NOTES

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Fluorescence of 9-Aminoacridine Bound to Polynucleotides

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Synopsis. The fluorescence of 9-aminoacridine is completely quenched when bound to poly (dG)·poly (dC), the quantum yield of fluorescence being nearly equal to that of free dye when bound to poly d(A–T). The dye bound to DNA shows a weak fluorescence for which the AT pair is responsible.

The interaction of 9-aminoacridine (9-AA) with DNA is of special interest because of its strong mutagenic activity.[1] In a previous paper,[2] it was reported that the fluorescence of 9-AA bound to DNA is almost completely quenched. In order to elucidate the interaction between 9-AA and the binding sites, fluorescence properties of 9-AA bound to DNA, poly d(A–T) and poly (dG)·poly (dC) were examined as a function of nucleotide to dye (P/D) ratio.

Experimental

9-AA (Tokyo Kasei) was purified by repeated crystallization and chromatography. Calf thymus DNA (Worthington Biochemical Corporation), poly d(A–T) and poly (dG)·poly (dC) (Miles Laboratories) were used.

Fluorescence and fluorescence-excitation (FE) spectra were measured with a Hitachi MPF-2A fluorescence spectrophotometer. Both spectra were corrected for the sensitivity of the detector system and the spectral-energy distribution of the exciting light. The fluorescence quantum yields were measured according to the method of Parker and Rees.[3] Fluorescence lifetimes were determined by the phase-shift measurements.[2] All the measurements were carried out in a 0.005 M phosphate buffer (pH 6.9) at 25 °C.

Results and Discussion

The results of fluorescence lifetimes (τ) and quantum yields (Φ_F) of 9-AA bound to DNA, poly d(A–T) and poly (dG)·poly (dC) are summarized in Table 1. The fluorescence of 9-AA is remarkably quenched when bound to DNA or poly (dG)·poly (dC), whereas the fluorescence quantum yield is approximately equal to that of free dye when bound to poly d(A–T). The fluorescence and FE spectra of 9-AA bound to polynucleotides were examined to obtain information on the fluorescing species. The fluorescence spectra of 9-AA bound to DNA and poly d(A–T) are almost the same, showing a red shift when compared to that of free dye (Fig. 1). We see that the fluorescence and FE spectra of 9-AA bound to poly (dG)·poly (dC) are superimposable on the corresponding fluorescence and FE spectra of free dye, respectively. Each FE spectrum was the same as the corresponding absorption spectrum except for 9-AA–poly (dG)·poly (dC) system. The fluorescence lifetimes of 9-AA–poly (dG)·poly (dC) system are almost the same as the lifetime of free dye (Table 1). In view of these findings, the following

Table 1. Fluorescence lifetimes (τ) and quantum yields (Φ_F) of 9-AA bound to polynucleotides

P/D	DNA		Poly d(A–T)		Poly (dG)·poly (dC)	
	τ(ns)	Φ_F	τ(ns)	Φ_F	τ(ns)	Φ_F
0	17.5	0.98	17.5	0.98	17.5	0.98
590	14.9	0.03				
250			34	0.82		
200	15.1	0.03				
100	15.4	0.03	37	0.81	15.3	0.00_6
50	14.8	0.03_5	38	0.81	15.7	0.01
20	15.0	0.03_5	38	0.77	17.0	0.04_5
10[a]	15.5	0.07	29	0.74	17.5	0.14

a) The contribution of free dye is not negligible.

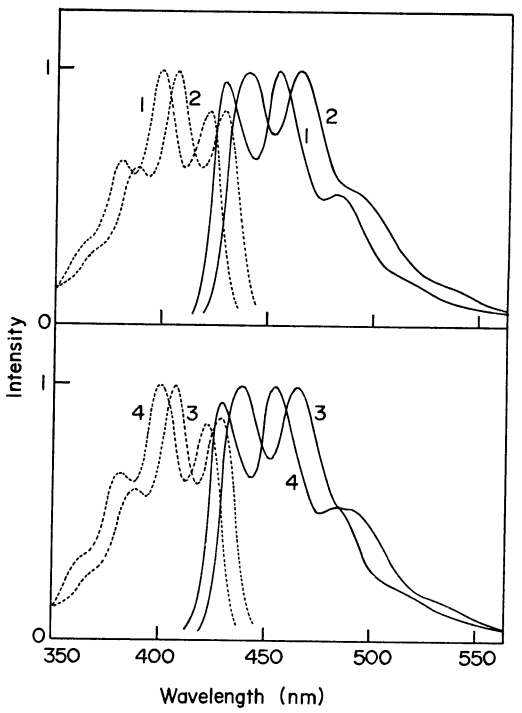

Fig. 1. Normalized corrected fluorescence (——) and FE (------) spectra of 9-AA (6.0×10^{-6} M) in 0.005 M phosphate buffer (pH 6.9) at 25 °C: (1) free, (2) bound to DNA (P/D=590), (3) bound to poly d(A–T) (P/D=250) and (4) bound to poly (dG)·poly (dC) (P/D=50). The excitation and emission wavelengths were 390 and 460 nm, respectively.

conclusions can be drawn: (1) the fluorescence of 9-AA–poly (dG)·poly (dC) system originates from free dye present in solutions;[4] the GC pair completely quenches the fluorescence of bound dye; (2) the AT pair is responsible for a weak fluorescence of 9-AA bound to DNA.

On the other hand, the fluorescence lifetime (34—38 ns) of 9-AA bound to poly d(A–T) is about twice that of free dye (Table 1). Our value is in agreement with the value obtained by nanosecond pulse fluorometry.[5] Such a long lifetime, however, seems to be reasonable on the basis of the strong hypochromicity of bound dye. The area under the absorption spectrum of bound dye plotted against wave number is reduced to about half the corresponding value of free dye. According to the method of Strickler and Berg,[6] the radiative lifetimes (τ_0) of free 9-AA and 9-AA bound to poly d(A–T) were calculated to be 17 and 37 ns, respectively. The τ/τ_0 value is approximately equal to the corresponding Φ_F value for both free and bound dye.

The results of 9-AA bound to DNA are somewhat surprising when compared to those of proflavine (PF). If PF is intercalated between adjacent AT pairs of DNA, the fluorescence quantum yield is nearly equal to that of PF bound to poly d(A–T).[2,7–9] However, the GC pair in the vicinity of bound PF gives rise to an almost complete quenching of the fluorescence.[2,7–9] From the results, it seems that 9-AA behaves similarly to PF with respect to the fluorescence properties. If this is the case, the fluorescence quantum yield of 9-AA bound to DNA should be about 0.3 in the case of calf thymus DNA (GC content of 42%).[2,10] Contrary to expectation, the observed quantum yield is much smaller (Table 1). The reason why the behavior of 9-AA is different from that of PF is not clear.

References

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10) Φ_F (bound to adjacent AT pairs)$=\Phi_F$ (bound to poly d(A–T))$\times$(mole fraction of AT: AT pair). The mole fraction of AT: AT pair is 0.3364 on the assumption that the base pairs are randomly distributed. Then the fluorescence quantum yield of 9-AA bound to adjacent AT pairs can be estimated to be about 0.3 (0.82×0.3364) by assuming that the bound dye molecules are randomly distributed.