

Synthesis, Characterization, and Binding of $[Cr(naphen)(H_2O)_2]^+$ with DNA: Experimental and Modeling Study

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(Received October 4, 2001)

A chromium(III) complex of naphen, where naphen denotes 1,2-bis(naphthylideneamino)ethane having the basic salen structure, with an extended aromatic system, has been synthesized and characterized using UV–visible, infrared and ESI mass spectra. Absorption titration and thermal denaturation studies revealed that $[Cr(naphen)(H_2O)_2]^+$ binds to DNA with moderate strength. Relative viscosity measurements clearly indicate that it binds to DNA by an intercalative mode. This observation is in contrast to an observation with the parent complex, $[Cr(salen)(H_2O)_2]^+$, which prefers groove binding. Intercalation of the complex results in a change in the helicity of CT DNA, which is reflected in the CD spectrum of DNA in the presence of $[Cr(naphen)(H_2O)_2]^+$. The binding constant of the complex to CT DNA has been estimated to be $(0.51 \pm 0.07)\times10^4\ M^{-1}$ (1 M = mol dm^{-3}), which is lower than the values reported for classical intercalators. Modeling studies with the dodecamer $d(GCGCAATTGCGC)_2$ show that as a result of the intercalation of $[Cr(naphen)(H_2O)_2]^+$ the vertical separation of the bases increases by 2.67 Å in one strand and by 1.89 Å in the other.

Studies pertaining to the interaction of transition metal ions with nucleic acid are an important area of research. One of the main reasons for sustained interest in this area is because the unique spectroscopic signature of transition-metal ions can be exploited for developing efficient probes for nucleic acid structure and conformation.[1] A large number of octahedral complexes of first-row transition-metal ions have been found to bind to the minor or major groove of DNA.[2] A few metal complexes have also been found to coordinate to heterocyclic nitrogen of the DNA bases.[3] On the other hand, there are only a few reports on the intercalative mode of binding of the octahedral metal ion complexes to DNA.[4] The cationic metal ions compensate the negative charges of the sugar phosphate backbone; the nature of these cations has been shown to play an important role in the folding of RNA[5] and in the formation of unusual DNA structures, such as Z-DNA, G-quartets and cruciforms.[6] One of the main benefits of studies carried out to understand transition metal ion–DNA interaction has been the development of DNA foot printing agents, which are extremely useful in molecular biology.[7] The redox properties and photophysical properties of the transition-metal ions like Ru(II), Ru(III), Co(III), and Cu(II), have been exploited for developing DNA cleaving agents.[8–11] Metal complexes that bind covalently to DNA have been known to function as anti-tumor agents.[12]

In recent times there has been a renewed interest in the biological role of Cr(III), because of the use of Cr(III) complexes as dietary supplements, and because of their function as biotransformation products of carcinogenic Cr(VI).[13–15] Hence, this study is important because chromium is an significant industrial element, and there has been much environmental concern regarding the discharge of Cr(VI) and Cr(III)-bearing effluents by the electroplating and tanning industries. The biotoxicity of Cr(VI) has been well established, and various mechanisms have been put forward concerning the observed toxicity of Cr(VI). The involvement of intermediate oxidation states of chromium viz., Cr(V) and Cr(IV), as well as radical species in the observed toxicity of chromium(VI) have been postulated.[16] Chromium(III) at trace levels has been believed to be nontoxic, since it is an essential trace element for glucose metabolism. This perception, however, is changing with the realization that for Cr(III), being a d^3 transition metal ion, many of its properties are largely dictated by the coordinating ligand. One of the apt examples to demonstrate this is the aqua ligand substitution behavior of Cr(III). Although chromium(III) is generally inert towards aqua ligand substitution, the coordination of a ligand like salen or EDTA makes the aqua ligand labile towards substitution when compared to hexaaquachromium(III). Hence, such complexes can readily bind to biomolecules, like DNA. Substitutionally inert Cr(III) complexes have been known to bring about protein DNA cross-linking.[17]

As a result of the interaction of Cr(III) with biomolecules, transformations can be brought about in the biomolecules, depending on the coordinating ligand. Yamini et al.[18] have recently demonstrated the ability of certain Cr(III) complexes to catalyze the nonspecific cleavage of BSA in the presence of peroxide, which demonstrates that the catalytic ability of the Cr(III) ion depends on the coordinated ligand. The influence of the ligand structure on the cell damage caused by Cr(III) has also been demonstrated. The Cr(III) Schiff-base complex, $[Cr(salen)(H_2O)_2]^+$, brings about apoptosis of the lymphocytes, whereas $[Cr(edta)(H_2O)]^-$ and $Cr(ox)_3^{3-}$ do not cause any cell damage.[19] Vijayalakshmi et al.[20,21] initiated a study on

the interaction of Cr(III) complexes with DNA to rationalise the role of the ligand structure on the biotoxicity of Cr(III). It has been demonstrated that the Cr(III) complex of a Schiff-base ligand with a donor–acceptor group exhibits nuclease activity.[20] On the other hand, $[Cr(salen)(H_2O)_2]^+$ cleaves DNA only in the presence of peroxide.[21] In this communication, we describe the synthesis of the Cr(III) Schiff-base complex **1** (Chart 1) containing fused benzene rings and their effect on the DNA binding properties of the complex.

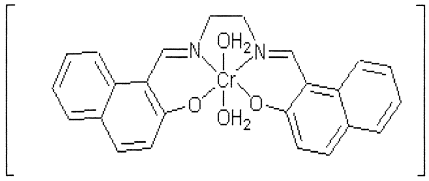

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Chart 1.

