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Interaction of chromium(III) complex of chiral binaphthyl tetradentate ligand with DNA

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Abstract—Since conformation of the molecule plays a vital role in the activity of drug, we have investigated the DNA interaction of a chromium(III) complex with ligands in two conformations. Chromium(III) complexes derived from chiral binaphthyl Schiff base ligands, viz. *R*- and *S*-2,2′-bis(salicylideneamino) 1,1′-binaphthyl, have been synthesized and characterized by mass, IR, and electronic spectra. The interaction of these *R*- and *S*-binaphthyl Schiff base chromium(III) complexes with CT–DNA was investigated with the goal of examining whether the chirality has an influence on the chromium(III)–DNA binding properties. The difference in chirality of the ligand did not show any striking difference in binding properties. The binding constants for *R* and *S* conformers were estimated to be 18 (± 0.4) $\times 10^3$ and 9.4 (± 0.3) $\times 10^3$ M^{-1}, respectively, through spectroscopic titrations. All the experimental results are suggestive that both the isomers are DNA groove binders. The results of steady-state as well as time-resolved fluorescence experiments, however, suggest that the *R* conformer has restricted mobility when bound to DNA because it is more deeply buried in the groove of DNA compared to the *S* isomer.

1. Introduction

Binding studies of small molecules to DNA on a molecular level are very important in the development of novel chemotherapeutics and highly sensitive diagnostic agents.[1–6] The interaction of transition metal complexes with DNA has attracted much attention during past decade. The metal complexes can interact non-covalently with nucleic acids by intercalation when the ligand contains planar ring systems, groove binding for large molecules, or external electrostatic binding for cations. The binding modes are dependent on the sizes and stereochemical properties of the metal complexes. In order to understand the role of ligand structure in determining the biotoxicity of chromium(III), study on the influence of ligands on the interaction of chromium(III) complexes with DNA is being investigated by us.[7–14] The precise roles played by chromium(III) in the biotoxic functions of the metal ion are difficult to analyze and also the transport of several chromium(III) complexes into the cells may be difficult under physiological pH conditions. The interaction of chromium(III) Schiff base complexes, $[Cr(salen)(H_2O)_2]^+$ where salen = *N*,*N*′-ethylenebis(salicylideneimine) and $[Cr(salprn)(H_2O)_2]^+$ where salprn = *N*,*N*′-propylenebis(salicylideneimine) with calf thymus DNA, has already been reported.[7] These chromium(III) Schiff base complexes have been shown to bring about apoptosis in lymphocyte cell cultures[15] as well as protein damage in the presence of peroxide.[16]

The design and synthesis of chiral ligands that upon coordination with metal ions can induce high stereoselectivity for organic transformations constitute an important issue in modern coordination chemistry.[17] These chiral centers add a new dimension and open up interesting perspectives for synthetic active site analogues of metal enzymes. The DNA double helix has a chiral structure, and complexes with enantiomeric amines as ligands may therefore lead to different diastereomeric interactions with DNA.[18,19] A simple method to introduce chiral moiety into the ligand scaffold is to use the 1,1′-binaphthylene residue. Recently, there has been increasing interest in utilizing a 'hybridized' form of binaphthyl ligands and salens, that is, binaphthyl Schiff base ligand as a chiral auxiliary for metal-mediated stereoselective catalytic processes. Aux-

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iliary disymmetric 1,1′-binaphthyl moiety has proved to be a highly desirable asymmetry-inducing unit because of its structural rigidity, resistance to racemization and, above all, effectiveness of chiral recognition.[20]

In continuation of our studies on synthesis, characterization, and structure–reactivity correlation of Cr(III) complexes of 'salen' type ligands, [21,22] we extended our research to the influence of chirality of Cr(III) complexes on their DNA binding properties. The binaphthyl Schiff base ligands bearing a C2-chiral auxillary have been employed for a number of metal-catalyzed asymmetric reactions.[23–27] There are reports on the enantioselective binding of metal complexes to DNA where asymmetry is induced after the formation of metal complexes[4,28,29] but very few reports have appeared on the interaction of ligand-based chiral complexes with DNA.[30] In the present investigation, we have attempted an elucidation of the binding strength of a chiral binaphthyl Schiff base chromium(III) complex with natural polynucleotide. Our approach is to investigate the enantioselectivity of *R* and *S* enantiomers of chiral Schiff base complexes during the interaction of calf thymus DNA. The various techniques used are absorption, steady-state emission, time-resolved fluorescence and CD spectroscopic studies, and viscosity measurements.

