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Electrochemical Behaviors of Neutral Red on Single and Double Stranded DNA Modified Electrode

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The interaction of neutral red (NR) with single or double stranded DNA was investigated on ssDNA or dsDNA modified gold electrode. DNA was covalently immobilized on the surface of mercaptoethanol self-assembled gold electrode through the carboxylate ester as linkage between the 5'-NH_2 end of DNA and -OH group of mercaptoethanol self-assembled monolayer with the help of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-hydroxysuccinimide (NHS). The electrochemical behaviors of NR on DNA modified electrode were carefully investigated by cyclic voltammetry. NR was bound with ssDNA by electrostatic force but intercalation with dsDNA. The binding constant was calculated as 8.95×10^3 M^{-1} and 2.15×10^4 M^{-1} respectively. The different responses of NR with ssDNA and dsDNA were further proved by UV-Vis absorption spectrophotometry and the results suggested that NR could be served to distinguish the ssDNA and dsDNA.

Keywords: Neutral red; DNA; gold electrode; self-assembled monolayer; interaction

1. INTRODUCTION

DNA electrochemical biosensor, which consists of a DNA chemical recognition element coupled to electrode that converts the recognition reaction into a measurable electronic signal, has aroused great potential application in disease diagnosis, drug screening and clinical test [1]. The interaction of small organic molecules with DNA can also be used for investigation the structure information of DNA or used as indicator for DNA biosensor [2-5]. Many different analytical methods such as UV-Vis spectrophotometry, fluorescence, resonance light-scattering techniques, electrochemical method and so on [6-10], were employed for the studies on the interaction. The small molecules studied include

organic dyes [11-12], drugs [13-14] and metal complexes [15-16]. Thought the changes of spectroscopic or electrochemical response of the interaction with DNA, the structure and functions of DNA can be explained.

Electrochemical method is a useful tool for the investigation for its high sensitivity, small dimensions, low cost and wider dynamic range. It can provide the information of the electron transfer process of DNA and act as an effective transducer for the analytical signal. Many organic molecules are electroactive and can be used as special electrochemical indicator for DNA biosensor. The interaction mechanism can be directly investigated in the solution by mixing the small molecular and DNA and record the changes of analytical response. Bard et al [17] had studied the interaction of metal chelates of $ML_3^{3+/2+}$ (M=Fe, Co; L=1,10-phenanthroline, 2,2'-bipyridine) with double-stranded calf thymus DNA by cyclic voltammetry and established a redox current equation for intercalator-DNA complex. Jiao et al [18-20] had applied some organic substances such as toluidine blue, methyl violet, brilliant cresyl blue etc. for the electrochemical detection of DNA in solution. The interaction can be also investigated on DNA modified electrode, which can save the usage of DNA and provide the surface electrochemistry of DNA biosensor. Ju et al [21-22] had studied the electrochemical behaviors of methylene blue or ferrocerium hexafluorophsphate on DNA modified electrode and further applied to the detection of the PCR product of hepatitis B virus.

In this paper the electrochemical behavior of neutral red (NR) on different DNA modified electrode was carefully investigated. NR is a phenazine dye with the molecular structure shown in figure 1. It had been used as a spectroscopic and voltammetric probe for the detection of DNA in solution [23-24]. The DNA was covalent immobilized on the electrode by using the gold as a basal electrode, which was modified with mercaptoethanol to form a self-assembled monolayer (SAM). With the help of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-hydroxysuccinimide (NHS), ssDNA and dsDNA were immobilized onto the surface of the SAM/Au electrode. The electrochemical behaviors of NR on the DNA modified electrode were studied by cyclic voltammetry. The binding constant was calculated and the interaction mechanism was discussed. The results showed that NR could distinguish ssDNA and dsDNA efficiently.

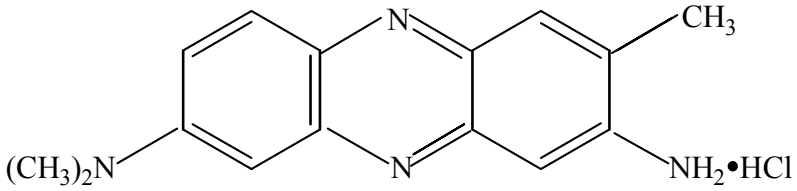


Figure 1. The molecular structure of neutral red (NR)

2. EXPERIMENTAL

2.1. Apparatus

All the electrochemical experiments were performed on a LK 98A electrochemical workstation (Tianjin Lanlike Chemical and Electron High Technology Co. LTD, China). The three-electrode

system is composed of a DNA modified gold electrode as working electrode, a saturated calomel electrode (SCE) as reference electrode and a platinum wire counter electrode. UV-Vis absorption spectra were recorded by a Cary 50 probe UV-Vis spectrophotometer (Australia Varian Company). A pHS-25 acidimeter (Shanghai Leici Instrument Factory) was used to measure the pH of buffer. All the experiments were carried out at 25 ± 2ºC.

