

Voltammetric Behavior and Detection of DNA at Electrochemically Pretreated Glassy Carbon Electrode

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Received: October 5, 2000

Final version: December 15, 2000

Abstract

Electrochemical oxidation of thermally denatured DNA at an electrochemically pretreated glassy carbon electrode was studied by means of adsorptive stripping voltammetry. The sensitivity for thermally denatured DNA detection at GCE was improved greatly by modifying the electrode with a simple and effective electrochemical oxidation at +1.75 V (vs. SCE) for 300 s in pH 5.0 phosphate buffer. Thermally denatured DNA was preconcentrated at pretreated GCE by its adsorption in open circuit for 5 min or at the potential of +0.3 V for 90 s, and produced two well-defined oxidation peaks of guanine and adenine residues at +0.80 V and +1.11 V in pH 5.0 phosphate buffer, respectively, while the native DNA almost did not appear at the same condition. This adsorptive stripping method can be used to study the properties of DNA using simple CV technique. A convenient quantitation of the low level of thermally denatured DNA was proposed. The detection limit of thermally denatured DNA was ca. $2.0 \mu\text{g mL}^{-1}$ when the accumulation time is 150 s at the potential of +0.3 V. As comparison, the acid-denatured DNA (treated in HClO_4 solution) was tested at the same condition and a detection limit of $0.10 \mu\text{g mL}^{-1}$ was obtained.

Keywords: DNA, Adsorptive stripping voltammetry, Electrochemically pretreatment, Glassy carbon electrode

1. Introduction

Deoxyribonucleic acid (DNA) is the genetic material of all organisms. The study on the electrochemical behavior of DNA is of important theoretical and practical significance in life science. There have been many reports on the electrochemical study of DNA over the past few decades. The studies of direct electrochemistry of DNA at mercury electrodes led to a deep understanding of the DNA double helix [1, 2]. Their main objects were focused on the reduction of DNA at mercury electrodes and the interaction between mercury electrode and native or denatured DNA. However, only a few works on the direct electrochemical behavior of DNA at solid electrodes were reported [3–9]. The electroactive groups for the electrochemical oxidation of DNA at GCE [3, 4] or graphite electrodes [5, 6] were guanine and adenine residues. The sensitivity of DNA detection in these works is not satisfactory and the shape of cyclic voltammograms is also very poor. Recently, new techniques based on nucleotide acid-pretreated electrodes have been developed [10–14]. The sensitivity of voltammetric analysis of DNA has been improved by several orders of magnitude. To our knowledge, the working electrodes in these methods were almost mercury electrodes. Remarkably, Wang et al. [15] reported a method to quantitate nanogram quantities of ss-DNA using adsorptive stripping potentiometry at electrochemically pretreated carbon paste electrode. The extraordinary low (ng/mL) detection limit is analogous to those obtained at the HMDE. The cyclic voltammogram of 2 mg/L ss-DNA at their pretreated CPE gave one anodic peak at about +1.05 V corresponding to the oxidation of the guanine residue in pH 4.8 acetate buffer at the potential range of +0.1 V to +1.3 V. Various carbon electrode materials for use in potential stripping analysis of nucleic acids have been compared. Carbon paste, pyrolytic graphite, highly oriented pyrolytic graphite and carbon strip gave low detection limits of 20, 30, 40 and 50 ng/mL for oligo(dG)₂₀, respectively, but no response was observed for trace measurements at the glassy carbon electrode [16]. The electrochemical pretreatment of glassy carbon electrodes is a widely used procedure to improve electrode response [17–21].

But the electrochemical behavior and detection of DNA at a pretreated GCE have not been reported.

