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Multispectroscopic DNA-binding studies of a tris-chelate nickel(II) complex containing 4,7-diphenyl 1,10-phenanthroline ligands

Nahid Shahabadi *, Azadeh Fatahi

Department of Chemistry, Faculty of Science, Razi University, Kermanshah, Iran

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ABSTRACT

A tris-chelate nickel(II) complex, $[Ni(DIP)_3](NO_3)_2 \cdot 3H_2O$ in which DIP = 4,7-diphenyl 1,10 phenanthroline, was synthesized and characterized by spectroscopic (^1H NMR and UV–vis) and elemental analysis techniques. Binding interaction of this complex with calf thymus-DNA (CT-DNA) was investigated using emission, absorption, circular dichroism (CD), viscosity and DNA thermal denaturation studies. In absorption spectrum of the complex, as the concentration of DNA increased, appearance of an isobestic point proved the new $[Ni(DIP)_3]^{2+}$–DNA complex formation. The intrinsic binding constant ($K_b = 4.34 \times 10^4\ M^{-1}$) is comparable to groove binding complexes. In fluorimetric studies, the dynamic enhancement constants (k_D) and bimolecular enhancement constant (k_B) were calculated and showed that the fluorescence enhancement was initiated by a static process in the ground state. Furthermore, the thermodynamic studies showed that the reaction is exothermic and enthalpy favored ($\Delta H = -58.41$ kJ/mol). A strong CD spectrum in the UV and visible region develops upon addition of CT-DNA into the racemate solution of Ni(II) complex (Pfeiffer effect). This has revealed that a shift in diastereomeric inversion equilibrium takes place in the solution to yield an excess of one of the DNA complex diastereomers. Finally, all of the experimental results prove that the minor groove binding must be predominant.

1. Introduction

Understanding the binding of small molecules to DNA is potentially useful in developing principles to guide the synthesis of new improved drugs which can recognize a specific site or conformation of DNA [1–3]. Generally, metal complexes upon binding to DNA are stabilized through a series of weak interactions such as the π-stacking interactions of aromatic heterocyclic groups between the base pairs (intercalation), hydrogen bonding and van der Waals interactions of functional groups bound along the groove of the DNA helix [2]. It is believed that the non-covalent interactions of these types take place in at least three distinct modes, i.e. binding along the outside of the helix, binding along the major and/or minor grooves, and intercalation of a planar molecule or a planar aromatic ring system between base pairs [3]. Identifying which of the above interaction modes is operative for the molecule concerned, as well as which is predominant among the series of the weak interactions, is essential in designing an effective new drug and a DNA-probe molecule.

Metal complexes of the type $[M(LL)_3]^{+n}$, where LL is either 1,10-phenanthroline (phen) or modified phen, are particularly attractive because they can effectively bind to DNA in different modes of interaction [4–7]. Additionally, tris-phenanthroline metal complexes and their analogs have attracted much attention for the chiral recognition of DNA double helices with the enantiomeric complexes and for the photochemical electron transfer reaction initiated by the complex bound to DNA [8–10]. Since, DNA is an optically active molecule, the specific binding sites may prefer one of the enantiomeric forms of the metal complexes [11,12]. For that reason, metal complexes with phenanthroline and related ligands, in particular ruthenium(II) complexes have been studied extensively as structural probes [13–18]. A singular advantage in using tris-chelate metal complexes is that the central metal or the ligands in them may be varied in an easily controlled manner to facilitate a certain application, which provides an easy access for the detailed study of DNA-binding mechanism [19]. Moreover, the types of different metal ions and their flexible valances, which usually are responsible for the coordination geometry of complexes, have significant influences on the intercalating ability of transition metal complexes to DNA [20]. In addition, modification of the ligand may lead to subtle or substantial changes in the binding modes, location and affinities of the complexes to DNA. It has been found that complexes of phenanthroline lacking substitutions can be mutagenic (by DNA intercalation) while substituted in some positions are not [21].

For these reasons, we became interested in synthesis of a Ni(II) tris-chelate complex with 4,7-diphenyl-1,10-phenanthroline (DIP)

* Corresponding author. Tel./fax: +98 831 8360795.
E-mail address: nahidshahabadi@yahoo.com (N. Shahabadi).

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ligand (Fig. 1) as a means of better understand the effect of ligand substituents and the central metal on the behavior of this class of compounds with DNA.

