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Interaction studies of DNA binding with a new Cu(II) complex by spectrophotometric, spectrofluorometric, voltammetric and circular dichroism techniques

M. Bordbar[1,*],F. Khodaie[2], M. Tabatabaee[2], A.Yeganeh-Faal[3], Z. Mehri lighvan[4], S. Mohammad-Ganji[5]

[1]*Department of Chemistry, Faculty of Science, University of Qom, Qom 37185-359, Iran.*
[2] *Department of Chemistry, Yazd Branch, Islamic Azad University, Yazd, Iran.*
[3]*Department of Chemistry, Faculty of Science, Payame Noor University, Tehran, Iran*.
[4]*Young Researchers and Elites Club, Department of Chemistry, Isfahan University of Technology, Isfahan, 84156/8311*
[5]*National Institute for Genetic Engineering & Biotechnology (NIGEB), P. O. Box: 14965-161Tehran, Iran.*

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The binding ability of the water-soluble copper(II) complex with pyridine-2,6-dicarboxylate ($pydc^{2-}$) and 2-aminopyrimidine (amp) with the formula $[Cu(pydc)(amp)].2H_2O.H_2O$ (**1**) to calf thymus DNA (CT-DNA) was investigated. The binding ability of **1** was studied by measuring the effects on the electronic absorption spectra, thermal denaturation studies, fluorescence quenching studies using methylene blue (MB) as a fluorescent probe and circular dichroism (CD) spectra. All results suggest that the interaction mode between (**1**) and DNA takes place by intercalation with a binding constant of $(9.51\pm0.2)\times10^4$ M^{-1}.

Keywords: Intercalative Interaction, CT-DNA, Methylene Blue, Mixed Nitrogen Donor Ligands, Cu (II) Complex.

INTRODUCTION

Cis-platine is one of the widely utilized antitumor drugs [1]. In order to decrease the toxicity of this kind of platinum complexes, many kinds of amine-substituted compounds are employed in the research of antitumor agents [2-4]. Some natural products have been used as amine-substituted ligands in this research [5], such as D-glucosamine [6], chitosan [7] and their derivatives. These are non-toxic towards the human body and the amino group in the chain has special activity. However, one disadvantage of normal chitosan is its low solubility in water. Recently there has been considerable interest in the binding of small water-soluble molecules to DNA [8]. Investigations of the interaction between small molecules and DNA are basic works in the design of new types of pharmaceutical molecules. A small molecule which can interact with DNA can be assigned to several categories: metal ions and metal complexes, such as metal bipyridyl complex [9] and metal phenanthroline complex [10]; heavy metals which cause damage of DNA, such as chromium [11], antibiotics, organic dyes and organic pesticides [12], protein molecules and nanoparticle markers [13]. Studies of the interaction between transition metal complexes and DNA have been pursued in recent years [14]. Metal complexes are known to bind to DNA *via* both covalent and non-covalent interactions. In covalent binding, the labile ligands of the complexes are replaced by a nitrogen base of DNA such as guanine N7. On the other hand, non-covalent DNA interactions include intercalative, electrostatic and groove (surface) binding of cationic metal complexes both outside the DNA helix, and along major or minor grooves. Among these interactions, intercalation is one of the most important DNA binding modes, as it invariably leads to cellular degradation and is an enthalpically driven process resulting from the insertion of a planar aromatic ring system between ds-DNA base pairs with concomitant unwinding and lengthening of the DNA helix [15]. Copper is a cofactor essential for the tumor angiogenesis processes [16]. Among the metal complexes, copper (II) complexes containing heterocyclic bases have been developed as a result of their diverse applications following the discovery of the ‘‘chemical nuclease’’ activity of the $[Cu(phen)_2]^{2+}$ complex [17]. Furthermore, copper complexes have shown strong interactions with DNA *via* surface associations or intercalation [18]. The factors that may affect their association with DNA include their size, the type of ligands, the presence and the position of small lipophilic groups. On the other hand, copper complexes having strong association with DNA are also capable of inducing a hydrolytic cleavage [19], as

* To whom all correspondence should be sent:
E-mail: m.bordbare@gmail.com

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several copper-based synthetic nucleases have been reported [20]. Their importance is due to their capacity to cleave DNA *via* oxidative mechanisms resulting from copper's ability to adopt different oxidation states.