This article reports a cyclic voltammetric study on the electrochemical oxidation of DNA at a pretreated GCE. The pretreatment was carried out by electrochemically oxidizing at +1.75 V (vs. SCE) for 300 s under stirring followed by cyclic sweep between +0.3 V and +1.25 V in pH 5.0 phosphate buffer until a steady-state current–voltage curve was observed. The background current of GCE was almost no increase after the pretreatment. This pretreated electrode can adsorb the denatured DNA at +0.3 V or in open circuit and results in a 10-fold increase in the current signal. The results suggest that the oxidation of thermally denatured DNA at the pretreated GCE is an irreversible process and the two oxidation peaks can be used for the detection of thermally denatured DNA at low levels.

2. Experimental

2.1. Chemicals and Apparatus

Calf thymus DNA ($\text{OD}_{260}/\text{OD}_{280} > 1.8$) was obtained from Sigma and was used without further purification. Adenine and guanine were purchased from Sigma. All other chemicals were of analytical reagent grade. Double distilled water was used for all experiments.

Cyclic voltammetry studies were carried out using a 270 Electrochemistry System (EG&G, USA). The three-electrode system used in this study contained a glassy carbon working electrode, a platinum wire counter electrode and a SCE reference electrode.

2.2. Pretreatment of the Glassy Carbon Electrode

The glassy carbon electrode (GCE) (4 mm diameter) was polished to a mirror finish using the BAS-polishing kit with 0.3 and $0.05 \mu\text{m}$ alumina paste. After it was rinsed with distilled water thoroughly, a potential of +1.75 V was applied to the

electrode under stirring in 0.1 M pH 5.0 PB for 300 s. Then the electrode was scanned between +0.3 V and +1.25 V until a steady-state current–voltage curve was obtained. After this treatment, a thin blue film could be observed on the electrode surface. When the potential exceeds +1.75 V or the time exceeds over 300 s, the color of the film becomes golden yellow to brownish and the adsorptive ability of thermally denatured DNA at the electrode decreases. According to the results of Kepley and Bard [17], the activation of GCE involves the formation of a new phase, which contains a significant amount of microcrystallinity and graphite oxide. The new phase is very porous, hydrated and nonconductive, in which the aromatic molecules intercalate quite well. Accordingly it appears that the pretreatment affects primarily the accumulation process and not the charge transfer of the guanine and adenine residues.

2.3. Denaturation of DNA

Thermally denatured DNA was produced by heating native DNA solution in a boiling water bath (100 °C) for about 10 min followed by rapid cooling in an ice bath. Generally, thermal denaturation involves the rupture of hydrogen bonds, the disturbance of stacking interaction but not any breakage of covalent bonds [22]. So thermally denatured DNA may act as ss-DNA.

Acidic denaturation of DNA was done according to literature [4]. 3.0 mg DNA was dissolved in 0.5 mL concentrated perchloric acid and stirred for 10 min, 0.5 mL 9 M NaOH was added to neutralize the solution followed by adding 5 mL of 0.2 M pH 5.0 phosphate buffer solution. This solution was diluted with water to the final volume of 10.0 mL. Concentrations of DNA (per nucleotide phosphate) used to calculate the adsorptive coefficient were determined spectrophotometrically with $\epsilon_{260} = 6600 \text{ M}^{-1} \text{ cm}^{-1}$ [23].

2.4. Voltammetric Procedure

The electrochemical experiments were performed in 0.1 M pH 5.0 PBS with different concentrations of DNA. The accumulation of DNA at the working electrode by adsorption was done in a stirred solution at +0.3 V for 90 s or in open circuit for 5 min. After a 5 s quiet period, the anodic stripping voltammograms were recorded from +0.3 V to +1.25 V at 100 mV s^{-1} . The electrode can be used for next measurement after a continuous sweep for three cycles at the same potential range.

