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FLUORESCENCE OF PROFLAVINE AND 9-AMINOACRIDINE BOUND TO DNA: EFFECT OF DNA BASE COMPOSITION

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The analysis of fluorescence quantum yields reveals that bound proflavine does not fluoresce unless it is intercalated between two adjacent AT pairs. The results of 9-aminoacridine suggest that two adjacent AT pairs next to GC pairs may be still quenching sites for the dye or the heterogeneity of the dye binding may occur.

The interaction of proflavine (PF) and 9-aminoacridine (9-AA) with DNA is of special interest because of their strong mutagenic activity.[1] It is well known that the fluorescence of both acridine dyes is highly quenched when bound to DNA.[2-6] This phenomenon probably is the result of a specific interaction between the dye molecules and binding sites. It is possible that a similar interaction plays an important role in the biological actions of acridine dyes.[7] In order to elucidate the interaction between acridine dyes and the binding sites, fluorescence properties of PF and 9-AA bound to DNAs of various base compositions have been examined as a function of the GC content of DNA.

PF (British Drug Houses) and 9-AA (Tokyo Kasei) were purified by repeated crystallization and chromatography. Bacteriophage T2 DNA was prepared by the method of Mandell and Hershey.[8] The following DNAs and synthetic polynucleotides were commercial products: Clostridium perfringens DNA (Worthington), calf thymus DNA (Worthington), Micrococcus luteus DNA (Miles), poly d(A-T) (Miles), and poly (dG)·poly (dC) (Miles).

Fluorescence quantum spectra were measured with a Hitachi MPF-2A spectrophotofluorometer calibrated by using a standard tungsten lamp. Fluorescence quantum yields were determined according to the method of Parker and Rees;[9] quinine sulfate in 1 N H_2SO_4 was used as the standard reference.[10] For measurements of dye-DNA complexes from which fluorescence was polarized, the artifact due to the polarization was considered.[11] A total intensity of fluorescence (I_F) at a given wavelength is defined by

$$I_F = I_{VV} + 2I_{VH}(I_{HH}/I_{HV}), \qquad (1)$$

where I_{VV}, I_{VH}, I_{HH}, and I_{HV} are the intensities of the four components of the fluorescence light. The subscripts denote the directions of polarization; the first letter for the exciting light and the second for the fluorescence. The measurements were made at all wavelengths, and then corrected spectra were constructed according to Eq. 1.

All the measurements were carried out in 5 mM phosphate buffer (pH 6.9) at room temperature (23±1°C). The molar ratio of DNA phosphate to dye (P/D) ranged from 200

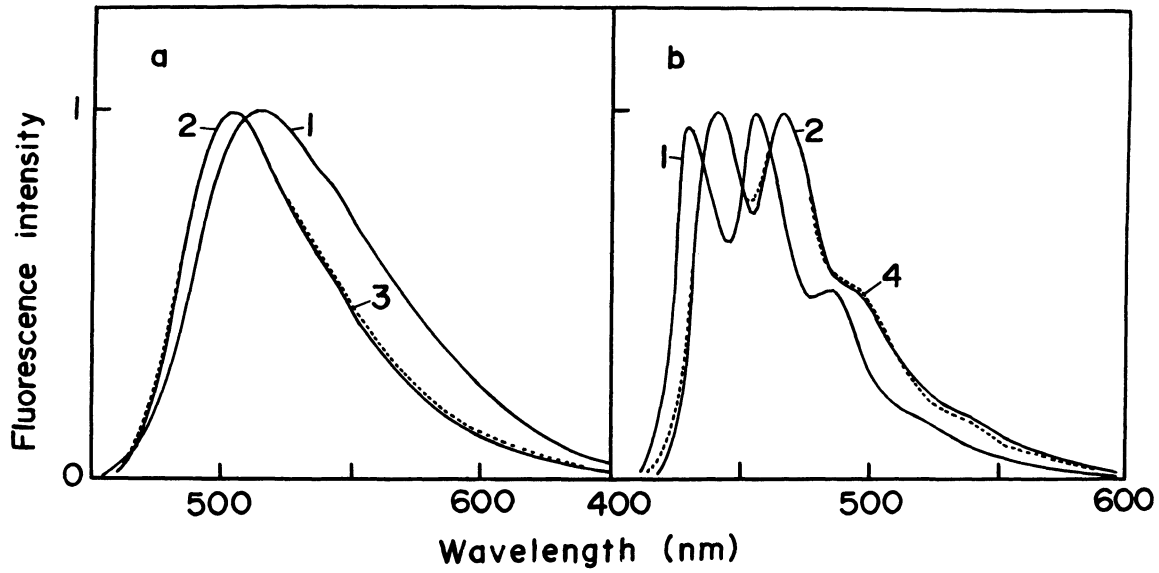

Fig. 1. Normalized fluorescence quantum spectra of (a) PF (2.2×10^{-6} M) and (b) 9-AA (4.3×10^{-6} M): (1) free, (2) bound to poly d(A-T) (P/D=200), (3) bound to M. luteus DNA (P/D=400) and (4) bound to Cl. perfringens DNA (P/D=400). The excitation wavelength was 400 nm.