Experimental

Chemicals. 2-Hydroxy-1-naphthaldehyde was obtained from Fluka chemical company and used as received. Hepes and Tris were obtained from SRL chemicals, Mumbai. Calf thymus DNA was obtained from Sigma Chemical Co. USA. A stock solution of DNA was prepared by stirring a sample dissolved in 10 mM (1 M = mol dm^{-3}) Hepes buffer (pH 7.0) at 4 °C. The solution was dialyzed exhaustively against a buffer for 48 h, and was filtered using a membrane filter obtained from Sartorius (0.45 μm). The filtered DNA solution in the buffer gave a UV absorbance ratio, (A_{260}/A_{280}) of about 1.9, indicating that the DNA was sufficiently free from protein.[22] The concentration of DNA was determined using an extinction coefficient of 6600 M^{-1} cm^{-1} at 260 nm.[23] A stock solution of Cr(III) complex was prepared in methanol, and the concentration was estimated by an earlier procedure.[24] All of the solvents used were of analytical grade received from Ranbaxy, Mumbai, and were used without further purification. All of the experiments were carried out in Hepes buffer at pH 7.0 in Milli Q triply deionized water.

Methods. UV–visible spectra of the complex and DNA binding studies were recorded on a Shimazdu-160A model double-beam spectrophotometer at 25 °C. Elemental analysis was performed using a Heraeus-CHN-Rapid Analyzer at RSIC, IIT, Madras. The emission spectra were recorded on a Hitachi 650-40 spectrofluorimeter. The electrospray ionization (ESI) mass spectrum of the complex was recorded with Hewlett Packard-1100 mass spectrometer equipped with an electron spray source. The infrared spectrum of the complex was recorded on a Perkin Elmer FT-IR spectrometer.

Synthesis. A Schiff-base complex of chromium(III), $[Cr(naphen)(H_2O)_2](ClO_4)$ (naphen = 1,2-bis(naphthylideneamino)ethane), was synthesised in situ. A solution of 2-hydroxy-1-naphthaldehyde (20 mmol, 3.44 g) in 15 mL methanol was added to 1,2-diaminoethane (10 mmol, 0.66 mL) in 10 mL of methanol. The reaction mixture was deaerated with oxygen-free N_2 gas. To this deaerated solution, $[Cr(H_2O)_6](ClO_4)_2$[25] (10 mmol) was added. The reaction mixture was stirred under N_2 gas for 10 min. Subsequently, it was exposed to atmospheric oxygen to convert the Cr(II) complex to Cr(III) complex. The resulting dark-brown solution was concentrated in a hot-water bath to one-third of its original volume. Upon the addition of few drops of 1 M $HClO_4$, a brown precipitate was obtained, which was filtered and washed with diethyl ether, and then vacuum dried. The compound was recrystallized from methanol-water (80:20). Found: C, 52.02; H, 4.00; N, 5.05; Cr, 9.39% Calcd for $C_{24}H_{22}ClCrN_2O_8$: C, 51.93; H, 3.89; N, 5.12; Cr, 9.21%; IR (KBr pellet) 3425, 3014, 2919, 1615, 1414, 1103 cm^{-1}.

Caution! Although we did not face any difficulty during our work, perchlorate salts are potentially explosive! Utmost care should be exercised in handling them.

DNA Binding Studies. The electronic spectra of the Cr(III) complex were monitored in both the presence and absence of DNA. The binding constant for the interaction of Cr(III) complex with DNA was obtained from absorption titration data. A fixed amount of the complex (10 μM) was titrated with increasing amounts of DNA over a range of 200–1100 μM. The binding constant was determined using

$$[DNA]/(\varepsilon_A-\varepsilon_F) = [DNA]/(\varepsilon_B-\varepsilon_F) + 1/K_b\,(\varepsilon_A-\varepsilon_F), \qquad (1)$$

where ε_A, ε_F , and ε_B correspond to A_{obsd}/[Cr], the extinction coefficient for the free chromium complex, and the extinction coefficient for the chromium complex in the fully bound form, respectively. A plot of $[DNA]/(\varepsilon_A-\varepsilon_F)$ vs [DNA], gives K_B as the ratio of the slope to the intercept.

DNA melting experiments were carried out using a spectrophotometer connected with a thermostat. The absorbance of DNA (75 μM) at 28–80 °C in both the absence and presence of 7.5 μM of the complex was recorded at 260 nm. The melting temperature (T_m) was calculated by plotting the temperature vs the relative absorption intensity (A/A_0).

Viscometric experiments were carried out using an Ostwald-type viscometer of 2 mL capacity, thermostated in a water bath maintained at (25 ± 1) °C. The flow rates of the buffer (10 mM), DNA (500 μM) and DNA in the presence of Cr(III) complex at various concentrations (5–75 μM) were measured with a manually operated timer at least three times to agree within 1 s. The relative specific viscosity was calculated according to the relation $\eta = (t-t_0)/t_0$, where t_0 is the flow time for the buffer and t is the observed flow time for DNA in the presence and absence of the complex. A plot of $(\eta/\eta_0)^{1/3}$ vs $1/R$ (R= [DNA]/[Complex]) was constructed from viscosity measurements.

