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ORIGINAL PAPER

Spectroscopic Characterization of the Interaction of Phenosafranin and Safranin O with Double Stranded, Heat Denatured and Single Stranded Calf Thymus DNA

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Abstract Interaction of phenosafranin and safranin O with double stranded, heat denatured and single stranded calf thymus DNA has been studied by fluorescence, absorbance and circular dichroic techniques. Binding to the double stranded and heat denatured DNA conformations induced strong quenching in the fluorescence spectra of both dyes. Linear Scatchard plots indicated the binding to be of one type and the affinity evaluated to be of the order of 10^5 M^{-1} with double stranded and heat denatured DNAs. Fluorescence quenching was much weaker with the single stranded DNA and the binding affinity was one order lower. Ferrocyanide quenching studies revealed that the fluorescence emission of the dye molecules bound to the double stranded and heat denatured DNAs was quenched much less compared to that bound to the single stranded DNA. Further, there was significant emission polarization for the bound dyes and strong energy transfer from the DNA base pairs to the dye molecules indicating intercalative binding. Salt dependence of the binding phenomenon revealed that electrostatic forces have significant role in the binding process. The intercalation of these molecules to double stranded and heat denatured DNA and simple stacking to single strands was proved by these fluorescence techniques. Support to the fluorescence results have been derived from absorption and circular dichroic results. Phenosafranin was revealed to be a stronger binding species compared to safranin O.

Keywords Phenosafranin · Safranin O · DNA binding · Fluorescence techniques

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Introduction

The binding of planar aromatic molecules with DNA and elucidation of the structural features of such bindings have been an active topic that has attracted several researchers across the world for a long time [1–6]. Such studies have remarkable relevance and implications in several biological processes including cancer chemotherapy for the design and development of efficient drugs targeted to DNA. Strong and highly specific binding of such molecules with the genomic DNA and consequent structural damages may be effective in preventing further information retrieval from DNA leading to arrest of cell division. Photophysical behaviour linked intercalative binding properties of such molecules can also be harnessed as diagnostic probes for DNA structure and DNA directed therapeutics [7]. For these and several other pharmacological utility molecular aspect of the intercalative interaction of aromatic molecules with DNA has been the subject matter of a large number of investigation since the discovery of the DNA structure. Nevertheless, to a large extent, an understanding of the nature, specificity and energetics of such interactions and the development of effective DNA targeted chemotherapeutic agents remained an elusive goal and elucidation of the molecular aspects of small molecule-DNA interaction continues to remain as an exciting field of research activity.

Cationic phenazinium dyes have attracted attention for their use in semiconductors, as energy sensitizer and as a probe for investigating various microenvironments including micells and polymeric matrices [8–10] Phenosafranin and safranin O, (Fig. 1), two most important phenazinium dyes, have found use in various biological applications including antimalarial activity and microdetection of DNA [11–17] The intercalative double stranded DNA binding was first reported from our laboratory [18] using spectro-

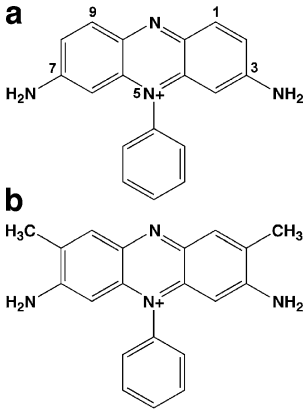

Fig. 1 Chemical structure of (**a**) phenosafranin and (**b**) safranin O

scopic, viscometric and calorimetric techniques and was subsequently confirmed by other workers [19]. Herein, we use extensive fluorimetric techniques for the characterization of the binding reaction of phenosafranin and safranin O with double stranded, heat denatured and single stranded calf thymus DNA. Due to the high sensitivity, and non invasiveness, fluorescence spectroscopy has been widely used to characterize drug-DNA interactions. Here we have exploited the strength of various fluorimetric tools like steady state, Stern-Volmer quenching, polarization anisotropy, energy transfer from base pairs to dye etc to characterize and analyze the interaction of phenosafranin and safranin O to calf thymus DNA in the double stranded, heat denatured and single stranded helical conformations.

Experimental

Apparatus

Fluorescence spectral measurements were made either on a Hitachi F4010 unit (Hitachi Ltd. Tokyo, Japan) or Shimadzu RFPC unit (Shimadzu Corporation, Kyoto, Japan) using 1 cm path length fluorescence free quartz cuvettes. Excitation and emission band passes of 5 nm were used. Polarization measurements were performed on the Hitachi unit equipped with excitation and emission polarizers.

Absorbance measurements and melting profiles of DNA samples were determined on a Jasco V 660 spectrophotometer (Jasco International Co, Tokyo, Japan) as described earlier [20, 21]. For circular dichroic measurements a Jasco J815 unit was used.

