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FLUORESCENCE LIFETIME STUDIES ON THE INTERACTION OF DNA WITH 9-AMINOACRIDINE

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The interaction of DNA with 9-aminoacridine has been studied by measuring fluorescence decay curves. The emitting sites of the dye on DNA (AT-rich regions) are found to exhibit fluorescence decay curves which can be resolved into three-exponential components corresponding to lifetimes of 1.6–2.4, 11–13, and 27–29 ns.

The interaction of acridine dyes such as proflavine and 9-aminoacridine (9AA) with DNA is of special interest because of their strong mutagenic activity.[1] From fluorescence quenching studies of DNA-proflavine and DNA-acriflavine complexes, it has been shown that there are two classes of binding sites.[2–7] One class (GC base pair) almost completely quenches the fluorescence of a bound dye, while the other (AT base pair) does not alter its fluorescence quantum yield; the first is called quenching sites and the second emitting sites.[3] This heterogeneity of binding sites may be important for the understanding of the biological actions of acridine dyes.[6] In order to elucidate the nature of binding sites, we have investigated systematically the interaction of DNA with acridine dyes by measuring fluorescence decay curves and fluorescence quantum yields.[7,8] In this paper, fluorescence decay curves of DNA-9AA complexes are studied. We show that the emitting sites of 9AA on DNA consist of at least three classes.

9AA (Tokyo Kasei) was purified by repeated crystallization and chromatography; any trace of impurity was not detected by thin-layer chromatography on silica gel. Bacteriophage T2 DNA was prepared by the method of Mandell and Hershey.[9] The following DNAs and synthetic polynucleotides were commercial products: Clostridium perfringens DNA (CP DNA; Worthington), calf thymus DNA (CT DNA; Worthington), Escherichia coli DNA (EC DNA; Worthington), poly (dG)·poly (dC) (Miles), and poly d(A-T) (Miles).

Steady-state fluorescence quantum spectra were measured with a Hitachi MPF-2A spectrophotofluorometer calibrated by using a standard tungsten lamp. Fluorescence quantum yields were determined by considering the artifact due to the polarization;[8] quinine sulfate in 1 N H_2SO_4 was used as a standard reference.[10] Fluorescence decay curves and nanosecond time-resolved fluorescence spectra were measured with an ORTEC Model 9200 nanosecond fluorescence spectrophotometer.[11] Excitation light (an air flash lamp) was passed through a Corning 7-60 filter and focussed on the sample with a lens. Emission from the sample cuvette was viewed at right angles through a monochromator (Applied Photophysics Ltd.) by an RCA 8850 photomultiplier tube. When fluorescence decays of the DNA-9AA complexes were measured, care was taken to eliminate

anisotropic contributions to the observed decay.[12] This was done by exciting with an unpolarized beam of light and observing the fluorescence through a Polacoat polarizer whose axis was at 54.7° to the excitation observation plane. Observed decay curves were analyzed by the method of non-linear least-squares.[13] The parameters obtained by analysis were convolved with the observed lamp flash and the fit between the observed and calculated decay curves was evaluated from the weighed residuals, the autocorrelation function of the residuals, and the reduced χ^2.[13,14]

All measurements were made in 5 mM phosphate buffer (pH 6.9) at room temperature (24±1°C). The molar ratio of DNA phosphate to dye (P/D) ranged from 100 to 1300, and the dye concentrations were 1.1–1.3 x 10^{-5} M. Under these conditions, the concentration of free dyes and energy transfer between bound dye molecules were negligible.[15]

In harmony with the results obtained previously,[7,16] it was found that the fluorescence spectrum of 9AA bound to DNA is almost identical with that of 9AA bound to poly d(A-T), regardless of the GC content of DNA, and that fluorescence quantum yield (ϕ_B) of the bound 9AA decreases with an increase in the GC content of DNA (Table 1) and is almost zero in the case of poly (dG)·poly (dC) (ϕ_B<0.003 at P/D=102). These findings suggest that AT base pairs of DNA are responsible for the fluorescence of the bound 9AA.

