

Synthesis, Spectral Characterization, and DNA Binding Studies of *trans*-[Dichlorobis(2,3-dicyanodipyrido-$N^{8,9}$-[3,2-*f*:2′,3′-*h*]-quinoxaline)chromium(III)] Perchlorate Dihydrate

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A new dichlorochromium(III) complex with the 2,3-dicyanodipyridoquinoxaline ligand has been synthesized and characterized by UV–visible, mass spectroscopy, and cyclic voltammetry. The DNA binding properties of this Cr^{III} complex to CT DNA were demonstrated by absorption titration, melting temperature and viscosity measurements. Hypochromism in the absorption spectra, an increase in the denaturation temperature and changes in the viscosity in the presence of the complex indicate that the complex binds to DNA through the intercalative mode. Based on the data obtained from molecular-mechanics calculations, an intercalative mode of binding to $d(GC)_{12}$ through a minor groove is favored. The title complex promotes the cleavage of plasmid pBR322 DNA in the presence of peroxide, and a slight retardation in the mobility of plasmid DNA.

The interaction of metal complexes with biomolecules has attracted much attention for the past few years. Stable, inert and water-soluble transition-metal complexes with spectroscopically active metal centers have been found to be extremely valuable candidates as probes of biological systems.[1] Several metal complexes, which bind to DNA through different modes, have also been used as agents for the mediation of strand scission of duplex DNA and as chemotherapeutic agents.[2] The DNA binding ability of polypyridyl transition metal complexes has attracted considerable current interest. Most attention has centered upon metal complexes that are capable of binding to DNA by intercalation.[3–5] In recent years, ligands derived from modifications of 2,2′-bipyridine (bpy) and 1,10-phenanthroline (phen) family have enjoyed certain applications.[6,7] The aggregation behavior of a surfactant, like SDS, has been studied in the presence of Cr^{III} complexes derived from bpy, phen, en (en = ethylene diamine), and Schiff-base ligands.[8] The dppz complexes (dppz = dipyrido-[3,2-*a*:2′,3′-*c*]phenazine), with their expansive aromatic surface area, form impressive candidates in developing novel probes for DNA, and are useful in biological systems.

Recently, we have focused our attention on the binding of chromium(III) complexes to DNA[9–12] and the photo cleavage of proteins[13] by introducing additional ligands capable of coordinating metal ions, thereby exploiting new structural topologies with tunable optical, redox, and electronic properties. In a continuation of our efforts in this direction, we have made an attempt to synthesize a new chromium(III) complex of dicyano subunit-appended ligand belonging to the dppz family, viz. 2,3-dicyanodipyrido[3,2-*f*:2′,3′-*h*]quinoxaline, dicnq. The photochemical functions and DNA interactions of Ru^{II}, Co^{III}, and Ni^{II} complexes of dicnq have already been reported.[14,15] This paper describes the synthesis, characterization, DNA binding, and cleavage studies of $[CrCl_2(dicnq)_2]ClO_4$, the structure of which is shown in Chart 1.

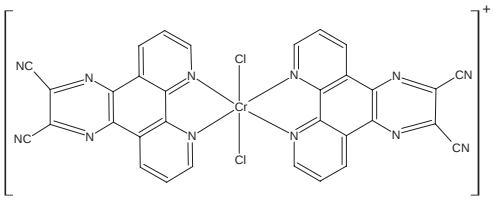

Chart 1.

Experimental

Materials and Methods. The following commercial reagents of analytical grade were used without further purification, unless otherwise noted. Diaminomaleonitrile was purchased from Aldrich. Calf thymus DNA and Plasmid (pBR322) DNA were purchased from Bangalore Genei, India. HEPES, Tris buffer, EDTA, Agarose (molecular biology grade), and Ethidium bromide were received from SRL chemicals, Mumbai. Dialysis membrane was obtained from Sigma Chemical Co. USA. Solvents used in this study were obtained in highest available purity, and used without purification. The solvents utilized for spectroscopic and electrochemical work were purified before use according to reported[16] procedures. The CT DNA obtained from SRL Chemicals, Mumbai, was suspended in a 10 mM (1 M = 1 mol dm^{-3}) HEPES buffer and kept for stirring at 4 °C for 24 h. The solution was then taken in a dialysis bag and dialyzed exhaustively against a 10 mM buffer for 48 h, and filtered through a membrane filter purchased from Sartorius (0.45 μM). The concentration of DNA was determined by monitoring the absorbance at 260 nm using a molar extinction coefficient of 6600 cm^{-1}. The absorbance gave a ratio of >1.8 at A_{260}/A_{280}, indicating that the DNA was sufficiently free from protein.[17] The stock solution was stored at −20 °C.