3. Results and Discussion

3.1. Cyclic Voltammograms of DNA at Glassy Carbon Electrode

Figure 1 shows the cyclic voltammograms of $120 \mu\text{g mL}^{-1}$ thermally denatured DNA at the unpretreated GCE (dotted line) and pretreated GCE (solid line) and $6.0 \mu\text{g mL}^{-1}$ acid-denatured DNA at the pretreated GCE (dashed line). At the pretreated GCE, the thermally denatured DNA and acid-denatured DNA gave two well-defined oxidation peaks in the first anodic sweep at about +0.80 V (peak 1) and +1.11 V (peak 2), while the response is very poor at the unpretreated GCE. The two anodic peaks at about +0.80 V (Peak1) and +1.11 V (peak 2) correspond to

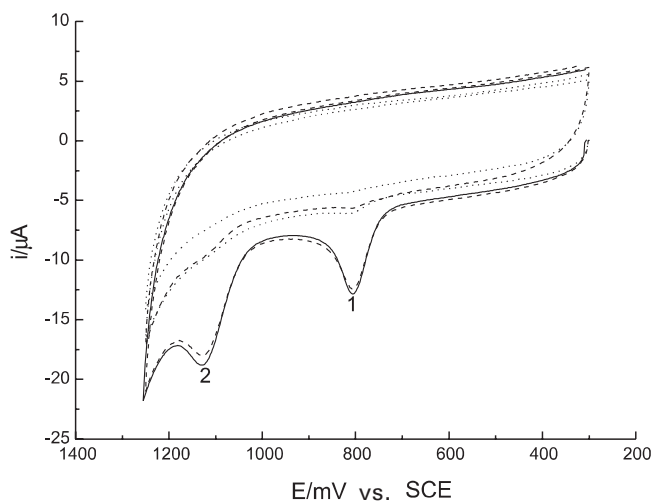


Fig. 1. Cyclic voltammograms of $120 \mu\text{g mL}^{-1}$ thermally denatured (ss-) DNA in 0.1 M pH 5.0 PB at unpretreated GCE (dotted line) and pretreated GCE (solid line) at 100 mV s^{-1} . The dashed line is the voltammogram of $6.0 \mu\text{g mL}^{-1}$ acid-denatured DNA at the pretreated GCE. Preconcentration potential and time: +0.3 V and 90 s.

electrochemical oxidation of guanine and adenine residues in denatured DNA molecules, respectively. This can be verified by comparison with the cyclic voltammogram (Fig. 2) of $0.20 \mu\text{g mL}^{-1}$ guanine and adenine at the pretreated GCE under the same condition as in Figure 1. The peak potential of +0.80 V, corresponding to the oxidation of guanine residue at the pretreated GCE, is more negative than that (+1.05 V) obtained at the pretreated CPE in pH 4.8 acetate buffer [15]. The peak current of the pretreated GCE is 10-fold more than that of the unpretreated GCE. No reduction peak is observed in the cathodic sweep, thus, the electrochemical oxidation of denatured DNA on the pretreated GCE is an entirely irreversible process rather than a quasireversible process reported by Brett et al. at an unpretreated GCE [4]. Both Brett et al. [4] and Brabec et al. [6] reported that there is a small oxidation peak of denatured DNA at about +0.4 V at the unpretreated GCE. This small peak has not been observed in our studies.

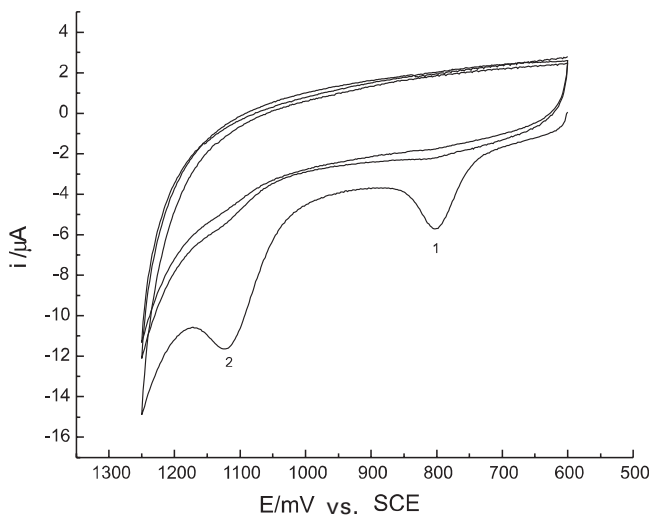


Fig. 2. Cyclic voltammogram of $0.20 \mu\text{g mL}^{-1}$ guanine and adenine solution at pretreated GCE. Accumulation potential is +0.6 V. Other conditions were the same as in Figure 1.

