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Synthesis, characterization and interaction studies of 2,7-naphthalenediol,2-[(4-Bromophenyl)]azo, with calf thymus deoxyribonucleic acid (ct-DNA)

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Abstract

In this study, at first, an azo dye, 2,7-naphthalenediol,2-[(4-Bromophenyl)azo (BPAND) as a ligand has been synthesized by addition of p-Bromoaniline to the modified montomorillonite K10 clay. This ligand was characterized using ^{1}H-NMR, UV-Vis and IR spectroscopies. Subsequently, its interaction with calf thymus deoxyribonucleicacid,ct-DNA was investigated in 5 mM phosphate buffer solution, pH=7 using UV-Vis absorption, thermal denaturation and viscosity measurement. From spectrophotometric titration experiments, the binding constant of BPAND with ct-DNA was found to be $(2.89\pm0.2)\times10^{7}$ M^{-1} at 25°C. In order to determine changes in thermodynamic properties such as binding constant, Gibbs free energy, binding enthalpy and binding entropy, this experiment was done at various temperatures. The enthalpy and entropy changes were -2.852±0.634 kJ/mol and -814.065 ±2.52 J/mol.K, respectively.Thermal denaturation experiments show the increasing of melting temperature of ct-DNA (about 0.06°C less than 0.6) due to binding of BPAND ligand. From these results, the mechanism and the stoichiometry of binding were determined. The results revealed that this interaction is exothermic (negative enthalpy change) along with negative entropy change. Therefore, the driving force in these interactions is enthalpy and the process is enthalpy-driven and suggests that the main driving force for the ct-DNA-BPAND complex formation is being Van der Waals or hydrogen binding.

Keywords: Deoxyribo nucleic acid; azo dye; thermodynamic; UV/Vis spectroscopy.

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Introduction

Dyes have been known as important industrial chemicals. These synthetic compounds, when exposed to sweat, light, water and many chemical substances include oxidizing agents and have a bad effect on health [1,2]. Almost all the dyes used by the textile industry are azo dyes, and they are also widely used in the printing, food, paper making and cosmetic industries. It was reported that they show toxic, carcinogenic and mutagenic effects. These are related the fact that many dyes are made from known carciogens such as benzidine[3,4]. It can also affect the aquatic ecosystem,decreasing the passage of light penetration and gas dissolution in lakes, rivers and other bodies of water[5].Dyes have been shown to interact with DNA, for example Tartrazine which is a food additive, interacts with ct-DNA *via* a groove interaction mode[6]. Another example, Indigo Carmine (IC) has been widely used as a coloring agent in food, such as bread, fruit, juices, dairy and tomato, interacts with DNA with outside binding mode(hydrophobic interaction and hydrogen bonds)[7].

The interaction of nucleic acids with small molecules such as drugs, organic dyes and metal complexes has been studied intensively as it provides knowledge on the screening and design of the novel and more efficient drugs targeting DNA, and can potentially speed on the drug discovery and development processes[8-11].The study of anti-carciogenic medicines and their interactions with DNA are also significantly important to develop new cancer therapy treatments[12,13].These studies on molecular interactions between drugs and DNA have been an active research subject in recent years. DNA is vital for all the living beings.There are several kinds of binding drugs to DNA:(i)intercalation(between two strains),(ii) minor groove,(iii)in the major groove, and (iv) on the outside of double helix(vander Waals or hydrogenic interactions),(v)electrostatic or interaction with phosphate group[14].

In view of all above, it was thought worthwhile to study the interaction of azo dyes with DNA.In the present study,the interaction of ,2,7- naphthalenediol,2-[(4-Bromophenyl)azo (BPAND, (Scheme 1) with native calf thymus deoxyribonucleicacid(ct-DNA) in 5 mM phosphate buffer aqueous solution at netural pH 7.00 have been investigated in different temperatures in view of thermodynamic by electronic absorption, thermal denaturation and viscosity measurements.

