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FLUORESCENCE LIFETIMES AND QUANTUM YIELDS OF ACRIDINE DYES BOUND TO DNA[1)]

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The fluorescence lifetimes and quantum yields of various acridine dyes bound to DNA have been measured at a high ratio of DNA phosphate to dye (P/D ratio). The nature of binding sites are discussed on the basis of the values of the observed lifetimes, quantum yields, and the calculated natural lifetimes.

It is well known that the fluorescence of acridine orange ($Ac[NMe_2]_2$) bound to DNA is enhanced at a high P/D value, while that of proflavine ($Ac[NH_2]_2$) is quenched.[2)] This phenomenon is of interest in connection with the mutagenic action of these dyes; $Ac[NH_2]_2$ is much more effective than $Ac[NMe_2]_2$.[3)] In general, the biological actions of acridine dyes are closely related to a specific interaction between dye molecules and DNA bases; in some cases, small changes in the dye structure lead to marked changes in the biological activity.[3)] In order to clarify this specific interaction, the fluorescence lifetimes and quantum yields of various acridine dyes bound to DNA were measured. As it is well known, the quantum yield (Φ_F) is equal to the ratio of the actual lifetime (τ) to the natural lifetime (τ_0):

$$\Phi_F=\tau/\tau_0 \qquad (1)$$

The value of τ_0 can be calculated from the data of absorption and fluorescence spectra by the aid of Eq. (2) presented by Strickler and Berg[4)]:

$$1/\tau_0=2.88\times10^{-9}n^2\langle\tilde{\nu}_F^{-3}\rangle_{Av}^{-1}\int\varepsilon d\ln\tilde{\nu}_A \qquad (2)$$

where ε is the molar extinction coefficient, $\tilde{\nu}_A$ and $\tilde{\nu}_F$ the wavenumbers of the absorption and fluorescence spectra, respectively, and n the refractive index of the medium. If Eq. (1) does not hold for the experimental and calculated results, the existence of the heterogeneity of binding sites is expected.[5)] In this communication, we wish to report on the relationship between the fluorescence properties of bound dye and the nature of binding sites or the dye structure.

Experimental

Calf thymus DNA was obtained from Worthington Biochemical Corporation, poly dAT and poly dG·dC from Miles Laboratories. The following acridine dyes were used: 10-methylacridinium chloride (10-Me), 9-aminoacridine (9-AA), 3-aminoacridine ($AcNH_2$), 3-dimethylaminoacridine ($AcNMe_2$), $Ac[NH_2]_2$, $Ac[NMe_2]_2$, 3,6-bis-methylaminoacridine ($Ac[NHMe]_2$), 3,6-bis-ethylaminoacridine ($Ac[NHEt]_2$), and 3,6-bis-diethylaminoacridine ($Ac[NEt_2]_2$). Acridine dyes except 9-AA (Tokyo Kasei), $Ac[NH_2]_2$ (British Drug Houses),

and $Ac[NMe_2]_2$ (Chroma) were prepared according to the methods described by Albert.[6] All dyes were purified by repeating recrystallization and chromatography.

Absorption spectra were measured with a Shimadzu MPS spectrophotometer. Fluorescence spectra were obtained with a Hitachi MPF-2A spectrophotofluorometer; they were corrected for a spectral sensitivity of an optical system consisting of lenses, a monochromator, and a photomultiplier (Hamamatsu TV, R446UR). Fluorescence quantum yields were determined in the way described by Parker and Rees[7]; 9-AA and acriflavine were used as the standard materials.[8] Fluorescence lifetimes were measured with a JASCO FL-10 phase fluorometer[9]; the lifetime of each system was measured several times, and the results averaged. The measurements of optical properties of dyes bound to DNA were made at 25°C and at P/D=200 in 5 mM phosphate buffer solution (pH 6.8). Dye concentrations were 10^{-6}–10^{-5} M. Data of equilibrium dialysis and absorption spectra[10] showed that, under the present experiment conditions, almost all dye molecules are bound to DNA as the monomeric form.

Results and Discussion

Typical absorption and fluorescence spectra are shown in Fig. 1 for the DNA-$Ac[NMe_2]_2$ system. In all systems both spectra showed a rather good mirror image relationship. In general, the maximum of absorption spectrum of the bound dye shifted towards longer wavelength and that of fluorescence spectrum towards shorter wavelength, compared with those of the free dye (Fig. 1; Table 1). The values of $\langle\tilde{\nu}_F^{-3}\rangle_{Av}^{-1}$ were of the same order of magnitude for both free and bound dyes, but the maximum of the molar extinction coefficient of the bound dye was smaller than that of the free dye. Therefore, the bound dye gave a larger value of τ_0 than the value of the free dye. The observed lifetimes, quantum yields, and the calculated natural lifetimes are summarized in Table 1.

