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ORIGINAL PAPER

Kangbing Wu · Junjie Fei · Wen Bai · Shengshui Hu

Direct electrochemistry of DNA, guanine and adenine at a nanostructured film-modified electrode

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Abstract A nanostructured film electrode, a multi-wall carbon nanotubes (MWNT)-modified glassy carbon electrode (GCE), is described for the simultaneous determination of guanine and adenine. The properties of the MWNT-modified GCE were investigated by scanning electron microscopy (SEM) and cyclic voltammetry. The oxidation peak currents of guanine and adenine increased significantly at the MWNT-modified GCE in contrast to those at the bare GCE. The experimental parameters were optimized and a direct electrochemical method for the simultaneous determination of guanine and adenine was proposed. Using the MWNT-modified GCE, a sensitive and direct electrochemical technique for the measurement of native DNA was also developed, and the value of (G+C)/(A+T) of HCl-digested DNA was detected.

Keywords Carbon nanotubes · Guanine · Adenine · DNA · Modified electrode

Abbreviations *MWNT* Multi-wall carbon nanotubes · *G* Guanine · *A* Adenine · *GCE* Glassy carbon electrode · *DHP* Dihexadecyl hydrogen phosphate · *SEM* Scanning electron microscope

Introduction

Direct electrochemistry of nucleic acids (NAs) has attracted much attention among analytical chemists. Nucleic acids analysis presently encompasses a wide range of fields, from molecular pathology to environmental or forensic sciences. This fact has encouraged chemists to develop faster, simpler, and more sensitive techniques to meet the various demands. In 1960, electroactivity of NAs was discovered [1], and electrochemical devices have been considered as promising tools for these purposes. The analysis of nucleic acid is highly desirable if contamination derived from sample handling and cumbersome labeling procedures are to be avoided. Within this aim, two major approaches involving direct and indirect methods have been developed.

Indirect methods are based on the determination of electroactive indicators that intercalate or otherwise associate with double-stranded DNA (dsDNA) [2, 3, 4, 5]. Direct methods mainly rely on the intrinsic electrochemical activity of the nucleobases [6], although sugar residues have recently been reported to be oxidized at a copper microelectrode [7]. Nucleobases undergo reduction at mercury electrodes and oxidation at different solid electrode such as carbon, gold, silver, platinum and copper, however, the use of these processes as analytical signals is limited, because their voltammetric peaks are poorly developed with very high overpotential that the peaks merge into the background discharge current. Recently, a number of works have been reported to overcome this drawback and many articles concerning direct measurement of nucleic acids or guanine and adenine have been published [8, 9, 10, 11, 12, 13, 14, 15]. To our knowledge, the direct electrochemical measurement of DNA or guanine and adenine at the MWNT-modified electrode has not been reported.

Since their discovery in 1991 [16], carbon nanotubes have attracted considerable attention, because they combine unique electronic structures, high specific surface area, electrical conductivity, and excellent strength. They show great potential for materials application and may be used as scanning probes [17, 18], electron field emission sources [19, 20], actuators [21], nanoelectronic devices [22], batteries [23], nanotube-reinforced materials [24], potential hydrogen storage material [25] and chemical sensors [26]. The subtle electronic behavior of carbon nanotubes has been used in electrode components in electrochemistry. For example, the electrochemical behavior of dopamine [27] and protein [28] at carbon nanotune electrodes have been reported. More recently, single-wall carbon nanotube (SWNT) film-coated glassy carbon electrodes [29], which exhibit electrocatalytic activity to the

K. Wu · J. Fei · W. Bai · S. Hu (✉)
Department of Chemistry, Wuhan University,
430072 Wuhan, P.R. China
e-mail: sshu@whu.edu.cn

oxidation of dopamine, ascorbic acid and epinephrine, MWNT-modified electrodes [30], with catalytic ability toward the oxidation of nitric acid, and carbon nanotube-modified electrodes for NADH detection [31], have been reported.

