Journal of Fluorescence, Vol. 15, No. 6, December 2005 (© 2005)
DOI: 10.1007/s10895-005-0002-7

Fluorescent Properties of Pentamethine Cyanine Dyes with Cyclopentene and Cyclohexene Group in Presence of Biological Molecules

M. Yu. Losytskyy,[1,2] K. D. Volkova,[1] V. B. Kovalska,[1] I. E. Makovenko,[1] Yu. L. Slominskii,[3] O. I. Tolmachev,[3] and S. M. Yarmoluk[1,4]

Received March 11, 2005; accepted August 23, 2005
Published online: November 8, 2005

A series of pentamethine cyanine dyes with cyclohexene or cyclopentene group in polymethyne chain, assumed as DNA groove-binders, were studied as fluorescent probes for nucleic acids as well as for native and denatured proteins. It was revealed that the presence of methyl or dimethyl substituent in 5 position of the cyclohexene group hinders the formation of dye–DNA fluorescent complex, while the methyl substituent in 2 position leads to the increasing of the dye–DNA complex fluorescence intensity. The dyes SL-251, SL-1041, and SL-1046 containing methyl group in the 2 position of the cyclic group, are reported as bright DNA-sensitive dyes. The study of the dyes DNA-binding specificity demonstrated significant AT-preference that points to the groove-binding interaction mode. At the same time, the dyes SL-251, SL-377, and SL-957 with the 2-methyl substituted cyclohexene group were shown to be sensitive fluorescent dyes both for nonspecific (in SDS presence) proteins detection and for native BSA.

KEY WORDS: Fluorescent probes; pentamethine cyanine dyes; nucleic acids detection; groove binding.

INTRODUCTION

Polymethine (tri- and pentamethine) cyanine probes are widely used for biomedical application due to their spectral characteristics, namely longwave absorption and emission as well as high emission intensity [1]. The pentamethine cyanine dyes have their absorption and fluorescence maxima shifted for about 200 nm to the longwave region as compared to monomethines, that permits to reach the region where the probes could be excited with the long-wave emission of nonexpensive semiconductor lasers. Besides, such spectral characteristics make pentamethine dyes suitable for the methods that include multicolor detection techniques [2].

Pentamethine cyanine dyes are successfully used in research investigations and clinical diagnostics as bright labels for oligonucleotides and nucleic acids in multicolor labelling (fluorescence *in situ* hybridization (FISH) assays, microarray-based expression analysis) [2], for *in vivo* DNA labelling, etc. Pentamethines are useful for proteins detection as well [3].

The cyanine dyes are known to bind to DNA *via* two basic mechanisms, namely intercalation [4–7] and groove-binding [5,8,9]. The binding mechanism may depend on both dye structure and DNA nucleotides content, as well as on the dye–DNA concentrations ratio [5,7]. While intercalators generally demonstrate slight specificity to GC-containing nucleotide sequences, in the case of the groove-binding molecules strong preference to AT-regions of the double-stranded DNA takes place [9].

Designing of dyes which interact with DNA *via* groove-binding is actual for the techniques that require

[1] Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine, 150 Zabolotnogo Street, 03143 Kyiv, Ukraine
[2] Kyiv Taras Shevchenko National University, Physics Department, Acad. Glushkova Avenue 2, 03680 Kyiv, Ukraine
[3] Institute of Organic Chemistry, National Academy of Sciences of Ukraine, Murmans'ka Street 5, 02094 Kyiv, Ukraine
[4] To whom correspondence should be addressed. E-mail: sergiy@yarmoluk.org.ua

selective detection of double-stranded regions of DNA [10]. On the other hand, groove binding becomes more prevalent for the cyanine dyes with more than one methine group in polymethine chain [5]. Thus, the specificity to dsDNA binding increases in case of polymethynes, in comparison with monomethynes.

In our previous works, the trimethine cyanine dyes were deeply studied and proposed to be used as high-efficient fluorescent probes for nucleic acids detection [11] and the interaction of trimethine cyanines with nucleic acids was studied [12–15]. Later, we investigated the bridged trimethine cyanines and showed that these dyes, also considered by us as groove-binders, are sensitive fluorescent dyes specific for nucleic acids [16].

Since pentamethines are more elongated than trimethines, it could be supposed, that the preference of groove-binding over intercalation should be stronger for these dyes as compared to trimethines. Thus, pentamethine cyanines could be even more selective to dsDNA than trimethine cyanines.

The presented paper is aimed on the study of the series of benzothiazole and benzoxazole pentametine dyes (Fig. 1) as fluorescent probes for DNA and RNA as well as for native and denatured proteins. The influence of the substituents in the cyclic group on the dye sensitivity to biopolymers would be studied. The study of the dyes specificity to AT- or GC-sequences could clarify the matter of DNA-binding mechanism of pentamethines.

EXPERIMENTAL

Material

Methanol, anhydrous dimethylformamide (DMF) distilled under reduced pressure and 0.05 M Tris–HCl buffer (pH 8.0) were used as solvents. Since the DNA double helix is the most stable at pH values from 4.0 to 11.0 [17], the selected pH value of the buffer that is in the middle of the mentioned interval is appropriate for our studies.

Total deoxyribonucleic acid (DNA) from chicken erythrocytes, total yeast ribonucleic acid (RNA), poly(dA-dT)-poly(dA-dT) and poly(dG-dC)-poly(dG-dC) polynucleotides, bovine serum albumin (BSA), and sodium-dodecylsulfate (SDS) were purchased from "Sigma" (USA).

