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Studies on the Spectral-Luminescent Properties of the Novel Homodimer Styryl Dyes in Complexes with DNA

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Series of homodimer styryls containing on (*p*-dimethylaminostyryl) pyridinium residues that are connected with aliphatic linkage group was synthesized. Spectral luminescent properties of obtained dyes in free state and in nucleic acids presence were studied. It was shown that DNA binding affinity of the novel homodimers exceeds that of parent monomer (*p*-dimethylaminostyryl)pyridine iodide. For homodimers with the linkage 4–10 carbon atoms preference in binding to DNA than to RNA was observed. It could be concluded that parent monomer has different mechanisms of binding to nucleic acids than corresponding homodimer dye.

KEY WORDS: Nucleic acid; fluorescent probes; homodimer styryl dyes.

INTRODUCTION

Due to their sensitivity to supramolecular assemblies, biomembranes and liposomes styrylic dyes are widely used in different biological techniques. Today styryles are known among mostly sensitive probes for unspecific fluorescent detection of proteins [1]. These dyes were also proposed as fluorescent probes for nucleic acids visualization [2], cell imaging [1] and DNA sequencing on gels [3]. Recently synthesis of a series of novel styryls as DNA fluorescent sensors with cell-permeable properties and their cell-based screening were reported [4]. These dyes interact with DNA with the increasing of emission intensity upon the binding.

In previous researches we used the method of polarization fluorescent spectroscopy to study and compare peculiarities of the binding of styryl dye F ((*p*-dimethylaminostyryl)pyridinium iodide) and monomethine cyanine dye Thiazole Orange (TO) to the dsDNA [5]. Well known, that interaction of small molecules with dsDNA could occur *via* intercalation or groove binding mechanism [6]. Intercalators are typically planar aromatic molecules while groove-binders contain fused ring system. For monomethine cyanine TO preference of the intercalation in the presence of DNA excess was shown [7,8]. Our studies have shown that value of polarization coefficient for TO–DNA complexes exceeds that for corresponding complexes of styryl dye F [5]. Such data could be an evidence of realization of groove-binding fixation for the styryl–DNA complexes.

For the first time we synthesized the series of homodimer styryls based on (*p*-dimethylaminostyryl)pyridinium residue with linkage aliphatic group of the length from 3 to 10 carbon atoms. Spectral-luminescent characteristics of the dyes in free form and in nucleic acids presence were studied. The dependence of the fluorescent properties of homodimer dye–DNA complexes on the length of the linkage group was investigated. It was concluded that homodimer styryls bind with DNA and RNA by different mechanisms than parent monomer styryl.

EXPERIMENTAL

The styryl dimer dyes were synthesized (Fig. 1) according to the procedures described below. Structure and purity of obtained compounds were confirmed with

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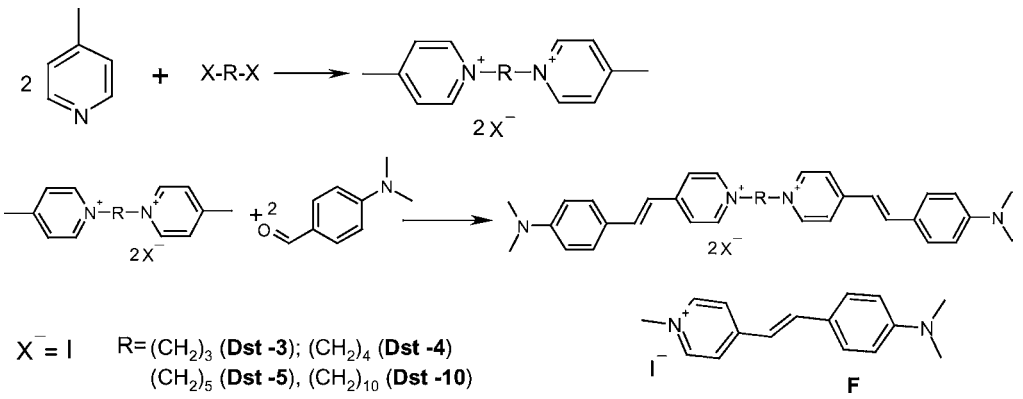

Fig. 1. Synthesis and structures of novel styryl dimeric dyes and structure of parent monomer dye F.

