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Fluorescence and binding properties of phenazine derivatives in complexes with polynucleotides of various base compositions and secondary structures

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Abstract

The interactions of two phenazine derivatives, one with a neutral chromophore (glycoside) and the other with a cationic one (quaternary salt), with various synthetic single- and double-stranded polynucleotides and natural DNA were studied by fluorescence techniques, conducting measurements of steady-state fluorescence intensity and polarization degree as well as fluorescence lifetime. These dyes show fluorescence quenching upon intercalation into the G · C sequences of the double-stranded nucleic acids and an increase in fluorescence emission and lifetime upon incorporation into the A · T and A · U sequences. G · C base pairs in continuous deoxynucleotide sequences were found to be preferred as binding sites for both phenazines, in contrast to A · T base pairs. On the contrary, the continuous ribonucleotide G · C sequence binds the phenazines more weakly than does the A · U sequence. With regard to the interaction of the phenazines with single-stranded polynucleotides, a stacking interaction of the dye chromophores with the nucleic bases was observed. In that case the guanine residue quenches the cationic phenazine fluorescence, while the stacking interaction with the other bases results in an increase in the fluorescence quantum yield. Unlike the cationic dye, the fluorescence of the neutral phenazine was quenched by both purine bases.

Keywords: Phenazines; Dye–nucleic acid interaction; Polarized fluorescence; Dye fluorescence lifetime

1. Introduction

The method of molecular hybridization of nucleic acids currently continues to be developed in order to detect and inactivate specific nucleotide sequences of genes. It is known that intercalating drugs covalently attached to antisense oligonucleotides stabilize their complexes with complementary sequences of the targets. Derivatives of acridine [1], phenazine [2,3], anthracycline [4] and other dyes have been used for

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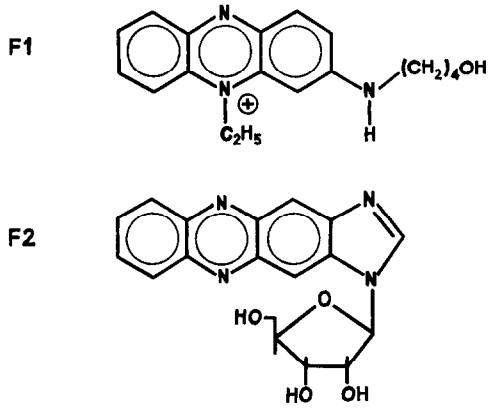

Fig. 1. Molecular structure of phenazine derivatives.

this purpose. Phenazines, like other dyes, enhance the oligonucleotide hybridization stability [3], but the chemistry of their attachment is simpler. Two authors of this paper previously suggested two phenazine derivatives (Fig. 1) for the modification of antisense oligonucleotides [5]. One of the phenazines (**F1**), with a cationic chromophore, is covalently linked to the terminal phosphate group via the polymethylene chain, and the second one (**F2**), with a neutral chromophore, is incorporated into the oligonucleotide chain, substituting a base.

Fluorescence techniques are very suitable for the study of molecular hybridization of oligonucleotides modified by intercalating dyes. The linked dye chromophore is a sensitive fluorescence probe. Besides indicating the complex formation, it is able to show its location in the complex structure, which allows one to judge the exactness of the complementary coupling. However, this requires a knowledge of how the fluorescence parameters of a dye change upon its incorporation into the structural elements of single- and double-stranded nucleic acids of various nucleotide compositions and on the interaction with separate nucleic acid bases. Information on the binding strength and specificity of the dye is also needed.

In this paper we report such data for the phenazines **F1** and **F2**, obtained as a result of studies of their binding to various single- and double-stranded synthetic polynucleotides and natural DNA. These data will be used in another study devoted to the hybridization of oligonucleotides modified with the phenazine dyes.

2. Materials and methods

The phenazine derivatives were synthesized in the Institute of Molecular Biology and Genetics, Ukrainian Academy of Sciences: *N*5-ethyl-3-(4-oxybutyl)aminophenazinium (**F1**) was synthesized by the method of oxidizing amination [6] and *N*1-β-D-ribofuranoside-2-methylimidazo(2,3-*d*)phenazine (**F2**) was obtained by condensation of the silylated heterocycle with peracylribofuranose in the presence of appropriate catalysts. Chicken erythrocyte DNA was purchased from Reanal, Hungary. All polynucleotides used were from Sigma (Deisenhofen, Germany). The polynucleotide concentrations were determined from absorbances, using the following extinction coefficients (M^{-1} cm^{-1}) at the UV band maxima: poly(A) 10100; poly(U) 9600, poly(C) 6200, poly(G) 9900, poly(A,G) 10000, poly(A)·poly(U) 7100, poly(C)·poly(G) 7900, poly(dA)·poly(dT) 6600, poly(dG)·poly(dC) 7400.

