Direct electrochemistry of thermally denatured calf thymus DNA on a poly(methyl methacrylate)–graphite microcomposite electrode

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Remarkable direct electrochemical behaviors of thermally denatured calf thymus DNA (ssDNA) on the poly(methyl methacrylate)–graphite powder microcomposite electrode (PMMA/GME) were observed and investigated. The result indicated that the PMMA/GME showed great promotion of the electrochemical response towards the ssDNA oxidation due to the specific characteristics of the nanostructured interface on the resultant composite electrode. Two irreversible oxidation peaks at +1.04 and +0.76 V with obvious negative movement of oxidation potential and increase of the oxidation current (*vs.* Ag/AgCl) were observed on the PMMA/GME, which corresponded to the oxidation of adenine and guanine residues in the ssDNA molecules. Therefore, detection of ssDNA was then performed at the PMMA/GME. Under the optimal conditions, a good linear relationship was obtained between the oxidation peak currents of guanine or adenine residues and the ssDNA concentration in the range of 5.9×10^{-3} to *ca.* 1.1 mg mL^{-1} with a detection limit of 1.0 μg mL^{-1}. This PMMA/GME exhibits some outstanding advantages, such as ease of fabrication, high stability, renewable surface, excellent stability, mechanical rigidity and high electrochemical reactivity, which holds huge promise for further DNA biosensor design.

1. Introduction

Identification of specific nucleic acid sequences of viral, bacterial pathogens, hereditary diseases and genetic abnormalities is of widespread interest in the areas of medicine, biotechnology and homeland security. Therefore, the relative research about the direct electrochemistry about nucleic acids has aroused enormous interest. Till now, many methods have been reported for the quantitative analysis of nucleotides and DNA.[1–3] Electrochemical study on DNA has been widely reported over the past few years.[4–8] There have been various kinds of electrodes devised for studying DNA electrochemistry.[9–14] However, the direct electrochemistry of DNA is associated with the oxidation of guanine and adenine residues,[5] and usually suffers from a high electro-oxidation potential. And also the microcosmic construction of the solid electrode utilized in the experiment affects obviously the electrochemical behavior of DNA. To overcome this drawback, some modified electrodes, such as carbon nanotubes (CNTs),[15] poly[$Ru(vbpy)_3^{2+}$] film,[16] fullerene-C_{60}-modified glassy carbon electrode,[17] boron-doped diamond electrode[18,19] and ionic liquid modified carbon paste electrode (CILE)[14,20] have been developed to lower the overpotential and increase sensitivity.

Carbon paste electrodes (CPEs) have been largely used for the preparation of biosensors due to the facility of modifying the entire bulk material with different organic, inorganic or biological compounds. They are characterized by a decreased residual current and a better reproducibility of currents compared to the pure carbon material.[21–23] We have fabricated a novel poly(methyl methacrylate)–graphite powder microcomposite electrode (PMMA/GME) and investigated its electrochemical behaviors towards some biomolecules. It has been found that this modified electrode showed a significant improvement in electrochemical reactivity and reversibility, which can effectively promote the electrochemical reactions of some biomolecules, such as NADH, ascorbic acid, uric acid, acetaminophen, norepinephrine, adrenalin and serine.[24]

In order to explore further application of the PMMA/GME in the electrochemistry of biomacromolecules, this paper reports the electrochemical oxidation behavior of ssDNA on this composite electrode. The results suggested that the ssDNA can be adsorbed on the surface of the PMMA/GME and exhibited an apparent negative shift of the oxidation peak potential and a great enhancement of the oxidation peak current.

2. Experimental

2.1. Chemicals

Calf thymus DNA (ctDNA) was purchased from Sigma. Methyl methacrylate (MMA), and 2,2-azobis-isobutyronitrile (AIBN) were purchased from SinoPharm (Shanghai, China). The graphite powder was purchased from Aldrich. All other chemicals were analytical grade or better. Phosphate buffer solution (PBS) was prepared by mixing suitable amount of 0.1 mol L^{-1} NaH_2PO_4/Na_2HPO_4 and adjusted to the pH with 0.1 mol L^{-1} H_3PO_4 or 0.1 mol L^{-1} NaOH. All solutions were prepared with ultrapure water obtained from a Milli-Q water purification system.

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2.2. Instruments

Electrochemical studies were carried out using a CHI620 electrochemical workstation (CHI, USA) with a conventional three-electrode electrochemical cell using PMMA/graphite paste electrodes, KCl-saturated silver–silver chloride (Ag|AgCl) and a platinum wire as the working, reference and counter electrodes, respectively.