H^1 NMR. Data of H^1 NMR analysis are presented in Appendix. Purity of all the dyes was stated as 95% or more.

Synthesis of the 4-Methyl-1-(4-Methyl-1-Pyridiniumylmethyl)pyridinium Dihalogenate

Mixture of 0.011 mol of picoline and 0.005 mol of dihalogenalkane was kept during 3 hr at 120°C. To the obtained alloy 5 mL of isopropanole was added, next mixture was refluxed during 3 hr. Product was filtrated, washed with water and alcohol.

Synthesis of the Dyes SD-3, SD-4, SD-5, SD-10

Mixture of 0.001 mol of quaternary salt, 0.002 mol *p*-dimethylaminobenzaldehyde and 10 drops of pipyridine in 5 mL of *n*-butanole was refluxed during 30 min. Crystalline precipitate was washed with alcohol and ether.

Preparation of Working Solutions

The dye stock solutions were prepared in DMS. Concentration of dye (in chromophores) in working solutions was 2.0×10^{-5} M. A 0.05 M Tris–HCl (pH = 7.5) buffer was used for the measurements. Total DNA from chicken erythrocytes and total yeast RNA were purchased from Sigma. The nucleic acids concentrations in the working solutions were 1.2×10^{-4} M (b.) for DNA and 2.4×10^{-4} M (b.) for RNA. Titration of dyes with nucleic acids was carried out by the addition of DNA or RNA aliquots to the buffer solution containing 2×10^{-5} M of dye (concentration in chromophores). Concentrations of nucleic acids were increased from 6×10^{-5} M (b.) to 7.2×10^{-4} M (b.) for DNA and from 1.2×10^{-4} M (b.) to 1.2×10^{-3} M (b.) for RNA.

Spectral-Luminescent Experiments

Absorption spectra were obtained with Specord M40 spectrophotometer (Germany). Fluorescence excitation and emission spectra were obtained with a Cary Eclipse fluorescence spectrophotometer (Australia). All measurements were carried out at room temperature.

To calculate the quantum yields of the free dyes (φ) a rhodamine 6G solution in ethanol was used as reference, the quantum yield of which is known to be equal to 0.97 [9].

RESULTS AND DISCUSSION

Spectral-luminescent characteristics of the studied dyes in free state and in presence of nucleic acids are presented in Table I. Absorption maximum (λ_{abs}) of monomeric styryl F in water buffer is observed on 450 nm. Obtained homodimers have their absorption maxima shifted to the longwave region on 10–20 nm relatively to the parent dye. Absorption spectra for styryl monomer F and homodimer with shortest linkage group Dst-3 are presented in Fig. 2. Some changes of the absorption spectra profiles and decreasing of their half-width occurs for homodimer styryl comparing with spectra of parent monomer dye F. All studied dyes in water buffer demonstrate low level of fluorescence intensity (I), values of fluorescence quantum yield (φ) are between 0.0005 for homodimer Dst-3 and 0.003 for monomer F. Maxima of fluorescence excitation spectra (λ_{ex}) for the dyes are shifted on 10–30 nm relatively to the maxima λ_{abs} of corresponding absorption spectra. Profiles of fluorescence spectra for free styryls are broad and have their maxima (λ_{em}) on 610–620 nm. Stokes shifts values (determined as $\Delta S = \lambda_{em} - \lambda_{abs}$) for the dyes are large (between 151 and 160 nm) that is typical for styryls. The maximum changes

Table I. Spectral-Luminescent Characteristics of Styryl Dyes in Free State and in Nucleic Acids Complexes

	Free dye					In dsDNA presence					In RNA presence				
Name	λ_{abs}	λ_{ex}	λ_{em}	I_0	φ	λ_{abs}	λ_{ex}	λ_{em}	I	I/I_0	λ_{abs}	λ_{ex}	λ_{em}	I	I/I_0
F	450	461	610	3.5	0.003	452	498	610	56	16	452	510	614	60	17
Dst-3	470	500	620	1.6	0.0005	489	518	611	50	35	490	530	620	40	25
Dst-4	463	480	616	5.4	0.0025	485	498	616	400	74	485	520	617	200	37
Dst-5	460	485	611	2	0.001	483	510	596	528	264	470	516	614	51	25
Dst-10	465	470	613	4.9	0.002	480	492	597	437	89	479	510	614	130	26