Sodium cacodylate buffer (1 mM, pH 7) with 0.5 mM Na_2EDTA was used as a solvent. Studies were carried out in solutions with Na^+ concentrations of 0.002 M (buffer) and 0.1 M (NaCl added to the buffer).

Absorption spectra were recorded on a UV–visible spectrophotometer (Cary 4E, Varian, Australia) and corrected fluorescence spectra on an SLM 48000 Aminco fluorimeter (SLM Aminco, Urbana, USA) at the IMB, Iena. Quantitative steady-state fluorescence measurements were carried out on a laboratory spectrofluorimeter, constructed on the basis of a double monochromator DFS-12 (Russia) and equipped with a photon counting electronic device system, at the ILTPE, Kharkov. Dark noise of the photomultiplier was at a level of 2 pulses s^{-1}.

The fluorescence was registered at the emission band maxima (emission slit 2 nm). Polarized components of the fluorescence intensity with the electric vector parallel ($I_{\parallel}$) and perpendicular ($I_{\perp}$) to the vector of the exciting polarized light beam were measured. The contribution from Rayleigh light scattering found in dye-free polymer solutions was subtracted from the measured intensities. Corrections were also made for the inner filter effect. The experimental error of the measured fluorescence intensity was about 0.5%. The total intensity I and polariza-

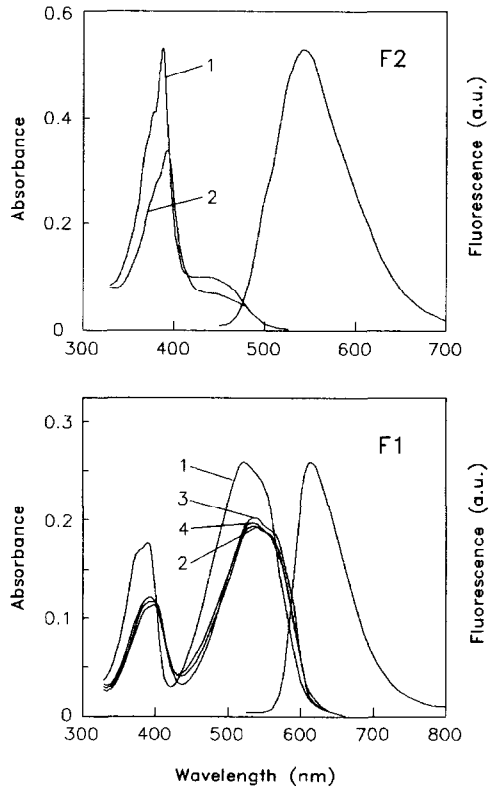

Fig. 2. Visible absorption (left) and fluorescence (right) spectra of free and bound phenazines in 1 mM cacodylate buffer, pH 7, 0.5 mM EDTA; 1 cm cell, temperature 25°C. For **F1**: dye alone (1), with DNA at P/D = 320 (2) and with poly(A)·poly(U) at P/D = 380 (3), $[Na^+] = 0.1$ M; with poly(C)·poly(G) at P/D = 500 (4), $[Na^+] = 0.002$ M (dye concentration, $C = 20$ μM). For **F2**: dye alone (1) and with DNA at P/D = 350 (2), $[Na^+] = 0.002$ M ($C = 24$ μM). Fluorescence spectra are shown for the free dyes.

tion degree ρ of fluorescence were calculated from the relations:

$$I = I_{\parallel} + 2I_{\perp} \quad \rho = (I_{\parallel} - I_{\perp})/(I_{\parallel} + I_{\perp}) \qquad (1)$$

The fluorescence lifetime measurements were performed at the IMB, Iena, using an SLM 48000 M4F Fourier transform spectrofluorimeter (SLM instruments Inc., Illinois, Urbana, USA) upgraded with a phase fluorimeter unit.

