 1°C.

The scanning electron microscope (SEM) image was obtained
using a JSM-6301F microscope. X-ray photoelectron spectroscopy
(XPS) measurement was performed using a PHI 5300 ESCA
electron spectrometer. UV spectroscopy measurement was per-
formed using a UV-3100 spectrometer. Fourier transform (FT)
IR spectra were recorded on a Nicolet MagnaIR 750 spectrometer.

Preparation of Single-Wall Carbon Nanotubes. The SWNTs
were prepared by a direct current arc-discharge method with
Y–Ni alloy as the catalyst33 and purified using the method of
oxidation in air.26 The nanotubes were of the armchair (n, n) type
(n = 8, 9, 10, 11) with a typical distribution of the tube diameter
ranging from 1.1 to 1.4 nm as determined from transmission
electron microscopy. Most SWNTs existed as SWNT bundles, and
each bundle consisted of tens of SWNTs as the SWNT “rope”.34

Preparation and Activation of the SWNT Film-Modified
Electrode. One milligram of purified SWNTs was dispersed with
the aid of ultrasonic agitation in 10 mL of DMF to give a 0.1 mg
mL–1 black suspension. The glassy carbon electrode was abraded
with emery paper (No. 1500), polished with 0.3μm alumina slurry,
and then washed ultrasonically in distilled water and ethanol
for a few minutes, respectively. The GC electrode was coated by
casting 15 μL of suspension of the SWNT (0.1 mg mL–1) in DMF
and dried under an infrared lamp.

The experiments indicate the activating potential range has a
great influence on the electrochemical response of cytochrome c
at the SWNT film-modified electrode. At potential range from +0.5
to −0.3 V, the electrochemical response is worse. With wider
potential range, +0.8 to −0.6 V, the reversibility of cytochrome c
is improved. At potential range from +1.5 to −1.0 V, the best
response of cytochrome c is obtained; beyond this potential range,
+1.8 to −1.3 V, the response of cytochrome c becomes worse
again. The SWNT film-modified electrode could be damaged when
the activating potential range is from +2.0 to −1.5 V. So the
activating potential range from +1.5 to −1.0 V was chosen for
our experiments.

A well-defined and stable CV of cytochrome c was obtained at
an activating time of 1.5 min at a scan rate of 1 V s −1. Shorter
activating time could result in insufficient activation, whereas
longer time might lead to damage to the SWNT film-modified
electrode. Thus, the activating time of 1.5 min at a scan rate of 1
V s −1 was selected.

To sum up, the SWNT film-modified electrode was put in fresh
0.1 M phosphate buffer solution (pH 6.24) for activation pre-
treatment. The potential was cyclically scanned between +1.5 and −1.0
V at a scan rate of 1 V s −1 for 1.5 min. The electrode was
thoroughly washed with triply distilled water and put in water
before use. The above activating condition was optimized for our
purpose.

RESULTS AND DISCUSSION

Physical Characterization. The SEM image of the activated
SWNT film on a pretreated glassy carbon disk is shown in Figure
1. It can be seen that the carbon nanotubes are formed as bundles
with individual nanotubes arranged in parallel to each other. The
diameters of these bundles are 15–60 nm, and the length
is unmeasurable. A small portion of amorphous carbon impurities
is also found in the SWNT sample.

Direct Electrochemistry of Cytochrome c at an Activated
SWNT Film-Modified Electrode. The CVs of cytochrome c at

---

1372–1374.


18379–18386.


675–681.

42, 551–556.

Cathodic peak potential, with the difference between the anodic peak potential and the cytochrome weak (Figure 2b). However, a pair of well-defined redox waves of electrode with the pretreatment of activation is believed to result in the removal of surface contaminants or inhibitory layers that hinder electron transfer and the increase in surface roughness and surface-bound oxygen groups.35–37

In 0.1 M phosphate buffer blank solution (pH 6.24), a pair of surface peaks is observed at the activated SWNT film-modified electrode (Figure 2d). The appearance of surface waves is attributed to the increment of the concentration of surface functionalities.38 While in 0.1 M phosphate buffer blank solution (pH 6.24), there is no electrochemical response at an unactivated SWNT film-modified electrode (Figure 2e).

