Short Communication

Redox Couple of DNA on Multiwalled Carbon Nanotube Modified Electrode

Hongxia Luo,[a]* *Shana Li*,[a] *Zhi-Xin Guo*,[a] *Nan He*,[a] *Liming Dai*[b]

[a] Department of Chemistry, Renmin University of China, Beijing 100872, China
[b] Department of Chemical and Materials Engineering, University of Dayton, Dayton, OH 45469-0240, USA
*e-mail: luohx@ruc.edu.cn

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Abstract
It has been envisioned that carbon nanotubes could promote electron-transfer reactions when used as electrode materials in electrochemical cells. In the present study, calf thymus DNA was electrochemically oxidized at an electrode modified with multiwalled carbon nanotubes. The potentials for DNA oxidation at pH 7.0 were found to be 0.71 and 0.81 V versus SCE, corresponding to the oxidation of guanine and adenine residues, respectively. An initial oxidation of adenine was observed in the first scan, which was followed by a quasi-reversible redox process of the oxidation product in the subsequent scans.

Keywords: Carbon, Nanotubes, DNA, Cyclic voltammetry, Redox chemistry

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Owing to its unique structure and property, carbon nanotube (CNT) has attracted much publicity and generated a great deal of research interest [1, 2]. Carbon nanotubes can be divided into single-walled carbon nanotubes (SWNTs) with one cylindrical graphene sheet that forms the nanotube structure and multiwalled carbon nanotubes (MWNTs) with additional cylindrical graphene sheets around the SWNT hollow core. Depending on their diameter and the orientation of graphenes along the nanotube length, carbon nanotubes can display unusual electrical conductivity, chemical stability, and excellent strength and stiffness [3]. These superior properties have made carbon nanotubes very attractive for various applications, including field-effect transistors [4, 5], nanoelectronic devices [6–9], batteries [10], tips for scanning probe microscopy [11, 12], and chemical sensors [13, 14]. The subtle electronic properties of carbon nanotubes could also enable them to promote electron-transfer reactions when used as electrode materials in electrochemical reactions. Since the first report of the oxidation of dopamine on a MWNT-modified electrode [15], the electrocatalytic behaviors of carbon nanotubes towards many biomolecules, such as protein [16], hemoglobin [17, 18], myoglobin [19], cytochrome *c* [20], glucose oxidase [21, 22], have been investigated.

Deoxyribonucleic acid (DNA) is the genetic material in living organisms. During the last decade or so, there is an ever increasing interest in studying the electrochemistry of DNA and its adsorption onto various electrodes. At the surface of a mercury electrode, DNA was found to exhibit cathodic signals, arising from the electrochemical reduction of adenine and cytosine residues. This followed by a specific anodic signal, resulting from the reoxidation of the newly reduced guanine residues at highly negative potentials [23, 24]. Irreversible oxidation of guanine and adenine residues can also occur during the electrochemical oxidation of DNA at the solid electrode surfaces [24–27].

Calf thymus DNA (CT DNA) is a type of nucleic acid with double strands. Native CT DNA has few guanine and adenine residues, which cannot easily be oxidized at electrode surfaces. When CT DNA is incubated at 100 °C, the thermal denaturation process takes place, which results in the dissociation of the DNA base pairs and severing of the DNA duplexes so that the long strands become short chains. These short DNA chains cannot be recovered after cooling to room temperature. Thus, more fresh guanine and adenine residues are produced. Generally, the denaturation of DNA involves the rupture of hydrogen bonds and the disturbance of stacking interactions, but not the breakage of covalent bonds [28].

Recently, the electrochemistry of DNA on the MWNT-modified [29–32], SWNT-modified [33], and CNT-paste [34] electrodes was reported by several research groups. In all of these studies, DNA was absorbed onto the CNT electrode surfaces, giving two irreversible oxidation peaks of adenine and guanine residues. These results were similar to those obtained from nanotube-free solid electrodes [24–27], albeit with much weaker signals. In this work, the electrochemical properties of thermally denatured CT DNA were investigated at a MWNT- modified glassy carbon electrode. For the first time, a redox couple was formed from the oxidation of DNA.