The adsorption of thermally denatured DNA on the pretreated GCE surface could be identified by the following procedure. Immersing the electrode gently into a quiet phosphate buffer (pH 5.0) containing thermally denatured DNA, the cyclic voltammogram shows very low response. After a potential of +0.3 V was applied for 90 s or in open circuit for 5 min under stirring, however, the electrode shows higher anodic peak current. Rinsing the electrode and transferring it to a pH 5.0 PB without DNA, two peaks can be observed. The voltammetric response is about half of that obtained in thermally denatured DNA solution. This suggests that the voltammetric currents of the pretreated electrode in thermally denatured DNA solution were partly contributed by the diffusion of DNA from the solution to the electrode. It can be seen from Figure 1 that the peaks of denatured DNA almost disappeared at the second cycle, which is in agreement with previous work [4, 6, 15], and is attributed to adsorption of the oxidation product at the pretreated GCE surface.

Figure 3 shows the voltammograms of DNA adsorbed electrodes in 0.1 M pH 5.0 PB. The electrodes were obtained by immersing GCE in DNA solution for 5 min and rinsing with water. It can be seen that the response of native (ds-) DNA at both unpretreated and pretreated GCE was very poor with only one small hump at about +0.99 V and +0.80 V, respectively (Fig. 3A), and the peak currents have no significant difference. The thermally denatured (ss-) DNA and acid-denatured DNA show obvious different responses at pretreated and unpretreated GCE (Fig. 3B, C). This illustrated that the denatured DNA adsorbed at pretreated GCE is more than that at unpretreated GCE. It can be seen also from Figure 3 that the responses of native (ds-) and thermally (ss-) DNA at the pretreated GCE have a large differ-

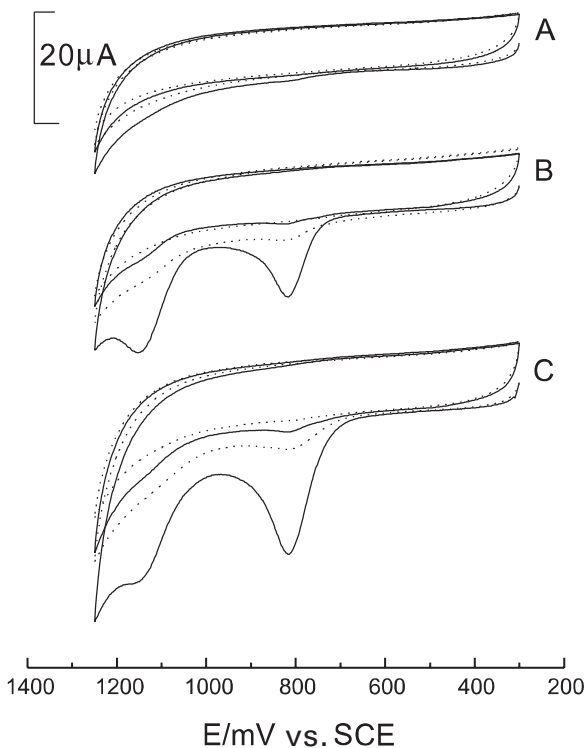


Fig. 3. Continuous voltammograms of native DNA (A), thermally denatured DNA (B) and acid-denatured DNA (C) at unpretreated GCE (dotted line) and pretreated GCE (solid line) for 2 cycles in 0.1 M pH 5.0 PBS at 100 mV s⁻¹. The concentration of native, thermally denatured and acid-denatured DNA is 1.0, 1.0, and 0.30 mg mL⁻¹, respectively.