Experimental

Chemical and materials

Sodium salt of calf thymus deoxyribonucleic acid, ct-DNA was purchased from sigma. All other reagents and solvents used were supplied by Merck chemical company and were used without further purification. Double distilled water was used throughout the experiments. The stock solution of ct-DNA was prepared by dissolving ct-DNA in 5Mm phosphate buffer at pH=7 and stirred 24h overnight. The concentration of base-pair ct-DNA was determined by monitoring the UV absorbance at 260nm using ε_{260}=6600 $M^{-1}cm^{-1}$[15]. The concentration was 1.268×10^{-3} M and stock solution was stored at -4°C.The solution of ct-DNA gave an UV absorbance ratio (260/280 nm) of more than 1.8, indicating that the DNA was sufficiently free of protein. The fresh ct-DNA solution was prepared throughout the measuments.

Synthesis of the ligand, 2,7-naphthalenediol,2-[(4-bromophenyl)azo (BPAND)

This ligand (BPAND) was prepared in a similar manner in the literature [16] in order to use in interaction with ct-DNA. In order to prepare BPAND, we added 0.64 gr equal to 5 mmole of parabromoaniline dissolved in 15 mL water to 25 mg of modified montomorillonlite K10 clay. After stirring for 30 min, 0.7 gr $NaNO_2$(10 mmol in 10 mLwater was added dropwise for a period of 30 min. After that, 5mmol of 2,7-dihydroxy naphthalene was added to the diazonium-clay complex and was slowly brought to room temperature with constant stirring and keeping at room temperature for 1h. The reaction mixture was then filtered and the precipitated dye was washed three times with cool water. Finally, the azo dye was extracted with methanol to separate from modified clay and, for more purification, it was recrystallized from water-methanol mixture.

Selected data for BPAND

yeild:75%,m.p:132-134, IR (KBr cm-1)3418(br, O-H), 1660 (C=C), 1509(C=C), 1485 (C=C), 1020(C-Br),UV(CH_3OH)(λ_{max} nm) 466.1H NMR (400MHz, DMSO-d6) δ (ppm):11.2 (1H, OH), 8.04 (2H, d, J=8.4 Hz), 7.64(2H,d, J=8.4Hz), 7.43 (1H, s),7.17(1H, s) 2.72(3H, s), 2.21 (3H, s), 13C NMR (100 MHz, DMSO-d6) δ (ppm):162.78, 151.07, 150.31, 137.59, 136.38, 136.02, 129.97, 129.72,128.57, 127.o2, 124.69, 122.18, 20.41, 15.86. EIMS (70eV) m/z 260(M^+), 148, 139, 121,111.UV.Visible (DMF-phosphate buffer):λ_{max}=378nm.

Absorption spectral studies

The absorbance measurements were made by UV/Vis. Perkin Elmer Lambda 25 double beam Spectrophotometer, operating from

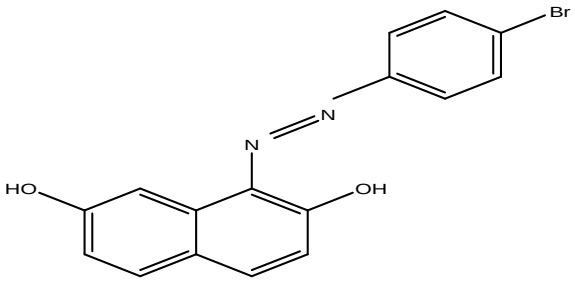
200-700 nm in 1.0 cm quartz cells. This spectrophothometer was equipped with thermostat bath. The absorbance titrations were performed at a fixed concentration of BPANDand the concentration of ct-DNA was changed. In order to prevent interferences due to ct-DNA absorption, the data were obtained by keeping the same concentration of ct-DNAin the reference cuvette.In all of the experiments, for the pH measurement, we used a potentiometer (Metrohm model, 744).

Viscosity measurement

The viscosity of ct-DNA solutions was determined at room temperature using an Ostwald viscosimeter. At first, 5mM phosphate buffer solution, pH 7, was introduced to the viscometer to obtain the reading of flow time. For measuring the solution viscosity, 10 mL of buffered solution of ct-DNA (1.26×10^{-5} M) was taken to the viscometer and a flow time reading was obtained. Defined values of BPAND were then added to the viscometer to give a certain r(r=[BPAND] /[ct-DNA]) value while keeping the ct-DNA constant and the flow time was read. The flow times of samples were measured after the achievement of thermal equilibrium (10 min). Each point measured was the average of at least three readings. The obtained data were shown as relative viscosity, $(\eta/\eta_0)^{1/3}$ against r, where η is the reduced specific viscosity of ct-DNA in the presence of BPAND ligand and η_0 is the reduced specific viscosity of ct-DNA alone [17,18].