The MWNT-modified GC electrode was immersed in a phosphate buffer solution (pH 7.0) containing 1 mg mL^{-1} thermally denatured DNA. After 5 minutes of accumulating

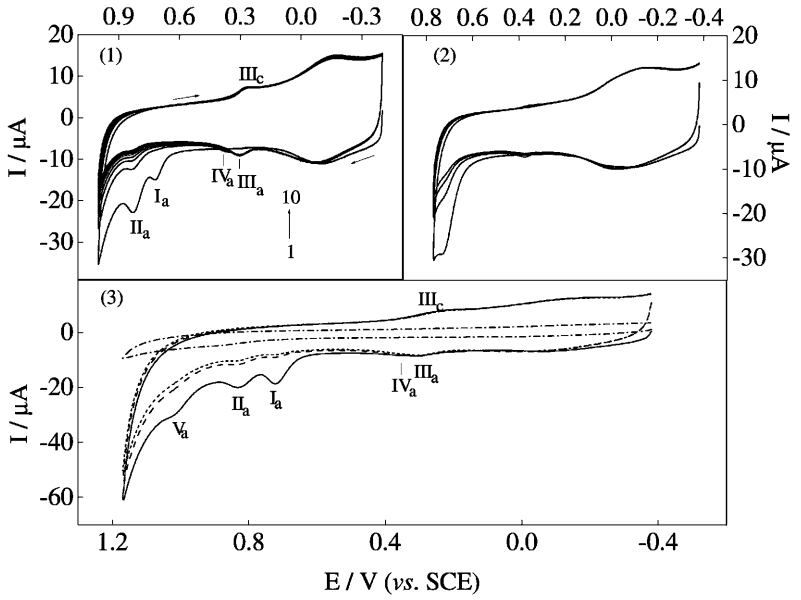

Fig. 1. Cyclic voltammograms of DNA (1 mg/mL) at a MWNT film modified electrode in 0.1 M phosphate buffer solution (pH 7.0) at the scan rate of 0.1 V/s in the scan range of −0.4 to 0.9 V for ten successive scans (1) and −0.4 to 0.75 V for four successive scans (2) after 5 minutes accumulation. Shown in (3) are the cyclic voltammograms of DNA (1 μg/mL) at the MWNT film modified electrode for three successive (a to c) and at a bare GC electrode (d) in the scan range of −0.4 to 1.15 V after 5 minutes of accumulation. a) (—); b) (----); c) (·····); d) (-·-·-·-).

DNA in the magnetically stirred solution in an open circuit, the cyclic voltammograms were recorded from −0.4 to 0.9 V. As shown in Figure 1 part 1, two irreversible anodic peaks (I_a and II_a) were obtained in the first anodic scan. In the reverse scan, a cathodic peak (III_c) was observed. In the second sweep towards positive potentials, two new anodic peaks (III_a and IV_a) appeared, among which III_a formed a chemically reversible couple with the cathodic peak III_c. The peak current for I_a, II_a, and IV_a decreased with the number of cycles. After 4 or 5 cycles, only the pair of peaks $III_c - III_a$ remained and their intensity was constant upon further scanning. If the accumulation was performed again with the same electrode in the same DNA solution, peaks I_a and II_a reappeared while the pair of peaks $III_c - III_a$ remained the same. At the scan rate of 0.1 V s^{-1}, the peak potential for I_a, II_a, III_a, III_c and IV_a was 0.71, 0.81, 0.30, 0.27, and 0.37 V (vs. SCE), respectively. Additionally, another pair of redox peaks at about −0.1 V was observed, which is attributable to the oxidation and reduction of MWNTs [35].

The cyclic voltammetric behavior of DNA on the MWNT-electrode was tested over scan ranges. In the scan range of −0.4 to 0.6 V, no peak related to DNA was observed except the redox peaks of MWNTs. When the reversal potential was controlled at 0.75 V, only peak I_a was observed at the first anodic scan. In the first reverse scan, a small wave appeared at 0.35 V. In the second sweep, a new anodic peak appeared at 0.37 V, which was defined as IV_a (Fig. 1 part 2). These peaks soon disappeared upon further scanning. Therefore, the absence of the pair of peaks $III_c - III_a$ in the initial voltammetric scan indicates that it is necessary to do a preliminary scan with potentials sufficiently positive to carry out the oxidation process II_a. Thus, the appearance of peaks III_c and III_a was correlated with the redox transformation of some product of primary DNA oxidation II_a. If the reversal potential was extended to 1.15 V, another irreversible peak V_a was obtained at 1.0 V in the first anodic scan (Fig. 1 part 3). The current of this peak decreased quickly with the number of cycles in a similar fashion for peak I_a and II_a. By comparison, the cyclic voltammogram of denatured DNA at a nanotube-free (bare) electrode was also recorded. At the first cycle, two irreversible, weak peaks at 0.72 and 0.88 V was observed (Fig. 1 part 3d), corresponding to the oxidation of guanine and adenine residues, respectively [36]. Compared with the electrochemical behavior of DNA at various solid electrodes reported before [24–27, 29–34], the two new anodic peaks III_a and IV_a were observed by using the MWNT-modified glassy carbon electrode. As only the pair of peaks $III_a - III_c$ were stable and chemically reversible, these were the focus in this work.