The studied dyes (Fig. 1) were synthesised as described in [18–21].

Pentamethine cyanines containing the cyclopentene group in the polymethine chain were obtained by the condensation of cyclopentane-1,3-diones (Fig. 2, I) with the quaternary salts of 2-methylbenzothiazolium (Fig. 2, II) at the temperature 210°C with the next processing of the reaction mass by triethylamine in the polar solvent. Further, the anion exchange by the action of either sodium perchlorate or tetrabutylammonium bromide in the solution of alcohol or acetonitryle was carried out [20].

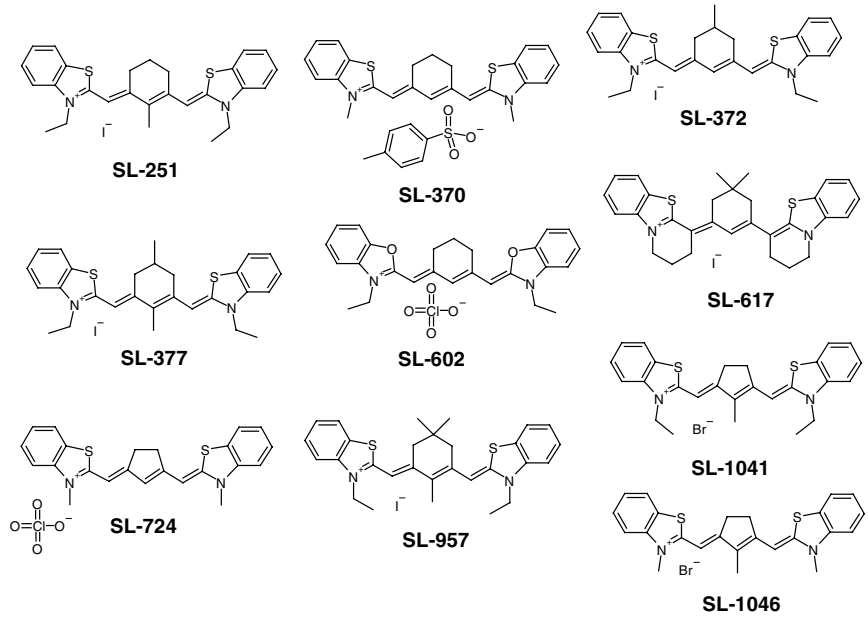

Fig. 1. Structures of the studied pentamethine dyes.

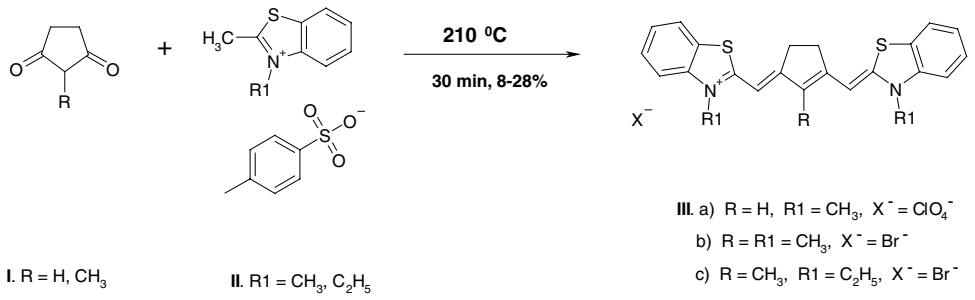

Fig. 2. Synthesis of pentamethine cyanines containing the cyclopentene group in the polymethine chain.

For the synthesis of pentamethine cyanines with the cyclohexene group in the chromophore, the condensation of the quarternary salts of nitrous heterocycles (benzothiazole or benzoxazole) containing the active methyl group, with the 1,5-dimethoxy-1,4-cyclohexadienes (Fig. 3, I, a–d) [18,19] or with 1,3-diethoxy-5,5-dimethyl- or 1,3-diethoxy-2,5,5-trimethyl-1,3-cyclohexadienes (Fig. 3, II, a, b) was carried out [21].

The reaction is performed either by the fusion of reagents, or in the benzonitryle solution at 120–130°C, the intermediate products (e.g. compounds III, Fig. 3) are then separated. Further, the intermediate products are heated with the necessary quaternary salt of the nitrous heterocycle in the polar solvent (in the case of benzoxazole derivatives, in the aprotonic solvent) in the presence of the organic base. Thus, the symmetric and unsymmetric pentamethine cyanines are obtained with the yield 24–61%. When obtaining symmetrical pentamethine cyanines, the separation of the intermediate product (III, Fig. 3) is not necessary.

The structures of the dyes were confirmed by the data of element analysis and ^{1}H NMR spectroscopy.

The Preparation of Stock Solutions

The dyes stock solutions were prepared by dissolving the dyes in DMF, the concentration was equal to 2×10^{-3} M. Stock solutions of nucleic acids (DNA, RNA, and polynucleotides), BSA, SDS, and BSA–SDS were prepared by their dissolving in 0.05 M Tris–HCl buffer (pH 8.0). The concentrations of nucleic acids, BSA, and SDS in stock solutions were 6×10^{-3} M base pairs (bp) for DNA, 1.2×10^{-2} M bases (b) for RNA, about 3×10^{-3} M bp for polynucleotides, 0.2 mg/mL for BSA, and 0.05% for SDS.