Melting experiments

Melting curves were made using an UV/Vis double beam spectrophotometer Perkin-Elmer Lambda 25 model equipped with a thermal bath. The measurements were carried out in 5 mM phosphate buffer, pH 7. The temperature inside the cuvette was measured with a platinum probe and was increased over the range 25-86 °C at a heating rate 0.5 °C/min. The melting temperature, T_m,was obtained from the midpoint of the melting curve.



Scheme 1. The structure of 2,7-naphthalenediol,2-[(4-Bromophenyl)azo (BPAND)

Results and discussion

Electronic absorption study

TheUV/Visible spectrophotometry method is usually used to determine the binding

constant of drugs or ligands to DNA. The absorption spectrum of BPAND in the absence and at various concentrations of ct-DNA is shown in Figure1. In the absence of DNA, BPAND has a peak at 378 nm in 5 mM phosphate buffer due to π-π* transition of the chromophore [19]. A spectral change of the BPAND due to addition of ct-DNA was shown in Figure 1. For obtaining these spectra, the fixed amount of BPAND in phosphate buffer solution, pH 7, was titrated with a stock solution of ct-DNA.It exhibited the low hyperchromism in all spectral regions and negligible red shift due to the incremental addition of ct-DNA. Hypochromism happens when the ct-DNA-binding mode of ligand has an electrostatic effect or an intercalation which stabilizes the DNA duplex [20-22]. Hyperchromism can probably be due to dissociation of aggregated ligand or external contact with DNA [23,24].The apparent binding constant, K_b for the interaction between BPAND and ct-DNA can be determined by analysis of absorption spectrophotometric titration data at room temperature using Eq. 1:

$$[DNA]_{total}/(|\varepsilon_{app}-\varepsilon_f|)=[DNA]_{total}/(|\varepsilon_b-\varepsilon_f|)+1/K_{app}(|\varepsilon_b-\varepsilon_f|) \quad (1)$$

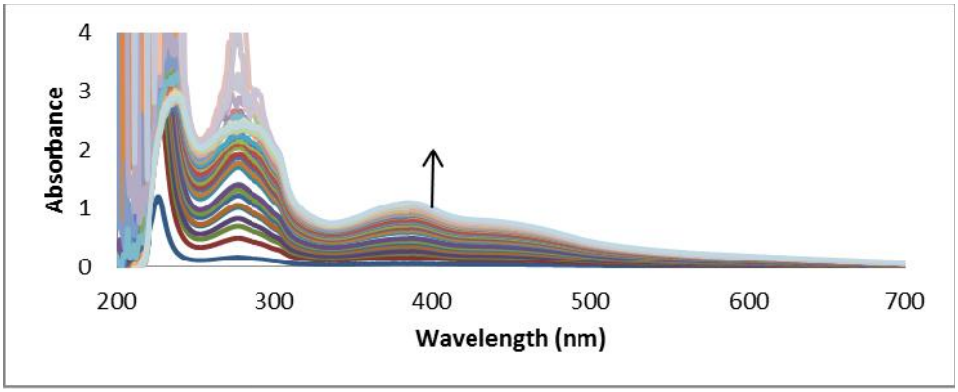


Figure 1. Electronic absorption spectra of BPAND ligand [1.2226×10^{-5} M] in the absence and in the presence of increasing amount of ct-DNA concentrations [at 25°C]. The arrow shows the absorbance changes upon increasing ct-DNA concentrations.