The absorption and fluorescence were measured in quartz cuvettes at room temperature, 24 ± 2°C. In the experiments the dye solution was titrated at a concentration $C = 20$ μM with increasing amounts of polynucleotide solution containing the same dye concentrations. The aim of the titrations was to obtain the dependence of the fluorescence properties on the molar phosphate-to-dye ratio, P/D.

3. Results

3.1. Spectroscopic properties of phenazine derivatives

The visible absorption and fluorescence spectra of the phenazines are shown in Fig. 2. The neutral phenazine **F2** shows an intense absorption band at 384 nm and a broad longwave shoulder. The cationic dye **F1** has two intense absorption bands at 388 and 520 nm. Non-structured fluorescence spectra of **F2** and **F1** have maxima at 543 and 615 nm, respectively.

The concentration dependences of the dye absorption properties are given in Fig. 3. The neutral form **F2** undergoes strong dimerization in aqueous solu-

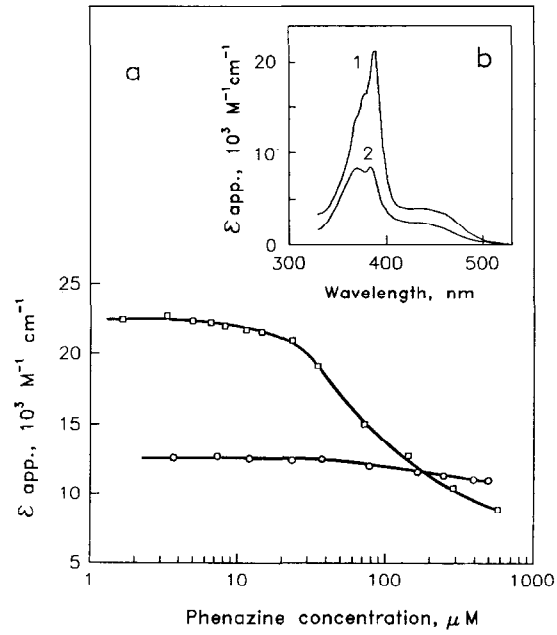

Fig. 3. Concentration dependence of dye absorption properties of phenazine derivatives in solutions with $[Na^+] = 0.002$ M. Other conditions as in Fig. 2. (a) Apparent extinction coefficients of **F1** (○) and **F2** (□) measured at the maxima of the absorption bands, at 520 and 384 nm respectively. (b) Absorption spectra of 3 μM (1) and 570 μM (2) **F2**.

tion. This is indicated by the hypochromism of its absorption spectrum and the appearance of a new absorption band with maximum at 370 nm (Fig. 3(b)) which belongs to dye dimers. The value of the dimerization constant for **F2**, $K_d = 5000 \pm 1000$ M^{-1}, was estimated according to Schwarz et al. [7] using the concentration dependence of the extinction coefficient at 384 nm shown in Fig. 3(a). The dimerization of **F1** is much smaller and its extinction coefficient has been found not to change essentially up to the concentration of 40 μM.

The essential difference between the optical properties of **F1** and **F2** is observed in the fluorescence parameters. The fluorescence decay of **F2** is biexponential with time constants $\tau_1 = 20$ ns and $\tau_2 = 5$ ns. The slow decay results in the low steady-state fluorescence polarization degree, ρ, that has been found to be 0.004. In contrast to **F2**, the fluorescence of **F1** molecules is characterized by the strictly monoexponential decay having small $\tau = 0.87$ ns and accordingly greater $\rho = 0.08$.

The optical properties of the phenazines are collected in Table 1.

3.2. Fluorescence properties of the cationic phenazine complexes

3.2.1. *F1–double-stranded polynucleotide complexes*

On **F1** binding to DNA and double-stranded polynucleotides, its absorption spectrum displays hypochromism and a red shift, and the fluorescence spectrum undergoes a blue shift (Fig. 2 and Table 1), typical of dye intercalation. The curves of fluorescence titration are shown in Fig. 4 as dependences of the changes in the fluorescence intensity and polarization degree on P/D. As can be seen, A · T and A · U sequences increase the dye fluorescence intensity, but G · C sequences cause fluorescence quenching. The quenching action of poly(C) · poly(G), occurring in solutions with 0.1 M Na^+ ions, is rather small, obviously due to the weak binding. The titration of **F1** with this polymer, performed at a low ionic strength (0.002 M Na^+ ions), revealed nearly the same quenching effect as in the case of poly(dG) · poly(dC) (Fig. 4(a)).