Reagents

All chemicals and reagents were of analytical grade or better. Double stranded (ds) calf thymus (CT) DNA was obtained from Sigma-Aldrich Corporation (St. Louis, MO, USA). The absorbance ratio of the sample A_{260}/A_{280} was in the range 1.80–1.90 and indicated the protein free nature of the sample. A molar extinction coefficient value of 6600 M^{-1} cm^{-1} was used for estimating the concentration by absorbance measurements at 260 nm. Heat denatured (hd) CT DNA was prepared by heating the double stranded DNA solution at 100°C in a boiling water bath for 10 min. and suddenly cooling in ice [22]. The hd DNA sample had about 25% residual hyperchromicity as determined by optical thermal melting studies. Single stranded (ss) CT DNA was prepared by heating the ds sample of CT DNA in presence of formaldehyde for 10 min. and plunging in ice bath. All stock solutions were stored in a refrigerator at 4°C. Phenosafranin (PSF) and safranin O (SO) (hereafter described as dyes or ligands) obtained from Sigma-Aldrich were used without further purification. Solutions were freshly prepared each day in the experimental buffer and kept protected in the dark till use. Molar extinction coefficient (ε) values of 33,000 M^{-1} cm^{-1} and 25,000 M^{-1} cm^{-1}, respectively at 520 nm for PSF and SO were used for concentration estimation. Beer's law was found to be obeyed in the concentration range used in this study. All experiments were conducted in filtered 10 mM cacodylate buffer, pH 7.0, prepared in deionised and triple distilled water. All other reagents used in this study were procured from Sigma-Aldrich.

Methods

Absorption Spectra

All absorbance spectra were measured on a Jasco V 660 double beam double monochromator spectrophotometer at 20±0.5°C in 1 cm path length quartz cuvettes using the methodologies described in details earlier [20, 21]. Melting profiles of the DNA samples were recorded on a Shimadzu Pharmaspec 1700 unit (Shimadzu Corporation, Kyoto, Japan) equipped with a TMSPC melting accessory in eight chambered quartz cuvettes of 1 cm path length as described earlier [20, 21].

Steady State Fluorescence Spectra

Steady state fluorescence measurements were performed on a Shimadzu RF5301PC spectrofluorimeter in fluorescence free quartz cuvettes of 1 cm path length as described previously [23, 24].

Ferrocyanide Fluorescence Quenching Measurements

Fluorescence quenching studies were carried out with the anionic quencher $K_4[Fe(CN_6]$. The quenching experiments were performed by mixing, in different ratios, two solutions, one containing KCl, the other containing $K_4[Fe(CN_6]$, in

addition to the normal buffer components, at a fixed total ionic strength. Fluorescence quenching experiments were performed at a constant P/D (DNA nucleotide phosphate/dye molar ratio) monitoring fluorescence intensity as a function of changing the concentration of ferrocyanide as described previously [24]. At least four measurements were taken for each set and averaged out. The data were plotted as Stern-Volmer plots of relative fluorescence intensity (F_o/F) versus $[Fe(CN_6]^{4-}$ concentration according to the Stern-Volmer equation

$$F_o/F = 1 + K_{SV}[Q] \quad (1)$$

where F_o and F denote the fluorescence emission intensities in the absence and presence of the quencher and [Q] is the quencher concentration. K_{SV} is the Stern-Volmer quenching constant, which is a measure of the efficiency of quenching by the quencher.

Measurement of Fluorescence Energy Transfer

Energy transfer from the DNA base pairs to the bound dye molecules was measured from the excitation spectra of the DNA-dye complexes in the wavelength range 220–310 nm [25, 26]. Excitation spectra were recorded keeping the emission wavelength at 570 nm for the dyes. The ratio $Q = q_b/q_f$, where q_b and q_f are the quantum efficiencies of bound and free dye, respectively, was calculated for each wavelength using the equation $Q = q_b/q_f = I_b\varepsilon_f/I_f\varepsilon_b$, where I_b and I_f are the fluorescence intensities in the presence and absence of the DNA, respectively, and ε_b and ε_f are the corresponding molar extinction coefficients of the dye. The ratio, Q_λ/Q_{310} was then plotted against wavelength. The normalization wavelength of 310 nm was chosen because DNA has very little absorbance at this wavelength.

Fluorescence Polarization Anisotropy

Fluorescence polarization anisotropy measurements of the dyes and their complexes with the three DNA conformations were carried out as suggested Larsson and colleagues [27] and described earlier [28]. Steady state polarization anisotropy 'A' is defined as

$$A = (I_{vv} - I_{vh}G)/(I_{vv} + 2I_{vh}G) \quad (2)$$

where I_{vv}, I_{vh}, I_{hv} and I_{hh} represent the fluorescence signal for excitation and emission with the polarizer positions set at (0°, 0°), (0°, 90°), (90°, 0°) and (90°, 90°) respectively. G is the ratio I_{hv}/I_{hh} used for instrumental correction.

Binding Stoichiometry: Job Plot

Continuous variation method of Job [29] was employed to determine the binding stoichiometry of binding in each case from fluorescence spectroscopy. At constant temperature, the fluorescence signal was recorded for solutions where the concentrations of both DNA and dyes were varied while the sum of their concentration was kept constant. The difference in fluorescence intensity (ΔF) of the dyes in the absence and presence of the various DNA conformations was plotted as a function of the input mole fraction of each dye. Break point in the resulting plot corresponds to the mole fraction of the bound dye in the complex. The stoichiometry was obtained in terms of DNA-dye $[(1- \chi_{dye}) / \chi_{dye}]$ where χ_{dye} denotes mole fraction of the respective dye. The results reported are average of at least three experiments.