In our previous work, MWNT were dispersed in redistilled water via ultrasonication in the presence of hydrophobic surfactant, and a MWNT film-coated electrode for the simultaneous determination of uric acid (UA) and xanthine (XA) was reported [32]. In this work, a sensitive electrochemical technique for the simultaneous determination of guanine and adenine based on the MWNT film-modified GCE is described. It was found that this MWNT film-coated GCE shows electrocatalytic activity in the oxidation of guanine and adenine, and based on this a direct electrochemical method was developed to determine trace levels of DNA.

Experimental

Apparatus and reagents

All the electrochemical measurements were performed with a 263A Potentiostat/Galvanostat (EG&G, USA). The active surface area of the GCE was 0.071 cm^2. A three-electrode system, consisting of a modified GC working electrode, a platinum wire counter-electrode, and a saturated calomel reference electrode (SCE), was employed. All potentials were referred to the SCE. The scanning electron microscopy (SEM) was performed with an Hitachi X-650 microscope.

The MWNT used in this work (obtained from the Institute of Nanometer, Huazhong Normal University) were synthesized by the catalytic pyrolysis method and then refluxed for 10 h in HNO_3 [33, 34]. It has been known that this treatment causes segmentation and carboxylation of MWNT at their terminus. Guanine, adenine, calf thymus DNA (Sigma) and dihexadecyl hydrogen phosphate (DHP) (Aldrich) were used as received without further purification. Aqueous solutions were prepared with redistilled water and other chemicals used were of analytical grade.

Native calf thymus double-stranded DNA (dsDNA) solution (1 mg mL^{-1}) was prepared by dissolution in 10 mmol L^{-1} pH 8.0 Tris-HCl buffer containing 1 mmol L^{-1} EDTA (TE buffer) prior to use.

Preparation of modified GCE

MWNT (5 mg) and DHP (5 mg) were dispersed in 5 mL of redistilled water by ultrasonic agitation for about 20 min to give a 1 mg mL^{-1} blank suspension. Prior to modification, the GCE was polished with a 0.05 μm alumina slurry (CH Instruments, USA), rinsed thoroughly with redistilled water, then sonicated in redistilled water for 1 min. Finally, the GCE was coated with 5 μL of 1 mg mL^{-1} MWNT-DHP suspension and left for the water to evaporate at room temperature in the air. The DHP-modified electrode was prepared by the same procedure explained above, but without MWNT.

Preparation of DNA samples

A general treatment of DNA with 1 mmol L^{-1} HCl leads to the selective removal of its purine bases by cleavage of purine glycoside bonds [35]. The calf thymus dsDNA was hydrolyzed as follows for quantification of guanine and adenine. Three milligrams of dsDNA was digested using 1 mL of 1 mol L^{-1} HCl in a 10-mL glass tube. After heating in a boiling water bath for 80 min, the pH of the solution was adjusted with 1 mL of 1 mol L^{-1} NaOH. Then the solution was diluted to 10 mL using 0.1 mol L^{-1} phosphate buffer (pH 7.0).

Experimental procedure

Phosphate buffer (0.1 mol L^{-1}, pH 7.0, 10 mL) was added into the electrochemical cell and the MWNT-DHP film-coated GCE was activated by means of cyclic voltammetric sweeping from 0 V to 1.1 V until the voltammograms were stable. After that, the desired volume of standard guanine, adenine or DNA solution was added by micropipette, and the voltammograms were recorded from 0.5 V to 1.1 V. For repetitive measurements, the MWNT-DHP-modified GCE undergoes successive cyclic sweeping between 0.5 V to 1.1 V in the blank phosphate buffer (pH 7.0) to give a fresh electrode surface.

The best parameters for differential pulse voltammetry (DPV) on the MWNT-DHP-modified GCE are accumulation time=2 min; pulse amplitude=50 mV, scan rate=20 mV s^{-1}, pulse width=50 ms.

Results and discussion

SEM images of DHP-film and MWNT-DHP film-coated GCE

Figure 1 shows the SEM images of the DHP film (left) and MWNT-DHP film (right) on the surface of the GCE.