In this work, the Cu (II) complex of 2-aminopyrimidine (amp) and pyridine-2,6-dicarboxylate (pydc) (**1**) was synthesized [21]. Interactions of this complex with calf thymus DNA (CT-DNA) were studied by UV–Vis, fluorescence using MB as a fluorescence probe [22], depressing emission quenching of this complex by $K_4[Fe(CN)_6]$ in presence of DNA [23], circular dichroism spectroscopic methods and cyclic voltammetry (CV) measurements in order to gain a better understanding of the binding mechanism of this complex [24].

EXPERIMENTAL

Reagents

The reagents and chemicals were purchased from commercial sources and used as received without further purification. Calf thymus DNA (CT-DNA) was obtained from Sigma. The stock solution of CT-DNA gave a ratio of UV absorbance at 260 and 280 nm (A_{260}/A_{280}) of 1.89 to check DNA purity and making sure that the DNA was sufficiently free from protein contamination [25]. The DNA concentration was determined by UV absorbance (ε =6600M^{-1} cm^{-1} at 260 nm) [26]. The stock solutions were stored at 4° C and used within 4 days. All experiments involving interaction of the complex with DNA were carried out in doubly distilled water buffer containing 5mM Tris-HCl [Tris(hydroxymethyl)-aminomethane] and 50 mM NaCl, adjusted to pH 7.3 with hydrochloric acid.

Electronic Absorption Spectra

The UV–Vis spectra were recorded on a Varian Cary-100 UV–Vis spectrophotometer. Absorption titration experiments were conducted by fixing constant concentration of the complex at 783 μM while varying the concentrations of CT-DNA or by fixing constant concentration of the (amino) and (pydc) ligands at 70 μM while varying the concentration of Cu(II) metal cation ([Cu(II)]/[L]=0-5). All experiments were conducted in a buffer containing 5 mM Tris–HCl (pH 7.3) and 50 mM NaCl. CT-DNA being an absorbing species in the absorption range of the complex, the titrations were carried out by adding the same amounts of CT-DNA solution to both reference and measuring cell to eliminate the absorbance of DNA itself.

Fluorescence Spectra

The fluorescence spectra were recorded on a Varian Cary Eclipse spectrofluorometer. The complex at a fixed concentration (19.6 μM) was titrated by increasing amounts of CT-DNA. Excitation wavelength of the complex was 296 nm, scan speed = 100 nm/min, slit width 5/5 nm. All experiments were conducted in a buffer containing 5 mM Tris–HCl (pH 7.3) and 50 mM NaCl. The binding mode of the complex with CT-DNA was studied by using methylene blue (MB) as a fluorescence probe. Also, depressing the emission quenching of this complex by $K_4[Fe(CN)_6]$ in the presence of DNA was studied.

Circular Dichroism Measurements

Circular dichroism measurements were carried out on a Jasco-810 spectropolarimeter at room temperature. A rectangular quartz cell of 1 cm path length was used to obtain spectra from 320 to 220 nm with a scanning speed of 100 nm/min and a response time of 4 s. Each spectrum was accumulated at least three times and results were expressed as molar ellipticity (H). The optical chamber of the CD spectrometer was deoxygenated with dry nitrogen before use and kept in a nitrogen atmosphere during the experiments. Scans were accumulated and automatically averaged. CD spectrum was generated which represented the average of three scans from which the buffer background had been subtracted. In the CD spectrum the concentrations of CT-DNA and complex were 100 and 50 μM, respectively.

Thermal Denaturation Experiments

Thermal denaturation experiments were carried out on a Varian Cary-500 UV-Vis double beam spectrophotometer. The absorbance of 75 μM DNA at 260 nm was monitored in the absence and presence of 37.5 μM complex with temperature ranging from 55 to 100 °C.

Cyclic Voltammetry Experiments

Cyclic voltammetry experiments were performed at room temperature with a conventional three-electrode electrochemical cell, using a Metrohm Autolab potentiostat/galvanostat. The three-electrode system used in this research consisted of a gold electrode as a working electrode, an Ag/AgCl reference electrode and a Pt foil auxiliary electrode. The solution was prepared by dissolving the complex in aqueous buffer containing 5 mM Tris–HCl (pH 7.3) and 50 mM NaCl.