Circular Dichroic Spectra

Circular dichroic (CD) spectra were acquired on a PC controlled spectropolarimeter Jasco J815 model equipped with a Jasco temperature programmer (model PFD 425 L/15) at 20±0.5°C as described previously [21, 24, 26]. A strain free rectangular quartz cell of 1 cm path length was used. Each spectrum was averaged from four successive accumulations at a scan rate of 100 nm/ min, keeping a bandwidth of 1.0 nm at a sensitivity of 100 millidegree, was baseline corrected and smoothed within permissible limits using the inbuilt software of the unit and normalized to nucleotide concentration in the region of intrinsic CD of the DNA. The molar ellipticity (θ) is expressed in deg. cm^2 $dmol^{-1}$.

Results and Discussion

Melting Characterization of the ds, hd and ss Calf Thymus DNA

The optical melting profiles of the three helical states of CT DNA are presented in Fig. 2. It can be seen that the ds conformation melts cooperatively with about 40% hyperchromicity while the hd DNA had a hyperchromicity of about 25%. The ss DNA did not show any appreciable enhancement in the absorbance on heating revealing the absence of any base paired segments in its structure.

Fluorescence Titration Studies

The fluorescence spectra of the phenazinium dyes are in the region 530–700 nm range with maximum at 570 nm when excited at 520 nm [18]. This property was exploited to monitor the interaction phenomena with the three conformations of the DNA. Binding to the three DNA conformations resulted in quenching of the fluorescence intensity, but to different extents, that eventually led to saturation of the

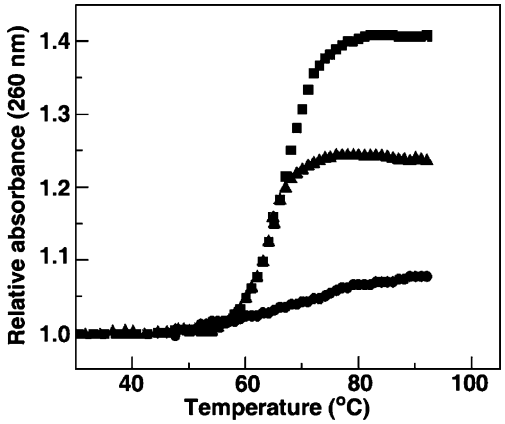

Fig. 2 Optical thermal melting profiles of ds (■), hd (▲) and ss CT DNA (●)

binding sites in each case. Representative fluorescence patterns of the complexation of PSF and SO with the three DNA conformations are presented in Fig. 3. The fluorescence quenching was large in ds DNA, followed by hd DNA and least in ss DNA indicating the strong association of the dye molecules to the ds regions of the structures due to an effective overlap of the π electron cloud of the bound molecules with the base pairs. This result proposes the location of the bound dye molecules to be in a hydrophobic environment similar to an intercalated state. The Scatchard binding isotherms revealed linear plots (inset of Fig. 3). The binding constants for ds, hd and ss DNA calculated from the fluorescence data as per the Scatchard analysis yielded values of 4.77×10^5, 2.60×10^5, 0.55×10^5 M^{-1} for PSF and 3.15×10^5, 2.39×10^5 and 0.41×10^5 M^{-1} for SO and these values are depicted in Table 1. It can be seen that both the dye molecules bind strongly with the ds DNA conformation followed by a closely strong binding to hd DNA and very weak binding to ss DNA. The number of binding sites in terms of nucleotides as evaluated from the Scatchard plots were around 2.5 for ds and around 3.2 for hd and above 4.1 for ss DNA (Table 1).

To establish the stoichiometry of binding of these dye molecules with the three DNA conformations, continuous variation analysis procedure (Job plot) was performed in fluorescence. The plots (not shown) of the difference fluorescence intensity versus dye mole fraction revealed single binding mode in each case. The intersection of the least square fitted lines at χ=0.289, 0.243, and 0.200 for PSF and χ=0.270, 0.234, and 0.171 for SO corresponds to a site size of 2.46, 3.10, and 4.0 nucleotides for PSF and 2.70, 3.20 and 4.90 nucleotides for SO complexing with ds, hd and ss DNAs. These values are close to the values obtained from Scatchard plots derived from fluorimetric titration data (*vide supra*).

