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ORIGINAL PAPER

Spectrophotometric Study on the Binding of Two Water Soluble Schiff Base Complexes of Mn (III) with ct-DNA

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Abstract In this work, binding of two water soluble Schiff base complexes: Bis sodium (5-sulfosalicylaldehyde) o- phenylendiiminato) Manganese (III) acetate (Salophen complex) and Bis sodium (5-sulfosalicylaldehyde) 1, 2 ethylendiiminato) Manganese (III) acetate (Salen complex) with calf thymus (ct) DNA were investigated by using different spectroscopic and electrometric techniques including UV-vis, Circular dichroism (CD) and fluorescence spectroscopy, viscommetry and cyclic voltammetry (CV). Both complexes have shown a hyperchromic and a small bathochromic shift in the visible region spectra. A competitive binding study showed that the enhanced emission intensity of ethidium bromide (EB) in the presence of DNA was quenched by the addition of the two Schiff base complexes indicating that they displace EB from its binding site in DNA. Moreover structural changes in the CD spectra and an increase in the CV spectra with addition of DNA were observed. The results show that both complexes bind to DNA. The binding constants have been calculated using fluorescence data for two complexes also K_b was calculated with fluorescence Scatchard plot for Salophen. Ultimately, the experimental results show that the dominant interactions are electrostatic while binding mode is surface binding then followed by hydrophobic interactions in grooves in high concentration of complexes.

Keywords Schiff base · ct-DNA · UV-Vis · Circular dichroism · Scatchard plot · Fluorescence

Introduction

Over the past decade, there has been substantial interest in metal complexes binding to DNA in order to develop novel probes for DNA structure determination and new therapeutic reagents that are potentially useful in molecular biology and in the design of putative drugs [1–3].

Specifically, the interactions among DNA and transition—metal complexes that contain multi dentate planar aromatic ligands are currently an active area of research. Complexes of particular interest are Schiff bases that may cause binding or cleavage of DNA [4–8]. Many factors affect the affinity and selectivity of Schiff bases binding to DNA [9]. The complexes should be stable and inert in biological environment, also soluble in aqueous media. These requirements are fulfilled by Planar, aromatic and charged Schiff bases [10–12]. These compounds have specific spectral characteristics that enable us to follow their binding process with DNA, such as, marked changes in their UV-Vis and fluorescence spectra, due to the excitation of the charge transfer transitions [13]. These properties together with the analysis of thermal behavior by viscometry and cyclic voltammetry of DNA in the presence of these complexes provide a convenient way for monitoring the binding process. Binding of the complexes could occur at different types of sites in the DNA molecule, such as: (i) between two base pairs (intercalation), due to π-stacking interactions associated with intercalation of

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aromatic heterocyclic groups between the base pairs, (ii) in the minor or major groove due to hydrogen bonding and Van der Waals interactions of functionalities bound along the groove of the DNA helix, (iii) on the outside of the helix (external electrostatic) due to the coordination cation interacting with the nucleic acid anion through electrostatic interactions [14]. Thus understanding the type of interaction of complex with DNA is essential to design effective chemotherapeutic agents and provides insights for action mechanism of antitumor antibiotics [15]. Also, determining the binding constants of the Schiff bases to DNA is of paramount importance in the development of cleavage agents for probing nucleic acid structure and other application [16, 17]. Therefore in the present work, we have selected two Manganese Schiff base complexes, because Manganese Schiff bases complexes were used as catalysts for dismutation of superoxide and catalytic antioxidants in preclinical trials and Manganese Salen complexes are the most efficient of them [18]. Several Manganese complexes with different functionalized Salen and Salophen derivatives synthesized and the interaction of them with DNA were investigated [19, 20]. Gravert and Griffin have demonstrated that the presence of substituents with different steric and electronic feature on the salen moiety has significant impact on their DNA binding/cleavage activity [21]. But in spite of the extent investigation on Manganese complexes, no work has been done on the binding of two selected complexes. Especially the anionic compounds have been considered less than other neutral or cationic compounds because, at first glance, it seems that negative charges on the phenyl rings prevent the interaction of complexes with DNA. So the focus of our work is on the interaction of ct- DNA with two negatively charged water soluble Schiff bases complexes of Mn(III): Bis sodium (5-sulfosalicylaldehyde) o- phenylendiiminato) Manganese (III) acetate (Salophen complex) and Bis sodium (5-sulfosalicylaldehyde) 1,2- ethylendiiminato) Manganese (III) acetate (Salen complex). The chemical structure of Salophen and Salen complexes are shown in Figs. 1 and 2, respectively. Both manganese complexes are 5-coordinate (square pyramidal) with axial acetate. The axial ligand and sulfonate groups on the complexes make them water soluble [22]. Understanding how the two Schiff base complexes

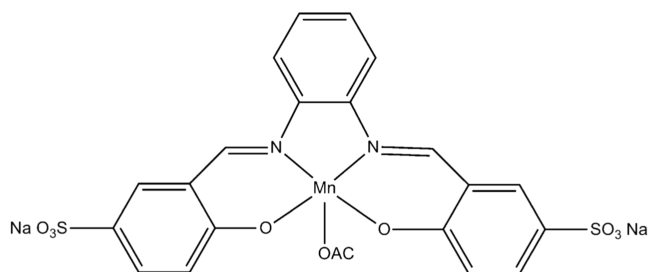

Fig. 1 Bis sodium (5-sulfosalicylaldehyde) o- phenylendiiminato) Manganese (III) acetate (Salophen complex)

