Full Paper

Voltammetric Behavior of the Alizarin Red S Interaction with DNA and Damage to DNA

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Abstract
The electrochemical behavior and the interaction of alizarin red S (ARS) with calf thymus DNA was investigated on a bare glassy carbon electrode (GCE) and DNA modified GCE (DNA/GCE), respectively. ARS showed a pair of redox peaks at −0.445 V and −0.414 V on a bare GCE. On addition of DNA into the ARS solution, the peak current of ARS decreased and the peak potential positively shifted, but without new redox peaks appeared. The ARS reduction peak current increased with immersion time on a DNA/GCE. The results showed that ARS could interact with DNA molecules by intercalative binding mode. The equilibrium constant, binding number and the ratio of binding constant for oxidized and reduced ARS forms were obtained. The DNA damage was directly detected by appearance of guanosine and adenosine bases oxidation signal. The influence of experimental conditions on DNA damage extent was discussed in detail.

Keywords: Alizarin Red S, DNA modified electrode, Interaction, DNA damage

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1. Introduction

Deoxyribonucleic acid (DNA) has an important function in life processes, because it bears heritage information and instructs the biological synthesis of proteins and enzymes through the replication and transcription of genetic information in living cells. Some works have been made to study the electrochemical properties of DNA and to take advantage of them for practical purpose, such as detection of nucleic acid [1], interaction between DNA and other molecules (including proteins) [2], DNA damage [3], DNA hybridization [4] and so on [5]. Recently, more attention has been attracted into the study of recognizing special sequence of DNA and measuring DNA damage [6, 7].

The interaction of DNA with various small molecules has been an intensive topic that fascinated scientists for the past few tens of years [8–10]. As being bound in various ways (covalent, non-covalent, intercalative, electrostatic binding), some small molecules could change DNA conformations and lead to changes of its structure and functional activity. These researches were of great help to understand the structural properties of DNA, the mutation of genes, the origins of some diseases, and the action mechanism of some antitumoral and antiviral drugs [11, 12]. These researches also supplied some important information in designing new and more efficient DNA-target drugs to deal with genetic disease. The interaction of some small molecules with DNA could cause DNA damage. Some chemical agents frequently involved interruption of the DNA strands [13]. By measuring changes of DNA structure or a special indicator’s electrochemical behavior, the DNA damage could be detected [14–17].

Alizarin red S (ARS) is a 3-substituted derivative of 1,2-dihydroxy-9,10-anthraquinone, which belong to the group of most durable dyes in textile wastewaters [18]. It is one representative compound of 1,2-dihydroxy-9,10-anthraquinone. It has a planar heterocyclic ring structure and has been used as a ligand in the adsorptive stripping determination of copper, scandium, zirconium ion and so forth [19, 20]. A. M. Oliverira-Brett studied voltammetric behavior of its analogous compound mitoxantrone using a DNA-biosensor. Mitoxantrone has a planar heterocyclic ring structure and basic side groups. It could interact with DNA by intercalation way [21]. Some study work reported that anthraquinone possess preventive effect on the bacterial mutagenicity caused by the heterocyclic amine [22] and the DNA damage caused by carcinogens in Drosophila [23]. On the other

\[
\begin{align*}
(ARS)_{ox} + 2 e^- + 2 H^+ & \rightarrow (H_2ARS)_{red} & E_f^{0} \\
K_1 & & \\
ARS-DNA + 2 e^- + 2 H^+ & \rightarrow H_2ARS-DNA & E_f^{0}
\end{align*}
\]

Scheme 1. The oxidized and reduced forms of alizarin red S associated with the third specie DNA in solution.
hand, some experimental evidences showed that anthraquinone and its sulfonated derivatives could cause cytotoxicity, genotoxicity and DNA strand breakage [24]. However, the mechanism of anthraquinone compounds interaction with DNA and its effect on biological organism are not yet fully understood.

Due to a relatively low cost, simplicity, direct monitoring and high sensitivity, electrochemical method has been successfully used for the study of biological compounds. Moreover, it is an efficient way to mimic the electron transfer process occurring in biological organism [25]. In the present paper, an electrochemical approach was used to explore the ARS interaction with DNA. The electrochemical behavior of ARS and its interaction with natural calf thymus DNA were examined with cyclic voltammetry, differential pulse voltammetry using a bare and DNA modified GCE, respectively. The relative parameters of DNA interaction with ARS, such as the equilibrium constant, binding number and the ratio of binding constant for oxidized and reduced ARS forms were determined. After ARS was reduced on the DNA modified GCE, the oxidation peak of purine bases existing in DNA molecule appeared, which was attributed primarily to conformational changes of DNA molecule. The research work provides a convenient and sensitive method for exploring the mechanism of electroactive compound interaction with DNA and determining DNA damage.