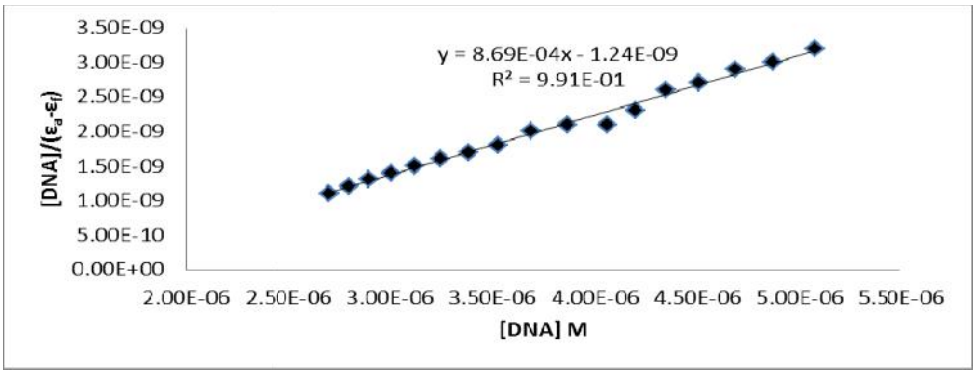


Figure 2. The plot of [ct-DNA] /(|$\varepsilon_{app} - \varepsilon_f$|) versus [ct-DNA] at 25°C

Where [DNA] is the total concentration of ct-DNA in base pairs, the apparent absorption coefficient ε_a, $\varepsilon_f$andε_b corresponds to Aobs/[BPAND], the extinction coefficient for the free BPAND, and the extinction coefficient of the BPAND in fully bound form, respectively. In plots of $\frac{[DNA]}{(\varepsilon a-\varepsilon f)}$ versus [DNA] that was shown in Figure2, K_b is given by the ratio of slope to the intercept [25-27]. The apparent binding constant of BPAND ligand was estimated and used for calculation of standard Gibbs free energy change of reaction at various temperatures.

Thermodynamics of ct-DNA-BPAND interaction

The thermodynamic of DNA-BPAND equilibrium can be conveniently characterized by three parameters, standard Gibbs free energy,ΔG°, standard enthalpy, ΔH° and standard entropy changes,ΔS°. ΔG° can be calculated from the equilibrium constant, *K*, of the reaction using the familiar relationship, ΔG°= -RT ln*K*, in which R and T refer to the gas constant, and the absolute temperature, respectively. Furthermore, *K* is the apparent equilibrium constant and consequently ΔG° is the apparent Gibbs free energy change. If heat capacity changes for the reaction are essentially zero, the van't Hoff equation (Eq.2) gives a linear plot of ln*K*versus 1/T (Figure 3) [28].

$$d\ln K/d(1/T) = -\Delta H°/R \qquad (2)$$

The apparent standard enthalpy change, ΔH°, can be calculated from the slope of the straight line, - ΔH°/R and the apparent standard entropy change from its intercept,ΔS°/R. The van't Hoff plots for interaction of the BPAND ligand with ct-DNA is shown in Figure3 and the calculated thermodynamic parameters with their uncertainties are reported in Table 1. It has been revealed that the standard Gibbs free

energy changes for ct-DNA-BPAND interaction is negative, representing the relative affinity of the CoL ligand to ct-DNA. It has also been indicated that the binding process is endothermic disfavored ($\Delta H°>0$) and entropy favored ($\Delta S°>0$). As proposed by Ross [29], when $\Delta H°<0$ or $\Delta H°\approx 0$,$\Delta S°>0$, the mainly acting force is electrostatic; when $\Delta H°<0$, $\Delta S°<0$, the mainly acting force is van der Waals or hydrogen bonding and when $\Delta H°>0$, $\Delta S°>0$, the mainly force is hydrophobic. From the thermodynamic data, it was quite clear that the interaction processes were exoothermic favored, but entropy disfavored ($\Delta H°< 0$, $\Delta S°< 0$). The value of *K*, the interaction constants of ct-DNA-BPAND, was ~ 10^4, which was at least 100 times smaller than reported examples of traditional intercalating mode, such as daunomycine [30], cryptolepine [31], and chlorobenzylidine [32]. These results furtherly illuminated that the interactions between ct-DNA and BPAND complex did not follow the traditional intercalating mode, while the conformation changes of ct-DNA structure may be realized *via* enthalpy driven non-classical intercalation interaction. The mainly force is hydrogenic or vanderwaals.

