J Fluoresc (2008) 18:139–147
DOI 10.1007/s10895-007-0252-7

ORIGINAL PAPER

The Mechanism of Benzothiazole Styrylcyanine Dyes Binding with dsDNA: Studies by Spectral-Luminescent Methods

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Received: 7 May 2007 / Accepted: 4 September 2007 / Published online: 28 September 2007

Abstract In the presented work studies of the interaction mode of monomer and two homodimer benzothiazole styryl dyes containing spermine-like linkage/tail group with the double stranded (ds) DNA are reported. For these dyes, equilibrium constant of dye binding to DNA (K_b), as well as the number of dsDNA base pairs occupied by one bound dye molecule (n) were determined. The data obtained show that the presence of spermine-like group containing quaternary nitrogen (Bos-5) results in increase of K_b value as compared to this of unsubstituted analogue (Sbt). Besides, for the dimer dyes containing benzothiazole styryl chromophores, the K_b value is either five times higher (DBos-13) or almost the same (DBsu-10) as compared to this of corresponding monomer Sbt, depending on the position in the benzothiazole ring where the linker is attached. Moreover, the n values for both dimers are significantly different as well, pointing to the bis-intercalative binding mechanism for DBos-13 and for the groove-binding one for DBsu-10. The conclusion about the dimer dyes-dsDNA binding mechanisms is also supported by the study of the fluorescent response of these dyes on the presence of AT- and GC-containing polynucleotides.

Keywords Styrylcyanine dyes · DNA · Fluorescent detection · Binding constant · Interaction mechanism

Introduction

For today styrylcyanines are among the fluorescent dyes widely used for biomolecules detection. These dyes are successfully applied as sensitive probes for unspecific fluorescent staining of proteins in gels [1]. Due to the ability of styrylcyanines to interact with DNA with up to hundred times fluorescent enhancement and ability to penetrate into the cell these dyes were proposed for intra cellular DNA imaging [2, 3].

Recently benzothiazolium styrylcyanines were proposed as probes for DNA detection upon two-photon excitation (TPE). Styryl dyes are known as having high two-photon absorption cross-section values [4, 5]; one of the benzothiazolium styryls was shown to give strong fluorescence upon TPE by 1064 nm irradiation [6]. On the other hand significant fluorescent response on the double-stranded (ds) DNA presence is observed for these dyes. Thus series of monomer and homodimer styrylcyanines was synthesized and studied for their efficiency as TPE excited nucleic acids sensitive dyes. It was shown that dyes modified with spermine-like linkage/tail group demonstrate increased sensitivity to DNA. They have low intrinsic emission intensity and enhance their fluorescence intensity up to three orders of magnitude in presence of DNA. Complexes of studied dyes with DNA also demonstrate intensive emission upon the TPE. The values of two-photon

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absorption cross section for these complexes were in the range $4.7–7.4\times10^{-50}$ cm^4 s [7].

It is known that small molecules can reversibly bind to dsDNA by three main modes: (1) intercalation; (2) major or minor groove-binding; and (3) external binding [8]. The intercalation suggests that the planar chromophore of the molecule becomes inserted between the adjacent base pairs of DNA. This interaction is usually independent of the DNA sequence context (small preference for GC sequences has been observed). The molecules interacting with dsDNA by fixation in its groove (groove-binders) generally show a strong preference for AT regions. Crescent shaped, cationic ligands are often minor groove binders. External electrostatic binding of ligands to the double helix generally does not dependent on DNA sequence [9–11]. Earlier, the styrylcyanine dye trans-4-[4-dimethylamino)styryl]methylpyridinium iodide was shown to be a minor groove binder with high affinity to dsDNA [12]. But generally mechanisms of styrylcyanines-dsDNA interaction are poorly studied for today.

Earlier homodimer dyes having linkage group bound to nitrogen atoms of benzothiazole rings were synthesized and studied as potential probes for DNA detection [2, 7]. In present work styryl homodimer connected with linkage group through the 6-positions of benzothiazole heterocycles (DBos-10) is firstly proposed for this purpose and studied (Fig. 1).

This research is aimed on the clarification of the interaction mode of benzothiazole styrylcyanine monomer and two various structure homodimers modified with spermine-like linkage/tail group (Fig. 1) with the dsDNA. For this purpose the specificity of the dyes binding to poly $(dA\text{-}dT)_2$ and poly$(dG\text{-}dC)_2$ polynucleotides will be studied. Calculation of the binding constants and the numbers of dsDNA base pairs occupied by one bound dye molecule will be carried out basing on the results of fluorescent titration. Non-substituted benzothiazole styryl dye Sbt (Fig. 1) will be used for the comparison that will allow us to estimate influence of the presence of spermine-like substituents on dye-DNA affinity. Dependence of dye-DNA binding mode on the position of linkage group connecting two chromophores of homodimer will be studied.

Experimental

Chemicals

Dyes Bos-5, Sbt, DBos-13, and DBsu-10 were synthesized according to [2, 13]. Stock solutions of the dyes (2×10^{-3} M) were prepared in DMSO and kept at 4°C.

Dimethylsufoxide (DMSO) and tris-(oxymethyl-)amino methanehydrochloride (Tris) were used without purification. 0.05 M Tris–HCl solution (pH 8.0) was used as a buffer in all experiments. Salmon tastes DNA, poly(dA-dT)$_2$ and poly(dG-dC)$_2$ were obtained from Sigma Chemical Co. The concentration of DNA stock solution in Tris–HCl buffer was 6×10^{-3} M bp (moles of base pairs per liter).