2. Results and discussion

2.1. On synthesis

Treatment of chiral 2,2′-diamino-1,1′-binaphthyl with salicylaldehyde afforded a pure chiral binaphthyl Schiff base ligand in good yield. The free ligand could be obtained as yellow needles and reacted with chromium(II) perchlorate. The pure complexes could be obtained easily for both (*R*)-(+) and (*S*)-(−) conformers using an appropriate ligand and through recrystallization. Like the Schiff base ligands salens, chiral binaphthyl Schiff base ligands usually function as a dianionic, tetradentate N_2O_2 ligand in forming complexes with metal ions. The proposed formula of the diaqua chromium(III) complex was consistent with the elemental analysis based on the percentage of C, H, N, and Cr. The complex was isolated with one ligand per chromium(III) and one anion. The authenticity of the complex was further confirmed by recording the mass spectrum. Previous reports[31] on the mass spectra of similar type of diaqua complexes show that the molecular ion for these complexes is with loss of two water molecules. Thus, the mass of the parent ion is $M-2H_2O = 538$, which was observed as the base peak in the mass spectrum. Octahedral complexes of the Schiff base derived from diamino-binaphthyl and salicylaldehyde are known to prefers diaquo-cis-configuration.[17] Since no good quality single crystals for these chiral binaphthyl Schiff base complexes could be grown, in order to arrive at the structure of the molecule, molecular modeling has been carried out. Energy optimization studies show that cis-configuration of the Cr(III) complex is more stable than the trans-configuration by 40 kcal/mol. This is in contrast to the corresponding metal complexes of salens, in which case, the planar N_2O_2 arrangements can be readily maintained and the trans-diaqua-octahedral species are very common.[21,22] Since the synthesized complex prefers a cis-configuration, one can expect Δ and λ conformations for both the complexes (with ligands having *R* and *S* conformations). However, we were unable to separate the two conformers.

2.2. Electronic absorption

The absorbance spectra of both *R* and *S* conformers consist of transitions associated with both 1,1′-binaphthyl and salicylidene chromophores. Both the complexes exhibit multiple absorbance between 200 and 800 nm. The absorbance at 238 and 305 nm is mostly due to π–π^* transitions of the binaphthyl moiety. The multicomponent bands at 272 and 276 nm may be attributed to a salicylidene chromophore and the absorbance at 358 nm may be due to the charge transfer transitions. The broad d–d band observed at 589 nm may be assigned to $^4A_{2g}$–$^4T_{2g}$ transition. The corresponding band for salen type homologue Cr(III) complexes appeared in the same spectral range viz. 560–590 nm.[21] The compounds are electrochemically active and the Cr^{III}/Cr^{II} reduction potential for *R* and *S* enantiomers is found to be − 0.824 and −0.867 V (vs SCE), respectively.

In order to compare quantitatively the affinity of each enantiomer toward DNA, the absorption spectra were recorded for a fixed amount of the complex with varying amounts of DNA. The absorption spectral band at 305 nm showed hypochromism in the presence of DNA and there was no appearance of redshift or the appearance of an isosbestic point. Figure 1 shows a plot of $[DNA]/(\varepsilon_A - \varepsilon_B)$ against [DNA] for *R* with a straight line. The binding constant K_b was calculated as 18.0 $(\pm 0.4) \times 10^3\ M^{-1}$ for *R* and 9.40 $(\pm 0.3) \times 10^3\ M^{-1}$ for *S* enantiomers, respectively. The observed binding constants have been found to be higher than that for the parent compound, $[Cr(salen)(H_2O)_2]^+$, for which K_b has been reported to be 2.5 $(\pm 0.4) \times 10^3\ M^{-1}$.[7] The *R* enantiomer binds two times more strongly than the *S*

