ELSEVIER

Biophysical Chemistry 118 (2005) 42 – 50

Biophysical Chemistry

http://www.elsevier.com/locate/biophyschem

Spectrometric and voltammetric investigation of interaction of neutral red with calf thymus DNA: pH effect

Xiue Jiang, Li Shang, Zhenxin Wang, Shaojun Dong*

State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, Jilin, 130022, China

Received 4 January 2005; accepted 16 March 2005
Available online 26 July 2005

Abstract

The interaction of neutral red (NR) with calf thymus DNA (CT DNA) was investigated by spectrometric (UV-vis, circular dichroism and fluorescence) and voltammetric techniques. It was shown that the interaction of NR with DNA depended on the values of R (R is defined as the ratio of the concentration of NR to that of CT DNA) and pH of the solution. NR intercalated into CT DNA base pairs at lower R value ($R<2.4$) and following by NR aggregating along the helical surface of DNA at higher R value ($R>2.4$) in pH 6.0 solution. Interestingly, we found that at lower R value, NR intercalated into CT DNA with its long axis perpendicular or parallel to the dyad axis of DNA in the solution of pH 6.0. While in pH 7.0 solution, NR bound with CT DNA through intercalation and electrostatic interactions. The electrochemical inactive complexes, NR-2CT DNA, 3NR-CT DNA, and NR-CT DNA were formed when NR interacted with nucleic acids in pH 6.0 and 7.2 solutions, respectively. The corresponding intrinsic binding constants for these complexes were obtained by UV-vis and fluorescence spectrometric methods, respectively. The CD spectra showed that the conformation of CT DNA was converted from right-handed B-DNA to left-handed Z-DNA due to the aggregating of NR along the surface of DNA in pH 6.0 solution, whereas a conversion from B-DNA to C-DNA was induced due to the interaction of DNA with NR in pH 7.2 solution. Finally, two binding modes of NR with CT DNA in aqueous with different values of pH were shown in the scheme.
© 2005 Elsevier B.V. All rights reserved.

Keywords: Neutral red; Calf thymus DNA; Binding mode; Binding constant; Conformational changes of CT DNA

1. Introduction

The interactions of cationic dyes with synthetic and natural nucleic acid have been studied widely [1–9], which involves the studies of structure and dynamics of nucleic acid or potential of pharmaceutical use, such as, DNA intercalating agents disrupt the normal function of cellular DNA and can lead to interfere with gene expression, gene transcription, mutagenesis, carcinogenesis, and cell death [10,11]. The interaction has been studied using various spectrometric techniques [1–9], such as NMR, ESR, circular dichroism (CD), fluorescence, resonance Raman, UV-visible and Fourier transform infrared (FTIR) spectroscopy, and also electrochemical method [12,13]. Among these spectra, the signals of the absorption and induced CD (ICD) spectra of small molecules bound to nucleic acid provide conveniently a signature for the binding mode to DNA [5,6,14].

NR is a basic azine dye whose chemical structure shows interesting similarities with other planar cationic dyes belonging to the acridine, thiazine, and xanthene groups [15–17], and has been applied to assay of nucleic acid [17]. Moreover, NR is an efficient anticancer drug of parent compound targeted to DNA [18]. Although there are already some research performed on the studying of the binding of DNA with NR [19,20]. But, to the best of our information, the interaction of NR with DNA, and the extent and nature of NR induced alterations in DNA structure have not been studied thoroughly. Especially, NR has pH dependent structural equilibrium between the protonated form (acid form) and the alkanolamine form (neutral form) as revealed

* Corresponding author. Fax: +86 431 5689711.
E-mail address: dongsj@ns.ciac.jl.cn (S. Dong).

0301-4622/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.bpc.2005.03.015

by UV-visible spectroscopy [16], so its interaction mechanisms with DNA should be different in different pH solutions.

Thus, in this paper, combining sensitive spectrometric and voltammetric techniques, the interactions of DNA with NR in different pH phosphate buffer solutions have been studied in detail. Our results showed that binding of NR with CT DNA accompanied with DNA conformational changes was sensitive to the concentration ratio of the two complexes and the pH value of the solution. Our results also provided the information about the interaction mechanism of biological probe with DNA.

2. Experiment

2.1. Materials

Calf thymus DNA (CT DNA) was obtained from Sigma Chemical Co. (USA), and used as received. Stock solution of DNA was prepared by dissolving nucleic acids in buffer solution, stored at 4 °C more than 24 h with gentle shaking occasionally to get homogeneity, and used within 2 days. The concentrations of CT DNA per nucleotide phosphate ([DNA]) were calculated according to the absorbance at 260 nm by using $\varepsilon_{DNA}=6600\ M^{-1}\ cm^{-1}$. Neutral red was bought from Shanghai No.1 Chemical Co. (Shanghai, China), and used after recrystallization from ethanol.

Phosphate buffer solutions (PBS) with different ionic strength used in all experiments were prepared by $Na_2HPO_4{\cdot}12H_2O$, $NaH_2PO_4{\cdot}2H_2O$, NaCl (analytical grade), and doubly purified water from Milli-Q system. The pH of each solution was measured before experiment.

2.2. Methods

Cyclic voltammograms were recorded using Mode 630 electrochemical analyzer (CH Instrument, U.S.A.) with a home-made electrochemical cell. A glassy carbon electrode (GCE) was used as the working electrode, a twisted platinum wire as auxiliary electrode, and a Ag/AgCl (saturated KCl) as the reference electrode. Before experiment, the GCE was mechanically polished with 1.0, 0.3, and 0.05 μm α-Al_2O_3 slurry, successively, and then washed ultrasonically in water followed by ethanol for a few minutes at each step.

