Spectroscopic and Spectroelectrochemical Studies of Interaction of Nile Blue with DNA

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Nile Blue can strongly bind to DNA and its affinity for DNA has been investigated by spectroscopy and spectroelectrochemistry. At low DNA concentrations, Nile Blue can bind to DNA(per nucleotide phosphate) to form a 1:1 association complex with the binding constant of 4.7×10^4 L/mol and the major binding model of Nile Blue to DNA is "electrostatic binding". However, the major binding model changes into "intercalative binding" at high DNA concentrations. In order to confirm which part of Nile Blue intercalating into DNA, the inclusion action of β-cyclodextrin (β-CD) has been used to study the interaction. Nile Blue can be included into the hydrophobic cavity of β-CD to form an inclusion complex with a stability constant of 1.9×10^3 L/mol. The experimental results indicate that the naphthalene ring part of Nile Blue is included into β-CD's hydrophobic cavity and the inclusion complex decomposes at high DNA concentrations. It may be deduced reasonably that the naphthalene ring part of Nile Blue molecule intercalates into DNA helix Strand.

Keywords Nile Blue, DNA, interaction, β-CD inclusion complex, spectroscopy, spectroelectrochemistry

Introduction

It is generally accepted today that there are three kinds of binding models for small molecules to DNA, which refer to intercalative binding, groove binding and electrostatic binding. In these binding models, the intercalative binding is thought as to be the strongest binding because it is a type of binding in which the intercalative molecule surface is sandwiched between the aromatic, heterocyclic base pairs of DNA.[1] In this type of binding, it is an important factor that intercalator can provide a planar structure for efficient intercalation into the DNA strand.[2] For an intercalator, neither whole molecule nor all planar parts can intercalate into the helix of DNA. For example, the isoquinoline part of berberine intercalates into the DNA double helix, but its benzenoid part is stick out of the helix of DNA.[3] For a small molecule, which part of molecule intercalating into DNA strand is of interest because it can provide useful message for design new and efficient drugs as disease diagnosis and chemotherapeutic agents.

Besides spectroscopic method, electrochemical, as well as spectroelectrochemical studies are of potential importance to probing the interaction of small molecule with DNA and the detection of DNA.[4,5] Several studies have been reported on small DNA-binding molecules such as dyes[6-8] and metal coordination compounds.[9,10] However, in previous investigation, the interest of researchers was focused only on the change of small molecule's behaviors, while the effects of small molecules structure had received little attention.

The structure of Nile Blue (NB) is similar to other dyes such as Methylene Blue, Toluidine blue. To our knowledge, the binding behavior of NB to DNA has not

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Received March 19, 2001; revised June 22, 2001; accepted August 18, 2001.

Project supported by the National Natural Science Foundation of China (No. 29835110) and the Doctoral Foundation of the National Education Ministry of China (No. 98028435).

been reported yet and the kinetic data of the interaction are lacking. In this paper, the interactions of NB with DNA was investigated by UV spectroscopic and spectroelectrochemical method. We utilized the inclusion action of β-CD to NB to determine the binding part of NB to DNA. Firstly, let NB be included by β-CD to form a NB/β-CD inclusion complex and then compared the difference between the interactions of both NB and NB/β-CD inclusion complex with DNA to reveal the affinity of NB binding to DNA. By using this method, the interaction of NB with DNA was studied and the interaction models under different DNA concentrations were discussed.

Experimental

Ultraviolet-visible spectra were recorded on a UV-265 Spectrophotometer (Shimadzu, Japan). A homemade optically transparent thin-layer cell (OTTLCell), with three-electrode system: a piece of platinum grid working electrode, Ag/AgCl reference electrode and a platinum sheet as counter one was used to carry out spectroelectrochemical measurements.