Physical Measurements. The electronic spectra were record-

Published on the web February 11, 2005; DOI 10.1246/bcsj.78.270

ed using a Perkin Elmer-Lambda 35 spectrophotometer. A Fast Atom Bombardment Mass Spectrometric (FAB-MS) analysis was carried out using a Harvard Apparatus (model 11). The mass spectra were recorded in the +ve ion mode in acetonitrile, and data were collected using a scan time of 4 s in the continuum channel acquisition mode. The presented data are the average of 10–12 scans. The ion assignment was aided by comparing the experimental and calculated isotopic fragmentation patterns. Cyclic voltammetric studies were carried out in acetonitrile employing *tert*-butylammonium perchlorate (TBAP) as the supporting electrolyte. A Princeton Applied Research (PAR) 173 Potentiostat, interfaced to a universal programmer (Model 175), was employed along with platinum working, platinum counter and standard calomel electrodes. ESR measurements were made using a Bruker-EMX spectrometer with 100 KHz magnetic field modulation.

Synthesis of $[CrCl_2(dicnq)_2]ClO_4$. The ligands, 1,10-phenanthroline-5,6-dione (phen-dion),[18] and 2,3-dicyanodipyrido[3,2-*f*:2′,3′-*h*]quinoxaline (dicnq)[14] were synthesized following literature procedures. The synthesis of a chromium(III) complex, $[CrCl_2(dicnq)_2]ClO_4$ was as follows. Hydrated chromium(III) chloride (0.18 g, 0.7 mM) and dicnq (0.6 g, 2.1 mM) were refluxed in 50 mL of methanol with zinc metal for 5 h. The resulting reaction mixture was cooled to room temperature and rotary-evaporated until its total volume was reduced to 20 mL. When the concentrate was cooled to 5 °C, a reddish-brown product crystallized out. The crude product was dissolved in 0.05 M perchloric acid at 50 °C, and the slurry was filtered while hot. To the filtrate, concentrated perchloric acid was added dropwise until the solution turned cloudy. The mixture was heated to 60 °C when the precipitate redissolved. Then, the clear solution was first cooled to room temperature, and then to 5 °C in an ice bath. The desired complex crystallized out as a reddish-brown precipitate. (Calcd: Cr, 6.32; N, 20.44; Cl, 8.63; C, 46.72; H, 1.46%. Found: Cr, 6.14; N, 20.12; Cl, 8.50; C, 46.52; H, 1.39%.)

Caution! Perchlorate salts are potentially explosive! Care should be taken in handling them.

DNA Binding and Cleavage Experiments. The absorbance spectra were obtained by keeping the complex concentration constant (20 μM) and varying the DNA concentration (0 to 1.1 mM). The absorbance values were recorded for the complex after the successive addition of DNA and 10 min equilibration. The intrinsic binding constant (K_b) was determined according to the following Eq. 1.[19]

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

where ε_A, ε_F, and ε_B correspond to $A_{obsd}/[Cr]$, the extinction coefficient for the free chromium complex and 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. Thermal-denaturation studies were carried out for DNA (200 μM) in a 10 mM HEPES buffer, pH 7.4, and in the presence of the chromium(III) complex (10 and 20 μM). The data were collected for every degree rise in the temperature, and plotted against the relative absorbance value. Viscosity measurements were carried out using an Ostwald viscometer, immersed in a thermostated water bath maintained at 25 °C. The DNA concentration was maintained at 400 μM while the complex concentration was varied (0–60 μM). The flow time was measured with a digital stop watch. The flow time of each sample was measured three times, and the average flow time was calculated. The relative viscosities for DNA in the presence (η) and absence (η_0) of the complex were calculated using the relation $\eta = (t - t^0)/t^0$, where t and t^0 are the observed flow time for each sample and buffer. The values of relative viscosity were plotted against $1/R$ (R = [DNA]/[complex]).

For the DNA nicking experiment, supercoiled plasmid DNA (67.5 ng) in a 10 mM HEPES buffer (pH 7.4) was treated with the metal complex (0–45 μM), and the mixture was incubated for 4 h at room temperature (36 °C). Hydrogen peroxide (12.5 mM) was then added and kept for 30 min incubation. The samples were analyzed by 0.8% agarose gel electrophoresis with 1X TBE buffer (pH 8.0) at 50 V for 2 h at 25 °C. The gel was then stained with ethidium bromide (0.5 μg/mL) and illuminated under a UV Gel Documentation system. The ESR spin-trapping method was employed to detect short-lived free radical intermediates. The spectrum was recorded in a 10 mM HEPES buffer (pH 7.4) with 1.5 mM of Cr(III) complex, 2.5 mM H_2O_2, and 15 mM DMPO.