2. Experimental

2.1. Materials

Commercially pure chemicals such as 4,7-diphenyl-1,10-phenanthroline (Merck), Tris–HCl (Sigma Co., Madrid, Spain), were purchased and used without purification. All experiments were carried out in Tris–HCl buffer solutions that prepared in double distilled water (10 mM, pH 7.2). Highly polymerized calf thymus-DNA (CT-DNA) was purchased from Sigma Co. All DNA samples were dissolved in 50 mM NaCl/5 mM Tris–HCl buffer, pH 7.2. Solutions of CT-DNA gave an UV absorbance ratio (260 over 280 nm) of more than 1.8, indicating that the DNA was sufficiently free of protein [22]. The DNA concentration (monomer units) of the stock solution (1×10^{-2} M per nucleotide) was determined by UV spectrophotometry in properly diluted samples using a molar absorption coefficient of 6600 $M^{-1}\,cm^{-1}$ at 258 nm [23]. The stock solutions were stored at 4 °C and used within 4 days. Stock solutions of $[Ni(DIP)_3]^{2+}$ complex were prepared by dissolving the complex in the 50 mM NaCl/5 mM Tris–HCl buffer to a final concentration of 0.5 mg ml^{-1} and after mixing with DNA, incubated at 37 °C for 24 h.

2.2. Synthesis of rac-[Ni(4,7-diphenyl-1,10-phenanthroline)$_3$](NO$_3$)$_2$·3H$_2$O

The [Ni(4,7-diphenyl-1,10-phenanthroline)$_3$](NO$_3$)$_2$ complex ($[Ni(DIP)_3]^{2+}$) (Fig. 1) was prepared by modification of the method which used ethylene diamine as ligand [24]. $Ni(NO_3)_2{\cdot}6H_2O$ (0.356 g) and 4,7-diphenyl-1,10-phenanthroline (1.495 g) were dissolved in 50 ml 1-butanol and refluxed for 5 h until the blue precipitate was formed. The solution was filtered and the precipitate was washed with 1-butanol and diethyl ether. The product appeared as blue powders with a high yield (92–95%) and had high solubility in water (0.1% DMSO). Anal. Calc. for $C_{72}H_{54}N_8O_9Ni$: C, 70.08; H, 4.41; N, 9.08. Found: C, 70.3; H, 5.0; N, 9.3. ^{1}H NMR: δ = 8.75 (d, 2H atoms of phen ligand), δ = 7.36 (d, 2H atoms of phen ligand), δ = 7.38 (d, 2H atoms of phen ligand), δ = 7.12 (s, 10H atoms of phenyl groups).

In the ^{1}H NMR spectrum of $[Ni(DIP)_3]^{2+}$, the signals due to the various protons of 4,7-diphenyl-1,10-phenanthroline ligand shifted to the high field (≈−0.2 ppm) with corresponding free ligand and confirmed complexation.

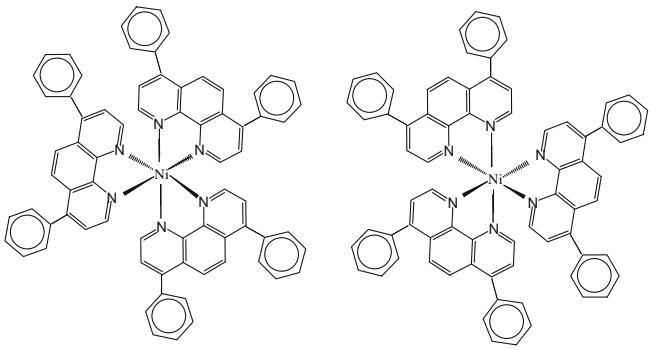

Fig. 1. The chemical structure of Ni(II) enantiomers used for enantioselective DNA binding study.

2.3. Instrumentation

^{1}H NMR spectra were recorded with using a Bruker Avance DPX200 MHZ (4.7 T) spectrometer using d_2-D_2O as solvent. The elemental analysis was performed using Heraeus CHN elemental analyzer. Absorbance spectra were recorded using hp spectrophotometer (agilent 8453) equipped with a thermo stated bath (Huber polysat cc1). Absorption titration experiments were carried out by keeping the concentration of $[Ni(DIP)_3]^{2+}$ complex constant (5.0×10^{-5} M) while varying the DNA concentration (R = [DNA]/[NiL$_3$] = 0.0 − 1). Absorbance values were recorded after each successive addition of DNA solution and equilibration (Ca. 40 min). To determine the intrinsic binding constant (K_b) and the stoichiometry of the $[Ni(DIP)_3]^{2+}$–DNA system, the quantity $(a^* - \varepsilon_f)(\varepsilon_b - \varepsilon_f)$ at 318 nm has been plotted as a function of the molar concentration of DNA, in monomeric units ($DNA_{phosphate}$). ε_b, ε_f and a^* are, respectively, the molar extinction coefficients of free $[Ni(DIP)_3]^{2+}$ complex, of $[Ni(DIP)_3]^{2+}$ complex bound to DNA and of the solution containing both free and bound $[Ni(DIP)_3]^{2+}$ complex. In particular, ε_f was determined by calibration curve of the isolated metal complex in aqueous solution, following the Beer–Lambert law. ε_b was determined from the plateau of the DNA titration, where addition of DNA did not result in further changes in the absorption spectrum. Finally, a^* was determined as the ratio between the measured absorbance and $[Ni(DIP)_3]^{2+}$ analytical molar concentration. Using Eq. (1) [25,26] was obtained the values of the intrinsic binding constant (K_b) and of the binding size in base pair(s) of the $[Ni(DIP)_3]^{2+}$–DNA complex.