As the electrochemically active species of DNA are guanine and adenine residues, which were studied alone and compared to their counterparts in the DNA structure. Figure 2 shows the cyclic voltammograms of guanine at the MWNT-modified electrode. At the first anodic scan, a single well-defined oxidation peak appeared at 0.70 V, corresponding to peak I_a of DNA. In the reverse scan, a cathodic peak at 0.35 V was observed. The subsequent anodic scanning led to a new oxidation peak at 0.37 V which formed a redox pair with the cathodic peak at 0.35 V. The reduction peak at 0.35 V quickly disappeared in the subsequent cathodic scanning, and the initial oxidation peak at 0.70 V and the new oxidation peak at 0.37 V decreased with the number of cycles. After the fifth scan, the

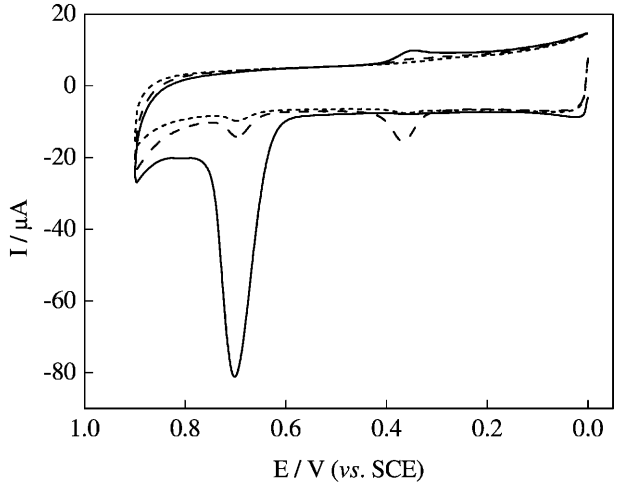

Fig. 2. Cyclic voltammograms of guanine (5 μg/mL) on MWNT modified GC electrode in 0.1 M phosphate buffer solution (pH 7.0) after 5 minutes of accumulation. Scan rate 0.1 V/s. a) First cycle (—); b) second cycle (----); c) the fifth cycle (·····).

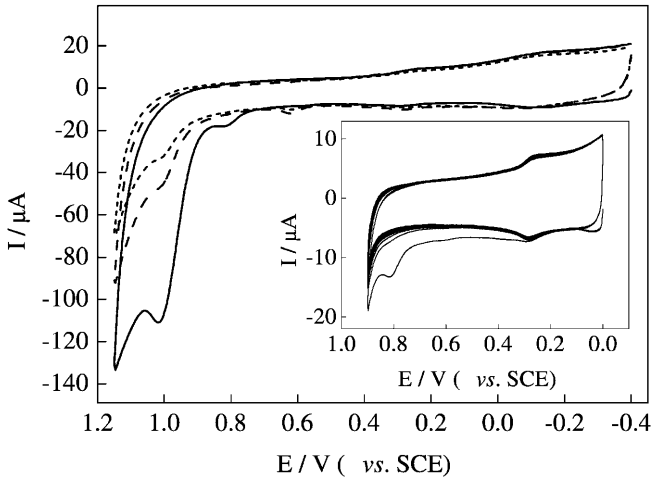

Fig. 3. Cyclic voltammograms of adenine (5 μg/mL) at a MWNT film modified electrode in 0.1 M phosphate buffer solution (pH 7.0) after 5 minutes of accumulation. Scan rate 0.1 V/s. a) First cycle (—); (b) second cycle (----); (c) third cycle (·····). Inset shows the continuous cyclic voltammograms of the same solution and the same electrode after another 5 minutes of accumulation, ten successive scans.

initial oxidation peak could be observed while the oxidation peak at 0.37 V was greatly reduced.

It was reported that guanine exhibited similar peaks at a polycrystalline gold electrode, but the new redox peaks could only be observed at a high scan rate (>1 V s^{-1}) [37]. The appearance of this pair of peaks was thought to be correlated with redox transformation between some products from the primary guanine oxidation. This pair of peaks was observed at the MWNT-modified GC electrode with a lower scan rate. The electrochemical oxidation of guanine on MWNT-modified electrode might follow a similar oxidation pathway as that on gold electrode [37]. The specific adsorption mode provided additional stabilization of the oxidized forms against their subsequent chemical transformation, which enabled observation of the electrochemical processes non-detectable with the bare GC electrode. As of poor stability, the redox pair of guanine generated on MWNT electrode was not studied further.