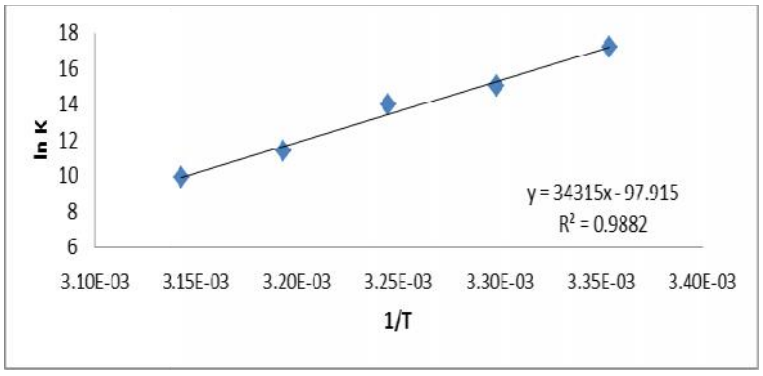


Figure 3. The Van't Hoff plot BPAND ligand binding to ct-DNA

Table 1. Thermodynamic properties of interaction of BPAND with ct-DNA

T (K)	ln K	ΔH° (kJ/mol)	ΔG° (kJ/mol)	ΔS° (J/mol K)
298.15	17.1785	-2.852 ±0.634	-42.58±0.117	-814.065±2.52
303.15	15.0304	-2.852 ±0.634	-38.51±0.130	-814.065±2.52
308.15	13.9553	-2.852 ±0.634	-34.44±0.142	-814.065±2.52
313.15	11.4076	-2.852 ±0.634	-30.37±0.155	-814.065±2.52
318.15	9.9431	-2.852 ±0.634	-26.30±0.168	-814.065±2.52

Viscosity measurement

Photophysicalspectroscopy measurements provide necessary, but not sufficient, evidence to support the binding mode of metal complexes with ct-DNA. Hydrodynamic data provide perhaps the most critical test for intercalative binding in the absenceof X-ray and NMR structural data [32]. A classical intercalation mode causes a significant increase in the viscosity of the ct-DNA solution due to the increase in separation of the base pairs at intercalation sites and hence to an increase in overall DNA contour length [33]. A partial or non-classical intercalation mode of binding could bend or kink the DNA helix, which reduces its effective length and its viscosity [34]. The values of relative specific viscosity *vs* [ct-DNA]/[BPAND] was plotted.

Thermal denaturation measurements

Another strong evidence of the binding mode between the metal complexes and ct-DNA was obtained from ct-DNA melting studies. The intercalation of small molecules into the double helix DNA is known to significantly increase the melting temperature of ct-DNA, at which the double helix denatures into single-stranded ct-DNA [35]. However, the T_m will lightly increase (<0.6°C) on the interaction of small molecules with ct-DNA through nonspecific electrostatic or vanderwaals interactions with the phosphate backbone of ct-DNA [36]. The extinction coefficient of ct-DNA bases at 260 nm in the double helical form is much less than that in the single stranded form [37], hence the melting of the helix leads to an increase in the absorption at this wavelength (Figure 6). Thus, the helix to coil transition temperature can be determined by monitoring the absorbance of ct-DNA at 260 nm as a function of temperature (T_m). Obtained data

show that the interaction of the [BPAND] complex with ct-DNA leads to relatively moderate stabilization of duplex structure (Table 2) Moreover, the increase in [BPAND] to [ct-DNA] concentration ratio, r, in the range $0.013 \leq r \leq 0.026$, weakly affects T_m of the melting curve. At greater [BPAND] /[ct-DNA] ratios, $0.052 \leq r \leq 0.104$, the T_m value increase with increasing concentration of [BPAND] ligand. At $r \geq 0.2$ aggregation effects are observed, which hinder acquisition of the melting curve. The obtained results specify that at low [BPAND]/[ct-DNA] ratios ($r \leq 0.026$) the external binding mode is more reasonable [37-40]. The results of thethermal denaturation experiments presented are consistent with the absorption spectral profiles, which demonstrate a non-intercalative mode.

This indicates that BPAND ligand binds strongly to ct-DNA mostly in the outside-binding and hydrogenic or vander Waals interaction mode.