It is known that in many cases of nucleic acid interaction with dyes having three-cyclic chro-

Table 1
Optical properties of the complexes between phenazine derivatives and nucleic acids [a]

Substance	P/D	Absorption [b]		Fluorescence						
		λ_{max}/nm	ϵ_{max}/m^{-1} cm^{-1}	λ_{max}/nm	I_b/I_0 [c]	ρ [c]	τ_1/ns	τ_2/ns	A_1 [d]	A_2 [d]
F1		520	12600	615	1	0.08	0.87		1	
F1–DNA	320	533	9400	611	0.15	0.42	0.75		1	
F1–poly(dA) · poly(dT)	450			609	2.1	0.39				
F1–poly(A) · poly(U)	380	535	10300	611	1.7	0.39	1.5		1	
F1–poly(dG) · poly(dC)	260			610	0.02					
F1–poly(C) · poly(G)	500	534	9500	610	0.05					
F1–poly(G)	260	529	9000	610	0.005	0.46				
F1–poly(A)	530	529	10400	613	1.47	0.25	1.0		1	
F1–poly(A,G)	1000	530	9500	611	0.035	0.33				
F2		384	22400	543	1	0.004	20	5	0.7	0.3
F2–DNA	350	389	14300	539	0.09		19	2	0.9	0.1
F2–poly(dA) · poly(dT)	440			538	2.7	0.085				
F2–poly(A) · poly(U)	480	390	15000	538	1.75	0.085	22	9	0.6	0.4
F2–poly(dG) · poly(dC)	400			539	0.03					
F2–poly(G)					≤ 0.005					
F2–poly(A,G)					≤ 0.01					
F2–poly(A)					≤ 0.08					

[a] For measurement conditions see captions to Figs. 4–6.
[b] The parameters of longwave absorption bands.
[c] Data were obtained by means of a graphical extrapolation of the I/I_0 and ρ versus D/P plots to D/P = 0.
[d] A_1 and A_2 represent normalized pre-exponential terms for each decay component: $A_1 + A_2 = 1$.

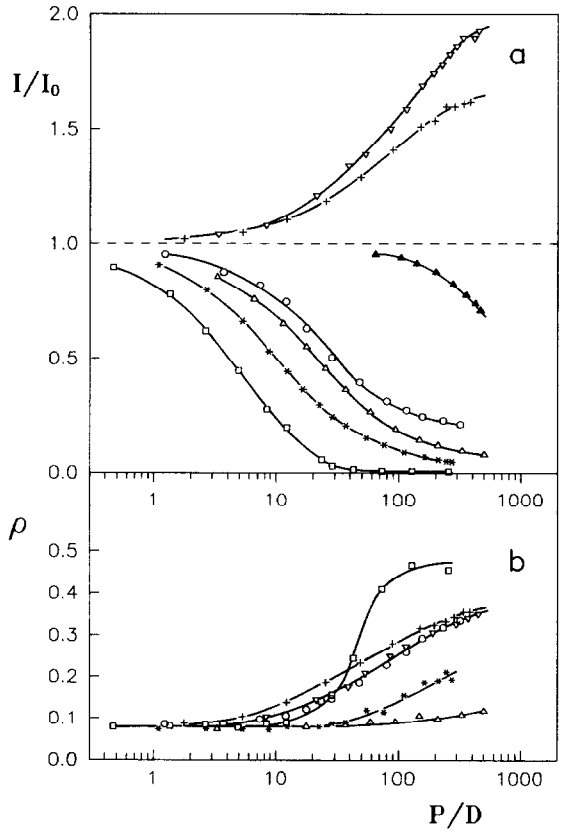

Fig. 4. Changes in (a) the relative fluorescence intensity I/I_0 and (b) the polarization degree ρ of cationic phenazine **F1** on titration by: poly(C)·poly(G) (△) and poly(G) (□) in solutions with $[Na^+] = 0.002$ M; DNA (○), poly(dA)·poly(dT) (▽), poly(dG)·poly(dC) (*), poly(A)·poly(U) (+) and poly(C)·poly(G) (▲) in solutions with $[Na^+] = 0.1$ M. Dye concentration, $C = 20$ μM, was constant. Excitation wavelength, $\lambda_{ex} = 530$ nm; I_0 is the fluorescence intensity of the free dye. Measurements were carried out at 24 ± 2°C in 1 mM cacodylate buffer, pH 7, 0.5 mM EDTA.