Molecular-mechanics calculations were performed on an SGIO2 workstation using the Biosym molecular simulation package (San Diego Inc., CA, USA). Models of both the *cis* and the *trans* configurations of $[CrCl_2(dicnq)_2]^+$ were constructed with the builder module of Insight II, and energy optimization was performed using an extensible systematic force field (ESFF) with the Discover_3 program. The validation of ESFF has been discussed previously.[12] The B-DNA system chosen for the study was constructed using the Biopolymer program of the Insight II package. The torsion angles were fixed and defined. This program kept the DNA structure fixed, rotated the torsion angle within the fixed values and then computed the coordinates of the resulting structure. The interaction energy of Cr–DNA complex was estimated from the difference between their total energies and the sum of lowest energies found for the optimized structures of the free DNA and the Cr(III) complex. The negative of the interaction energy gives the binding energy:

$$\text{I.E} = \text{T.E} - (\text{sum of the individual energy}), \quad (2)$$

$$\text{B.E} = -\text{I.E}, \quad (3)$$

where I.E is the interaction energy, T.E the total energy of the Cr–DNA complex and B.E the binding energy.

Results and Discussion

Synthesis and Characterization. The ligand, dicnq, was prepared according to a known method. The authenticity of the compound was confirmed by its IR vibration frequencies and ^{1}H NMR data.[14] The chromium(III) complex was initially prepared by a direct reaction of chromium(II) perchlorate with the ligand in methanol under a nitrogen atmosphere and its subsequent oxidation to the chromium(III) complex. Since the yield was very poor, the compound was made using chromium(III) chloride, and the ligand in methanol under reflux conditions in the presence of Zn metal as the catalyst. The thus obtained chloride salt was dissolved in 0.1 M $HClO_4$ at 40 °C. The mixture was cooled to 5 °C, and the complex was precipitated as its perchlorate salt. We isolated the complex with two dicnq units with two chloride atoms and one anion. The proposed formula of the complex, as shown above, was consistent with an elemental analysis based on the percentages of C, H, N, Cl, and Cr. The elemental-analysis data indicate the dihydrate nature of the Cr^{III} complex synthesized in this study. The authenticity of the compound was ascertained from its FAB mass spectra (Fig. 1). The compound shows a molecular ion peak at $m/z = 686$, which is also the base peak for the complex ion. The electronic spectral data for the ligand, dicnq

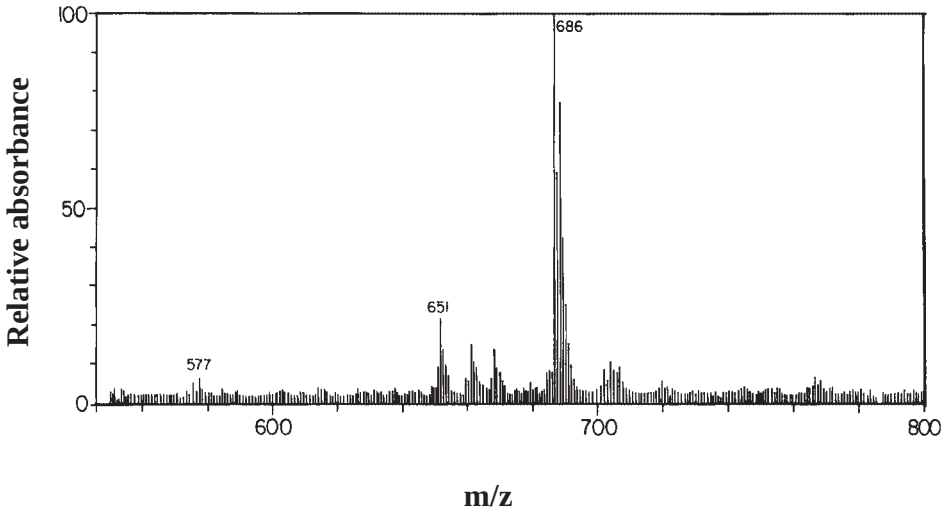

Fig. 1. The FAB mass spectrum of $[CrCl_2(dicnq)_2]ClO_4 \cdot 2H_2O$.