Viscometry Measurements

The viscosity was determined using a digital Brookfield circulating bath Ultra DV III viscometer maintained at 25.0 ± 0.1 °C in a circulating water-bath. Data were analyzed as $(\eta/\eta_0)^{1/3}$*versus* the ratio of the concentration of (**1**) and DNA, where η is the viscosity of DNA in presence of the complex and η_0 is the viscosity of DNA alone.

Synthesis of the Complex

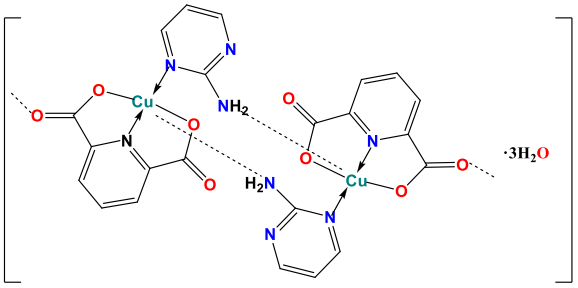
The copper complex was prepared as described by our group [27]. Pyridine-2,6-dicarboxylic acid (0.167 g, 1 mmol) was dissolved in 10 ml of deionized water containing 0.08 g (2 mmol) of NaOH and was stirred for 30 min at room temperature. An aqueous solution of 0.241 g (1 mmol) of $Cu(NO_3)_2.3H_2O$ and 0.095 g (1 mmol) of 2-aminopyrimidine was added to pyridine-2,6-dicarboxylic acid solution. The reaction mixture was placed in a Parr-Teflon lined stainless steel vessel, sealed and heated at 130˚C for 4 h. Blue crystals of the complex were obtained upon slow cooling (yield 91%) [28].

RESULTS AND DISCUSSION

Characterization of the Complex

Recently, synthesis and characterization of {[Cu(pydc)(amp)].$3H_2O$}n by elemental analysis, IR spectroscopy, thermal analysis and X-ray diffraction studies have been reported by our group [28]. As reported in ref. [21], each Cu (II) ion is coordinated by an O,N,O-tridentate $pydc^{2-}$ ligand (bound *via* pyridine nitrogen atom and two carboxylate oxygen atoms) and one heterocyclic nitrogen atom of the 2-aminopyrimidine ligand (Scheme 1). The same coordination mode was found for the nickel complex [29]. The six-coordination was completed by two water molecules in the nickel complex, while each metal ion in the copper complex is weakly connected to two neighboring ones, through two carboxylate bridging groups of dipicolinate and the amino-nitrogen of the NH_2 group of 2-aminopyrimidine (Scheme 1).

The FTIR spectrum of the crystals shows broad strong bands in the region 3275–3523 cm^{-1}, which could be related to the existence of O–H···O hydrogen bonding between the water molecules. It must have been coupled by other indicative peaks such as N–H and O–H stretching frequencies and the stretching frequencies due to the aromatic rings which originally fall within this region.



Scheme 1. Structure of the Cu (II) mixed ligand complex

Absorption Spectra

Electronic absorption spectroscopy is universally employed to examine the binding mode of DNA with metal complexes [30]. The absorption spectra of (**1**) in the absence and presence of CT-DNA are given in Fig.1. In the absence of DNA, the absorption spectrum of (**1**) has a strong π–π* transition band at 268 nm. Increasing DNA concentration, the absorption band of the complex at 268 nm shows hypochromism of 85.5% and bathochromism of about 8 nm; at 220 nm it shows 51% hypochromism and bathochromism about 9 nm. These phenomena indicate that the complex probably interacts with CT-DNA by intercalation mode, involving strong p π-stacking interactions between the aromatic rings of the complex and DNA base pairs.

The apparent binding constant, K_b, for the interaction between the complex and CT-DNA can be determined by analysis of the spectrophotometric titration data at room temperature using Eq. (1) [31].

$$[DNA]/(|\varepsilon_a-\varepsilon_f|)=[DNA](\varepsilon_b-\varepsilon_f)+ 1/K_b(\varepsilon_b-\varepsilon_f) \quad (1)$$

where [DNA], ε_a, ε_f and ε_b correspond to the total concentration of CT-DNA base-pair, A_{obsd}/[Com], the extinction coefficient for the free complex and the extinction coefficient for the complex in the fully bound form, respectively. In the plot of [DNA]/($|\varepsilon_a-\varepsilon_f|$) *versus* [DNA], K_b is given by the ratio of the slope to the intercept.