Preparation of Working Solutions

All working solutions were prepared immediately before the experiments. Working solutions of free dyes were prepared by dilution of the dye stock solution in either buffer or methanol. Working solutions of dye–DNA (or RNA) complexes were prepared by mixing of an aliquot of the dye stock solution and an aliquot of DNA/RNA stock solution in a buffer. Working solutions

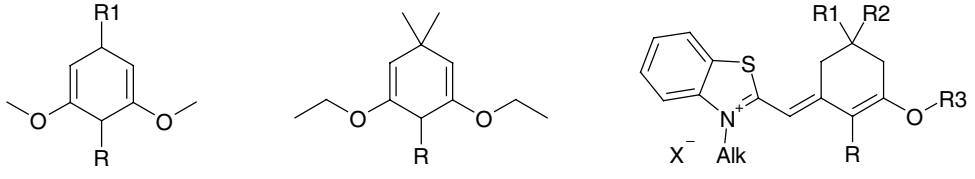

Fig. 3. Initial compounds for the synthesis of pentamethine cyanines containing the cyclohexene group in the polymethine chain (I, II) and intermediate product obtained during this synthesis (III).

Table I. Spectral-fluorescent Characteristics of the Studied Dyes in Methanol, Buffer, and in the Presence of DNA and RNA

	Methanol	Buffer				In DNA presence					In RNA presence				
Name	λ_{abs}	λ_{abs}	λ_{ex}	λ_{em}	I_0	λ_{abs}	λ_{ex}	λ_{em}	I^{DNA}	I^{DNA}/I_0	λ_{abs}	λ_{ex}	λ_{em}	I^{RNA}	I^{RNA}/I_0
SL-251	646	570[a], 643	645	659	55	561[a], 651	655	667	1182	21.5	564[a], 658	662	672	753	14
SL-370	640	636	644	654	285	651	667	675	2455	8.6	557[a], 643	660	668	818	2.9
SL-372	643	638	647	659	401	594[a], 636	651	666	674	1.7	559[a], 638	660	667	687	1.7
SL-377	648	569[a], 644	649	662	36	548[a], 654	664	672	360	10	546[a], 661	664	673	705	19.6
SL-602	583	580	582	596	2127	581	597	600	2623	1.2	583m	599	606	2782	1.3
SL-617	670	667	667	683	9	668	683	726	37	4.1	617[a], 681	690	702	186	20.7
SL-724	597	539[a], 592	598	609	655	529[a], 601	605	614	3000	4.6	511[a], 608	611	621	724	1.1
SL-957	655	584[a], 649	653	670	73	656, 791[a]	667	676	152	2.1	590[a], 658	672	678	615	8.4
SL-1041	607	547[a], 604	606	616	738	611	612	625	11455	15.5	538[a], 616	620	630	1136	1.5
SL-1046	606	491[a], 600	606	616	247	610	612	622	7727	31.3	521[a], 617	621	629	772	3.1

Note. λ_{abs} (λ_{ex}, λ_{em}): maximum wavelength of absorption (fluorescence excitation, fluorescence emission) spectrum. Only the monomeric maxima and the most intensive aggregate maxima (in the case it was more intensive than the monomeric one) are presented; I_0 (I^{DNA}, I^{RNA}): fluorescence intensity of dye in buffer in free form (in presence of DNA, RNA).

[a]The most intensive maximum, in the case that this maximum does not belong to dye monomers.

of dyes in the presence of BSA, SDS, and BSA–SDS were prepared by dilution of the dye stock solution in BSA, SDS, or BSA–SDS stock solution, respectively. The working solutions of the dyes in presence of SDS and BSA–SDS mixture were incubated for about 20 min until the equilibrium is reached and the fluorescence intensity achieves saturation. The concentrations of dye, DNA, RNA, BSA, and SDS in working solutions were equal to 5×10^{-6} M, 6×10^{-5} M bp, 1.2×10^{-4} M bases, 0.2 mg/mL, and 0.05%, respectively.

Working solutions of the dyes in the presence of polynucleotides were prepared in the same way as dye–DNA or dye–RNA solutions. But in order to decrease dye aggregation, the dye concentration was substantially decreased and was equal to 5×10^{-7} M. The polynucleotides concentration was controlled spectrophotometrically and was equal to 6×10^{-5} M bp.

Spectroscopic Measurements

Absorption spectra were recorded on Specord M-40 spectrophotometer (Carl Zeiss, Germany). Fluorescence excitation and emission spectra were registered on Cary Eclipse fluorescence spectrophotometer (Varian, Australia). Fluorescence emission was excited at the maximum of the fluorescence excitation spectrum. Both excitation and emission slits had the width equal to 5 nm. The fluorescence measurements were performed using the quartz cell (1 cm × 1 cm). The quantum yield value for SL-251 and SL-370 in the presence of DNA was determined using Nile Blue solution in ethanol as the reference (quantum yield value 0.27) [22]. In order to decrease the dye aggregation, for the quantum yield measurement of dyes SL-251 and SL-370, the dye concentration was equal to 10^{-6} M and 2×10^{-6} M, respectively, while the DNA concentration was 2.4×10^{-4} M bp.

All the measurements were performed at room temperature.

RESULTS AND DISCUSSION

Spectroscopic Characterization of Free Dyes in Methanol and Buffer

Spectroscopic characteristics of 10 pentamethine cyanine dyes in methanol and buffer are presented in Table I.