Electrochemical experiments were performed with a CHI660 Electrochemical Workstation (CHI Co. USA). All electrochemical experiments were carried out in a single-compartment cell. The working electrode was a platinum disk electrode. A saturated calomel electrode (SCE) and platinum wire served as the reference and counter electrodes, respectively. Supporting electrolyte was Tris-HCl (0.02 mol/L) + phosphate (0.2 mol/L) buffer (pH 7.2) and was deaerated *via* purging with pure nitrogen for 5 min prior to measurements.

Calf thymus DNA was purchased from Sigma Co. and used without further purification. Purity of DNA was checked by monitoring the absorption spectrum and the ratio of the absorbance at 260 nm to 280 nm. The ratio was 1.89, indicating the DNA was free from protein.[11] The concentrated stock solutions of DNA were directly prepared in doubly-distilled water. The concentration of DNA (in nucleotide phosphate) was determined by the absorbance at 260 nm, at which extinction coefficient is 6600.[11] The stock solutions were stored at 4 ℃ and used in 5 days. NB was of analytical grade. The β-CD was twice recrystallized before used. All other chemicals were of reagent grade and used as received. All experiments were carried out at room temperature.

Results and discussion

Formation of β-CD inclusion complex

As well known, cyclodextrins are a kind of polysaccharides made up of six to eight *D*-glucose monomers connected at 1 and 4 carbon atoms, and they can provide a hydrophobic cavity in aqueous solution for the hydrophobic molecules or groups to form inclusion complexes, which have been widely used in chemical analysis. In our experiments, β-CD was used as a recognition reagent to identify the interaction of NB with DNA.

Spectroscopic characterization

The variation of the absorption spectra of NB with the increasing concentration of β-CD is showed in Fig. 1.

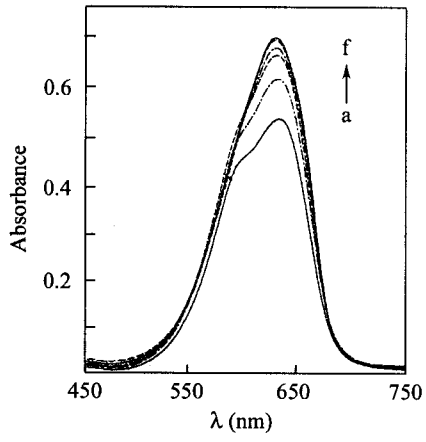


Fig. 1 Absorption spectra of NB in the presence of β-CD (C_{NB}: 1.0×10^{-5} mol/L, 0.1 mol/L pH 7.0 phosphate buffer; $C_{\beta\text{-CD}}$: (a) 0, (b) 2.5×10^{-4} mol/L, (c) 5×10^{-4} mol/L, (d) 1.5×10^{-3} mol/L, (e) 2.5×10^{-3} mol/L, (f) 4.0×10^{-3} mol/L).

It was observed that the absorbance of NB at 635 nm increased with the increasing concentration of β-CD. When the concentrations of β-CD was reached 4.0×10^{-3} mol/L, the increase of absorbance at 635 nm became slowly and tended to be constant, suggesting that NB was included fully. The dependence of the absorbance at 635 nm on the concentration of β-CD obeyed the following equation:[1]

$$C/\Delta\varepsilon_{ap} = C/\Delta\varepsilon + 1/(\Delta\varepsilon k) \quad (1)$$

where, C is the concentration of β-CD, $\Delta\varepsilon_{ap} = (\Delta\varepsilon_a - \Delta\varepsilon_f)$ and $\Delta\varepsilon = (\Delta\varepsilon_b - \Delta\varepsilon_f)$, ε_a (the apparent extinction coefficient) is obtained by calculating $A_{abs}/[NB]$, ε_b and ε_f correspond to the extinction coefficient of the bound and free form of NB, respectively. The stability constant k can be obtained from the ratio of the slope and the y-intercept.

From the experimental data, the regression equation of Eq. (1) was $C_{\beta\text{-CD}}/\Delta\varepsilon_{ap} = 3.2 \times 10^{-5} C_{\beta\text{-CD}} + 1.68 \times 10^{-8}$ and the linear correlation coefficient was 0.9952. According to the equation, stability constant k was calculated to be 1.9×10^3 L/mol.