Spectral studies

Sbt, Bos-5, DBos-13, and DBsu-10 of 5×10^{-6} M (homodimers concentration was calculated in chromophores) were titrated by dsDNA. Aliquots (5–525 μl) of the DNA stock solutions were added to the 5×10^{-6} M dye buffer solution to obtain the mixing dye-base pair (dye-bp) ratios from 1:2.4 to 1:200. To avoid the dye concentration decrease as the result of dissolving, the DNA stock solution contained 5×10^{-6} M dye as well. Absorption spectra were recorded by the Specord M 40 spectrophotometer (Carl Zeiss, Germany). Fluorescence excitation and emission spectra were recorded by a Cary Eclipse fluorescence spectrophotometer (Varian Inc., Australia). All measurements were carried out at room temperature.

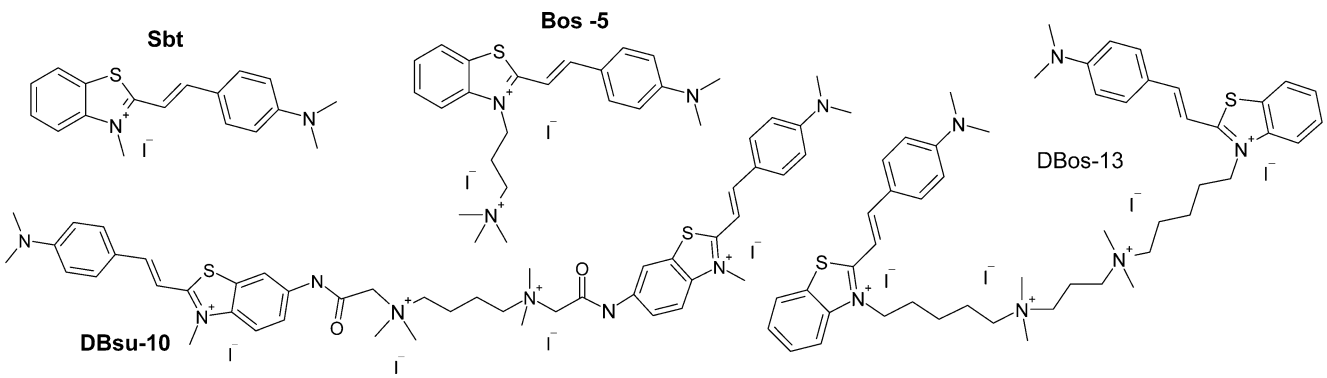

Fig. 1 Chemical structures of benzothiazole styryls

Determination of the binding constant (K_b) and the number of dsDNA base pairs occupied by one bound dye molecule (n)

The binding constant, K_b, indicates the stability of ligand-DNA complex. This is a useful measure to describe the strength of binding (or affinity) between DNA and their ligands. In the case there is the following equilibrium in the solution:

$$\text{dye} + \text{DNA} \leftrightarrow \text{dye} - \text{DNA} \tag{1}$$

the law of mass action has the following form [14]:

$$\frac{C_{bd}}{C_{fDNA} \times C_{fd}} = K_b \tag{2}$$

C_{bd}, C_{fd} and C_{fDNA} being concentrations of the dye bound to DNA, dye in free form and DNA binding sites unoccupied by dye molecules, respectively. One of the most difficult points in the K_b study is the determination of C_{fDNA}, taking into account the fact that the dye could occupy several DNA base pairs upon binding. J.D. McGhee and P.H. von Hippel reported a detailed theoretical study of ligand-DNA interactions accounting for the above mentioned difficulty, which have advantages over linear Scathard plots [14]. In the case of the single mode of ligand-DNA interaction and non-cooperative binding (i.e., neighboring ligands do not influence one another), the following expression of the mass action law (Eq. 2) was obtained [14]:

$$\frac{v}{L} = K_b(1 - nv)\left(\frac{1 - nv}{1 - (n-1)v}\right)^{n-1} \tag{3}$$

where L is the free ligand concentration, ν is the binding density (i.e. the number of the bound dye molecules per one DNA base pair), and n is the number of dsDNA base pairs occupied by one bound dye molecule.

Assuming only one type of interaction between dye and DNA the binding being non-cooperative, K_b and n values can be calculated from fluorescent titration curves by modifying the formula (Eq. 3). Since the DNA presence leads to the sharp increase in the dye fluorescence intensity, intrinsic fluorescence of free dye could be neglected. Thus for the case of dye fluorescent titration by dsDNA, one could express the percent of the bound dye as:

$$\frac{C_{bd}}{C_{dye}} = \frac{I}{I_{max}} \tag{4}$$

C_{dye} and C_{bd} being the total dye concentration and the concentration of the dye bound to DNA, respectively; the maximum value of the fluorescence intensity (I_{max}) is obtained when all the dye molecules are bound. The parameters L and ν from the Eq. 3 could be thus expressed using the Eq. 4 as follows:

$$L = C_{dye} - C_{bd} = C_{dye}\left(\frac{I_{max} - I}{I_{max}}\right) \tag{5}$$

$$v = \frac{C_{bd}}{C_{DNA}} = \frac{C_{dye}}{C_{DNA}} \cdot \frac{I}{I_{max}} \tag{6}$$

Substituting Eqs. 5 and 6 into the Eq. 3, we can obtain the required expression to calculate K_b and n:

$$Y = I_{max} - \frac{X}{C_{dye}K_b} \frac{\left(1 - \left[(n-1)\frac{X}{I_{max}}\right]\right)^{n-1}}{\left(1 - n\frac{X}{I_{max}}\right)^{n}} \tag{7}$$

where $Y=I$ and $X=I\times C_{dye}/C_{DNA}$. Therefore, K_b, n and I_{max} values can be calculated as approximation parameters of fitting by the Eq. 7 the experimentally obtained data plotted as the dependence of Y on X. For the homodimer dyes, the calculation was performed with respect to the whole homodimer molecule.