Until now, the binding constant reported in most papers has been calculated at a single wavelength since different parts of a compound do not have similar interaction with DNA. In this case, the absorption spectrum at different wavelengths is differently affected by this interaction and is expected to give different binding constants at different wavelengths. The calculated binding constants for DNA-(**1**) at some of the selected wavelengths are reported in Table 1. It can be inferred that different values for the binding constant are obtained at different wavelengths. It

seems that the comparative binding constant calculated at a single wavelength for the DNA-compound interaction may not be correct. This probably may be true if the binding constant is reckoned as an average of the binding constants at all wavelengths. Herein, we calculated the binding constant K_b for the DNA-(**1**) at all wavelengths in MATLAB and the mean result is $9.51(\pm 0.2)\times10^4$ L mol^{-1}.

Table 1. K_b values at different wavelengths

Wave length (nm)	280	270	260	220	202
K_b (M^{-1})	5.46 $\times10^4$	1.00 $\times10^5$	3.21 $\times10^4$	9.52 $\times10^5$	4.34 $\times10^6$

The K_b value obtained for our copper(II) complex is considerably higher than those for any other known copper(II) complexes including complexes such as $[Cu(phen)_2Cl_2]$ (K_b, 2.70×10^3 M^{-1})[32], $[Cu(phen)(L\text{-}Thr)(H_2O)]ClO_4$ (K_b, 6.35×10^3 M^{-1}) [33], [Cu (II) Schiff base complexes] (K_b, 3.20×10^3 and 9.60×10^3 M^{-1}) [34] $[Cu(phen)(L\text{-}Gly)(H_2O)]$ (K_b, 4.68×10^3 M^{-1}) [35]

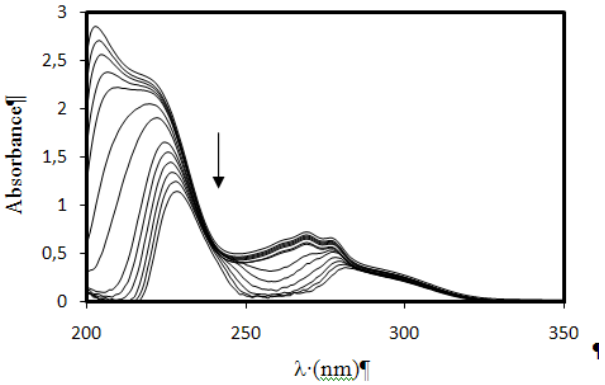


Fig. 1. UV–Vis absorption spectra of the complex (70 μM) in the presence of increasing amounts of CT-DNA; [DNA]=(0-950) μM The arrow shows the changes upon increasing the amount of CT-DNA.

Fluorescence Spectra

The complex showed fluorescence emission in Tris buffer at room temperature, with a maximum at about 363 nm. As shown in Fig. 2, the fluorescence intensity of the complex was steadily quenched with the increasing concentration of CT-DNA.

The interaction mode of the complex binding to DNA can be determined according to the classical Stern–Volmer equation [36]:

$$F_0/F=1+K_q[Q] \qquad (2)$$

where F_0 and F represent the emission intensity in the absence and presence of quencher, respectively, K_q is a linear Stern–Volmer quenching constant and [Q] is the quencher concentration. The Stern–Volmer quenching plots from the fluorescence titration data are shown in the inset of Fig. 2.

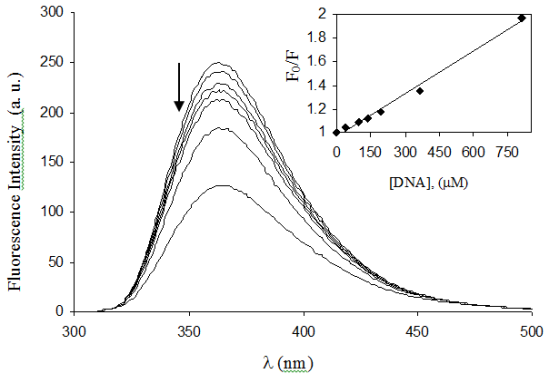


Fig. 2. Emission spectra of the complex (a) in Tris–HCl buffer upon addition of CT-DNA. [Complex]= 20 μM, [DNA]= (0–809) μM. Arrow shows the intensity change upon increasing CT-DNA concentration. The inset is a Stern-Volmer quenching plot of (**1**) with increasing concentrations of CT-DNA.