Table 1. The Room Temperature Absorption Spectral Data of the Compounds in Acetonitrile

Compound	λ_{max}/nm (log ε)
Dicnq[a)]	305 (4.40) 347 (3.93), 365 (3.83)
$[CrCl_2(dicnq)_2]^{+}$ [b)]	312 (4.16), 337 (3.96), 354 (3.88), 413.5 (2.71), 527 (2.31)

a) Ref. 14. b) This work.

Table 2. The Electro Chemical Data of the Compounds in Acetonitrile

Compound	E_{red}/V
Dicnq[a)]	−0.66 (in DMF)
Dppz[b)]	−1.62
$[Cr(bpy)_3]^{3+}$ [c)]	−0.209
$[Cr(phen)_3]^{3+}$ [c)]	−0.237
$[Cr(terpy)_2]^{3+}$ [c)]	−0.110
$[CrCl_2(dicnq)_2]^{+}$ [d)]	−0.505, −0.680, −1.304

a) Ref. 14. b) N. Komatsuzaki, R. Katoh, Y. Himeda, H. Sugihara, H. Arakawa, and K. Kasuga, *J. Chem. Soc., Dalton Trans.*, **2000**, 3053. c) M. C. Hughes and D. J. Macero, *Inorg. Chem.*, **15**, 2040 (1976). d) This work.

and its chromium(III) complex are summarized in Table 1. The n–π^* and π–π^* transitions[20] of the ligand moiety observed at 305, 347, 365 nm shifted to 312, 337, and 354 nm, respectively, in the case of the chromium(III) complex. The visible region of the spectrum is characterized by the appearance of broad bands at 413.5 and 527 nm due to the d–d type transition.

The electro-chemical properties of the compound were studied in acetonitrile. Since the new Cr^{III} complex is based on a derivative of a fully conjugated and planar ligand, like dppz, the half-wave potentials of the ligand dppz and dicnq are presented in Table 2 along with the $[Cr(phen)_3]^{3+}$ and $[Cr(bpy)_3]^{3+}$ complexes. By analogy with similar complexes, the wave centered at $E_{red} = -0.505$ V vs SCE is associated with the metal localized $Cr^{III/II}$ reduction. It should be noted that the $Cr^{III/II}$ couple in this case is irreversibile, unlike that in the cases of $Cr(bpy)_3{}^{3+}$ and $Cr(phen)_3{}^{3+}$. The irreversible nature of the $Cr^{III/II}$ wave is not surprising, since the $Cr^{III/II}$ nuclear reorganization barrier is expected to be large because of the Jahn–Teller effect in the Cr^{II} species. Moreover, the axial chloride ligand may be replaced by the solvent once Cr^{III} is reduced to Cr^{II}. This, again, would lead to an irreversibility of the $Cr^{III/II}$ reduction wave. The $Cr^{III/II}$ wave reported here is more –ve compared to that observed for $[Cr(bpy)_3]^{3+}$, $[Cr(phen)_3]^{3+}$, and $[Cr(terpy)_2]^{3+}$, reported in the literature, indicating that the corresponding Cr^{II} complex is more reducing. The ligand dicnq is known to show an irreversible reduction wave at −0.66 V. The reduction wave appearing at −0.68 V in the case of the chromium(III) complex is also irreversible, which may be due to the coordinated dicnq ligand. A third quasi-reversible reduction wave has been observed for the complex at −1.304 V, which can be attributed to the $Cr^{II/I}$ couple. No oxidation wave was discernible for the title compound in acetonitrile upto +1.8 V. From the data given in Table 2, we can observe that the electron abstraction from the metal center is more difficult in the title compound. This may be due to the electron-withdrawing nature of the CN groups present in the ligand.

The assignment of the configuration was made with the help of molecular-mechanics (MM) calculations. Molecular mechanics is a well-accepted model used to calculate both the structure and the energies of the metal complexes. The general principles and the inherent limitations of the application of molecular mechanics methods to inorganic and bio-inorganic systems have been immensely discussed.[21,22] Since it is an interpolative method, the results depend on the database to which the force field has been fitted. Strain energies are relative quantities, and hence MM methods provide reasonable estimates of the energy difference between two conformers. The electronic effects are taken into consideration by apparently defining atom types in the ESFF database in terms of the metal-ion charge, the M–L distance and the L–M–L angle, respectively. The same methods were recently employed by us to predict the preferred configuration of diaqua Cr^{III} salhex,[23]