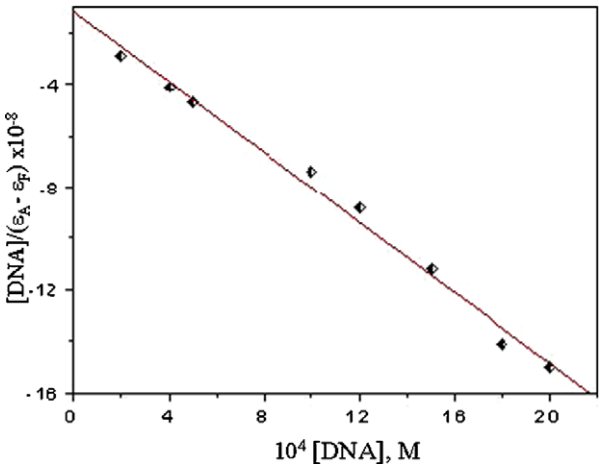


Figure 1. Plot of [DNA] versus $[DNA]/(\varepsilon_A - \varepsilon_F)$ for Cr(III) complex of *R* conformer.

enantiomer. The analogue $[Cr(salen)(H_2O)_2]^+$ complex has previously been shown to bind to the minor groove of DNA.[9] Hence, it is reasonable to assume that the binaphthyl Schiff base chiral complex described here too preferentially binds to the minor groove of DNA. To obtain further support for the DNA binding mode, the binding energies of the complex were calculated with the Dickerson sequence, $d(GCGCATATGCGC)_2$. Since energy minimization studies show the cis-isomer to be more stable than the trans-isomer, the cis-complex was manually docked at different sites of the B-form DNA. Several starting geometries for intermolecular complexes were selected by a structure-based docking strategy and by considering all possible steric factors. The complex was positioned in all possible modes of the binding viz. minor groove, major groove, and intercalation through both the minor and major grooves. After docking minimizations were performed on the intermolecular complexes to remove short contacts. The results presented in this study are only for DNA–metal complexes that have a positive binding energy. It is evident from previous reports on the interaction of various Cr(III) complexes with DNA that extensible systematic force field (ESFF) provides reliable estimates of the binding energy, and a possible comparison with the experimental results can be rationalized. The binding energies computed for various modes of interaction of chromium complexes with DNA in the B conformation are given in Table 1. The values presented in Table 1 suggest that binding of the complex through the minor groove of DNA is more favorable by 11 kcal/mol (Fig. 2). The other noteworthy feature is that the energy for the binding of the *S* chiral metal complex with the Dickerson sequence is less than that of *R* chirality. The intercalation mode of interaction is not at all favorable for these complexes. It is possible that the diaquo complex allows a significant aromatic–aromatic overlap, whereas the complex with a shorter binaphthyl ligand is forced to bind in a more 'head-on' fashion, which is favored from the minor groove.

Table 1. Calculated binding energies of Cr complex with polynucleotides through molecular modeling

Conformation	Mode	Binding energy (kcal/mol)
R	Major groove	28.87
	Minor groove	46.11
S	Major groove	27.43
	Minor groove	34.86

2.3. Steady-state emission

The fluorescence emission spectra are sensitive to changes in the fluorophores' environment. Both *R* and *S* enantiomers emit at 495 nm when excited at its charge transfer band (375 nm). Addition of DNA causes a gradual increase in a fluorescence intensity and emission maximum also shifts by 5 nm to a longer wavelength (500 nm). The results of the emission titration for *R* and *S* conformers are illustrated in Figure 3. The emission intensity of *R*-[Cr-(binaphthyl SB)$(H_2O)_2]^+$ in HEPES buffer increased much more significantly than for *S*-[Cr-(binaphthyl SB)$(H_2O)_2]^+$. This effect arises because the metal complex is bound in a relatively nonpolar environment compared to water in the presence of DNA. However, the increase in fluorescence intensity is less than that for the intercalators.[32–34] The Stern–Volmer plots of the emission curve go upward with increase of DNA concentration. From the figure