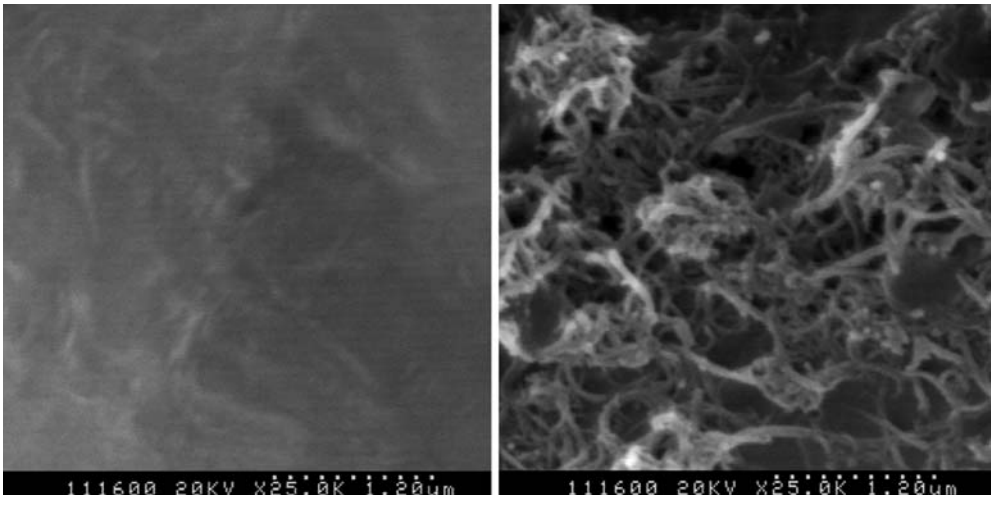

Fig. 1 SEM image of the DHP film (*left*) and the MWNT-DHP film (*right*) on a GC disk with magnification 25000×

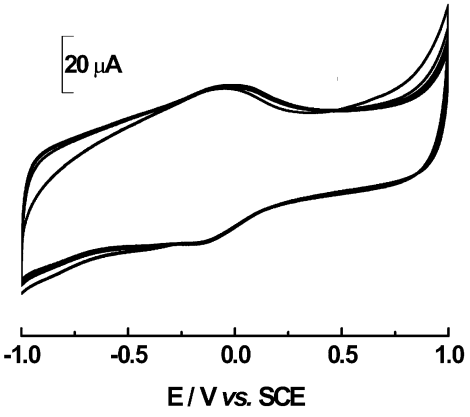

Fig. 2 Cyclic voltammograms of the MWNT-DHP-modified GCE in 0.1 mol L^{-1}, pH 7.0, phosphate buffer (removal of oxygen). Scan rate: 0.1 V s^{-1}

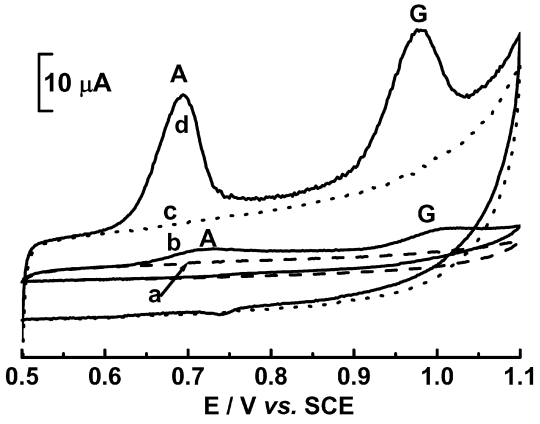

Fig. 3 Cyclic voltammograms of 1×10^{-5} mol L^{-1} guanine (*G*) and adenine (*A*) in pH 7.0 phosphate buffer (0.1 mol L^{-1}) at three different electrodes without accumulation: (*a*) DHP-modified GCE; (*b*) bare GCE; (*d*) MWNT-DHP film-coated GCE. Curve (*c*): cyclic voltammogram of MWNT-DHP film-coated GCE in 0.1 mol L^{-1}, phosphate solution (pH 7.0) without guanine and adenine. Scan rate: 0.1 V s^{-1}

As can be seen, DHP forms a thin and uniform film on the GCE surface. From the SEM images of MWNT-DHP cast film, many nanocarbon tubes with average diameters ranging from 30–40 nm were observed. It also can be seen that the negligible carbon particle impurities were observed. From comparison of these two images it is very clear that the GCE surface is cast with MWNT.