Fluorescence measurements utilized a Hitachi model 650-40 spectrofluorimeter. The concentration of the complex was fixed (20 μM), while the concentration of DNA was varied from 200–1400 μM. The Cr(III) complex was excited at 370 nm and the emission intensity was monitored at 465 nm. The Stern–Volmer quenching constant was determined from

$$F_0/F = 1 + k_q\tau_0[Q] = 1 + K_D[Q], \qquad (2)$$

where F_0 and F are the fluorescence intensities in the absence and presence of DNA, respectively; k_q is the bimolecular quenching rate constant, τ_0 the lifetime of the fluorophore in the absence of quencher, [Q] the concentration of quencher and $K_D = k_q\tau_0$ the Stern–Volmer quenching constant. The quenching data are presented as a plot of F_0/F vs [Q].

Time-resolved fluorescence of complex **1** was determined using a picosecond laser-excited, TCSPC spectrometer. The excitation

source was a tunable Ti–Sapphire laser (Tsunami, Spectrophysics, USA) with a pulse width of < 2 ps and a repetition rate of 82 MHz. The sample was excited at 375 nm and the emission was monitored at 465 nm using a MCP-PMT (Hamamatsu-C 4878) detector. Decay traces were deconvulated using a non- linear least-squares analysis using IBH software. The lifetimes of a 25 μM Cr(III) complex were measured in the presence of different amounts of DNA (200 μM to 1000 μM).

Circular dichroic spectra of DNA in the presence and absence of Cr(III) complex were obtained by using a J-715 Spectropolarimeter at 25 °C along with a 0.1 cm pathlength cuvette. The spectra were recorded for 100 μM DNA in the presence of a 0.87–4.35 μM of Cr(III) complex in the 220–320 nm region.

Molecular modeling was performed on a Silicon Graphics O2 workstation using the Biosym Modeling Package from Molecular Simulation Inc. (San Diego, CA). The dodecamer Dickerson B-DNA, $d(GCGCAATTGCGC)_2$ was chosen to study the interaction of the Cr(III) complex. Models of 12 mer oligonucleotide were constructed using the Biopolymer program of the Insight II package. The chromium(III) complex was also modeled using coordinates from the Insight II library. An energy refinement of the starting structure was carried out using an ESFF force field with a non-bonded cut off of 10 Å and a sigmoidal distant dependent dielectric function ($\varepsilon = 4r_{ij}$). For a molecular mechanics energy refinement, a diffuse charge distribution in which the +3 charge of the metal atom is delocalized over the whole ligand was used. In all of the calculations, a steepest descent and conjugate gradient energy minimization algorithm was applied for the helix and complex–helix interaction geometry. The minimization was considered to be convergent when a rms of 0.3 (kcal/mol Å) was achieved. The interaction energy of the Cr–DNA complex was estimated by calculating the differences between the total energy of the DNA–Cr complex adduct structure (E_{int}) and the sum of the lowest energies found for the optimized structures of free DNA (E_{DNA}) and free Cr(III) complex ($E_{complex}$). The binding energy was calculated as the negative of the interaction energy. Modeling studies based on a molecular mechanics approach were performed by interacting Cr(III) complex **1** with the dodecamer. Complex **1** was partially inserted centrally between A_7T_6 of one strand and T_6A_7 of the complementary strand of the duplex. This approach is expected to lead to a complete understanding and interpretation of the experimental observation of Cr(III) complex binding to DNA.

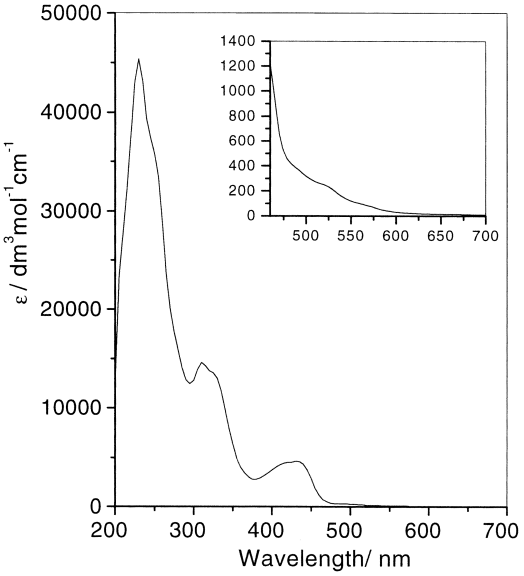

Fig. 1. UV–visible spectrum of $[Cr(naphen)(H_2O)_2]^+$ in methanol.

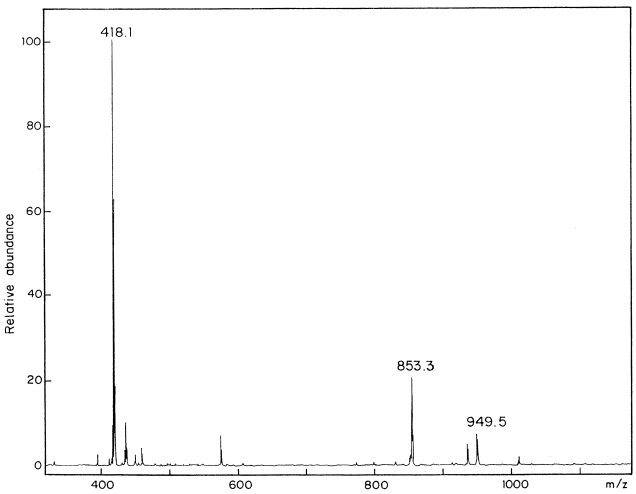

Fig. 2. ESI Mass spectrum of $[Cr(naphen)(H_2O)_2]^+$ in methanol.