UV-vis absorbance spectra were measured with Cary 500 UV-vis-NIR spectrometer (Varian Co., USA) using a 1 cm path length cell and the fluorescence spectra were recorded on a PerkinElmer LS-55 luminescence spectrometer. The absorption and fluorescence quenching titrations with DNA were performed by keeping the NR concentration constant and varying the nucleic acid concentrations. This was done by adding different concentrations of DNA into appropriate amount of NR, while maintaining the total volume of the solution constant. Fluorescence emission spectra were recorded in the wavelength range of 550–750 nm by exciting the NR at 537 and 462 nm in pH 6.0 and 7.2 PBS, respectively, with the excitation and emission slit widths of 10 nm. The intrinsic binding constants of NR with CT DNA were determined by absorption and fluorescence titrations, respectively. In case of the former, the absorbance at λ_{max} is recorded after each addition of nucleic acid. The intrinsic binding constant K_a is determined by Eq. (1) [21]:

$$C/\Delta\varepsilon_a = C/\Delta\varepsilon + 1/(\Delta\varepsilon K_a) \quad (1)$$

Where C is the concentration of CT DNA, $\Delta\varepsilon_a=[\varepsilon_a-\varepsilon_f]$, $\Delta\varepsilon=[\varepsilon_b-\varepsilon_f]$, and ε_a, ε_b and ε_f correspond to the apparent extinction coefficient of NR, the extinction coefficient of binding form of NR and free NR, respectively. In fluorescence quenching experiments, the data were plotted according to the Stern–Volmer equation [2]

$$I_0/I = 1 + K_q[Q]. \quad (2)$$

Where, I_0 and I are the fluorescence intensities in the absence and presence of DNA, respectively. K_q is the Stern–Volmer fluorescence quenching constant. $[Q]$ is the concentration of quencher. Because it is the static quenching of DNA to NR, the quenching constant is considered as the formation constant of NR and DNA [20], i.e., the binding constant of NR with DNA.

Circular dichroism spectra were measured using a 62A DS CD spectrometer (AVIV, USA) with a 1.0 cm path length rectangular quartz cell controlled by a thermoelectric cell holder (AVIV). The CD absorption titrations of CT DNA with NR were conducted by keeping the concentration of CT DNA constant, and varying concentrations of NR. The induced CD absorption titrations of NR with CT DNA were conducted by keeping the concentration of NR constant, and varying concentrations of nucleic acid.

3. Results and discussion

3.1. Voltammetric behavior of CT DNA-NR complexes

The interactions of NR with CT DNA in different pH solutions were monitored with cyclic voltammetry (CV). Shown in Fig. 1A and B the free NR exhibits one couple of redox peaks with $E_{1/2}=(E_{pa}+E_{pc})/2=-0.57$ V (Fig. 1A, curve 1) and −0.5 V (Fig. 1B, curve 1) in pH 7.2 and 6.0 solutions, respectively. In the presence of CT DNA, no new redox peaks appear and the peak currents decrease with the increasing of the concentration of CT DNA, indicating that NR interacting with CT DNA forms an electrochemically inactive complex. Comparing with Fig. 1A and B, at the same concentration of CT DNA, the decrease of the redox peak currents of NR are larger in pH 6.0 solution than that in pH 7.2 solution, indicating that acidic form of NR facilitates the interaction of DNA with NR. When the concentration of

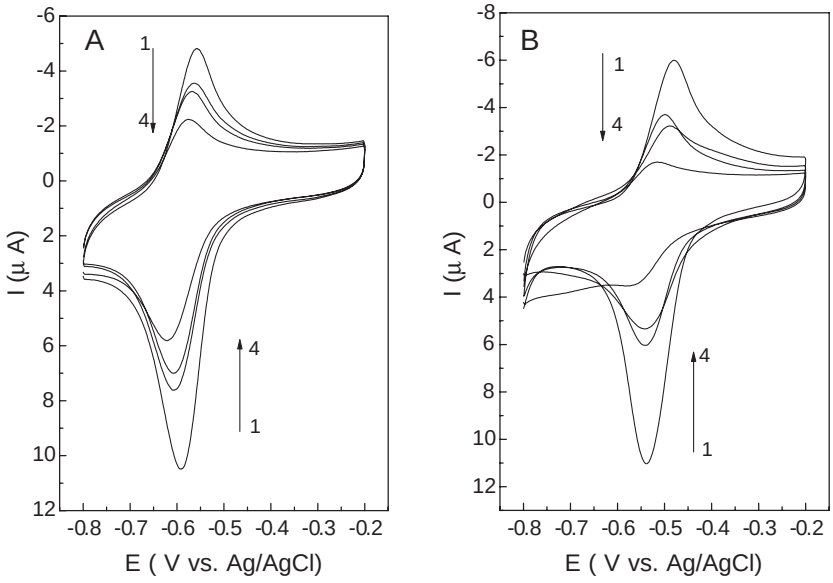


Fig. 1. (A) Cyclic voltammograms of 1.1×10^{-5} M NR in 0.1M PBS (pH 7.2) in the presence of (1) 0, (2) 0.11, (3) 0.22, (4) 0.5 mg/ml CT DNA. (B) Cyclic voltammograms of 1.1×10^{-5} M NR in 0.1M PBS (pH 6.0) in the presence of (1) 0, (2) 0.11, (3) 0.22, (4) 0.5 mg/ml CT DNA.

CT DNA is kept constant and that of NR varied, according to the reported method [12],

$$\text{DNA} + \text{nNR} \rightleftharpoons \text{DNA} - \text{nNR} \quad (3)$$

the equilibrium constant is expressed as,

$$\beta_s' = ([\text{DNA} - n\text{NR}])/([\text{DNA}][\text{NR}]^n) \quad (4)$$

then n can be obtained according to,

$$lg(\Delta I/(\Delta I_{\text{MAX}} - \Delta I)) = lg\beta_s' + nlg([\text{NR}]) \quad (5)$$

here I represents the cathodic peak current I_{pc}.

The plot of $\lg(\Delta I/(\Delta I_{\text{MAX}}-\Delta I))$ vs. lg[NR] shows a linear line with the slope $n=0.96$ in pH 7.2 solution (Fig. 2A), indicating the interaction of DNA with NR can form a single complex, NR-CT DNA. However, a non-linear relationship between $\lg(\Delta I/(\Delta I_{\text{MAX}}-\Delta I))$ and lg[NR] is obtained in pH 6.0 solution (Fig. 2B). Two n values of 0.58 at lower R and 3.3 at higher R are obtained, showing formation of two kinds of complexes, NR-2CT DNA and 3NR-CT DNA.