mophore structures, the guanine residue is the cause of the fluorescence quenching. To ascertain the extent of **F1** fluorescence quenching by the guanine residue the titration of this dye with four-stranded poly(G) was carried out. Due to the low ionic strength of the solution, a high degree of dye binding was achieved, confirmed by the fact that the P/D dependences of the fluorescence parameters had reached steady levels (Fig. 4(a) and (b), poly(G) curves). The residual fluorescence intensity is 0.5% of the free dye intensity. The very high $\rho = 0.46$ signifies that the fraction of the free dye is infinitesimal. For the other systems studied such complete **F1** binding has not been achieved and the values of the relative fluorescence intensity I_b/I_0 and polarization degree ρ for polynucleotide-bound **F1** were estimated by means of a graphical extrapolation of their D/P dependences to D/P = 0. They are collected in Table 1.

The lifetime measurements were conducted for **F1**–DNA and **F1**–poly(A) · poly(U) complexes. The fluorescence decay was found to remain monoexponential with the τ value somewhat decreased in the case of DNA and increased about 1.7-fold in the case of poly(A) · poly(U). The latter correlates with the increased fluorescence emission (Table 1).

3.2.2. *F1–single-stranded polynucleotide complexes*

The results of fluorescence titration of **F1** with four single-stranded polynucleotides poly(A), poly(U), poly(C) and poly(A,G) are shown in Fig. 5. The experiments were carried out in solutions of low ionic strength (0.002 M Na^+ ions) in order to obtain

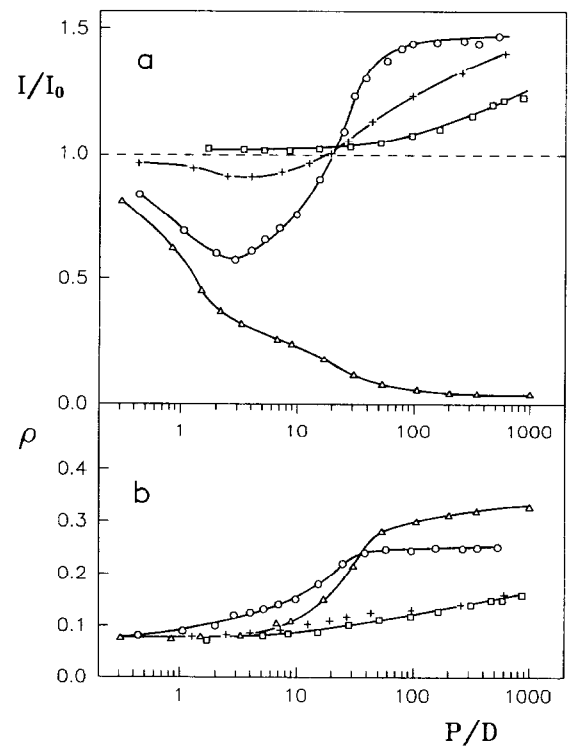

Fig. 5. Fluorescence titration of **F1** by single-stranded polynucleotides: poly(A) (○), poly(U) (+), poly(C) (□) and poly(A,G) (△) in solutions with $[Na^+] = 0.002$ M. $\lambda_{ex} = 530$ nm. Other conditions and explanations as in Fig. 4.

a high degree of binding. The form of the curves of I/I_0 versus P/D plots shows the existence of two types of binding. The electrostatic cooperative binding of the dye to the polyanionic chain of the polynucleotides predominates at low P/D, which is characterized by fluorescence quenching as a result of the stacking interaction of the dye chromophores. It is displayed by the appearance of the minima in the I/I_0 versus P/D plots, which is most pronounced in the case of poly(A). This kind of effect was observed for other cationic dyes on interaction with linear polyanions [8–11]. However, electrostatic binding is essential only at low ionic strengths and disappears at moderate ionic strength due to the competitive binding of the Na^+ ions [10,11].

At high P/D the dye stacks fall apart and the chromophores of the monomeric dye join the stacking interaction with the nucleic acid bases. Hence, ρ increases, essentially indicating a limited dye chromophore mobility. The interaction with adenine, uracil and cytosine residues increases **F1** fluorescence, but the interaction with the guanine residue quenches it. Since the copolymer poly(A,G) quenches **F1** fluorescence powerfully (see Fig. 5(a)), this fact signifies that the nucleotide sequence of this polynucleotide is alternating to a considerable extent and the guanine contact with the dye on one side is sufficient for realization of the quenching action.

