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To the studies on (*p*-dimethylaminostyryl)pyridinium based homodimer to dsDNA binding mechanism

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Summary. With the aim to study the interaction mode of (*p*-dimethylaminostyryl)pyridinium homodimer dye Dst-6 with dsDNA, the equilibrium constant of dye-dsDNA binding (K) and the number of dsDNA base pairs occupied with one bound dye molecule were estimated. Obtained data support the previously made suggestion about realization of groove-binding mechanism of dye-DNA interaction.

Keywords: styrylcyanine dye, DNA, fluorescence, groove-binding, binding constant.

Introduction. It has been shown recently that homodimer styrylcyanine dyes could be considered among the most promising classes of compounds for fluorescent detection of nucleic acids [1-4]. Some of these dyes for today are the most efficient two-photon excited fluorescent probes for DNA detection [5]. They possess high values of fluorescence intensity in the presence of DNA, moreover, their fluorescence intensity increases by orders of magnitude upon DNA addition.

At the same time, the behavior of homodimer dyes and mode of their interaction with DNA strongly depend on the nature and the position of a linker connecting two chromophores. As it was shown recently [6], two similar homodimer styryl dyes based on the same benzothiazole chromophore monomer, bind to DNA differently when connected by different linkers and in different positions.

Thus the detailed study of the interaction between styrylcyanine dyes and dsDNA is very important, both for understanding the processes taking place in the dye-dsDNA solution and for the development of novel dsDNA probes based on these dyes.

In one of the recent papers [3], we demonstrated that Dst-6, the (*p*-dimethylaminostyryl)pyridinium based homodimer dye with aliphatic linker (Fig. 1), increases the fluorescence intensity by two orders of magnitude in the presence of dsDNA. In addition, this dye demonstrated specificity to poly(dA-dT) · poly(dA-dT) as compared to poly(dG-dC) · poly(dG-dC) that points to the groove-binding mechanism of this dye interaction with dsDNA. In this study, equilibrium constant of dye-dsDNA binding (K) and the number of dsDNA base pairs occupied by one bound dye molecule are estimated to support our suggestion about the dye binding with the dsDNA minor groove.

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Materials and methods. ***Chemicals.*** The dye Dst-6 was synthesized according to [1]. Stock solution of the dye ($2x10^{-3}$ M, the concentration was calculated in (*p*-dimethylaminostyryl)pyridinium chromophore units) were prepared in DMSO and kept at 4 ˚C.

Dimethysufoxide (DMSO, purchased from Chimlaborreaktiv Ltd.) and tris-(oxymethyl)-amino methane hydrochloride (Tris, purchased from Sigma) were used without purification. 0.05 M Tris-HCl buffer (pH 8.0) was used as a buffer in all experiments. Salmon testes DNA was purchased from Sigma. The concentration of DNA stock solution in Tris-HCl buffer was $6x10^{-3}$ M b.p. (moles of base pairs per liter).

Spectral studies. $5x10^{-6}$ M solution of Dst-6 in tris-HCl buffer was titrated by dsDNA. Aliquots (5-250 μl) of the DNA stock solutions were added to the $5x10^{-6}$ M dye buffer solution to obtain the mixing dye/base pair (dye/b.p.) ratios from 1:2.4 to 1:107. To avoid the dye concentration decrease as the result of dissolving, the DNA stock solution contained $5x10^{-6}$ M dye as well. Absorption spectra were recorded on Specord M 40 spectrophotometer (Carl Zeiss, Germany). Fluorescence excitation and emission spectra were recorded on a Cary Eclipse fluorescence spectrophotometer (Varian Inc., Australia). All the measurements were carried out at room temperature.