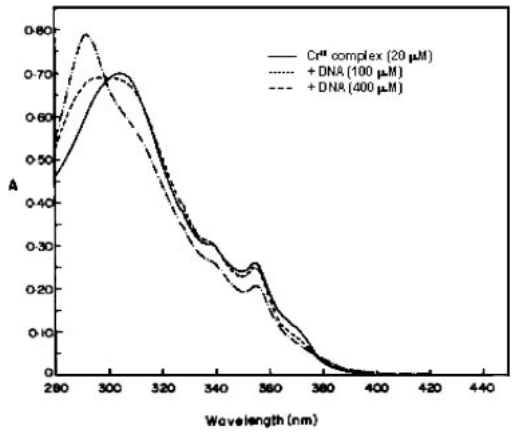

Fig. 2. Absorption spectra of $[CrCl_2(dicnq)_2]^+$ in the absence of (——) and in presence of 100 μM (----), 400 μM (–•–•–) CT-DNA in 10 mM HEPES buffer, pH 7.4.

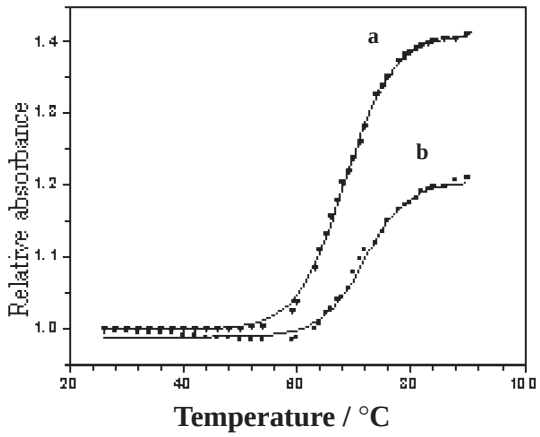

Fig. 3. Denaturation curves of CT-DNA (200 μM) in the absence (a) and in presence of $[CrCl_2(dicnq)_2]^+$ (20 μM) (b) in 10 mM HEPES buffer at pH 7.4.

Mn^{III} salhex[24] and Cu^{II} salen,[25] where salhex is a long-chain Schiff-base ligand and salen is a bis(salicylideneimine) ligand. In the present investigation, we used molecular mechanics to calculate the minimum energy configuration of both the *cis*- and the *trans*-dichloro compounds and the total strain energy for both configurations have been calculated. The calculated energy for the *cis*-configuration was found to be 448 kJ/mol. whereas that for the *trans*-configuration, the minimum energy, was estimated to be 259 kJ/mol. This indicates that $[CrCl_2(dicnq)_2]^+$ prefers the *trans*-configuration over the *cis*-configuration.

The biotoxicity of Cr^{III} complexes have been attributed to the DNA cleaving ability of Cr^{III} complexes in certain ligand environments.[9,10] A coordinated ligand has also been shown to play a role in determining the mode of the binding of the complex to DNA.[9,12] The binding of the synthesized complex to calf thymus DNA has been investigated to understand the preferential role played by 2,3-dicyanodipyrido[3,2-*f*:2′,3′-*h*]-quinoxaline, dicnq ligand in the mode of binding of its Cr^{III} complex to DNA.

DNA Binding Studies. Electronic Absorption Spectra: The absorption spectra of $[CrCl_2(dicnq)_2]^+$ in the presence of different concentrations of calf thymus DNA are depicted in Fig. 2. The absorption maximum at 355 nm due to the π–π^* transition shows a decrease in the peak intensity upon the addition of DNA. A continuous decrease in the absorbance of the chromium complex was followed by saturation at a high concentration of DNA. The absorbance was reduced by 35% in the end. Generally, hypochromism in the electronic absorption spectra is due to a strong interaction between the DNA bases and the chromophore.[26,27] In addition to the decrease in the absorbance intensity, a small red shift of 1–2 nm in the absorbance maximum and an isosbestic point at 376 nm are also observed. These spectral data support the idea that the Cr^{III} complex binds to DNA by an intercalative mode. The binding constant (K_b) were calculated from a linear plot of [DNA] vs $[DNA]/(\varepsilon_A - \varepsilon_F)$, and found to be $(1.2 \pm 0.2) \times 10^3$ M^{-1}. The moderate binding constant for this Cr^{III} complex to DNA is comparable to those observed for similar DNA-intercalating polypyridyl complexes, $[Ru(phen)_2(dicnq)]^{2+}$ and $[Ru(dicnq)_3]^{2+}$, for which the binding constants have been found to be $(3.0 \pm 0.5) \times 10^4$ M^{-1}, $(9.7 \pm 0.5) \times 10^3$ M^{-1}, respectively,[14] but lower than that observed for $[CrCl_2(dppz)_2]^+$, for which the binding constant has been found[28] to be of the order 10^6 M^{-1}. This is because an extended aromatic dppz system binds very strongly to DNA bases.

