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ORIGINAL PAPER

Studies on interaction of porphyrin and its complexes with DNA at interface on gold electrode modified by thiol-porphyrin self-assembled monolayer

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Abstract The interaction of 5-[p-(mercaptopropyloxy)-phenyl]-10, 15, 20-triphenylporphyrin (H_2MPTPP) and its metalloporphyrin (Co, Ni-MPTPP) with calf thymus deoxyribonucleic acid (DNA) has been studied on gold electrode modified by thiol-porphyrin self-assembled monolayer (SAM). The mode and characteristics of their interaction with DNA have been studied by cyclic voltammetry, scanning electrochemical microscope (SECM), and alternating current (AC) impedance. Some electrochemical parameters have been determined, i.e., apparent heterogeneous reaction rate constant (k_{eff} from SECM and k_f from AC impedance) and the hindrance (B) of electrode. $K_3[Fe(CN)_6]$ was used as probe to obtain some electrochemical information of electrode interface. SECM images obtained from interface on SAM interacted with DNA showed very good resolution with different topography. Based on a comparison with the results from experiments, a reasonable agreement between SECM and AC impedance can be obtained, which means a conjunction of them. It is proposed to be electrostatic interaction of H_2MPTPP, Co-MPTPP and Ni-MPTPP with DNA, and the attractive force between porphyrins and DNA follows the order Ni-MPTPP>Co-MPTPP>H_2MPTPP.

Keywords Calf thymus DNA · Porphyrin · Self-assembled monolayer (SAM) · Scanning electrochemical microscope (SECM) · AC impedance · Electrostatic interaction

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Introduction

Since the first observation of the preferential accumulation of hematoporphyrin in neoplastic tissues by Policard in 1924, porphyrins and their derivatives have roused people's much greater concern. Water-insoluble and water-soluble porphyrinyl-nucleosides containing adenosine and thymidine have strong tumoricidal activity against human malignant melanoma [1–3]. Their deoxyribonucleic acid (DNA)-binding interactions have long been of interest because of the potential for therapeutic applications and the novel binding interactions observed with DNA. Porphyrin–DNA interactions have been extensively studied using a wide variety of techniques [3–7]. In general, three binding modes for the porphyrin derivatives–DNA complex have been accepted, namely, intercalation [4–6], outside stacking along the helix [4], and outside random binding involving either placement of the porphyrin in the minor groove or electrostatic interaction with the backbone [5, 6]. Kim et al. [4] have studied the binding mode of cationic monomer and dimer porphyrin with native and synthetic polynucleotides by polarized light spectroscopy. The results indicated that some of them intercalated into the DNA, and others were stacked along the polynucleotide stem. Nyarko et al. [5] have studied the interaction of Au(III), Pt(II), and Pd(II) porphyrins with DNA by the ultraviolet–visible (UV–Vis) and circular dichroism (CD), and the results indicate that $H_2(TMPyP)^{4+}$, $Pt(II)(TMPyP)^{4+}$, and $Pd(II)(TMPyP)^{4+}$ intercalate into the DNA bases, while $Au(III)(TMPyP)^{5+}$ interacts with DNA via the outside binding mode but with partial intercalation at high concentrations. Tjahjono et al. [6] used visible absorption spectroscopy, CD, and magnetic circular dichroism to determine the binding mode of porphyrin–DNA interactions. Metallopor-

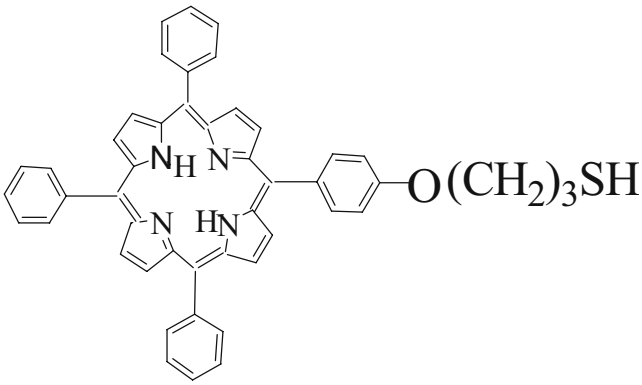
phyrins NiPzP and CuPzP are intercalated into the calf thymus (ctDNA). MnPzP is bound edge-on in the minor groove of ctDNA, while ZnPzP is bound face-on at the step of the major groove of ctDNA. The binding constants of the metalloporphyrins to ctDNA were obtained. Most of these detective methods are spectral. The electrochemistry methods used are rare in the reported study [7]. S.M. Chen and S.V. Chen [7] studied the electrochemistry of water-soluble iron porphyrins (Fe(n-TMPyP)) as an electrochemically active film on DNA modified glassy carbon, gold, platinum, and transparent semiconductor tin oxide electrodes in solutions of various pH values. The electrocatalytic reduction in p-nitrophenol by Fe(2-TMPyP)/DNA film are also discussed.

In these studies, porphyrins that interact with DNA are almost water-soluble porphyrin [1–7], while the interaction of water-insoluble porphyrins with DNA have rarely been seen in a report [3]. We provide the method of self-assemble monolayers (SAMs) of water-insoluble porphyrins on gold electrode to study the interaction of porphyrins and DNA at the electrode/solution interface, using a new measurement—the scanning electrochemical microscope (SECM), which is an electrochemical tool that can be used to study immobilized biomolecules and chemical or biological reactions at various interfaces with high spatial resolution and can obtain rate constants of homogeneous and heterogeneous redox reactions precisely [8–10]. We provide insight into the interaction of DNA with porphyrins and metalloporphyrin SAMs on gold electrode by means of cyclic voltammetry (CV), SECM, and alternating current (AC) impedance methods using a redox couple as a probe. With the SECM experiments, we hope to find an electrochemical microscopic view of the layer that is not possible to obtain with conventional techniques. Moreover, CV and AC impedance methods can well be utilized to prove the result of the SECM.

Experimental section

Chemicals and synthesis of porphyrin

Potassium ferricyanide and iron trichloride were purchased from Beijing Chemical (China). 7,7,8,8-Tetracyanoquinodimethane and tetrabutylammonium chloride were purchased from Aldrich. Potassium chloride, trichloromethane, nitrobenzene, and all acetate salts were purchased from Tianjing Chemical (China). All the other reagents were of analytical grade. The synthesis of the 5-[p-(mercaptopropyloxy)-phenyl]-10, 15, 20-triphenylporphyrin(H_2MPTPP) (the structure of porphyrin is shown in Scheme 1) was carried out according to the methodology reported previously [11].



