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Fluorescence Properties of Intercalating Neutral Chromophores in Complexes with Polynucleotides of Various Base Compositions and Secondary Structures

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Changes in the relative quantum yield and polarization degree of steady-state fluorescence of daunomycin (DM) on its binding to six synthetic single- and double-stranded polynucleotides of various nucleotide compositions were measured over a wide range of molar polymer-to-dye ratios, in solutions of low ionic strength. Guanine base was found to be an effective quencher of DM fluorescence [in the DM–poly(G) complex the intensity of residual emission was ~0.5% of the free dye intensity]. The quenching of DM fluorescence by another purine base, i.e., adenine, was also revealed. But, unlike guanine, adenine exhibits quenching activity when it is in close contact with the DM chromophore, as realized in the complex with single-stranded poly(A). In intercalative complexes with double-stranded nucleic acids, where such contact is lacking, the quenching ability of adenine does not manifest itself, which has been demonstrated with the DM–poly(A) · poly(U) complex. It was found that the interaction with pyrimidine bases does not substantially change the DM quantum yield. The quenching feature of DM fluorescence is identical to that observed earlier by us for a glycoside phenazine dye containing, like DM, a neutral chromophore.

KEY WORDS: Neutral chromophores; daunomycin; dye-DNA interaction; polarized fluorescence.

INTRODUCTION

Covalent attachment of intercalating dyes to antisense and antigene oligonucleotides is known to be used for stabilization of their double- and triple-helix formations [1]. Rapid assay of these formations can be performed by fluorescence techniques using the sensitivity of the fluorescence emission of the linked dye chromophore to the oligonucleotide hybridization. Besides its an ability to indicate complex formation at very low reagent concentrations, fluorescence spectroscopy can also yield certain structural information, for instance, on the location of the dye chromophore in the complex. However, this requires knowledge of how various nucleotides included in binding sites of single- and double-stranded nucleic acids affect dye fluorescence characteristics.

Not so long ago, dyes containing neutral chromophores were conjugated with oligonucleotides, namely, daunomycin [2,3] and imidazophenazine glycoside [4] (Fig. 1). Earlier we studied the fluorescence behavior of the latter phenazine derivative in complexes with various polynucleotides [5] and revealed that it differs from that of cationic dyes. The fluorescence of this neutral dye was found to be quenched by both purine bases, i.e., guanine and adenine, unlike cationic dyes, the fluorescence of which is known to be quenched by the guanine residue alone.

Therefore it would be interesting to examine whether the fluorescence of other intercalating dyes containing a

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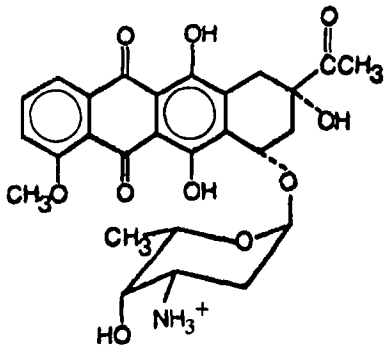
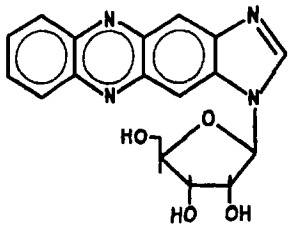


Daunomycin



Imidazophenazine glycoside

Fig. 1. Molecular structure of daunomycin and phenazine derivative.

neutral chromophore is quenched by the adenine base. In the present work the anthracycline antibiotic daunomycin (DM) was investigated in that light. This molecule (Fig. 1) has a positive charge on the amino sugar moiety but the chromophore is in the neutral form. Effects of adenine as well as other nucleic acid bases on parameters of DM steady-state fluorescence were ascertained from the results of DM titration by some synthetic polynucleotides of various nucleotide compositions and secondary structures.

EXPERIMENTAL

Daunomycin hydrochloride and the polynucleotides poly(A) · poly(U), poly(C) · poly(G), poly(G), poly(A), poly(U), and poly(C) were obtained from Sigma Chemical Co. (Deisenhoven, Germany). For all experiments 1 m*M* sodium cacodylate buffer, pH 7, with 0.5 m*M* EDTA, prepared from deionized distilled water, was used as a solvent. The low ionic strength of solutions was chosen in order to achieve a high degree of binding. Polynucleotide concentrations were determined by UV absorption using known extinction coefficients [5]. An extinction coefficient of 11,500 M^{-1} cm^{-1} at 480 nm was used for determination of the DM concentration.

The spectrofluorimeter and method of measurement of fluorescence parameters were described earlier [5]. Fluorescence was excited with steady-state polarized radiation from a He–Cd laser at a wavelength of 441.6 nm, whose intensity was stabilized to within ±0.2%. The laser beam was significantly attenuated with a light filter to prevent DM photodecomposition. To calculate the relative quantum yields, the absorbances at the laser wavelength were measured on a SPECORD M-40 spectrophotometer (VEB Carl Zeiss, Jena).