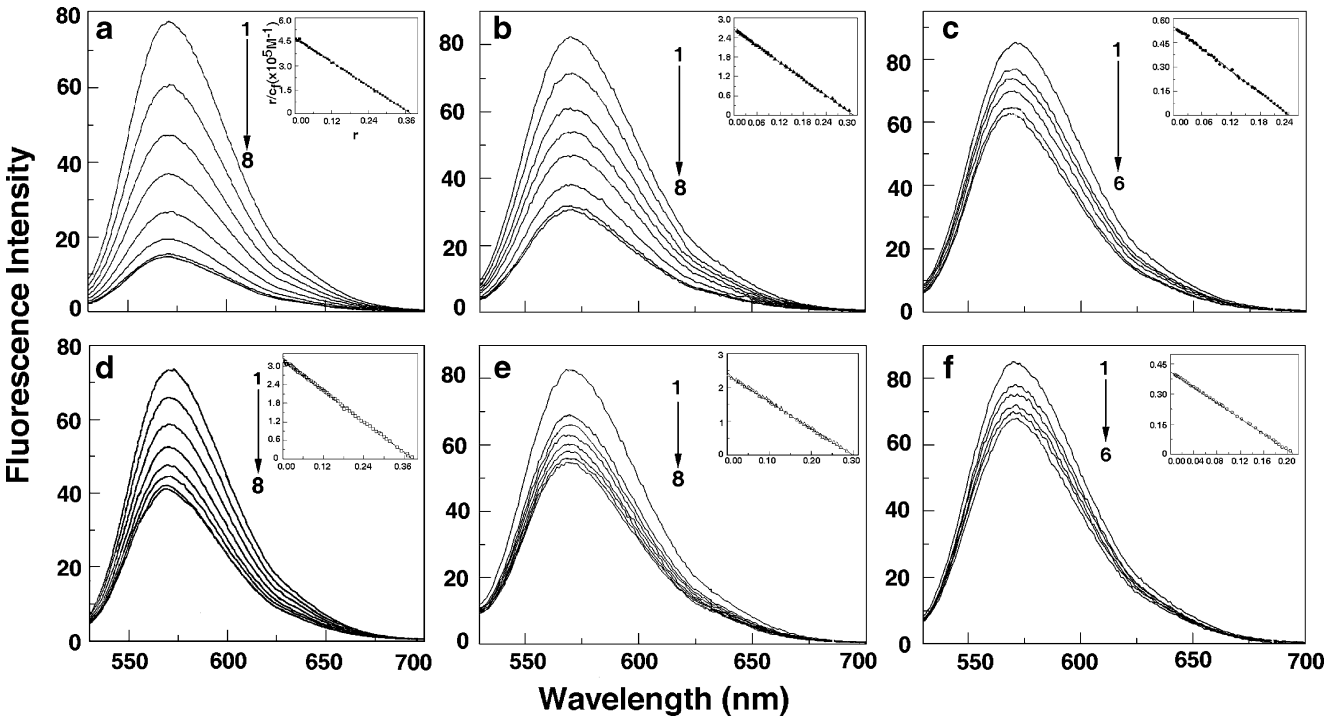

Fig. 3 Representative steady state fluorescence emission spectrum of PSF (0.6 μM) treated with (**a**) 0, 3, 6, 9, 12, 15, 18, and 24 μM (curves 1–8) of ds CT DNA, (**b**) 0, 6, 9, 15, 18, 21, 24, and 25.2 μM (curves 1–8) of hd CT DNA, (**c**) 0, 9, 12, 18, 24, and 30 μM (curves 1–6) of ss CT DNA. Representative steady state fluorescence emission spectrum of SO (0.6 μM) treated with (**d**) 0, 3, 6, 9, 12, 18, 24, and 25.2 μM (curves 1–8) of ds CT DNA, (**e**) 0, 9, 12, 15, 18, 21, 24, and 27 μM (curves 1–8) of hd CT DNA, (**f**) 0, 9, 12, 24, 30, and 36 μM (curves 1–6) of ss CT DNA. All experiments were done in 10 mM sodium cacodylate buffer of pH 7.0. Inset: representative Scatchard plots of the binding

Table 1 Binding parameters for the complexation of phenosafranin and safranin O to ds, hd, and ss calf thymus DNA evaluated from Scatchard analysis of the fluorescence titration data

DNA conformation	Phenosafranin		Safranin O	
	$K_i\times10^{-5}(M^{-1})$[a]	*n*	$Ki\times10^{-5}(M^{-1})$	*n*
Double stranded	4.77±0.20	2.58	3. 15±0.20	2.59
Heat denatured	2.60±0.30	3.24	2.39±0.30	3.25
Single stranded	0.55±0.013	4.11	0.41±0.30	4.67

Average of four determinations

[a] Binding constants (*Ki*) and the number of binding sites (n) refer to solution conditions of 10 mM cacodylate buffer, pH 7.0 at 20°C

Fluorescence Quenching Studies by Ferrocyanide Ions

The mode of binding of the phenazinium dyes to ds DNA was suggested previously to be intercalative [18, 19]. Here we probed the same in comparison to the binding with hd and ss DNAs using fluorescence quenching experiments in presence of $[Fe(CN)_6]^{4-}$ ions. Quenching experiments are quite straight forward and may indicate the location of the bound molecules to be either on the outside or inside of the helix. An anionic quencher like $[Fe(CN)_6]^{4-}$ would not be able to penetrate the negatively charged barrier around the double helix and if the bound molecules are buried within the ds or hd DNA helix by intercalation little or no change in fluorescence is expected. Linear Stern-Volmer plots obtained for the quenching of the fluorescence of PSF and SO by the three DNA helical states are shown in Fig. 4. Results revealed that the free dye molecules were quenched efficiently by the $[Fe(CN)_6]^{4-}$. Very little quenching was observed for complexed PSF and SO molecules with ds DNA. Quenching to some extent was observed for the dye molecules bound to the hd DNA and profuse quenching close to that observed for free molecules was found for ss DNA bound PSF and SO molecules. This result indicated that the PSF and SO molecules bound to the ds DNA and to a considerably large extent those bound to the hd DNA are located in a relatively more protected environment, presumably more deeply and or strongly intercalated, compared to that bound to the ss DNA. It is not surprising that the ss DNA bound dye molecules are easily accessible to the quencher because of the lack of a double helical structure for the ss DNA. The Stern-Volmer quenching constants calculated for PSF and SO and their complexes with the three DNA helical states were 29.0, 14.51, 18.32, 25.46 and 32.48 19.01, 22.12, 29.16 L/mol, respectively (Table 2). From these results it can be clearly inferred that the dye molecules bound to the ds DNA are more protected than those bound to the hd DNA but both are