$$\frac{a^* - \varepsilon_f}{\varepsilon_b - \varepsilon_f} = \frac{b - (b^2 \frac{2K_b^2 C_t[DNA]}{s})^{1/2}}{2K_b C_t} \qquad b = 1 + K_b C_t + \frac{K_b[DNA]}{2s} \tag{1}$$

where C_t is the total concentration of the metal complex. The K_b and s values, obtained by nonlinear fitting of the experimental data by Eq. (1), are $K_b = (4.34 \pm 0.02) \times 10^4\ M^{-1}$ and $s = 0.43 \pm 0.01$.

Thermal denaturation experiments were carried out by monitoring the absorption of CT-DNA at λ = 260 nm at various temperatures, both in the absence and presence of different amounts of the complex ($1/R = [Ni(DIP)_3]^{2+}/[DNA]$ = 0.0, 0.25, 0.5, 1.0) ($[DNA] = 5.0 \times 10^{-5}$ M). The melting temperature (T_m, the temperature at which 50% of double-stranded DNA becomes single-stranded) was calculated as reported [27,28].

Circular dichroism (CD) spectra of the racemic $[Ni(DIP)_3]^{2+}$ complex in the absence and presence of CT-DNA at various $[Ni(DIP)_3]^{2+}$/[DNA] ratios ($1/R$ = 0–0.2) were recorded on a JASCO (J-810) spectropolarimeter operating at room temperature (ca. 25 °C). The region of wavelength between 200 and 700 nm was scanned for each sample using 1 cm path quartz cell.

Viscosity measurements were made using a viscosimeter (SCHOT AVS 450) which thermo stated at 25.0 ± 0.5 °C by a constant temperature bath. The DNA concentration was fixed at 5.0×10^{-5} M and flow time was measured with a digital stop watch, the mean values of three replicated measurement were used to evaluate viscosity η of the samples. The value of relative specific viscosity $(\eta/\eta_o)^{1/3}$ where η_o and η are the specific viscosity contributions of DNA in the absence (η_o) and in the presence of the complex (η), were plotted against $1/R$ ($R = [DNA]/[Ni(DIP)_3]^{2+}$ [29].

Fluorescence measurements were carried out with a JASCO spectrofluorimeter (FP6200) using a fixed complex concentration to which increments of the DNA stock solution were added.

3. Results and discussion

3.1. Absorption spectroscopy

The electronic absorption spectroscopy is one of the most useful techniques in DNA-binding studies [13]. The band at 260 nm of DNA arises due to the π–π^* transitions of DNA bases. Stacking pattern changes, disruption of the hydrogen bonds between complementary strands, covalent binding of DNA bases and intercalative mode involving a strong stacking interaction between aromatic rings of molecules and the base pairs of DNA lead to the hypochromism, red and/or blue shifts of this band [30,31]. The absorption spectra of the $[Ni(DIP)_3]^{2+}$ complex in the absence and in the presence of increasing amounts of CT-DNA are shown in Fig. 2. The electronic spectrum of this $[Ni(DIP)_3]^{2+}$ complex in water (0.1% DMSO) is characterized by an intense ligand-centered transition in the UV region at 284 nm and a metal-to-ligand charge transfer (MLCT) in the visible region at 318 nm. The visible band around 318 nm attributed to the overlap of $Ni(d\pi) \rightarrow DIP\ (\pi^*)$ and the ultraviolet band around 284 nm can be attributed (DIP) $\pi \rightarrow \pi^*$. The UV–vis absorption of the $[Ni(DIP)_3]^{2+}$ complex is significantly perturbed by the addition of increasing amounts of CT-DNA. In details, the absorption band of the complex at λ = 318 nm shows a red shift about 3 nm and hypochromism (Fig. S1). Moreover, the intensity of the absorption band at λ = 284 nm is lowered by the addition of DNA in the range $0 \leqslant [DNA]/[complex] \leqslant 0.2$ while it increases at higher molar ratios. The DNA-binding constant of metal complexes containing dinitrogen ligands are usually determined by the MLCT band because no light absorption for DNA is found in the range of wavelength over 300 nm and thus no interference exists. The extent of hypochromism in the visible MLCT band is commonly consistent with the strength of intercalative interaction [13]. The extent of hypochromism of the spectra at λ = 318 nm was measured by Eq. (2) ($H\%$ = 15%).