To obtain further information on the interaction between 9AA and the binding sites, fluorescence decay curves were measured by the single-photon counting method. Fluorescence decays of 9AA and the poly d(A-T)-9AA complex were single-exponential, while those of the DNA-9AA complexes clearly deviated from a single-exponential decay law. Typical decay curves obtained with the CT DNA-9AA complex (P/D=1311) are shown in Figs. 1 and 2. Figure 1 shows the best fit for a two-component exponential decay law, while Fig. 2 indicates the best fit for a three-component exponential decay law. The fit for the two-exponential components is seen to be poor, whereas that for the three-exponential components is excellent. The decay data obtained with the DNA-9AA complexes are thus consistent with a three-exponential decay law within the present criteria.[17] It should be emphasized that other decay laws are not precluded since, for example, it is hard to analyze decay data in which two lifetimes are very close.[19] Typical sets of the decay parameters obtained and the fluorescence quantum yields (ϕ_B) are shown in Table 1.

Next, nanosecond time-resolved fluorescence spectra were measured in order to study any time-dependent interactions between 9AA and its environment at binding

Table 1. Fluorescence decay parameters* and fluorescence quantum yields

System	GC(%)	P/D	τ_1(ns)	α_1	τ_2(ns)	α_2	τ_3(ns)	α_3	χ^2	ϕ_B
9AA	—	—	15.8	1.000	—	—	—	—	1.03	0.96
poly d(A-T)-9AA	0	136	31.3	1.000	—	—	—	—	1.11	0.73
CP DNA-9AA	30	402	2.3	0.384	12.6	0.191	29.0	0.425	1.31	0.12_9
T2 DNA-9AA	34	202	2.4	0.517	13.1	0.166	28.4	0.317	1.42	0.09_0
CT DNA-9AA	42	219	1.6	0.571	12.8	0.165	28.2	0.264	1.20	0.04_3
	42	1311	1.7	0.587	11.4	0.131	28.3	0.282	1.61	0.04_5
EC DNA-9AA	50	209	1.6	0.638	13.3	0.173	27.4	0.190	1.27	0.02_8

* The emission was observed at 455 nm. The pre-exponents are normalized to unity.

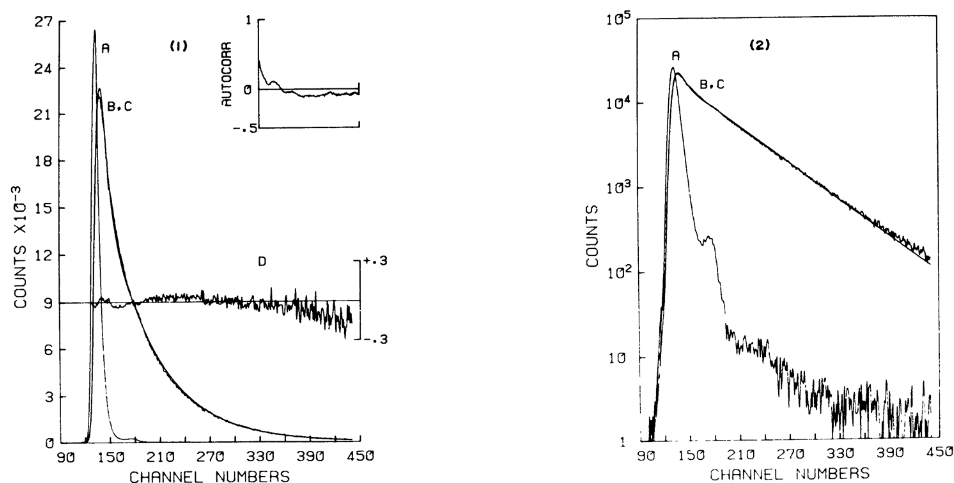

Fig. 1. (1) Two component analysis of the decay of the CT DNA-9AA complex (P/D=1311) in 5 mM phosphate buffer (pH 6.9) at 24°C. The emission wavelength was 455 nm. Curve A is the lamp flash profile. Curve B is the observed decay curve. The smooth curve C shows the computed decay curve. Curve D is the weighed residuals. The inset is the autocorrelation function of the residuals. Timing calibration was 0.453 ns/channel. Parameters obtained: τ_1=2.5 ns, τ_2=26.5 ns, α_1=0.219, α_2=0.131, and χ^2=3.85. The pre-exponents normalized to unity are α_1=0.626 and α_2=0.374. (2) Logarithmic representation of the same data.