Results and Discussion

Synthesis and Characterisation. The UV–visible spectrum of complex **1** is dominated by the ligand centered and charge-transfer transitions. The ligand field transitions were observed as shoulders at 515 and 556 nm (Fig. 1, inset) and the MLCT band was observed at 421 nm. The homologue complex $[Cr(salen)(H_2O)_2]^+$ shows ligand field bands at 485 and 584 nm and a MLCT band at 378 nm. The ESI mass spectrum of the complex shows the base peak at *m/z* 418 (Fig. 2) for the cation $[Cr(naphen)]^+$ formed by the loss of two coordinated water molecules. The Schiff-base complex, $[Cr(salprn)(H_2O)_2]^+$, is also known to readily lose coordinated water molecules under the conditions employed for obtaining the ESI mass spectrum to produce the cation $[Cr(salprn)]^+$.[26] The peak at *m/z* 853 can be attributed to the formation of a dimer, $[Cr(naphen)\text{-}OH\text{-}Cr(naphen)]^+$. The mass spectral data confirm the molecular weight of the synthesized compound. The complex exhibits emission at 465 nm when excited at 370 nm. It is of interest to note that the homologue complex $[Cr(salen)(H_2O)_2]^+$, does not show any emission. On the other hand, a higher homologue of $[Cr(salen)(H_2O)_2]^+$ i.e. $[Cr(salprn)(H_2O)_2]^+$ shows emission at 417 nm.[21]

Absorption Titration. The nature of the binding modes, like electrostatic or intercalation, can induce changes in the electronic spectrum of molecules binding to DNA.[27] The electronic spectrum of $[Cr(naphen)(H_2O)_2]^+$ in the presence and absence of DNA was monitored at a wavelength of 420 nm, which arises due to a metal-to-ligand charge transfer (MLCT) transition in the Cr(III) complex. Upon the addition of DNA, a considerable drop in the absorptivity of the MLCT band was observed. There was a bathochromic shift from 5 nm to 9 nm of the MLCT band upon increasing the concentration of DNA. The spectrum of the complex when recorded as a function of the DNA concentration shows an isosbestic point at 463 nm

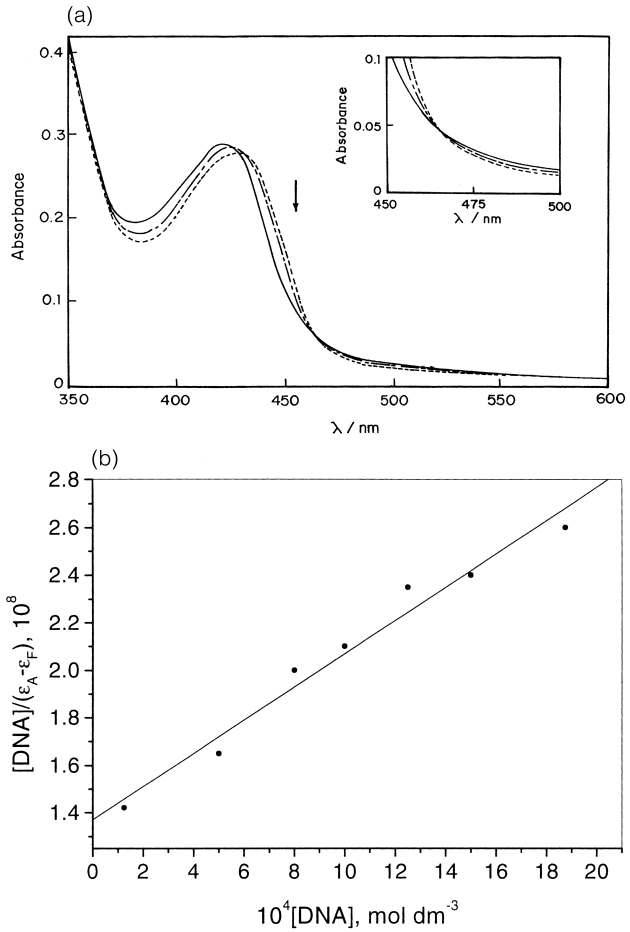

Fig. 3. (a) UV–visible spectra of [Cr(naphen)(H$_2$O)$_2$]$^+$ (10 μM) in the absence (—) and presence of increasing additions of CT DNA (0.30 mM, - — - and 0.35 mM, ---) in Hepes buffer Inset. Portion of the graph showing isosbestic point at 467 nm.
(b) Plot of [DNA] vs [DNA]/($\varepsilon_A - \varepsilon_F$).

(Fig. 3a). Such a single isosbestic is indicative of clean one-step binding of the complex to DNA. Even though the Cr(III) complex **1** contains two aqua ligands, binding of the complex to the heterocyclic nitrogen of DNA base is very unlikely, since such a binding is expected to give rise to a marked change in the ligand field transition of the complex. The homologue complex, [Cr(salen)(H$_2$O)$_2$]$^+$ also does not bind to the nitrogen atom of the DNA base.[21] The intercalative binding of small molecules to a DNA helix has been characterized by large changes in the absorbance (hypochromism) and an appreciable shift in the wavelength (red shift) due to the interaction of a DNA π stack and a complex π system.[28] The percentage hypochromic shift of the MLCT band of **1** upon binding to DNA was found to be 20% (hypochromicity, H% = [(($\varepsilon_f - \varepsilon_b$)/$\varepsilon_f$)×100], where ε_f and ε_b are the molar absorption coefficients for the free and bound form of complex). The observed hypochromism in the visible absorption of the complex **1** bound to DNA, however was lower in value when compared to that of classical intercalators (such as ethidium bromide). The intrinsic binding constant for the interaction of [Cr(naphen)(H$_2$O)$_2$]$^+$ with DNA was found to be (0.51 ± 0.07)×10^4 M^{-1} from the absorption titration data (Fig. 3b). The binding constant was also estimated by monitoring the spectral changes at 400 nm, and was found to be (0.48 ± 0.05)×10^4 M^{-1}. The binding constant value observed here for the Cr(III) complex is comparable to that reported for some intercalators, like AQS (0.35×10^4 M^{-1}) [29] and [Ru(NH$_3$)$_4$(DPPZ)]$^{2+}$ (1×10^5 M^{-1}).[30] The classical intercalator, however, shows a much higher binding constant of 1×10^7 M^{-1}.[31]