3.2. Spectroscopy study

3.2.1. Electronic absorption spectroscopy study

Fig. 3A and B shows the electronic absorption spectra of NR after addition of different concentrations of DNA in pH

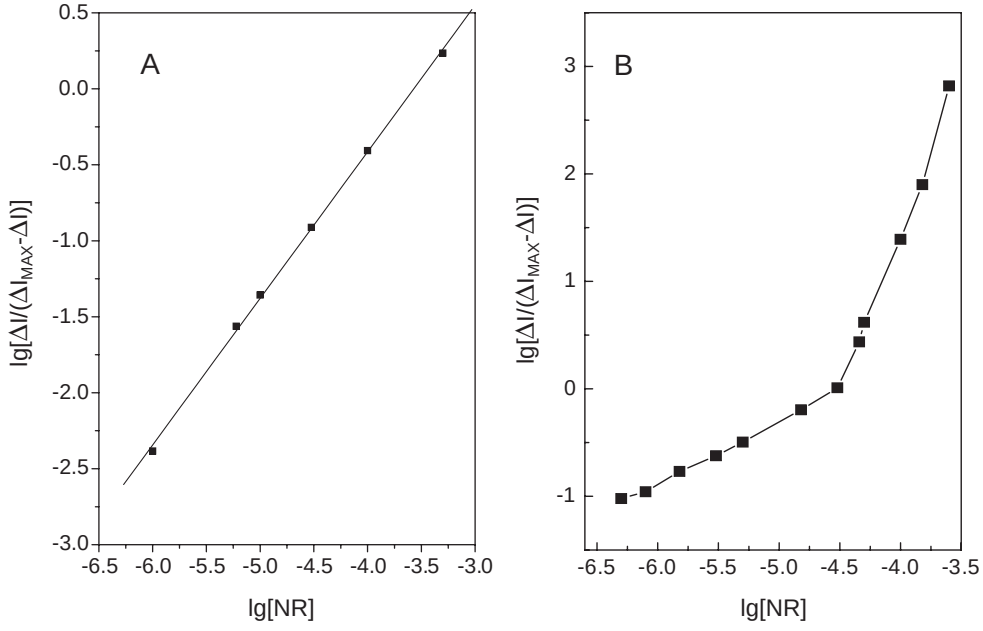


Fig. 2. (A) Relationship between $\lg[\Delta I/(\Delta I_{\text{MAX}}-\Delta I)]$ and lg[NR] in pH 7.2 PBS, CT DNA 5.51×10^{-4} M. (B) Relationship between $\lg[\Delta I/(\Delta I_{\text{MAX}}-\Delta I)]$ and lg[NR] in pH 6.0 PBS, CT DNA 5.51×10^{-4} M.

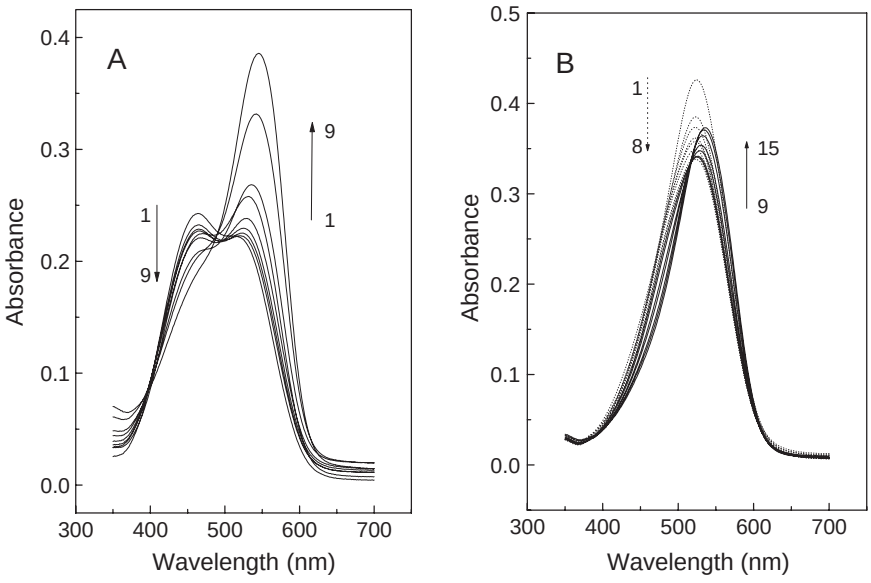


Fig. 3. (A) Absorption of 3.0×10^{-5} M NR in 0.1 M PBS (pH 7.2) after addition of CT DNA. The values of R are (1) the concentration of CT DNA is 0, (2) 3.7, (3) 1, (4) 0.7, (5) 0.5, (6) 0.3, (7) 0.2, (8) 0.1, (9) 0.06. (B) Absorption of 3.0×10^{-5} M NR in 0.1 M PBS (pH 6.0) after addition of CT DNA. The values of R are (1) the concentration of CT DNA is 0, (2) 10, (3) 6.67, (4) 5, (5) 4, (6) 3.3, (7) 2.86, (8) 2.4, (9) 2, (10) 1.6, (11) 1, (12) 0.8, (13) 0.57, (14) 0.44, (15) 0.36.

7.2 (A) and pH 6.0 (B) PBS, respectively. As reported [21], a stronger absorption at 463 nm for neutral form NR and a weaker absorption at 522 nm for acidic form NR can be seen (Fig. 3A, curve 1) in 0.1 M PBS (pH 7.2). With the R values shifting from 3.7 to 0.06 (Fig. 3A, curve 2∼9), the absorption intensity of NR at 463 nm decreases accompanied with a large bathochromic shift (∼24 nm) and a stronger hyperchromic effect of the absorption at 522 nm, indicating that NR behaves as an intercalator within the R values studied in pH 7.2 PBS [1,22]. Whereas in pH 6.0 PBS, the absorbance changes of NR upon the addition of CT DNA (Fig. 3B) are distinctly different from those in pH 7.2 PBS. It can be seen that the changes of spectra depend on the value of R. At higher R value (Fig. 3B, curve 2∼8), the interaction of NR with increasing concentration of CT DNA produces a hypochromicity but without any obvious shift of the maximum wavelength (λ_{max}) of NR at $R \geq 2.4$, while at lower R value (Fig. 3B, curve 9∼15), the absorption at λ_{max} increases with a red-shift (∼13 nm) from 526 to 539 nm. The subsequent spectra changes related with the R values reflect that the binding mode of NR with CT DNA is dependent on the ratio of the two compounds in pH 6.0 PBS. NR is aggregated on the surface of CT DNA helix [1,5,6,22–25] at higher R value, and intercalates into CT DNA base pairs [1,22,25] at lower R value.