2. Experimental

2.1. Chemicals and Apparatus

Calf thymus DNA (sodium salt) was purchased from Sigma and used without further purification. Stock solutions of DNA were prepared by dissolving an appropriate amount of the DNA in double-distilled water. It was stored at 4 °C and used not more than 5 days. Alizarin Red S (ARS) was received from Shanghai Chemical Reagent Company. The stock solution was prepared by directly dissolving it in double-distilled water (oxygen being removed using N2) and diluted with buffer solution according to the demands. A 0.10 mol L−1 pH 4.5 acetate buffer solution was prepared by directly dissolving 2.80 g of 2.80 mol L−1 acetate buffer solution according to the following formula [29]:

\[ I_p = n^2 F^2 A \Gamma_T v/4 RT \]

2.2. Preparation of DNA Modified GCE

The DNA modified glassy carbon electrode (DNA/GCE) (Ø = 3 mm) was prepared as follows: It was polished using a piece of 1500 diamond paper, and then polished to mirror smoothness with about 0.05 μm alumina water slurry on silk. Afterward, it was washed with absolute alcohol and double-distilled water in an ultrasonic bath to remove the adsorbrates on the electrode. After electrode was electrochemically pretreated (as described by Pang et al. [26]) and dried in air, a 20 μL DNA solution (2.0 mg mL−1) was dropped onto the surface of the clean glassy carbon electrode and solvent was evaporated at 4 °C for 6 hours. The DNA/GCE was subsequently transferred into the previously deaerated ARS or background solution.

2.3. Determination of DNA Damage

The reagent solution (containing ARS or not) was purged with high purity nitrogen gas for 15 min. Nitrogen gas was maintained over the solution by continuing with a flow of the pure gas during the voltammetric experiment. Then, the DNA/GCE was transferred into the solution. The deposit potential \( E_d \) or repetitive cathodic cycling (from –0.20 V to –0.60 V) was adjusted and started after DNA/GCE was immersed in solution for 60 min. After a given time, the circuit was open, and then differential pulse voltammetry was performed.

3. Results and Discussion

3.1. Electrochemical Behavior of ARS

ARS is a complex molecule and different groups can be oxidized and reduced [27]. The typical cyclic voltammograms of 2.80 × 10−3 mol L−1 ARS were shown in Figure 1 curve a. There was a pair of redox peaks in the range −0.20 to −0.60 V (vs. SCE) on a glassy carbon electrode. The cathodic peak potential (\( E_{pc} \)) was at −0.445 V and the anodic peak potential (\( E_{pa} \)) was at −0.414 V with a scan rate of 100 mV s−1.

The formal potential of the reversible reduction of the anthraquinone functionality varied linearly with increasing pH range from 3 to 10, resulting in a slope corresponds to 59 mV per pH unit (data not show). This result showed that the reduction mechanism was pH dependent and the same number of the protons and electrons participated in the reduction of ARS. This result was similar to previous work reported by Dai [28]. Furthermore, the cathodic peak current \( I_{pc} \) of ARS varies linearly with \( v^{1/2} \) (data not show) at scan rate from 20 to 500 mV s−1. This result indicates that the electrode process is controlled by adsorption step. According to the following formula [29]:

\[ I_p = n^2 F^2 A \Gamma_T v/4 RT \]
the number of electron transfer per ARS molecule could be determined to be \( n = 2 \).

### 3.2. Interaction of ARS with DNA

With respect to adsorption of ARS on the surface of electrode, after recording a cyclic voltammetric curve of the ARS in the presence of DNA, the surface of electrode was re-polished in order to record a next cyclic voltammetric curve of the ARS in the presence of another concentration DNA. Figure 1 shows the changes of the ARS cyclic voltammetric curve in absence and in the presence of different concentration DNA. With increase of DNA concentration, the redox peak current of ARS decreased and the peak potential positively shifted. The cathodic current decreased more than the anodic current. The dependence of the cathodic peak current on the concentration of DNA is shown in Figure 2. However, there is no new redox peaks appeared in the presence of DNA. The redox peak current of ARS decreasing and the peak potential shifting in the presence of DNA indicated that ARS could interact with DNA to form electrochemically non-active complex [30].

The CVs of ARS in the presence of DNA, both the cathodic and anodic peak potentials gradually shifted to more positive values versus that in absence of DNA. The formal potential \([E^0 = (E_{pa} + E_{pc})/2]\) shifting to the positive direction indicated that the ARS interacted with DNA in solution by intercalative mode [31, 32]. The limiting positive shift of +14 mV was observed when the concentration of DNA was 0.40 mg mL\(^{-1}\).