Fluorescence Studies. The excitation of **1** at 370 nm results in an emission spectrum with a maximum at 465 nm. In the presence of DNA, the fluorescence emission of complex **1** is quenched with no evident shift in the emission maximum. Measurements of the fluorescence emission of a fixed amount of the Cr(III) complex in both the presence and absence of DNA were taken until no further change in the luminescence was observed. The decrease in the emission intensity for complex **1** upon binding to DNA is due to the change in the environment around the metal complex. Hydrophobic interactions between the complex and DNA may also induce changes in the excited-state properties. Generally, there is an enhancement in the fluorescence emission when the molecule binds to DNA through the intercalative mode.[4] However, this observation does not hold for all systems. Luminescence quenching has been reported for an intercalator, like daunomycin, when bound to DNA.[32] A Stern–Volmer plot was constructed from the emission intensity vs. concentrations of DNA. The Stern–Volmer plot for **1** was linear and the quenching constant for **1** in the presence of DNA was found to be 241 M^{-1}.

Fluorescence lifetime measurements were monitored for complex **1** by single-photon counting. Complex **1** was excited at 375 nm and the emission lifetime was measured at 465 nm. The lifetime measured for complex **1** was found to be 5.75 ns in aqueous methanol at pH 7.0, and the luminescence of the complex was found to show single exponential decay. In the presence of DNA, the emission lifetime was found to decrease with increasing concentrations of DNA. Using the lifetime data, the bimolecular quenching rate constant was determined. For the Cr(III) complex **1**, k_q (K_D/τ_0) was found to be 4.19×10^{10} M^{-1} s^{-1} in the absence of DNA. In the presence of DNA, k_q was found to increase with increasing concentrations of DNA (4.25×10^{10}, 4.35×10^{10}, and 4.44×10^{10} M^{-1} s^{-1} at 0.25, 0.625, and 1.0 mM DNA, respectively).

Melting Studies. The interaction of small molecules with a double helix is known to increase the melting temperature (T_m), the temperature at which the double helix denatures into single-strand DNA.[33] The melting temperature can be determined by monitoring the absorbance of the DNA at 260 nm as a function of the temperature. A T_m experiment carried out for CT DNA in the absence of any added complex revealed a T_m of 65.5 ± 0.5 °C under our experimental condition. The addition of complex **1** to DNA showed a ΔT_m of 5 ± 0.5 °C when compared to native calf thymus DNA. The melting curve for complex **1** is shown in Fig. 4. An intercalative mode of binding was reported to give rise to a small as well as a large change in T_m.[30,31] The melting temperature has been found to reflect the binding affinity of the complex to DNA. The binding of [Ru(tpy)(bpy)H$_2$O]$^{2+}$ to CT DNA increases the T_m of DNA by 4.2 °C and its DNA binding constant has been reported to be 660 M^{-1}. On the other hand, [Ru(tpy)(DPPZ)H$_2$O]$^{2+}$ increas-

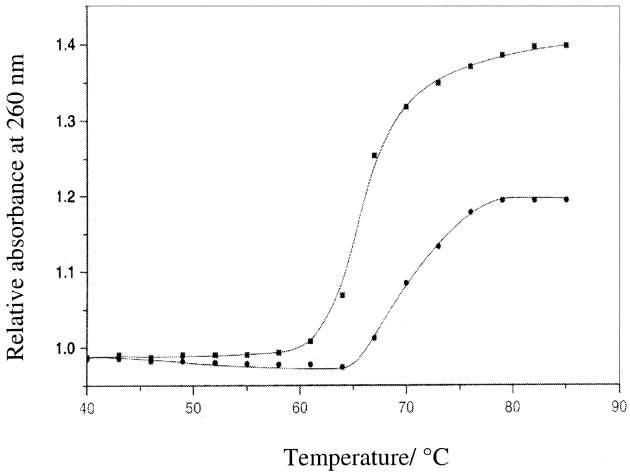

Fig. 4. Melting curves of CT DNA (75 μM) in the absence (■) and in the presence of $[Cr(naphen)(H_2O)_2]^+$ (7.5 μM) (●).