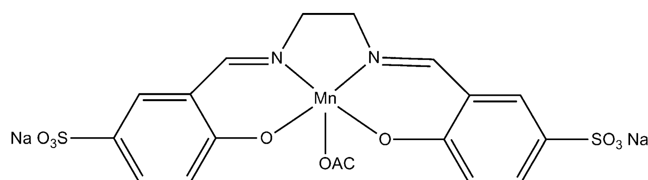

Fig. 2 Bis sodium (5-sulfosalicylaldehyde) 1,2ethylendiiminato) Manganese (III) acetate (Salen complex)

interact with DNA even though both are negatively charged was interesting for us. So, In order to understand the biological activity of these two complexes, some valuable techniques such as UV-Vis, fluorescence, thermal denaturation, viscometry, cyclic voltammetry and Circular dichroism were used. The analysis of experimental data reveals the details of binding such as the binding constants, binding mode and the changes in conformational stability of DNA. The experimental results suggest that two complexes have a potential for binding to DNA and Salophen interact with DNA stronger than Salen complex.

Experimental Section

Materials

Double stranded calf thymus DNA (ct-DNA, activated and lyophilized) was purchased from Sigma (sodium salt, average molecular weight). Ct-DNA stock solutions (2 mg/mL) were prepared in 10 mM Tris/HCl, pH 7.2 buffer. Water was purified to 18 MΩ, Millipore- MilliQ, Millipore Inc. France. The Schiff bases were synthesized, purified and characterized according to literature methods [23]. Other reagents were purchased commercially and used without further purification. All experiments were carried out in a 10 mM Tris–HCl aqueous buffer at pH 7.2, and 100 mM NaCl. The DNA concentration of the stock solution (2 mg/mL) was determined by spectrophotometry, using the molar absorption coefficient of 6,600 $M^{-1}cm^{-1}$ at 260 nm [24]. The ratio of A_{260}/A_{280} for solution of ct-DNA in 10 mM Tris- HCl, pH 7.2 was 1.8–1.9 that represents the free protein DNA sample [25].

DNA Binding Studies

Absorption titration was performed using a UV-Vis, Carry-500 double beam spectrophotometer, operating from 200 to 800 nm, using 1 cm path-length cuvettes. The absorbance titrations were performed at a fixed concentration of the complexes (10 μM) while varying DNA concentration of (0–180 μM) base pairs. In order to eliminate the absorbance of DNA, an equal amount of DNA was added to both compound solution and reference cuvette and absorbances

were recorded after each successive addition of DNA solution and equilibration (i.e. when there was no further change).

Fluorescence measurements were carried out on a Shimadzu spectrofluorometer model RF-5000 coupled to a data recorder and a Peltier system to control the temperature inside the cuvettes, using a quartz cell of 1 cm path length. All the curves presented were baseline-corrected using the application included in the RF-5000 digital station software. Both excitation and emission slits were set as 5 nm. In competition binding complexes with Ethidium Bromide (EB)-DNA, DNA and EB concentrations were 80 μM and 12 μM, respectively while complexes varied from 0 to 220 μM for Salen and 0–380 μM for Salophen complex. The 515 nm was chosen as the excitation wavelength and the emission spectra were recorded from 520 to 700 nm. The shape of Stern–Volmer plot can be used to characterize the quenching as being predominantly dynamic or static where; the plots of F_0/F versus $[Q]$ appear to be linear. This plot has been defined according to the classical Stern–Volmer equation [26]:

$$F_0/F = Kq[Q] + 1$$

Where F_0 is the emission intensity in the absence of quencher, F is the emission intensity in the presence of quencher, K_q is the quenching constant, and $[Q]$ is the quencher concentration.

Melting experiments were performed using an UV-Vis Carry-500 double beam spectrophotometer in conjunction with a thermostated cell compartment. The measurements were carried out in 10 mM Tris / HCl buffer, pH 7.2 (100 mM NaCl) containing 30 μM DNA. The temperature inside the cuvette was determined with a platinum probe and was increased over the range of 20–90 °C at a heating rate of 1 °C/min (Thermal software). The melting temperature, T_m, was obtained from the mid-point of the hyperchromic transition.

Viscosity measurements were carried out using an Ostwald viscometer immersed in a thermostatic water-bath maintained to 25.0 °C. The flow time were recorded with a manually operated timer for different concentration of two complexes (10–100 μM), keeping DNA concentration constant (40 μM). The buffer flow time was recorded as t_0. Data were presented as $(\eta/\eta_0)^{1/3}$ versus the ratio of the concentration of the compound to DNA, where η (η=(t−t_0)/t_0) is the viscosity of DNA in the presence of complex and η_0 is the viscosity of DNA alone [27]. Every experiment was repeated three times and the results were recorded.

Electrochemical measurements were performed on Potentiostat/Galvanostat Autolab PGSTAT 30 in a 2 mL cell containing gold disk as working electrode, a platinum wire was used as an auxiliary electrode, and an Ag/AgCl/3 M KCl was used as a reference electrode. Potentials were reported versus the Ag/AgCl. All experiments were performed at room temperature and solutions purged with nitrogen gas. The gold disk electrode (0.0962 cm^2) was polished using aqueous slurries of alumina (0.30 down to 0.05 μm), sonicated in water/chloroform/water for 5 min, and then cleaned electrochemically by cycling the electrode potential between 0.00 and +1.50 V in 0.5 M sulfuric acid, until reproducible oxidation peaks of gold was obtained. Concentration of Salophen and Salen complexes were 550 μM and 1,500 μM, respectively, in 10 mM of Tris-HCl buffer (pH 7.2) in the absence and presence of different concentrations of ct- DNA. The cyclic voltammetry of two complexes at a rate scan of 100 mVS^{-1} were performed.