2.3. Denaturation of ctDNA

Thermal denaturation involves the rupture of hydrogen bonds and the disturbance of stacking interaction; no covalent bond is broken through this process, so thermally denatured dsDNA may act as single-stranded DNA (ssDNA). The temperature-denatured DNA was made by the following procedure: ctDNA stock solution was incubated at 100 °C for 15 min then cooled rapidly, and under this condition DNA bases were decomposed.[25,26]

2.4. Procedure

The graphite/PMMA composite electrodes were prepared following the procedure proposed in our previous report.[27] The surface of the resulting paste electrodes was smoothed and rinsed carefully with water prior to each measurement.

The electrochemical experiments were performed in 0.1 mol L^{-1} PBS with certain concentrations of ssDNA. The preconcentration potential and time were carefully optimized. Typically, the preconcentration process of ssDNA at the PMMA/graphite was carried out at the potential of +0.25 V for 300 s. Cyclic voltammograms were recorded from +0.3 to +1.1 V at the scan rate of 100 mV s^{-1} after a 2 s quiet period.

3. Results and discussion

3.1. Morphology of PMMA/GME

In order to further investigate the reason why this electrochemical platform exhibits favorable electrochemical activity towards biological molecules, FSEM images were determined.[24] In the SEM image, most of the PMMA/GME interface is with considerable lacunose form and rich in surface defects. Its edge plane site is only about 18 nm (Fig. 1) on the interface of PMMA/GME. The good conductivity and outstanding electrocatalysis may be due to its abundant edge plane site like defective sites which may provide a great deal of active sites for electron transfer to biological species.

3.2. Adsorption feature of ssDNA on PMMA/GME

The investigation reported that the direct electrochemistry of DNA including guanine and adenine at a sensing platform is favorable for the fabrication of the sensitive electrochemical biosensors for native DNA. Fig. 2 shows the typical cyclic voltammograms on the PMMA/GPE in pH 5.5 PBS containing 0.2 mg mL^{-1} ssDNA at the scan rate of 100 mV s^{-1} after preconcentration at +0.25 V for 300 s. Two irreversible oxidation peaks appear on the PMMA/GME at the potential of +0.76 and +1.04 V (*vs.* Ag/AgCl), which are corresponded to the oxidation of guanine and adenine residues on the ssDNA molecules,

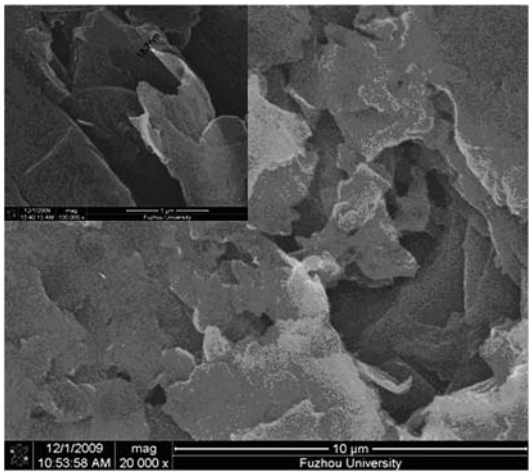

Fig. 1 FSEM images of PMMA/GME.

respectively.[5] While, no oxidation peak appears on PMMA/GME in blank PBS solution. Compared with those on other modified electrodes,[5,14] these oxidation potentials were slightly shifted to the negative direction. The result indicated that the PMMA/GME shows great promotion to increase the electrochemical response towards the ssDNA oxidation. It corresponded to our previous work[24] that the PMMA/GME could provide some decrease in the over-potential of biomolecules, which might be attributed to the conducting network within the polymer matrix developed by PMMA and graphite powder. The intrinsic conductivity and the nanostructure construction of graphite powder is probably the key factor governing the electrical conductivity of the graphite powder and PMMA composite film. Similar to our previous result, the rough and lacunose network construction of graphite powder and PMMA polymer matrix could act as an efficient electron conducting tunnel between the matrix interface and electrolyte.[24] However, such a special architecture of PMMA and graphite powder composite electrode interface could further be employed for high quantity load of the immobilization of biomacromolecules. Additionally, coupled with the high-performance of the proposed conducting