Scheme 1 The scheme of porphyrin (H_2MPTPP) used in this experiment

The final product was characterized by hydrogen nuclear magnetic resonance.

ctDNA obtained from Huamei Chemical (China) was used as received. Solutions of DNA (about 10^{-5} mol·l^{-1} in nucleotide phosphate NP) in 50 mM NaCl/5 mM Tris pH= 6.8 gave a ratio of UV absorbance at 260–280 nm, A_{260}/A_{280} of about 1.7–1.8, indicating that the DNA could be used [12, 13].

Supporting electrolyte for all experiments was 0.1 mol·l^{-1} Tris HCl buffer (pH=6.8). The pH was chosen after a series of experiments. All solutions were deoxygenated via purging with N_2 for 15 min before each measurement. Other chemicals were of analytical reagent grade. Water was double distilled.

Preparation of modified electrode and metalloporphyrins

A 3-mm diameter gold electrode was polished with alumina powder (0.3 and 0.05 μm) and rinsed with pure water. Then, the gold electrode was immersed in the trichloromethane solution containing 0.1 mol/l porphyrin for different times, and before experiments, the modified electrode was rinsed with absolute ethanol and pure water, respectively. The metalloporphyrins were prepared using the methods reported by Nishimura et al. [14] The insertion of metal ions was performed by refluxing the metal salt (0.5 g) solution of chloroform (50 ml) and methanol (5 ml) for 3 h in which the free-base porphyrin monolayer-coated gold electrodes was dipped. The metal salts were cobalt (II) acetate and nickel (II) acetate. Then, the modified gold electrode was obtained.

Interaction of the modified electrode with DNA

After the modified gold electrode was obtained, it was put into a DNA solution for some time. Then rinsed with pure water, it was performed with other measurements. All experiment were carried out at room temperature of 25±2 °C.

Electrochemical Measurements

SECM apparatus

Electrochemical experiments were carried out using a CHI 900 scanning electrochemical microscopy (CH Instruments, Austin, USA) with a three-electrode cell. The gold electrode (or modified), a platinum wire, and a KCl saturated Ag/AgCl acted as the working, counter, and reference electrodes, respectively. The SECM tip is a 25-μm diameter Pt ultramicroelectrode. Before each experiment, the tip was polished with 0.3-μm alumina and rinsed with pure water, and the solutions were purged with pure nitrogen for 15 min before each run. The main quantitative operation was obtained from the feedback mode. Tip current (i_T) was normalized to the steady-state current ($i_{T,\infty} = 4nFDC^*a$, F is the Faraday constant, n is the number of electrons transferred in the tip reaction, D is the diffusion coefficient of electroactive species, C^* is the bulk concentration of the species, and a is the tip radius) and distance (d) to tip electrode radius (a). $i_T/i_{T,\infty}$ is called normalized current. The approach curve is presented in from i_T vs L ($L=d/a$, and d is the distance between the tip and the substrate and are independence of disk diameter, diffusion coefficient, and solute concentration).

The electron transfer (ET) rate were calculated according to the corresponding formulas. [15–17]

CV measurements

The CV experiment was carried out with a CHI 660 electrochemical workstation (CH Instrument) in a one-compartment three-electrode system at room temperature of 25±2 °C. A bare gold electrode or ctDNA-SAM/Au was used as the working electrode (CH Instrument). An Ag/AgCl electrode was used as a reference electrode, and a platinum wire served as the counter electrode.

AC impedance

AC impedance measurements were performed with a CHI 660 electrochemical workstation (CH Instrument) in a one-compartment three-electrode system at room temperature of 25±2 °C. The potential was controlled at the open-circuit value, and the frequency was varied over the range 10^5–1 Hz, with the amplitude of 5 mV. A bare gold electrode or ctDNA-SAM/Au was used as the working electrode (CH Instrument). An Ag/AgCl electrode was used as a reference electrode, and a platinum wire served as the counter electrode. The ET rate was calculated according to the corresponding formulas. [18, 19]

Results and discussion

The interaction of the H_2MPTPP SAMs of the gold electrode with DNA

Successful self-assembly process usually requires a relatively strong bond between the substrate and atom or moiety in the molecule. The strength of head group–substrate bonds, the lateral interactions, and the density of packing result in the sufficient stableness; the monolayer resists removal by a solvent rinse. Because of excellent affinity between gold and sulfur and some other advantages, such as easy purification and smoothness, the gold is used as substrate electrode modified with thiol-porphyrin.

The modification of gold surface with thiol-porphyrin is characterized by CV, and the redox behavior of a reversible couple, $Fe(CN)_6^{3-/4-}$, can be used to probe the packing structure of the monolayer. Figure 1 shows the cyclic voltammograms of the bare and modified gold electrode with H_2MPTPP at different times. From top to bottom, the modified times were 0 (bare gold), 12, and 24 h, respectively. There is a reversible electrochemical behavior on the bare electrode (curve a). The reaction is controlled by diffusion of 3–$Fe(CN)_6^{4-}$. After being modified by H_2MPTPP, the curves become flat with a rise of the adsorption times. According to the method reported in our previous paper [20], we can calculate that the hindrance ($B = 1 - \left[i_p^f(H_2MPTPP)/i_p^f(Au)\right]$) of the electrode ($i_p^f(H_2MPTPP)$ and $i_p^f(Au)$ are the forward (reduction in ferricyanide) peak currents measured at the modified and the bare electrode, respectively. After 24 h, a value of

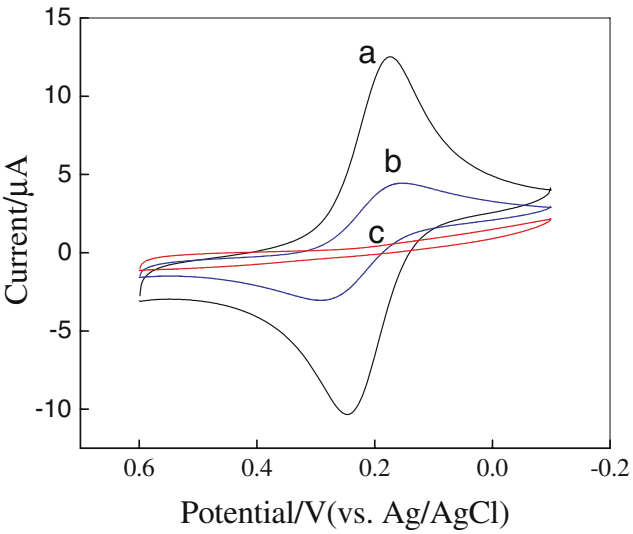


Fig. 1 Cyclic voltammograms of 1 mM $K_3Fe(CN)_6$ in 0.1 M KCl at a gold electrode modified with H_2MPTPP at different times. *From top to bottom*, the modified times were 0, 12, and 24 h, respectively. Sweep rate 10 mV/s

B=0.91 is reached, indicating practically a totally covered Au surface, so the totally modified SAM/Au was obtained.