Electrochemical behavior of MWNT-DHP-modified GCE

The electrochemical properties of the MWNT-DHP film-coated GCE were examined by cyclic voltammetry (CV). Figure 2 shows a successive cyclic voltammograms of MWNT-DHP-modified GCE in 0.1 mol L^{-1}, pH 7.0 phosphate buffer (removal of oxygen). A redox couple, with cathodic and anodic peak potentials at –0.12 V and –0.02 V, was observed. The peak potentials and peak currents remained very stable during the successive potential sweeps. The redox peak currents and potentials are closely related to the solution pH. When the potential is positive to 0.8 V or negative to –0.8 V, the background current increased greatly. Similar results were observed in previous work [29, 30]. The overall stability of the MWNT-DHP-modified GCE was evaluated by repeating the cyclic voltammetric experiments after it was exposed to the air for 24 h. Negligible change in the peak currents and peak potentials was observed, suggesting that the MWNT-DHP film on the GCE surface exhibited remarkable stability.

Voltammetric responses of guanine, adenine and DNA

The cyclic voltammograms of 1×10^{-5} mol L^{-1} guanine (G) and adenine (A) in pH 7.0 phosphate buffer on three different electrodes are illustrated in Fig. 3. The responses at the bare GCE are very poor with two small humps at 0.73 V (peak G) and 1.02 V (peak A) (curve b). At the DHP-film-coated GCE the voltammetric signals of G and A disappear because the DHP film blocks their electron transfer with the GCE (curve a). However, the oxidation peak currents of G and A at the MWNT-DHP film cast GCE increase significantly resulting in two huge and well-defined peaks at 0.68 V (peak G) and 0.97 V (peak A) (curve d). The remarkable enhancement in the peak current and the lowering of oxidation overpotential are clear evidence of the catalytic effects of MWNT toward guanine and adenine oxidation.

Figure 4 shows the cyclic voltammograms of 50 μg mL^{-1} calf thymus DNA in pH 7.0 phosphate buffer. There is no electrochemical response corresponding to calf thymus DNA at the bare GCE (curve a) and the DHP-modified GCE (curve b). However, two well-defined oxidation peaks at 0.68 V (peak G) and 0.97 V (peak A) appear at the MWNT-DHP film-coated GCE; these are attributed to oxidation of the G and A content of the DNA (curve c).

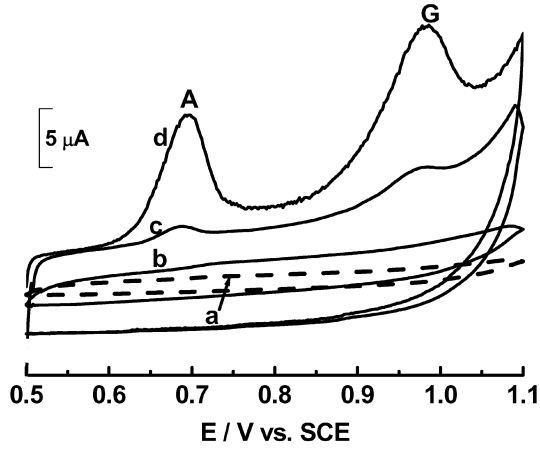

Fig. 4 Cyclic voltammograms of 50 μg mL^{-1} calf thymus DNA in 0.1 mol L^{-1} phosphate buffer (pH 7.0). (*a*) bare GCE; (*b*) DHP-modified GCE; (*c*) MWNT-DHP-modified GCE; and (*d*) MWNT-DHP-modified GCE for an accumulation time of 2 min. Scan rate: 0.1 V s^{-1}. *G*, guanine; *A*, adenine

After 2 min of accumulation, the oxidation signals increase significantly at the MWNT-DHP-modified GCE (curve d). The separation between the oxidation potential of G and A is about 290 mV, indicating that the signals of G and A do not interfere with each other.