Determination of the binding constant (K) and the number of dsDNA base pairs occupied by one bound dye molecule (n). The nonlinear least squares fitting of the experimentally obtained data plotted as the dependence of Y=I on X= IxC_{dye}/C_{DNA} with the McGhee and von Hippel equation [7] was performed as described in [6]. The fitting was performed with the Origin 5.0 program, fitting method being based on the Levenberg-Marquardt algorithm. The McGhee and von Hippel equation was modified to the following one:

$$Y = I_{max} - \frac{X}{C_{dye}K}\frac{\left(1-\left[(n-1)\frac{X}{I_{max}}\right]\right)^{n-1}}{\left(1-n\frac{X}{I_{max}}\right)^{n}} \quad (1).$$

C_{dye} and C_{DNA} being total dye and DNA concentrations, I being the dye fluorescence intensity at the DNA concentration C_{DNA}, I_{max} being the dye intensity after reaching saturation. The values of binding constant (K), number of dsDNA base pairs occupied with one bound dye molecule (n), and I_{max} were calculated as approximation parameters of fitting procedure. The experiment was repeated four times, and the mean values of K and n were calculated. The mean value error was calculated with the confidence interval equal to 0.7.

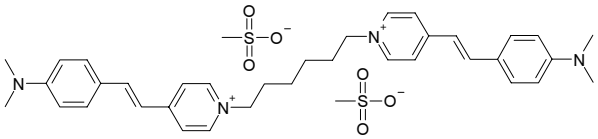

Fig. 1. Structure of the dye Dst-6.

Results and discussion. To characterize the stability of homodimer styrylcyanine dye Dst-6 complex with dsDNA, absorption and fluorescence spectra of the dye in the presence of different DNA concentrations were studied. The absorption spectra of the dye in the presence of $0\div2.3x10^{-4}$ M b.p. DNA are presented at the Fig. 2.

The spectrum of free Dst-6 and that of the dye at maximum DNA concentration (maxima at 459 and 485 nm, respectively) correspond to absorption of the non-bound and DNA-bound monomer forms of dye chromophore units, respectively [1]. It could be suggested that absorption spectra of the dye in the presence of different DNA concentrations is a superposition of these two overlapping bands. At the same time, the isobestic point is not observed for the mentioned absorption spectra. Thus, it is possible that some part of dye chromophores in DNA

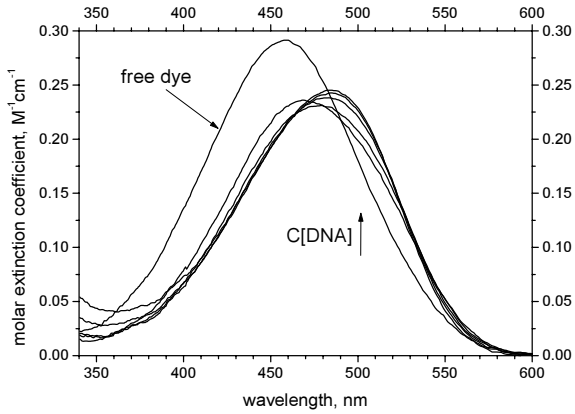

Fig. 2. Absorption spectra of the dye Dst-6 in Tris-HCl buffer in free state and in the presence of $0.12x10^{-4}$, $0.24x10^{-4}$, $0.6x10^{-4}$, $1.2x10^{-4}$ and $2.3x10^{-4}$ M b.p. DNA (the arrow shows the increasing of DNA concentration). The dye concentration used for the measurements was $5x10^{-6}$ M.

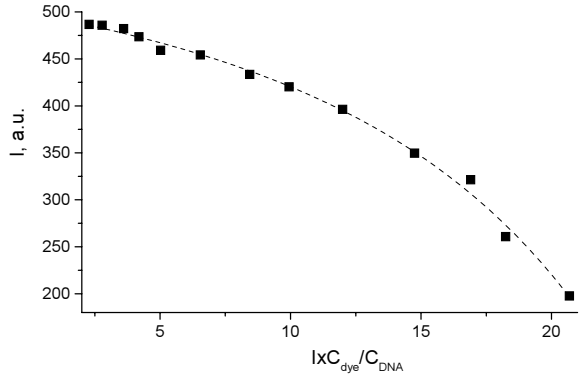

Fig. 3. Plot of fluorescence intensity as a function of IxC_{dye}/C_{DNA} ratio (■) and its approximation by the equation (1) (- - -) for Dst-6.