In 0.1 mol/L phosphate buffer, NB showed two absorption bands (at 325 and 278 nm) in UV region (Fig. 2). The absorbance at 325 nm increased with increasing β-CD concentration, but the location of absorption peak did not change. The absorbance at 278 nm increased and the absorption peak obviously shifted to short wavelength region in the presence of β-CD. This suggested that the hydrophobic interaction between NB and β-CD mainly affected the absorption band at 278 nm.

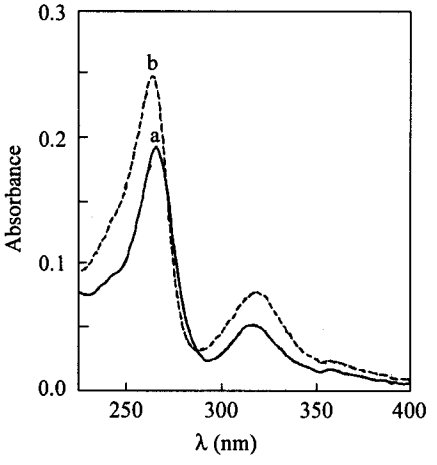


Fig. 2 UV absorption spectra of NB (a, phosphate buffer solution), in the presence of β-CD (b, phosphate buffer solution + 1.0×10^{-2} mol/L β-CD), (C_{NB}: 1.0×10^{-5} mol/L, 0.1 mol/L phosphate buffer pH 7.0).

Electrochemical characterization

Fig. 3 shows the typical cyclic voltammograms of NB without β-CD (a) and with β-CD (b). A couple of redox peaks of NB were observed at Pt electrode in the range of −0.15 V—−0.60 V (*vs.* SCE), in which cathodic peak potential (E_{pc}) and anodic peak potential (E_{pa}) were −0.431 V and −0.370 V, respectively. This was a quasi-reversible 2-electron redox process[12] and its formal potential ($E^{0'}$) was −0.400 V. However, both E_{pc} and E_{pa} shifted to more positive potential and the peak currents (both i_{pa} and i_{pc}) decreased in the presence of β-CD. Although the $E^{0'}$ shifted to more positive potential by 20 mV in the presence of 1.0×10^{-3} mol/L β-CD, the ΔE_p (the difference of E_{pa} and E_{pc}) was almost unchanged. The shift of $E^{0'}$ and the decrease of peak currents are evidences of forming an β-CD inclusion complex.[13-15] The stability constant obtained was 1.2×10^3 L/mol according to the dependence of peak currents on the β-CD concentrations,[15] which was close to that obtained from spectroscopic method.

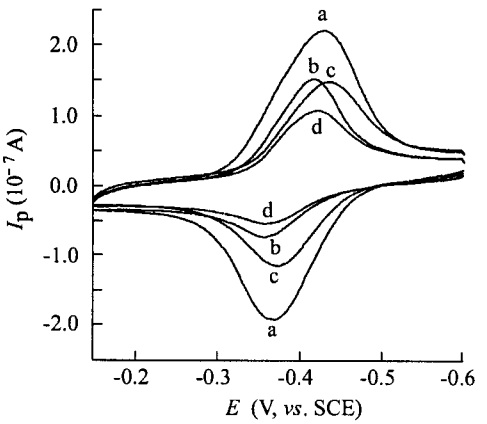


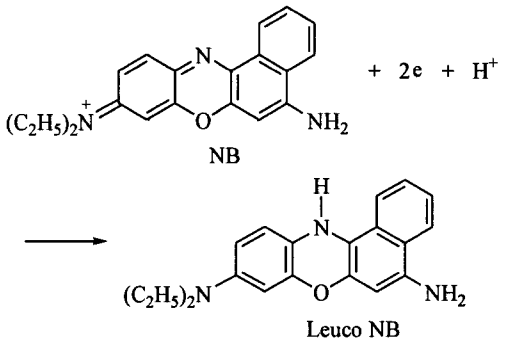
Fig. 3 Cyclic voltammograms of NB in Tris-HCl (0.02 mol/L) + phosphate (0.2 mol/L) buffer, pH 7.2 at 50 mV/s; (a) NB (5×10^{-5} mol/L), (b) a + β-CD (1×10^{-3} mol/L), (c) a + DNA (5×10^{-5} mol/L), (d) b + DNA (5×10^{-5} mol/L).