In order to examine Eq. (1), the values of Φ_F are plotted against τ/τ_0 in Fig. 2. It can be noted from this figure that Eq. (1) holds for all the free dyes except $Ac[NH_2]_2$, while it fails for $AcNH_2$ and $Ac[NH_2]_2$ when bound to DNA. The reason why

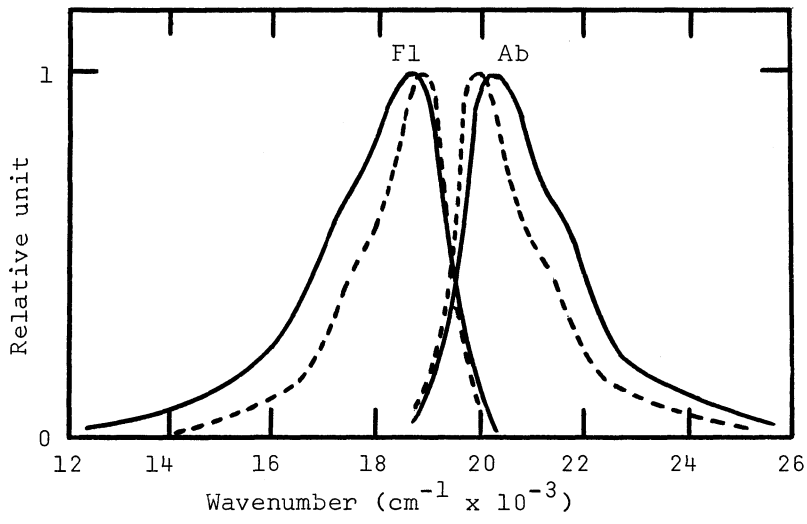

Fig. 1. Absorption (Ab) and fluorescence (Fl) spectra of the DNA-$Ac[NMe_2]_2$ system at 25°C.

—— $Ac[NMe_2]_2$ (1.9×10^{-6} M)

--- DNA-$Ac[NMe_2]_2$ (P/D=200)

Table 1. Fluorescence lifetimes and quantum yields

No	System	$\tilde{\nu}^{A}_{max}$ a) ($cm^{-1} \times 10^{-3}$)	$\tilde{\nu}^{F}_{max}$ a) ($cm^{-1} \times 10^{-3}$)	τ (nsec)	τ_0 (nsec)	Φ_F
1	10-Me	24.0 b)	20.4	54.80	52.55	1.00 c)
2	10-Me+DNA	23.3 b)	20.2	d)	86.70	<0.01
3	9-AA	24.9	21.9	17.48	16.90	0.98 c)
4	9-AA+DNA	24.6	21.4	d)	35.03	≃0.01
5	$AcNH_2$	22.1	18.4	3.68	17.58	0.21
6	$AcNH_2$+DNA	21.4	18.4	6.25	24.34	0.07
7	$AcNH_2$+poly dAT	21.2	18.4	7.48	25.44	0.22
8	$AcNH_2$+poly dG·dC	21.2	18.4	d)	26.92	<0.01
9	$AcNMe_2$	20.8	17.6	2.60	16.07	0.13
10	$AcNMe_2$+DNA	20.3	17.6	3.40	22.28	0.12
11	$Ac[NH_2]_2$	22.5	19.4	4.98	6.22	0.44
12	$Ac[NH_2]_2$+DNA	21.7	19.8	6.10	7.07	0.15
13	$Ac[NH_2]_2$+poly dAT	21.7	19.8	6.91	7.19	0.47
14	$Ac[NH_2]_2$+poly dG·dC	21.7	19.6	d)	7.29	≃0.01
15	$Ac[NMe_2]_2$	20.3	18.7	1.68	5.58	0.25
16	$Ac[NMe_2]_2$+DNA	19.9	18.9	5.20	6.40	0.75
17	$Ac[NMe_2]_2$+poly dAT	19.9	18.9	5.10	6.38	0.77
18	$Ac[NMe_2]_2$+poly dG·dC	19.9	18.9	5.50	6.32	0.81
19	$Ac[NHMe]_2$	21.6	19.8	4.32	5.76	0.73
20	$Ac[NHMe]_2$+DNA	21.1	19.9	3.96	6.47	0.57
21	$Ac[NHEt]_2$	21.5	19.6	4.25	5.80	0.74
22	$Ac[NHEt]_2$+DNA	21.0	19.9	3.98	6.47	0.57
23	$Ac[NEt_2]_2$	20.0	18.8	0.91	5.40	0.15
24	$Ac[NEt_2]_2$+DNA	19.7	18.8	4.05	6.30	0.63

