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Interactions of Cytochrome *c* with DNA at glassy carbon surface

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Abstract

Cyclic voltammetry (CV) was used to investigate the interactions of Cytochrome *c* (Cyt *c*) with deoxyribonucleic acid (DNA) at glassy carbon (GC) electrodes. The results indicate that there are strong interactions between Cyt *c* and DNA. The binding constant (k_A) and binding free energy ($\Delta_r G$) of Cyt *c* with dsDNA are $(1.69 \pm 0.38) \times 10^5$ L·mol^{-1} and $-(29.76 \pm 0.56)$ kJ·mol^{-1}, respectively; and those of Cyt *c* with ssDNA are $(3.35 \pm 0.50) \times 10^5$ L·mol^{-1} and $-(31.49 \pm 0.37)$ kJ·mol^{-1}, respectively. The binding sites are achieved to be 3.3 bp per Cyt *c* molecule with dsDNA and 4.0 nucleotides (ssDNA) binding one Cyt *c* molecule. This experiment affords a valid method for investigating the interactions between DNA and proteins by electrochemical techniques.

Keywords: Cytochrome *c*; Interaction; DNA; Modified electrode

1. Introduction

It is recognized that DNA plays a determinative role in biological genetic information transfer and protein biosynthesis. Proteins, saccharide and nucleic acid have relations with each other of inter-mutation, inter-dependence and inter-restriction. For example, DNA and some redox proteins are coexistent in the mitochondrion. Such proteins are generally located on or in the biological membranes and play a basic function of electron transport in the electron transport chain. Therefore, it is wondrously significant to investigate the electron transport between redox proteins and DNA for apprehending the biological oxidation, energy production and conversion in respiratory chain, etc.

Cytochrome *c*, whose crystallographic dimension is $3.0 \times 4.0 \times 3.0$ nm, is a kind of basic redox protein embedding in inner membranes of mitochondrion and carries out electron transport by virtue of the redox reaction of iron ion in its iron-porphyrin prosthetic group. On the external surface of Cytochrome *c*, there bestrew quite a lot of electriferous amino acid residues which make the oxidated form (ferricytochrome *c*) and the reduced form (ferrocytochrome *c*) own an overall charge of +9 and +8 at neutral pH conditions, respectively. So interactions can take place between the phosphoric radicles of DNA carrying negative charge and Cyt *c* with positive charge through electrostatic attraction [1–4]. Recently, the direct electrochemical behaviors of Cyt *c* with nucleic acid as the electron transfer promoter have been reported [5–7]. Nevertheless, few articles are about such kinds of interactions of Cyt *c* with DNA. In this article, DNA modified GC electrodes prepared by air-drying/adsorption method are used to study the interactions between Cyt *c* and DNA.

2. Experimental

2.1. Reagents

Cyt *c* of horse heart was purchased from Roche, and the Cyt *c* solution used in experiments was attained by dissolving some fixed amount of Cyt *c* into the 1 mM

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phosphate buffer solution (pH 7.0). Triply distilled water was used for preparing all of the solutions. Other chemicals were of analytical reagent grade.

2.2. Apparatus and method

Electrochemical experiments were carried out with a CHI660A software-controlled electrochemistry workstation (CHI660A, Shanghai CH Instruments, Inc.). Three-electrode-architecture was adopted, which consisted of a working electrode of GC (effective area is 0.054 cm^2 as our previous work (Y.H. Bi, B. Liang, Z.L. Huang, Y.D. Zhao and Z.N. Gao, Interactions between DNA and metal chelates at nanometer-scale size interface, Indian J. Chem. (in press).)), dsDNA or ssDNA modified GC electrode that was prepared according to the method of reference [8] and denoted as dsDNA/GC or ssDNA/GC; a platinum wire counter electrode and a saturated calomel reference electrode (SCE). All solutions were purged with high purity nitrogen for more than 15 min prior to experiments and kept under a nitrogen atmosphere during all experiments. Experiments were carried out at room temperature (25 °C).

3. Results and discussion

3.1. Direct electrochemistry of Cyt c at DNA/GC electrodes

In the range of −0.2 to +0.2 V, CV examinations were performed in PBS (pH7.0) solutions at GC, dsDNA/GC and ssDNA/GC electrodes, and no redox peaks appeared in the cyclic voltammograms. DNA is non-electroactive within that range.