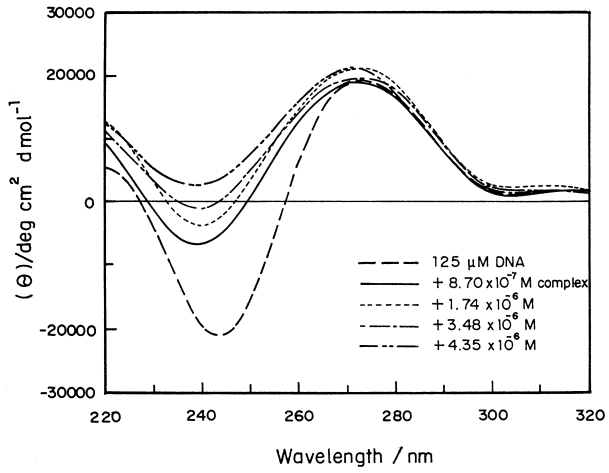

Fig. 6. CD spectra of CT DNA in the presence (0.87–4.35 μM) and in the absence (100 μM) of $[Cr(naphen)(H_2O)_2]^+$.

es the T_m of CT DNA by 14.1 °C and its DNA binding constant has been reported to be 7×10^5 M^{-1}.[34] The small change in the T_m of DNA observed here in the presence of complex **1** is indicative of the fact that the interaction of the complex with DNA is not very strong. This is also reflected in the binding constant.

Viscometric Measurement. The classical method to determine the mode of binding of DNA with small molecules has been viscometric titration. Generally, intercalating agents are expected to destack the base pairs, thus causing an elongation of the double helix, and resulting in an increase in the viscosity of DNA, whereas non-intercalative binding is expected to produce no change or decrease in the viscosity of DNA.[35] Figure 5 shows the results of a comparative viscosity study designed to explore the mode of binding of the Cr(III) complex to DNA. The relative specific viscosities increases with increasing concentration of the complex. The increase in the relative specific viscosity shows a lengthening of the DNA helix resulting from intercalation. The relative viscosity of DNA in the presence of the homologue complex $[Cr(salen)(H_2O)_2]^+$, however, shows a different trend, depending on the concentration of the complex.[21] $[Cr(salen)(H_2O)_2]^+$ has been found to show predominantly a groove binding characterisitic. The introduction of a fused ring in the ligand changes the mode of the binding of the complex from the groove mode to the intercalative mode.

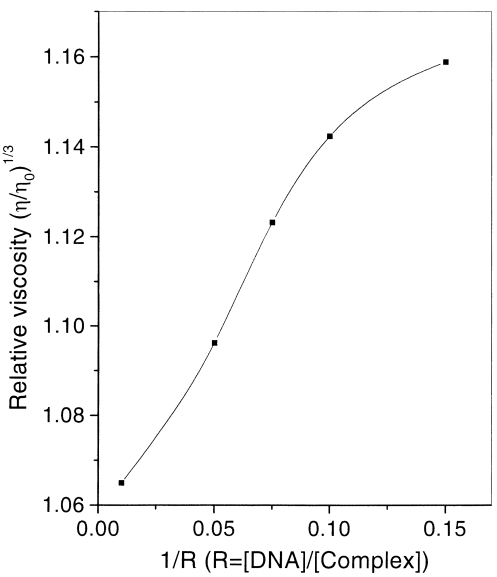

Fig. 5. Plot of relative viscosities $(\eta/\eta_0)^{1/3}$ of CT DNA vs $1/R$ (R = [DNA]/[Complex]).

Circular Dichroism. The Cr(III) complex **1** is achiral, and hence does not exhibit any band in the CD spectra. The calf thymus DNA in the B form conformation shows two conservative CD bands, a positive band at 275 nm (due to stacking) and a negative band at 245 nm (due to helicity) in the ultraviolet region. In Fig. 6, the changes in the CD spectrum of CT DNA in the presence of increasing concentrations of complex **1** are depicted. The positive band in the CD spectrum was perturbed with increasing concentrations of complex **1**. The enhancement in the molar ellipticity was accompanied by a slight red shift of the band maximum. At higher concentrations of the complex, the positive band was marginally shifted towards longer wavelength. The negative band (at 245 nm) in the CD spectra of DNA was perturbed progressively with increasing concentration of the complex. The molar ellipticity of the negative band is shifted towards the positive region upon increasing the concentration of the complex. At a particular concentration of the Cr(III) complex (4.35 μM), the band was shifted completely towards the positive region with no shift in the band maximum. These observations suggest that the binding of the complex increases the stacking and decreases the helicity of double helical DNA, which is consistent with the intercalative mode of interaction. This would be because in the intercalative mode of binding the molecules stack in between the base pairs of DNA, resulting in an unwinding of the helix.

Molecular Modeling. The flexibility of DNA and the intercalation of drugs with DNA have been well studied by both experimental and theoretical methods.[36] An advantage of the molecular modeling approach is that it allows a detailed energetic analysis of all the structural models, which are assumed to be close to those which occur in real systems. Using molec-

ular mechanics, a systematic search for lower energy structure has been carried out for the free DNA structure with a specified sequence and its complex with compound **1**. The analysis of initial models of DNA and Cr(III) complex **1** has shown that partial intercalation is the probable mode of interaction due to its octahedral geometry and the presence of aqua ligands in the complex. The flanking of the DNA base pairs and change in the C1′–C1′ distance due to the interaction of complex **1** were determined using molecular modeling. The starting Cr–DNA complex was constructed by placing the Cr(III) complex in between the AATT base pairs of the DNA helix.