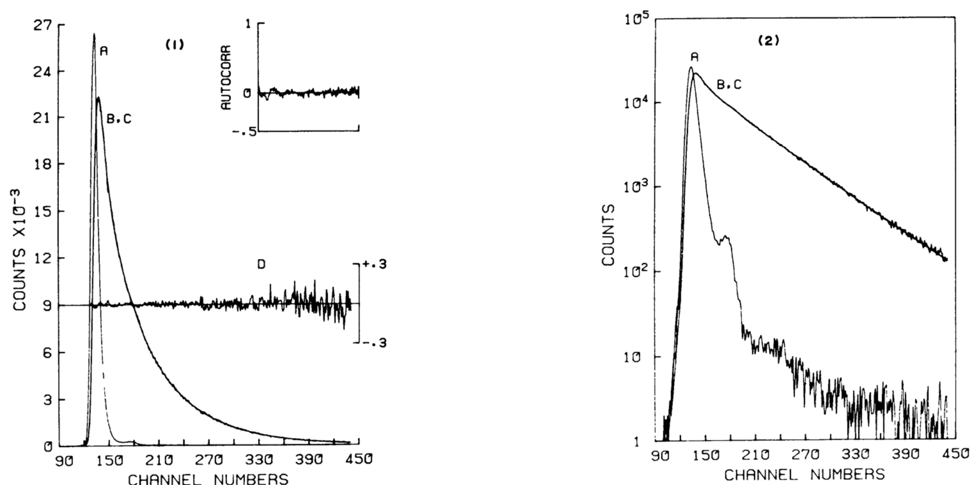

Fig. 2. (1) Three component analysis of the decay of the CT DNA-9AA complex (P/D=1311) in 5 mM phosphate buffer (pH 6.9) at 24°C. Conditions are the same in Fig. 1. The computed decay curve is based on the following decay parameters: τ_1=1.7 ns, τ_2=11.4 ns, τ_3=28.3 ns, α_1=0.225, α_2=0.050, α_3=0.108, and χ^2=1.61. The pre-exponents normalized to unity are α_1=0.587, α_2=0.131, and α_3=0.282. (2) Logarithmic representation of the same data.

sites. It was found that the spectra are independent of time and almost identical with steady-state fluorescence spectra.[15] This finding suggests that the structure of the DNA-9AA complex is not substancially altered during the lifetime of the excited singlet state of 9AA. Further, the shapes or the maxima of the absorption and fluorescence spectra of the bound 9AA and the molar extinction coefficient exhibited no dependence on the GC content of DNA. This result indicates that the radiative lifetime of 9AA is constant under a variety of circumstances.[15]

On the basis of these results, it is concluded that the emitting sites of 9AA on DNA are composed of at least three classes having different fluorescence quantum yields: (I) τ_1=1.6–2.4 ns, ϕ_1=0.05–0.07, (II) τ_2=11–13 ns, ϕ_2=0.31–0.37, and (III) τ_3=27–29 ns, ϕ_3=0.77–0.82, where each ϕ value was calculated by using the radiative lifetime, 35.0 ns.[5] As is seen in Table 1, the ϕ_3 and τ_3 values are almost the same as the quantum yield and the lifetime of 9AA bound to poly d(A-T) and the α_3 value increases with decreasing GC content of DNA. Therefore, it seems reasonable to conclude that class III is ascribed to 9AA bound to the AT-rich regions of DNA. Since the α_1 value increases with increasing GC content of DNA, class I may be attributed to 9AA bound to the sites on which the GC base pairs exert a certain effect, for example, AT:AT sites adjacent to a GC pair. The nature of class II is still not clear from this work. Further investigations are in progress.

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