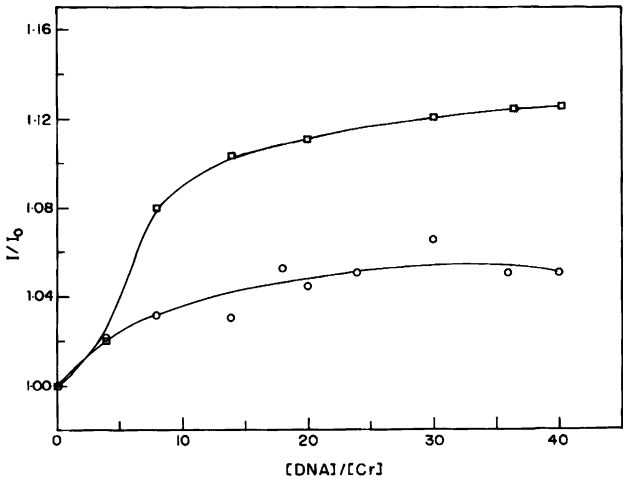


Figure 3. Plot of emission intensity versus [DNA]/[Cr] ratio *R* (□) and *S* (○) enantiomers of Cr(III) complex.

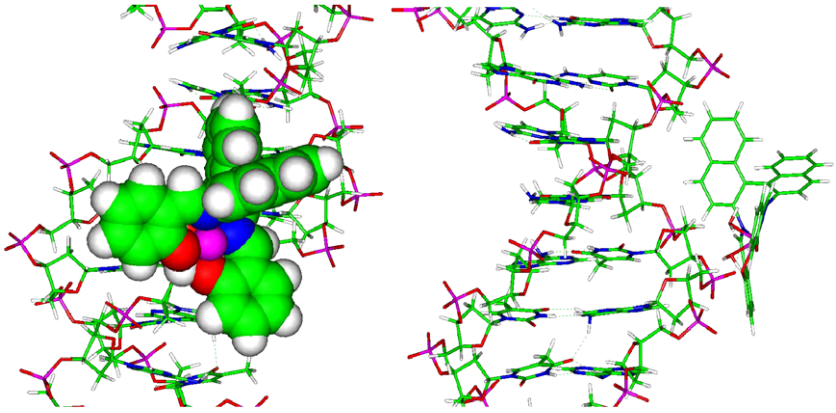


Figure 2. Optimized structure of Dickerson sequence with *R* enantiomer interacted in between the base pairs through the minor groove.

it is observed that the luminescence intensity of *R* conformer grows much more quickly in comparison with the *S* conformer in the presence of DNA, implying a relatively weak association to DNA in the case of *S* conformer. The emission enhancement and lifetime increase are related to the extent to which the complex gets into the hydrophobic environment inside the DNA and avoids quenching effects of solvent water molecules. The emission intensity grows up to 1.12 for *R* and 1.02 for *S* conformers and saturates when the DNA/[Cr] ratio reaches 20. The relatively weaker association of *S* conformer may be explained by visualizing the *R* conformer to be more deeply buried in the DNA groove, compared to the *S* conformer. As a result, the excited state of *S* conformer will be more accessible to a solvent water molecule compared to the excited state of *R* isomer. This fact is also evident from the minor differences in their emission behavior in the presence of DNA.

2.4. Time-resolved emission

The lifetime measurements were monitored by single-photon counting. Each enantiomer was excited at 375 nm and the lifetime emission was measured at 495 nm. The luminescence of *R* and *S* enantiomer shows mono-exponential decay in the absence of DNA and the lifetime has been found to be 0.303 for *R* and 0.301 ns for *S* enantiomer. In the presence of DNA, the chiral complexes showed bi-exponential emission decay. This clearly indicates that along with the Cr(III)–DNA species, free Cr(III) complex is also present in the solution. One of the lifetimes extracted from the decay profile of both the isomers matched the values for the free Cr(III) complex (0.3 ns) and the value remained the same irrespective of the DNA concentration. The second lifetime value extracted from the decay profile of *R* isomer increased from 6.02 to 10.8 ns as the concentration of DNA was varied from 400 to 1500 μM. In the case of *S* isomer, a similar behavior was obtained with the lifetime value increasing from 4.4 to 4.81 ns. A plot of τ/τ_0 shown in Figure 4 reveals that for the *R* isomer the value of τ/τ_0 increases from 5 to 28 as the [DNA]/[Cr] ratio increases from 5 to 30. Any further increase in [DNA]/[Cr] ratio leads to only a marginal increase in τ/τ_0 value. In the case of *S* isomer, the value of τ/τ_0 increases only marginally with increase in [DNA]/[Cr] ratio. These results are in agreement with the steady-state emission results where the emission intensity of the *R* isomer has been found to increase more in the presence of DNA than that of *S* isomer. The higher lifetime (10.8 ns) of the *R* isomer compared to that of the *S* isomer (4.81 ns) in the presence of DNA indicates that the *R* isomer is buried more deeply in the groove than the *S* isomer.