2.2. Reagents

Natural fish sperm DNA (dsDNA, Beijing Jingke Biochemical Co.), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, Sigma), N-hydroxysuccinimide (NHS, Shanghai Chemical Reagent Company), Neutral red (NR, Shanghai No.3 Chemical Reagent Factory) were used as received without further purification.

Stock solution of dsDNA was prepared by dissolving it in doubly distilled water directly. The purity of DNA is checked by measuring the absorbance at 260 nm and 280 nm. The value of A_{260}/A_{280} was got as 1.85, which indicated that dsDNA was sufficiently pure without the protein. The single-stranded DNA (ssDNA) was obtained by heating native dsDNA in 100ºC water bath for 10 min and then cooled in an ice-water bath. The following buffers were used: phosphate buffer solution (PBS, 20 mmol/L pH 7.0), Britton-Robinson (B-R) buffer solution (0.2 mol/L pH 6.0). Other chemicals were of analytical reagents grade and used without further purification. Doubly distilled water was used throughout.

2.3. Preparation of DNA modified electrode

DNA modified electrode was prepared with the following procedure: the gold electrode was first polished with 0.05 μm albumin/water slurry on the polishing cloth and then ultrasonicated in acetone, ethanol and water for 5 min each, respectively, to get a mirror-like surface. Then the gold electrode was immersed into a 10.0 mmol/L mercaptoethanol solution for 24 h in the dark to get a mercaptoethanol self-assembled monolayer on the surface of gold electrode (SAM/Au). The SAM/Au electrode was dipped into a phosphate buffer solution (PBS) containing 5.0 mmol/L EDC and 8.0 mmol/L NHS for 30 min to get an active surface, which was further immersed in a PBS solution with ssDNA or dsDNA for 24 h at 25ºC. Based on the covalently immobilization of ssDNA or dsDNA via carboxylate ester linkage between 5'-NH_2 end of DNA and -OH end of mercaptoethanol monolayer. ssDNA or dsDNA was tightly fixed on the surface of self-assembled monolayer to form a ssDNA or dsDNA modified electrode. The whole procedure can be expressed in the following scheme:

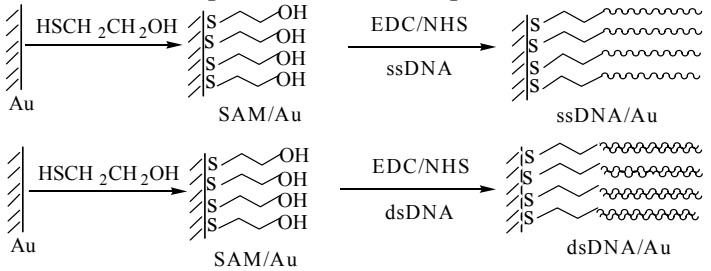


Figure 2. Scheme of procedure of immobilizing ssDNA or dsDNA on Au electrode

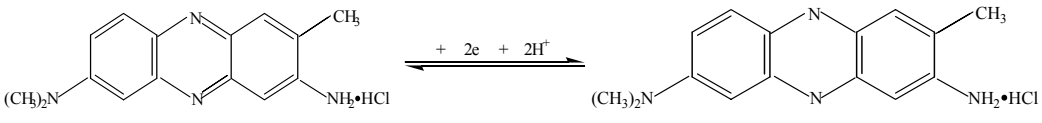
2.4. Electrochemical measurements

Cyclic voltammetry (CV) was used for the investigation by putting DNA modified electrode in a 1.5×10^{-4} mol/L NR solution at pH 6.0 B-R buffer. After the 10 min accumulation in an open circuit, the cyclic voltammogram of NR on DNA modified electrode was recorded in the potential range from -350 to -750 mV (vs. SCE) with the scan rate as 100 mV/s. The changes of electrochemical response of NR on different DNA modified electrode were used to compare the different binding process.