Figure 2 shows the cyclic voltammograms of the totally modified SAM/Au after being immersed in the solution of DNA (pH=6.8) for 12 h (curve c). Comparing a, b, and c, it can be seen that the curves b (the totally modified SAM/Au) and c (the totally modified SAM/Au interacted with DNA after 12 h) is almost superposed, but curve c is much flatter. After interacted with DNA, value of B of the SAM/Au reached 0.96. Because the curve became too flat to measure the peak current, the values are approximations. But the results indicate that H_2MPTPP has interacted with DNA. For this reason, SECM image was employed to study the interaction process of the SAMs with DNA visually. The modified electrode was used as the substrate, and the potential was chosen at 0.5 V [20]. The feedback current increased (a positive feedback) on the bare gold electrode (Fig. 3d), whereas it decreased (a negative feedback) at the totally SAMs covered electrode (the SAMs are formed for about 24 h; see the Fig. 3e curve e). The changes prove that the ET has been blocked, and without redox center, the feedback current on the porphyrin monolayer could only be attributed to direct electron tunneling (i.e., ET through the monolayer between $Fe(CN)_6^{4-}$ and Au) and pinholes and defects. [21] The process of partly and totally modified electrode has been discussed previously [20].

After the totally modified SAMs electrode interacting with DNA for 12 h, the corresponding normalized currents (see Fig. 3e curve f) are much lower than without DNA (curve e). With the increase in interaction time, the normalized currents decreased. The rate constants (k_{eff}s) of the ET through the monolayer are measured using $Fe(CN)_6^{3-}$ as the mediator in solution (Table 1). The tendency confirms that k_{eff}s decrease with the increase in interaction times, but after 12 h, the normalized current almost did not decrease, thus, 12 h of interaction time was chosen in this study. There is an interesting phenomenon in the experiments. After the modified electrode interacted with DNA, if it was ultrasonically treated in twice-distilled water for a minute, the normalized currents (see Fig. 3e curve g) are lower than if it was only rinsed by twice-distilled water (curve f). It indicates that the interaction force between porphyrin monolayer and DNA is very strong, which cannot be removed by being ultrasonically treated and becomes more compact.

Curves a, b and c in Fig. 4 show the complex plane AC impedance frequency spectrum at bare, totally modified electrodes, and the totally modified SAMs interacted with DNA for 12 h, respectively. We can see that the semicircle in the high frequency region at the DNA/SAM is larger, and the AC resistance R_{ct} is apparently larger than without DNA, which indicates that porphyrin monolayer has

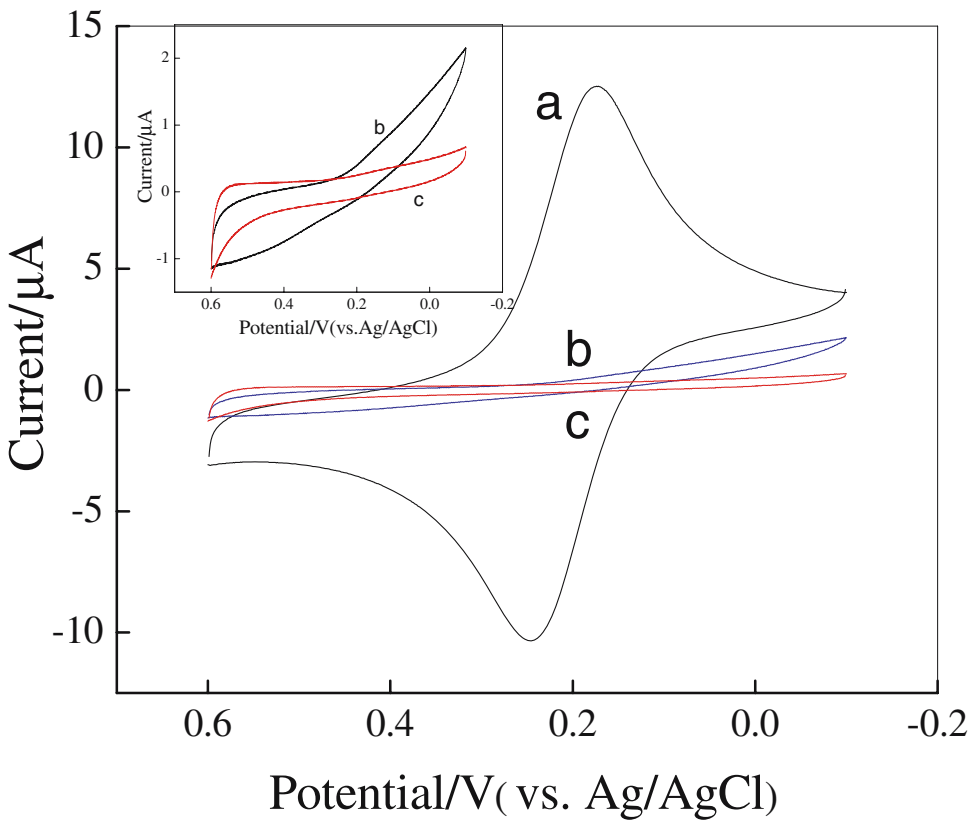


Fig. 2 Cyclic voltammograms of 1 mM $K_3Fe(CN)_6$ in 0.1 M KCl: *a* the bare gold electrode, *b* the totally modified H_2MPTPP SAM, and *c* the totally H_2MPTPP SAM after interacted with DNA for 12 h, respectively. Sweep rate 10 mV/s. The amplified *b* and *c* (*inset*)