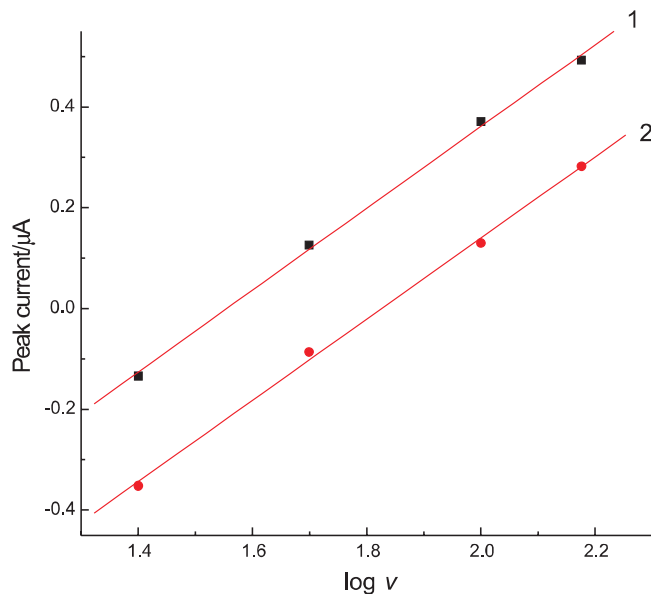


Fig. 4. Plots of log i_p - log v . For the oxidation of guanine (1) and adenine (2) residues in 30 $\mu\text{g mL}^{-1}$ thermally denatured DNA. Other conditions were as in Figure 1.

ence. On the other hand, the pretreated GCE has a higher sensitivity to the changes in DNA double helix than unpretreated GCE. This is an important advantage of electrochemical analysis of nucleic acid with pretreated GCE. This is probably attributed to the fact that the electroactive adenine and guanine residues in native DNA are inaccessible to the electrode surface and most bases in denatured DNA can freely interact with the surface group of GCE. On the other hand, the hydrogen-bonded bases in native DNA are hidden within the double helix. This represented a serious steric barrier to electron transfer between the purine and the GCE [6]. Palecek et al. [24] has shown that the DNA dena-

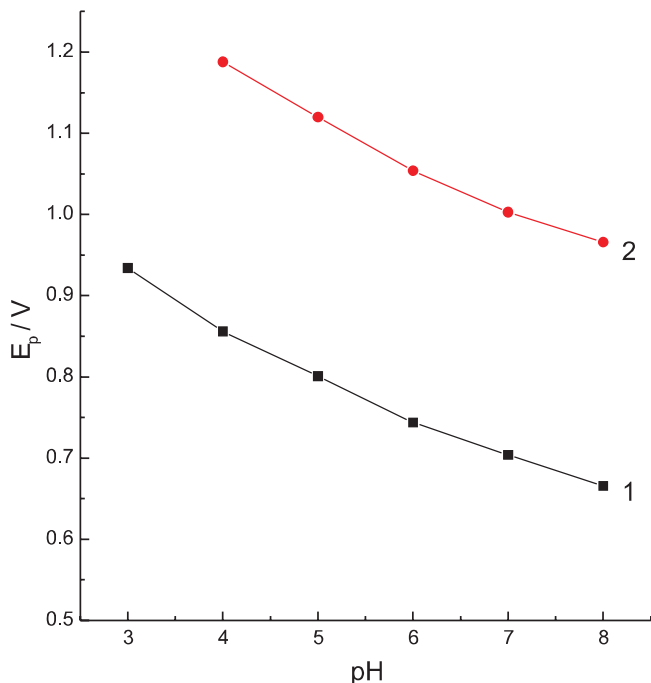


Fig. 5. Effect of pH on peak potentials in phosphate buffers at 100 mV s⁻¹. Other conditions were as in Figure 4.