According to Eq. (1) and the absorption of NR after addition of different concentrations of CT DNA, the plots of $C/\Delta\varepsilon_a$ vs. C_{DNA} in pH 7.2 (Fig. 4A) and pH 6.0 (Fig. 4B) PBS can be drawn, respectively. The plot of $C/\Delta\varepsilon_a$ vs. C_{DNA} exhibits a linear line in pH 7.2 PBS, while, two linear lines are obtained in pH 6.0 PBS, which also indicates that the interaction mode of NR with CT DNA depends on the value of the solution pH. According to the linear regression equation, the intrinsic binding constants, 1.0×10^5 for pH 7.2 and 1.3×10^5, 6.2×10^3, for pH 6.0 at $R > 2.4$ (Fig. 4B, curve a) and $R < 2.4$ (Fig. 4B, curve b) are obtained, respectively. Such binding constants are consistent with that of the similar type of molecules, such as acridine orange and safranine T [2]. The value of 6.2×10^3 for pH 6.0 at $R < 2.4$ is relatively smaller, which may due to the steric effect caused by the substituted group on the azine plane [2].

3.2.2. Fluorescence spectroscopy study

The interactions of NR with CT DNA in different pH solutions were also monitored by changes in the intrinsic fluorescence of NR at varying DNA concentrations. Fig. 5A and B shows the representative fluorescence emission spectra of NR upon addition of CT DNA in pH 7.2 (A) and pH 6.0 (B) PBS, respectively. It can be seen from Fig. 5(A) that the fluorescence intensity increases with decreasing of R values from 7.39 to 0.048 accompanied with the blue shift of the emission maximum ($\lambda_{em,\ max}$) from 632 to 625 nm in pH 7.2 PBS. Obviously, NR binding to DNA can change the average local environment of NR chromophores [29]. However, the change of the R value has different effects on the fluorescence intensity of NR in pH 6.0 PBS (Fig. 5B). The fluorescence of NR is efficiently quenched by CT DNA at $R \geq 2.86$. Then, the fluorescence intensity increases upon continually decreasing of R values but without any perceptible shifts in fluorescence $\lambda_{em,\ max}$. This further proves that the binding mode of NR with CT DNA is affected by the values of pH and R. Considering the results obtained by UV-vis spectra, the aggregation of NR on the surface of CT DNA helix reduces the intrinsic fluorescence of NR, whereas the intercalation makes its fluorescence increase in intensity. This may be due to the masking or exposure of NR chromophores upon aggregation of NR on the surface of CT DNA or intercalation into CT DNA base

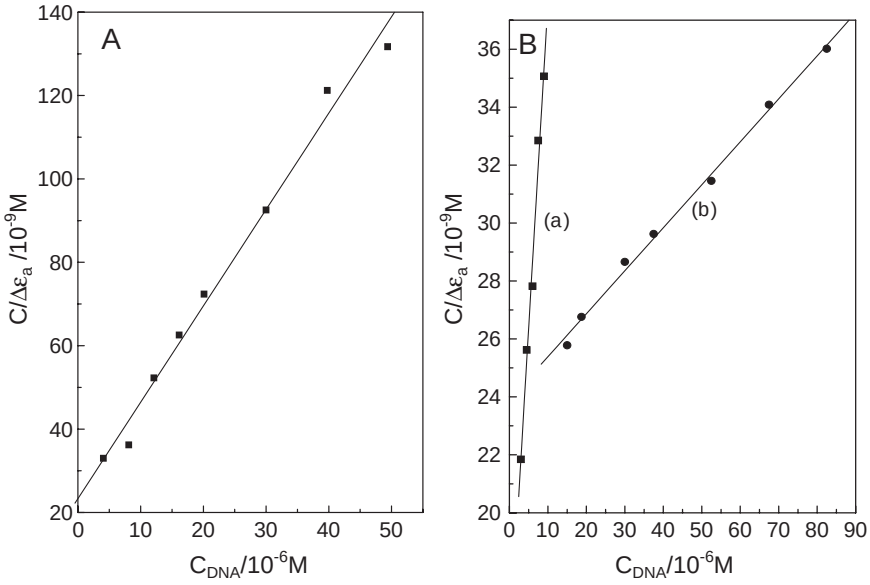


Fig. 4. (A) The relation between the absorbance change of NR at λ_{max} and the concentration of nucleic acids added in 0.1 M PBS (pH 7.2). (B) The relation between the absorbance change of NR at λ_{max} and the concentration of nucleic acids added in 0.1 M PBS (pH 6.0), (a) $R>2.4$, (b) $R<2.4$.

pairs [30]. The Stern–Volmer plots provide the binding constants as 4.6×10^5 for pH 7.2 (Fig. 6A), 1.5×10^5 for pH 6.0 at $R>2.86$ (Fig. 6B, curve a), and 1.6×10^3 at $R<2.86$ (Fig. 6B, curve b), respectively.

3.2.3. CD spectroscopy study

The ICD of NR can provide the important information for identifying the binding mode of NR with CT DNA. Free NR has no intrinsic CD activity, while, the ICD activity may appear upon binding to CT DNA as shown in Fig. 7A and B. Fig. 7(A) shows the ICD spectrum of NR with one small negative band at 519 nm at $R=0.1$ in pH 7.2 PBS.