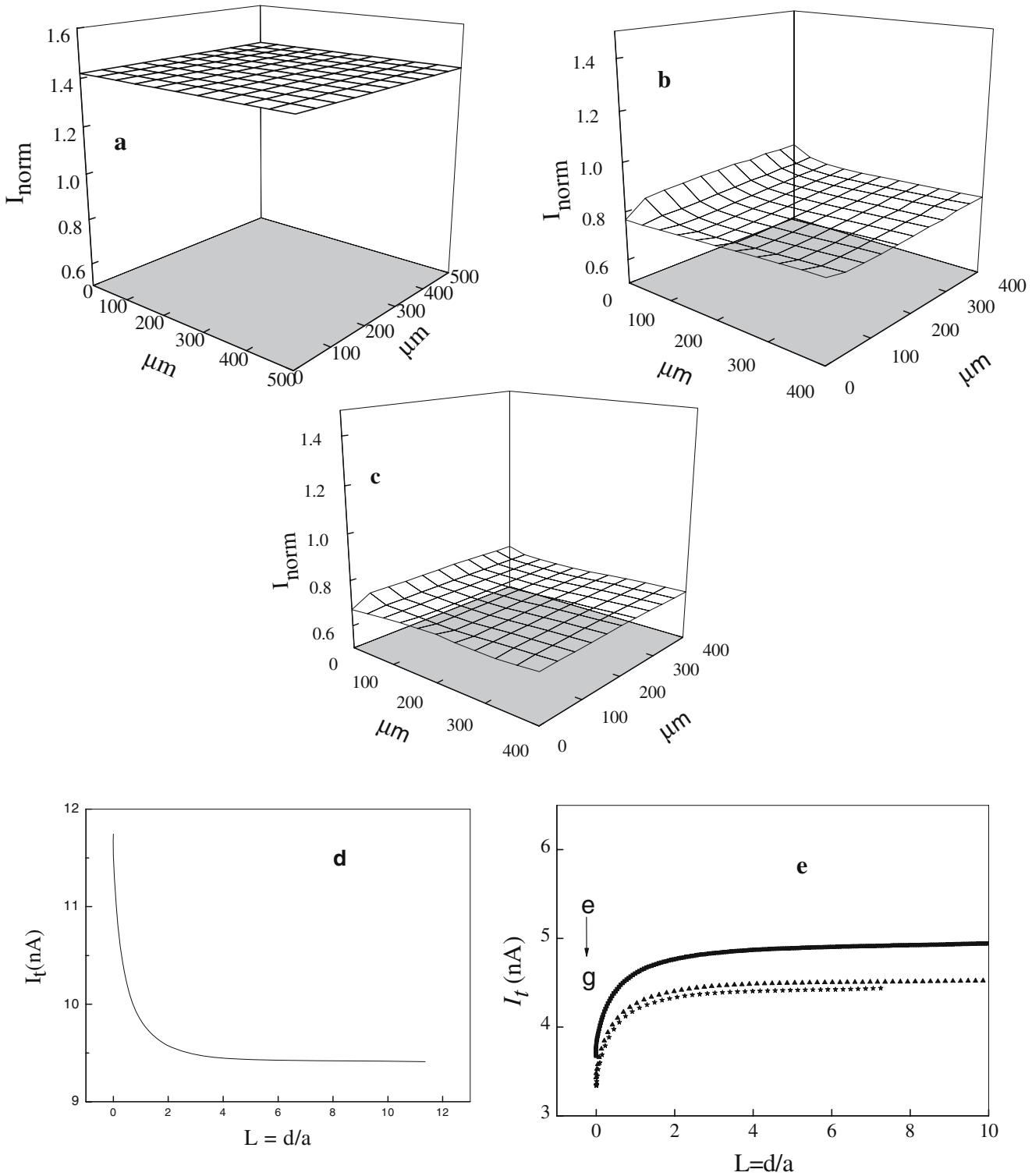


Fig. 3 The images of modified gold electrode using $K_3Fe(CN)_6$ as mediator in 0.1 M KCl solution, E_{tip}=−0.1 V, E_{sub}=0.5 V. **a** The bare gold electrode, **b** the totally modified H_2MPTPP SAM, and **c** the totally H_2MPTPP SAM after interacted with DNA for 12 h, respectively. *Inset*: **d** the probe approach curve of the bare gold electrode; **e** probe approach curves: *e* the totally modified H_2MPTPP SAM, *f* the totally modified H_2MPTPP SAM after interacted with DNA for 12 h, and *g* the totally modified H_2MPTPP SAM after interacted with DNA for 12 h was ultrasonically treated in twice-distilled water for a minute. The solution is 1.0 mM $K_3Fe(CN)_6$ and 0.1 M KCl

Table 1 The apparent heterogeneous rate constants (k_{eff} from SECM and k_f from AC impedance) and the corresponding coverage after different interaction times of gold electrode totally modified by porphyrin monolayer with DNA

	0 min	5 min	15 min	200 min	300 min	12 h
k_{eff} (10^{-4}) cm/s	3.26	3.12	2.08	1.16	1.07	0.92
k_f (10^{-4}) cm/s	1.25	1.02	0.87	0.48	0.41	0.25
B	0.91	0.91	0.92	0.94	0.94	0.96

interacted with DNA, that is, DNA must have been adsorbed onto the surface of the porphyrin monolayer to block the ET much more between indicator of $Fe(CN)_6^{3-}$ and electrode surface [22]. The apparent reaction rate constants (k_f) are calculated for different interaction times and listed in Table 1. Compared to the values of k_{eff} obtained with SECM, there is a good agreement between them, which means both methods could be associated and used as the novel method to detect the process of ET at solid/liquid interface. The results show that, after the SAM interacted with DNA, DNA must have been adsorbed on the porphyrin and increased the inhibition of ET.

To observe the interaction of porphyrin SAMs with DNA furthermore, the partly modified electrode for 6 and 10 h also was investigated. Figure 5 shows the cyclic voltammograms of the 6 h partly modified with H_2MPTPP (curve a) and interacted with DNA for 12 h (curve b) at gold electrode. The current of curve b decreased apparently in comparison with those of curve a, and the peak separation (ΔE) was increased, indicating the interaction of the SAM and DNA, which is more visible than in Fig. 2 (the interaction of totally modified electrode with DNA), and the hindrance (B) of the SAM increased to 0.88 from 0.72. Figure 6a and b show the SECM images obtained from 6-h partly modified Au electrode and interacted with DNA for 12 h, respectively. The topography after interacted with DNA is more regular and flat except the decrease in the normalized current. The image of 6-h partly modified SAM Au electrode is very irregular, which means there are more defects and pinholes. After interacted with DNA, defects and pinholes reduce, so the image is becoming regular and flat, and the normalized current decreases gradually. Furthermore, after interacted with DNA, DNA must have been adsorbed on the porphyrin, and electrostatic repulsion between deoxyribose-phosphate of DNA and $Fe(CN)_6^{3-}$ is capable of modulating the transfer of mediator molecules towards the SAM [23]. From Fig. 6c, the feedback tip currents on curve e (interacted with DNA) are much lower than those on curve d (without DNA), which also indicates that ET was blocked by DNA. Figure 7 shows the electrochemical impedance spectroscopy of 6-h partly modified SAM Au electrode (a) and interacted with DNA (b). We can see apparently the larger semicircle in the high-frequency region at the DNA-SAM/Au. The AC