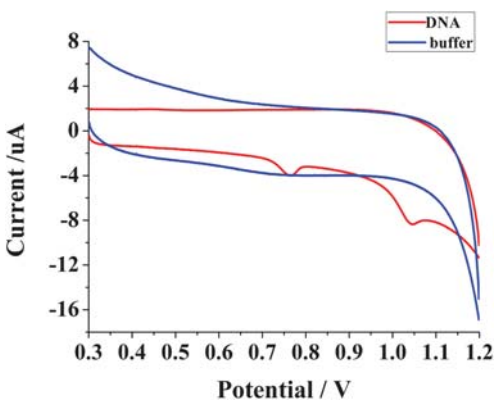

Fig. 2 Cyclic voltammograms of PMMA/GME in pH 5.5 PBS containing 0.1 mg mL^{-1} ssDNA (red line) and in the buffer solution (blue line) after preconcentration at +0.25 V for 300 s with a scan rate of 100 mV s^{-1}.

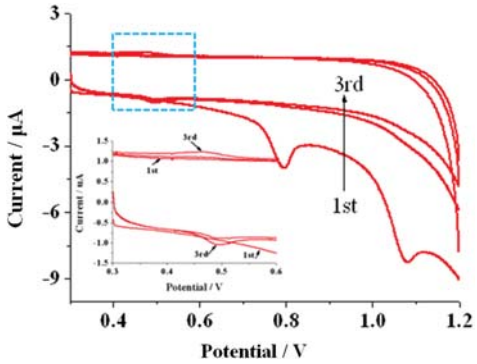

Fig. 3 Adsorption of ssDNA on PMMA/GME, electrochemical behavior of ssDNA on P PMMA/GME with successive scans; other conditions were the same as those of Fig. 1.

network, such PMMA/GME thus could be an ideal sensing interface for biomolecules, especially for immobilized biomacromolecules. In successive scans (Fig. 3), the oxidation current becomes lower with the scanning, and in the second scan the oxidation peaks nearly disappear. This was due to the adsorption of the oxidation product at the electrode surface to impede the nucleic acid to further reach to the electrode surface, which was in agreement with previous work.[5–8,28] However, a pair of new peaks appeared at the potentials of +0.46 and +0.49 V (see the insert of Fig. 3), respectively, which corresponds to the oxidation of free guanine base.[29]

3.2. Influence of the scan rate

Fig. 4 shows the voltammograms of ssDNA at the PMMA/GME in 0.1M PBS (pH 5.5) at various scan rates range from 10 to 100 mV/s. The inset of Fig. 4A shows the linear dependence of the peak currents on scan rate. It can be seen that both the oxidation peak currents of adenine and guanine residues increase with the scan rate and the oxidation peak potential shifts towards a more positive position. A good linear relationship was obtained between the oxidation peak currents and the scan rate (υ) in the range of *ca.* 10–100 mV s^{-1} with the linear regression equations of I_{pa} (μA) = −0.1227 − 0.023υ (mV s^{-1}) (n = 6, γ = 0.9950) for guanine residues and I_{pa} (μA) = −0.6104 − 0.523υ (mV s^{-1}) (n = 6, γ = 0.9940) for adenine residues, respectively. These

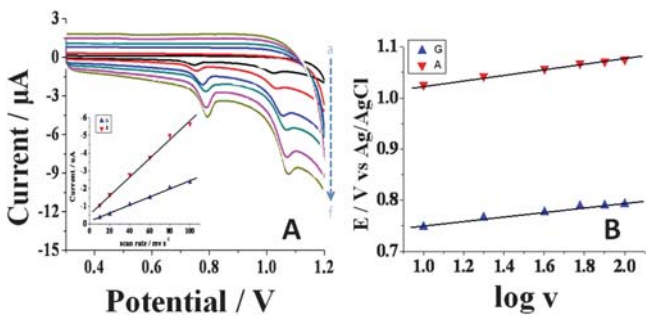

Fig. 4 (A) Cyclic voltammograms of 1 mg mL^{-1}ssDNA in pH 5.5 PBS with different scan rates (from a to f: 10, 20, 40, 60, 80, 100 mV s^{-1}). The inset was the relationship between the oxidation current and scan rate. (B) Plot of the oxidation peak potentials (E_{pa}) *versus* log υ.

results indicated that the electrode process was adsorption controlled.