a) $\tilde{\nu}^{A}_{max}$ and $\tilde{\nu}^{F}_{max}$ denote the peak wavenumbers of absorption and fluorescence spectra, respectively.
b) The peak position was estimated by separating the absorption intensity in the concerned wavelength region into two parts belonging to the strong and weak bands.
c) Taken from Ref. 8.
d) Reproducible results were not obtained because the intensity of fluorescence was very weak.

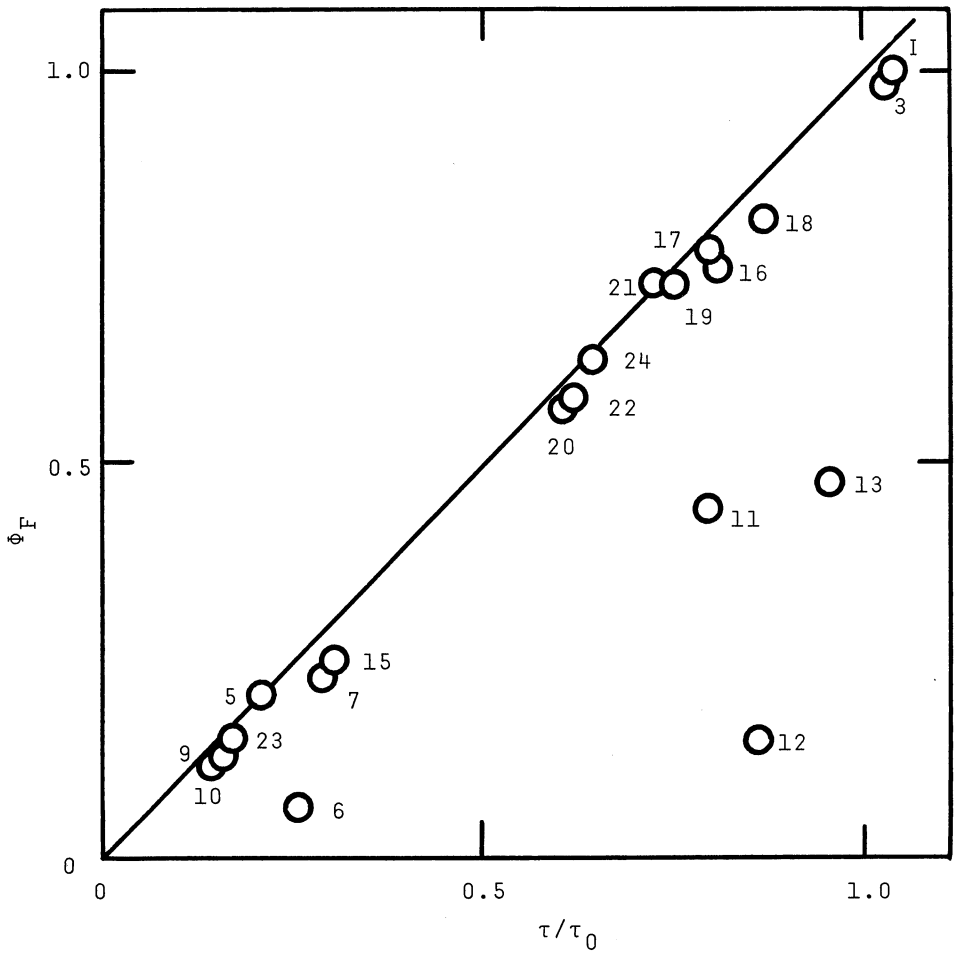

Fig. 2. Plots of Φ_F against τ/τ_0. Each number corresponds to that in Table 1.