3. RESULTS AND DISCUSSION

3.1. Cyclic voltammograms of NR on DNA modified electrode

Figure 3 shows the cyclic voltammograms of NR on bare gold (1), ssDNA/Au (2) and dsDNA/Au (3) electrode, respectively. As shown in curve 1, NR had a pair of redox peaks on bare gold electrode with the Epa= -542 mV and Epc= -586 mV, which was attributed to the electrochemical redox process of C=N group in NR structure. The electrode reaction process can be expressed with the following equation:



(1)

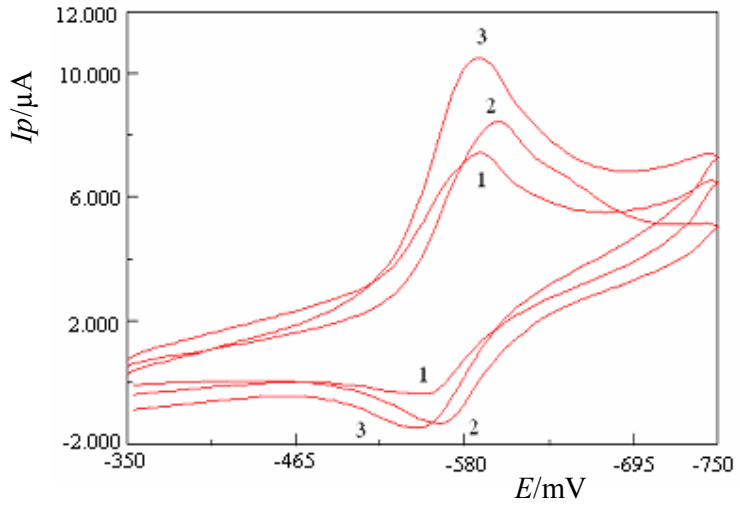


Figure 3. Cyclic voltammograms of NR on bare gold (1), ssDNA/Au (2) and dsDNA/Au (3) electrode in pH 6.0 B-R buffer solution with the scan rate as 100 mV/s.

On the ssDNA/Au electrode (curve 2), the redox peaks increased slightly and the peak potentials changed to -566 mV and –589 mV, respectively, which indicated that NR could bind to ssDNA fragment on the surface of electrode and resulted in the accumulation of NR on the electrode. On curve 3, after interaction of NR with dsDNA, a significant increase of reductive peak current

appeared, which indicated NR had stronger interaction with dsDNA and more NR molecules were accumulated on the electrode surface. The difference of electrochemical behavior of NR on ssDNA or dsDNA modified electrode indicated NR had different binding process with ssDNA or dsDNA and it could be served as an effective electrochemical indicator.

3.2. Effect of NR concentration on the peak current

The influence of NR concentration on the peak current was investigated and the cyclic voltammograms of different concentration of NR on dsDNA modified electrode were recorded. As shown in figure 4, the reductive peak current increased gradually and then reached its saturation value at the concentration of NR more than 1.5×10^{-4} mol/L, which indicated the binding of NR with dsDNA had reached its equilibrium. So a 1.5×10^{-4} mol/L NR solution was used for further investigation.

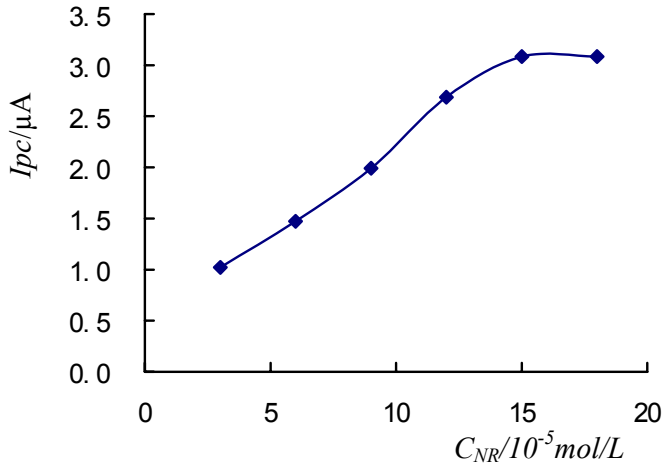


Figure 4. The influence of NR concentration on dsDNA modified electrode

Since the binding of NR with DNA modified electrodes indicate a trend to a Langmuir adsorption, the adsorption constants of NR at different DNA modified electrode surfaces were calculated according to the following Langmuir adsorption thermodynamic equation [25]:

$$\frac{c}{Ip}=\frac{1}{KIp_{\max}}+\frac{c}{Ip_{\max}} \qquad (2)$$

Where Ip represents the cathodic or anodic peak current, Ip_{max} the maximum of peak current, c the concentration of NR and K the binding constant of NR on the DNA modified electrode. From the equation it could be seen that the relationship of c/Ip and c should be a line. From the slope and intercept the value of Ip_{max} and K can be obtained.