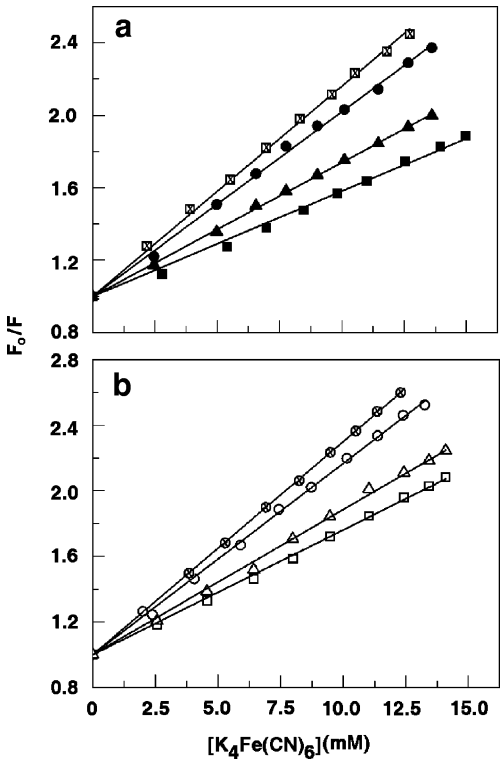

Fig. 4 Stern-Volmer plots for the quenching of (**a**) PSF (⊠) and complexes of PSF-ss CT DNA (●), PSF-hd CT DNA (▲) and PSF-ds CT DNA (■), and (**b**) SO (⊗), SO-ss CT DNA (○), SO-hd CT DNA(Δ) and SO-ds CT DNA (□) in 10 mM sodium cacodylate buffer of pH 7.0

sequestered away from the solvent indicating strong intercalative binding. Those molecules bound to the ss DNA are accessible to the quencher to the maximum extent and the binding appears to be on the periphery of the helix as intercalation is not feasible. This experiment clearly proves that PSF and SO have intercalative mode of binding to ds DNA and to the ds regions of hd DNA.

Table 2 Various binding parameters for the complexation of phenosafranin and safranin O to ds, hd, and ss calf thymus DNA evaluated from the fluorescence data

DNA conformation	Phenosafranin		Safranin O	
	K_{sv}(L. M^{-1})[a]	A	K_{sv}(L. M^{-1})	A
Free	29.00	0.035	32.48	0.032
Double stranded	14.51	0.192	19.01	0.181
Heat denatured	18.32	0.176	22.12	0.172
Single stranded	25.46	0.092	29.16	0.087

Average of four determinations

[a] Stern-Volmer constants (*Ksv*) refer to solution conditions of 10 mM cacodylate buffer, pH 7.0 at 20°C

Fluorescence Polarization Anisotropy

Fluorescence polarization anisotropy measurements also provided evidence for the varying extents of binding of the two dyes with the three DNA conformations. It has been found that fluorescence polarization anisotropy values of free PSF and SO were 0.035 and 0.032 respectively. Bound PSF and SO molecules had anisotropy values of 0.192 and 0.181 with ds DNA and 0.176 and 0.172 with hd DNA (Table 2). With ss DNA, the anisotropy values for PSF and SO were 0.092 and 0.087, respectively. The results suggested strong binding mode for these dyes to ds and hd DNAs and a weak binding to the ss DNA conformation.

Fluorescence Energy Transfer

The specificity of binding of these dyes to the three DNA conformations was also verified from contact fluorescence energy transfer. When a ligand molecule intercalates into double stranded regions of DNA, DNA molecules can efficiently transfer energy to the excited fluorophore due to the favourable close contact and orientation of the donor-acceptor dipoles. Fluorescence energy transfer from DNA base pairs to bound dye molecules is manifested by an increase in the fluorescence quantum yield of the bound dye in the wavelength range corresponding to DNA absorbance [25, 26]. This can be used as an unambiguous evidence for intercalative binding since energy transfer can occur efficiently only if the bound dye molecules are in close contact with, and oriented parallel to the DNA base pairs. Figure 5a and b depicts plots of variation of Q_λ/Q_{310} against wavelength at various P/D (nucleotide phosphate / dye molar ratio) values showing an increase in quantum yield in the region of DNA absorbance. The increase in quantum yield was much higher for the complexes with ds and hd conformation in comparison to the ss DNA conformation. This result indicated that binding to the ds and hd conformations resulted in substantially higher energy transfer for PSF and SO complex compared to the ss complexes providing strong evidence for a true intercalative binding to ds and hd helical states.