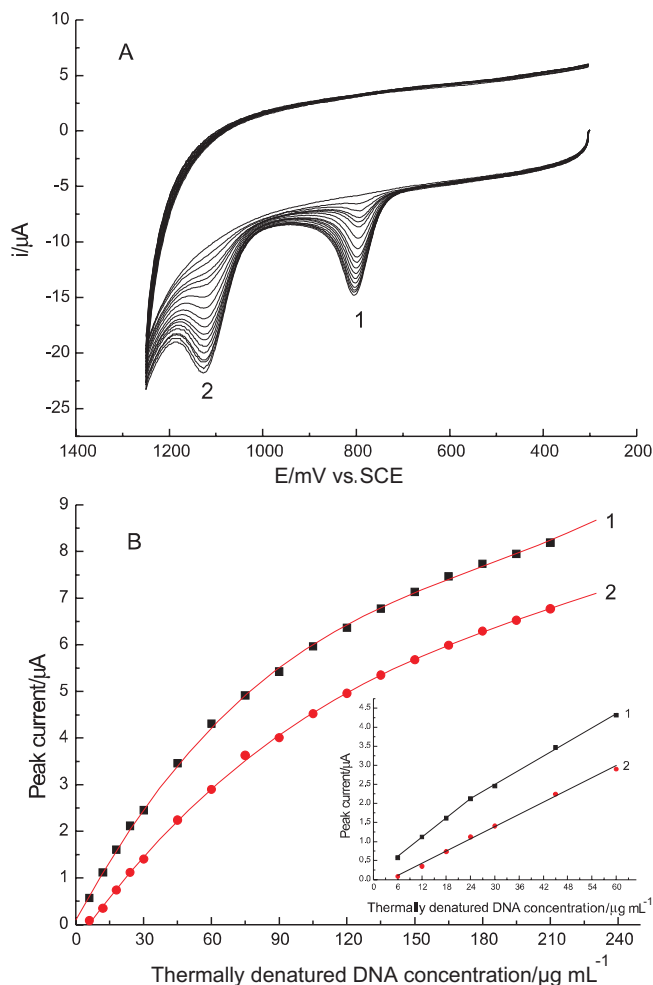


Fig. 6. Voltammograms of thermally denatured DNA solutions with increasing concentrations from 0–210 $\mu\text{g mL}^{-1}$ (A), and calibration plots of responses vs. concentration (B). Conditions were as in Figure 1. Inset in B: calibration plots in the range of 6.0–60 $\mu\text{g mL}^{-1}$ thermally denatured DNA. 1 and 2 are corresponding to the Peak 1 and 2 of the voltammograms.

tured in concentrated HClO_4 actually produced a deep DNA degradation involving DNA depurination and reduction of the molecular mass of the depurinated DNA. So the acid-denatured DNA gives larger signals than the thermally denatured DNA at the same concentrations. The data of Figure 1 and Figure 3 clearly demonstrated that the electrochemical activation of GCE surface could greatly enhance the signal of thermally denatured (ss-) DNA, thus increasing the difference between native (ds-) and thermally denatured (ss-) DNA.

3.2. Effect of Scan Rates (ν)

At the pretreated electrode, there is no linear relation between the peak currents of thermally denatured DNA in pH 5.0 PB and the scan rate, ν , or $\nu^{1/2}$. In the scan rate range of 25–150 mV/s, the plots of $\log i_p$ vs. $\log \nu$ for two peaks (Fig. 4) follow the equations $\log i_p = -1.262 + 0.812 \log \nu$ (Peak 1, $r = 0.9992$) and $\log i_p = -1.471 + 0.805 \log \nu$ (Peak 2, $r = 0.9991$) (ν in mV/s and i_p in μA). The slopes of 0.812 and 0.805 indicate that the electrode processes were simultaneously controlled by surface adsorption and heterogeneous electron transfer rate. With increasing scan