$$H\% = \frac{A_{Free} - A_{Bounded}}{A_{Free}} \times 100 \quad (2)$$

Similar spectral characteristics, i.e. bathochromic shift and hypochromism in the presence of DNA, observed for ruthenium(II) complexes, have been attributed to a mode of binding which involves a stacking interaction of aromatic chromophores with the base pairs of DNA [22].

The intrinsic binding constant, K_b, of the $[Ni(DIP)_3]^{2+}$ complex was determined to be $(4.34 \pm 0.02) \times 10^4\ M^{-1}$ using Eq. (1). This value is comparable to those reported by Barton and coworkers [12] for related ruthenium complex ($[Ru(phen)_2(DIP)]^{2+}$–DNA; $(1.15 \times 10^4\ M^{-1})$. Furthermore, the K_b value also is rather similar to the binding constants of the delta and lambda isomers of $[Ru(o\text{-phen})_3]^{2+}$ with binding constants of $4.9 \times 10^4\ M^{-1}$ and $2.8 \times 10^4\ M^{-1}$, respectively, which were assigned as non-intercalators [32].

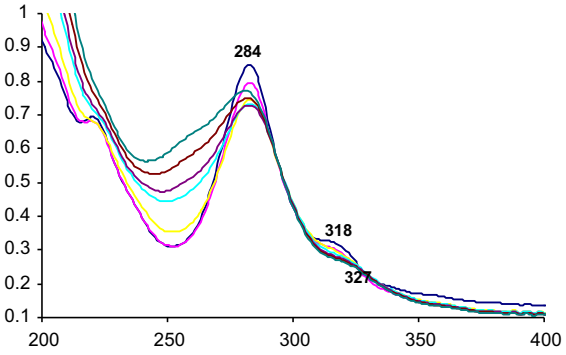

Fig. 2. Absorption spectra of $[Ni(DIP)_3]^{2+}$ complex in Tris–HCl buffer upon addition of CT-DNA. [Ni] = 2×10^{-5} M, [DNA] = $(0 - 1) \times 10^{-5}$ M.

3.2. Fluorescence studies

As the $[Ni(DIP)_3]^{2+}$ complex is luminescent in the absence of DNA, it does show appreciable increase in emission upon addition of CT-DNA. (Fig. 3). This figure shows that a regular increase in the fluorescence intensity of the $[Ni(DIP)_3]^{2+}$ complex with a shift of fluorescence emission maximum (414–416 nm) took place upon increasing the concentration of DNA at 25.0 °C and at pH 7.2. These fluorescence enhancements indicate that the $[Ni(DIP)_3]^{2+}$ complex interacted with DNA and the quantum efficiency of $[Ni(DIP)_3]^{2+}$ was increased. Like to quenching process, the enhancement constant can be obtained by Eq. (3) [33]:

$$\frac{F_0}{F} = 1 - K_E[E] \quad (3)$$

If a dynamic process is part of the enhancing mechanism, the above equation can be written as follows [33]:

$$\frac{F_0}{F} = 1 - k_D[E] = 1 - K_B\tau_0 \quad (4)$$

where k_D is the dynamic enhancement constant (like to a dynamic quenching constant), k_B is the bimolecular enhancement constant (like to a bimolecular quenching constant) and τ_0 is the lifetime of the fluorophore in the absence of the enhancer. The dynamic enhancement constants of $[Ni(DIP)_3]^{2+}$ complex at different temperatures were calculated using Eq. (4) (Fig. 4, Table 1). Since fluorescence lifetimes are typically near 10^{-8} s, the bimolecular enhancement constant (k_B) was calculated from $k_D = k_B\tau_0$ (Table 1). By considering the equivalency of the bimolecular quenching and enhancement constants, it can be seen that the latter is greater than the largest possible value ($1.0 \times 10^{10}\ M^{-1}\ s^{-1}$) in aqueous medium. Thus, the fluorescence enhancement is not initiated by a dynamic process, it is suggested that a static process involves complex formation in the ground state [33].