Denaturation Temperature: A melting-temperature experiment was carried out for calf thymus DNA in a HEPES buffer (pH 7.4) in both the presence and absence of $[CrCl_2(dicnq)_2]^+$ by monitoring the absorbance at 260 nm as a function of the temperature. Thermal denaturation curves for DNA in the presence of 20 μM and the absence of Cr^{III} complex are given in Fig. 3. The T_m of DNA was found to be 69 ± 1 °C under our experimental conditions. Under the same set of conditions, the addition of $[CrCl_2(dicnq)_2]^+$ ([DNA]/[complex] = 10 and 20) increased the T_m value by 4 ± 1 °C. A similar increase in the denaturation temperature of DNA was observed[15] in the presence of $[Ni(phen)_2(dicnq)]^{2+}$. Since the Cr(III) complex exhibits only moderate binding to DNA, as evident from the binding-constant value, only a marginal change in the melting temperature of DNA is expected in the presence of the Cr(III) complex. At higher concentrations of Cr(III) complexes one can expect some significant change in the T_m values of DNA. However, at [DNA]/[Complex] below 8, the solution became turbid due to some precipitation, and hence the T_m value could not be measured under these conditions.

Viscosity Measurements: Photophysical measurements generally provide necessary, but insufficient, results to support an intercalative binding model. In the absence of crystallographic structural data, hydrodynamic measurements, such as sedimentation and viscosity, are regarded as being the least ambiguous and the most critical experiments for the binding model in solution.[29] The intercalation of the ligand to DNA is known to cause a significant increase in the viscosity of a DNA solution due to an increase in the separation of the base pairs at the intercalation site. In contrast, the groove binders cause a less-pronounced change (positive or negative), or no change, in the viscosity of the DNA solution.[30,31] The change in the viscosity of calf thymus DNA upon the addition of $[CrCl_2(dicnq)_2]^+$ is shown in Fig. 4. As the concentration of the complex increases, the relative viscosity increases. Such

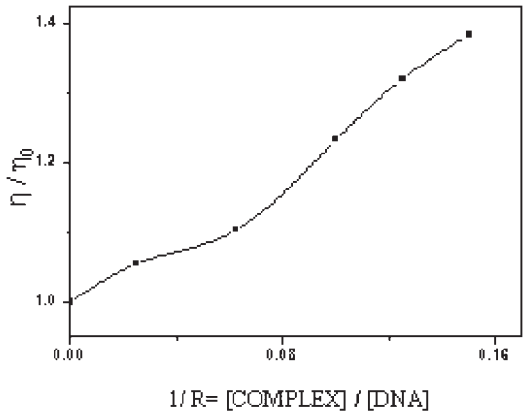

Fig. 4. Effect of increasing amounts of $[CrCl_2(dicnq)_2]^+$ (0–60 μM) on the relative viscosities of CT-DNA (400 μM) at 25 °C.

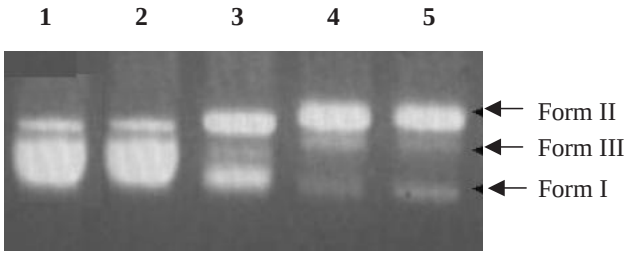

Fig. 5. 0.8% agarose gel electrophoresis of pBR322 plasmid DNA (67.5 ng) in the presence of $[CrCl_2(dicnq)_2]^+$ (14.8 μM), lane 1, plasmid DNA (67.5 ng) with H_2O_2 (12.5 mM), lane 2, plasmid DNA (67.5 ng) incubated with different concentrations of $[CrCl_2(dicnq)_2]^+$, 14.8 μM, 29.6 μM, and 44.4 μM (lanes 3–5) respectively in the presence of H_2O_2 (12.5 mM).

a behavior is similar to that observed for intercalators (ethidium bromide, $[Ru(phen)_2(dppz)]^{2+}$).[32,33] The viscosity values suggest an intercalative mode of binding of the $[CrCl_2(dicnq)_2]^+$ complex to DNA. However, it is interesting to note that in the presence of Cr(III)–Schiff base complexes the viscosity of DNA shows a concentration dependence on the metal complex, and they show a biphasic mode of binding to DNA.[10] The design of the planar, extended aromatic system drastically changes the nature of binding of the complex to DNA.