Salt Dependence Studies

PSF and SO are cationic dyes whose binding to anionic DNA is expected to be salt dependent. To ascertain the extent of electrostatic interaction, salt dependent fluorescence titrations were performed at three [Na^+] ion concentrations viz. 10, 50 and 100 mM [Na^+]. The binding affinity of PSF to the ds DNA varied from 4.77×10^5 M^{-1} at 10 mM to 0.68×10^5 M^{-1} at 100 mM

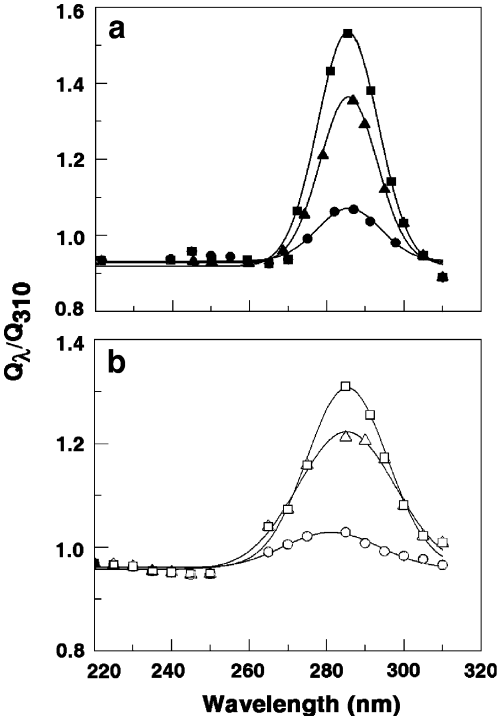

Fig. 5 Variation of the relative fluorescence quantum yield of (**a**) PSF in the presence of ss CT DNA (●), hd CT DNA (▲), ds CT DNA (■) and (**b**) SO in presence of ss CT DNA (○), hd CT DNA (Δ), ds CT DNA (□) in 10 mM sodium cacodylate buffer of pH 7.0 at 20°C as a function of excitation wavelength

while that of hd DNA varied from 2.60×10^5 M^{-1} at 10 mM to 0.61×10^5 M^{-1} at 100 mM. In the case of ss DNA, the binding affinity variation was from 0.55×10^5 M^{-1} at 10 mM to 0.39×10^5 M^{-1} at 100 mM. Almost similar results were obtained for SO binding also (not shown). It can be observed that the change is maximum with the ds DNA, intermediate with hd DNA and least with the ss DNA indicating that as the salt concentration was increased, binding in ds and hd DNA was affected drastically compared to the simple electrostatic binding in ss DNA.

Absorbance Spectral Study

The effect of the three DNA helical conformations on the absorbance spectra of PSF and SO was also studied. PSF and SO have characteristic visible absorption spectra in the 450–700 nm region with a maximum at 520 nm that is convenient to monitor the interaction. Pronounced hypochromic and bathochromic effects were observed in this spectral region of the dyes when mixed with increasing concentrations of ds and hd DNA (Fig. 6). Hypochromic effect of 27 and 25% and bathochromic shift of 19 and 16 nms, respectively, were revealed with PSF and SO on binding to the ds DNA