The fluorescence quenching constant (K_q) evaluated using the Stern–Volmer equation is 1.12×10^3 M^{-1}. When the Stern–Volmer plot is linear, it indicates that only one type of quenching process occurs and the obtained linear relationship could be applied to determine DNA. This phenomenon of the quenching of luminescence of the complex by CT-DNA may be attributed to the photoelectron transfer from the guanine base of DNA to the excited metal-to-ligand charge-transfer (MLCT) state of the complex [37].

DNA–MB Displacements

Further support for the binding of the complexes to DNA by intercalation mode was given by the competitive binding experiment. MB has long been used as a planar dye molecule for biological straining and diagnosis of diseases including carcinoma [38]**.** The interaction of methylene blue with DNA has been studied with various methods [39]. Most studies indicated that (at low ionic strength buffer and low concentration of DNA) the major binding mode of MB with DNA was through intercalation. The experiment involves the addition of the present complex CT-DNA pretreated with MB as a fluorescence probe ([DNA]/[MB]=10) and the measurement of emission intensities of DNA-bound MB. Interestingly, the emission intensity of MB is quenched on adding CT-DNA. This emission quenching phenomenon reflects the change in the excited state structure in consequence of the electronic interaction in the MB–DNA complex [40], expected from the strong stacking interaction (intercalation) between the adjacent

DNA base pairs. The emission spectra of the MB–DNA solutions in the presence of increasing amounts of (**1**) is shown in Fig. 3, which clearly reveals a gradual increase in the fluorescence intensity of the probe molecule (MB) by adding (**1**). The increase in fluorescence intensity is due to the release of free MB molecules from the DNA–MB complex. Therefore, the formation of metal complex–DNA prevents the binding of MB. The complete metal complex–DNA formation occurs when the probe fluorescence intensity is sufficiently close to the corresponding pure MB fluorescence intensity. Complete recovery of MB fluorescence intensity is indicative of an intercalative mode of binding, but in this experiment, the recovery of MB was 6% in [complex]/[DNA]=3 and 37% in [complex]/[DNA]=92, which confirms that the intercalative binding strength of the Cu (II) complex to DNA is weaker than to MB.

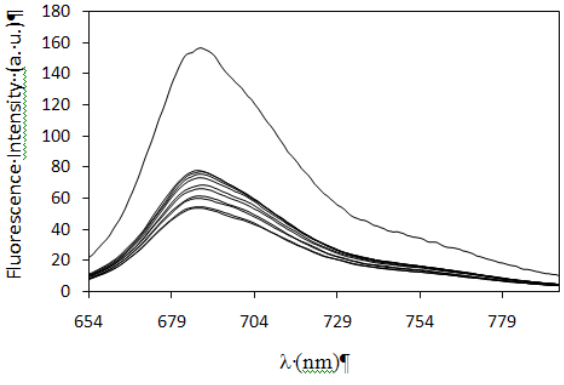


Fig. 3. Emission spectra of the competitive reaction between Cu (II) complex and methylene blue with DNA. $C_{complex}$ = 0.0, 144.0, 283.0, 420.0, 550.0, 680.0, 1048.0, 1765.0, 2080.0, and 3129.0 μM, C_{MB} =5μM and C_{DNA} = 50 μM in Tris–HCl buffer (5 mM, pH 7.3) plus 50 mM NaCl.

Emission Quenching Titration with $K_4Fe(CN)_6$

In aqueous solution, iodide and ferrocyanide anions quench the fluorescence of (**1**) very efficiently, so we used potassium ferrocyanide as the quencher to determine the relative accessibilities of the free and bound (**1**).The highly negatively charged quencher is repelled away from the negatively charged phosphate backbone of DNA, the bound copper cation should be protected from anionic quenchers, while the emission from free complexes should be readily quenched by an anionic quencher. Fig. 4 shows the linear Stern–Volmer plots with ferrocyanide anion as a quencher for (**1**), at [DNA]/[Complex] =0 or 40, as expected for a single-component donor–acceptor system.