Investigations were carried out to research the electron transport of Cyt *c* at the bare GC, dsDNA/GC and ssDNA/GC working electrodes, when the ionic strength was lower (1 mM). No redox peak was observed at bare GC electrode in the cyclic voltammograms until the concentration of Cyt *c* was raised up to 15 μM, at this moment, a pair of peaks appeared, just as indicated in Fig. 1, curve a. When the dsDNA/GC electrode was immersed in 15 μM Cyt *c* solution, the electrochemical responses were recorded by consecutive cyclic voltammetry, then it could be seen that the redox peak currents become higher and higher with the scan time extending, indicating that the average surface coverage of Cyt *c* adsorbed onto the DNA/GC electrodes increases continuously. 30 circles later, the peak current tends to be stable (Fig. 1, curve b), indicating that Cyt *c* at the electrode surface covered with dsDNA attains the balance of adsorption and desorption. In the same way, putting the ssDNA/GC electrode into 15 μM Cyt *c* solutions, when the peak current was stable, then a pair of reversible redox peaks could also be observed (Fig. 1, curve c). All of the above demonstrate that DNA modified on the surface of glassy carbon electrode has relatively strong interaction with Cyt *c*.

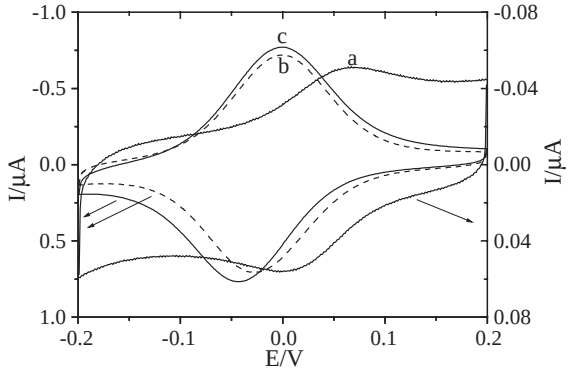

Fig. 1. CV voltammograms for 15 μM Cyt *c* solution containing 1 mM PBS at (a) bare GC, (b) dsDNA/GC and (c) ssDNA/GC. Scan rate is 20 mV s^{-1}.

From Fig. 1, the formal potential $E^{o\prime}$ of Cyt *c* at bare GC electrode can be obtained as +32 mV, and that at ssDNA/GC is −21 mV with shifting 53 mV in the negative direction comparing with that in solution. At dsDNA/GC, a pair of peaks are obtained with a formal potential $E^{o\prime}$ of −14 mV with a shift of −46 mV in $\Delta E^{o\prime}$. So it can be seen that the formal potentials of Cyt *c* at the surface of DNA/GC shift negatively with that in the solution, just as the reports [9–11] indicate that the electrostatic interaction between DNA and some molecules could bring on the formal potential shifting negatively at lower ionic strength, the interaction between Cyt *c* and DNA is electrostatic interaction.

From the magnitudes of the shifts ($\Delta E^{o\prime}$), a ratio $\left(\frac{K_{\mathrm{Cytc(Ox)}}}{K_{\mathrm{Cytc(Red)}}}\right)$ of the binding constants for the oxidized and reduced forms of Cyt *c* at dsDNA- and ssDNA-surfaces can be calculated out to be 6.0 and 8.0, respectively, according to Eq. (1). These results show that the oxidized form has a larger binding constant, indicating that the interaction of the oxidized form with DNA modified on the surface of GC electrode is stronger than that of reduced form, which also accords with the electrostatic interaction.

$$\Delta E^{o\prime} = \frac{RT}{nF} \ln \frac{K_{\mathrm{Cytc(Ox)}}}{K_{\mathrm{Cytc(Red)}}} \quad (1)$$

where, *T*, *R*, *n* and *F* have their usual meanings.