Table 1. Fluorescence quantum yields of PF and 9-AA bound to DNA[a)]

Dye-DNA	GC (%)	Φ_B	$\Phi_{(AT)_2}$[b)]	$\Phi_{(AT)_4}$[c)]	$\Phi_{(AT)_5}$[d)]
PF-poly (dG)·poly (dC)	100	0.01			
PF-DNA (M. luteus)	72	0.038	0.48		
PF-DNA (calf thymus)	42	0.171	0.51		
PF-DNA (Cl. perfringens)	30	0.254	0.52		
PF-poly d(A-T)	0	0.52	0.52		
9-AA-poly (dG)·poly (dC)	100	0			
9-AA-DNA (M. luteus)	72	<0.001[e)]			
9-AA-DNA (calf thymus)	42	0.034	0.10	0.30	0.52
9-AA-DNA (bacteriophage T2)	34	0.090	0.21	0.47	0.72
9-AA-DNA (Cl. perfringens)	30	0.125	0.25	0.52	0.74
9-AA-poly d(A-T)	0	0.71	0.71	0.71	0.71

a) The fluorescence quantum yields of PF and 9-AA were 0.44 and 0.96, respectively.
b) The calculated fluorescence quantum yield of a dye bound to AT:AT sites.
c) The calculated fluorescence quantum yield of a dye bound to AT:AT:AT:AT sites.
d) The calculated fluorescence quantum yield of a dye bound to AT:AT:AT:AT:AT sites.
e) The accurate value was not obtained because the fluorescence intensity was very weak.

to 400, and the low dye concentrations were used to avoid the inner filter effect. In such conditions, the concentration of free dye and energy transfer between bound dye molecules were negligible.[2,12]

Figure 1 shows typical fluorescence spectra of PF and 9-AA. Fluorescence spectrum of each dye bound to DNA was almost identical with that of the dye bound to poly d(A-T), irrespective of the GC content of DNA. This finding suggests that AT pairs of DNA are responsible for the fluorescence of the bound dye. Table 1 summarizes the fluorescence quantum yields (Φ_B) of the dye bound to DNAs of various base compositions. As is clearly seen in Table 1, the value of Φ_B is almost zero when bound to poly (dG)·poly (dC) and Φ_B increases as the GC content of DNA decreases. This phenomenon may be associated with changes in the content and arrangement of DNA base pairs.

It is now significant to examine how AT and GC pairs of DNA interact with the bound dye. If we do not distinguish AT pairs from TA pairs and GC pairs from CG pairs, there are three kinds of sites in DNA; AT:AT, AT:GC and GC:GC sites. The mole fraction of each site can be calculated by assuming that the base pairs are randomly distributed. Chan and McCarter[13] found that Φ_B of acriflavine (10-methylated PF) bound to DNA increases in proportion to an increase in the mole fraction of AT:AT sites in DNA, and they concluded that the bound acriflavine does not fluoresce unless it is bound to two adjacent AT pairs. In the case of PF, we also assume that the dye bound in proximity of GC pairs is non-fluorescent.[5] Then we can calculate the fluorescence quantum yield of a dye bound to AT:AT sites ($\Phi_{(AT)_2}$) according to the equation derived previously.[5,13] The calculation is based on the assumption that dye molecules are not selectively bound to particular sites and that bound dye molecules are randomly distributed. This assumption seems reasonable in view of theoretical and experimental results; the former[14] shows that most intercalation sites have almost equal binding affinity for the dye and the latter shows that the binding constants of acriflavine[13,15] and PF[16] are independent of the GC content of DNA. As is seen in Table 1, the value of $\Phi_{(AT)_2}$ for PF is almost the same as the Φ_B value of the dye bound to poly d(A-T). The results obtained here confirm the previous findings[3–5,7,11] which show that AT pairs of DNA are responsible for the fluorescence of the bound PF, but GC pairs almost completely quench its fluorescence.

The same calculation was made on 9-AA. The results in Table 1 show that $\Phi_{(AT)_2}$ is very small compared to the Φ_B value of the dye bound to poly d(A-T). This implies that 9-AA may interact with binding sites in ways different from those of PF. Two possible explanations can be proposed for interpretation of the results. One of those would assume that two adjacent AT pairs next to GC pairs may be still quenching sites for 9-AA. If we calculate the probability of AT:AT:AT:AT or AT:AT:AT:AT:AT sequence in DNA and then the fluorescence quantum yield ($\Phi_{(AT)_4}$ or $\Phi_{(AT)_5}$) of a dye which occupies one of three or four AT:AT sites, the results in Table 1 are obtained. In agreement with the above assumption, the values of $\Phi_{(AT)_4}$ and $\Phi_{(AT)_5}$ are very close to the Φ_B value of the dye bound to poly d(A-T). Another explanation would assume the heterogeneity of the dye binding. That is, the dye may bind more preferentially to GC-rich regions (quenching sites) than AT-rich regions (fluorescing sites).

It should be emphasized that at this point we have no information on why the

fluorescence yield characteristics of 9-AA differ from those of PF and acriflavine. Experiments to elucidate this point are now in progress.

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