DM solution with a starting concentration of 10 μ*M* was titrated with increasing amounts of concentrated polynucleotide solutions. The DM concentration was reduced by two to three times at the close of the titration. The goal was to measure the fluorescence properties at different polynucleotide-to-daunomycin ratios (Pol/DM), expressed as moles of bases per moles of DM, up to high values. The fluorescence and absorbance were registered alternatively in the same 1-cm-path length quartz cuvette at room temperature, 24 ± 2°C.

RESULTS

The results of titration of DM with the double-stranded polynucleotides poly(A) · poly(U), poly(C) poly(G), and poly(G) are shown in Fig. 2, and with single-stranded poly(A), poly(U), and poly(C) in Fig. 3, as the dependences of the fluorescence relative quantum yield, Q/Q_0, and polarization degree, p, on the molar ratio Pol/DM. Q/Q_0 was calculated from the relation

$$Q/Q_0 = FA_0C_0/F_0AC$$

where F_0 and F are the fluorescence intensities of the total amount of DM in the free state and in the mixture with a polymer, respectively, measured at the emission band maxima (the latter have been found to be in the range of 592–607 nm); A_0 and A are the absorbances of these samples at the excitation wavelength, 441.6 nm; and C_0 and C are the starting concentration of free DM and the concentration in the mixture, respectively.

The form of the titration curves, obtained in solutions of low ionic strength (in the buffer), shows the existence of two types of DM binding. The electrostatic cooperative binding of DM to the polyanionic chains of polynucleotides predominates at Pol/Dye ≤2 and is characterized by fluorescence quenching owing to a stacking interaction of DM chromophores [6]. The quenching is complete since the fluorescence polarization, being equal to that of the free DM ($p \approx 0.095$), shows that only the unbound

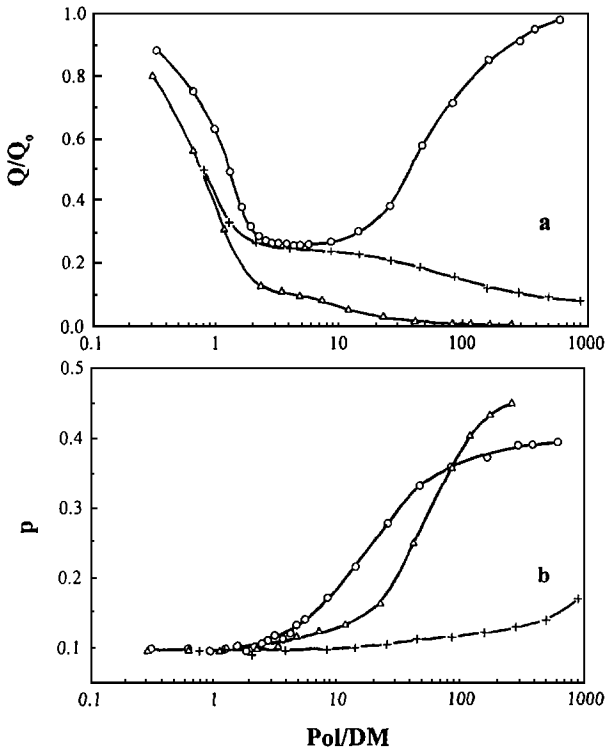


Fig. 2. Relative quantum yield, Q/Q_0, and polarization degree, p, of daunomycin (DM) fluorescence on titration with poly(A)·poly(U) (○), poly(C)·poly(G) (+), and poly(G) (△). Measurements were carried out at ±24°C in 1 m*M* sodium cacodylate buffer, pH 7, 0.5 m*M* EDTA. The initial DM concentration was 10 μ*M*, which was decreased by two to three times at the close of titration. The fluorescence was excited by a He–Cd laser at a wavelength of 441.6 nm. Q/Q_0 is defined in the text. Pol/DM is the polynucleotide-to-daunomycin ratio, expressed as moles of bases per moles of DM.