As illustrated in Fig. 4, the emission of (**1**) in the presence of DNA is difficult to be quenched. This may be explained by the fact that the bound cations of the complex are protected from the anionic water-bound quencher by the array of negative charges along the DNA phosphate backbone [41]. The obtained quenching constants were 3.5 and 2.1 M^{-1} with and without CT-DNA, respectively. The quenching of (**1**) was in fact enhanced by a factor of 1.6 when (**1**) was bound to the DNA helix.

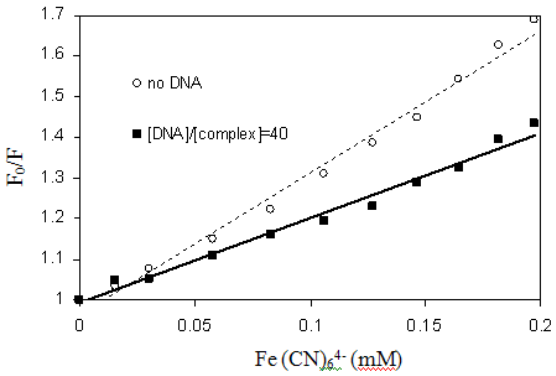


Fig. 4. Emission quenching with Fe $(CN)_6^{4-}$ for free and DNA-bound (**1**).

CD Spectroscopy

Circular-dichroism (CD) studies were conducted to determine the extent of change in conformation of DNA upon complexation. CD spectral variations of CT-DNA were recorded by addition of the complex to CT-DNA. The CD spectrum of CT-DNA consists of a positive band at 277 nm due to base stacking and a negative band at 248 nm due to helicity, which is characteristic of DNA in the right-hand B form and is quite sensitive to the mode of DNA interactions with small molecules [24]. Thus simple groove binding and electrostatic interaction of small molecules show less or no perturbation on the base stacking and helicity bands, while intercalation enhances the intensities of both bands, stabilizing the right-hand B conformation of CT-DNA as observed for the classical intercalator methylene blue [42]. Fig. 5 shows the CD spectra of CT-DNA which was added to the complex. In these CD data the intensities of both negative and positive bands decreased significantly similar to that induced by the other Cu (II) complex [43]. This alteration suggests that the DNA binding of the complex induces certain conformational changes, such as the conversion from a more B-like to a more Z-like structure within the DNA molecule [44]. In addition, (**1**) binds DNA mainly by intercalation mode and indicates an unwinding of the DNA helix upon complex formation and loss of helicity [45].

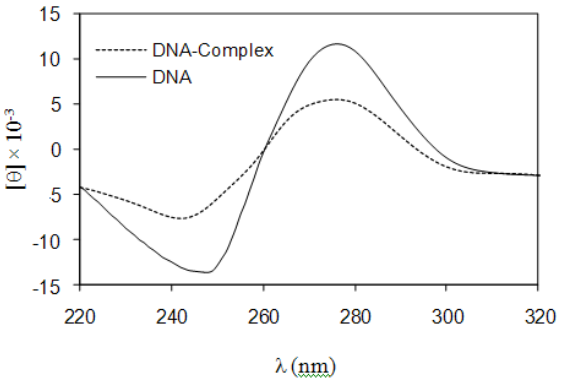


Fig. 5. Circular dichroism spectra of DNA (50 μM) in Tris–HCl (5 mM) in the presence of increasing amounts of copper complex (100 μM).

Thermal Denaturation Studies

Thermal denaturation studies of CT-DNA with the complex provide evidence for the ability of the complex to stabilize the double-stranded DNA [46]. It is well-accepted that when the temperature of the solution increases, the double-stranded DNA gradually dissociates to single strands, generating a hyperchromic effect in the absorption spectra of the DNA bases (λ_{max} = 260 nm). So the transition temperature of double strands to single strands can be determined by monitoring the absorbance of the DNA bases at 260 nm as a function of temperature [26]. According to the previous reports [47], intercalation normally facilitates base stacking in DNA and increases the melting temperature T_m of DNA.

The effect of (**1**) on the melting temperature (T_m) of CT-DNA in the buffer is shown in Fig. 6. In this experiment, the T_m of CT-DNA alone was 81.62 °C. After adding the complex, ΔT_m increased by 3.95°C for the mole ratio of [Cu]/[DNA] = 0.5. It has been reported that ΔT_m for metallointercalators (>10 °C) [48] and the organic intercalator EB (13 °C) [49] were higher than for this complex. These results indicated that the binding strength between the complex and DNA was only moderate [46]. The observed small change in the T_m of CT-DNA in the presence of (**1**) suggests that the dominating interaction of this complex with DNA is intercalative binding [50, 51].