The energy-minimized Dickerson model with complex **1** in the intercalative mode is shown in Fig. 7. The binding energy was calculated for complex **1** bound to DNA, and found to be 24.93 kcal/mol (Table 1). The vertical separate distance of the base pairs (rise of base pairs) of DNA upon minimization leads to an increase in the distance between the base pairs when compared to the distance of the minimized structure of DNA alone. The vertical rise of the central base pairs (AATT) and the distance between C1′–C1′ of both strands were measured before and after interaction of the Cr(III) complex with the Dickerson sequence. For the minimized structure of DNA before the interaction with complex **1**, the distance between T_6N3 : A_7N1 of one strand is 4.23 Å, and for the complementary strand the distance between A_{7B}N1 : T_{6B}N3 is 4.43 Å, whereas the distance measured after the interaction of DNA with complex **1** was found to be 6.90 Å for T_6N3 : A_7N1 bases of one strand and 6.32 Å for A_{7B}N1 : T_{6B}N3 bases of the complementary strand. Hence, it has been observed that the base pairs are flanked due to a rise in the vertical separation of 2.67 Å of one strand and 1.89 Å of the other strand after an interaction with complex **1**. The distances between C1′–C1′ of the sugar moiety of both strands of the central base pairs (A_{7B}:T_6:$T_{6B}A_7$) was measured and compared with the optimized conformation after an interaction with complex **1**. The distances measured for the central base pairs were found to be 10.79 Å and 10.81 Å. The observed changes in the C1′–C1′ distance after the interaction were found to be 10.93 Å and 10.59 Å. The elongation of the molecule is in keeping with the intercalative mode of binding of complex **1** to the DNA model.

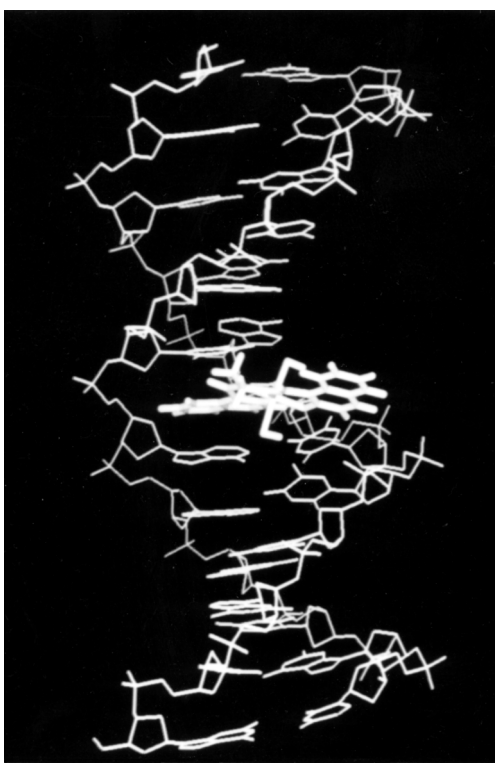

Fig. 7. Optimized structure of dodecamer Dickerson sequence interacted with [Cr(naphen)$(H_2O)_2$]$^+$.

Table 1. Energy Decomposition of the [Cr(naphen)-$(H_2O)_2$]$^+$-Dickerson Complex (kcal/mol)

1.	*E* (Total)	−123.435450
2.	*E* [d(CGCGAATTCGCG)$_2$]	134.403025
3.	*E* [Cr(naphen) $(H_2O)_2$]$^+$	−232.912418
4.	*E* (2+3)	−98.509393
5.	*E* (Interaction Energy, (1–4))	−24.926057

Octahedral metal ion complexes have generally been shown to interact electrostatically with DNA. Classical intercalation needs an extended π system. The introduction of fused rings on the basic Cr-salen structure not only creates an extended π system, but also changes the mode of binding from the groove mode to the intercalative mode. The homologue complex [Cr(salen)$(H_2O)_2$]$^+$ and its higher homologue [Cr(salprn)-$(H_2O)_2$]$^+$ show groove binding, whereas [Cr(naphen)$(H_2O)_2$]$^+$ shows an intercalative binding. Intercalation of the complex leads to an increase in the vertical separation of 2.67 Å in one strand of model DNA and 1.89 Å in the other strand. The results from measurements of the melting temperature, absorption titration, and viscosity measurements also suggest that the complex binds to DNA with moderate strength through the intercalative mode. The results from a CD study also show a change in the helicity of the DNA structure.

The authors thank National Centre for Ultrafast processes for providing the facility to carry out the time-resolved fluorescence work. V.G.V. acknowledges CSIR for the research fellowship.

References

1 a) A. M. Pyle and J. K. Barton, *Prog. Inorg. Chem.*, **38**, 413 (1990). b) P. B. Dervan, *Science*, **232**, 464 (1986). c) H. E. Moser and P. B. Dervan, *Science*, **38**, 645 (1987). d) J. K. Barton, *Science*, **233**, 727 (1986). e) C. S. Chow and J. K. Barton, *Methods Enzymol.*, **212**, 219 (1992). f) H. Y. Mei and J. K. Barton, *J. Am. Chem. Soc.*, **108**, 7414 (1986). g) M. R. Kirshenbaum, R. Tribolet, and J. K. Barton, *Nucleic Acids Res.*, **16**, 7943 (1988).

2 R. T. Watson, N. Desai, J. Wildsmith, J. F. Wheeler, and N. A. P. Kane-Maguire, *Inorg. Chem.*, **38**, 2683 (1999).

3 a) T. Tanaka, K. Tsurutani, A. Komatsu, T. Ito, K. Iida, Y. Fujii, Y. Nakano, Y. Usui, Y. Fukuda, and M. Chikira, *Bull. Chem. Soc. Jpn.*, **70**, 615 (1997). b) D. J. Gravert and J. H. Griffin, *Inorg. Chem.*, **35**, 4837 (1996).