The relationships of c/Ip with c on ssDNA/Au and dsDNA/Au electrode are constructed and shown in figure 5. Two lines are got and from the linear regression equations the binding constants of NR on ssDNA/Au and dsDNA/Au electrode are calculated with the results as 8.95×10^{3} M^{-1} and 2.15×10^{4} M^{-1}, respectively. The differences of binding constants of NR on different DNA modified electrode indicated that NR had more affinity for dsDNA than ssDNA. So the complex of NR-dsDNA was more stable than that of NR-ssDNA.

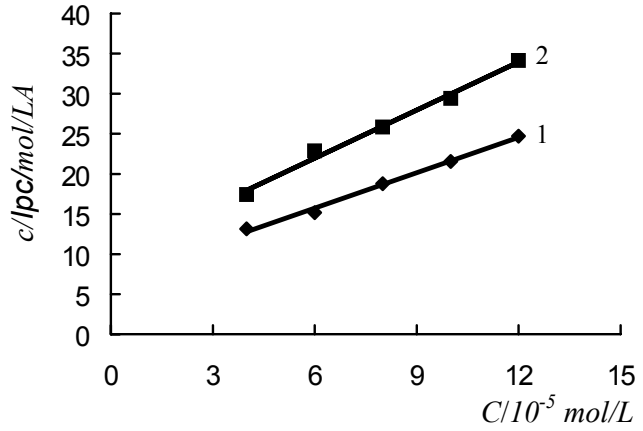


Figure 5. Plot of *c/Ipc* vs. *c* at ssDNA/Au (1) and dsDNA/Au (2) electrode

3.3. Influence of scan rate

The influences of scan rate on the peak current of NR at bare gold, ssDNA/Au and dsDNA/Au electrodes were also investigated in the range of 50 to 500 mV/s. With the increase of scan rate, the peak current was also increased but no linear relation was got at all these three electrodes. The plots of log*Ip* vs. log*v* at different electrodes are shown in figure 6. Three lines are got with the slope as 0.77, 0.84 and 0.91, respectively. The different slopes are between 0.5 and 1. Since the theoretical slope value of log*Ip* vs. log*v* is 0.5 for ideal diffusion-controlled electrode process and 1 for ideal surface-controlled process, respectively. So the electrode process of NR on the three electrodes is the combination of surface-controlled and diffusion-controlled processes, which resulted in the mixture of free and bound NR on the electrode surface. The slopes increased gradually, which indicated that the contribution of surface-controlled process on dsDNA modified electrode was more than that on ssDNA modified electrode. More NR molecules were bound to dsDNA on the surface of dsDNA modified electrode than on ssDNA modified electrode.

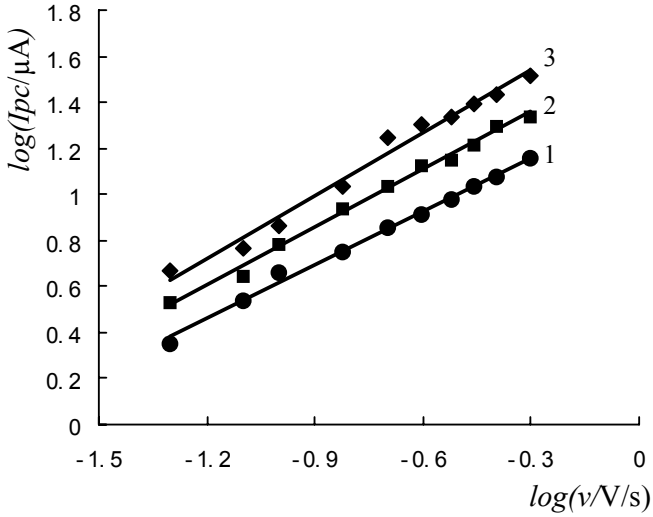


Figure 6. Plots of log*Ip* vs log*v* at bare gold (1), ssDNA/Au (2) and dsDNA/Au (3) electrode

3.4. Interaction of NR with DNA in solution

The interaction of NR with ssDNA and dsDNA was also investigated in solution by electrochemical and UV-Vis spectrophotometric method.

In pH 6.0 B-R buffer solution NR has a well-defined redox peaks on the bare gold electrode and the electrochemical parameters are listed in Table 1. The results indicate that NR takes place quasi-reversible electrode process on gold electrode. After the addition of 200.0 mg/L ssDNA and dsDNA into 1.5×10^{-4} mol/L NR solution, the electrochemical results are also recorded and listed in Table 1. Compared with NR solution, a significant decrease of oxidation peak current was appeared, which resulted from the decrease of free NR concentration in the solution due to the formation of NR-DNA complex. Since dsDNA has higher affinity to NR than ssDNA, the change of electrochemical response is more distinct, which can be further used for distinguish the ssDNA and dsDNA in solution.