Circular dichroism was recorded with a Chirascan CD spectrometer (AppliedPhotophysics, UK). The wavelength interval was 200–600 nm, bandwidth 1 nm with steps of 1.0 nm. The time-per-point was 0.5 s. The spectra were recorded in a 1 cm cell and each spectrum is the average of 4 scans from 200 to 600 nm. The samples were incubated for 24 h.

Result and Discussion

UV–Vis Absorption Spectroscopy and DNA Binding Interaction

The spectra of Salophen and Salen complexes at various concentration of DNA are shown in Figs. 3 and 4, respectively. Salophen exhibits an intense absorption band at about

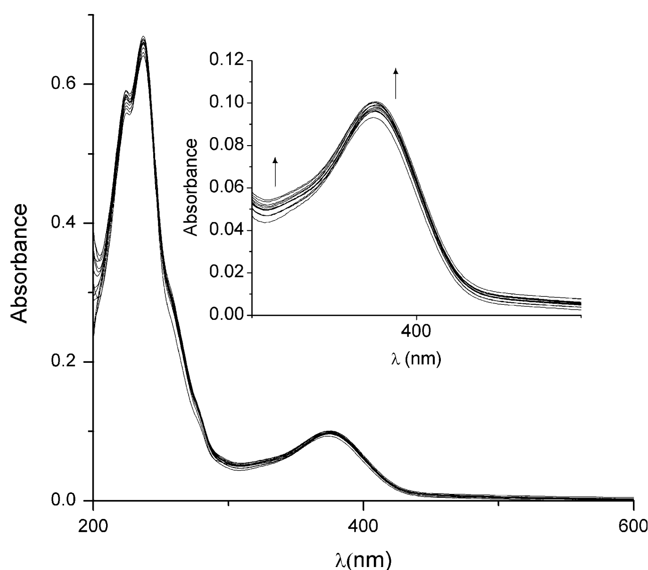

Fig. 3 Absorption titration spectra of Salophen complex (10 μM) in the presence of increasing amounts of ct-DNA in 10 mM Tris-HCl / 100 mM NaCl, pH=7.2, [DNA]=0–180 μM. *Arrow shows* the absorbance changes upon increasing DNA concentration

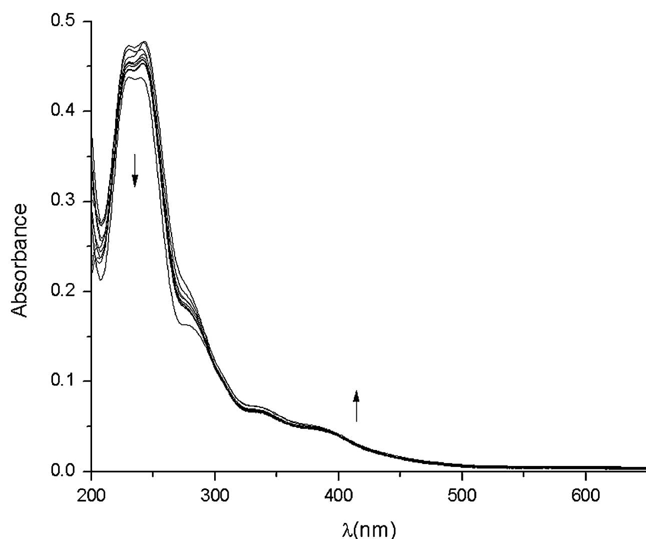

Fig. 4 Absorption titration spectra of Salen complex (10 μM) in the presence of increasing amounts of ct-DNA in 10 mM Tris-HCl / 100 mM NaCl, pH=7.2, [DNA]=0–140 μM. *Arrow shows* the absorbance changes upon increasing DNA concentration

237 nm and a medium absorption band at 375 nm. The Salen complex shows two intense bands at 232 and 243 nm and two shoulders around 340 and 400 nm. After adding DNA to a fixed concentration of Salophen hyperchromism (8%) was observed in all spectral regions with a small red shift about 2 nm for Salophen and hypochromism (9.1%) in UV region and hyperchromism (6.3%) in the visible region for Salen was observed as reported for similar systems [28].

Hypochromism and hyperchromism are both spectral feature of DNA concerning changes in its double helix structure. Hypochromism happens when the DNA-binding mode of a complex has an electrostatic effect or an intercalation which stabilizes the DNA duplex [29, 30]. While hyperchromism may probably be due to dissociation of aggregated ligand or external contact with DNA [9, 31]. A similar hyperchromism has been observed for the soret bands of certain porphyrins when they interact with DNA [32].

As stated before, the two complexes have a replaceable acetate ligand in solution, which may be replaced with a sulfonate group in the phenyl ring of neighbor molecule or with H_2O molecules in the solution [20]. Thus we have a complex that contains a positive charge on the manganese in the middle and negative charges on the phenyl rings. Interactions of two complexes with DNA would be with two possible binding pathways. One pathway is interaction of sulfonate groups with sodium cation in Stern layer of DNA (with respect to 100 mM of NaCl in buffer) and coordination of Mn^{3+} with DNA and the other pathway interaction of sulfonate groups with cations in the Stern layer and also engaging in hydrogen binding with suitable donors on the base pairs thus repulsion between solfunate group and phosphate backbone would be decreased and interaction of two anionic complexes with DNA would be possible and considerable.