If electrostatic interaction plays an important role in the incorporation process of Cyt *c* carrying negative charge and DNA with positive charge, it can be expected that the electrochemical behaviors of Cyt *c* would be strongly affected by the ionic strength of the solution [6]. The influence of the ionic strength on the electrochemistry of Cyt *c* was examined in different solutions containing 4.5 μM Cyt *c*. Fig. 2 shows the cyclic voltammograms of 4.5 μM Cyt *c* at dsDNA/GC in different PBS solutions. It can be seen that the peak current decreases quickly with the increase of the electrolyte concentration when the electrolyte concentration is over 1 mM. This indicates that the interaction of Cyt *c* with DNA is electrostatic interaction, i.e. the competitive adsorption of cationic ions on the

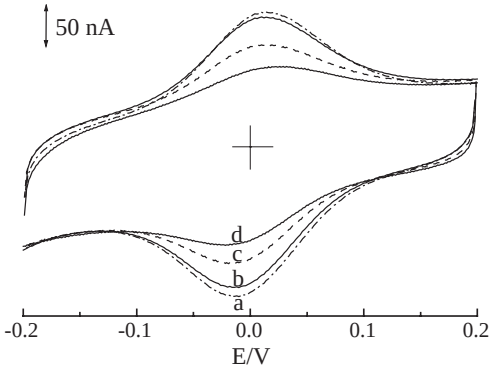

Fig. 2. Cyclic voltammograms of 4.5 μM Cyt *c* at dsDNA/GC in different PBS solutions: (a)1, (b)2, (c)5, (d)12 mM. Scan rate is 20 mV s^{-1}.

negative sites of DNA weakens the electrostatic adsorbing interaction.

The results above reveal that a strong electrostatic interaction exists between Cyt *c* and DNA.

The contrast of the incorporation process of Cyt *c* with DNA on surface to that in solution is the base of the study on dependability of modified electrode. So we also examined the interaction of Cyt *c* with DNA in solution, as shown in Fig. 3. It can be seen that, when the concentration of Cyt *c* is constant, the formal potential $E^{o\prime}$ shifts negatively with the increasing of DNA concentration, just as curve b (8.9×10^{-5} M) and curve c (1.2×10^{-4} M) shown. Comparing with the formal potential $E^{o\prime}=+32$ mV of Cyt *c* at bare GC electrode, $E^{o\prime}$ of curve b is +8 mV with shifting 24 mV in the negative direction and $E^{o\prime}$ of curve c is +3 mV with a shift of −29 mV in $\Delta E^{o\prime}$. So it can be seen that, there is a strong interaction between Cyt *c* and DNA in solution.

As shown in Fig. 4, the peak current decreases quickly with the increase of electrolyte concentration (curve a 1 mM→curve b 14 mM), indicating the increase of ionic strength, i.e. the competitive adsorption of cationic ions on the negative sites of DNA weakens the electrostatic adsorbing interaction, and then induces to the peak

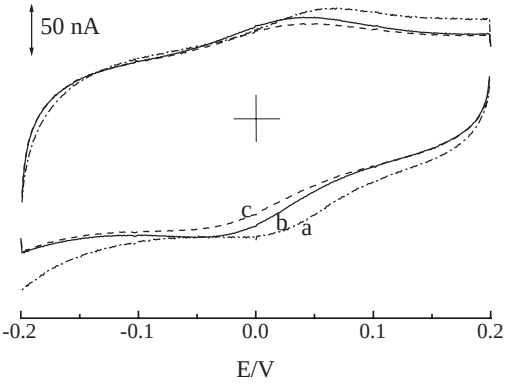

Fig. 3. Cylic voltammograms of bare GC electrode at: (a)30 μM Cyt *c* solution, (b)solution containing 30 μM Cyt *c* and 8.9×10^{-5} M dsDNA, (c)solution containing 30 μM Cyt *c* and 1.2×10^{-4} M dsDNA. Scan rate is 20 mV s^{-1}.