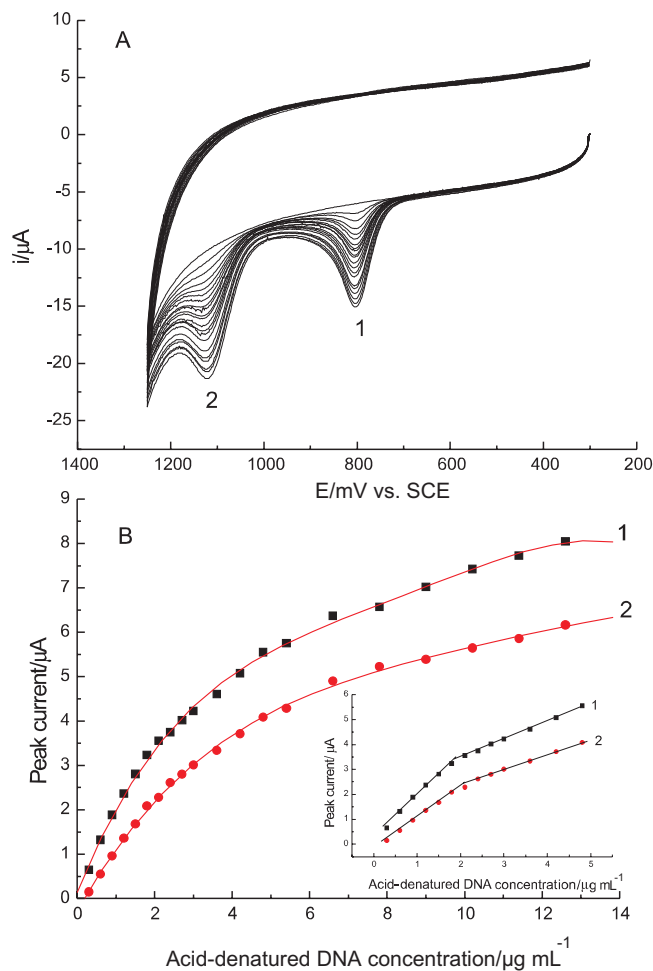


Fig. 7. Voltammograms of acid-denatured DNA solutions with increasing concentrations from 0–12.6 $\mu\text{g mL}^{-1}$ (A), and calibration plots of responses vs. Concentration (B). Conditions were as in Figure 1. Inset in B: calibration plots in the range of 0.30–4.8 $\mu\text{g mL}^{-1}$ acid-denatured DNA. 1 and 2 are corresponding to Peak 1 and 2 of the voltammograms.

Table 1. Thermodynamic parameters of adsorption process of denatured DNA adsorbed at pretreated GCE.

| | Γ_m (mol cm^{-2}) | β (L mol^{-1}) | ΔG^0 (kJ mol^{-1}) |
|-------------------------|-------------------------------------|---------------------------------|---------------------------------------|
| Acid-denatured DNA | 1.75×10^{-10} | 9.87×10^4 | -28.49 |
| Thermally denatured DNA | 7.68×10^{-11} | 1.47×10^4 | -23.77 |

rate, both anodic peak potentials shifted to more positive values. The equations of peak potential E_p vs. $\log \nu$ are as follows (ν in mV/s): $E_p = 0.726 + 0.039 \log \nu$ (Peak 1, $r = 0.9961$) and $E_p = 1.026 + 0.045 \log \nu$ (Peak 2, $r = 0.9932$).

3.3. Effect of pH on E_p

The experimental results show that the pH dependence on the oxidation peak potential of thermally denatured DNA obeys the equations $E_p = 1.115 - 0.063 \text{ pH}$ (peak 1) and $E_p = 1.455 - 0.067 \text{ pH}$ (peak 2) in pH 3.0–6.0. When $\text{pH} > 6.0$, the slopes $-\partial E_p / \partial \text{pH}$ become smaller as show in Figure 5. The oxidation of guanine and adenine themselves has been shown to follow a two-step mechanism involving the total loss of four electrons. In

both cases, the first $2e^-$ loss is rate determining step [25]. The slopes of 63 mV pH^{-1} and 67 mV pH^{-1} show that two protons take part in the rate determining step.