According to the reported results, an intercalated chromophore centered near the helix axis of DNA should exhibit negative ICD for a transition polarized perpendicular to the long axis of the base pair [1,5,6,19,22–28], so the intercalation is a binding mode in the system. But the small ICD band implies that another binding mode also exists in the system, such as electrostatic interaction because this interaction gives no ICD signal. To determine the contribution of electrostatic interaction in the overall binding of NR with CT DNA, the absorption spectra of NR-CT DNA complex ($R=0.1$) were measured in solutions of varying ionic strengths (Fig. 7A, insert). The results show that the absorption intensity decreases with small blue shift upon

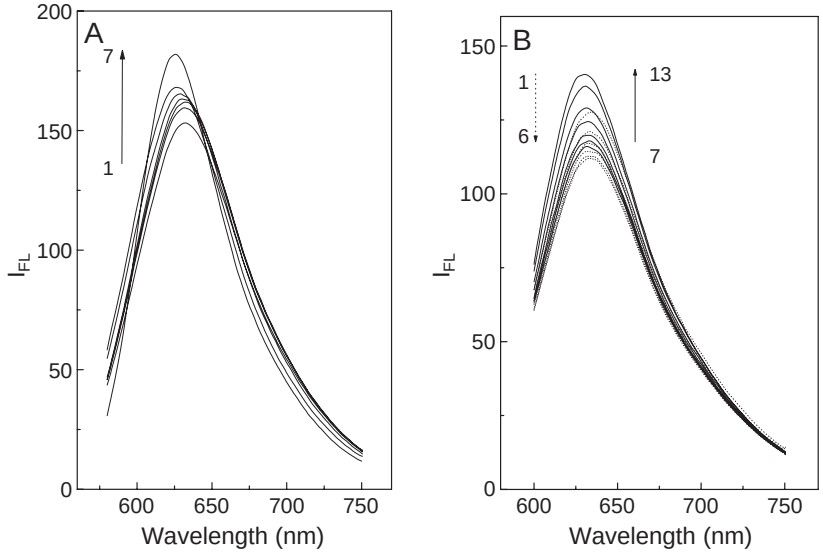


Fig. 5. (A) Fluorescence spectra of 3.0×10^{-5} M NR in 0.1 M PBS (pH 7.2) after addition of CT DNA. The values of R are (1) the concentration of CT DNA is 0, (2) 7.3, (3) 2.5, (4) 0.7, (5) 0.3, (6) 0.08, (7) 0.048. (B) Fluorescence spectra of 3.0×10^{-5} M NR in 0.1 M PBS (pH 6.0) after addition of CT DNA. The values of R are (1) the concentration of CT DNA is 0, (2) 10, (3) 6.7, (4) 5, (5) 4, (6) 2.86, (7) 2.4, (8) 2, (9) 1.6, (10) 1.3, (11) 0.8, (12) 0.57, (13) 0.44.

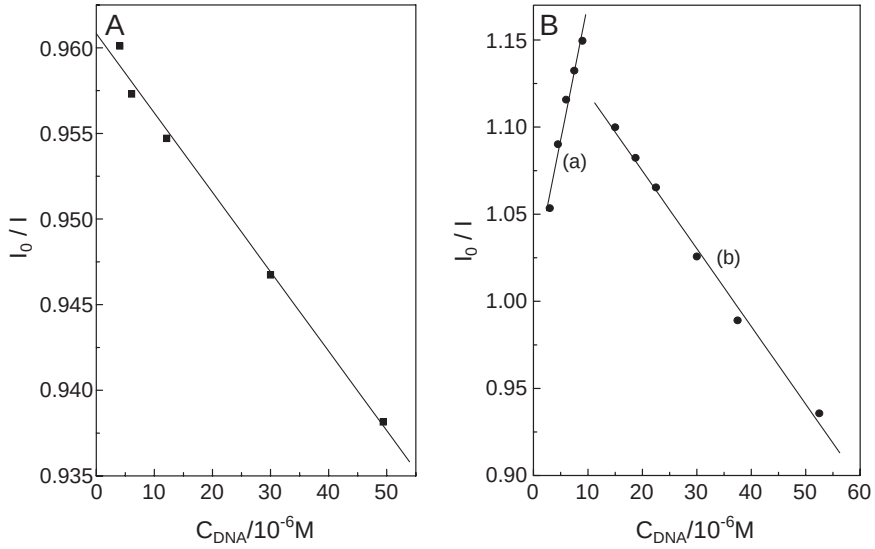


Fig. 6. (A) Stern–Volmer quenching plot of NR with increasing concentrations of DNA in 0.1 M PBS (pH 7.2). (B) Stern–Volmer quenching plot of NR with increasing concentrations of DNA in 0.1 M PBS (pH 6.0), (a) $R>2.86$, (b) $R<2.86$.

increasing of NaCl concentration, indicating the presence of electrostatic interaction force in the NR-CT DNA complex [2] in pH 7.2 PBS. Fig. 7B shows the ICD spectra of NR in pH 6.0 PBS. Two ICD bands, a positive band at 477 nm and a negative band at 524 nm, are observed in the ICD spectrum at $R=3$, just like an exciton CD spectrum (Fig. 7B curve b). Such an exciton CD can be explained as, one partially intercalated NR molecule could couple with second externally bound NR molecule, which interacts with the first intercalated NR to form an aggregating complex that stacking along the surface of helix [11,5,6,19,22–28] at higher value of R. When R value decreases to 0.1, the negative peak decreases with small blue shift and a positive band appears at 574 nm, resembling the exciton-like spectrum (Fig. 7B, curve c). However, if it is a true exciton spectrum, the chromophore arrangement may indicate external stacking of a second ligand with the first intercalated molecule. But this exciton-like spectrum in our system is preserved even at lower R value, which is inconsistent with external stacking interaction hypothesis. Another possible explanation may involve the presence of two different intercalation modes in intercalation pocket [31]. Actually, an intercalated chromophore oriented with its long axis perpendicular to the dyad axis ($\gamma=90°$) will induce