Note. λ_{abs}, λ_{ex}, λ_{em}, maximum wavelengths of absorption; fluorescence excitation and emission spectra (nm); I_0, dye intrinsic fluorescence intensity (arbitrary units); I (DNA/RNA), dye fluorescence intensity in the DNA/RNA presence (arbitrary units); I/I_0, enhancement of fluorescence intensity in DNA/RNA complexes; φ, fluorescence quantum yield.

in both absorption and fluorescence spectra profiles and maxima positions comparing with parent monomer F are observed for the homodimer styryl Dst-3, which has shortest linkage group.

Novel homodimers and parent styryl F were studied for their spectral-luminescent characteristics in the presence of dsDNA and RNA (Table I). Absorption spectra of homodimeric dyes in the presence of biopolymers are bathochromically shifted relatively to the spectra of free dyes. Absorption maxima of dyes–nucleic acid complexes

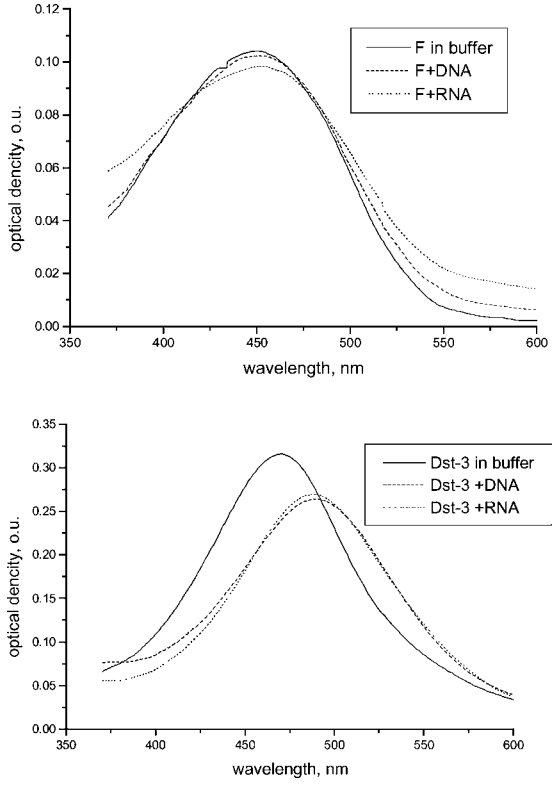

Fig. 2. Profiles of absorption spectra of monomer styryl dye F and homodimer styryl Dst-3 in free state and in nucleic acids presence.

maxima are situated on 470–490 nm. Highest value of the long-wave shift in complexes with nucleic acids (up to 20 nm) is observed for Dst-3. In opposite to the homodimer styryls, addition of nucleic acids to the solution of monomer styryl F slightly changes absorption characteristics of the dye (Fig. 2).

Similar to the spectra of free dye, fluorescence excitation spectra of styryls in the DNA and RNA presence are shifted to the long-wave region relatively to corresponding absorption spectra. However the values of the shifts in nucleic acids complexes (12–58 nm) could significantly exceed such shifts observed for the free styryls. The most significant shift between the maxima of absorption and excitation spectra among studied styryls in nucleic acid complexes was detected for the monomeric dye F. Fluorescence maxima of the dyes in DNA and RNA presence are situated on 596–620 nm. It is necessary to note, that for the studied dyes position of fluorescence spectra maxima in RNA complexes are shifted on 1–20 nm to long-wave spectral region relatively to that of DNA complexes. Addition of nucleic acids to the dye solutions also resulted in decreasing of Stokes shift values. The Stokes shifts observed for the styryl DNA/RNA complexes are between 86 and 118 nm.