In 0.1 M phosphate buffer blank solution (pH 6.24), one couple of peaks corresponding to the redox of the carboxylic acid groups is obtained at an unactivated electrode modified with SWNTs functionalized with carboxylic acid groups; this is in accordance with the existing literature.25 The electrochemical response in 0.1 M phosphate buffer blank solution (pH 6.24) remains almost unchanged after the modified electrode has been activated (Figure 3c). On the basis of the results, we speculate that surface peaks at the activated SWNT film-modified electrode (Figure 2d) might be the redox of the carboxylic acid groups introduced on the SWNT film-modified electrode due to activation, because the same electrochemical response is obtained at the unactivated electrode modified with SWNTs functionalized with carboxylic acid groups.

Having a catalytically active surface and very high aspect ratio (length over diameter), SWNTs can increase the surface area of the electrode, so the background voltammetric response for the SWNT-coated surface is stronger than that for the bare surface. This is consistent with the result obtained from oxidation of dopamine at the MWNT electrode.16

**Effect of pH on the Peak Potential of Cytochrome c at an Activated SWNT Film-Modified Electrode.** The response of cytochrome c is well-behaved in 0.1 M phosphate buffer solution in the range of pH 5.29–8.04. At about pH 6.24, $\Delta E_p$ reaches the

---


least and the best reversibility of cytochrome c is achieved. So 0.1 M phosphate buffer solution (pH 6.24) was chosen for our experiments.

Stability of the Activated SWNT Film-Modified Electrode. One GC electrode modified with the SWNTs five times and activated in the same manner, then immersed in $5.0 \times 10^{-4}$ M cytochrome c solution, with a scan rate of 0.02 V s$^{-1}$, gave an average cathodic peak current of 1.6 $\mu$A with a relative standard deviation (RSD) of 4.0%. The peak current obtained in $5.0 \times 10^{-4}$ M cytochrome c solution decreases 11% after 30 continuous cyclic scannings. Furthermore, $\Delta E_p$ increases with continuous cyclic scanning. These might be due to the adsorption of cytochrome c on and within carbon nanotubes, which hinders the electron transfer of cytochrome c at the electrode surface. To confirm this hypothesis, a comparison experiment was carried out by XPS spectroscopy. Two identical activated SWNT film-modified electrodes, one not cyclically scanned in cytochrome c solution (a), and the other cyclically scanned in cytochrome c solution and then thoroughly washed with triply distilled water (b), were characterized by XPS. The results indicate that the amount of N (whose binding energy is 400.6 eV) increases after cyclic scanning in cytochrome c solution (cytochrome c contains element N) (the concentration of N1s in (a) is 0.82% while that of N1s in (b) is 3.78%). The absorbance of N in (a) is attributed to the unevaporated solvent of DMF within carbon nanotubes (DMF also contains element N). The above results verify that cytochrome c can be adsorbed within carbon nanotubes.

Working Curve. The dependence of cathodic peak current on the concentration of cytochrome c is a linear relationship in the range from $3.0 \times 10^{-5}$ to $7.0 \times 10^{-4}$ M. The linear regression equation is expressed as $I_p/\mu$A $= 0.138 + 0.00308 c/\mu$M (correlation coefficient $r = 0.9987$). The detection limit is $1.0 \times 10^{-5}$ M.

Effect of Scan Rate on the Peak Current at an Activated SWNT Film-Modified Electrode. The influence of scan rate on the electrochemistry of cytochrome c at the activated SWNT film-modified electrode was investigated by cyclic voltammetry. The reduction peak current of cytochrome c at the electrode increases linearly with the square root of the scan rate in the range from 0.05 to 0.32 V s$^{-1}$ with a correlation coefficient of 0.9979. The linear regression equation is expressed as $I_p/\mu$A $= -3.226 + 26.84 (V/Vs)^{1/2}$). The result suggests that the electrochemical reaction of cytochrome c is a diffusion-controlled process, though cytochrome c can be adsorbed on the electrode surface, resulting in the decrease of the electrochemical response of cytochrome c.