3.2.1. Equilibrium binding titration

The binding constant (K_f) and the binding stoichiometry (n) for the complex formation between $[Ni(DIP)_3]^{2+}$ complex and DNA were measured using the Eq. (5) [22,34].

$$\log\frac{(F - F_0)}{F} = \log K_f + n\log[DNA] \quad (5)$$

here F_0 and F are the fluorescence intensities of the fluorophore in the absence and in the presence of different concentrations of

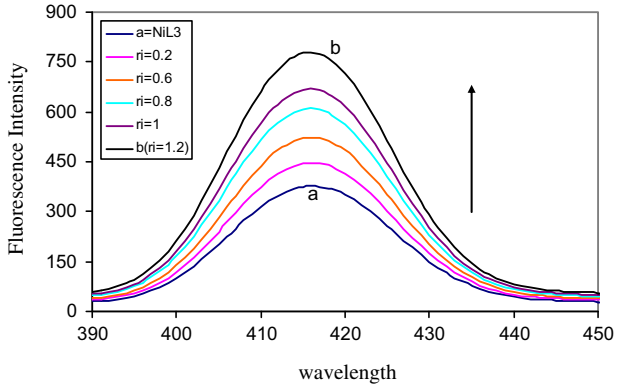

Fig. 3. Fluorescence spectra of the $[Ni(DIP)_3]^{2+}$ complex in the absence and presence of the increasing amounts of DNA (solid lines) in aqueous solution at 25 °C. R = [DNA]/$[Ni(DIP)_3]^{2+}$ = 0.0, 0.2, 0.6, 0.8, 1, 1.2.

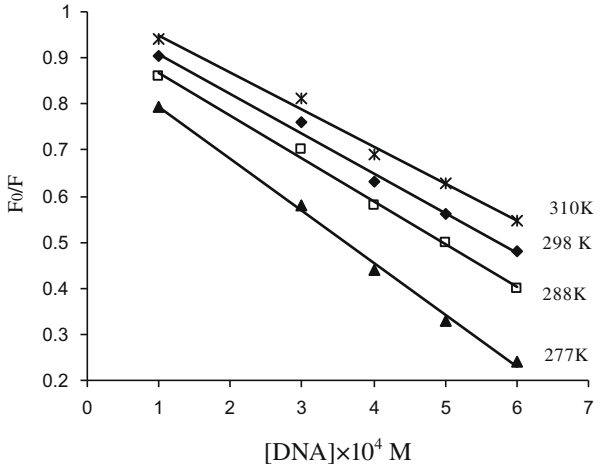

Fig. 4. Stern–Volmer plot for the observed fluorescence enhancement of $[Ni(DIP)_3]^{2+}$ complex upon addition of CT-DNA at different temperatures.

Table 1
Dynamic enhancement and bimolecular enhancement constants of $[Ni(DIP)_3]^{2+}$ complex at different temperatures.

Temperature (K)	Linear equation	k_D	k_B
277	$Y = 0.999 - 0.115X$	1150	1.15×10^{11}
288	$Y = 0.99 - 0.094X$	940	9.4×10^{10}
298	$Y = 0.988 - 0.0866X$	866	8.6×10^{10}
310	$Y = 1 - 0.0798X$	798	7.9×10^{10}

DNA, respectively. The linear equations of log $(F - F_0)/F$ vs. log [DNA] at different temperature are shown in Table 2 (Fig. S2). The values of K_f clearly underscore the remarkably high affinity of $[Ni(DIP)_3]^{2+}$ to DNA.

3.2.2. Thermodynamic parameters of DNA binding

To have a better understanding of thermodynamics of the reaction between $[Ni(DIP)_3]^{2+}$ complex and DNA, it is useful to determine the contributions of enthalpy and entropy of the reaction. Therefore, the evaluation of formation constant for the $[Ni(DIP)_3]^{2+}$–DNA complex at four different temperatures (277, 288, 298, and 310 K) allows determining the thermodynamic parameters such as enthalpy (ΔH) and entropy (ΔS) of $[Ni(DIP)_3]^{2+}$–DNA formation by van't Hoff equation and plotting log K_f vs. $1/T$. The positive slope of the plot ($-\Delta H/R$, R is the gas constant) indicates that the reaction of DNA with $[Ni(DIP)_3]^{2+}$ complex is exothermic and enthalpy favored. The ΔH and ΔS values of the $[Ni(DIP)_3]^{2+}$–DNA complex were -58.41 ± 0.6 kJ/mol and -122.75 ± 2 J/mol K, respectively. In general, electrostatic interactions exhibit small enthalpy and positive entropy changes. Hydrophobic interactions are generally indicated by positive enthalpy and entropy changes. Hydrogen bonding and van der Waals interactions are usually characterized by negative standard enthalpies of interaction [34,35]. From the thermodynamic data, it is quite clear that while complex formation is enthalpy favored, it is also entropy disfavored. Formation of the complex therefore results in a more ordered state, possibly due to the freezing (fixing) of the motional freedom of both the NiL_3 and DNA molecules. These thermodynamic data are lower than those of previously reported for an intercalator [36] and confirmed the non-intercalating interaction mode.