4 a) Y. Jenkins, A. E. Friedman, N. J. Turro, and J. K. Barton, *Biochemistry*, **31**, 10809 (1992). b) A. Ambroise and B. G. Maiya, *Inorg. Chem.*, **39**, 4256 (2000).

5 G. J. Quigley, M. M. Teeter, and A. Rich, *J. Mol. Biol.*, **75**,

64 (1978).
6 E. Palecek, *Crit. Rev. Biochem. Mol. Biol.*, **26**, 151 (1991).
7 a) W. K. Pogozelski, T. J. Mcneese, and T. D. Tuilius, *J. Am. Chem. Soc.*, **117**, 6428 (1995). b) D. S. Sigman, T. W. Bruice, A. Mazumder, and C. L. Sutton, *Acc. Chem. Res.*, **26**, 98 (1993). c) B. Meunier, *Chem. Rev.*, **92**, 1411 (1992).
8 A. E. Friedman, J. C. Chambron, J. P. Sauvage, N. J. Turro, and J. K. Barton, *J. Am. Chem. Soc.*, **112**, 4960 (1990).
9 N. J. Turro, J. K. Barton, and D. A. Tomalia, *Acc. Chem. Res.*, **24**, 332 (1991).
10 Q. L. Zhang, J. G. Liu, H. Chao, G.-Q. Xue, and L.-N. Ji, *J. Inorg. Biochem.*, **83**, 49 (2001).
11 R. Ren, P. Yang, W. Zheng, and Z. Hua, *Inorg. Chem.*, **39**, 5454 (2000).
12 J. Stubbe and J. W. Kozarich, *Chem. Rev.*, **87**, 1107 (1987).
13 M. J. Tsapakos and K. E. Wetterhahn, *Chem. Biol. Interact.*, **46**, 265 (1983).
14 R. P. Farell, R. J. Judd, P. A. Lay, N. E. Dixon, R. S. U. Baker, and A. M. Bonin, *Chem. Res. Toxicol.*, **3**, 227 (1989).
15 M. A. Billadeau and H. Morrison, *J. Inorg. Biochem.*, **57**, 249 (1990).
16 a) R. N. Bose, S. Moghaddas, and E. Gelerinter, *Inorg. Chem.*, **31**, 1987 (1992). b) R. Codd, C. T. Dillon, A. Levina, and P. A. Lay, *Coord. Chem. Rev.*, **216-217**, 537 (2001).
17 T. C. Tsou, R. J. Lin, and J. L. Yang, *Chem. Res. Toxicol.*, **10**, 962 (1997).
18 H. Yamini Shrivastava, M. Kanthimathi, and B. U. Nair, *Biochem. Biophy. Res. Commun.*, **265**, 311 (1999).
19 R. Rajaram, B. U. Nair, and T. Ramasami, *Biochem. Biophy. Res. Commun.*, **210**, 434 (1995).
20 R. Vijayalakshmi, V. Subramanian, and B. U. Nair, *Biochem. Biophy. Res. Commun.*, **271**, 731 (2000).
21 R. Vijayalakshmi, M. Kanthimathi, V. Subramanian, and B. U. Nair, *Biochim. Biophy. Acta*, **1475**, 157 (2000).
22 J. Marmur, *J. Mol. Biol.*, **3**, 208 (1961).
23 R. Reichmann, S. A. Rice, C. A. Thomas, and P. Doty, *J. Am. Chem. Soc.*, **76**, 3047 (1954).
24 K. Lingane and R. Peesok, *Anal. Chem.*, **20**, 425 (1948).
25 R. J. Balahura and A. J. Johnston, *Inorg. Chem.*, **22**, 3309 (1983).
26 K. P. Madhusudhanan, S. B. Katti, R. Vijayalakshmi, and B. U. Nair, *J. Mass Spectrom.*, **34**, 880 (1999).
27 V. A. Bloomfield, D. M. Crothers, and I. Tinoco, Jr., "Physical Chemistry of Nucleic Acids," Harper and Row, New York (1974), p. 432.
28 J. M. Reddy, C. J. Murphy, D. J. McConell, and C. OhUigin, *Nucleic Acid Res.*, **13**, 167 (1985).
29 D. T. Breslin, C. Yu, D. Ly, and G. B. Schuster, *Biochemistry*, **36**, 10463 (1997).
30 R. B. Nair, E. S. Teng, S. L. Kirkland, and C. J. Murphy, *Inorg. Chem.*, **37**, 139 (1998).
31 a) M. Cory, D. D. Mckee, J. Kagan, D. W. Henry, and J. A. Miller, *J. Am. Chem. Soc.*, **107**, 2528 (1985). b) M. J. Waring, *J. Mol. Biol.*, **13**, 269 (1965).
32 J. B. Chaires, N. Dattagupta, and D. M. Crothers, *Biochemistry*, **21**, 3933 (1982).
33 W. Saenger, "Principles of Nucleic acid and structures," Springer Advanced Texts in Chemistry Series, Springer, New York (1984), p. 143.
34 G. A. Nehyart, N. Grover, S. R. Smith, W. A. Kalsbeck, T. A. Fairley, M. Cory, and H. H. Thorp, *J. Am. Chem. Soc.*, **115**, 4423 (1993).
35 G. Yang, F. Z. Wu, L. Wang, L. NianFi, and X. Tina, *J. Inorg. Biochem.*, **66**, 141 (1997).
36 a) M. T. Record, C. F. Anderson, and T. M. Lohman, *Quart. Rev. Biophys.*, **11**, 103 (1978). b) G. S. Manning, *Quart. Rev. Biophys.*, **11**, 179 (1978).