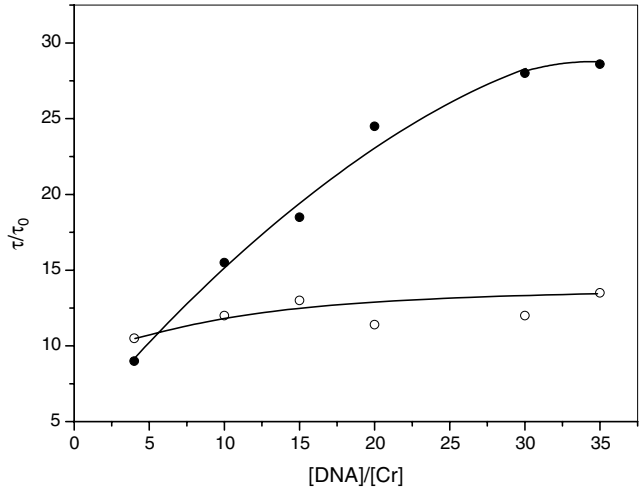


Figure 4. Plot of lifetime versus [DNA]/[Cr] ratio *R* (●) and *S* (○) enantiomers of the Cr(III) complex.

2.5. Circular dichroism

The CD spectrum is known to be very sensitive to the environment of the metal complex. The appearance of induced CD for optically inactive complexes or changes in intrinsic CD of chiral molecules reflect the binding geometry and binding mode of the complex as well as the DNA bases. The Cr(III) complexes with *R* and *S* enantiomer ligands exhibit an intrinsic CD spectrum and the spectrum is expected to change upon the addition of DNA. The CD spectra of plain DNA solutions were subtracted from that of the DNA/Cr(III) mixture for ease of comparison. Figure 5 depicts the CD spectra of *R* and *S* isomers in the presence of 200 μM DNA. The CD spectra of two complexes as expected are mirror images of one another. The Cr(III) complex of *R* enantiomer exhibits CD spectra with a positive band at 296 nm and a negative band at 246 nm, and in the case of *S* isomer, the positive band occurs at 246 nm and negative band at 296 nm. In the presence of DNA, the 246 nm band is perturbed more in the case of *R* isomer. This shows that the *R* isomer interacts more strongly with DNA, when compared to the *S* isomer.

2.6. Viscosity measurements

Photophysical probes generally provide necessary but insufficient clues to support intercalative binding model. Hydrodynamic methods that are sensitive to DNA

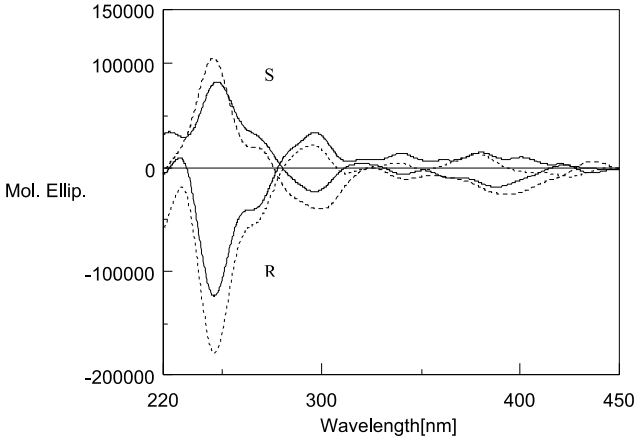


Figure 5. CD spectra of *R* and *S* enantiomers (25 μM) in 10 mM Hepes buffer pH 7.0, in the absence of (solid curves) and in the presence of (dashed curves) DNA (200 μM). The CD spectrum of CT–DNA was subtracted from that of the corresponding mixture for easy comparison.