Electrophoresis: The DNA cleavage activity by cationic $[CrCl_2(dicnq)_2]^+$ was examined by 0.8% agarose gel electrophoresis using supercoiled pBR322 plasmid DNA. The gel pattern is shown in Fig. 5. The incubation of plasmid DNA with the Cr^{III} complex (lane 1) did not result in any DNA cleavage. Similarly, the incubation of plasmid DNA with hydrogen peroxide (H_2O_2) without the Cr^{III} complex also did not result in DNA cleavage (lane 2). However, the incubation of plasmid DNA containing different concentrations of $[CrCl_2(dicnq)_2]^+$ in the presence of H_2O_2 promoted the conversion of supercoiled form (Form I) to the nicked circular form (Form II) and the linear form (Form III) while increasing the concentrations of the complex (lanes 3–5). It is of interest to note that Cr^{III} complexes like $[Cr(bpy)_3]^{3+}$, $[Cr(salen)(H_2O)_2]^+$, and $[Cr(salprn)(H_2O)_2]^+$ have been shown to bring about DNA

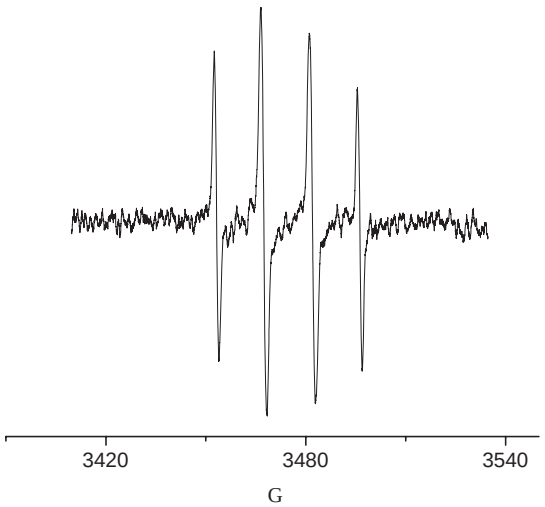

Fig. 6. ESR spectrum recorded for the mixture containing 1.5 mM $[CrCl_2(dicnq)_2]^+$, 2.5 mM H_2O_2 and 15 mM DMPO in 10 mM HEPES buffer at pH 7.4. The spectrometer settings were: receiver gain, 1.12×10^6; modulation amplitude 1 G; field 3472 ± 100 G.

damage in the presence of peroxide and hydroxyl radicals have been implicated in their observed DNA cleaving action.[10,34] In order to ascertain the formation of radicals produced during the reaction of the Cr^{III} complex and H_2O_2, which in turn is responsible for DNA cleavage, a spin-trapping ESR experiment was carried out using 5,5-dimethyl-1-pyrroline *N*-oxide, (DMPO) as the spin-trapping agent. Figure 6 shows a typical spin adduct ESR spectrum obtained from a mixture of the Cr^{III} complex, H_2O_2 and DMPO. The hyperfine splittings of the spectrum are very characteristic of DMPO/$^\bullet$OH, and are representative of virtually identical coupling of free electrons to the nitroxide nitrogen and beta hydrogen of the pyrroline ring ($a_N = a_H = 14.9$ G respectively). However, the intensity of the spectrum is not 1:2:2:1 as expected for the intensity of the DMPO/$^\bullet$OH signal. It is possible that some other radical species is also produced that stems the spectrum. H_2O_2 alone in the presence of DMPO did not generate any detectable DMPO/$^\bullet$OH signal. The results from the above study suggest that the cleavage mechanism of plasmid DNA in the presence of the Cr^{III} complex follows radical generation from hydrogen peroxide. The electrophoretic mobility of DNA is determined by many factors, including the molecular weight, shape and charge. An alteration of any of these factors may affect the migration of DNA through the gel. The intercalation and covalent binding of molecules to DNA has been shown to retard the electrophoretic mobility of DNA.[35] The retardation in the mobility observed in our gel pattern again confirms the intercalative binding of the Cr^{III} complex to DNA.