This declaration could be proved by the observed hyperchromism in the absorption spectra. Since, columbic interaction of sulfonate group with Sodium cations would decrease electron density on the phenyl ring and MLCT transitions happen easier than free complex. So with addition of DNA to fix concentration of complexes we will see hyperchromism due to MLCT transitions.

Thus, above results show that two complexes bind to DNA and their interaction is stronger than other anionic compound $[Fe\,(CN)_6]^{-4}$ with DNA [16] and we have to state that the location of negative charge on the complex is important in the binding process. If the negative charge is on the ligand domain, like the copper Phenanthroline complexes [28], the complex can be bound easier than when the negative charge is on metal and interaction with DNA will be more extensive.

In order to determine the mode of binding using absorbance spectroscopy, EB was used as a typical indicator of intercalation [33]. The spectrum of EB, EB-DNA and EB-DNA-Salophen and Salen are shown in Fig. 5. The comparison of spectra in Fig. 5 represents a considerable decrease in the absorbance of EB at 479 nm and a red shift with the addition of DNA which is characteristic of intercalation. However, when Salophen or Salen are added to the solution of EB-DNA, there is an increase in the absorbance. This increase in absorbance could be due to the interaction of EB with the Complexes and the releasing of EB from DNA, or competition of the complexes with EB for occupation of the same binding sites. We investigated the effect of the complexes on the absorption spectrum of EB and there were no changes in spectrum or new absorption peaks thus we have to neglect the former reason.

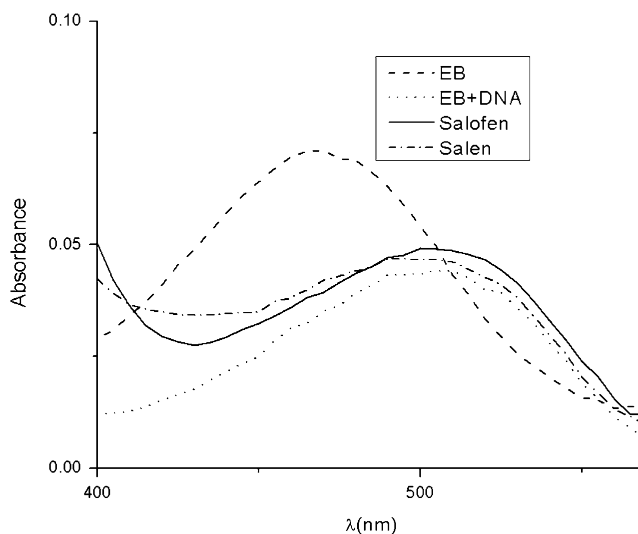

Fig. 5 The visible absorption spectra of EB (4 μM) and EB+DNA (100 μM) and EB+DNA+Salophen and Salen complex (4 μM) in Tris buffer (10 mM, 100 mM NaCl, pH 7.2) solution

Fluorescence Studies

To investigate further the binding of complexes to DNA by intercalation, an emission quenching experiment has been carried out. EB is a common fluorescent probe for DNA structure determination and has been employed in the examinations of the binding mode and process of metal complex binding to DNA [33, 34]. The decrease of (42.5% for Salophen and 25% for Salen) EB-DNA emission due to the addition of the Salophen and Salen complexes can be from the competition of the complexes with EB for the same binding sites on DNA. It is worth stating that adding Salen to EB-DNA solution until 20 μM increased the emission spectra, but after this concentration there was a decrease in the spectra. Also adding the complexes to EB did not affect the absorbance spectrum of EB and there is no interaction between EB and Schiff base complexes.

Plots of F_0/F versus $[Q]$ appear to be linear for Salophen and Salen complex (Figs. 6 and 7) and the values of $2\pm 0.1\times10^3$ M^{-1} and $1.6\pm0.1\times10^3$ was obtained for Stern-Volmer constants.

Measurement of Binding Curves

In order to investigate, Salophen's competition with EB to DNA, fluorescence Scatchard plots for the binding of EB to ct- DNA in the presence of varying concentrations of Salophen were obtained, as described previously [35, 36]. Fluorescence Scatchard plots of the binding of EB to ct-DNA in the absence and the presence of various amount of Salophen complex were investigated. Each sample containing DNA and the complex was titrated with EB stock solution. Then the samples were excited at 515 nm and its emission observed at 580 nm. All measurements were made at room temperature. Binding isotherms were determined in the presence of the Salophen complex depicted in Fig. 8 and the corresponding Scatchard plots constructed and we attempted to determine the type of binding between the salophen and DNA. From depicting the Fluorescence Scatchard plots, we may achieve four classes of behavior for binding of complex with DNA. In this experiment, from analyzing the data type D behavior was observed. Non-competitive inhibition of EB binding (type D behavior) produces a Scatchard plot in which the slope is almost constant in the presence of increasing amounts of Salophen