Table 2
Linear equations of log $(F - F_0)/F$ vs. log [DNA], and K_f of $[Ni(DIP)_3]^{2+}$ complex with DNA at different temperatures.

Temperature (K)	Linear equation	K_f
277	$Y = 0.71X + 4.61$	4.1×10^4
288	$Y = 0.69X + 4.24$	1.74×10^4
298	$Y = 0.68X + 3.87$	7.4×10^3
310	$Y = 0.67X + 3.43$	2.7×10^3

The analogous $[Ru(phen)_3]^{2+}$ [35,37] which is suggested to bind electrostatically to DNA, has been reported to exhibit a positive enthalpy change, but Mudasir et al. [38] found that the mode of binding of CT-DNA with $[Fe(phen)(DIP)_2]^{2+}$ ($\Delta H = -33.1$ kJ mol^{-1}) was intercalation. Furthermore, Chaires studies of the thermodynamic parameters of drug–DNA interactions revealed that for all the intercalators except actinomycin, the binding enthalpy changes are large and negative [1].

When cationic binding agent such as $[Ni(DIP)_3]^{2+}$ binds to DNA, it is likely replace a countercation from the compact inner (stern) layer or the defuse outer layer surrounding DNA. This counter ion release process is believed to be nearly entirely entropic [39] and the signs of ΔH and ΔS obtained for DNA binding of complex should be positive. Since $[Ni(DIP)_3]^{2+}$ is also a cationic binding agent and its structure is similar to other complexes which were previously studied, [38] it is expected that counter ion release, hydration and hydrophobic interaction, should occur in this complex. Evidently, the overall negative enthalpy and entropy changes are obtained for DNA binding of this complex. In addition to counter ion release there should be another type of molecular interaction process in the DNA binding of $[Ni(DIP)_3]^{2+}$ complex from which large negative enthalpy and entropy changes are produced. Intercalation of the phenyl group and partial phenanthroline ring of one DIP ligand in $[Ni(DIP)_3]^{2+}$ complex which involves π-stacking interaction between these planar substituents and DNA base pairs seems to be consistent with the enthalpy and entropy changes. Intercalation, in which two phenyl rings of the ligand are inserted into the DNA base pairs, may be less favorable, because the sheer size of the DIP ligand would preclude simultaneous intercalation of both phenyl rings as the diameter of the ligand is much larger than the width of base pairs. In addition, the crystal structure data of solid $[Ru(DIP)_3]^{2+}$ [17] and $[Cu(bcp)_2]$ BF4 (bcp = 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline) [40] complexes show that all the phenyl groups are skewed about 40° out of phenanthroline plane owing to steric effect between hydrogen atoms in the phenyl and phenanthroline rings. Intercalation of the entire DIP moiety thus would require that the phenyl groups rotate into the plane of the phenanthroline moiety in order to minimize stacking. This may produce unfavorable intermolecular interaction between the complex and DNA. In order to explain such steric hindrance, a model of partial intercalation of the phenanthroline ligand with only one phenyl substituent and partial phen ring inserted between the base pairs in the major groove (an asymmetric docking) has been proposed [41]. In this model, the other phenyl group and non-intercalating DIP ligands are aligned along the major groove and involved in hydrophobic non-stacking interactions with the base pairs along the helical major groove. This model does not require any rotation of the phenyl groups and takes into account the participation of the non-intercalated phenyl group and the other DIP ligands in supporting the binding. Furthermore, even when the model is not operating, intercalative binding of this complex could still be possible if the binding energy is large enough to overcome the barrier against rotation of the phenyl group into the plane.

3.3. Thermal denaturation experiments

The T_m experiments were carried out for DNA in the absence and presence of different amounts of $[Ni(DIP)_3]^{2+}$ complex. The melting plot of DNA (5.0×10^{-5} M) monitored by plotting the UV

maximum absorption of DNA at 260 nm vs. the temperature, in the absence and presence of $[Ni(DIP)_3]^{2+}$ complex and at molar ratios $1/R = [NiL_3]/[DNA] = 0.0, 0.25, 0.5, 1.0$. The T_m for DNA in Tris–HCl buffer and in the absence of any added of $[Ni(DIP)_3]^{2+}$ complex was obtained 70 ± 0.5 °C. An increase in the DNA melting temperature by 2, 5 and 7 °C for the above mentioned molar ratios was observed. In comparison with other studies [41,42], our finding results suggest that the $[Ni(DIP)_3]^{2+}$ complex interacted with DNA via groove binding.