All studied dyes demonstrate enhancement of fluorescent intensity in the presence of nucleic acids. For homodimer styryls Dst-4, Dst-5 and Dst-10 moderate emission intensity was observed in DNA presence (Table I). Fluorescence intensity values of other dye/nucleic acids complexes were significantly lower. The least value of emission intensity increase in the DNA presence is observed for monomer styryl F ($I/I_0 = 16$), for homodimer dyes value of I/I_0 depends on the length of linkage group and changes from 35 to 264. For the studied dyes in complexes with RNA rather close values of fluorescent enhancement (17–37 times) are observed. However values of emission increasing in RNA presence for all homodimeric dyes exceed that for parent monomer F.

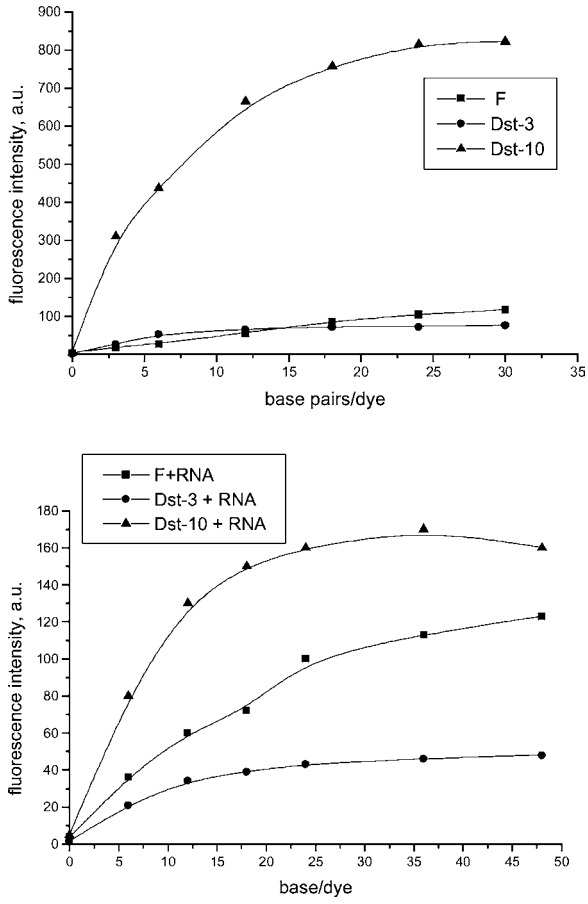

Fig. 3. Dependence of fluorescent intensity of the styryl dyes F, Dst-3 and Dst-10 from concentration of dsDNA and RNA. Dye concentration 2×10^{-5} M (concentration is calculated in chromophores). Concentrations of nucleic acids were increased from 6×10^{-5} M (b.) to 7.2×10^{-4} M (b.) for DNA and from 1.2×10^{-4} M (b.) to 1.2×10^{-3} M (b.) for RNA.

It could be concluded that homodimeric styryl dyes have higher affinity to DNA than parent monomer and behavior of these dyes in the presence of DNA significantly depends on the length of linkage group. Lengthening of the carbon chain from 3 to 5 atoms results in the increasing fluorescence intensity of homodimer styryl–DNA complex. Dye Dst-10 that has longest linkage group (10 atoms) also demonstrates bright emission in the DNA presence. Also styryls with linkage length 4, 5 and 10 carbon atoms demonstrate preferences in binding to DNA than to RNA, while monomer dye F does not show preference in binding to DNA or RNA.

Dependence of fluorescent intensity of the styryl dye F, homodimers with shortest (Dst-3) and longest (Dst-10) linkage group on dsDNA and RNA concentration are presented in Fig. 3. As could be seen from titration curves, emission intensity of Dst-10–DNA complex significantly exceeds corresponding values for dyes F and Dst-3.

Despite significant difference of fluorescence intensities of homodimeric dyes Dst-3 and Dst-10 in DNA presence for both dyes similar shapes of titration curves were obtained. In used concentration range saturation points for these dyes were determined at about one dye molecule per 8 base pairs. It is necessary to note that saturation for dye F–DNA complex was not achieved for the concentrations ratio range used (one dye molecule per up to 30 base pairs).

The same behavior was observed for these styryls in complexes with RNA. In used concentration range homodimeric styryls Dst-3 and Dst-10 have close values of fluorescence saturation point (about dye molecule per 14 bases), while for monomer styryl F this point could not be clearly determined. Such difference in the behavior of studied styryl dyes in presence of nucleic acids could be and evidence for the realization of different interaction modes for studied homodimer styryl and parent monomer.