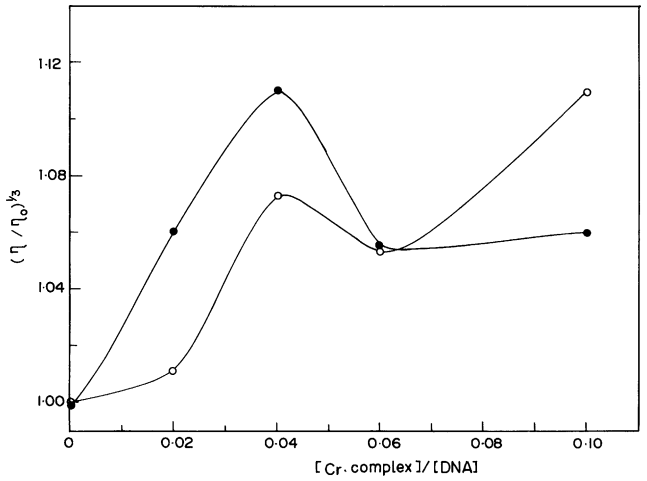


Figure 6. Effect if increasing amounts of *R* (●) and *S* (○) enantiomers of the Cr(III) complex on the relative viscosity of CT–DNA (500 μM) at 25 (±0.5) °C.

length changes are the most critical test of the classical intercalation model and, therefore, offer the most definitive method of inferring the binding mode of DNA binding agents. Intercalating agents are expected to destack the base pairs causing lengthening of the double helix resulting in an increase in the viscosity of DNA. In contrast, non-classical intercalation or groove binding of the complex could bend or kink the DNA helix, reduce its effective length and concomitantly its viscosity.[35] The effect of *R* and *S* enantiomers of the Cr(III) complexes on the viscosity of DNA is depicted in Figure 6 and it shows that there are at least two phases of binding between the Cr(III) complex and DNA. At lower loading of the *R* enantiomer, the viscosity increases and then decreases at higher loading of the complex. The viscosity data suggest lengthening of the DNA base pairs at a lower metal complex concentration but when the complex concentration is increased the helix might be kinked or bend and hence decrease in the viscosity. The effect of increase in the viscosity of DNA in the presence of *S* enantiomer is comparatively lesser than the *R* enantiomer at a lower concentration of the complex. At higher loading ([complex]/[DNA] = 0.1), the *S* complex increases the viscosity of DNA which is in contrast with the *R* enantiomer. This could be explained in terms of a bridged structure to promote the extension of duplexes. Here, the binding may involve an inner sphere complex formation via phosphate oxygen. It would indicate that the binding of Cr(III) complex with DNA could be surface binding or by forming bridged adducts. Similar results have been reported for Ru(II) and Cu(II) complexes[36] which bind to DNA by a non-intercalative mode.

3. Conclusion

We report here the synthesis and characterization of chromium(III) complexes derived from chiral binaphthyl Schiff base ligands, viz. *R* and *S*-2,2′-bis(salicylideneamino) 1,1′-binaphthyl ligands and their DNA binding properties. Both the complexes of *R* and *S* enantiomers bind to the minor groove of DNA. Even though the two complexes have enantiomeric Schiff base as ligands, the complexes did not show any striking differences in their DNA binding behavior. Steady-state as well as time-resolved emission studies suggest that *R* isomer is more deeply buried in the minor groove of DNA, when compared to the *S* isomer. This observation is supported by molecular modeling studies, which reveal that binding energy of *R* isomer to the minor groove of DNA is greater than that of *S* isomer by 11 kcal/mol.