Fig. 4 showed the UV spectra of NB (a) and Leuco NB (b) (after electrolysis 5 min in OTTLCell at the potential of −0.60 V). Being reduced, the NB absorption band at 325 nm disappeared and the absorption band at 278 nm shifted to 260 nm. The reduction mechanism of NB can be described as Scheme 1.[12]

It can be observed that the conjugated planar structure of NB was damaged after reduced but its naphthalene ring was kept. So it is reasonable to suggest that the absorption band at 278 nm mainly comes from the naphthalene ring. Thus, the part of NB molecule included into β-CD cavity may be the part of the stronger hydrophobic naphthalene ring, because the hydrophobic action of β-CD mainly affected the absorption band at

278 nm, as described in section of *Spectroscopic characterization*.

Scheme 1



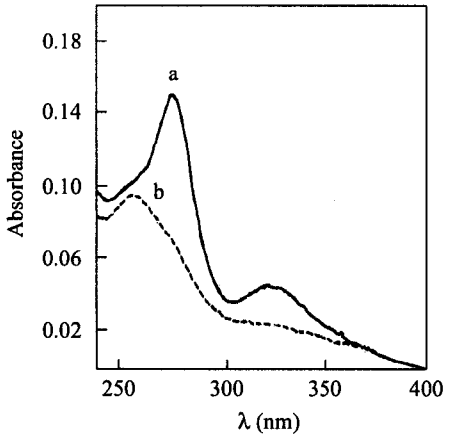


Fig. 4 Thin-layer UV absorption spectra of NB before (a) and after (b) electrolysis 5 min at − 0.60 V (vs. Ag/AgCl). C_{NB}: 1.0×10^{-4} mol/L in Tris-HCl (0.02 mol/L) + phosphate buffer (0.2 mol/L).

Binding of NB *to* DNA

UV *spectroscopic investigation*

Hypochromism and red shifts of the absorption bands were used to characterize the binding of small molecules to DNA.[15] The variation of NB spectra in the presence of DNA is shown in Fig. 5. It was observed that the absorbance at 635 nm greatly decreased with increasing DNA concentration, but no obvious red shift was observed in the range of 0—5×10^{-5} mol/L DNA (Fig. 5 curves a—d). However, when the DNA concentration was higher than 5×10^{-5} mol/L, the absorption band obviously red shifted by 20 nm eventually and the absorbance of NB increased with increasing amount of DNA (Fig. 5 curves e—j). Among the whole concentration range of DNA, the absorption peak firstly decreased without red shift and lastly increased with obvious red shift. Strictly speaking, the change of absorption peak underwent an approach to the same as sharp "V" type, which gave a turning point 644 nm (*ca.* 5×10^{-5} mol/L DNA). So it is reasonable that the interaction models at high and low DNA concentrations are different.