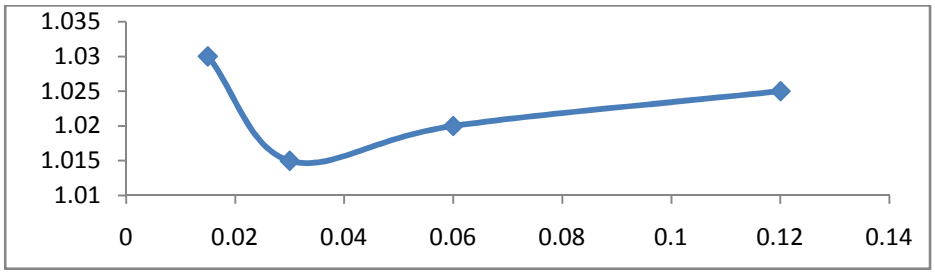


Figure 4. Relative viscosity of ct-DNA (9.4×10^{-5} M) in the presence of increasing amounts of [BPAND] at stoichiometric ratios r = [BPAND]/[ct-DNA] = 0.0- 0.12, plotted as $(\eta/\eta_o)^{1/3}$ vs. r. Measurements were done in 5 mM phosphate buffer, pH 7 and at 25 ^oC.

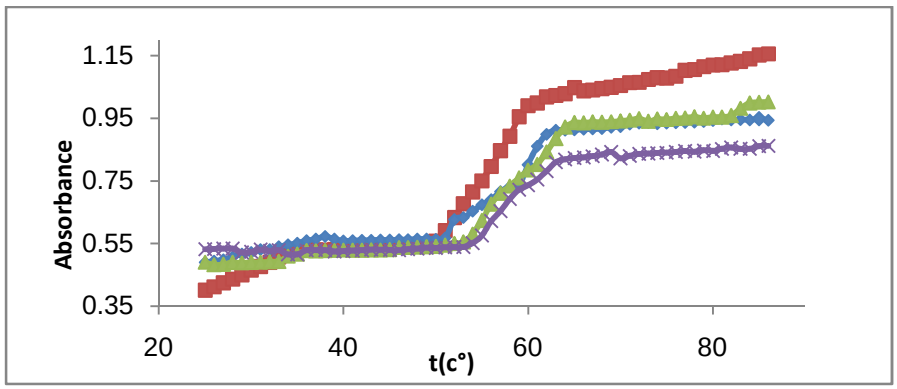


Figure 5. Melting profiles (λ = 260 nm) at various molar ratios (r=[BPAND]/[ct-DNA]), r_1= 0.0 (♦), r_2= 0.013 (▪), r_3= 0.026 (Δ), r_4= 0.052(○) and r_5= 0.104 (*) in 5 mM phosphate buffer, pH 7 and in range of temperature 25°C- 86°C.

Table 2. Melting temperatures of ct-DNA in absence and presence of BPAND

[BPAND]/[ct-DNA]	0	0.028	0.055	0.11
T_m (K)	333.171	330.301	330.179	330.118

Conclusion

In this work, the interaction of ct-DNA with an azodye(BPAND) as a ligand, was studied in view of thermodynamic. The mode of interaction of BPAND with ct-DNA was investigated using UV/Vis spectroscopy, thermal denaturation and viscosity measurements. The binding constant of this interaction was calculated using spectroscopic titration experiment and determined $(-2.89\pm0.2)\times10^7$ M^{-1} at 25°C. The enthalpy and entropy changes were 2.852±0.63 kJ/mol and -814.065 ±2.52 J/mol. K, respectively. Thermal denaturation experiments show the increasing of melting temperature of ct-DNA (about 0.06 less than 0.6°C) due to the binding of BPAND ligand. Using these results,the mechanism and the stoichiometery of binding was determined. The results revealed that this interaction is exothermic (negative enthalpy change) along with a negative entropy change. Therefore, the driving force in this interaction is enthalpy and the process is enthalpy-driven. These results suggest that the main forces in this interaction are van der Waals or hydrogen binding. This research could be valuable for seeking the cancinogenic dyes, as well as for understanding the mode of the azo dyes binding to ct-DNA.

Acknowledgments

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