The surface coverage Γ of the adsorbed ssDNA can be calculated with the aid of the equation,[30]

$$\Gamma = Q/nFA$$

where the charge Q is the total area of adenine and guanine oxidation of ssDNA, A is the geometric area of the modified GPE and n is the electron transfer number (the value of 4 was taken according to the results of Palecek[31]). According to this method, the value of Γ was calculated to be $(2.42 \pm 0.07) \times 10^{-10}$ mol cm^{-2}. This surface density of oligonucleotides on PMMA/GME was considerable higher than that reported for other DNA modified surfaces.[32–34] Furthermore, as observed from Fig. 4A, the oxidation peak potential shifted gradually towards a more positive position. A good linear relationship between the oxidation peak potentials and the log υ was found to be obey the linear regression equations of E_{pa} (V) = 0.0506log(υ/V s^{-1}) + 0.9755 (n = 6, γ = 0.9940) for adenine residues and E_{pa} (V) = 0.0434log(υ/V s^{-1}) + 0.708 (n = 6, γ = 0.9910) for guanine residues (Fig. 4B). The charge transfer coefficient (α) was estimated to be 0.75 (for adenine residues) and 0.70 (for guanine residues) by cyclic voltammetry with Laviron's method.[35]

3.3. Influence of pH

As can be seen in Fig. 5A, the oxidation potentials of ssDNA were pH dependent. With increasing pH of the supporting electrolyte, the two potentials shift negatively. It can be seen that two linear relationships were found in the pH range from 4.5 to 7 with a slope of −65 mV/pH for adenine residues and −61 mV/pH for guanine residues. The values were close to the ideal value of 59.0 mV/pH. Since the oxidation of guanine and adenine themselves has been proved to follow a two-step mechanism involving the total loss of four electrons and the first $2e^-$ loss was the rate

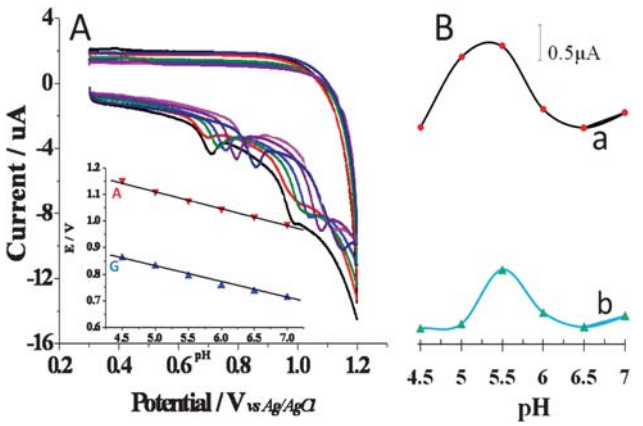

Fig. 5 Influence of pH. (A) Cyclic voltammograms of PMMA/graphite electrode in PBS with various pH values containing 1 mg mL^{-1} ssDNA after preconcentration at +0.25 V for 300 s with a scan rate of 100 mV s^{-1}. The inset was the relationship between the oxidation potentials and pH values. (B) The current response of guanine (a) and adenine (b) residues of ssDNA on PMMA/graphite electrode with various pH PBS containing 1 mg mL^{-1} ssDNA after preconcentration at +0.25 V for 300 s with a scan rate of 100 mV s^{-1}.

determining step, the slope values indicated that two protons took part in the rate determining step. This result corresponds to the reports by Sun *et al.*[20] and Wang *et al.*[15] The oxidation peak currents were also influenced by pH values and the maximum one appeared at pH 5.5 (seen Fig. 5B). So pH 5.5 PBS buffer was chosen in the following experiments.

3.4. Influence of the preconcentration potential and time

Since the oxidation of ssDNA on the PMMA/graphite paste electrode was an adsorption-control process, the preconcentration conditions were optimized and the results were shown in Fig. 6. The peak current reached its maximum at the preconcentration potential of +0.25 V. The influence of the preconcentration time on the cyclic voltammetric response was also investigated in the range of *ca.* 200–550 s. It was found that the maximum peak current was obtained at the preconcentration time of 300 s. Hence, the preconcentration potential of +0.25 V and preconcentration time of 300 s were employed in the following procedure.