There is a long history on the study of the electrochemical oxidation of adenine. Early in 1968, Dryhurst and Elving [38] reported the electrochemical oxidation of adenine, where two irreversible peaks were obtained. In the study of Goyal et al. in 1991 [39], adenine was demonstrated to display an oxidation peak at the first anodic sweep, together with two redox peaks in the reverse and subsequent scans. However, the products of the adenine oxidation were very complicated. In recent years, the oxidation of adenine-containing compounds were investigated comprehensively by Tuñón-Blanco group. They found that at carbon paste electrode or pyrolytic graphit electrode, the electrochemical oxidation of the adenine moiety in nicotinamide adenine dinucleotide, adenosine, adenosinemonophosphate, adenosinediphosphate, adenosinetriphosphate, polyadenylic acid and even underivatized oligonucleotides such as deoxyadenylic acid icosanucleotide, gave rise to the redox-active products which strongly adsorbed in the electrode surface [40–44].

Similarly, the electrochemical behavior of adenine at the MWNT-modified electrode was studied and the results are shown in Figure 3. At the first anodic scan, two irreversible oxidation peaks were observed at 0.81 and 1.00 V, respectively, which decreased significantly in the subsequent scans. If the accumulation process was performed again and the scan range was restricted to 0–0.9 V, a redox pair at 0.27 and 0.30 V was clearly observed clearly, which was exactly the same as the III_a–III_c pair of DNA (Fig. 3, inset).

On the basis of the above findings, we can conclude that both adenine and guanine in denatured DNA form redox couples after their initial oxidation on MWNT-modified electrode. At the first anodic scan, guanine exhibited one oxidation peak (I_a). In the reverse and subsequent scan, a pair of new peaks related to guanine appeared, but all of them disappeared after several scanning cycles. On the other hand, adenine showed two oxidation peaks in the first anodic scan (II_a and V_a), which decreased with the number of cycles. A new reduction peak (III_c) related to adenine was obtained in the reversed scan, which formed a reversible couple with the oxidation peak III_a observed after the second scan. The pair of peaks III_c–III_a remained unchanged after many time voltammetric scans, and were therefore extensively studied.

After several cyclic voltammetric scans, the accumulation process was performed again and then the DNA-MWNT-modified electrode was removed from the DNA solution, rinsed thoroughly with water. The water-washed electrode was then immersed in a blank phosphate buffer solution before performing the electrochemical scan. As can be seen in Figure 4 part 1, peaks I_a, II_a, III_c and III_a were all observed at the first cycle. From the second cycle, peaks I_a and II_a

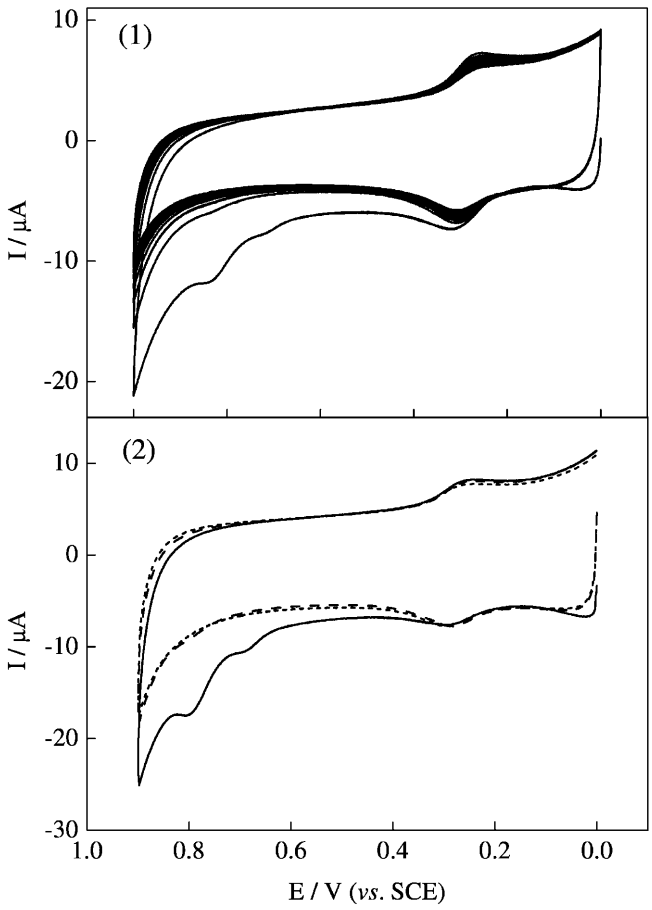

Fig. 4. 1) Cyclic voltammograms of DNA-MWNT modified electrode in 0.1 M phosphate buffer solution (pH 7.0) at the scan rate of 0.1 V/s, ten successive scans. 2) Cyclic voltammograms of DNA-MWNT modified electrode in 0.1 M phosphate buffer solution (pH 7.0) at the scan rate of 0.1 V/s after removing from DNA solution for a) 0 h (—), b) 1 h (----), and c) 24 h (·····).