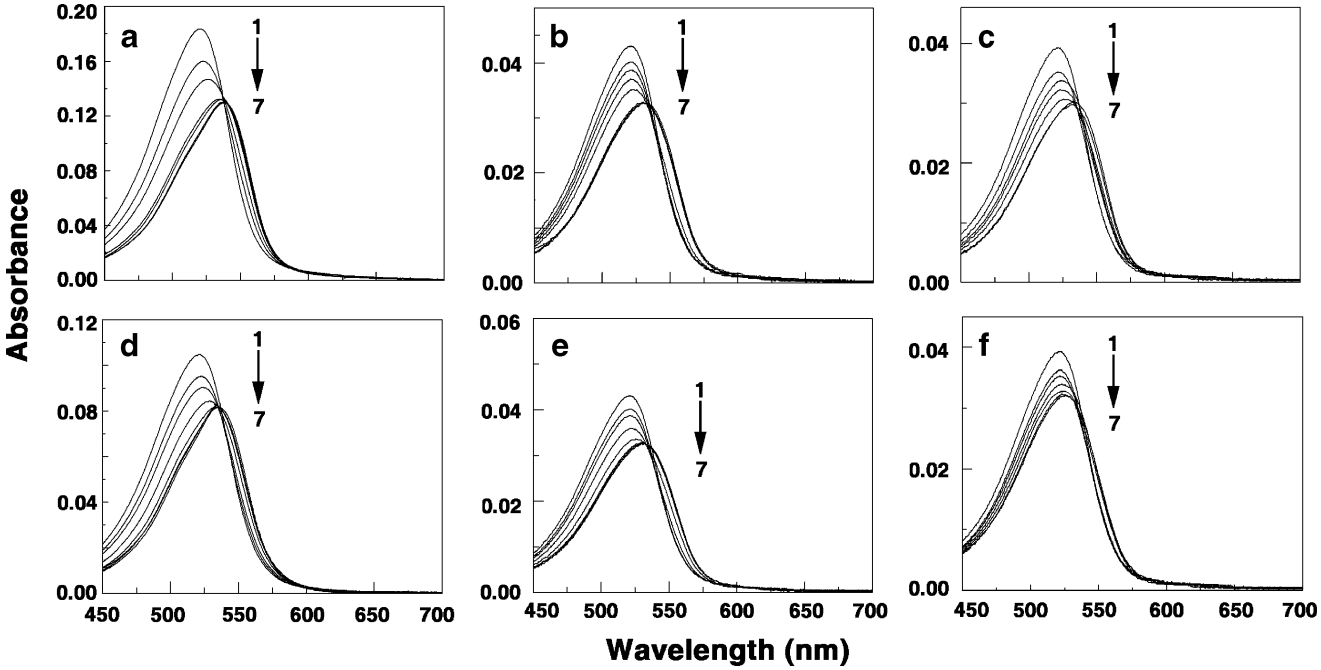

Fig. 6 Representative absorption spectra of (**a**) PSF (5.5 μM) treated with 0, 27.2, 54.5, 109.1, 136.3, 163.6, and 174.5 μM (curves 1–7) of ds CT DNA, (**b**) PSF (1.5 μM) treated with 0, 7.5, 15.1, 22.7, 37.8, 48.4, and 57.5 μM (curves 1–7) of hd CT DNA, (**c**) PSF (1.1 μM) treated with 0, 11.5, 17.3, 23, 28.8, 40.3, and 48.34 μM (curves 1–7) of ss CT DNA (**d**) SO (4 μM) treated with 0, 20, 40, 80, 100, 120, and 160 μM (curves 1–7) of ds CT DNA (**e**) SO (1.7 μM) treated with 0, 8.4, 16.8, 42, 58.8, 67.2, and 75.6 μM (curves 1–7) of hd CT DNA, and (**f**) SO (1.5 μM) treated with 0, 15.2, 30.4, 45.6, 60.8, 68.4, and 76.0 μM (curves 1–7) of ss CT DNA. All experiments were performed at 20°C in 10 mM sodium cacodylate buffer at pH 7.0

structure. Binding to the hd DNA resulted in 23 and 19% hypochromicity and 12 and 10 nm bathochromic effects for PSF and SO. The changes observed with the ss DNA with a hypochromicity of 16 and 15% and bathochromic effect of 8 and 5 nm, respectively, for PSF and SO were the lowest. Another important feature in the absorbance titration was that while sharp isosbestic point was observed with ds and hd conformations for both dyes, this was not apparent on binding to ss DNA revealing strong intermolecular association with ds and hd DNAs. Such spectral changes observed with ds and hd forms have been usually interpreted to arise from a strong interaction between the π electron cloud of the interacting dye and the base pairs presumably due to intercalation [30].

Spectroscopic Study Using Circular Dichroism

The circular dichroic spectral pattern of the different DNA helical forms displayed a B-form conformation characterized by a large positive band in the 270–280 nm region and a negative band around 245 nm although there were differences in the ellipticity and wavelength maxima. Conformational changes associated with the binding were investigated from circular dichroic studies. The CD bands of the ds and hd forms are caused due to the stacking interactions between the bases and the helical structure that provide an asymmetric environment for the bases. PSF and SO are achiral molecules and do not have any optical activity but may acquire optical activity (induced CD) on binding to the helical organization of DNA. To record dye induced conformational changes in DNA, the CD spectra in the 210–400 nm region were recorded in presence of varying D/P (dye/nucleotide phosphate molar ratio) values. In presence of both PSF and SO, the ellipticity of the long wavelength positive band of all the DNA conformations increased and as the interaction progressed a small red shift in the wavelength maximum was also observed. The ellipticity changes were maximum with ds and hd structures and marginal with the ss structure. The CD spectral data are presented in Fig. 7a–f. Further, strong induced CD bands appeared on either sides of the wavelength maximum of absorbance of PSF and SO (520 nm) with each of the DNA conformation with the positive wavelength maximum around 550 nm and the negative maximum around 500 nm. This may be attributed to an exciton splitting type mechanism arising due to an effective interaction of the transition moments of intercalated dye molecules with that of the base pairs whereby the isohelical arrangement of the dye molecules on the DNA helix results in an asymmetric arrangement of the dye chromophores. The positive induced CD band for PSF with ds DNA system had an