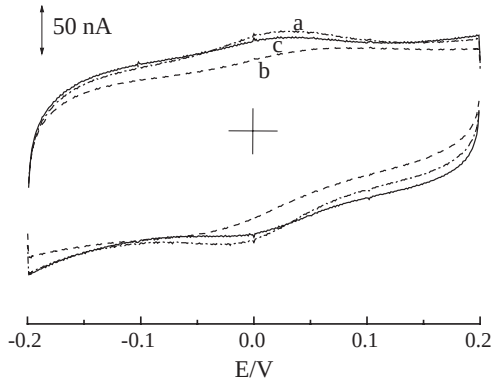

Fig. 4. Cyclic voltammograms of bare GC electrode at (a) containing 30 μM Cyt *c* and 1 mM PBS, (b) containing 30 μM Cyt *c* and 14 mM PBS, (c) containing 8.6 μM Cyt *c* and 4 mM PBS. Scan rate is 20 mV s^{-1}.

currents decrease. Then adds distilled water into the solution, which leads to the electrolyte concentration decrease to 4 mM and the concentration of Cyt *c* to 8.6 μM, however, the peak currents increase quickly. These show that although the concentration of Cyt *c* decreased, the decrease of ionic strength increases the interaction of Cyt *c* with DNA.

All the results indicate that the interaction of Cyt *c* with DNA on the electrode surface is electrostatic interaction, which is the same as that obtained in the solution.

3.2. Electrochemical behavior of Cyt c absorbed on the surface of DNA/GC electrodes

The dsDNA/GC and ssDNA/GC electrodes were immersed into the 20 μM Cyt *c* solution and took cyclic voltammetric experiments at the same time. When steady curves were obtained, took out the electrodes and rinsed them lightly with triply distilled water, and then transferred them into the blank solutions rapidly, and at the same time, took CV scan over the range of −0.2 to +0.2 V. A pair of well-defined redox peaks could still be observed in the cyclic voltammograms. Next, the relations between the peak current and scan rate were examined within a short period of time. Fig. 5(A) shows the CV voltammograms for Cyt *c*-dsDNA/GC at different scan rate in PBS (the figure of Cyt *c*-ssDNA/GC electrode is omitted). The results illustrate that, when the scan rate is lower than 0.5 V·s^{-1}, the relation between the peak current and scan rate inclines to a linear tendency, as shown in Fig. 5(B). This is consistent with the surface reaction dynamical model.

After the Cyt *c*-dsDNA/GC and Cyt *c*-ssDNA/GC electrodes having being immersed in the blank solution for a long period of time, it could be seen that the peak currents decreased gradually with the time's prolonging. This suggests that Cyt *c* dissociates gradually from the surface of the DNA modified electrodes. Fig. 6(A) is the cyclic voltammograms of Cyt *c*-dsDNA/GC electrodes soaked in PBS solution for different time (the figure of Cyt *c*-ssDNA/GC electrode is omitted). The potential

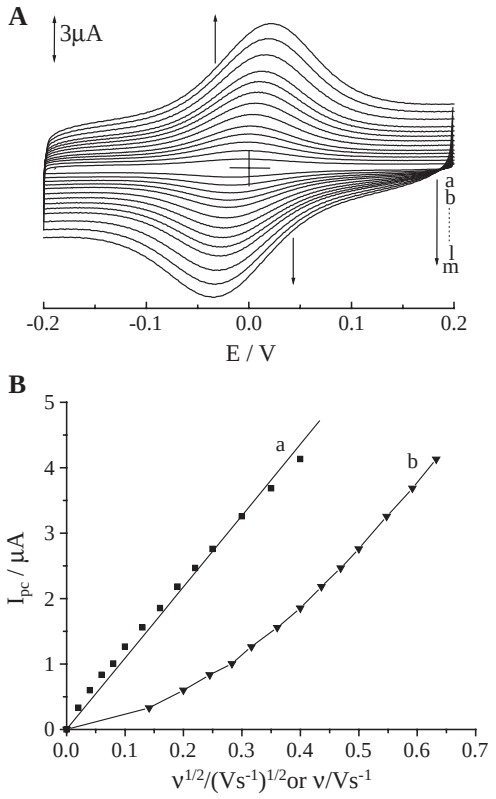

Fig. 5. (A) CV voltammograms for Cyt *c*-dsDNA/GC at different scan rates in PBS: a—20, b—40, c—60, d—80, e—100, f—130, g—160, h—190, i—220, j—250, k—300, l—350, m—400 mV s^{-1}; (B) plots of (a) I_{pc} vs. ν and (b) I_{pc} vs. $\nu^{1/2}$.

changes a little with the time's prolonging, which may be caused by the following two factors: first, for the dissociation time is so long (about 600 min) that the structure of the modified electrode surface may be changed with the dissociating of Cyt *c*; second, the configuration of Cyt *c* changes with several times of redox.