In absorption spectra of the studied pentamethine cyanines in methanol, the band corresponding to the dye monomer form is observed. The maxima of absorption spectra for the benzothiazole dyes with the cyclohexene group (SL-251, SL-370, SL-372, SL-377, SL-617, and SL-957) are situated between 640 and 670 nm. At the same time the maxima of the benzothiazole dyes with the cyclopentene group (SL-724, SL-1041, and SL-1046) lie between 597 and 607 nm. The benzoxazole dye SL-602 containing the cyclohexene group has its absorption maximum at 583 nm.

In aqueous buffer significant changes in the absorption spectra of all the studied dyes take place. In absorption spectra of majority of the studied dyes, the band related to the monomer form of dye is accompanied by one or several additional bands, shifted to short- and long-wave region as compared to the monomer one (Figs. 4 and 5). These additional bands are most probably connected with the dyes aggregates, which are often formed by cyanines

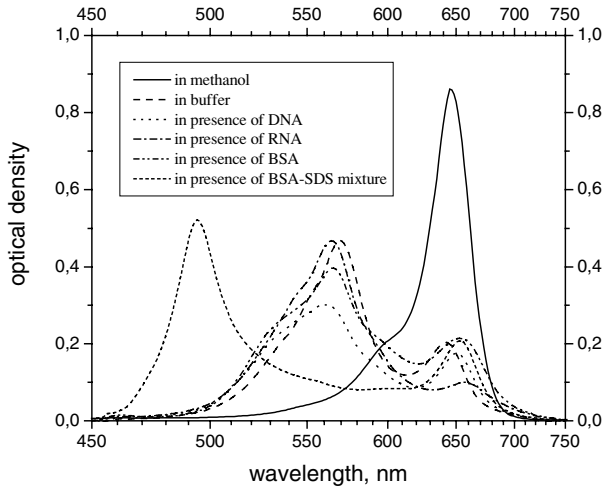

Fig. 4. Absorption spectra of the dye SL-251 in methanol, buffer, and in the presence of DNA, RNA, BSA, and BSA–SDS mixture.

in aqueous solutions [12,23]. Since the aggregation processes and their spectral manifestation are quite complicate for the studied dyes, we do not discuss association in details in this paper, and only the positions of "monomer" and most intensive aggregation absorption bands are presented. In the case of the dyes SL-251, SL-377, SL-724, SL-957, SL-1041, and SL-1046, the short-wave aggregate bands are more intensive than the monomer ones (Table I). The monomer absorption maxima of the studied dyes in buffer are shifted to the short-wave region by 3–6 nm in comparison with methanol solutions of the dyes.

The monomer maxima of fluorescence excitation spectra of the studied dyes in buffer lie between 582 and

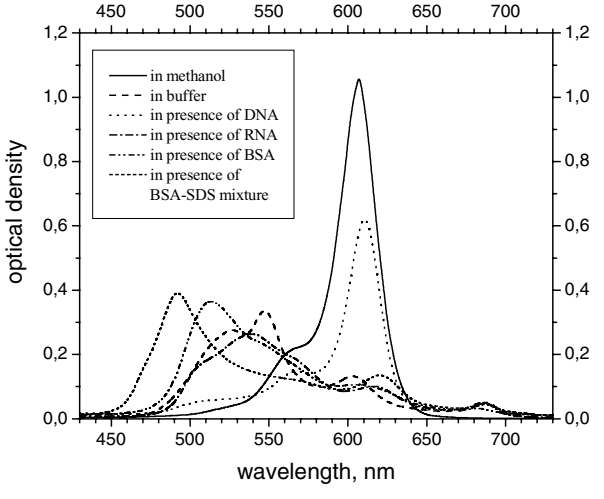

Fig. 5. Absorption spectra of the dye SL-1041 in methanol, buffer, and in presence of DNA, RNA, BSA, and BSA–SDS mixture.

667 nm, while the fluorescence emission maxima are situated at 596–683 nm (Table I). The aggregate fluorescence spectra that are observed for some of the dyes are less intensive than the monomer ones and are not presented.

The lowest intensity of intrinsic fluorescence was observed for dyes SL-251, SL-377, SL-617 and SL-957 and was equal to 9–73 a.u. (arbitrary units). Other dyes, with the exception of SL-602, demonstrate the comparatively moderate intrinsic emission intensity (247–738 a.u.), while for the benzoxazole dye SL-602, a very bright fluorescence is observed (2127 a.u.).

Spectroscopic Characterization of Pentamethine Cyanines in the Presence of Nucleic Acids

Spectroscopic characteristics of the studied dyes in the presence of DNA and RNA are presented in Table I. The addition of nucleic acids to the dye solutions results in the shifting of "monomer" absorption maximum up to 17 nm, as well as to redistribution of their monomer and aggregate band intensities as compared to the free dye in buffer (Figs. 4 and 5). Such changes point to the interaction of the dyes with the nucleic acids.

Positions of emission maxima of the studied dyes in DNA presence are shifted to the long-wave region by 4–21 nm as compared to the free dye buffer solution. The exception is SL-617, for which the shift is equal to 43 nm. Positions of fluorescence maxima of dye–RNA complexes are shifted to the long-wave region by 8–14 nm in comparison to the position of fluorescence maxima of corresponding free dyes in buffer.