On **F1** binding to poly(A) and poly(A,G), the absorption spectra of the dye change nearly in the same way as on binding to double-stranded polynucleotides (Table 1). Upon **F1** complex formation with poly(A) the fluorescence decay remains monoexponential with a somewhat increased value of τ (Table 1).

Purine polynucleotides bind **F1** more effectively than do pyrimidine polynucleotides. In the case of poly(A) and poly(A,G) a high degree of binding was achieved, as the plateaus of the titration curves show (Fig. 5), which made it possible to obtain the values of the fluorescence parameters of **F1** bound to the polynucleotides (Table 1).

3.3. Fluorescence properties of the neutral phenazine complexes

The character of the change in the **F2** absorption spectrum upon complex formation with DNA (Fig. 2) evidences the intercalative binding mechanism.

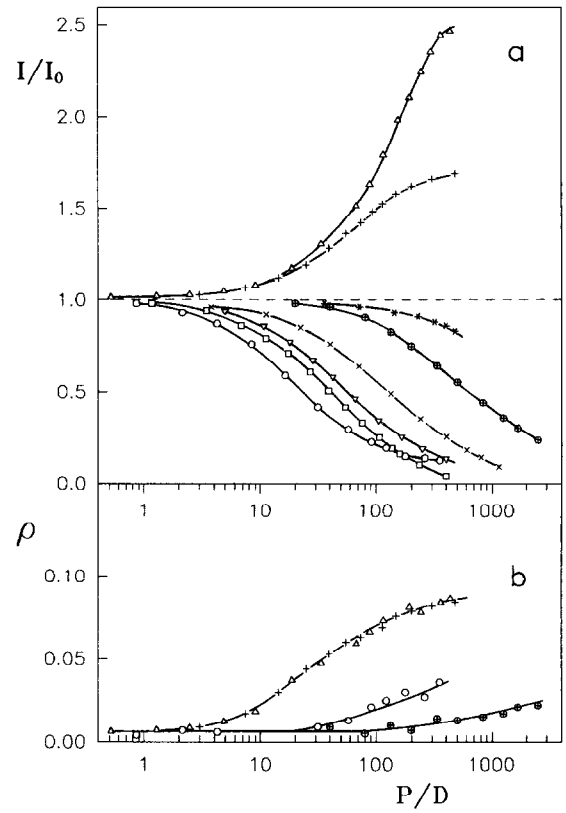

Fig. 6. Fluorescence titration of neutral phenazine **F2**: by poly(dA)·poly(dT) (△), poly(dG)·poly(dC) (▽), poly(A)·poly(U) (+) and poly(C)·poly(G) (*) in solutions with $[Na^+]=0.1$ M; by DNA (○), poly(G) (□), poly(A) (⊕) and poly(A,G) (×) in solutions with $[Na^+]=0.002$ M. $\lambda_{ex}=450$ nm. Other conditions and explanations as in Fig. 4.

The results of the fluorescence titrations are shown in Fig. 6. The I/I_0 values were calculated taking into account the dimerization of the free dye. As expected, the neutral molecules of **F2** bind to polynucleotides and DNA less effectively than the cationic molecules of **F1**. Complete dye binding has not been reached in the systems studied and the values of **F2** fluorescence parameters in the complexes were estimated in all cases by means of a graphical extrapolation of their D/P dependences to D/P = 0 (Table 1).

The picture of alteration of **F2** fluorescence properties upon binding to different polynucleotides is the following. On intercalation of this dye into double-stranded structures of A · T and A · U nucleotide

sequences, I increases. When the dye is inserted into G · C sequences, DNA and four-stranded poly(G), the fluorescence is quenched. A very small fluorescence emission is observed in the complex with poly(G), as has been found for **F1**. The realization of **F2** binding is confirmed by the increase in ρ, with the exception of the guanine-containing polynucleotides for which the change of values has not been registered. This can be explained by the fact that the highly polarized fluorescence of the complexes makes a negligible contribution to the total emission.