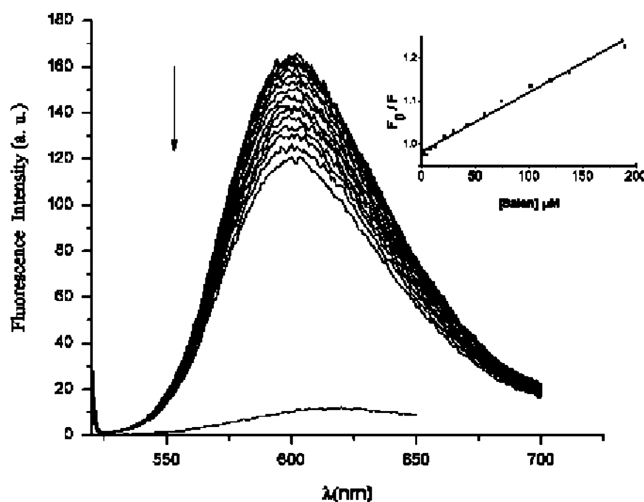

Fig. 7 The emission spectra of DNA-EB (80 and 12 μM), λ_{ex}= 515 nm, $\lambda_{max\text{-}em}$=600 nm, in the presence of 0–220 μM Salen complex. Intensity decreases by addition complex. Inset: Stern-Volmer plot of the fluorescence titration data of complex, $K_q=1.6\times10^3$ M^{-1}

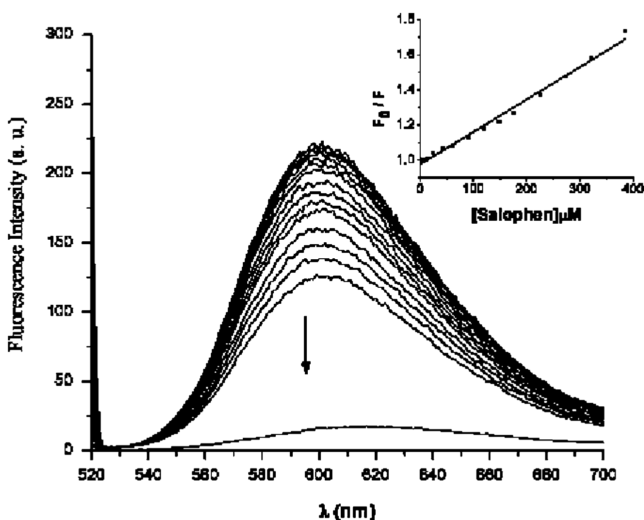

Fig. 6 The emission spectra of DNA-EB (80 and 12 μM), λ_{ex}= 515 nm, $\lambda_{max\text{-}em}$=600 nm, in the presence of 0–380 μM Salophen complex. Intensity decreases by addition complex. Inset: Stern-Volmer plot of the fluorescence titration data of complex, $K_q=2\times10^3$ M^{-1}

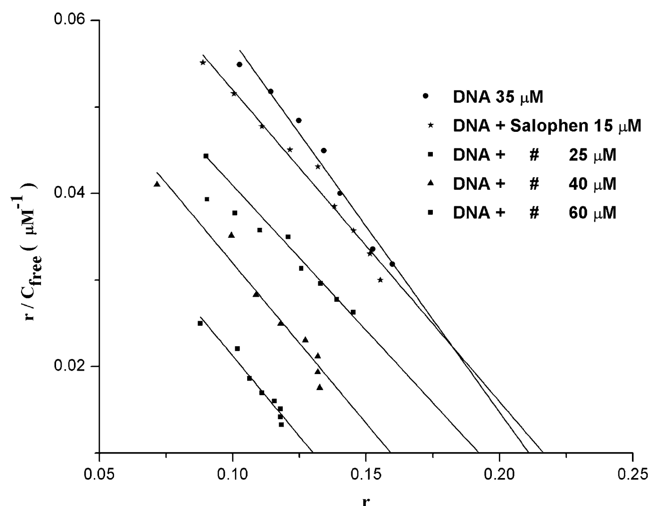

Fig. 8 Fluorescence Scatchard plots of the binding of EB to DNA in the absence and the presence of various value of Salophen complex. [DNA]= 35 μM and EB concentration varied from 1.5 to 11 μM and [Salophen] / [DNA]=0.4, 0.7 and 1 and 2; λ_{ex}=515 nm, λ_{em}=580 nm

complex and In this kind of binding curves, the complex is attached to DNA on the base of the groove / covalent or through electrostatic interactions and sterically prevents the binding of EB to the latter.

Also, Binding constants were estimated for Salophen complex and EB with using the equations that were stated previously [37]. The K_M value is 2×10^4 M^{-1} and K_{EB} in this experiment is 3.6×10^5 M^{-1} and n about 0.24.

Thus, with respect to the previous results electrostatic interaction has the main role for binding of complex and intercalation could not occur and fluorescence results show that Salofen interacts with DNA stronger than Salen.

Aggregation of Salophen Complex and NaCl Effect

Salophen shows fluorescence spectra and has an emission band with maxima centered at 480 nm when excited at 400 nm. Thus, aggregation of complex in solution in the extended range of concentration was investigated using the fluorescence titration experiment. In this regards, stock solution of Salophen was added consecutively to a specified volume of buffer in the cell and emission intensity of solution with different concentrations of complex was measured at 420–600 nm under 400 nm excitation wavelength. Figure 9 (Inset a) shows the fluorescence intensity increased to some extent (about 400 μM), then decreased due to the addition of more complex. This phenomenon can be related to the aggregation of the complex. In order to understand the aggregation we investigated the effect of NaCl salt on the emission spectrum of a Salophen solution. So we have selected fix concentrations of Salophen complex and have titrated the complex with stock solution of salt. It was found that addition of salt to the solutions with concentration higher than 400 μM, increase the emission intensity (inset b of Fig. 9). This increase in emission intensity can be related to dissociation of aggregated complex in the presence of salt.