A linear dependence of $\ln I_{pc}$ on dissociation time (t) ($r = 0.998$), as shown in Fig. 6(B), indicates that the dissociating behaviors of Cyt *c* from dsDNA/GC electrode obey first-order kinetics. The dissociating behavior of Cyt *c* from ssDNA/GC electrode is the same as that from dsDNA/GC (the figure of Cyt *c* dissociating from ssDNA/GC electrode is omitted). So the equations can be adopted as (2) and (3):

$$\ln I_p = k' + kt \tag{2}$$

$$t_{1/2} = \frac{\ln 2}{k} \tag{3}$$

where, I_p is the peak current, A; t is the dissociation time, min; k is the dissociation rate constant, min^{-1}; $t_{1/2}$ is the half-life, min.

From Fig. 6(B), the dissociation rate constant (k_1) for Cyt *c* dissociating from dsDNA/GC can be calculated as 0.00253 min^{-1} and the half-life ($t_{1/2}$) is 274.0 min. And the relevant dissociation rate constant (k_2) for Cyt *c* dissociating from ssDNA/GC is determined to be 0.00237 min^{-1} and the half-life is 292.5 min. These results suggest that, the interaction of Cyt *c* with ssDNA is appreciably stronger than that of Cyt *c* with dsDNA, resulting in the slower dissociating process from ssDNA than from dsDNA.

After Cyt *c* had completely dissociated in PBS solution/triply distilled water from DNA modified on the surface of electrode, we transferred the electrode into the former Cyt *c* solution and took CV scan again. Only some tiny changes of peak potentials and currents were observed, which indicated that the interactions between Cyt *c* and DNA at the surface of DNA/GC electrodes have preferable recurrence.

The average surface coverage (Ã) of Cyt *c* adsorbed onto the DNA/GC electrodes can be estimated from the coulometric charge obtained by integrating the cathodic (or anodic) peak area in the cyclic voltammograms and can be calculated out according to Eq. (4). Where Q is the amount of coulometric charge (C); n, the number of electrons transferred; F, Faraday's constant, 96 500 $C \cdot mol^{-1}$; A, the electrode area, cm^2.

$$\Gamma = \frac{Q}{nFA} \tag{4}$$

The plots of surface coverage (Γ) of Cyt *c* at modified electrodes versus the concentration of Cyt *c* in solution (C)

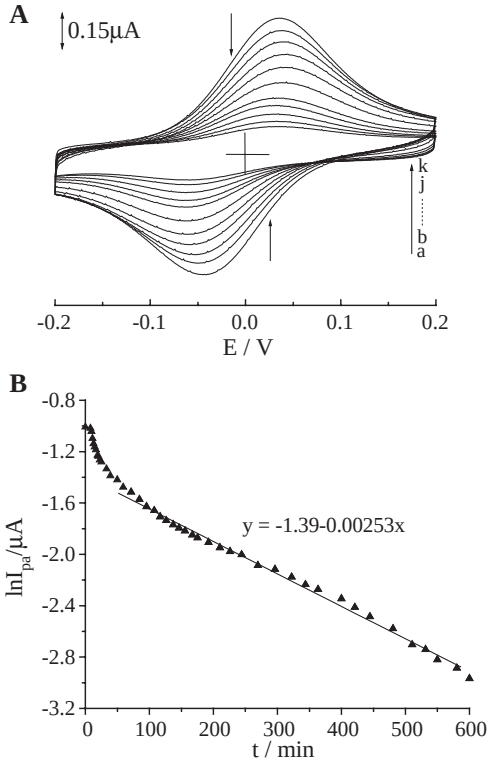

Fig. 6. (A) CV voltammograms for Cyt *c*-dsDNA/GC at different scan times in PBS: a—0, b—5, c—15, d—30, e—50, f—100, g—200, h—290, i—390, j—500, k—600 min; (B) plots of $\ln I_{pc}$ vs. t of Cyt *c*-dsDNA/GC in blank solution with a scan rate of 20 mV s^{-1}. Solid line is a linear fit.