The interaction of **F2** with the single-stranded polynucleotides poly(A) and poly(A,G) can be proved. Electrostatic binding is absent since the **F2** derivative has no positive charge. Undoubtedly, there is a stacking interaction of the dye chromophores with the polynucleotide bases. Both the polymers quench the dye fluorescence. It is interesting to note that the effect of poly(A) on **F2** is quite the opposite to the effect on **F1**, the emission of which is increased. The binding of **F2** to the pyrimidine sequences poly(C) and poly(U) was found to be negligible.

Fluorescence lifetime values were obtained for **F2** complexes with poly(A) · poly(U) and DNA. The decay remains biexponential with an essential change in τ_2 only (Table 1).

3.4. Binding constants

Binding data were obtained for the interaction of the phenazines with the double-stranded polynucleotides and DNA in solutions of the moderate ionic strength solutions. The fractions of bound dye, γ_b, and free dye, γ_f, were calculated, assuming the existence of a single type of complex (intercalative), from the relations [12]:

$$\gamma_b = (1 - I/I_0)/(1 - I_b/I_0) \quad \gamma_b + \gamma_f = 1 \qquad (2)$$

using the fluorescence titration data on I/I_0 and values of I_b/I_0 presented in Table 1. Then the concentrations of the free dye in moles per litre, $C_f = C \cdot \gamma_f$ (C is the total dye concentration in solution), and the binding densities of the dye on the polymer (in moles of ligand per mole of nucleic base pair), $r = 2\gamma_b/(P/D)$, were calculated for the construction of Scatchard plots (Fig. 7). The values of the apparent binding constants, K, were determined using the intercepts on the r/C_f axis by the experimental Scatchard curves and are given in Table 2. For the extremely weak binding of **F1** and **F2** to poly(C) · poly(G) only approximate K values are presented.

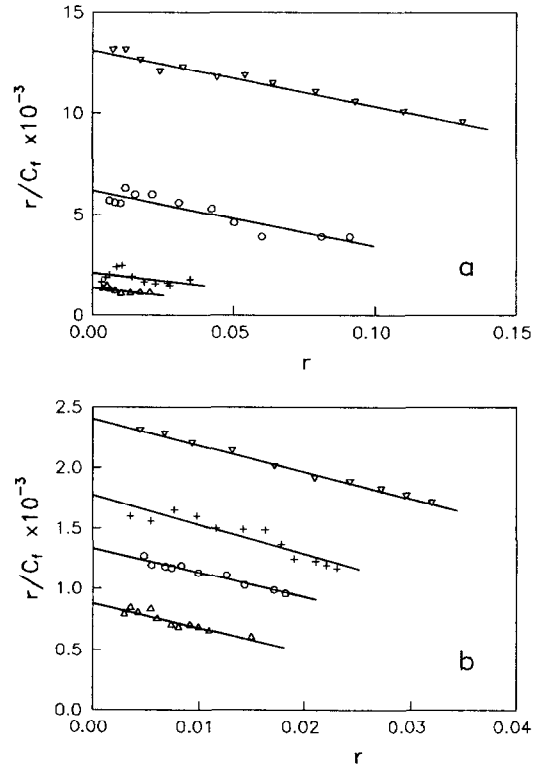

Fig. 7. Scatchard plots for the binding of **F1** (a) and **F2** (b) to DNA (○), poly(dG)·poly(dC) (▽), poly(dA)·poly(dT) (△) and poly(A)·poly(U) (+) in aquous solutions with $[Na^+] = 0.1$ M, pH 7. r is expressed as the number of bound dye molecules per polymer base pair, C_f in mol l^{-1}.

Table 2
Apparent binding constants K (M^{-1}) for the interaction of phenazine derivatives with DNA and polynucleotides in aqueous solutions with 0.1 M Na^+ ions at 24 ± 2°C

	F1	F2
poly(dG)·poly(dC)	13100	2400
DNA	6100	1300
poly(dA)·poly(dT)	1300	900
poly(A)·poly(U)	2100	1800
poly(C)·poly(G)	≈ 100	≈ 50

For the interaction of **F1** with poly(dG) · poly(dC) the K value is found to be an order of magnitude higher than that with poly(dA) · poly(dT). For **F2** this difference is less pronounced. In contrast, for the binding of phenazines to polyribonucleotides the K value is more than 20 times higher for poly(A) · poly(U) than for poly(C) · poly(G) (Table 2). These findings were confirmed for both dyes by the measurements of their fluorescence parameters upon interaction with mixed samples of poly(dA) · poly(dT) with poly(dG) · poly(dC) and poly(A) · poly(U) with poly(C) · poly(G) for equal concentrations of the polymers.