DNA-Binding Study with Cyclic Voltammetry

The application of cyclic voltammetry (CV) to the study of the binding of metal complexes to DNA provides a useful complement to the above methods of investigation [12, 52].

Typical cyclic voltammetric (CV) behavior of 48 μM (**1**) in the absence and presence of CT-DNA is shown in Fig. 7. The cyclic voltammogram of (**1**) in the absence of DNA (Fig. 7A) featured reduction of 2+ to the 1+ form at a cathodic peak potential, E_{PC} of -0.57 V *versus* SCE.

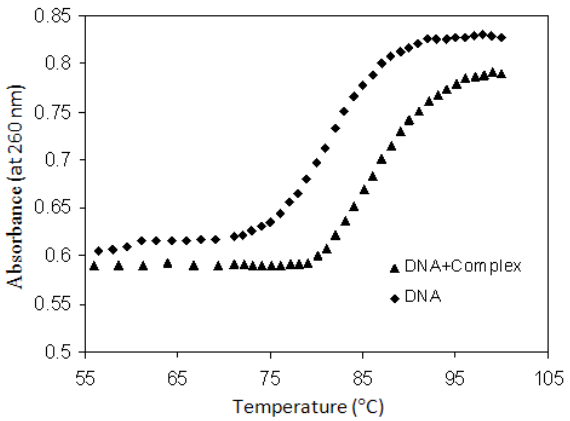


Fig. 6. Plots of the changes of absorbance at 260 nm of CT-DNA on heating in the absence and presence of the complex. C_{DNA} = 75 μM and $C_{complex}$ = 37.5 μM in 50 mM Tris–HCl with 50 mM NaCl.

Reoxidation of 1+ occurred upon scan reversal, at 0.18 V. The separation of the anodic and cathodic peak potentials, ΔE_P=255 mV, indicated an irreversible redox process. The formal potential $E^{0'}$(or voltammetric $E_{1/2}$), taken as the average of E_{Pc} and E_{Pa} is -195 mV, in the absence of DNA. The presence of DNA in the solution at the same concentration of (**1**) causes a decrease in the voltammetric current coupled with a slight shift in the E_{Pc} (-0.59 V), E_{Pa} (0.22 V) *versus* SCE and $E_{1/2}$ ($E_{1/2}$=-185 mV) to a less negative potential (Fig. 7B). The drop of the voltammetric currents in the presence of CT-DNA can be attributed to diffusion of the metal complex bound to the large, slowly diffusing DNA molecule.

Viscosity Study

Intercalation and groove binding modes can be distinguished by using hydrodynamic methods such as viscosity, flow dichroism measurements, and NMR [53, 54]. Intercalation of the molecules to the DNA resulted in unwinding and lengthening of the DNA. This DNA elongation causes the viscosity of the solution to increase.

The effect of (**1**) on the viscosity of DNA at 25.0±0.1 °C is shown in Fig. 8. It can be observed that the viscosity of DNA increases with increasing amounts of (**1**). Such behavior is in accordance with that of other intercalators, which increase the relative specific viscosity for the lengthening of the DNA double helix resulting from intercalation. This result indicates that (**1**) can intercalate between

adjacent DNA base pairs, causing an extension in the helix, and thus increases the viscosity of DNA.

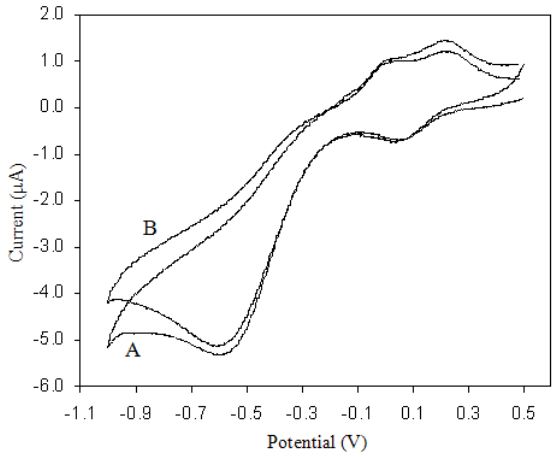


Fig. 7. Cyclic voltammograms of (**1**) in the absence (A) and presence (B) of DNA in 50 mM NaCl, 5 mM Tris, pH 7.3. [Cu]=122 μM, [DNA]/[Cu]=1, Scan rate, 50 mVs^{-1}.