The interaction of the studied pentamethine cyanines with nucleic acids results in the dyes fluorescence intensity increase for up to 31.3 times (Figs. 6 and 7). For the dyes SL-251 and SL-370 in DNA presence, the fluorescence quantum yield was measured, which is equal to 0.41 and 0.66, respectively. The highest enhancement of the fluorescence intensity in the presence of DNA was observed for SL-251, SL-1041, and SL-1046 (in 15.5–31.3 times). At the same time, in the presence of RNA the highest emission intensity increase was detected for the dyes SL-251, SL-377, and SL-617 (in 14–20.7 times). For the other dyes, the fluorescence intensity enhancement in the presence of nucleic acids was less then 10 times. It is worth noting that for SL-1041 and SL-1046 the I^{DNA} value is about 10 times higher than the corresponding I^{RNA} value. It should be mentioned that the dyes SL-251, SL-1041, and SL-1046 in presence of DNA manifest both the significant increase of fluorescence intensity and the high absolute value of the fluorescence intensity. Such properties make these dyes promising for application as

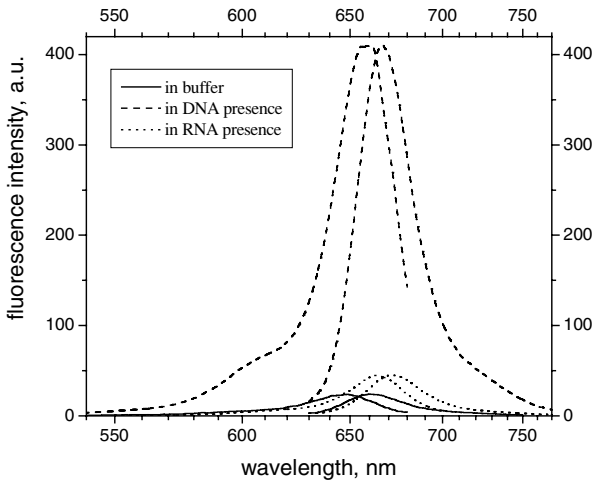

Fig. 6. Fluorescence excitation (*left*) and emission (*right*) spectra of the dye SL-251 in buffer, and in presence of DNA and RNA. Fluorescence excitation at 620 nm. Fluorescence emission at 690 nm (in buffer and in DNA presence) and at 710 nm (at RNA presence). Fluorescence excitation spectra normalised to corresponding emission spectra.

fluorescent probes for the DNA detection. It should be noticed that the studied pentamethines have as high quantum yield in DNA presence as the DNA-sensitive trimethine cyanines, though the fluorescence intensity increase in DNA presence is somewhat less for pentamethines than for trimethines [24]. But the main advantage of pentamethines is that they could be excited by the nonexpensive semiconductor lasers at about 635 nm.

The analysis of the data presented in Table I permits to make some conclusions about the influence of some substituents in the dyes structure on forming the fluorescent complexes with DNA by these dyes. First, it is seen that both I^{DNA} and I^{DNA}/I_0 values decrease in the series SL-251, SL-377, and SL-957. The only difference between these dyes is in the 5 position of the cyclohexene group, which is nonsubstituted for SL-251, substituted with methyl group for SL-377, and with two methyls for SL-957. Thus, the more bulky is the substituent in the 5 position of the cyclohexene group, the more it hinders the formation of dye–DNA fluorescent complexes. This conclusion is supported by the comparison of the I^{DNA} and I^{DNA}/I_0 values for the dye pairs SL-370 and SL-372, though these dyes have different *N*-alkyl substituents (methyl and ethyl, respectively) and thus the comparison cannot be strictly correct.

Second, the comparison of the dye pairs SL-377 and SL-372, SL-1046 and SL-724 shows that the I^{DNA}/I_0 values of the first dye of each pair is noticeably higher than for the second one. The difference between the dyes inside each pair is that the 2 position of cyclohexene or cyclopentene group of the first dye of each pair is methyl-substituted, while for the second one the same position is unsubstituted. Thus, we can suppose that the methyl substitute in the 2 position of cyclohexene or cyclopentene group enhances the stability of dye–DNA fluorescent complex. This conclusion is supported by the comparison of the I^{DNA}/I_0 values for the dye pairs SL-251 and SL-370, though these dyes have different *N*-alkyl substituents (ethyl and methyl, respectively) which could also influence the results of the comparison.

Thus, on the basis of the obtained results, we can suppose that the presence of one or two methyl substituents in the position 5 of cyclohexene ring prevents the dye–DNA fluorescent complex formation, while presence of methyl group in the position 2 leads to the stabilization of the dye–DNA fluorescent complex. Such results are consistent with the groove-binding mode of the dye–DNA interaction that is supposed for pentamethine dyes.

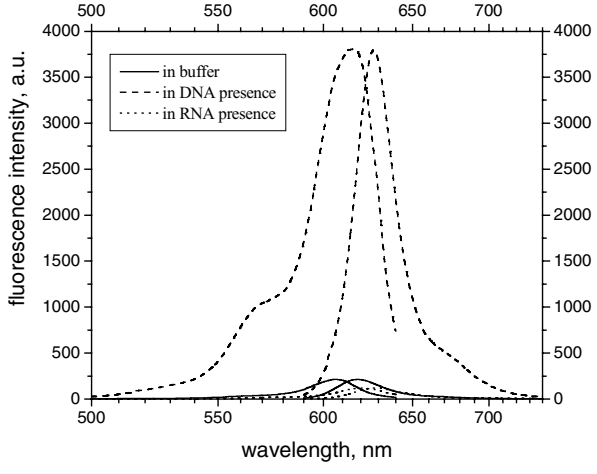

Fig. 7. Fluorescence excitation (*left*) and emission (*right*) spectra of the dye SL-1041 in buffer, and in presence of DNA and RNA. Fluorescence excitation at 580 nm, fluorescence emission at 650 nm. Fluorescence excitation spectra normalised to corresponding emission spectra.