4. Discussion and conclusions

The first aim of this paper was to ascertain how the fluorescence properties of the two different phenazine derivatives change on interaction with various structural elements of nucleic acids. The fluorescence quantum yield and lifetime of both phenazines were found to increase upon dye intercalation between A · T and A · U base pairs of double-stranded polynucleotides. That effect might be caused by the departure of the dye chromophore from an aqueous environment [13]. A similar explanation may be given for the case of the fluorescence enhancement by single-stranded polynucleotides, when cationic phenazine enters into the stacking interaction with single nucleic acid bases, with the exception of the guanine residues. In all cases when the phenazines interacted with various polynucleotides containing guanine residues, strong fluorescence quenching was observed, independent of the secondary structure of the polymers (Table 1). This provides evidence for the same mechanism for the fluorescence quenching.

Earlier, one author of this paper suggested that the quenching is caused by electron transfer from the purine residues in their singlet ground state to the dye molecules in their first excited singlet state [14]. This occurs when the reduction potential of the dye after excitation becomes more positive than the oxidation potential of guanine or adenine. Recently, this suggestion has been confirmed for the interaction of the guanine residue with methylene blue [15,16] and benzo(a)pyrene [17] by transient light absorption measurements. Evidently, the electron-transfer quenching mechanism is also realized in guanine–phenazine complexes.

The fluorescence of the neutral phenazine derivative was found to be quenched more than tenfold on interaction with poly(A), unlike that of the cationic phenazine, for which fluorescence emission enhancement was observed. This is one of a few observations of the quenching action of adenine. Note that the fluorescence increases upon **F2** intercalation into adenine-containing double-stranded polynucleotides. Obviously, adenine exhibits quenching activity when it is in close contact with the dye chromophore, as is realized in the **F2**–poly(A) complex. In intercalative complexes such contact is absent, as was shown by Quigley et al. [18] for daunomycin which has a molecular geometry similar to that of the **F2** molecule. The daunomycin chromophore is incorporated into DNA with its long axis approximately perpendicular to that of the Watson–Crick base pairs, which decreases its overlap with the planes of the nucleic bases.

The second aim of this paper was to establish the binding affinity and specificity of the phenazines to nucleic acids of various structures and nucleotide compositions. The results obtained show that both the dyes bind more strongly to the deoxynucleotide G · C sequence than to the A · T sequence. The G · C binding specificity becomes manifested in the complex with DNA. The phenazine fluorescence quenching by DNA confirms this conclusion. Indeed, using the value of $I_b/I_0 = 0.15$ for the **F1**–DNA complex (Table 1) and considering that DNA contains two kinds of binding sites, one of which increases the dye fluorescence intensity (the A · T base pairs, $I_b/I_0 = 2.1$) and the other quenches the fluorescence (the G · C base pairs, $I_b/I_0 = 0.02$) (Table 1), it is possible to calculate the dye fractions bound to the two types of sites. It turns out that the fraction of the fluorescent dye is 0.07, which is considerably smaller than the fraction of the binding sites in the gaps between adjacent A · T base pairs in the chicken erythrocyte DNA used, which is equal to the square of the A · T base pair content, $[A \cdot T]^2 = 0.336$, if random distribution of the nucleotides is assumed.

In contrast, the specificity of the phenazines binding to continuous sequences of polyribonucleotides is quite opposite to that of the polydeoxynucleotides:

the A · U sequence binds phenazines much stronger than does the C · G sequence. Note that a much higher binding strength of poly(A) · poly(U) in comparison with poly(C) · poly(G) has also been found for the interaction with ethidium bromide [19].

The observed interaction of the phenazines with the nucleic bases of single-stranded polynucleotides is caused mainly by hydrophobic forces. They not only bring about the insertion of the dye chromophores into the ordered structures of poly(A), poly(A,G) and poly(C) but also produce the stacking of disordered uracil bases of poly(U) with the dye chromophores. Since the area of a purine base is larger than that of a pyrimidine, **F1** binding to poly(A) is stronger than to poly(C). It is clear that the existence of a positive charge at the dye molecule increases the dye binding due to electrostatic attraction, as was observed for the cationic phenazine binding.

The results obtained in this work may be useful for investigation of the molecular hybridization of oligonucleotides modified with phenazines using fluorescence methods.

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