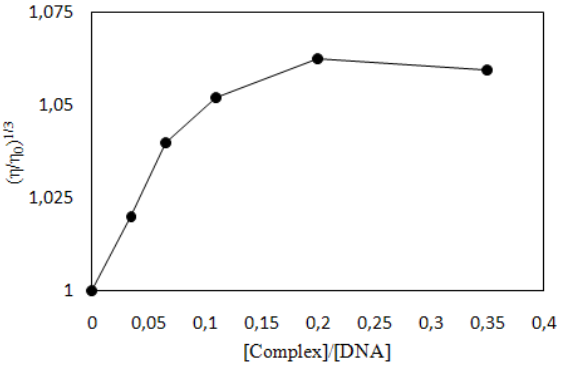


Fig. 8. Effect of increasing amount of (**1**) on the relative viscosity of CT-DNA at 25 ± 0.1 °C, [DNA]= 50 μM, pH 7.3.

CONCLUSIONS

It is clear that transition metal complexes offer a great potential as structure-selective binding agents for nucleic acids.

In this work the interaction between (**1**) and calf thymus DNA was investigated using UV, fluorescence and CD spectroscopy, cyclic voltammetry and viscosity measurements. The experimental results indicate that (**1**) could bind to DNA molecules mainly by intercalative mode, which correlates well with the extended aromatic ring system present in the ligand.

UV-Visible spectroscopy enabled us to find the Cu complex/DNA binding constant. The K_b of this complex is 9.51 (±0.2)×10^4 M^{-1}. The existence of hypochromicity, large red shift in the (**1**) spectra suggests an intercalation binding mode. Increase in DNA viscosity during its interaction with (**1**) is consistent with lengthening of DNA due to intercalation. Today many researchers focus their attention on the bioactivity of Cu(II) complexes. So further studies on anticancer activities of the complex are in progress, because non-covalent binding is reversible and is typically preferred over covalent adduct formation, keeping in mind the drug metabolism and toxic side effects.

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ИЗСЛЕДВАНЕ НА ВЗАИМОДЕЙСТВИЕТО НА ДНК С НОВ Cu(II) КОМПЛЕКС ЧРЕЗ СПЕКТРОСКОПСКИ, СПЕКТРОФЛУОМЕТРИЧНИ, ВОЛТАМПЕРОМЕТРИЧНИ МЕТОДИ И С КРЪГОВ ДИХРОИЗЪМ

М. Бордбар[1,*], Ф. Ходайе[2], М. Табатабаее[2], А. И. Фаал[3], З. Мехрилихван[4], S. Мохамад-Ганджи[5]

[1]Дерпартамент по химия, Научен факултет, Университет в Кум, Кум, Иран
[2] Департамент по химия, Клон Язд, Ислямски университет „Азад“, Язд, Иран
[3]Департамент по химия, Университет Паяме Нур, П.К. 37185-311, Иран
[4]Клуб на младия изследовател, Клон Северен Техеран, Ислямски университет „Азад“, Техеран, Иран
[5]Национален институт за генно инженерство и биотехнология (NIGEB), П.К. 14965-161, Техеран, Иран.

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(Резюме)

Изследвана е свързващата активност на водо-разтворими мед(II) комплекс с пиридин-2,6-диарбоацетат ($pydc^{2-}$) и 2-аминопиримидин (amp) с формула $[Cu(pydc)(amp)].2H_2O].H_2O$ (**1**) с DNA (CT-DNA) от телешки тимус. Свързващата активност на **1** е изследвана чрез ефекта й върху електронните абсорбционни спектри, ефекта на термична денатурация, флуресцентното закаляване (използвайки метиленово синьо като флуоресцентен маркер) и спектри на кръгов дихроизъм. Всички резултати внушават взаимодействие между (**1**) и ДНК чрез интеркалация с константа на свързване $(9.51\pm0.2)\times10^4$ M^{-1}.