4. Experimental

4.1. Materials

All reagents and solvents were purchased commercially (AR grade) and were used as received unless otherwise noted. HEPES, Tris, agarose, and ethidium bromide were purchased from SRL, Mumbai. Calf thymus DNA, (*R*)-2,2′-diamine-1,1′-binaphthyl, (*S*)-2,2′-diamine-1,1′-binaphthyl and dialysis tubings were received from Sigma (USA). Cellulose acetate membrane filter (0.45 μm) was obtained from Sartorius, Germany. The stock solution of DNA was prepared by dissolving DNA in 10 mM HEPES buffer at pH 7.0, dialyzing exhaustively against the same buffer for 48 h, and filtering using a membrane filter. The solution gave an absorption ratio of >1.8 at A_{260}/A_{280}, indicating that the DNA was sufficiently free from protein.[7] The concentration of DNA was determined by monitoring the UV absorbance at 260 nm using $\varepsilon_{260} = 6600\ cm^{-1}$. The stock solution was stored at −20 °C. All experiments were carried out in 10 mM HEPES buffer at pH 7.0 in triply ionized Milli-Q water. The stock solutions of Cr(III) complexes were prepared in water and their concentrations estimated by the wet oxidation method.[37]

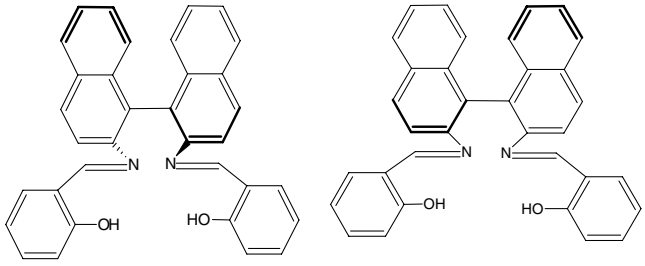
4.2. Instrumentation

Absorbance spectra were recorded at room temperature using a Perkin-Elmer Lambda 35 double-beam spectrophotometer. The elemental analysis was performed using Heraeus-CHN-Rapid Analyzer at RSIC, IIT, Madras. The electrospray ionization (ESI) mass spectrometric analysis was carried out on a MicroMass Quattro II triple quadrupole mass spectrometer. The ESI capillary was set at 3.5 kV and the cone voltage 40 V. The spectrum was collected in six scans. Infrared spectra of the ligand and complex were recorded as KBr pellets using a Perkin-Elmer RX-1 model FT-IR spectrophotometer. Emission spectra were recorded with a Perkin-Elmer LS 45 luminescence spectrometer. Time-resolved emission 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 CD spectra were measured on a Jasco-715 spectropolarimeter. Viscosity measurements were carried out using an Ostwald-type viscometer (1 mL capacity) maintained at a constant temperature of 25 ± 0.5 °C in a thermostat bath. Cyclic voltammetric investigations for these chiral complexes

have been carried out in dimethylsulfoxide employing *tert*-butyl ammonium perchlorate (TBAP) as the supporting electrolyte. A Princeton Applied Research (PAR) potentiostat interfaced to a universal programmer has been employed along with platinum working, platinum counter, and standard calomel electrodes.

4.3. Synthesis of binaphthyl Schiff base ligand, viz. 2,2′-bis(salicylideneamino) 1,1′-binaphthyl

The chiral ligands were prepared by refluxing commercially available (*R*)-(+)-2,2′-diamine 1-1′-binaphthyl (1 mmol) or (*S*)-(−)-2,2′-diamine 1-1′-binaphthyl (1 mmol) and salicylaldehyde (2 mmol) in ethanol for 2 h.[38] The yellow precipitate was collected by filtration and dried in vacuum. The ligand was characterized by IR and CD spectra, and the spectral data were matched with literature values.[39] The molecular structures of the chiral ligands used in this study are shown below:



(**R**) conformer (**S**) conformer

4.4. Synthesis of *cis*-diaqua-2,2′bis(salicylideneiminato) 1,1′-binaphthyl chromium(III) perchlorate dihydrate, *cis*-[Cr(binaphthyl Schiff base)$(H_2O)_2$]$ClO_4 \cdot 2H_2O$

The chromium(III) complexes were prepared by a direct reaction of 1 mmol of hexaaqua chromium(II) with 1 mmol of the chiral *R* or *S* binaphthyl Schiff base ligand dissolved in methanol under nitrogen atmosphere. During the addition of Cr(II) solution to the ligand, the reaction mixture turned brownish green. The Cr(II) complex thus obtained was then air oxidized to get Cr(III) complex. The volume of the reaction mixture was reduced to one-fourth of its original volume by rotary evaporation and the resulting mixture was allowed to cool to 0 ° C. The crude brownish green product thus obtained was dissolved in 0.05 M $HClO_4$ and heated to 50 ° C. The solution was filtered and allowed to cool to room temperature. It was dried and stored in a $CaCl_2$ containing desiccator. The pure, dried product was analyzed for $[Cr(C_{34}H_{18}N_2O_2)(H_2O)_2]ClO_4 \cdot 2H_2O$. Calcd: C, 57.46; H, 3.66; N, 3.94, and Cr, 7.32. Found: C, 57.34; H, 3.50, N, 3.90; and Cr, 7.12. The authenticity of the compound was confirmed by elemental and mass spectral analyses.