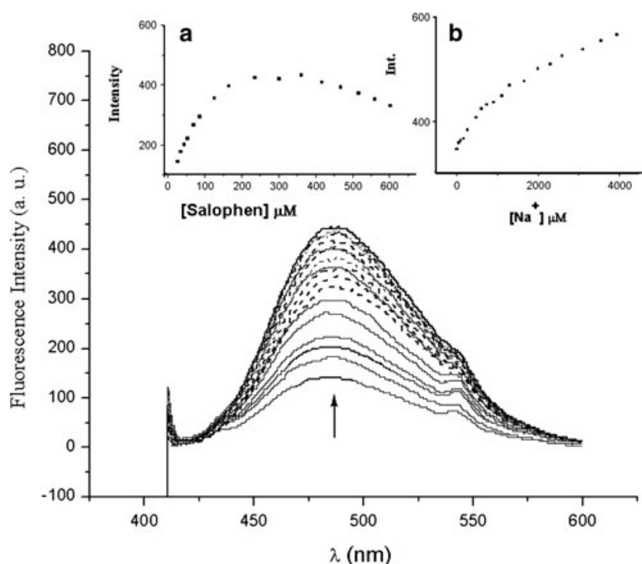

Fig. 9 Fluorescence Emission spectra of Salophen complex in different concentration, [Salophen]=0–600 μM, λ_{ex}=400 nm, Intensity increases with addition of Salophen (__ lines) and then decreases in high concentration (— lines). Inset **a** Fluorescence intensity as a function of Salophen concentration, λ_{ex}=400 nm and λ_{em}=485 nm. Inset **b** Effect of NaCl salt on emission intensity of Salophen complex, [Salophen]=560 μM and λ_{ex}=400 nm and λ_{em}=485 nm

Thermal Denaturation Study

Thermal behavior of DNA in the presence of the complexes provides information on the conformational changes and the strength of the DNA-complex interaction. The double stranded DNA gradually dissociates to single strands with increasing solution temperature and results in a hypochromic affect. T_m is strictly related to the stability of double helix, and the interaction of the molecules with DNA may alter the T_m by stabilizing or destabilizing the final complex. Moreover, it is also possible to obtain information on the strength of the interaction.

The stabilization of the DNA double helix from the stabilizing stacking interactions is followed by a considerable increase in the melting temperature of DNA. The low ΔT_m value suggests that the primary groove and / or electrostatic binding of the complexes to DNA stabilize the DNA double helix structure [38–40]. The thermal properties of DNA in the presence of various amounts of Salophen complex are shown in Fig. 10. The increase in thermal stability of DNA due to addition of Salophen and Salen complex is about 5 °C.

These low changes in T_m are due to electrostatic binding. Although, the amount of increase in T_m is dependent on the mode of binding, the increasing trend is independent of the

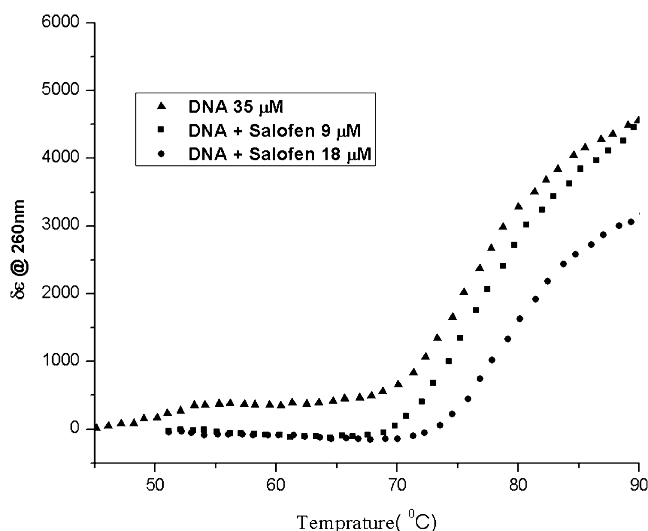

Fig. 10 Melting curve of ct-DNA (at 260 nm) in the absence and presence of Salophen

mode of binding. The observed changes in T_m in this case are indicative of non-intercalative binding.

Viscosity Measurments

Photophysical spectroscopy measurements provide necessary, but not sufficient evidence to support the binding mode of the metal complexes with DNA. The viscosity of a DNA solution is sensitive to the addition of metal complexes which bind to DNA. Hydrodynamic data provide perhaps the most critical test for intercalative binding in the absence X-ray and NMR structural data [17]. Intercalation leads to an increase in the DNA viscosity because the DNA base pairs are pushed apart to accommodate the binding ligand. The other non-classical, mode of binding could bend or kink the DNA helix, which reduces its effective length and its viscosity. To further confirm the interaction mode of these complexes with DNA, the viscosity of DNA with Salophen and Salen complex were investigated.

Viscometry studies showed no considerable changes at low concentrations of the complexes while at high concentrations, there was a slight increase in viscosity as shown in Fig. 11. This kind of change in hydrodynamic data may be explained by binding modes electrostatic and hydrophobic (groove binding) [28].

Electrochemistry Study

The application of electrochemical methods to study the metallointercalation and coordination of metal ion to DNA provides a complementary study to the other spectroscopic studies discussed and to understand the nature of DNA binding to metal complexes [41, 42]. The cyclic voltammograms of the complexes in the absence and presence of ct-DNA are shown in Figs. 12 and 13. It can be seen that for complexes the cathodic and anodic peak currents increased gradually by the addition of ct-DNA; however, the Salen complex has a weak anodic peak. In the absence of DNA, the anodic peak potential of Salophen and Salen complexes were +0.26 V and +0.24 V, respectively, while the cathodic peak was only observed for Salophen complex at +0.6 V.