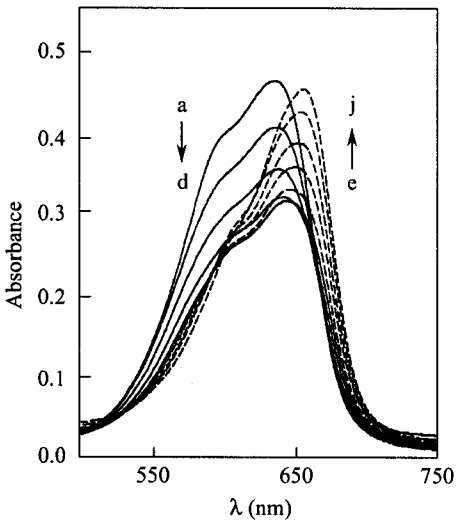


Fig. 5 Absorption spectra of NB in different concentrations of DNA. C_{NB}: 1.0×10^{-5} mol/L, 0.1 mol/L phosphate buffer pH 7.0; C_{DNA}: (a) 0, (b) 1.0×10^{-5} mol/L, (c) 2.0×10^{-5} mol/L, (d) 4.0×10^{-5} mol/L, (e) 6.0×10^{-5} mol/L, (f) 8.0×10^{-5} mol/L, (g) 1.2×10^{-4} mol/L, (h) 1.7×10^{-4} mol/L, (i) 2.5×10^{-4} mol/L, (j) 5.0×10^{-4} mol/L.

Experimental results showed that at low DNA concentration (lower than 5×10^{-5} mol/L), the change of the NB absorbance with DNA obeys the Eq. (1). According to experimental data, the binding constant (k) of NB to DNA was 4.7×10^4 L/mol and the binding number (n) was 1.0. It means that NB bound to DNA to form a 1∶1 association complex under this case. In three kinds of interaction model, the most possible model for forming a 1∶1 association complex is "electrostatic binding". In addition, the effect of the salt concentration of solution for binding constant was investigated. It was observed that the binding constant increased with the decrease of the salt concentration. When the salt con-

centration of solution decreased from 0.1 mol/L to 0.01 mol/L the binding constant increased from 4.7×10^4 L/mol to 7.1×10^4 L/mol. The salt effect is an important evidence for "electrostatic binding". Thus, at low DNA concentration the major interaction model of NB with DNA is "electrostatic binding", in which NB binds to DNA through its cationic group $=N^+H_2$ to PO_4^- in backbone of DNA. The red shift of absorption band is an important evidence for the intercalation of small molecules into DNA base stack.[16,17] And at high DNA concentration, the eventual red shifts of absorption band by 20 nm and the increase of absorbance strongly suggested that the major interaction model was "intercalative binding".

Spectroelectrochemical investigation

In the presence of DNA, the cathodic peak potential shifted to negative slightly and the anodic peak potential almost did not change, but their peak currents decreased with DNA concentration (as shown in Fig. 3c). The reason may be i) the NB-DNA complex does not respond electrochemically at this potential and/or ii) NB and Leuco NB have the same extent for binding to DNA.[18] To confirm above deduction, we used spectroelectrochemical method to investigate the interaction of NB with DNA. The absorption band of NB at 630 nm gradually decreased with working potential shifting to more negative value, which suggested that the NB was reduced to Leuco NB. In the presence of 7.5×10^{-5} mol/L DNA, the mixture solution was still reduced but the change extent of absorbance was smaller than that in the absence of DNA. However, in the presence of 7.5×10^{-4} mol/L DNA, the absorbance of mixture solution almost did not change with the working potential, which showed that no species was reduced in solution. The dependence of absorbance of solution on the working potential was shown in Fig. 6. It clearly suggested that NB-DNA complex had no electrochemical response. This revealed that NB has intercalated into DNA helix strand at high DNA concentration. When NB intercalated into DNA, its active center was enveloped by DNA molecule.

Interaction of inclusion complex with DNA

The change of absorption band of NB/β-CD inclusion complex with DNA is shown in Fig. 7. The decrease of absorption band and its bathochromic shifts, as well as the appearance of an isobestic point at 646 nm indicated that NB/β-CD also interacted with DNA. Hypochromism

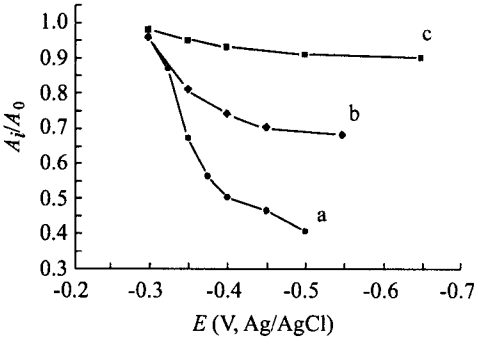


Fig. 6 Dependence of absorbance on the working potential. C_{NB}: 1.0×10^{-4} mol/L; A_0: the absorbance before electrolysis; A_i: the aborbance at applied potential; (a) without DNA, (b) DNA (7.5×10^{-5} mol/L), (c) DNA (7.5×10^{-4} mol/L).