disappeared and the peak currents of $III_c - III_a$ pair decreased slightly with the number of cycles, whereas the peak position of III_c became slightly more negative. If the scans were performed one by one (i.e. one cycle was done in one voltammetric scan), the peak currents and potentials of the $III_c - III_a$ pair were stable. As shown in Figure 4 part 2, the MWNT-modified electrode adsorbed with DNA was exposed to air for 24 hours in another experiment after removing it from the DNA solution, which resulted in little change in the peak potentials and peak currents.

The electrochemical behavior of the DNA-MWNT-modified electrode at different pH values was also studied. It was found that both peaks III_c and III_a shifted linearly to more negative potentials with increase in pH. In the pH range of 2.4 – 11.4, the potential shifts can be represented by: $E_{Pc} = 0.79 - 0.065$ pH and $E_{Pa} = 0.82 - 0.064$ pH, respectively. The slopes of the plots of E_{Pc} and E_{Pa} vs. pH (– 65 and – 64 mV/pH) were close to the theoretical value of – 58 mV/pH at 20 °C. This shows that, during the electrode reaction, one proton was corresponded to one electron transfer.

The potential scan rate also had an influence on the cyclic voltammetric behavior of the DNA-MWNT-modified electrode. A higher scan rate resulted in a higher current flow, as expected for a surface wave [45]. Both the reduction and oxidation peak currents were proportional to the scan rate over the range 0.01 to 1 V s^{-1}. When the scan rate was lower than 0.2 V s^{-1}, both the cathodic peak potential E_{Pc} and the anodic peak potential E_{Pa} remained unchanged despite increasing the scan rate. The peak width at half-height was > 90 mV; the peak separation was about 30 mV, which was larger than the ideal theoretical value of 0 mV for a monomolecular electroactive film on an electrode. Such behavior is characteristic of nonideal, quasireversible thin-layer voltammetry of redox species.

In conclusion, a pair of well-defined redox waves was obtained in a phosphate buffer solution containing calf thymus DNA at the electrode modified with a MWNT film. The observed redox waves were resulted from the initial oxidation product of adenine residues in DNA. The electrochemical behavior of DNA-MWNT-modified electrode was very stable and reversible.

Experimental

CT DNA (sodium salt), adenine and guanine were purchased from Sigma Chemical Co., USA, and used without further purification. All the other chemicals were analytical grade reagents. A phosphate buffer solution ($KH_2PO_4 + Na_2HPO_4$) was used as the supporting electrolyte. Double-distilled water was used throughout of the experiment.

To prepare the DNA solution, DNA (ca. 2 mg) was weighed and dissolved in 1 mL of water and stored at 4 °C for 24 hours to obtain a homogeneous solution. In order to denature DNA, the above DNA solution was heated in a boiling water bath for ca. 10 minutes, followed by rapid cooling in an ice bath.

Commercially available MWNTs (CVD method, Shenzhen Nanotech Port Co., Ltd, China) were purified via sonication in 1:3 concentrated nitric-sulfuric acid at ca. 50 °C, followed by filtration through a Teflon membrane with 0.45-μm pores. The resultant solid was washed thoroughly with double-distilled water until the pH value reaching ca. 6 and then dried in a vacuum oven at 50 °C overnight.

Cyclic voltammetry (CV) measurements were carried out on an ADI PowerLab/4sp potentiostat with PowerLab/Echem electrochemistry system. A normal three-electrode configuration consisting of a glassy carbon (GC) working electrode (3 mm in diameter), a saturated calomel reference electrode, and a platinum wire auxiliary electrode was used. All experiments were conducted at room temperature (about 20 °C).

To fabricate the MWNT modified electrode, 1.0 mg of the purified MWNTs was dispersed with the aid of ultrasonication in 10 mL of *N*, *N*-dimethylformamide (DMF) to give a 0.1 mg mL^{-1} black suspension. The GC electrode was first abraded with emery paper (No. 1500) and polished with 0.3 μm alumina slurry, then washed ultrasonically in distilled

water and ethanol, respectively. The GC electrode was coated by gradually casting 20 μL of the MWNT suspension prepared above and then dried under an infrared lamp.

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