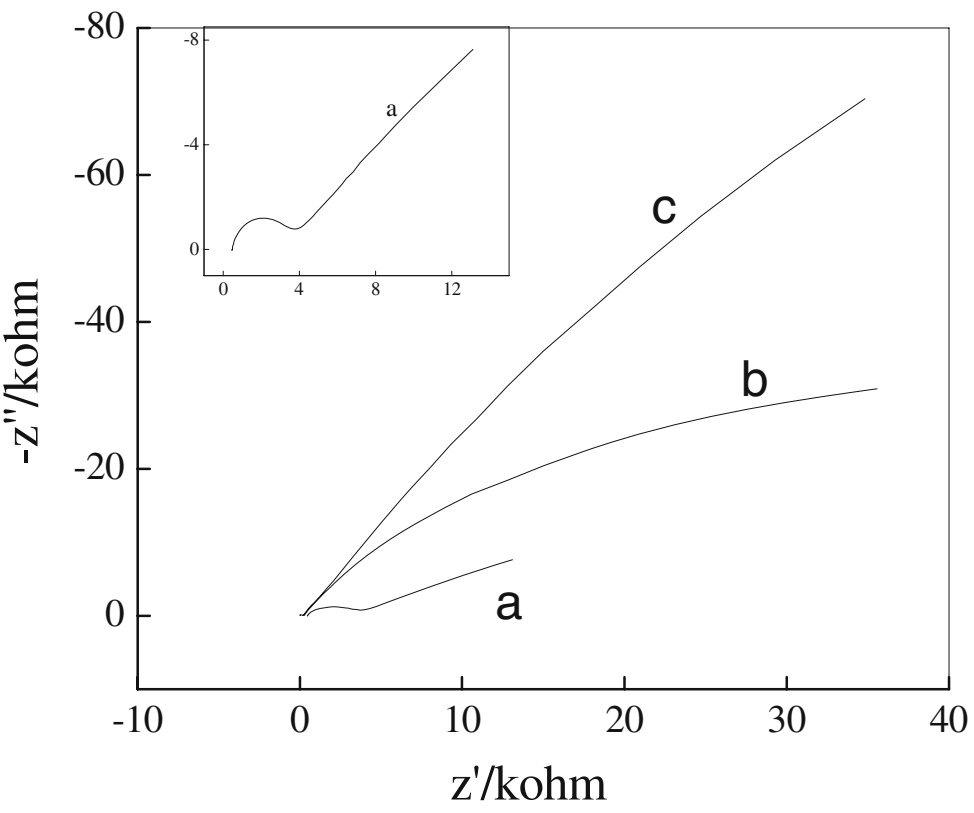


Fig. 4 Nyquist AC impedance frequency spectrum: *a* the bare gold electrode, *b* the totally modified H_2MPTPP SAM, *c* the totally modified SAM interacted with DNA for 12 h. Frequency range: 100 kHz–0.05 Hz, potential step: 0.4–0.9 V, pulse width: 0.5 s

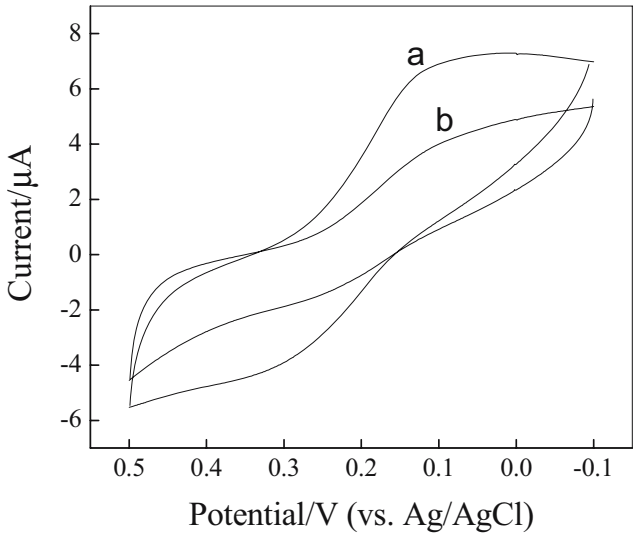


Fig. 5 Cyclic voltammograms of 1 mM $K_3Fe(CN)_6$ in 0.1 M KCl at a gold electrode: *a* the 6-h partly modified H_2MPTPP SAM and *b* the partly modified H_2MPTPP SAM after interacted with DNA for 12 h, respectively. Sweep rate 10 mV/s

resistance is larger than that without DNA. The rate constants k_{eff} (from SECM) and k_f (from AC impedance) and B (the hindrance parameter) are listed in Table 2. The decrease in k_{eff} or k_f values and increase in B values prove that DNA must have been absorbed onto the porphyrin SAMs and effectively increased the inhibition of ET at SAM/Au electrode. The 10-h partly modified SAMs interacted with DNA were also investigated. The result is similar to the 6-h partly modified one. The only difference is the approach curves. Figure 8 shows different behaviors ranging from a positive (curve a) to a negative (curve b) feedback current, which indicate that after interacted with DNA, ET at the SAM/Au has indeed been blocked. The mechanism of ET at the SAMs was discussed previously in detail [19, 24]. The totally modified electrode by H_2MPTPP is compact and has no defects so that electrons can be transported through the SAM only by tunneling. Compared with the totally modified electrode, the electrons can be transported through the pinholes and defects at the 6-h partly modified electrode; after it interacted with DNA, defects and pinholes reduced to block ET, and the current decreased. These results prove that DNA can interact with H_2MPTPP and must have been absorbed on the porphyrin SAM. From above results, we suggest that the mode of the interaction between H_2MPTPP and DNA is electrostatic interaction [25].