Results and discussions

The effect of interaction with DNA on the absorption, fluorescence excitation and emission spectra of dyes

Characteristics of absorption, fluorescence excitation and emission spectra of the studied dyes (5×10^{-6} M) in aqueous buffer solution and in the presence of rather high dsDNA concentration (6×10^{-4} M bp) are summarized in Table 1.

The absorption spectra of studied monomer styryls in Tris−HCl buffer were comprised of the only band corresponding to the dye monomer maximum. The maxima of absorption spectra are located at 511 and 529 nm for Sbt and Bos-5, respectively. Absorption maxima for these dyes in the presence of DNA are shifted to the long wavelength region on 30–31 nm comparing with the maxima in buffer and correspond to the dye monomer bound to DNA. Absorption spectra of these two dyes in dsDNA presence have the similar shape (Fig. 2a,b).

In absorption spectra of homodimer styryls dominating bands are blue-shifted as compared to that of the parent monomer Sbt; these bands are settled at 491 and 469 nm for DBos-13 and DBsu-10, respectively (Fig. 3a,b). Basing on the results of [7, 15], where the dimers with the same chromophore were regarded, we believe that the mentioned absorption bands belong to H-aggregates of the dimer dyes. In the presence of 6×10^{-4} M bp DNA, the absorption band of DBos-13 was strongly red-shifted (491 nm for free dye

Table 1 Characteristics of absorption, fluorescence excitation and emission spectra of styrylcyanine dyes (5×10^{-6} M) in buffer and in complexes with DNA (6.0×10^{-4} M bp.)

Name	Free dye				In DNA presence				
	λ_{abs}, nm	λ_{ex}, nm	λ_{em}, nm	*I, a.u.*	λ_{abs}, nm	λ_{ex}, nm	λ_{em}, nm	*I, a.u.*	I/I_0
Sbt	511	530	593	17.9	542	555	603	1,633.3	91.2
Bos-5	529	542	598	20.1	559	568	608	2,005.9	99.8
Dbos-13	491	540	596	1.5	556	560	606	1,899	1,266
Dbsu-10	469	560	613	9.1	452[a] 545	555	610	1,253.6	137.8

λ_{abs}, λ_{ex}, λ_{em}—absorption, fluorescence excitation and emission spectra maxima wavelengths
I – fluorescence intensity of free or DNA-bound dye at λ_{em}
[a] Band is manifested as a shoulder

and 556 nm for dye-DNA complex) and corresponds to the dye monomers bound to DNA. For DBsu-10 in presence of 6×10^{-4} M bp DNA the dominating absorption band is situated at 545 nm and corresponds to the dye monomers bound to DNA as well. Besides, H-aggregates band manifested as the shoulder is observed, being shifted to the short wavelength region on 17 nm relative to the corresponding band of dye in buffer. Basing on the shift of the H-aggregate band caused by DNA presence, one can conclude that the aggregates are formed on the DNA molecule.

The study of the fluorescence excitation and emission spectra of the studied monomer and homodimer dyes shows that these spectra of the free dyes as well as of the dyes bound to DNA belong to the dye monomer form (Figs. 4 and 5). Besides, no emission corresponding to H-aggregates was observed. In addition, the dyes binding to DNA results in the emission intensity increase in 2–3 orders of magnitude. Hence, the dye fluorescence intensity in DNA presence allows to estimate the percent of the dye molecules bound with DNA in monomer form (Eq. 4).

Monomer dyes

The styrylcyanine dye Bos-5 modified with spermine-like tail group as well as the unsubstituted dye Sbt were studied in comparison to characterize stability of DNA-monomeric dye complex and to study the influence of above mentioned substituent on the affinity of DNA-dye complexes.

Absorption and fluorescence spectra of the dyes in presence of different DNA concentrations were studied. The absorption spectra of the both dyes in presence of $0 \div 5.3 \times 10^{-4}$ M bp DNA (Fig. 2a,b) consist of the two bands corresponding to the non-bound (maxima at 511 nm for Sbt and at 529 nm for Bos-5) and DNA-bound (542 nm for Sbt and at 559 nm for Bos-5) monomer forms of dye.

Since for both Sbt and Bos-5 the absorption spectra of the dye in presence of different DNA concentrations cross