Table 2. Data of fluorescence quantum yields

Acridine	Φ_b	$\Phi_{\substack{AT \\ AT}}$	$\Phi_{\substack{GC \\ GC}}$	$\Phi_{\substack{AT \\ GC}}$
10-Me	<0.01	≃0	≃0	≃0
9-AA	≃0.01	≃0	≃0	≃0
$AcNH_2$	0.07	0.22	<0.01	≃0
$Ac[NH_2]_2$	0.15	0.47	≃0.01	≃0
$Ac[NMe_2]_2$	0.75	0.77	0.81	0.70

free $Ac[NH_2]_2$ does not satisfy Eq. (1) is not clear. It is expected from the results in Fig. 2 that there exist two or more types of binding sites in the cases of $AcNH_2$ and $Ac[NH_2]_2$ and only one type of site in the other dyes.

It is generally accepted that the bound dye molecules are intercalated between adjacent base pairs in the DNA helix at a high P/D value.[11] It is now significant to examine how AT and GC pairs interact with the bound dye. Therefore, we next investigated the fluorescence properties of acridine dyes bound to poly dAT and poly dG·dC. As can be seen from Table 1, the Φ_F values of $AcNH_2$ and $Ac[NH_2]_2$ bound to poly dG·dC are almost zero, while the Φ_F value of $Ac[NMe_2]_2$ bound to poly dG·dC is nearly equal to that bound to poly dAT. The results similar to those of $Ac[NMe_2]_2$ were also obtained in the cases of $AcNMe_2$, $Ac[NHMe]_2$, $Ac[NHEt]_2$, and $Ac[NEt_2]_2$. Further, the Φ_F values of 10-Me and 9-AA were almost zero in both cases of poly dAT and of poly dG·dC.

If we do not distinguish AT pair from TA pair, and GC pair from CG pair, there are three kinds of binding sites in DNA. On the assumption that dye molecules are not selectively bound to particular sites and that bound molecules are randomly distributed, the quantum yield of an intercalated dye (Φ_B) can be represented by[12]:

$$\Phi_B = \Phi_{\substack{AT\\AT}} X_{\substack{AT\\AT}} + \Phi_{\substack{AT\\GC}} X_{\substack{AT\\GC}} + \Phi_{\substack{GC\\GC}} X_{\substack{GC\\GC}} \qquad (3)$$

where $\Phi_{\substack{AT\\AT}}$ denotes the quantum yield of a dye intercalated between adjacent AT pairs and X the corresponding mole fraction. The above assumption seems reasonable on the basis of the theoretical and experimental results; the former[13] shows that most intercalation sites have almost equal binding strength and the latter shows that the binding constants of acriflavine[14] and $Ac[NH_2]_2$[15] are independent of the GC content of DNA. By substituting the values of Φ_B, $\Phi_{\substack{AT\\AT}}$, $\Phi_{\substack{GC\\GC}}$, and X into Eq. (3), $\Phi_{\substack{AT\\GC}}$ can be evaluated. The mole fractions for calf thymus DNA (GC content 42%)[16] are calculated on the assumption that the base pairs are randomly distributed. The results for some dyes are summarized in Table 2.

The results in Table 2 indicate that acridine dyes are classified in three groups with respect to the fluorescence properties:

(1) Group I; 10-Me and 9-AA. All binding sites almost completely quench the fluorescence of the dye.

(2) Group II; $AcNH_2$ and $Ac[NH_2]_2$. Data of the bound dye do not satisfy Eq. (1). The bound dye does not fluoresce unless it is bound to two adjacent AT pairs; that is, the GC pair almost completely quench the fluorescence of the dye.

(3) Group III; $AcNMe_2$, $Ac[NMe_2]_2$, $Ac[NEt_2]_2$, $Ac[NHMe]_2$, and $Ac[NHEt]_2$. Data of the bound dye satisfy Eq. (1). Fluorescence properties of the bound dye show no dependence on the kind of binding sites.

From comparison of Groups II and III, it can be noted that substitution by alkyl groups of one or both hydrogens of amino groups leads to a change of the dye from Group II to III. Recently, it was found that the fluorescence of bound acriflavine decreases with an increase in the GC content of DNA.[14,17] This dye has also two amino groups at 3 and 6 positions as well as $Ac[NH_2]_2$. In view of these, it seems likely that, in Group II, the quenching of the fluorescence is attributed to a specific interaction between the GC pair and amino groups of the dye. In Group I,

the mechanism different from that in Group II may be responsible for the quenching of the fluorescence, since all sites quench the fluorescence of the dye. Further studies are in progress to make the mechanism of the fluorescence quenching clearer.

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