Effects of scan rate and solution pH

The influences of scan rate on the oxidation peak currents and potentials of guanine and adenine at the MWNT-DHP-modified GCE in pH 7.0 phosphate buffer were studied by linear sweep voltammetry (LSV). The results from LSV showed that the peak current (i_p) was directly proportional to the scan rate (ν) and the E_p shifted to more positive values with increasing ν. The current function ($i_p/C\nu$) remained unchanged with ν indicating an adsorption process. Meanwhile, the electrode process was totally irreversible as confirmed by the fact that there was no reduction peak observed in the cyclic voltammograms and from the observation of the E_p shift with scan rate in LSV. The number of electrons involved in the oxidation of guanine and adenine was then evaluated. On the basis of the slope of E_p with log(ν) in LSV, the αn_α is calculated to be 1.03 for guanine and 0.97 for adenine. Assuming α is equal to 0.5, the value of n_α is 2. This is in good agreement with previous results that the oxidation of guanine and adenine follows a two-step mechanism involving the total loss of $4e^-$, and that the first $2e^-$ oxidation is rate-determining step [36].

Both the oxidation peak currents and peak potentials were dependent on the solution pH of the phosphate buffer. The oxidation peak currents of guanine and adenine at the MWNT-DHP film-coated GCE changed slightly over the pH range from 5.0 to 8.0. Unlike to peak currents, the oxidation peak potentials (E_p) of guanine and adenine obey the equations: E_p=1.102–0.056pH (r=0.998) and E_p=1.384–0.061pH (r=0.997), respectively. The slopes of 56 and 61 mV/pH suggest that two protons take part in the rate-determining step.

Adsorption of guanine and adenine

The relationship between i_p and ν indicates that the electrode process of guanine and adenine is adsorption-controlled. To infer more about the adsorption of guanine and adenine on three different electrodes, chronocoulometry was investigated. From the intercepts of the Anson's plots, the excess charge (Q_{ads}) corresponding to the adsorbed component (after background charge correction from the backward step) can be obtained, and the results are summarized in Table 1. Under identical conditions, the value of Q_{ads} of guanine and adenine at the DHP-modified GCE is much lower than that at the bare GCE, suggesting that the DHP film blocks the adsorption of guanine and adenine. Conversely, the value of Q_{ads} of guanine and adenine at the MWNT-DHP film-coated GCE increased significantly in comparison with that at the bare GCE, indicating that MWNT offers more effective accumulation of guanine and adenine. There is no doubt that the MWNT-DHP-modified electrode gives more sensitive oxidation signals to guanine and adenine.

Table 1 Q_{ads} of 2×10^{-5} mol L^{-1} guanine and adenine in 0.1 mol L^{-1}, pH 7.0, phosphate buffer at three different electrodes. Chronocoulometry experimental conditions: E_i=0.5 V, E_f=1.1 V, pulse width 10 s, sample interval=0.00025

	GCE (μC)	DHP-modified GCE (μC)	MWNT-DHP modified GCE (μC)
Q_{ads} of G	5.5	2.6	40
Q_{ads} of A	4.2	2.5	41

The oxidation peak currents of guanine and adenine after 2 min of accumulation at different potential were measured. The results revealed that the accumulation potential did not affect the oxidation peak currents, and open-circuit accumulation therefore was performed in this work.

The influence of accumulation time on the oxidation peak currents at the MWNT-DHP-modified GCE was investigated. The oxidation peak currents increased remarkably within the first 2 min, and then leveled off. This may be caused by the fact that the adsorption of guanine and adenine on the MWNT-DHP-modified electrode surface becomes saturated.