The Spectral Properties of the Dyes in the Presence of poly(dA-dT)-poly(dA-dT) and poly(dG-dC)-poly(dG-dC) Polynucleotides

It was supposed above that the studied dyes bind to DNA *via* the groove-binding mode. In order to find more evidences to this point, the fluorescent properties of the dyes SL-251, SL-372, and SL-1046 were studied in the presence of poly(dA-dT)-poly(dA-dT) and poly(dG-dC)-poly(dG-dC) polynucleotides. It is known [9] that the grove-binding dyes demonstrate significant specificity to AT-containing dsDNA sequences, while the intercalating dyes manifest slight preference to GC-containing ones.

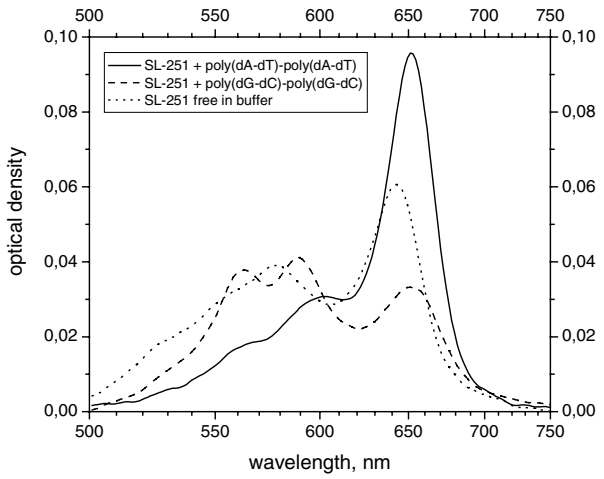

Fig. 8. Absorption spectra of the dye SL-251 (5×10^{-7} M) in buffer and in the presence of poly(dA-dA)-poly(dA-dT) and poly(dG-dC)-(dG-dC) polynucleotides (6×10^{-5} M bp).

In order to decrease aggregation and thus to deal only with dye monomers binding to polynucleotides, the dye-to-polynucleotide concentration ratio was about 1 dye molecule per 120 nucleotide base pairs. Nevertheless, only for SL-372, monomeric band was predominant in absorption spectra of the dye in free form and in the presence of both polynucleotides. At the same time, while the absorption spectrum of the dye SL-251 in the presence of poly(dA-dT)-poly(dA-dT) corresponds to the dye monomers, in the spectrum of the same dye in the presence of poly(dG-dC)-poly(dG-dC), two aggregate bands (near 560 and 590 nm) are even more intensive than the monomer one (near 650 nm) (Fig. 8). Since the above mentioned aggregate bands are not manifested in the free dye spectrum, they belong to the aggregates formed on the polynucleotide. The forming of dye aggregates on the polynucleotide at such a low dye-to-polynucleotide concentration ratios (1 dye molecule per 120 base pairs) could be caused by the hindrances of dye monomer interaction with the GC-containing polynucleotide. The behavior of the SL-1046 absorption spectra in free state and in the presence of both polynucleotides is the same as for SL-251.

The results of the study of fluorescence spectra are presented in Table II. It is seen from Table II that for the dyes SL-251 and SL-1046, the fluorescence intensity increase in the presence of poly(dA-dT)-poly(dA-dT) (I^{AT}/I_0) exceeds the corresponding increase in presence of poly(dG-dC)-poly(dG-dC) (I^{GC}/I_0) for an order of magnitude. On the basis of these results as well as of the results of the absorption study, one can conclude that poly(dA-dT)-poly(dA-dT) polynucleotide is much more favorable for forming the fluorescent complexes with SL-251 and SL-1046 monomers than the poly(dG-dC)-poly(dG-dC) polynucleotide. Such preference for AT-containing polynucleotide as compared to GC-containing one could be the evidence for the groove-binding of these dyes to dsDNA. Nevertheless, in order to finally establish the mechanism of dyes–DNA interaction, additional experiments should be performed.

The dye SL-372 was shown above to be less suitable for forming a fluorescent complex with DNA as compared to the structurally similar SL-251. The methyl substituent in 5 position of the cyclohexene group of SL-372 was supposed to hinder the binding with DNA. At the same time, this dye does not have the methyl substituent in 2 position, which seems to stabilize the dye–DNA fluorescent complex. It could be seen from Table II that the I^{AT}/I_0 value for SL-372 is small (3.1), being at the same time almost equal to the I^{GC}/I_0 value (2.9). Thus, we can suppose that the forming of the fluorescent complexes of SL-372 with dsDNA is really hindered.

Spectroscopic Characterization of Pentamethinecyanines in the Presence of BSA and BSA-SDS

Spectroscopic characteristics of the studied dyes in the presence of bovine serum albumin (BSA) and

Table II. The Fluorescent Properties of Dyes SL-251, SL-372, and SL-1046 in the Presence of poly(dA-dT)-poly(dA-dT) and poly(dG-dC)-poly(dG-dC) Polynucleotides

		poly(dA-dT)-poly(dA-dT)				poly(dG-dC)-poly(dG-dC)			
Dye	I_0, a.u	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
SL-251	21	655	664	1620	77	663	670	183	8.7
SL-372	157	662	670	494	3.1	655	665	450	2.9
SL-1046	43.6	612	621	5530	127	615	626	705	16.2

Note. λ_{ex} (λ_{em}): maximum wavelength of fluorescence excitation (fluorescence emission) spectrum; I_0 (I^{AT}, I^{GC}): fluorescence intensity of dye in buffer in free form (in presence of poly(dA-dT)-poly(dA-dT), poly(dG-dC)-poly(dG-dC) polynucleotides).