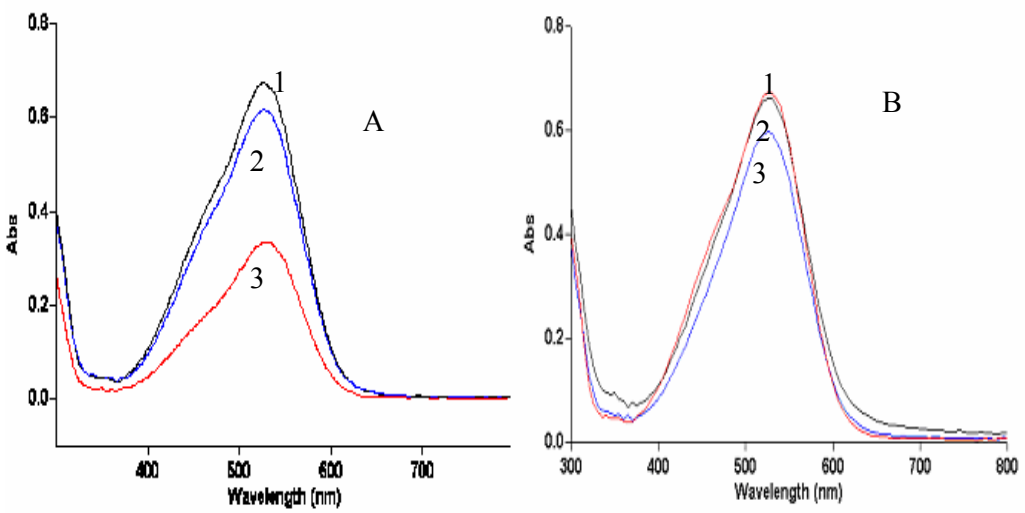


Figure 7. UV-Vis absorption spectra of NR interacted with dsDNA (A) and ssDNA (B)
A: (1), 4.0×10^{-5} mol/L NR in pH 6.0 B-R buffer; (2-3), 1+10.0, 50.0 mg/L dsDNA
B: (1), 4.0×10^{-5} mol/L NR in pH 6.0 B-R buffer; (2-3), 1+10.0, 20.0 mg/L ssDNA

Table 1. Changes in the electrochemical parameters of 1.5×10^{-4} mol/L NR in the absence and presence of ssDNA or dsDNA

	i_{pa}/μA	i_{pc}/μA	E_{pa}/mV	E_{pc}/mV	Decrease of i_{pa}	Decrease of i_{pc}	ΔE/mV
NR	-1.351	4.309	-542	-586			44
NR+ssDNA	-0.893	3.318	-550	-590	33.9%	23.0%	40
NR+dsDNA	-0.770	2.290	-554	-585	43.1%	46.9%	32

UV-Vis absorption spectra of NR and its interaction with ssDNA and dsDNA can be used to prove the different interaction model of NR with ssDNA and dsDNA. Figure 7 shows the UV-Vis absorption spectra of NR with different amount of dsDNA (A) and ssDNA (B) in pH 6.0 B-R buffer solution. NR had a maximum absorption wavenumber at 525.1 nm (curve 1 of A and B). After the addition of different amount of dsDNA in NR solution, the maximum absorption peak redshift with the greatly decrease of absorbance value (curves 2, 3 of figure 7A). According to the research of Borton [26], the increase of maximum absorption number and the decrease of absorbance value were the characteristics of intercalative model between the dsDNA and small molecular. So the result indicated NR bound to dsDNA through intercalative model. After the interaction of NR with ssDNA (figure 7B), only slightly decrease of absorbance can be observed without the movement of absorption number (curves 2, 3 of figure 7B). Although ssDNA don't have double-stranded structure and cann't intercalated with NR, the backbone of ssDNA is in negative charge and can easily attract positive charged NR molecules.

4. **CONCLUSIONS**

The direct electrochemical behaviors of neutral red (NR) on ssDNA or dsDNA modified electrode were carefully investigated. ssDNA or dsDNA were covalent immobilized on the mercaptoethanol self-assembled gold electrode, which had different binding ability with NR in pH 6.0 B-R buffer solution. The peak current was greatly increased on dsDNA/Au in comparison with that on ssDNA/Au. The binding constant was calculated as 8.95×10^{3} M^{-1} and 2.15×10^{4} M^{-1} for ssDNA and dsDNA, respectively, which indicated that NR has more affinity with dsDNA than ssDNA. The electrochemical results were coinciding with UV-Vis absorption spectra and NR can be further used to distinguish the dsDNA and ssDNA efficiently.

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