Interaction of Cytochrome c with Adenine. As reported in the literature, there exists an interaction of cytochrome c with DNA, which was studied by UV spectroscopy, gel permeation chromatography, and membrane diffusion techniques. In our experiments, we investigated the interaction of cytochrome c with the adenine residue in DNA. Figure 4a shows a CV of $5.0 \times 10^{-4}$ M cytochrome c at the activated SWNT film-modified electrode. After adding $1.6 \times 10^{-3}$ M adenine to the cytochrome c solution and placement for 24 h, the peak currents of cytochrome c increase and a little oxidation peak appears at potential of 0.363 V (Figure 4b). After placement for 48 h, the oxidation peak current at 0.363 V increases a little (Figure 4c). While in sole adenine solution, a small oxidation peak is found positioned at 0.663 V at the activated SWNT film-modified electrode. Based on the above results, there might be interaction of cytochrome c with adenine, and this interaction increases with the deposition time. Furthermore, this interaction might have some effect on the configuration of cytochrome c and adenine, although the exact nature of this effect is still unclear. To confirm the existence of the interaction, UV and IR experiments were also performed. Figure 5 shows typical UV spectra of the solutions of cytochrome c, adenine, and their mixture in comparison with the additive spectrum. The absorbance of a mixture of $2.0 \times 10^{-5}$ M adenine and $6.0 \times 10^{-6}$ M cytochrome c (the concentrations of cytochrome c and adenine in the mixed solution are $6.0 \times 10^{-6}$ and $2.0 \times 10^{-5}$ M, respectively) (Figure 5c) decreases in comparison with the additive spectrum (Figure 5d). The peak at 1653 cm$^{-1}$ corresponds to absorbance band I of amide on the microscopic FT-IR spectrum of solid cytochrome c (Figure 6, top) shifts to a higher wavenumber (1659 cm$^{-1}$) on the microscopic FT-IR spectrum of a mixture of cytochrome c and adenine hemisulfate salt (the mixture of cytochrome c and adenine hemisulfate salt was obtained by volatilizing the solvent of cytochrome c and adenine hemisulfate salt solution which was deposited for 48 h) (Figure 6, bottom). While the peak at 1546 cm$^{-1}$ corresponding
to the absorbance band II of amide (Figure 6, top) shifts to a lower wavenumber (1537 cm\(^{-1}\)) on the microscopic FT-IR spectrum of the mixture of cytochrome c and adenine hemisulfate salt. Furthermore, the peaks at 1709, 1616, 1573, 1478, 1224, and 1027 cm\(^{-1}\) on the microscopic FT-IR spectrum of solid adenine hemisulfate salt (Figure 6, middle) become invisible on the microscopic FT-IR spectrum of mixture of cytochrome c and adenine hemisulfate salt (Figure 6, bottom). However, the peaks at 1414, 945, 902, 803, and 714 cm\(^{-1}\) on the microscopic FT-IR spectrum of solid adenine hemisulfate salt (Figure 6, middle) still exist on the microscopic FT-IR spectrum of a mixture of cytochrome c and adenine hemisulfate salt; they shift to 1416, 942, 890, 798, and 716 cm\(^{-1}\), respectively. These suggest the interaction of cytochrome c with adenine.

CONCLUSION

The activated SWNT film-modified electrode has a good promotion toward the reduction/oxidation of cytochrome c due to better conductivity and high aspect ratios of the tubes. A pair of well-defined redox waves was obtained in cytochrome c aqueous solution at the activated SWNT film-modified electrode. The optimal condition for activating SWNT film-modified electrode was determined. The pretreatment of activation might result in the introduction of the carboxylic acid groups on the SWNT film-modified electrode surface, because the same electrochemical behavior of the activated SWNT film-modified electrode and the unactivated electrode modified with SWNTs functionalized with carboxylic acid groups is obtained in phosphate buffer solution. Electrochemical and spectral experiments indicate interaction of cytochrome c with adenine.

The preparation of the SWNT film-modified electrode was an economical, simple, and convenient way to utilize carbon nanotubes in electrochemistry in comparison with that of other carbon nanotube electrodes.\(^{16,18,19}\) This modified electrode might be used in biosensors to study the electrochemistry of biological systems because the high aspect ratios of the tubes may present a steric effect for more efficient redox reactions of biomolecules.

ACKNOWLEDGMENT

This work is supported by the National Natural Science Foundation of China (Grant 29835110).

Received for review September 6, 2001. Accepted February 28, 2002.

AC010978U