For further investigation, voltammograms for complexes and complex–DNA at different scan rates (υ) were done. The E and I changed with scan rate over the entire range 10 to 500 mVs^{-1} and it was found that the peak current is proportional to $υ^{1/2}$, suggesting the process is controlled by diffusion.

Therefore, both the increase in current and the shift in potential show that the complexes bind to DNA in spite of other similar works [43] that show a decrease in the current with the addition of DNA to complex. This behavior can be related to the aggregation. As it has been shown in section 'Aggregation of Salophen Complex and NaCl Effect', that Salophen forms aggregates at concentrations higher than 400 μM. It seems that the addition of DNA causes the dissociation of Salophen aggregates and forms smaller species. These smaller species interact with DNA and form a supramolecule. The Diffusion rate of small species is faster than supramolecules (DNA- Salophen complex) and can be the reason for the increasing of the current peak.

Circular Dichroism Studies

The Mn complexes are not optically active and therefore do not exhibit any CD spectra, but ct-DNA in the B form conformation shows two conservative CD bands in the UV region: a positive band at 278 nm due to base stacking and a negative band at 246 nm due to right handed helicity of DNA. Changes in the CD spectrum of DNA in the presence of complexes are depicted in Figs. 14 and 15. The CD

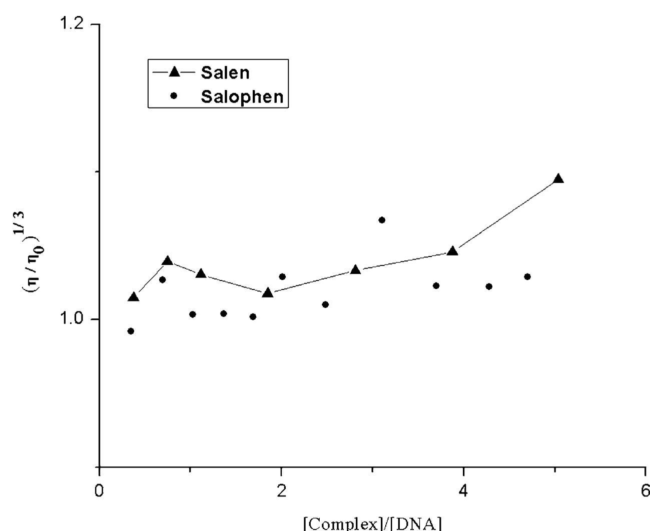

Fig. 11 plots of relative viscosities of 40 μM ct-DNA vs. [Complexes] / [DNA]

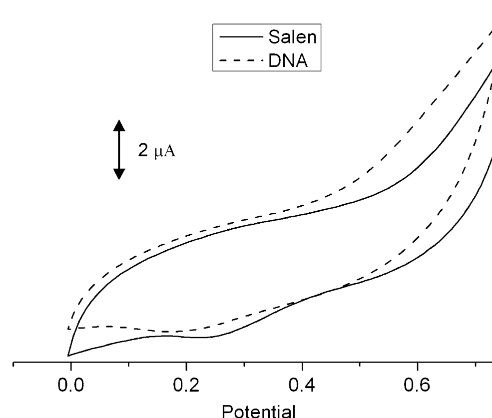

Fig. 12 Cyclic voltammograms of 1,500 μM [Salen complex] in the absence (-) and presence (–) of 290 μM DNA

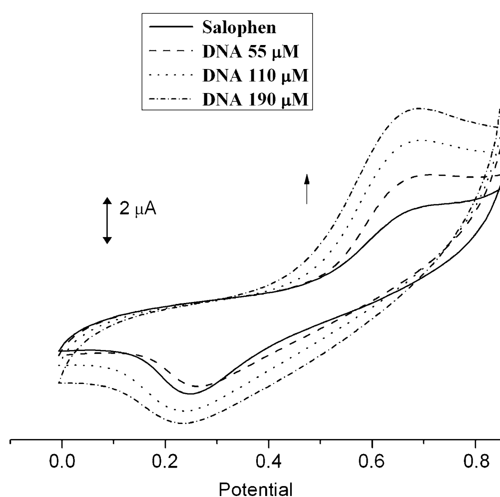

Fig. 13 Cyclic voltammograms of 550 μM [Salophen complex] in the absence (-) and presence of 56, 110, 190 μM DNA

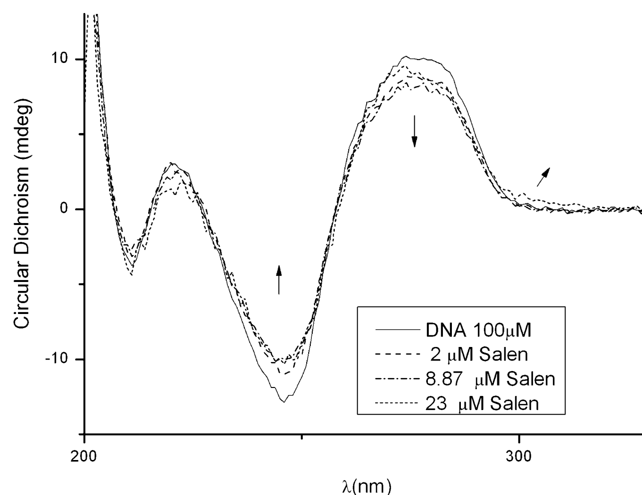

Fig. 15 CD spectra of ct-DNA in the absence and presence of Salen complex

spectra of DNA with the addition of complexes exhibit a decrease in both the positive and negative peaks. There was no indication of induced CD that could be detected in the visible region for both complexes. The shape of CD spectra is dependent on the concentration of added complexes, in a mole ratio of more than 1, the increase in the positive band and a decrease in the negative band was observed too, the inset of Fig. 14 and the changes in the CD spectra of Salophen are more than Salen.