3.4. Viscosity measurements

The hydrodynamic measurements are sensitive to length change of DNA in absence and presence of foreign molecules. A classical intercalation model demands that the DNA helix must lengthen as base pairs are separated to accommodate the binding ligand, leading to increase in DNA viscosity [1]. In contrast, a partial and/or non-classical intercalation ligand could bend (or kink) the DNA helix, reduce its effective length and concomitantly its viscosity, while ligands that bind exclusively in the DNA grooves (e.g., netropsin, distamycin), under the same conditions, typically cause less pronounced changes (positive or negative) or no changes in DNA solution viscosity [39]. The values of relative specific viscosity $(\eta/\eta_0)^{1/3}$ – here η_0 and η are the specific viscosity contributions of DNA in the absence and in the presence of the present complex were plotted against $1/R$ ($R = [DNA]/[[Ni(DIP)_3]^{2+}]$) (Fig. 5).

The results showed that the relative viscosity increases in relative low ratio, $[Ni(DIP)_3]^{2+}/[DNA] = 0–0.2$, while it decreases in high ratios. The increase at the beginning is the same as intercalators [43]. This equilibrium shift suggests $[Ni(DIP)_3]^{2+}$ complex bind to CT-DNA by intercalation in very low ratios, whereas at higher concentration it binds partial and or in a non-classical intercalation mode which can bend (or kink) the DNA helix reducing its effective length and concomitantly its viscosity. This non-linearity is probably due to the phenyl groups so that the streric affect play a prominent role in the DNA-binding process. It is also noteworthy that the racemic mixture of $[Ni(DIP)_3]^{2+}$ complex is relatively labile towards racemization and upon binding to DNA, diastereomeric shift occurs in the solution, resulting in an enrichment of one enantiomer which is less or more favorable to the DNA binding [38,44].

3.5. Determination of enantioselectivity in DNA binding

The labile metal complexes toward racemization, e.g. iron(II), nickel(II) and cobalt(II) complexes of phen and its derivatives have been well known to undergo enantiomeric equilibrium shift in the solution upon binding to optically active biopolymers such as DNA, RNA or proteins, which is called Pffeifer effect [35,45–47]. This equilibrium shift suggests that one of the enantiomers of the metal complexes is preferentially bound to the optically active biopolymers to afford their enantioselective binding. The shift in enantiomeric equilibrium due to the enantioselective binding of metal complexes to optically active biopolymers gives rise to the drastic CD spectral changes in the UV–vis region because of the enrichment of one enantiomeric conformation in the solution. This phenomenon can be readily monitored in the laboratory by CD spectroscopy. The enantioselective binding of optically active compounds, e.g. optically active drugs to DNA, RNA or protein has also been noticed as one of the reasons why one of the enantiomers of optically active drugs exhibits a biological activity significantly different from the other, although both have chemically very similar structures [48]. In order to understand such effects better at the level of molecular interactions, it is of vital importance to examine enantiomeric effects on the DNA binding of metal complexes. Here, we studied the CD spectroscopic behavior of racemic $[Ni(DIP)_3]^{2+}$ complex upon binding to CT-DNA to examine whether such selectivity also happens to this complex. The UV and visible CD spectra of racemic $[Ni(DIP)_3]^{2+}$ complex upon binding to CT-DNA and free CT-DNA are shown in Fig. 6.

It is clearly seen that a few strong CD signals develop in the UV and visible region of wavelength upon addition of CT-DNA to the racemic solution of $[Ni(DIP)_3]^{2+}$ complex. The characteristic bands of CT-DNA due to base stacking (275 nm) and that due to right handed helicity (248 nm) disappear and a biphasic CD signal with positive (261 nm) and negative (296 nm) with a cross over at 280 nm are observed. A positive signal (525 nm) is also observed and the intensities of all these signals increase with increasing in value of $[Ni(DIP)_3]^{2+}$ concentration. These observations suggest that diastereomeric shift occurs in the solution due to the enantioselective binding of $[Ni(DIP)_3]^{2+}$ complex to CT-DNA. Indeed, no CD signals are observed in the UV and visible region for the racemate solution of $[Ni(DIP)_3]^{2+}$ complex. Furthermore, the development of the CD spectra suggests that an enrichment of one of the complex-DNA diastereomers occurs in the solution, probably owing to a shift in the diastereomeric equilibria. Interestingly, the developed CD spectrum has an exactly mirror-shape (opposite sign) with respect to the Λ-enantiomers which were studied previously [38,49,50]. Therefore, it is immediately confirmed that the Δ-enantiomer of $[Ni(DIP)_3]^{2+}$ complex is preferentially bound to CT-DNA rather than Λ-enantiomer. Similar observation has been reported previously for the DNA binding of racemic iron(II) complexes of phen and bpy (bpy = 2,2'-bipyridine) [45,47,51], rac-$[Fe(phen)(DIP)_2]^{2+}$ complex [38] $[Co(phen)_2(dppz)]^{2+}$, [46] $[Fe(4,7\text{-}dmp)_3]^{2+}$ [52] and Δ-$[Ru(bpy)_2(pyip)](PF6)_2$ [53]. This result may be due to different matching between the enantiomers and DNA or to the different binding sites of the enantiomers, as DNA is a flexible