The interaction of the metal–MPTPP SAMs of the gold electrode with DNA

Some metalloporphyrins usually play important roles in nature as mentioned previously [7]. Investigation of the

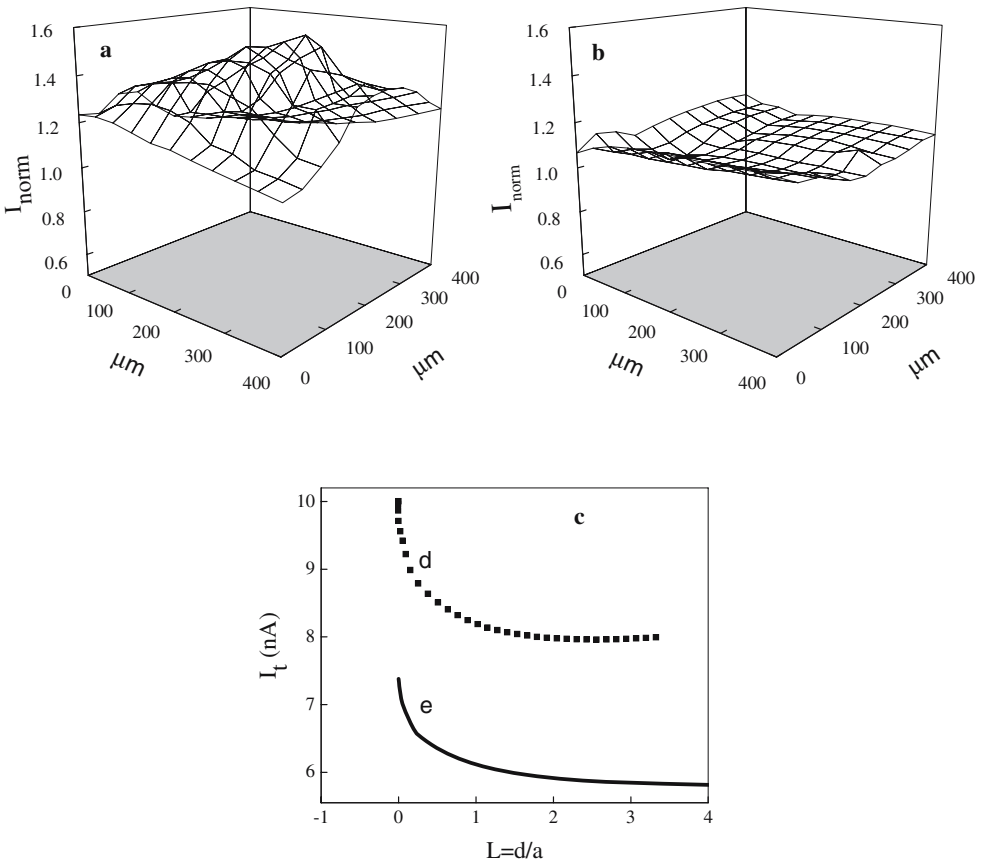


Fig. 6 The images of partly modified H_2MPTPP SAM at gold electrode using $K_3Fe(CN)_6$ as mediator in 0.1 M KCl solution, E_{tip}=−0.1 V, E_{sub}= 0.5 V. **a** 6-h partly modified gold electrode with H_2MPTPP, and **b** the 6-h partly modified H_2MPTPP SAM after interacted with DNA for 12 h, respectively. *Inset*: **c** probe approach curves: *d* the 6-h partly modified gold electrode and *e* the 6-h partly H_2MPTPP SAM at gold electrode after interacted with DNA for 12 h. The solution is 1.0 mM $K_3Fe(CN)_6$ and 0.1 M KCl

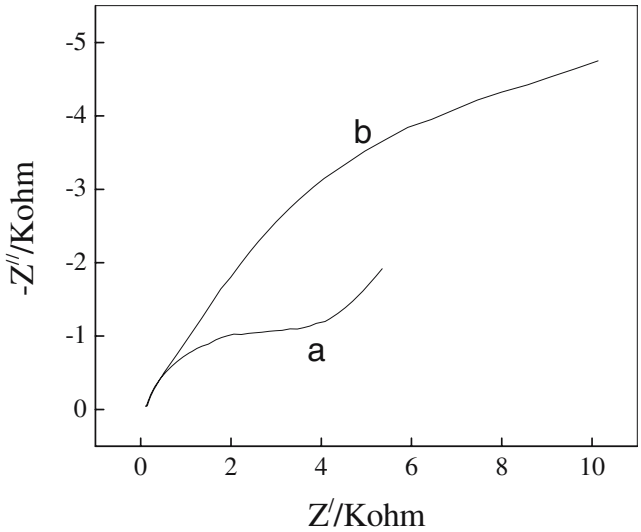


Fig. 7 Nyquist AC impedance frequency spectrum of different electrodes: *a* the 6-h partly modified H_2MPTPP SAM at gold electrode and *b* the 6-h partly H2MPTPP SAM at gold electrode after interacted with DNA for 12 h. Frequency range: 100 kHz–0.05 Hz, potential step: 0.4–0.9 V, pulse width: 0.5 s

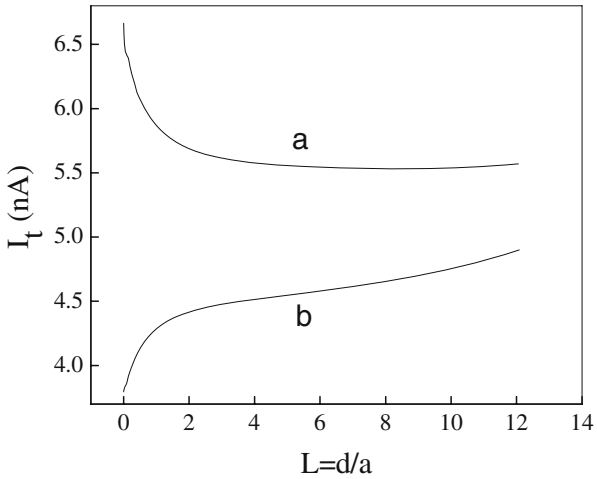


Fig. 8 Probe approach curves: *a*, the 10-h partly modified H_2MPTPP SAM at gold electrode and *b* the 10-h partly H_2MPTPP SAM after interacted with DNA for 12 h. The solution is 1.0 mM $K_3Fe(CN)_6$ and 0.1 M KCl, E_{tip}=−0.1 V, E_{sub}=0.5 V

interaction of metalloporphyrins and DNA may lead to medical applications in the inhibition of the AIDS virus, and in the photodynamic treatment of tumors [26–28]. Therefore, it is a very significant area of research.

Figure 9 shows the cyclic voltammograms of ferricyanide at the modified gold electrode with Co-MPTPP (curve b) and interacted with DNA (curve c). Comparing b and c, it can be seen that the current for the$Fe(CN)_6^{3-}$ after interacted with DNA decreased dramatically in comparison with those of curve b, and the oxidation peak I (at 0.668 V) potential shifted (E_{pa}) negatively by 100 mV (to 0.568 V) and the oxidation peak II (at 0.528 V) potential shifted (E_{pa}) negatively by 138 mV (to 0.390 V) and the reduction peak III (at 0.644 V) potential (E_{pc}) shifted positively by 56 mV (to 0.700 V). The result shows two main changes: the decrease in current and loss of reversibility of the redox reaction, which indicate that Co-MPTPP bind electrostatically to the negatively charged deoxyribose -phosphate backbone of DNA [25].