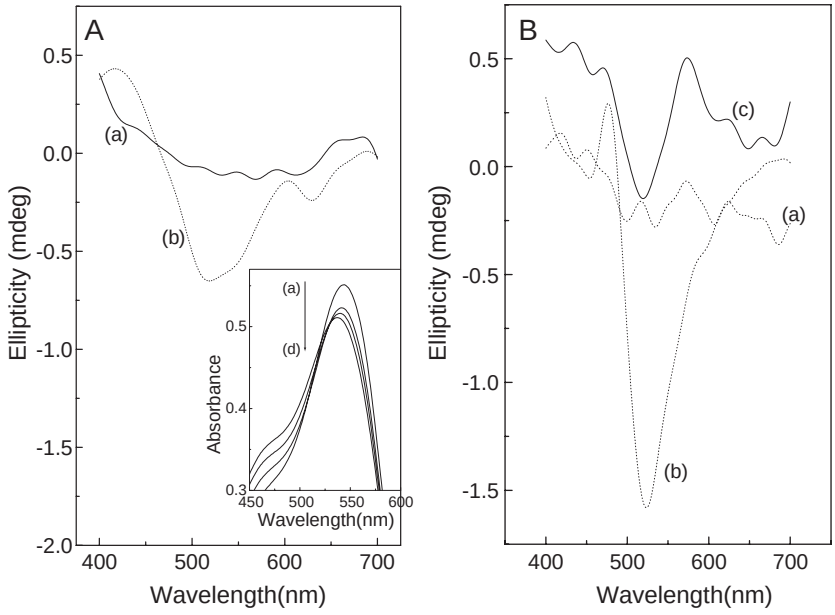
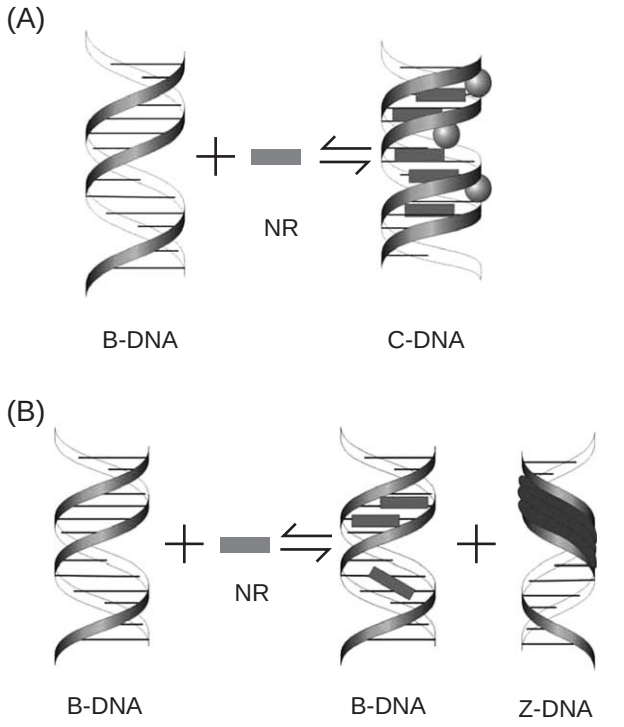


Fig. 7. (A) Induced CD spectra of 3.0×10^{-5} M NR in pH 7.2 (0.1 M) PBS after addition of CT DNA (a) 0, (b) 3.0×10^{-4} M. insert: the effect of NaCl concentration (a) 0, (b) 50, (c) 100, (d) 200 mM, on the absorption spectra of NR-CT DNA complex ($R=0.1$). (B) Induced CD spectra of 3.0×10^{-5} M NR in 0.1 M PBS (pH 6.0) after addition of CT DNA (a) 0, (b) 1.0×10^{-5}, (c) 3.0×10^{-4} M.

a negative Cotton peak, while the induced CD is positive when cationic dye being oriented parallel to the dyad axis ($\gamma = 0°$) of DNA double helix [25,27]. So the negative band should correspond to an intercalation mode of NR with the long axis perpendicular to the dyad axis ($\gamma = 90°$) while the positive ICD will reflect an intercalation geometry where NR being oriented parallel to the dyad axis ($\gamma = 0°$).

Fig. 8A and B shows the CD spectra of CT DNA in the presence and absence of NR in pH 7.2 PBS (A) and pH 6.0 PBS (B), respectively. The CD spectrum of free CT DNA (Fig. 8A, B curve a) exhibits one positive peak at 274 nm due to the base stacking and one negative band at 245 nm due to the helicity, which is the characteristic of DNA in the right-handed B form [32,33]. Increasing the concentration of NR ($R = 0.1$) in CT DNA solution (pH 7.2), the intensity of positive band decreases and negative band increases (Fig. 8A, curve b), suggesting that DNA bound with NR induces certain conformational changes, DNA double helix in solution is in a more helical state belonging to C form DNA [34,35]. Double helix form DNA is not really a rigid molecule, especially in solution, and water molecules are important to the conformations of DNA. The major and minor grooves are 'coated' by a unimolecular layer of water molecules which interact with the exposed C═O, N and NH functions of ribose and also extensively solvate the phosphate backbone [36,37]. While the NR binding to the DNA in electrostatic form, the cationic head groups of NR also interact with the ribose and phosphate by electrostatic attraction. There exists a competition between water molecules and NR. So water contents in the micro surrounding around the DNA may be decreased. This would make the helicity of DNA become stronger. Thus, the conformation of DNA is changed from the B-form to a more compact C form. While in pH 6.0 PBS, increasing the concentration of NR in CT DNA solution (at $R = 0.1$), the intensities of both positive and negative bands increase (Fig. 8B curve b), suggesting that DNA bound with NR just as increases the proportion of right-handed form of DNA in the solution. When increasing the concentration of NR continually, an apparent



Scheme 1. (A) Representation of binding mode of NR-CT DNA in pH 7.2 PBS. (B) Representation of binding mode of NR-CT DNA in pH 6.0 PBS.