4.5. DNA binding studies

The electronic spectra of Cr(III) complex in the presence and absence of DNA were recorded. The binding constant for the interaction of Cr(III) complex with DNA was obtained from absorption titration data. The titration was performed by keeping the concentration of the Cr(III) complex constant (50 μM) with increasing amounts of DNA (0–2 mM). A reference blank with DNA solution was used to eliminate the absorbance of DNA. The intrinsic binding constant K_b was determined according to the Eq. 1.[40]

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

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

4.6. Molecular modeling

Molecular modeling was carried out to confirm the geometry of the metal complex and its mode of binding to DNA. Molecular modeling was performed on a Silicon Graphics O2 workstation using the Biosym Modeling package from Molecular Simulation Inc. (SanDiego, CA). The B-DNA system chosen for the study was the dodecamer duplex of sequence d(CGCGAATTCGCG)2 and constructed using the Biopolymer program of the Insight II package. Models of both the cis- and the trans-configurations of diaqua-2,2′bis(salicylideneiminato) 1,1′-binaphthyl chromium(III) perchlorate 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 metal complexes and each metal/B-DNA complex were subjected to minimization using ESFF with a non-bonded cutoff of 10 Å and a sigmoidal distant-dependent dielectric function ($\varepsilon = 4r_{ij}$), which had been demonstrated to be an appropriate implicit treatment for the dielectric function in computing the electrostatic potential of nucleic acid. The charge of the complex was fixed as unipositive. In the energy minimization, the geometry of the whole complex was then refined until convergence (criterion of root mean square (rms) energy gradient of 0.01 kcal/mol per Å) was reached throughout. Electrostatic and van der Waals interactions were the dominant interactions, hydrogen bonding interactions having only a minor role. The interaction energy of the 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:

$$IE = (TE) - (\text{Sum of individual energy})$$

$$BE = -IE$$

where IE is the interaction energy, TE the total energy of the Cr–DNA complex, and BE the binding energy.

4.7. Circular dichroism

Circular dichroic (CD) spectra of *R* and *S* enantiomers of chromium(III) complex in the presence and absence

of DNA were recorded at 25 °C using a 0.1 cm path length cuvette cell. The spectra were recorded for each enantiomer (25 μM) in the presence of 200 μM DNA in the 220–500 nm region. The CD spectra of corresponding DNA were subtracted from that of the chromium(III) complex–DNA mixture for ease of comparison.

Emission spectra were recorded for a solution of the free enantiomer or a solution containing the enantiomer bound to DNA. Each enantiomer was excited at 375 nm and the emission intensity was monitored at 495 nm. The concentration of the complex was fixed (50 μM), while the DNA concentration was varied from 200 to 2000 μM. For the time-resolved fluorescence measurements, the sample was excited at 375 nm and the emission decay was monitored at 495 nm using a MCP-PMT (Hamamatsu-C 4878) detector. Decay traces were deconvulated using a nonlinear least-squares analysis using IBH software. The lifetimes of the *R* and *S* Cr(III) complex, (40 μM), were measured in the presence of different amounts of DNA (200–1500 μM), keeping [Cr(III) complex] constant (40 μM).

4.8. Viscosity measurements

Viscosity measurements were carried out using an Ostwald-type viscometer, thermostated in a water bath maintained at 25 ± 1°C. The viscosity for DNA was measured in the presence and absence of *R* and *S* enantiomers. The DNA concentration was maintained at 500 μM, while the complex concentration varied (10, 20, 30, and 50 μM). Flow time was measured with a digital stopwatch and each sample was measured three times and an average flow times was used. 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 $1/R$, where R = [DNA]/[complex].

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