These observations suggest that DNA binding of the complexes induce certain conformational changes, such as conversion from a more B-like conformation to a more C-like conformation within the DNA molecule. This conformational change is attributed to electrostatic interaction both along the phosphate back bone and between sites on the bases which bear partial negative charges and the negatively charged phosphate groups. Further transformation of the DNA structure proceeds by the removal of water from base sites and the grooves of the helix [44] and the role of dehydration is considered as a subsidiary force which facilitates the interaction of complexes with the weaker base sites. As stated in section 'UV–Vis Absorption Spectroscopy and DNA Binding Interaction' interaction of sulfonate group with sodium cation in the Stern layer following with coordination of Manganese with DNA backbone or with partial negative charges on the bases could be the reason of decreasing in the positive and negative CD band of DNA. After electrostatic binding to DNA and approaching the complex to DNA, hydrogen binding with sulfonate group and suitable donors on the

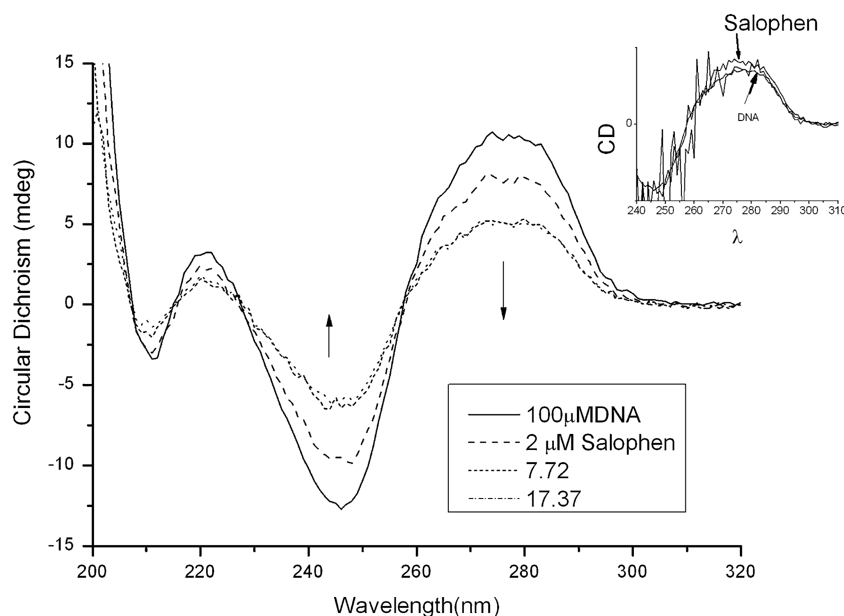

Fig. 14 CD spectra of ct-DNA in the absence and presence of Salophen complex the inset shows [complex]/ [DNA] =1 and 1.5

base pairs may happen through the grooves of DNA helix and removal the water from the grooves will cause. So, from the experimental result of CD, UV-vis and viscometry we can say that electrostatic interactions have the main role for binding and then at higher concentrations a portion of complex binds to DNA by hydrophobic interaction from the grooves.

Conclusion

This work reports on the interaction of two water soluble Schiff base complexes with ct-DNA. At first, we have used a lot of experiments for emphasis on the potential of two complexes for binding with DNA and after that determining the type of interaction was important for us. Because, type of interaction is essential to design effective chemotherapeutic agents and provides insights for action mechanism of drugs. Thus from the experimental results:

Hyper and hypochromism was observed from the absorption experiment and Binding constants has been determined with using fluorescence data for both complexes and emission data of Scatchard plot for the Salophen complex. It has been found that both complexes stabilize the ct-DNA thermally up to 5 °C. The competitive binding study shows that the enhanced emission intensity of EB in the presence of DNA was quenched by the addition of the two complexes indicating that they displaces EB from its binding site in DNA. Small changes in the DNA viscosity shows electrostatic or groove binding and the shift in potential and increase in current from the electrometry experiment confirm the interaction of two complexes with DNA. Also CD spectra indicate that the molecular structure of DNA has been changed but the base group stacking which makes the DNA structure stable is not significantly changed at low concentrations. All the experimental results suggest that dominate binding mode in the system is surface binding (electrostatic interaction) due to interaction of sulfonate groups with sodium cations in the Stern layer of DNA and also coordination of Manganese with DNA and hydrophobic interaction from grooves in high concentration of complexes may happen.

From comparing the experimental results of competitive binding between EB and complexes and CD spectra, it is obvious that interaction of Salophen complex is stronger than Salen due to the more planarity and aromaticity.

We have selected these complexes due to more solubility in water relative to other Manganese Schiff base complexes and we know that negative charge on the complex will prevent interaction of it with DNA but In this work, we found that the location of negative charge or anionic substitute on the ligand is more important and compare to other reported anionic compounds [3], these two complexes have shown relatively good interaction and their binding constants are comparable with the neutral or even cationic compounds.

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