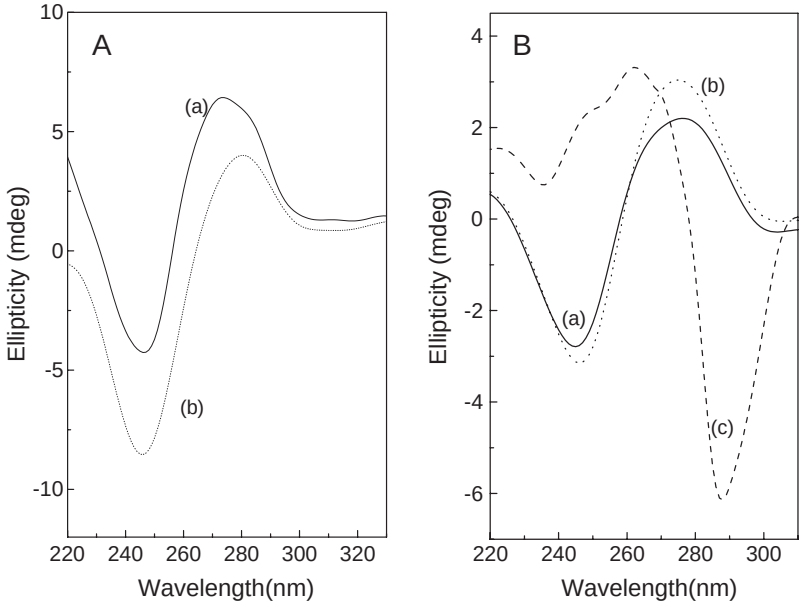


Fig. 8. (A) CD spectra of CT DNA (3.5×10^{-5} M) after addition of NR, (a) 0, (b) 3.5×10^{-6} M, in 0.1M PBS (pH 7.2). (B) CD spectra of CT DNA (2.3×10^{-5} M) after addition of NR, (a) 0, (b) 2.3×10^{-6}, (c) 5.8×10^{-5} M, in 0.1M PBS (pH 6.0).

change of CD spectral for DNA is induced, both of the intensities of positive band at 274 nm and negative band at 245 nm decrease, and a new negative peak and positive peak appear at 287 and 263 nm, respectively at $R=2.5$ (shown in Fig. 8B, curve c). The new CD spectrum corresponds to the characteristic CD spectrum of Z form of DNA. The results identify that NR induces the structural changes of CT DNA at higher R value. In natural system such as CT DNA, the Z form is normally escaped from detection because the alter length of native G-C stretches are rather small [32,33]. The B→Z transition induced by NR is depended on the value of R. Apparently, NR binds along the surface of CT DNA helix with aggregating, which changes the conformation of CT DNA. The original CD spectrum (free DNA) is partly recovered while the ionic strength increasing from 0.1 to 0.5 M, which shows the induced B Z transition is partly reversible. It is reasonable to think that the outer-sphere binding of some complexes can significantly alter the conformation of DNA, even to the extent of reversing the helical direction [32,33,38,39].

4. Conclusions

The present investigation provides a reliable evidence for the binding of NR to DNA. The interaction mechanism of NR with CT DNA was interpreted at a molecular level by using UV-vis, CD and fluorescence spectroscopic techniques in combination with voltammetric method. Two R-dependent electrochemically inactive complexes were formed when NR interacting with CT DNA in pH 6.0 PBS, while only one single complex was formed in pH 7.2 PBS within the investigated R values. NR intercalating into the DNA base pairs was through two different intercalation modes in intercalation pocket at lower R, and then followed aggregating along the surface of helix of DNA as the concentration of NR increasing in pH 6.0 PBS, whereas in pH 7.2 PBS, NR binding with DNA was by intercalation and electrostatic interactions. Though the Z form in such a natural DNA would normally escape from detection, in this paper, the conformation of DNA was significantly altered that the complete conversion of B to Z form in CT DNA could be observed while NR was aggregating along the surface of helix of CT DNA in pH 6.0 PBS, and the C form conformation was induced with the binding of NR into CT DNA in pH 7.2 PBS. Scheme 1A and B illustrates the interactions of NR with CT DNA in pH 7.2 and pH 6.0 PBS, respectively.

Acknowledgment

This work is supported by National Natural Science Foundation of China (Nos. 20275037, 20275036, 20211130506).

References

[1] D.H. Tjahjono, T. Akutsu, N. Yoshioka, H. Inoue, Cationic porphyrins bearing diazolium rings: synthesis and their interaction with calf thymus DNA, Biochim. Biophys. Acta 1472 (1999) 333–343.
[2] Y. Cao, X.W. He, Studies of interaction between safranine T and double helix DNA by spectral methods, Spectrochim. Acta, Part A 54 (1998) 883–892.
[3] J.K. Ghosh, A.K. Mandal, M.K. Pal, Energy transfer from thiacyanine to acridine orange facilitated by DNA, Spectrochim. Acta, Part A 55 (1999) 1877–1886.
[4] G. Pratviel, J. Bernadou, B. Meunier, Selective DNA cleavage by metalloporphyrin derivatives, in: H. Sigel (Ed.), Metal Ions in Biological Systems, vol. 33, Marcel Dekker, New York, 1996, pp. 399–426.
[5] T. Uno, K. Hamasaki, M. Tanigawa, S. Shimabayashi, Binding of *meso*-Tetrakis (N-methylpyridinium-4-yl) porphyrin to double helical RNA and DNA. RNA hybrids, Inorg. Chem. 36 (1997) 1676–1683.
[6] M. Monnot, O. Mauffret, V. Simon, E. Lescot, B. Psaume, J.M. Saucier, M.J. Charra, J. Belehradek Jr., S. Fermandjian, DNA-drug recognition and effects on topoisomerase II-mediated, a three-mode binding model for ellipticince derivatives, J. Biol. Chem. 33 (1991) 1820–1829.
[7] M.A. Sari, J.P. Battioni, D. Dupre, D. Mansuy, J.B. Le Pecq, Interaction of cationic porphyrins with DNA: importance of the number and position of the changes and minimum structural requirements for interaction, Biochemistry 29 (1990) 4205–4215.
[8] G. Dougherty, J.R. Pilbrow, A. Skorobogaty, T.D. Smith, Electron spin resonance spectroscopic and spectrophotometric investigation of the bonding of interactionic porphyrins and porphyrazines with calf thymus DNA, J. Chem. Soc., Faraday Trans. 81 (1985) 1739–1759.
[9] M.A. Medina, F.J. Ramirez, J. Ruiz-chica, DNA-chlorpheniramine interaction studies by spectroscopic techniques, Biochim. Biophy. Acta 1379 (1998) 129–133.
[10] E.C. Miller, J.A. Miller, Mechanisms of chemical carcinogenesis, Cancer 47 (1981) 1055–1064.
[11] E.C. Miller, J.A. Miller, Searches for ultimate chemical carcinogens and their reactions with cellular macromolecules, Cancer 47 (1981) 2327–2345.
[12] Q. Feng, N.Q. Li, Y.Y. Tian, Electrochemical studies of porphyrin interaction with DNA and determination of DNA, Anal. Chim. Acta 344 (1997) 97–104.
[13] Z. Wang, D. Liu, S. Dong, Voltammetric and spectroscopic studies on methyl green and cationic lipid bound to calf thymus DNA, Biophys. Chemist. 87 (2000) 179–184.
[14] M.A. Ismail, K.J. Sanders, G.C. Fennell, H.C. Latham, P. Wormell, A. Rodger, Spectroscopic studies of 9-hydroxyellipticine binding to DNA, Biopolymers 46 (1998) 127–143.
[15] R.H. Espelosin, J.C. Stockert, Neutral red fluorescence of chromatin: specificity and binding mechanism, Z. Naturforsch. 37c (1982) 139–141.
[16] F.G. Walz Jr., B. Terenna, D. Rolince, Equilibrium studies on neutral red-DNA binding, Biopolymer 14 (1975) 825–837.
[17] Y. Wang, F. Zhao, K. Li, S. Tong, Molecular spectroscopic study of DNA binding with neutral red and application to assay of nucleic acids, Anal. Chim. Acta 396 (1999) 75–81.
[18] Y. Cao, Y.J. Li, X.W. He, Studies on neutral red as interacting mode spectroscopic probe of DNA, Chem. J. Chinese Universities 20 (1999) 709–712.
[19] C.Z. Huang, Y.F. Li, P. Feng, A spectrophotometric study on the interaction of neutral red with double-stranded DNA in large excess, Talanta 55 (2001) 321–328.
[20] H. Heli, S.Z. Bathaie, M.F. Mousavi, Electrochemical investigation of neutral red binding to DNA at the surface, Electrochem. Commun. 6 (2004) 1114–1118.
[21] J.D. McGhee, P.H. von Hippel, Theoretical aspects of DNA-protein interactions: cooperative and non-cooperative binding of large ligands