The net shift in \( E_{1/2} \) can be used to estimate the ratio equilibrium constants for the binding of the oxidized and the reduced species to DNA. The general process can be described with the following Scheme 1 and Equation 3 proposed by Bard [31]. Here, \((\text{ARS})_{\text{ox}}\) and \((\text{H}_2\text{ARS})_{\text{red}}\) represent the oxidized and reduced form of the ARS, respectively and ARS-DNA or \(\text{H}_2\text{ARS-DNA}\) denotes the oxidized or reduced form of ARS bound to the DNA molecule. \(E_{pa}^0\) and \(E_{pc}^0\) are the formal potential of the ARS/\(\text{H}_2\text{ARS}\) couple in the free and bound forms, respectively. \(K_1\) and \(K_2\) are the corresponding binding constants for the \((\text{ARS})_{\text{ox}}\) and \((\text{H}_2\text{ARS})_{\text{red}}\) species to DNA.

\[
2(E_{pa}^0 - E_{pc}^0) = 0.059 \log \left(\frac{K_2}{K_1}\right)
\]

Thus, for a limiting shift of +14 mV, \(K_2/K_1\) for \((\text{H}_2\text{ARS})_{\text{red}}/\text{ARS})_{\text{ox}}\) is 3.0, i.e., the reduced form \((\text{H}_2\text{ARS})_{\text{red}}\) is bound ca. 3.0 stronger than the oxidized form \((\text{ARS})_{\text{ox}}\). This indicates that an additional hydrogen bond between \((\text{H}_2\text{ARS})_{\text{red}}\) and DNA is more stable than bond between the \((\text{ARS})_{\text{ox}}\) and DNA.

With reference to the method of Li et al. [33], the binding ratio and the binding constant of ARS-DNA can be determined. It is assumed that DNA and ARS only produce a single complex DNA-mARS.

\[
\text{DNA} + m\text{ARS} \rightarrow \text{DNA}-m\text{ARS}
\]

The equilibrium constant is expressed as

\[
\beta = \frac{[\text{DNA} - m\text{ARS}][\text{ARS}]^m}{[\text{DNA}]}
\]

Because of

\[
\Delta I_{\text{max}} = kC_{\text{DNA}}
\]
\[ \Delta I = k [\text{DNA} - \text{mARS}] \]  
\[ [\text{DNA}] + [\text{DNA}-\text{mARS}] = C_{\text{DNA}} \]  
Therefore

\[ \Delta I_{\text{max}} - \Delta I = k(C_{\text{DNA}} - [\text{DNA} - \text{mARS}]) = k[\text{DNA}] \]  

And the following equation can be deduced:

\[ \frac{1}{\Delta I} = \frac{1}{\Delta I_{\text{max}}} + \left( \frac{1}{\beta \Delta I_{\text{max}}} \right) \times \left( \frac{1}{[\text{ARS}]} \right) \]  

where, \( \Delta I_{\text{max}} \) represents the maximum difference of the cathodic peak current of ARS before and after adding DNA. If DNA and ARS form a single complex, the plot of \( 1/\Delta I \) vs. \( 1/[\text{ARS}] \) is linear. A linear curve of \( 1/\Delta I \) vs. \( 1/[\text{ARS}] \) could be obtained from \( m = 1 \) with a correlated coefficient of 0.997. \( \beta \) was evaluated to be \( 1.01 \times 10^4 \) by using the slope of the curve and its intercept. It also confirmed that the hypothesis of Scheme 1 is reasonable.

DNA-modified electrode was applied to explore interaction of ARS with DNA. Figure 3 showed the cyclic voltammetric response of ARS on the bare and DNA-modified GCE. Compared to the bare GCE, the DNA-modified electrode exhibits higher background current due to the thickness DNA layer coating on electrode surface. However, the peak potential using the DNA-modified electrode is 15 mV more negative than that observed at the bare GCE. The peak current obtained at this DNA/GCE was about 1.8 times higher than that obtained with the bare GCE. The negative shift of peak potential and the increase of peak current also indicated that ARS could interact with DNA immobilized on the GCE surface.

In addition, the reduction of ARS at the DNA modified electrode (Fig. 4) and the effect of the immersion time on cathodic peak current (inset of Fig. 4) were investigated with differential pulse voltammetry. The peak current was found to increase with time and almost reach saturation after 1 h of immersion, which indicated that it was possible to preconcentrate ARS on the DNA film. Following this, the electrode was gently washed with double-distilled water. It could observe that a magenta color layer appeared on the electrode surface after dryness. This is a confirmation that ARS could bind into DNA molecule [34].