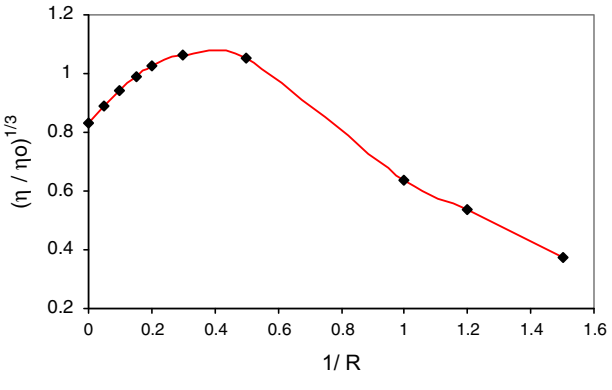

Fig. 5. Effects of increasing amounts of $[Ni(DIP)_3]^{2+}$ complex on the viscosity of CT-DNA (5.0×10^{-5} M) in 10 mM Tris–HCl. ($1/R = 0.0–1.5$).

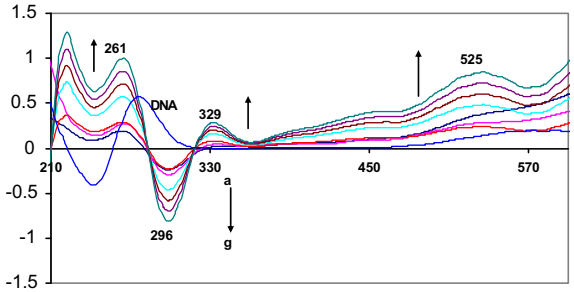

Fig. 6. Circular dichroism (CD) spectra of free CT-DNA (blue line) of 24.5 mM in UV and visible region at various $1/R$ ([Ni]/[DNA]) in Tris–HCl buffer (pH 7.2) and 50 ml NaCl at 25 °C. Each spectrum was recorded at about 40 min after each incremental addition of CT-DNA. The arrows for solid lines indicate the directions of CD spectral changes as a function of r_i. (For interpretation of color mentioned in this figure the reader is referred to the web version of the article.)

double helix, and the $[Ni(DIP)_3]^{2+}$ complex can interact with the DNA helix axis from any direction.

4. Conclusions

In summary, we have synthesized a tris-chelate nickel(II) complex, $[Ni(DIP)_3]^{2+}$, which exhibits high binding affinity to CT-DNA. Different instrumental methods were used to finding the interaction mechanism. The following results supported the fact that the $[Ni(DIP)_3]^{2+}$ complex can bound to CT-DNA by the mode of groove binding.

1. In absorption spectrum of the complex, as the concentration of DNA increased, appearance of an isobestic point proved the new $[Ni(DIP)_3]^{2+}$–DNA complex formation.
2. The intrinsic binding constant ($K_b = 4.34 \times 10^4\ M^{-1}$) is comparable to groove binding complexes.
3. Fluorescence studies showed appreciable increase in $[Ni(DIP)_3]^{2+}$ emission upon addition of DNA with a shift of fluorescence emission maximum (414–416 nm).
4. The positive slope in Van't Hoff plot indicated that the reaction of the Ni(II) complex and DNA was enthalpy favored ($\Delta H = -58.41$ kJ/mol).
5. Circular dichroism results suggest that diastereomeric shift occurs in the solution due to the enantioselective binding of $[Ni(DIP)_3]^{2+}$ complex to CT-DNA.
6. In comparison with other studies, an increase of DNA melting temperature by 7 °C for $r_i = 1$ demonstrated the groove binding mode.
7. Decrease of the relative viscosity of CT-DNA in the presence of the $[Ni(DIP)_3]^{2+}$ complex ($r_i > 0.5$) showed that the minor groove binding must be predominant.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2010.02.048.

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