Table III. Spectral-fluorescent Characteristics of the Studied Dyes in the Presence of BSA and in the Presence of BSA–SDS Mixture

	In BSA presence					In SDS presence			In BSA–SDS presence					
Name	λ_{abs}	λ_{ex}	λ_{em}	I^{BSA}	I^{BSA}/I_0	λ_{ex}	λ_{em}	I^{SDS}	λ_{abs}	λ_{ex}	λ_{em}	$I^{BSA-SDS}$	$I^{BSA-SDS}/I_0$	$I^{BSA-SDS}/I^{SDS}$
SL-251	566[a], 655	663	674	1318	24	672	688	67	500[a], 661	673	684	1410	25.6	21.0
SL-370	552[a], 641	662	669	598	2.1	662	674	214	476[a], 656	667	675	3430	12.0	16.0
SL-372	644m	663	670	1727	4.3	666	681	260	661	673	683	4570	11.4	17.6
SL-377	545[a], 656	665	673	1136	31.6	674	692	90.5	503[a], 665	674	687	1705	47.4	18.8
SL-602	580	598	604	3032	1.4	596	613	2070	597	602	614	9610	4.5	4.6
SL-617	679	684	701	729	81	694	715	15.7	684	693	709	304	33.8	19.4
SL-724	485[a], 592	615	623	582	0.9	613	624	1240	462[a], 611	616	626	2595	4.0	2.1
SL-957	661	673	682	1955	26.8	680	696	98.5	668	683	694	1280	17.5	13.0
SL-1041	513[a], 607	623	627	773	1.1	620	638	516	492[a], 620	625	634	8000	10.8	15.5
SL-1046	491[a], 627	625	631	253	1.02	621	631	955	472[a], 591	625	633	2195	8.9	2.3

Note. λ_{abs} (λ_{ex}, λ_{em}): maximum wavelength of absorption (fluorescence excitation, fluorescence emission) spectrum. Only the monomeric maxima and the most intensive aggregate maxima (in the case it was more intensive than the monomeric one) are presented; I_0 (I^{BSA}, I^{SDS}, $I^{BSA-SDS}$): fluorescence intensity of dye in buffer in free form (in presence of BSA, in presence of SDS, in presence of BSA–SDS mixture).
[a]The most intensive maximum, in the case that this maximum does not belong to dyes monomers.

BSA–sodium dodecylsulfate (BSA-SDS) mixture are presented in Table III. For all the studied pentamethine cyanines, the position of absorption maxima of their monomer form in BSA presence are shifted to the long-wave region up to 27 nm in comparison to those in aqueous buffer.

Absorption maxima of studied dyes in BSA–SDS mixture, with the exception of SL-1046, are shifted to the long-wave region for 16–23 nm with respect to free dye solution. As for SL-1046, in BSA–SDS presence the absorption maximum of this dye shifts to the short-wave region by 9 nm in comparison to the free dye solution.

For all the studied dyes, the maximum wavelength of the emission spectrum in the presence of BSA shifts to the long-wave region for 8–18 nm as compared to the free dye solution. For the dyes SL-251, SL-377, SL-617, and SL-957, the fluorescence intensity I^{BSA} increases to 24–81 times as compared to I_0. It should be mentioned that the value of I^{BSA} for the dyes SL-251, SL-377, and SL-957 is rather high as well, so these dyes could be used as probes for BSA detection. For the other dyes, the value of I^{BSA}/I_0 does not exceed 4.3.

The maximum wavelength of the fluorescence spectrum of all the studied dyes in BSA–SDS presence shifts for 17–26 nm to the long-wave region relatively to the free dye solution. The fluorescence intensity increase value $I^{BSA-SDS}/I_0$ for the dyes SL-251, SL-377, SL-617, and SL-957 is between 17.5 and 47.4, while for other dyes does not exceeds 12. At the same time, the value $I^{BSA-SDS}/I^{SDS}$ exceeds 13.0 for all the studied dyes except SL-602, SL-724, and SL-1046 ($I^{BSA-SDS}/I^{SDS}$ being between 2.1 and 4.6). We calculated both the values of $I^{BSA-SDS}/I_0$ and $I^{BSA-SDS}/I^{SDS}$, since the first one describes the signal-to-noise ratio of the dye in BSA–SDS system, when detergent molecules cover the protein one, while the second one characterizes the dye fluorescence intensity increase in SDS solution upon BSA addition. It is worth noting that for the dyes SL-251, SL-377, and SL-957, both I^{SDS} and I^{SDS}/I_0 values are high enough, so that these dyes demonstrate appropriate characteristics for nonspecific protein detection in the presence of SDS and are also potentially sensitive to biological membranes. It is interesting that mentioned dyes (SL-251, SL-377 and SL-957) contain methyl substituent in the 2-position of cyclohexene group.

It should be also mentioned that the dyes SL-1041 and SL-1046, which exhibit noticeable emission intensity and significant fluorescence intensity enhancement upon DNA binding, show week emission in the dye-BSA and dye-BSA-SDS complexes.