We consider that styryl homodimer dyes have increased binding affinity to nucleic acids comparing with parent monomer (*p*-dimethylaminostyryl)pyridinium iodide. Homodimer dyes with the linkage group length of 4 and above carbon atoms prefer binding with DNA than with RNA and interact with DNA with noticeable increasing of fluorescence intensity. It could be concluded that homodimer dyes bind with nucleic acids in other way than corresponding monomer. We can even suppose that one of the (*p*-dimethylaminostyryl)pyridinium residues of homodimer could bind with DNA through intercalation into the interbase space.

APPENDIX

Data of ^{1}H NMR analysis for novel dimer dyes.

Dst-3: 4-[2-(4-Dimethylaminophenyl)-1-ethenyl]-1-(3-4-[2-(4-dimethylaminophenyl)-1-ethenyl]-1-pyridiniumylpropyl)pyridinium diiodide.

Yield: 86%; m.p.: 274°C. ^{1}H NMR (DMSO-d_6) δ (ppm): 2.65 (2H, q, $J = 7.7$), 3.04 (12H, s), 4.55 (4H, d, $J = 7.7$), 6.78 (4H, d, $J = 9.7$), 7.20 (2H, d, $J = 17.7$), 7.60 (4H, d, $J = 10.0$), 7.95 (2H, d, $J = 18.0$), 8.10 (4H, d, $J = 7.7$), 8.77 (4H, d, $J = 7.7$). Anal. calcd. for $C_{33}H_{38}I_2N_4$: I, 34.09. Found: I, 34.16.

Dst-4: 4-[2-(4-Dimethylaminophenyl)-1-ethenyl]-1-(4-4-[2-(4-dimethylaminophenyl)-1-ethenyl]-1-pyridiniumylbutyl)pyridinium diiodide.

Yield: 89%; m.p.: 302–303°C. ^{1}H NMR (DMSO-d_6) δ (ppm): 1.92 (4H, br m), 4.52 (4H, br, t), 6.79 (4H, d, $J = 10$), 7.21 (2H, d, $J = 18.0$), 7.62 (4H, d, $J = 9.7$),

7.96 (2H, d, $J = 18.3$), 8.19 (4H, d, $J = 7.7$), 8.78 (4H, d, $J = 8.0$). Anal. calcd. for $C_{40}H_{52}I_2N_4$: I, 33.46. Found: I, 33.52.

Dst-5: 4-[2-(4-Dimethylaminophenyl)-1-ethenyl]-1-(5-4-[2-(4-dimethylaminophenyl)-1-ethenyl]-1-pyridiniumylpentyl)pyridinium diiodide.

Yield: 87%; m.p.: 285–286°C; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.23 (2H, m), 2.95 (4H, quint, $J = 7.3$), 3.02 (12H, s), 4.44 (4H, t, $J = 7.3$), 6.78 (4H, d, $J = 10.0$), 7.17 (2H, d, $J = 17.7$), 7.58 (4H, d, $J = 9.7$), 7.95 (2H, d, $J = 18.0$), 8.08(4H, d, $J = 7.7$), 8.77 (4H, d, $J = 7.7$). Anal. calcd. for $C_{35}H_{42}I_2N_4$: I, 32.85. Found: I, 32.74.

Dst-10: 4-[2-(4-Dimethylaminophenyl)-1-ethenyl]-1-(10-4-[2-(4-dimethylaminophenyl)-1-ethenyl]-1-pyridiniumyldecyl)pyridinium diiodide.

Yield: 88%; m.p.: 278–279°C; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.27 (12H, m), 1.88 (4H, t, $J = 6.7$), 3.03 (12H, s), 4.42 (4H, t, $J = 7.6$), 6.79 (4H, d, $J = 10.0$), 7.20 (2H, d, $J = 17.7$), 7.60 (4H, d, $J = 10.0$), 7.95 (2H, d, $J = 17.7$), 8.08 (4H, d, $J = 7.7$), 8.79 (4H, d, $J = 8.0$). Anal. calcd. for $C_{40}H_{52}I_2N_4$: I, 30.12. Found: I, 30.23.

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