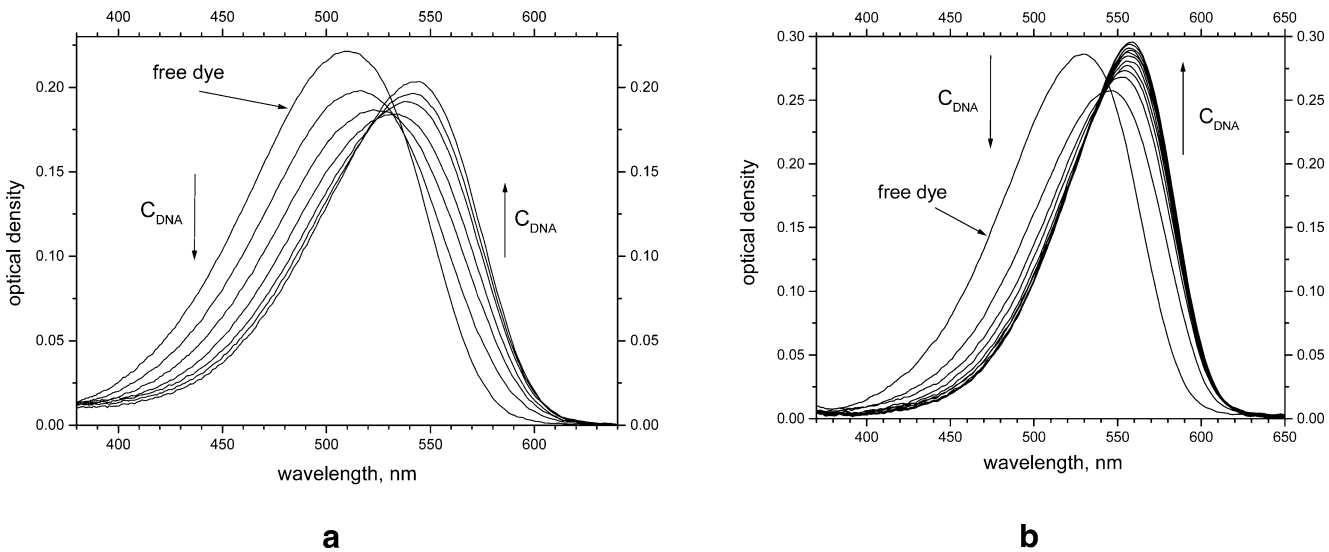

Fig. 2 Absorption spectra of the dyes Sbt (**a**) and Bos-5 (**b**) in Tris–HCl buffer in the presence of $0 \div 5.3 \times 10^{-4}$ M bp DNA. The dye concentration used for the measurements was 5×10^{-6} M

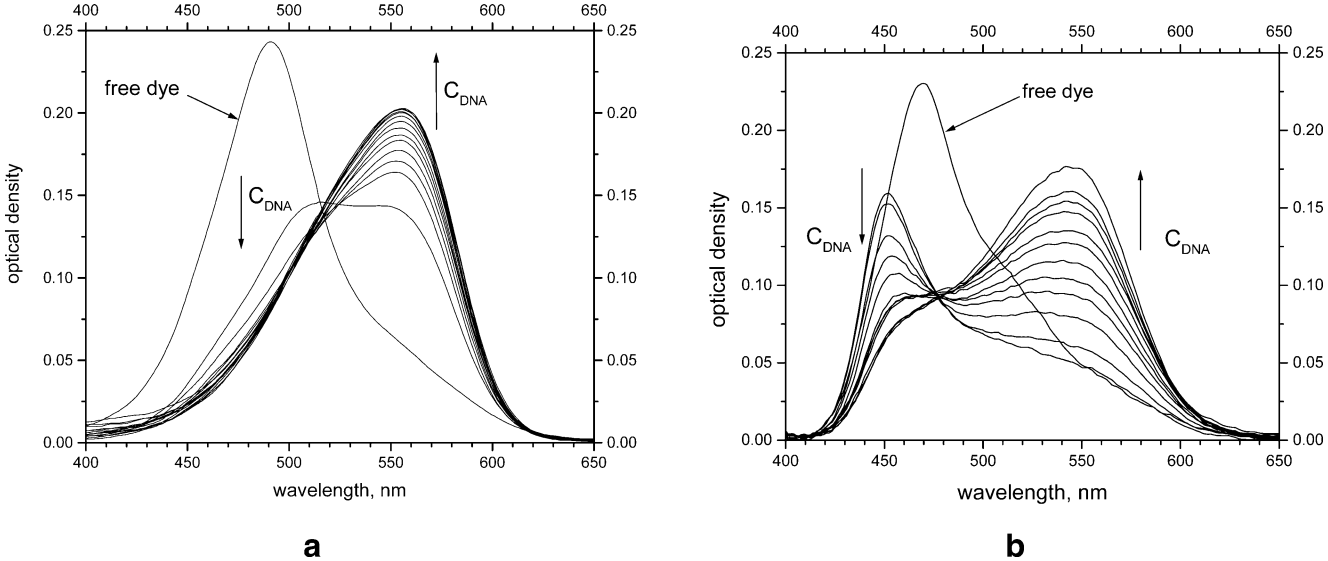

Fig. 3 Absorption spectra of the dyes DBos-13 (**a**) and DBsu-10 (**b**) in Tris–HCl buffer in the presence of $0 \div 5.3 \times 10^{-4}$ M bp DNA. The dye concentration used for the measurements was 2.5×10^{-6} M of dimer molecules

near the isobestic point, one can generally consider the equilibrium between the nonbound and DNA-bound dye molecules in monomer form in the solution according to Eq. 1.

The parameters of the equilibrium (Eq. 1) could then be found using the equation developed by McGhee and von Hippel [14].

The binding constants (K_b) and the number of dsDNA base pairs occupied by one bound dye molecule (n) for the dyes Sbt and Bos-5 were estimated by fluorescent titrations. The approximation of the experimental results with the McGhee and von Hippel equation (Eq. 7; Fig. 6a,b) shows that for the both dimer dyes the mentioned equation generally correctly describes the obtained results. The values of K_b and n for the studied dyes are summarized in the Table 2.

The average K_b values obtained for the dyes Sbt and Bos-5 were 1.8×10^4 M^{-1} and 6.2×10^4 M^{-1} respectively, the number of dsDNA base pairs occupied by one bound dye molecule (n) were correspondingly 3.4 and 2.3. Such values of K_b show that Sbt and Bos-5 form stable complexes with DNA. It should be noted that K_b values for Bos-5 is more than three times higher than that for Sbt. Since the structures of these two monomer styryls differ from each other by N-alkylammonium substituent (spermine tail group) we can notify that incorporation of this substituent leads to the increase of dye affinity to DNA.