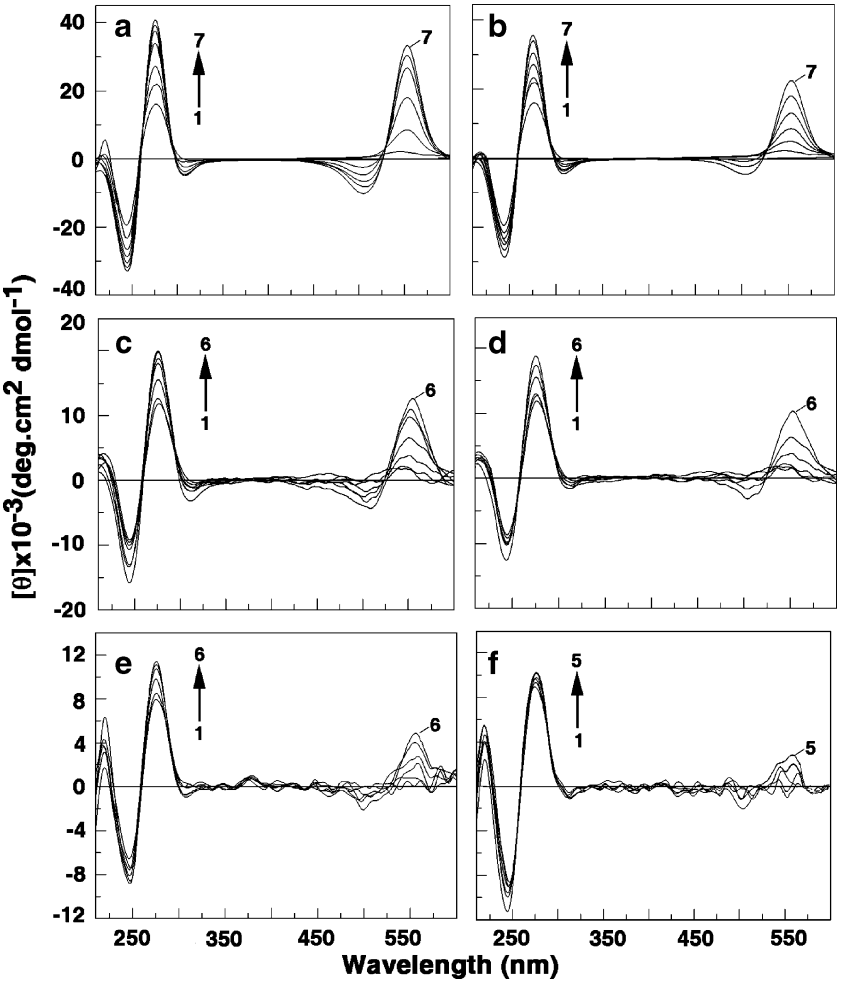

Fig. 7 Representative intrinsic circular dichroic spectra of 60 μM ds CT DNA treated with (**a**) 0, 6, 12, 18, 24, 30, and 36 μM of PSF (curves 1–7), and (**b**) 0,3, 18, 24, 30, 42, and 60 μM of SO (curves 1–7) 30 μM hd CT DNA treated with (**c**) 0, 1.5, 9, 15, 18, 21 μM of PSF (curves 1–6) and (**d**) 0, 6, 7.5, 15, 24, and 33 μM of SO (curves 1–6), 30 μM ss CT DNA treated with (**e**) 0, 6, 12, 18, 24, and 30 μM of PSF (curves 1–6), and (**f**) 0, 6, 18, 24, and 36 μM of SO (curves 1–5). All experiments were performed at 20°C in 10 mM sodium cacodylate buffer at pH 7.0

ellipticity value of 32,400 deg cm^2 $dmol^{-1}$, for hd DNA it was 13,050 deg cm^2 $dmol^{-1v}$, and for ss DNA conformation it was only 5525 deg cm^2 $dmol^{-1}$. The negative band ellipticity for PSF with ds, hd and ss DNA conformations were 10,050, 9125 and 2020 deg cm^2 $dmol^{-1}$, respectively. For SO with ds, hd and ss DNAs, the positive ellipticity values were 22,078, 10,885 and 2510 deg cm^2 $dmol^{-1}$ and the negative ellipticity values were 5150, 3235 and 2010 deg cm^2 $dmol^{-1}$, respectively. The induced CD band ellipticity thus was highest with ds DNA and varied as ds > hd>> ss DNA for PSF and SO complexation and the large differences in the intensities indicate that the binding is clearly sensitive to the duplex nature, being highest with ds and lowest with ss DNA structure. The magnitude of the induced CD spectral bands may also suggest differences in the strength of the bound molecules inside the helical organization of the DNA conformations. Based on this, the intercalation of PSF and SO to ds DNA structure appears to be stronger than with the hd DNA and the weakest binding was observed with the ss DNA conformation. This result is in confirmation with the results obtained from other spectroscopic experiments.

Conclusions

This study presents the structural aspects of the binding of two phenazinium dyes, phenosafranin and safranin O, with three helical conformations of CT DNA using fluorescence, absorption and circular dichroic techniques. Several conclusions have emerged from the data obtained from diverse fluorescence techniques used. At the first instant, the binding affinity of both the dyes to ds and hd DNA structures are remarkably higher than that with ss DNA structure. Secondly, intercalation of PSF and SO to ds and ds regions of hd DNA has been confirmed. Thirdly, PSF is a stronger DNA binder than SO. It is likely that the bulkier methyl groups of SO may be hindering the intercalation of SO compared to PSF. The binding also resulted in induction of optical activity in the bound dye molecules and the same was stronger with the ds conformation. Taken together, the results provide unequivocally evidences for the preference of PSF and SO to the double stranded DNA conformation and prove the ability of fluorescence techniques to clearly elucidate the DNA binding characteristics of these compounds.

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