### 3.3. Detection of DNA Damage

After the structure of ds-DNA molecules is damaged, the purine residues such as guanine and adenine residues released from the protection of double helix. The released purine residues could easily oxidized on the surface of electrode. According to appear oxidation signal of the purine residues, DNA damage could be determined. The irreversible damage by interaction of DNA modified on surface of electrode with reduction product of ARS was monitored by the appearance of these purine bases oxidation peak. Two methods were applied to examine DNA damage. In the first method, the differential pulse voltammetry was used to detection DNA damage after ARS was reduced by successive cathodic cycling from \(-0.20 \text{ V to } -0.60 \text{ V}\). In the second method, ARS was reduced using DNA modified electrode at constant potential \( (E_d) \) for different time \( (t_d) \). The differential pulse voltammetry was used to detect the oxidation signals of purine residues.

Differential pulse voltammetric curve of ARS oxidation process using a DNA modified GCE showed almost no
oxidation peak of the purine residues (Fig. 5a), except for ARS dihydroxyl oxidation peak at $E_p = +0.582\, \text{V}$. Since purine groups are folded inside the rigidity of the double helix DNA, and are distant from the electrode surface. It is interesting to observe appearance of two new oxidation peaks after ARS was reduced by repetitive differential pulse cathodic cycling (Fig. 5b) and constant deposit potential $E_d = -0.45\, \text{V}$ for 2 min (Fig. 5c). These two new oxidation peaks occurred at $+1.024\, \text{V}$ and the $+1.296\, \text{V}$, respectively. Comparing this behavior and the published picture of DNA bases as well as corresponding nucleosides [10, 35, 36], the observed DNA anodic signal can be described to the oxidation of guanosine and adenosine respectively. The results clearly demonstrated that the construction of double helix in DNA molecule was broken. Moreover, the ARS oxidation peak significantly increases after ARS was reduced by the above two reduction methods, which indicated that more ARS concentrated on the DNA film.

In order to obtain more information, the signal changes of the guanosine residues oxidation were examined, because the guanosine residues were more easily oxidized than the adenosine residues and the signal changes of guanosine residues oxidation was sensitive to test DNA damage [37]. The strength dependence of guanosine residues oxidation peak on the number of successive cathodic scans from $-0.20\, \text{V}$ to $-0.60\, \text{V}$ was explored. The oxidation peak current of guanosine residues gradually increases with the number of scan up to 15 scans and the current reaches constant value after 15 scans (seen Fig. 6a). In constant potential reduced ARS method, the deposit potential also affected the extent of DNA damage. The effect of deposit potential $E_d$ on the oxidation peak current of the guanosine residues was shown in Figure 6b. It could be observed that extent of DNA damage was enhanced with more negative potential up to $-0.45\, \text{V}$ that was applied to reduce ARS when deposit time was constant. At more negative potential than $-0.45\, \text{V}$, $I_{\text{Gua}}$ values became level off. It was corresponding to the reduction peak potential of ARS in cyclic voltammogram.

The dependence of the oxidation peak current of the guanosine residues on the deposit time was shown in Figure 6c. The extent of DNA damage was enhanced with increasing the deposit time at $E_d = -0.45\, \text{V}$. When the time was increased to 6 min, the oxidation peak current of guanosine residues tended to the biggest value. Then, the oxidation peak current of guanosine residues decreased with increasing deposit time. But, another new oxidation peak appeared at $E_p = +0.818\, \text{V}$, which could be attributed to the oxidation of guanine residues (see Fig. 5d) [35]. So the guanosine residues oxidative signal became decrease after electrochemical reduction ARS for 8 min. It was possible that the construction of DNA double helix was broken with

![Image](47x450) oxidation peak of the purine residues (Fig. 5a), except for ARS dihydroxyl oxidation peak at $E_p = +0.582\, \text{V}$. Since purine groups are folded inside the rigidity of the double helix DNA, and are distant from the electrode surface. It is interesting to observe appearance of two new oxidation peaks after ARS was reduced by repetitive differential pulse cathodic cycling (Fig. 5b) and constant deposit potential $E_d = -0.45\, \text{V}$ for 2 min (Fig. 5c). These two new oxidation peaks occurred at $+1.024\, \text{V}$ and the $+1.296\, \text{V}$, respectively. Comparing this behavior and the published picture of DNA bases as well as corresponding nucleosides [10, 35, 36], the observed DNA anodic signal can be described to the oxidation of guanosine and adenosine respectively. The results clearly demonstrated that the construction of double helix in DNA molecule was broken. Moreover, the ARS oxidation peak significantly increases after ARS was reduced by the above two reduction methods, which indicated that more ARS concentrated on the DNA film.

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4. Conclusions

In the present paper, the electrochemical behavior of ARS and its action with natural calf thymus DNA were explored with cyclic voltammetry, differential pulse voltammetry. The obtained experimental results gave evidence that ARS could interact with DNA by intercalating in double helix of DNA. The equilibrium constant, binding number, etc parameters were obtained. The appearing oxidation peak of DNA purine bases indicated that construction of DNA molecules changed after ARS was reduced. DNA damage could take place in ARS reduced process.

5. Acknowledgements

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6. References