3.4. Adsorption Feature and Detection of DNA

The effects of thermally denatured DNA concentration (from 6.0 to $210 \mu\text{g mL}^{-1}$) on the CV peak currents are shown in Figure 6. The peaks increase nearly linearly with the thermally denatured DNA concentration up to $60 \mu\text{g mL}^{-1}$, although the resulting calibration plot of peak current of guanine residues vs. thermally denatured DNA concentration show two linear sections with different slopes in the range of 6.0 – $60.0 \mu\text{g mL}^{-1}$. The relations become nonlinear and the current tends to the maximum values when the thermally denatured DNA concentration is more than $60.0 \mu\text{g mL}^{-1}$. A detection limit of $2.0 \mu\text{g mL}^{-1}$ thermally denatured DNA can be estimated when the accumulation time is 150 s at $+0.3 \text{ V}$. The reproducibility of the CV response is good. The relative standard deviation of 4.6% and 4.9% corresponding to Peak 1 and Peak 2 were obtained when a $30.0 \mu\text{g mL}^{-1}$ thermally denatured DNA solution was measured 6 times. The relationship of peak current with acid-denatured DNA concentrations is shown in Figure 7. The plots of peak currents of adenine and guanine residues vs. acid-denatured DNA concentration also show two linear sections with different slopes in the range of 0.30 – $4.8 \mu\text{g mL}^{-1}$ (inset in Fig. 7B). The detection limit of acid-denatured DNA is about $0.10 \mu\text{g mL}^{-1}$ with an accumulation time of 150 s at $+0.3 \text{ V}$. Six repetitive measurements of a $3.0 \mu\text{g mL}^{-1}$ acid-denatured DNA yield the relative standard deviation of 4.1% and 4.4% for Peak 1 and Peak 2, respectively. Therefore, the adsorptive stripping cyclic voltammetry can be used to study the electrochemical behavior and quantitate the thermally denatured DNA at low levels.

The peak area for DNA oxidation can be used to estimate the adsorbed amount of DNA at the pretreated GCE. With the aid of equation, $\Gamma_m = Q_m/nFA$, the maximum surface concentration, Γ_m , of the adsorbed DNA can be calculated from the value of the limiting charge Q_m (the total area of Peak 1 and Peak 2 of denatured DNA) and electron transfer number n (the value of 4 was taken according to the results of Palecek [26]). In order to eliminate the effect of diffusion, the Q_m and Q of the adsorbed DNA were measured in $\text{pH } 5.0$ PB without DNA after preconcentration. Supposing the denatured DNA is adsorbed on the GCE surface in a monolayer, the adsorption coefficient (β) can be calculated according to the Langmuir adsorption equation. Therefore, the adsorption free energy (ΔG^0) can be calculated with the equation, $\beta = \exp(-\Delta G^0/RT)$. The results are given in Table 1.

It can be seen from Table 1 that the maximum surface concentrations, Γ_m , of adsorbed denatured DNA are close to the theoretical value of a monolayer, and the Γ_m of thermally denatured DNA is less than that of acid-denatured DNA. It can be concluded that the acid-denatured DNA was degraded and more guanine and adenine residues can be adsorbed at the electrode surface.

4. Conclusions

The electrochemical pretreatment of GCE is simple and effective for the adsorption of thermally denatured DNA. The pretreated GCE gives more than 10-fold current responses of guanine and adenine residues compared with those at the unpretreated GCE. Furthermore, the pretreated GCE has a higher sensitivity to changes of the DNA double helix. So this study can provide a basis for detecting and immobilizing DNA, for probing DNA damage, interaction and structure of nucleic acids, and for the development of sequence-specific hybridization sensors.

5. Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 29835110 and 29975013) and the National Science Foundation of Jiangsu (No. BK99030).

6. References

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