The binding constant values obtained for the monomer dyes are typical for both intercalators and groove-binders as well. Binding constant (K_b) values for intercalators that

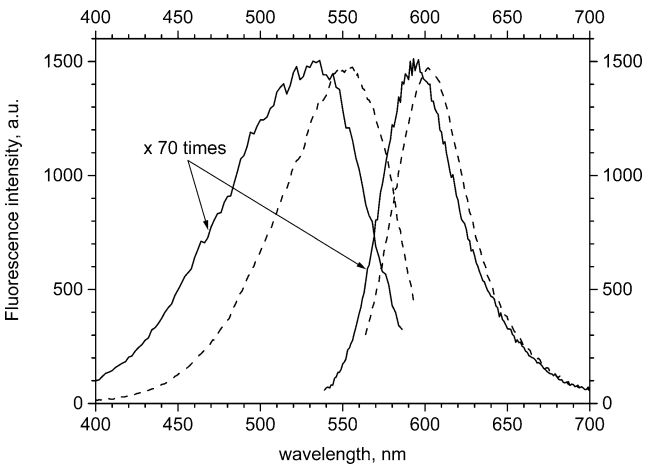

Fig. 4 Fluorescence excitation (*left*) and emission (*right*) spectra of the dye Sbt in Tris–HCl buffer (*solid line*) and in the presence of 6×10^{-4} M bp DNA (*dashed line*). The dye concentration was 5×10^{-6} M. The spectra of the free dye were multiplied in 70 times

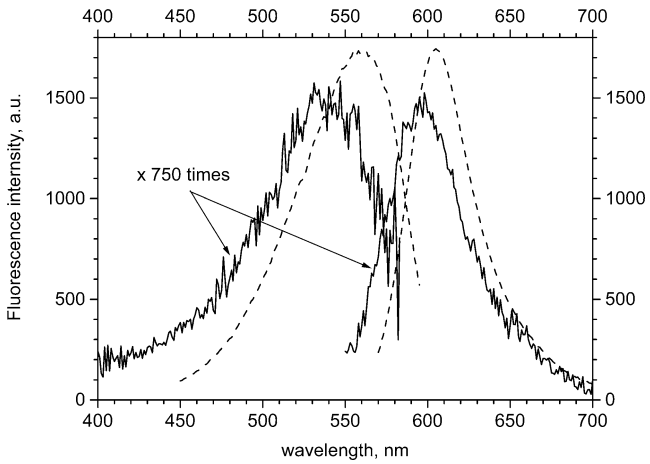

Fig. 5 Fluorescence excitation (*left*) and emission (*right*) spectra of the dye DBos-13 in Tris–HCl buffer (*solid line*) and in the presence of 6×10^{-4} M bp DNA (*dashed line*). The dye concentration was 2.5×10^{-6} M of dimer molecules. The spectra of the free dye were multiplied in 750 times

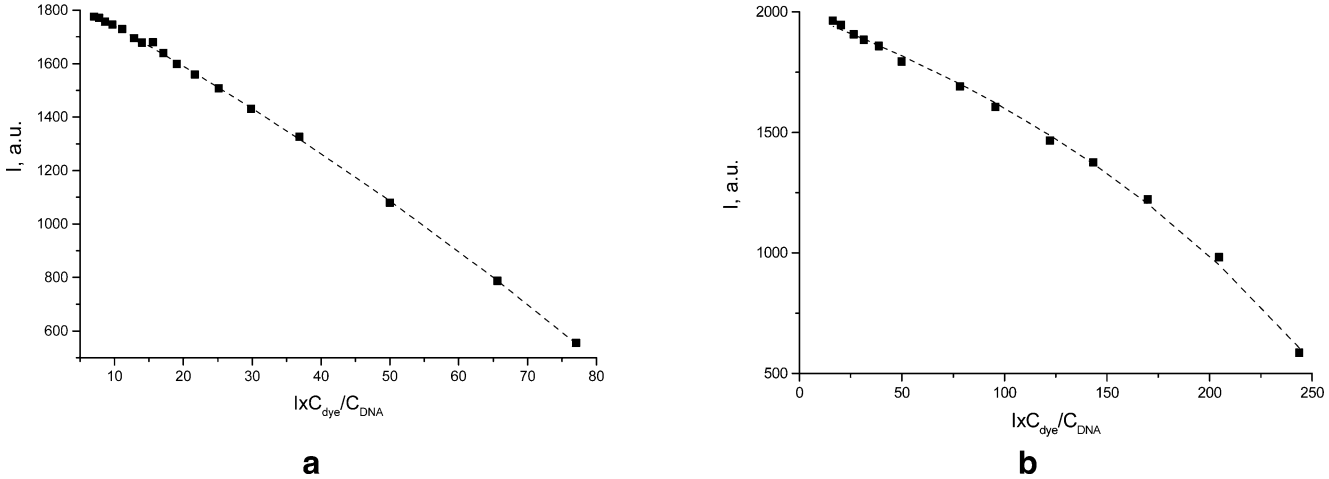

Fig. 6 Plot of fluorescence intensity dependence on $I \times C_{dye}/C_{DNA}$ ratio (*square*) and its approximation by the Eq. 7 (*broken line*) for Sbt (**a**) and Bos-5 (**b**)

bind to DNA usually do not exceed 10^7 M^{-1}. K_b values of Acridine orange (AO), thiazole orange (TO), and ethidium bromide are equal to 5.03×10^4, 10^6 and 1.5×10^5 M^{-1}, respectively [11, 16, 17]. At the same time, the *n* value for Bos-5 (2.3) implies that the dye can interact with DNA by intercalation mode, since in the case of groove-binding this dye would occupy at least 3 base pairs. Meanwhile, the same value for Sbt (3.4) could be consistent with both intercalation and groove-binding. In order to obtain more information about the binding mechanism of Sbt and Bos-5, the fluorescent properties of the dyes in the presence of AT- and GC-containing polynucleotides were studied (Table 3).