3.5. Electrochemical detection of ssDNA

Under the optimized conditions the determination of ssDNA on PMMA/GME was performed with cyclic voltammetry at the scan rate of 100 mV s^{-1} in pH 5.5 PBS buffer solution after preconcentration at +0.25 V for 300 s. As shown in Fig. 7, the oxidation peak current increased with the ssDNA concentration over the range of 5.9×10^{-3} to *ca.* 1.1 mg mL^{-1} with a detection limit of 1.0 μg mL^{-1} (3σ). The linear regression equations were:

$$I_{pa}\ (\mu A) = 3.9606C\ (\text{mg mL}^{-1}) + 3.747\ (\text{adenine residues};\ n = 8,\ \gamma = 0.9960)$$

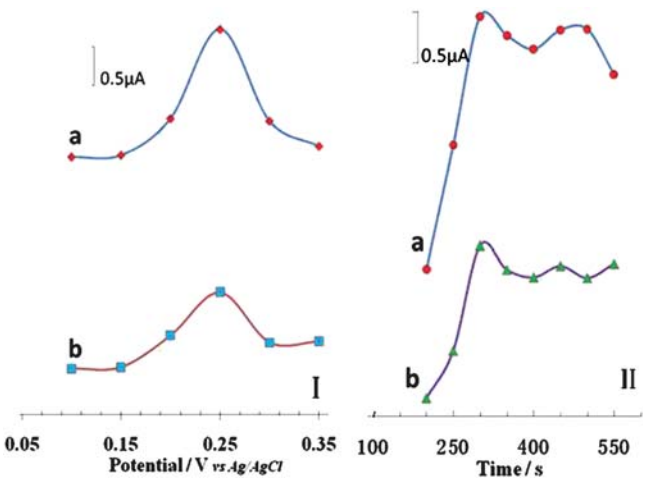

Fig. 6 Influences of preconcentration conditions on the electrochemical behavior of ssDNA on PMMA/graphite composite electrode. PBS pH 5.5, scan rate of 100 mV s^{-1}. (I) The effect of applied potential of preconcentration on the electrochemical response of ssDNA (guanine (a) and adenine (b)); preconcentration time: 300 s. (II) The effect of preconcentration time on the electrochemical response of ssDNA (guanine (a) and adenine (b)); preconcentration potential: +250 mV.

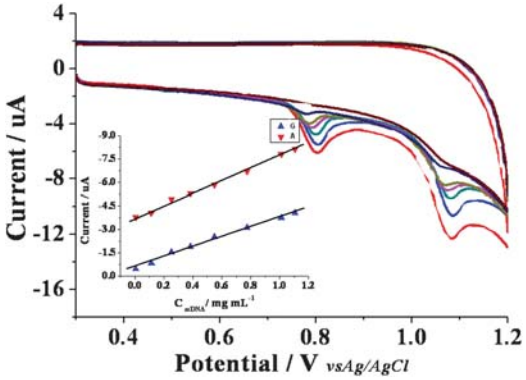

Fig. 7 Cyclic voltammograms of PMMA/GME in the presence of different concentrations of ssDNA in 0.1 M PBS (pH 5.5) with scan rate 100 mV/s. Inset was the calibration curve.

and $I_{pa}\ (\mu A) = 3.2156C\ (\text{mg mL}^{-1}) + 0.5856$ (guanine residue; $n = 8$, $\gamma = 0.9910$).

The electrode surface needs to be renewed after each detection, as the reaction product of ssDNA very easily accumulates on the electrode surface. This drawback limits the development of many modified electrodes to the applications of DNA biosensors. Fortunately, the PMMA/GME could be regenerated by simple rinsing with ultra-pure water to get a full fresh surface in the case of electrode fouling. The standard deviation in the peak currents for six successive rinsing works out to be <5% for adenine residues and <4% for guanine residues of the same electrode in 0.1 mg mL^{-1} ssDNA. Therefore, the proposed experimental results indicated that the PMMA/GPE sensing platform offers a simple, effective, sensitive and stable method for detecting ssDNA.

4. Conclusions

In summary, direct electrochemical behavior of single-stranded DNA (ssDNA) on the PMMA/GME were investigated. Such a PMMA/GME could lead to the decrease in the over-potential for some biomolecules including ssDNA, and amplify their electrochemical response to some extent, which might be attributed to the conducting network within the polymer matrix developed by PMMA and graphite. As a new kind of composite electrode which is similar to the ceramic carbon electrode, such PMMA/GME not only enlarges the species of the composite electrode, but also exhibits some outstanding advantages, such as easy fabrication, high stability, renewabile surface, excellent stability, mechanical rigidity and high electrochemical reactivity. The PMMA/GME offers a basic and promising electrochemical platform for monitoring DNA hybridization or damage for further research, which also allows the device miniaturization in practice.

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