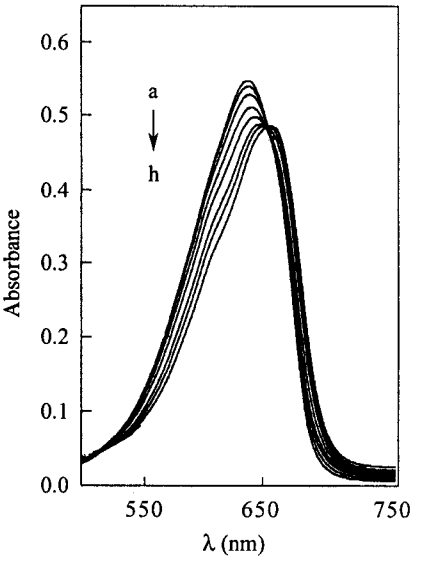


Fig. 7 Absorption spectra of NB in the presence of β-CD (1.0×10^{-2} mol/L) with various concentrations of DNA (1.0×10^{-5} mol/L in 0.1 mol/L phosphate buffer pH 7.0). C_{DNA}: (a) 0, (b) 1.0×10^{-5} mol/L, (c) 2.5×10^{-5} mol/L, (d) 5.0×10^{-5} mol/L, (e) 1.0×10^{-4} mol/L, (f) 2.0×10^{-4} mol/L, (g) 3.0×10^{-4} mol/L, (h) 5.0×10^{-4} mol/L.

and bathochromic shifts were observed but the increase of absorbance at high concentration of DNA was not appeared. It is different from that in the absence of β-CD (see Fig. 5). The decrease of absorbance and red shifts of absorption band suggested that NB/β-CD inclusion complex interacted with DNA. However, a question raised is whether the inclusion complex decomposes when NB/β-CD interacts with DNA. Comparing the absorption curve in the absence of β-CD with that in the presence of β-CD at high DNA concentration (as shown in Fig. 8), it can be observed that these two curves almost overlap each other. This revealed that NB/β-CD complex decomposed when it bound to DNA at high concentration. In other words, NB went out of the β-CD hydrophobic cavity and then intercalated into DNA strand. Therefore, it can be reasonably deduced that the part intercalating into DNA strand is just the one included into β-CD cavity.

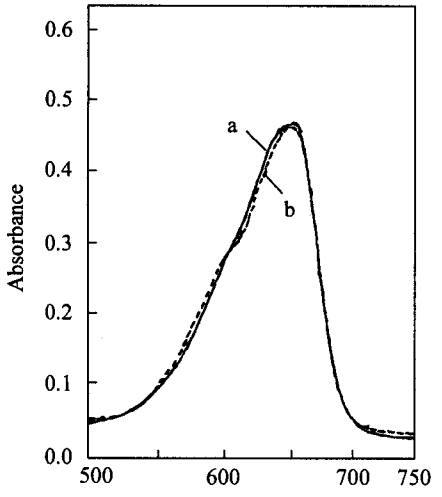


Fig. 8 In the presence of DNA, the absorption spectra of NB with β-CD (C_{DNA}: 1.0×10^{-3} mol/L, $C_{\beta\text{-CD}}$: 1.0×10^{-2} mol/L) (a), and without β-CD (C_{DNA}: 5.0×10^{-4} mol/L wthout β-CD) (b), C_{NB}: 1.0×10^{-5} mol/L in 0.1 mol/L phosphate buffer pH 7.0.

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(E0103193 SONG, J. P.; FAN, Y. Y.)