Both dyes Sbt and Bos-5 showed large enhancement of fluorescence intensity in the presence of both poly(dA-dT)$_2$ and poly(dG-dC)$_2$ polynucleotides. Small preference to GC-containing DNA sequences over the AT-containing ones observed for Sbt could evidence for its binding with dsDNA *via* intercalation. At the same time, for Bos-5 the preference to AT-sequence was observed. Nevertheless, the fluorescence intensity of Bos-5 in the presence of poly(dA-dT)$_2$ (I^{AT}) is only in 1.7 times higher than the same value in the presence of poly(dG-dC)$_2$ (I^{GC}), while for the groove-binding dyes I^{AT} generally exceeds I^{GC} for an order of magnitude. Thus taking into account the *n* value for Bos-5, we could consider the intercalation mode of interaction to be more consistent with the experimental results as compared to the groove-binding one.

Table 2 Binding constant (K_b) and the number of dsDNA base pairs occupied by one bound dye molecule (*n*) values of investigated dyes

Dye	K_b, 10^4 M^{-1}	*N*
Sbt	1.8±0.3	3.4±0.8
Bos-5	6.2±0.9	2.3±0.5
Dbos-13	10.0±1.4	4.9±2.2
Dbsu-10	2.7±0.8	14.5±3.2

Homodimer dyes

For the two benzothiazole styryl homodimers DBos-13 and DBsu-10 that differ from each other by the position of linkage group, the interaction with dsDNA and synthetic polynucleotides poly(dA-dT)$_2$ and poly(dG-dC)$_2$ was studied.

Absorption spectra of the both homodimers in the presence of different DNA concentrations are presented in the Fig. 3.

For the both dyes DBos-13 and DBsu-10 in free form, the absorption spectrum belongs to the aggregates of the H-type (maxima at 491 and 469 nm respectively). The addition of the small DNA ammount causes the appearing of new aggregate bands in absorption spectra of the both dyes, which could be attributed to the dye H-aggregates bound with the DNA. It should be mentioned that while in the case of DBsu-10 only one band (at 452 nm) corresponding to H-aggregates is observed, the behavior of the DBos-13 spectra is more complicated. Besides the maximum near 510 nm, in the absorption spectrum of DBos-13 in DNA presence one more aggregation band situated at about 460 nm and manifested as a shoulder could be observed. Further addition of DNA leads to the decrease of mentioned H-aggregate bands in the spectra of the both dyes and increase of the band corresponding to the dye monomers bound to DNA (556 nm for DBos-13 and 545 nm for DBsu-10).

Thus, in the studied solutions of the dimer dyes in presence of DNA, the equilibrium takes place between the dye H-aggregates formed on DNA and dye monomers bound to DNA; besides, in the case of DBos-13 at least two

Table 3 Characteristics of fluorescence excitation and emission spectra of styrylcyanine dyes in the presence of poly(dA-dT)$_2$ and poly(dG-dC)$_2$ polynucleotides

Dye	I_0	Poly(dA-dT)$_2$				poly(dG-dC)$_2$			
		λ_{ex} (nm)	λ_{em} (nm)	I^{AT}, *a.u.*	I^{AT}/I_0	λ_{ex} (nm)	λ_{em} (nm)	I^{GC}, *a.u.*	I^{GC}/I_0
Sbt	19.1	548	601	658	34.5	562	607	756	39.6
Bos-5	24.8	566	608	1695	68.3	575	612	996	40.2
Dbos-13	2.2	557	606	1455	661.4	562	608	1445	656.8
Dbsu-10	6.1	551	596	729	119.5	574	614	82	13.4

λ_{ex}, λ_{em}—fluorescence excitation and emission spectra maxima wavelengths; I_0 (I^{AT}, I^{GC})—fluorescence intensity of free dye (in presence of AT-, GC-polynucleotide) at λ_{em}

kinds of H-aggregates exist. Thus, the equilibrium equations should be much more difficult for both dimer dyes than the Eq. 1, and the McGhee and von Hippel equation can not be correctly applied to the binding of DBos-13 and DBsu-10 with dsDNA. Nevertheless, the McGhee and von Hippel equation (Eq. 7) turned out to give adequate approximation of the dependence of I on $I \times C_{dye}/C_{DNA}$ (Fig. 7). The values of K_b and n obtained as the approximation parameters are presented in the Table 2.

Significant difference in n values (Table 2) observed for DBos-13 and DBsu-10 dyes points that due to the distinctions of structures these homodimer dyes interact with DNA via different mechanisms. DBos-13 has a positively charged linkage group which is bound to the nitrogen atoms of benzothiazole ring. At the same time, in DBsu-10 the linkage group is attached in the 6-position of benzothiazole residues. The value of n for DBos-13 (4.9) points to the bis-intercalative dsDNA-binding mechanism for this dye, while the same value for DBsu-10 (n=14.5) clearly demonstrates the groove-binding mechanism of interaction of this dye with the dsDNA.

On the other hand, K_b values for the both dyes (10.0×10^4 M^{-1} for DBos-13 and 2.7×10^4 M^{-1} for DBsu-10) could be characteristic for both groove-binding and intercalation. Nevertheless, the exceeding of the K_b value for DBos-13 over this for DBsu-10 is consistent with the intercalation binding mode for the first dye and the groove-binding for the last one.