The cyclic voltammograms of the modified gold electrode with Ni-MPTPP (curve a) and interacted with DNA (curve b) are shown in Fig. 10. The difference from Co-MPTPP is that the peak currents of Ni-MPTPP interacted with DNA increased, and peak potentials all shifted negatively by 80 mV, which also indicate that the mode of interaction between Ni-MPTPP with DNA is electrostatic interaction [25]. The same with Co-MPTPP is the peak current of ferricyanide, which decreased after interacted with DNA, which indicates the negative charge on the phosphate groups of DNA [28] repelled the diffusion of Fe $[(CN)_6]^{3-/4-}$ at the electrode, so the k_f (k_{eff}) decreased

Table 2 The apparent heterogeneous rate constants (k_{eff} from SECM and k_f from AC impedance) and the corresponding coverage from interaction of different SAM gold electrode with DNA for 12 h

	6 h H_2MPTPP		10 h H_2MPTPP		Co-MPTPP		Ni-MPTPP	
	No-DNA	DNA	No-DNA	DNA	No-DNA	DNA	No-DNA	DNA
k_{eff} (10^{-4}) cm/s	19.3	8.31	7.82	5.16	9.51	5.89	9.64	8.41
k_f (10^{-4}) cm/s	13.1	4.02	3.51	2.03	4.83	2.14	4.96	4.02
B	0.14	0.39	0.54	0.77	0.35	0.72	0.30	0.40

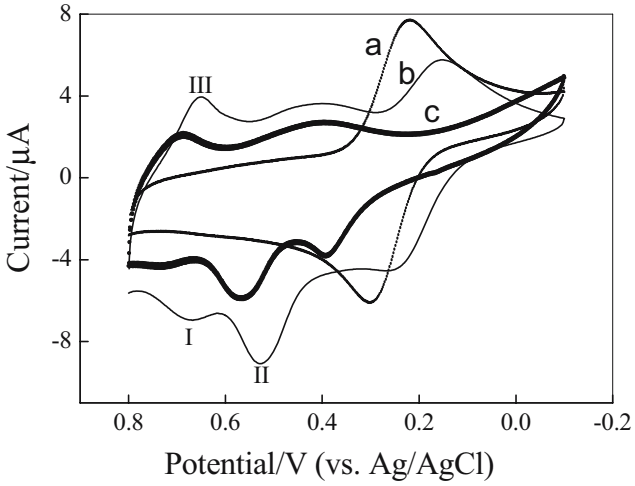


Fig. 9 Cyclic voltammograms of 1 mM $K_3Fe(CN)_6$ in 0.1 M KCl at a gold electrode: *a* the bare gold electrode, *b* the modified Co-MPTPP SAM at gold electrode, and *c* the Co-MPTPP SAM at gold electrode after interacted with DNA for 12 h, respectively. Sweep rate 10 mV/s

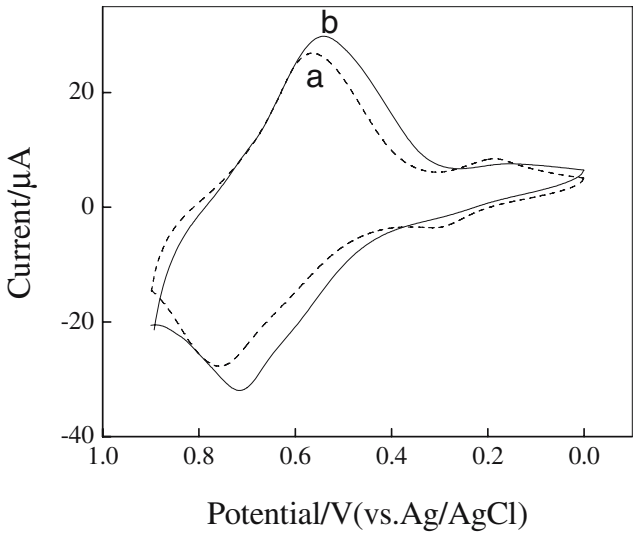


Fig. 10 Cyclic voltammograms of 1 mM $K_3Fe(CN)_6$ in 0.1 M KCl at a gold electrode: *a* the modified Ni-MPTPP SAM at gold electrode and *b* the Ni-MPTPP SAM at gold electrode after interacted with DNA for 12 h, respectively. Sweep rate 10 mV/s

slightly. The increased current of Ni-MPTPP after interacted with DNA may be because the electrostatic force between Ni-MPTPP and DNA is stronger, which makes Ni-MPTPP partly intercalated into the bones of DNA and decreased the capacitance current.

From the images of the metalloporphyrin SAMs (Fig. 11), some differences between Co-MPTPP and Ni-MPTPP interacted with DNA were observed on the surface such as the topographies became fluctuant and the normalized current increased with different extent. The approach curves changed behaviors ranging from a positive to a negative feedback current for Co-MPTPP after interacted with DNA, but for Ni-MPTPP, all were positive feedback current, and only the currents were lower slightly (not shown). The rate constants k_{eff} (from SECM) and k_f (from AC impedance) and B are listed in Table 2.

The attractive force between porphyrins and DNA is favorable for an electrostatic interaction because of the negative charge on the phosphate groups of DNA and the positive charges on the porphyrins. Compared with these electrochemical characteristics, the order of interaction force of DNA between them is Ni-MPTPP>Co-MPTPP> H_2MPTPP.