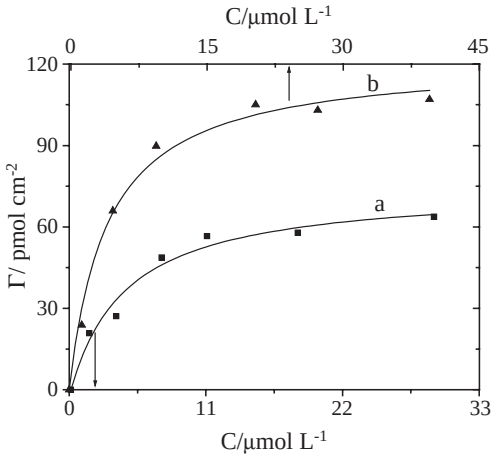

Fig. 7. Surface coverage of Cyt *c* bound to (a) dsDNA/GC and (b) ssDNA/GC vs. the concentration of Cyt *c* in 1 mM PBS solution. Solid line is a fit to the Langmuir model.

obey the Langmuir monolayer adsorbing model, as shown in Fig. 7, so Eq. (5) will be used:

$$\Gamma_A = \frac{\Gamma_\infty \cdot C_A}{1/k_A + C_A} \quad (5)$$

where Γ_A is the surface coverage of species A; k_A is the binding constant of A; C_A is the concentration of A; Γ_∞ is the saturated surface coverage.

In this way, the saturated surface coverage of Cyt *c* at dsDNA/GC ($\Gamma_{ds,\infty}$) and ssDNA/GC ($\Gamma_{ss,\infty}$) can be fitted as $7.46\times10^{-11}\pm5.02\times10^{-12}$ mol·cm^{-2} and $1.22\times10^{-10}\pm4.44\times10^{-12}$ mol·cm^{-2}, respectively; and similarly, the binding constant of Cyt *c* with dsDNA and ssDNA can be calculated as $k_{ds}=(1.69\pm0.38)\times10^5$ L·mol^{-1} and $k_{ss}=(3.35\pm0.50)\times10^5$ L·mol^{-1}, respectively.

The double helix's average diameter of dsDNA is 2 nm, the base stacking distance is 0.34 nm. Suppose that the array of DNA molecules at the electrode surface is a tight theoretical single-component surface structure, the saturated surface coverages of dsDNA and ssDNA can be calculated as $\Gamma_{dsDNA}=2.44\times10^{-10}$ mol·cm^{-2}, $\Gamma_{ssDNA}=4.89\times10^{-10}$ mol·cm^{-2}, respectively. Therefore, Γ_{dsDNA}:$\Gamma_{ds,\infty}$ can be calculated as 3.3, that is to say, 3.3 bp (dsDNA) bind 1 Cyt *c* molecule; analogously, Γ_{ssDNA}:$\Gamma_{ss,\infty}$ can be obtained as 4.0, namely, 4.0 nucleotides (ssDNA) bind 1 Cyt *c* molecule.

Two factors, which may introduce some windages into the calculations of the surface coverage of DNA, are as follows: first, the negative charges of DNA make it impossible to achieve ordered and saturated monolayer structure on the effective electrode areas, so this would make the calculations derive out greater values than the practical ones; second, part of DNA absorbed at the verge of the effective electrode areas or suspending in the solutions, while this might make the final data attained smaller than the actual values.

Cyt *c* combines DNA with electrostatic interaction. The binding free energy $\Delta_r G$ of Cyt *c* with dsDNA and ssDNA can be calculated out to be $-(31.49\pm0.37)$ kJ·mol^{-1} and $-(29.76\pm0.56)$ kJ·mol^{-1}, respectively, according to Eq. (6):

$$\Delta rG = -RT\ln k_A \quad (6)$$

where k_A is the binding constant.

4. Conclusions

In this paper, the cyclic voltammetry was used to investigate the interactions of Cyt *c* with DNA. The final studies demonstrate that Cyt *c* and DNA can interact with each other by electrostatic interaction. This study affords an effective method to investigate the interactions between DNA and proteins, making use of surface-based electrochemical methods.

Acknowledgements

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