CONCLUSIONS

1. The pentamethine benzothiazole cyanine dyes with cyclopentene (SL-1041 and SL-1046) and cyclohexene (SL-251) group were shown to be efficient DNA-binders, which increase the fluorescence intensity in DNA presence to 15.5–31.3 times, the quantum yield of SL-251 in DNA presence being equal to 0.41.
2. For the studied benzothiazole pentamethine dyes, methyl or dimethyl substitution in 5 position of the cyclohexene group hinders the formation of fluorescent complex with DNA. At the same time, methyl substitution in 2 position of cyclohexene or cyclopentene group makes a contribution to the additional increasing of the dye fluorescence

intensity in DNA complexes and perhaps points to the complex stabilization.

3. The study of the dyes specificity to the nucleotide AT and GC sequence shows that the benzothiazole pentamethine dyes containing cyclohexene (SL-251) or cyclopentene (SL-1046) group demonstrates significant AT preference that could be an evidence of interaction with DNA *via* the groove-binding mode.
4. The benzothiazole pentamethine dyes SL-251, SL-377, and SL-957 containing the 2-methyl substituted cyclohexene group increase the fluorescence intensity in BSA and BSA–SDS presence to 17.5–47.4 times. We consider that these dyes could be applied for fluorescent protein detection in the presence of SDS, as well as for detection of native BSA. On the basis of these dyes, the membrane fluorescent probes could be also constructed.

REFERENCES

1. R. Haughland (2002). *Molecular Probes. Handbook of Fluorescent Probes and Research Chemicals*, 9th ed., Molecular Probes, Inc., Eugene, OR.
2. L. G. Kricka (2002). *Ann. Clin. Biochem.* **39**, 114–129.
3. G. Patonay, J. Salon, J. Sowell, and L. Strekowski (2004). *Molecules* **9**, 40–49.
4. M. Petersen, A. A. Hamed, E. B. Pedersen, and J. P. Jacobsen (1999). *Bioconjugate Chem.* **10**, 66–74.
5. K. M. Sovenyhazy, J. A. Bordelon, and J. T. Petty (2003). *Nucleic Acids Res.*. **10**, 2561–2569.
6. L. K. Jensen, C. H. Gotfredsen, K. Bondensgaard, and J. P. Jacobsen (1998). *Acta Chem. Scand.* **52**, 641–650.
7. H. Zipper, H. Brunner, J. Bernhagen, and F. Vitzthum (2004). *Nucleic Acids Res.* **32**, e103.
8. H. J. Karlsson, M. Eriksson, E. Perzon, B. Akerman, P. Linkoln, and G. Westman (2003). *Nucleic Acids Res.* **31**, 6227–6234.
9. C. Kumar, R. Turner, and E. Asuncion (1993). *J. Photochem. Photobiol. A: Chem.* **74**, 231.
10. M. Bengtsson, H. J. Karlsson, G. Westman, and M. Kubista (2003). *Nucleic Acids Res.* **8**, 1–5.
11. S. M. Yarmoluk, V. B. Kovalska, S. S. Lukashov, and Y. L. Slominskii (1999). *Bioorg. Med. Chem. Lett.* **12**, 1677–1678.
12. T. Y. Ogulchansky, M. Y. Losytskyy, V. B. Kovalska, S. S. Lukashov, V. M. Yashchuk, and S. M. Yarmoluk (2001). *Spectrochim. Acta Part A: Mol. Biomol. Spectrosc.* **13**, 2705–2715.
13. V. B. Kovalska, I. V. Valyukh, S. S. Lukashov, Y. L. Slominskii, and S. M. Yarmoluk (2002). *J. Fluoresc.* **2**, 209–212.
14. S. M. Yarmoluk, S. S. Lukashov, M. Y. Losytskyy, B. Akerman, and O. S. Kornyushyna (2002). *Spectrochim. Acta Part A: Mol. Biomol. Spectrosc.* **14**, 3223–3232.
15. V. B. Kovalska, M. Y. Losytskyy, and S. M. Yarmoluk (2004). *Spectrochim. Acta Part A: Mol. Biomol. Spectrosc.* **1–2**, 129–136.
16. I. V. Valyukh, V. B. Kovalska, Y. L. Slominskii, and S. M. Yarmoluk (2002). *J. Fluoresc.* **1**, 105–107.
17. A. L. Lehninger (1972). *Biochemistry. The Molecular Basis of Cell Structure and Function*, Worth Publishers, Inc., New York.
18. Y. L. Slominskii, A. V. Kuleshin, and A. I. Tolmachev (1970). *Zh. Org. Khim.* **6**(9), 1936–1940 (in Russian).
19. Y. L. Slominskii, A. L. Skulbidenko, and A. I. Tolmachev (1974). *Ukr. Khim. Zh.* **40**(10), 1166–1173 (in Russian).
20. Y. L. Slominskii, N. I. Ephimenko, A. D. Kachkovskii, L. G. Kurkina, and A. I. Tolmachev (1986). *Ukr. Khim. Zh.* **52**(3), 301–307 (in Russian).
21. Y. L. Slominskii, N. N. Romanov, Z. N. Stetsenko, and A. I. Tolmachev (1979). *Zh. Org. Khim.* **15**(4), 865–868 (in Russian).
22. R. Sens and K. H. Drexhage (1981). *J. Luminesc.* **24**, 709–712.
23. T. Y. Ogulchansky, M. Y. Losytskyy, V. B. Kovalska, V. M. Yashchuk, and S. M. Yarmoluk (2001). *Spectrochim. Acta Part A: Mol. Biomol. Spectrosc.* **7**, 1525–1532.
24. S. M. Yarmoluk, M. Y. Losytskyy, and V. M. Yashchuk (2002). *J. Photochem. Photobiol. B: Biol.* **67**, 57–63.