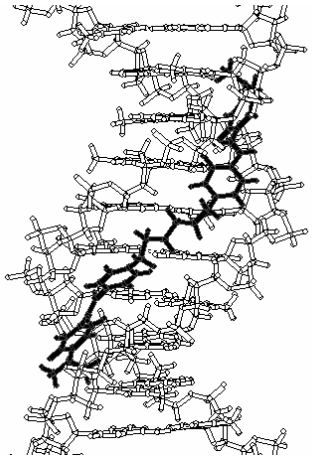

Fig. 4. The possible structure of Dst-6 groove-binding complex with dsDNA. The structure was built with HyperChem program package, Dst-6 geometry was optimized with PM3 method, and the dsDNA geometry was then optimized with AMBER method.

presence exists in aggregated form. Nevertheless, since the dominating bands of the spectra are those corresponding to the non-aggregated dye chromophores, for the rough estimation the existence of the following equilibrium between the free and DNA bound non-aggregated dye molecules in the solution could be generally considered: dye + DNA ↔ dye-DNA.

Thus, the constant (K) of the mentioned equilibrium, as well as the number of dsDNA base pairs occupied by one dye molecule (n) could be estimated using the equation developed by McGhee and von Hippel [7], transformed to the expression (1) [6]. The values of K and n for the dye Dst-6 were estimated by fluorescent titration. The approximation of the experimental results with the McGhee and von Hippel equation (1) (Fig. 3) shows that obtained results are generally correctly described with this equation. The values of K and n for the studied dyes are presented in the Table 1.

The average K value obtained for the pyridinium dye Dst-6 is equal to 6.8×10^4 M^{-1}, the number of dsDNA base pairs occupied by one bound dye molecule being equal to 8.1. The value of binding constant is of the same order of magnitude as these of the homodimer benzothiazole styrylcyanines studied by us earlier [6]. The estimated number of dsDNA base pairs occupied by one bound dye molecule is consistent with the molecular model of the minor groove-binding of the dye. The possible structure of the dye Dst-6 groove-binding complex with dsDNA is presented in the Fig. 4. It is seen from the Fig. 4 that the Dst-6 dimer dye molecule when bound to dsDNA minor groove occupies about 8 base pairs that is consistent with the n value obtained as the result of approximation. It should be mentioned that the evidence of the groove-binding mode of Dst-6 interaction with dsDNA corresponds to the results of the previous study [3] of Dst-6 fluorescent properties in the presence of AT- and GC-containing polynucleotides.

Table 1

Binding constant (K) and the numbers of dsDNA base pairs occupied by one bound dye molecule (n) values for the dye Dst-6

K, 10^4 M^{-1}	n
6.8±1.9	8.1±1.5

Conclusions. For the (*p*-dimethylaminostyryl)pyridinium homodimer dye Dst-6, the approximation of the fluorescence titration of the dye by DNA using the McGhee and von Hippel equation was performed. Thus the values of dye-dsDNA binding constant as well as the number of dsDNA base pairs occupied by one dye molecule were estimated. The earlier suggested mechanism of minor groove binding of Dst-6 to dsDNA was confirmed.

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До вивчення механізму взаємодії гомодимера на основі (*p*-диметиламіностирил)піридинію з дсДНК

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Резюме. З метою вивчення механізму взаємодії (*p*-диметиламіностирил)піридинієвого гомодимерного барвника Dst-6 з длДНК проведено дослідження, спрямовані на визначення константи зв'язування барвник-ДНК (K) і кількості пар основ ДНК, що займає молекула барвника (посадкових місць). Отримані дані підтверджують зроблене нами раніше припущення, що барвник зв'язується з ДНК за борозенковим механізмом.

Ключові слова: стирилціанінові барвники, ДНК, флуоресценція, зв'язування з борозенкою, константа зв'язування.

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