to a one-dimensional homogeneous lattice, J. Mol. Biol. 86 (1974) 469–489.
[22] P. Yang, Introduction of Bioinorganic Chemistry, Xi'An Jiao Tong Unversity Publisher, Xi' An, p. 152. Chinese.
[23] G.C. Zhao, J.J. Zhu, J.J. Zhang, H.Y. Chen, Voltammetric studies of the interaction of methylene blue with DNA by means of β-cyclodextrin, Anal. Chim. Acta 394 (1999) 337–344.
[24] J. Jurasová, V. Kubáň, Acid-base and optical properties of neutral red in the presence of ionic and nonionic tensides, Collect. Czechoslov. Chem. Commun. 52 (1987) 2401–2411.
[25] R. Lyng, T. Hard, B. Norden, Induced CD of DNA interactions electric dipole allowed transitions, Biopolymers 26 (1987) 1327–1345.
[26] J.L. Seifert, R.E. Connor, S.A. Kushon, M. Wang, B.A. Armitage, Spontaneous assembly of helical cyanine dye aggregates on DNA nanotemplates, J. Am. Chem. Soc. 121 (1999) 2987–2995.
[27] H.C. Becker, B. Norden, DNA binding mode and sequence specificity of piperazinylcarbonyloxyethyl derivatives of anthracene and pyrene, J. Am. Chem. Soc. 121 (1999) 11947–11952.
[28] N.E. Mukundan, G. Petho, D.W. Dixon, M.S. Kim, L.G. Marzilli, Interactions of an electron-rich tetracationic tentacle porphyrin with calf thymus DNA, Inorg. Chem. 33 (1994) 4676–4685.
[29] A.G. Krishna, D.V. Kumar, B.M. Khan, S.K. Rawal, K.N. Ganesh, Taxol-DNA interactions: fluorescence and CD studies of DNA groove binding properties of taxol, Biochim. Biophys. Acta 1381 (1998) 104–112.
[30] M.A. Khan, J. Musarrat, Interactions of tetracycline and its derivatives with DNA in vitro in presence of metal ions, Int. J. Biol. Macromol. 33 (2003) 49–56.
[31] B. Juskowiak, E. Galezowska, S. Takenaka, Spectral properties and binding study of DNA complexes with a rigid bisintercalator 1,4-bis((N-methylquinolinium-4-yl)vinyl)benzene, Spectrochim. Acta, Part A 59 (2003) 1083–1094.
[32] S. Nahadevan, M. Palauiandavar, Spectral and electrochemical behavior of copper(II)-phenanthrolines bound to calf thymus DNA. $[(5,6\text{-dimethy-OP})_2Cu]^{2+}$(5,6-dimethyl-OP=5,6-dimethyl-1,10-phenanthroline) induces a conformational transition from B to Z DNA, Inorg. Chem. 37 (1998) 3927–3934.
[33] A. Rich, A. Nordheim, A.H.J. Wang, The chemistry and biology of left-handed Z-DNA, Annu. Rev. Biochem. 53 (1984) 791–843.
[34] K. Tanaka, Y. Okahata, A DNA-lipid complex inorganic media and formation of an aligned cast film, J. Am. Chem. Soc. 118 (1996) 10679–10683.
[35] N.J. Zuidam, Y. Barenholz, A. Minsky, Chiral DNA packaging in DNA-cationic liposome assemblies, FEBS Lett. 457 (1999) 419–422.
[36] Z. Zhang, W. Huang, J. Tang, E. Wang, S. Dong, Conformational transition of DNA induced by cationic lipid vesicle in acidic solution: spectroscopy investigation, Biophys. Chemist. 97 (2002) 7–16.
[37] G.G. Prive, U. Heinmann, S. Chandrasegaren, L.S. Kan, M.L. Kopra, R.E. Dickerson, Helix geometry, hydration, and G.A mismatch in a B-DNA decamer, Science 238 (1987) 498–504.
[38] J. Klysik, S.M. Stirdivant, J.E. Larson, P.A. hart, R.D. Wells, Left-handed DNA in restriction fragments and a recombinant plasmid, Nature 290 (1981) 672–677.
[39] A. Jaworski, W.-T. Hsieh, J.A. Blaho, J.E. Larson, Left-handed DNA in vivo, Science 238 (1987) 773–777.