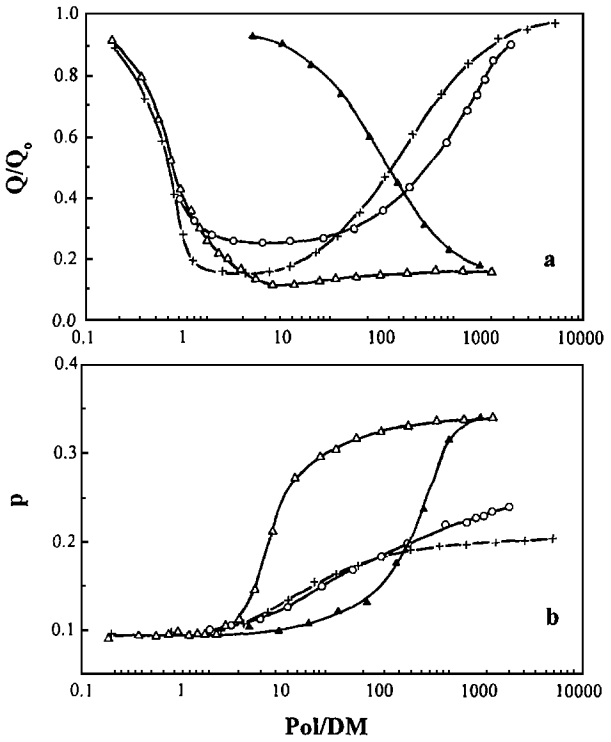


Fig. 3. Fluorescence titration of daunomycine with single-stranded polynucleotides: poly(U) (+), poly(C) (○), and poly(A) (△) in solutions of low ionic strength (buffer) and poly(A) (▲) in the solution with 0.1 *M* NaCl. Other conditions and explanations as in the legend to Fig. 2.

dye is fluorescent (Figs. 2b and 3b). With a molar ratio increase from the vaiue of 2, the dye stacks fall apart gradually and DM chromophores enter the interaction with nucleic acid bases, resulting in a p increase due to the appearance of higher polarized emission of the bound dye. The intercalative binding mechanism is realized in the case of double-stranded polynucleotides [7,8]. On binding to single-stranded polynucleotides, by all appearances, the DM chromophore joins with two adjacent bases, while the amino sugar is electrostatically attached to a phosphate group of the polymer.

To illustrate an effect of ionic strength on DM binding, the titration curves obtained with poly(A) in the solution containing 0.1 *M* NaCl are shown in Fig. 3. As can be seen, in this case the electrostatic cooperative binding is suppressed to a great extent by the competitive binding of Na^+ ions, but the interaction with adenine bases takes place, which is confirmed by the increase in p.

Reliable values of DM fluorescence parameters in the complexes with polynucleotides can be obtained only at very high Pol/DM ratios, at which fractions of the stacked and unbound dye become negligible. As shown in Figs. 2 and 3, under the indicated conditions Q/Q_0 for the systems with poly(A) poly(U) and poly(U) tends to be close to 1. Apparently, this is the case for poly(C). Thus it may be presumed that the fluorescence quantum yield of the DM chromophore does not change substantially on its incorporation into the structures of these biopolymers.

The known [9] strong quenching effect of guanine containing nucleic acids on DM fluorescence was also observed in this work (Fig. 2). At the highest molar ratio poly(G)/DM = 250 used, the Q/Q_0 value was 0.005. The high p (0.45) indicated the existence of a certain residual fluorescence of DM in the complex. The smaller quenching action of poly(C)·poly(G) observed (Q/Q_0 = 0.08 at the maximal Pol/DM reached, 900) is explained by the fact that a high degree of binding was not achieved due to the weak binding ability of this polymer. This is confirmed by the rather small increase in p. Quenching of DM fluorescence by poly(A) was also observed (Fig. 3). The effect quantity estimated at high poly(A)/DM molar

ratios, at which a high degree of DM binding to adenine bases was achieved, is characterized by $Q/Q_0 \approx 0.16$.

DISCUSSION

The causes of changes in the fluorescence of intercalating dyes on their binding to nucleic acids are well known. In intercalative complexes with non-guanine-containing polynucleotides, dye fluorescence usually is enhanced due to the departure of the chromophore from an aqueous environment [10]. The results obtained in the experiment with poly(A)· poly(U) show that this rule is violated in the case of DM, providing reason enough to conclude that the DM quantum yield does not change substantially. This was also observed when DM bound to bases of the single-stranded pyrimidine polynucleotides.

The fluorescence quenching of various dyes by guanine-containing polymers has been attributed to electron transfer from guanine to the singlet excited state of dyes [11,12], which has been confirmed experimentally [13, 14]. As shown above, this mechanism is very efficient at the DM quenching.

The fluorescence quenching ability of another purine base, i.e., adenine, was also predicted [11], but as far as I know, reliable experimental confirmation for the case of cationic dyes is absent. For instance, the quenching of fluoroquinacrine fluorescence by poly(A) observed in Ref. 15 undoubtedly occurred due to the cooperative stacking of the dye molecules at the polyanionic chain of the polymer, arising because of the low ionic strength of the solution used.

A quenching action of adenine on DM fluorescence was revealed. However, the effect was displayed in the single-stranded nucleotide sequence only. Obviously, the quenching occurs when DM chromophores enter into close contact with adenine rings, as is realized in the DM–poly(A) complex. In intercalative complexes with double-stranded nucleic acids, overlap of the DM chromophore with nucleic bases is slight [8]; this is why the fluorescence quenching was not observed in the experiment with poly(A) poly(U). A similar feature of fluorescence quenching by adenine was also observed earlier [5] for the glycoside phenazine dye with a neutral chromophore (Fig. 1).

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