Individual determination of guanine and adenine

The calibration curve for guanine in pH 7.0 phosphate buffer was measured by DPV. Two linear segments were observed. The initial linear portion increases from 0.02 to 2 μmol L^{-1} with a slope of 7.05 μA μmol^{-1} L (r=0.9991). The second linear segment up to 5.4 μmol L^{-1} with a regression equation of i_p=0.45+1.98×10^{6}C (r=0.997, C in mol L^{-1}). The initial linear portion is understandable due to the strong adsorption of guanine on the MWNT-DHP-modified GCE. The detection limit of 7.5×10^{-9} mol L^{-1} was obtained with accumulation for 2 min. The relative standard deviation (RSD) of 3.6% for 1 μmol L^{-1} guanine (n=10) showed excellent reproducibility.

As to adenine, similar studies were also done as in the case of guanine and the calibration curve yields a linear range from 0.01 to 5 μmol L^{-1} and is saturated at higher concentration. The detection limit (signal to noise=3) is 5×10^{-9} mol L^{-1} for 2 min accumulation. Similarly, to calculate the reproducibility of the MWNT-DHP film-coated electrode, 10 continuous measurements of 1 μmol L^{-1} adenine show good reproducibility with a 3.8% RSD.

Simultaneous determination of guanine and adenine

The voltammetric responses of guanine (or adenine) in the presence of large excess of adenine (or guanine) were examined by DPV in pH 7.0 phosphate buffer. The oxidation peak current of 5×10^{-7} mol L^{-1} guanine holds stable in the presence of adenine from 0 to 1×10^{-5} mol L^{-1}. Further increasing the adenine concentration caused a decrease in

Table 2 Guanine and adenine content of HCl-digested calf thymus DNA determined with the MWNT-DHP-modified GCE

	Digested by HCl
Guanine (mol%)	22.34
Adenine (mol%)	27.94
Molar ratio ((G+C)/(A+T))	0.80

the peak current. A similar phenomenon was observed for adenine. Although there is a competitive adsorption at the MWNT-DHP-modified electrode, the competitive adsorption equilibrium can be attained at suitable concentrations of guanine and adenine, and this proposed method is suitable for the simultaneous determination of guanine and adenine.

Analytical applications

Native double-stranded DNA (dsDNA) gives two well-defined peaks at the MWNT-DHP film-coated GCE due to the oxidation of guanine and adenine residues. The detection limit of 30 ng mL^{-1} with 2 min of open-circuit accumulation was obtained for calf thymus dsDNA. Ten continuous measurements of 5 μg mL^{-1} and 50 μg mL^{-1} calf thymus dsDNA show good reproducibility with a 4.1% and 3.2% RSD, respectively.

In this work, the MWNT-DHP-modified GCE was used to detect the guanine and adenine content of acid-digested calf thymus dsDNA. The determination of guanine and adenine concentrations was performed by standard addition method as follows. Fifty microliters of the HCl-digested DNA solution was added into a cell containing 10 mL of pH 7.0 phosphate buffer and the peak currents of guanine and adenine were measured. Then a certain quantity standard of solution of guanine and adenine was added and the peak currents of guanine and adenine were measured again. From the difference between the peak currents, the concentrations of guanine and adenine in DNA can be obtained. The results are summarized in Table 2.

Using the proposed method, the value (G+C)/(A+T) of 0.80 was obtained for HCl-digested calf thymus DNA sample, which accords with standard value of 0.77 [37].

Conclusion

The MWNT-DHP film-coated GCE exhibits remarkable enhancement effects on the oxidation peak currents of guanine and adenine. Due to its strong adsorptive property and high specific surface area, MWNT shows very highly effective accumulation of guanine and adenine and consequently, significantly improves the oxidation currents of guanine and adenine. The proposed method can be used to simultaneously determine guanine and adenine free or contained in ds DNA with the following advantages: direct detection, high sensitivity, rapid response, excellent reproducibility, and extreme simplicity.

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