The study of the fluorescent properties of the dyes in the presence of AT- and GC-containing polynucleotides (Table 3) showed almost the same I^{AT} and I^{GC} values for DBos-13. This result evidences for the dye intercalation into the dsDNA supporting the supposition made before. The possible structure of DBos-13 intercalation complex with dsDNA is presented in the Fig. 8a. This structure (Fig. 8a) corresponds to the case when the dye molecule occupies 5 base pairs (correspondingly to the nearest neighbor exclusion principle). Nevertheless, due to the flexible linking group DBos-13 chromophores could intercalate into dsDNA on different distances one to another.

At the same time, the dye DBsu-10 demonstrates the high specificity to AT-sequence (I^{AT} exceeds I^{GC} in almost

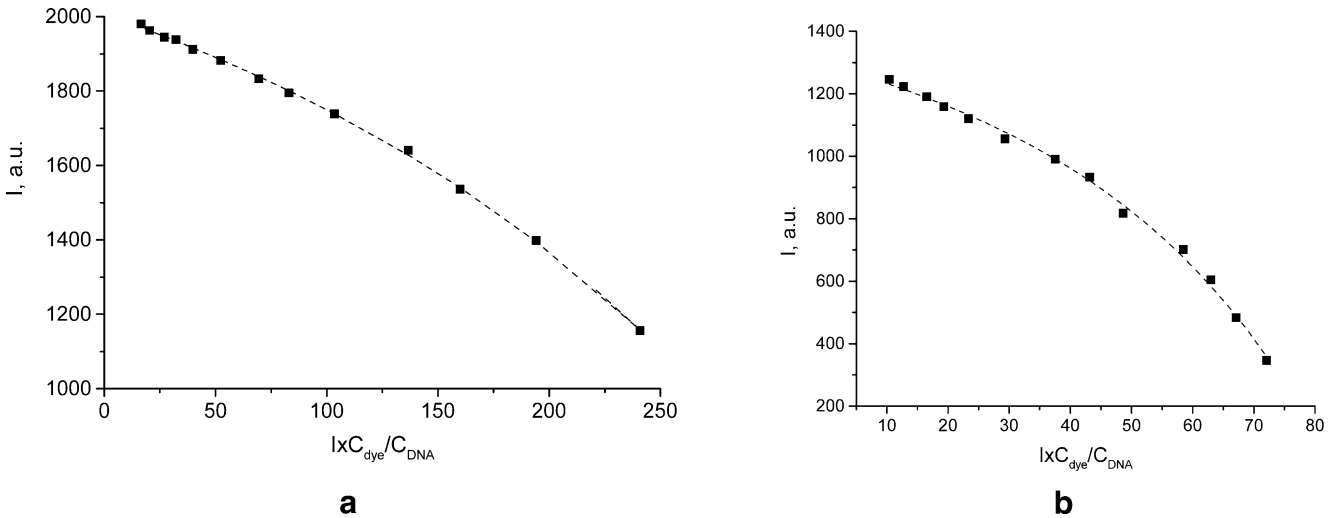

Fig. 7 Plot of fluorescence intensity dependence on $I \times C_{dye}/C_{DNA}$ ratio (*square*) and its approximation by the Eq. 7 (*broken line*) for DBos-13 (**a**) and DBsu-10 (**b**)

nine times) that points on the groove-binding mode of DBsu-10 interaction with dsDNA. This supposition is consistent with the high n value obtained for the dye. Besides, the intercalation seems to be spatially hindered for the molecule with the linkage group attached in the 6-position of the benzothiazole residue. The possible structure of DBsu-10 complex with dsDNA formed by groove-binding mode is presented in the Fig. 8b. As it is shown on Fig. 8b the DBsu-10 dye molecule occupies approximately 9–10 base pairs, that is generally consistent with the n value obtained as the result of approximation. Higher value obtained in the experiment (n=14.5) could be explained by the preference of the groove-binding molecules to AT-rich sequences.

Comparison of monomer and homodimer dyes

The study of the equilibrium parameters of dyes binding with dsDNA revealed several results. First of all, the presence of spermine-like tail group in the monomer molecule leads to the increase of K_b value in about three times, thus to stabilization of the dye-DNA complex. Second, for a dimer with the positively charged linkage group which is bound to nitrogen atoms of the benzothiazole ring (DBos-13) the estimated binding constant increases in 5 times as compared to the parent monomer Sbt. It should be also noticed that for the both monomers and the DBos-13 dimer, the study of dyes sensitivity for AT- and GC-sequences of DNA point to the intercalative mechainsm of dye-dsDNA binding, that is consistent with the obtained values of n. Finally, for the dimer dye with the linkage group containing quaternary nitrogen atoms and bound in 6-positions of benzothiazole heterocycle (DBsu-10), the value of K_b was close to this of the parent dye Sbt. It could be explained by the groove-binding mode of DBsu-10 interaction with DNA. The evidences for this mechanism are (1) the large value of binding site length (n=14.5 base pairs), and (2) the strong preference to the AT-containing dsDNA sequence over the GC-containing ones.