Conclusions

In this work, we have carried out the CV, SECM, and AC impedance methods to study the interaction of porphyrin (H_2MPTPP) and metalloporphyrin (Ni, Co-MPTPP) with DNA. After totally and partly modified Au electrode by H_2MPTPP interacted with DNA, the results are similar: the decrease in current and loss of reversibility of the redox reaction for $K_3[Fe(CN)_6]$, apparent heterogeneous reaction

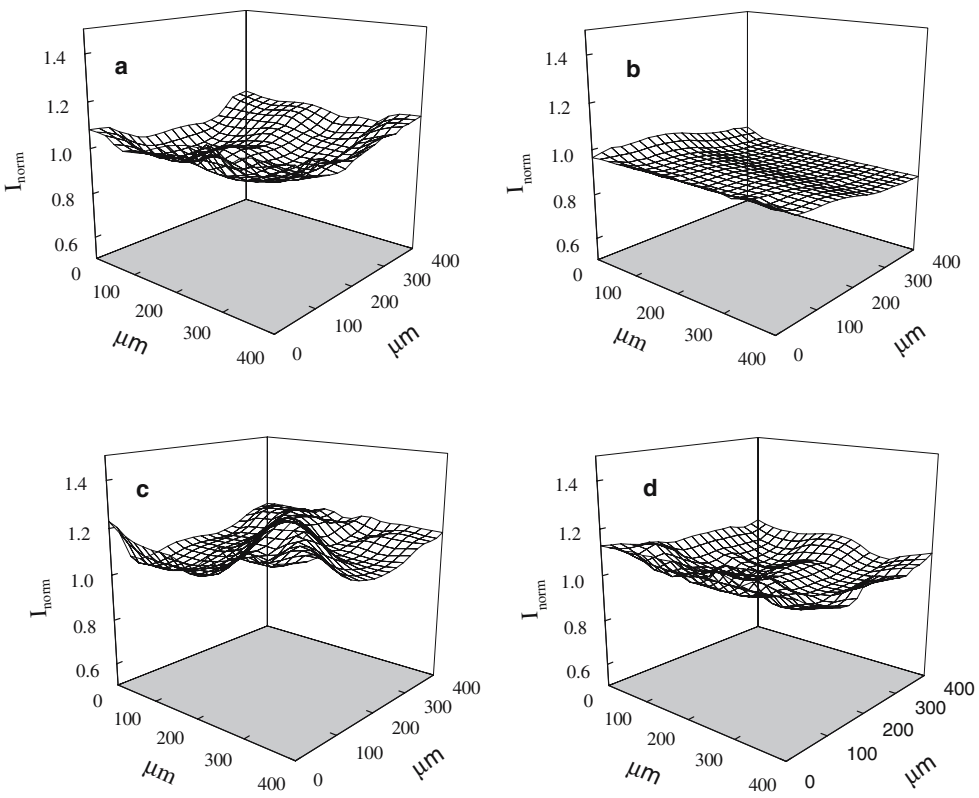


Fig. 11 The images of modified gold electrode by metalloporphyrins using $K_3Fe(CN)_6$ as mediator in 0.1 M KCl solution, E_{tip}=−0.1 V, E_{sub}=0.5 V. **a** The modified gold electrode with Co-MPTPP, **b** the modified Co-MPTPP SAM at gold electrode after interacted with DNA for 12 h, **c** the modified gold electrode with Ni-MPTPP, and **d** the modified Ni-MPTPP SAM at gold electrode after interacted with DNA for 12 h, respectively

rate constant (k_{eff} from SECM and k_f from AC impedance) was decreased, and the hindrance (B) of electrode increased, which show that DNA must have been adsorbed on the porphyrin and increased the inhibition of ET. And then the interaction of Ni, Co-MPTPP with DNA was studied. The difference from Co-MPTPP is that the current decreased after interacted with DNA, and the oxidation peak potential shifted (E_{pa}) negatively by 100 mV, and the reduction peak potential (E_{pc}) shifted positively by 56 mV, but the redox peak currents of Ni-MPTPP interacted with DNA increased, and redox peak potentials all shifted negatively by 80 mV. The k_{eff} from SECM and k_f from AC impedance and the hindrance (B) of electrode were also detected, which show DNA was adsorbed on the porphyrin. It is suggested to be electrostatic interaction of H_2MPTPP, Co-MPTPP, and Ni -MPTPP with DNA, and the order of attractive force between porphyrins and DNA is Ni-MPTPP> Co-MPTPP>H_2MPTPP.

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References

1. Czuchajowski L, Habdas J, Niedbala H, Wandrekar V (1992) J Heterocycl Chem 29:479
2. Czuchajowski L, Habdas J, Niedbala H, Wandrekar V (1991) Tetrahedron Lett 32:7511
3. Han GY, Yang P (2002) J Inorg Biochem 91:230
4. Kim JO, Lee YA, Jin B, Park T, Song R, Kim SK (2004) Biophys Chemist 111:63
5. Nyarko E, Hanada N, Habib A, Tabata M (2004) Inorg Chim Acta 357:739
6. Tjahjono DH, Mima S, Akutsu T, Yoshioka N, Inoue H (2001) J Inorg Biochem 85:219
7. Chen SM, Chen SV (2003) Electrochim Acta 48:4049
8. Kwak J, Bard AJ (1989) Anal Chem 61:1221
9. Cai CX, Liu B, Mirkin MV, Frank HA, Rusling JF (2002) Anal Chem 74:114
10. Wittstock G (2001) Fresenius J Anal Chem 370:303
11. Lu XQ, Geng ZX, Wang YS, Lv BQ, Kang JW (2002) Synth React Inorg Met-Org Chem 32(5):843
12. Bard AJ, Fan FRF, Pierce DT, Unwin PR, Wipf DO, Zhou F (1991) Science 254:68
13. Nassar AEF, Rusing JF, Nakashima N (1996) J Am Chem Soc 118:3043
14. Nishimura N, Ooi M, Shimazu K, Fujii H, Uosaki K (1999) J Electroanal Chem 473:75
15. Bard AJ, Mirkin MV (2001) Scanning electrochemical microscopy. Marcel Dekker Inc, New York, p 213
16. Shao Y, Mirkin MV (1998) J Phys Chem 102:9915
17. Forouzan F, Bard AJ, Mirkin MV (1997) Isr J Chem 37:155
18. Tsionsky M, Bard AJ, Mirkin MV (1996) J Phys Chem 100:17881
19. Liu J, Li QW, Luo GA, Sun HW, Feng J (2002) Journal of Instrumental Analysis 21:13
20. Lu XQ, Zhang LM, Li MR, Wang XQ, Zuo GF (2006) Chemphyschem 7:854
21. Liu B, Bard AJ, Mirkin MV, Creager SE (2004) J Am Chem Soc 126:1485
22. Laviron E (1979) Electroanal Chem 101:19
23. Turcu F, Schulte A, Hartwich G, Schuhmann W (2004) Biosens Bioelectron 20:925
24. Lu XQ, Li MR, Yang CH, Zhang LM, Li YF, Jiang L, Li HX, Liu CM, Hu WP (2006) Langmuir 22:3035
25. Zhao YD, Pang DW, Wang ZL, Cheng JK, Qi YP (1997) J Electroanal Chem 431:203
26. Lugo-Ponce P, McMillin DR (2000) Coord Chem Rev 208:169
27. Miller J (1999) J Chem Educ 76:592
28. Guliaec AB, Leontis NB (1999) Biochemistry 38:15425