Molecular Modeling: In order to support the experimental results, molecular modeling was carried out for the binding of *trans*-$[CrCl_2(dicnq)_2]ClO_4$ with the Dickerson model. The complex was positioned in all possible modes of the binding viz. minor groove, major groove, and intercalation through both the minor and major groove. The optimized energies for the Dickerson model, *trans*-$[CrCl_2(dicnq)_2]ClO_4$ and DNA-complex have been calculated. The computed binding

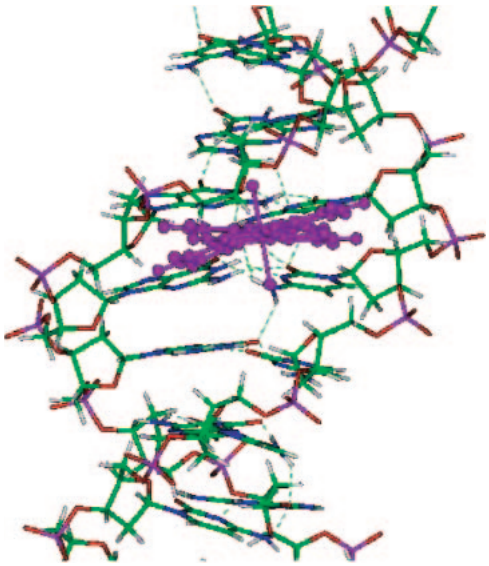

Fig. 7. Optimized structure of $d(GC)_{12}$ with $[CrCl_2(dicnq)_2]^+$ interacted in between the base pairs through minor groove.

energies suggest that intercalation through the minor groove is more favorable. In order to find the sequence specificity of the complex, the interactions of the Cr^{III} complex with $d(GC)_{12}$ and $d(AT)_{12}$ have been studied. The complex prefers $d(GC)_{12}$, and the binding energy was found to be 307.3 kJ/mol. The root-mean-square deviation (RMSD) with respect to all atoms between the normal and intercalated model of all the sequences was calculated by superimposing the optimized structures before and after interaction with the complex. The RMSD values for each dodecamer namely, Dickerson, $d(GC)_{12}$ and $d(AT)_{12}$ with the complex interacted through minor groove, were found to be 0.811, 0.966, and 0.367, respectively, with respect to all atoms. These observations indicate that the metal complex brings about more distortion and flanking of the base pairs in $d(GC)_{12}$ than in the Dickerson and $d(AT)_{12}$ models, and hence more RMSD for $d(GC)_{12}$. From a molecular-mechanics calculation, it is clear that the complex prefers the intercalative mode of binding through the minor groove and the GC sequence specificity. The optimized structure of the Cr^{III} complex with $d(GC)_{12}$ intercalated through the minor groove is shown in Fig. 7. Similar results have been reported for Δ-$[Ru(phen)_2dppz]^{2+}$ and Δ-$[Ru(phen)_2dpq]^{2+}$ (dpq = dipyrido quinoxaline), binding to dodecanucleotide from NMR and modeling studies.[36,37] The dpq ligand differs from dppz only by an aromatic ring on the quinoxaline moiety. The Δ-$[Ru(phen)_2dppz]^{2+}$ complex may bind to DNA with several intercalative geometries, including an asymmetric binding mode, where the complex is canted toward one strand.[5] It is possible that the dppz ligand allows a significant aromatic–aromatic overlap, whereas the shorter dpq ligand is forced to bind in a more "head-on" fashion, which is favored from the minor groove.

Conclusion

We report here the synthesis, spectral characterization and DNA binding and cleavage of $[CrCl_2(dicnq)_2]^+$. The above observations indicate that the complex binds to calf thymus DNA by intercalation with a planar dicnq ligand stacked between the base pairs of the DNA. The changes in the absorption spectra of $[CrCl_2(dicnq)_2]^+$ by the addition of DNA, increase in the melting temperature, and the changes in the viscosity show that the complex binds to DNA by intercalation. The electrophoretic mobility of plasmid DNA is retarded as the complex concentration increases, confirming the intercalative mode of the binding of the complex to DNA. The complex exhibits an effective cleavage of pBR322 DNA in the presence of H_2O_2. The cleavage mechanism involves radical generation by the complex in the presence of H_2O_2. The molecular modeling studies of the sequence specificity and the interaction of the Cr^{III} complex further support that the complex binds to DNA by intercalation through the minor groove, and prefers GC base pairs than AT base pairs.

The authors thank Central Drug Research Institute, Lucknow for providing FAB mass spectrum. RV thanks CSIR, Delhi for a research fellowship.

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