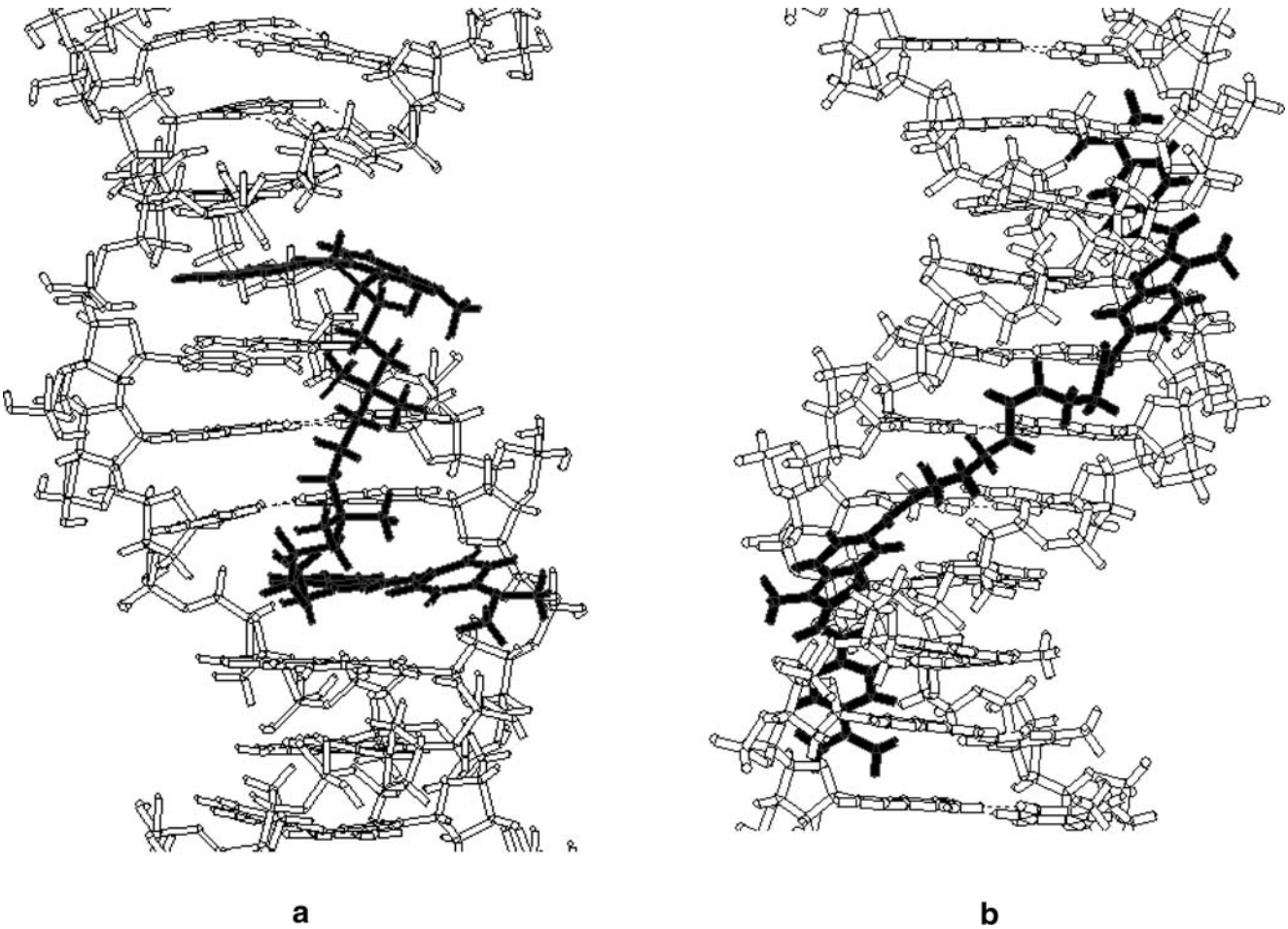

Fig. 8 The possible structures of DBos-13 intercalation complex (**a**) and DBsu-10 groove-binding complex (**b**) with dsDNA

Conclusions

1. It was shown that presence of spermine-like linkage/tail group leads to the increase of dye-DNA binding constant values. This value is 1.8×10^4 M^{-1} for parent dye Sbt; 6.2×10^4 M^{-1} for monomer Bos-5 and 10.0×10^4 M^{-1} for homodimer DBos-13. Despite the presence of linkage group for homodimer dye DBsu-10 the value of binding constant (2.7×10^4 M^{-1}) is close to that for dye Sbt. Values of binding constants recorded for the studied dyes are consistent with both groove-binding and intercalation mechanisms.
2. Values of dsDNA base pairs number occupied by one bound dye molecule calculated for monomer Bos-5 (n= 2.3) and homodimer DBos-13 (n=4.9) point on existing of intercalation binding mode, while for homodimer DBsu-10 this value (n=14.5) is the evidence of groove-binding mechanism. The value obtained for Sbt (n= 3.4) does not point unambiguously on one of these mechanisms.
3. Studies of dyes interaction with synthetic polynucleotides poly(dA-dT)$_2$ and poly(dG-dC)$_2$ has shown that parent dye Sbt, monomer Bos-5 and homodimer DBos-13 give comparable fluorescent response on the presence of both type of polynucleotides. This could be mentioned as an evidence of intercalation binding mode. Strong AT-binding preference demonstrated by homodimer DBsu-10 points on groove-binding mode.
4. It was shown that position of linkage group in homodimer dye molecule could significantly affect on the mode of dye interaction with DNA. Thus for a dimer DBos-13 having linkage group bound to nitrogen atoms of benzothiazole ring intercalation is proposed as binding mode. At the same time homodimer DBsu-10 with the linkage group bound in 6-positions of benzothiazole heterocycle is considered to be groove binder.

Acknowledgements This investigation was partially supported by The Scientific and Technological Research Council of Turkey. The study was also supported by Science and Technology Center in Ukraine (grant #U3104k). We are thankful to Prof. Valeriy M. Yashchuk for the valuable discussion.

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