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KINETICS OF FLUORESCENCE QUENCHING OF 9-AMINOACRIDINE AND 9-AMINO-10-METHYLACRIDINIUM BY PURINE MONONUCLEOTIDES

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A study on the kinetics of fluorescence quenching of 9-aminoacridine and 9-amino-10-methylacridinium by purine mononucleotides (adenosine-5'-monophosphate and guanosine-5'-monophosphate) is reported. A set of quenching parameters have been obtained on the basis of the kinetic scheme involving both dynamic and static quenching processes.

In a previous paper,[1] it was reported that the fluorescence of the mutagenic dye 9-aminoacridine (9AA) is markedly quenched by adenosine-5'-monophosphate (AMP) and guanosine-5'-monophosphate (GMP) and that fluorescence spectra of the 9AA-AMP system are dependent on the concentration of AMP. This paper describes further study on the steady-state and transient kinetics of fluorescence quenching of 9AA by AMP and GMP and also describes a comparison of results of 9AA with those of 9-amino-10-methylacridinium having no mutagenicity.

AMP and GMP, chromatographically pure, were obtained from Sigma Chemical Co. 9AA was the same sample as used before.[1] 9-Amino-10-methylacridinium chloride (10Me-9AA) was prepared according to the method described by Albert and Ritchie[2] and was purified by repeated recrystallizations from hot water.

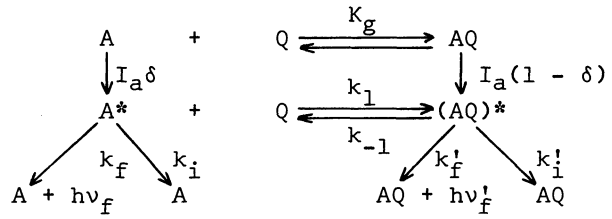
Absorption and fluorescence spectra were recorded with a Shimadzu UV-200S spectrophotometer and a Hitachi MPF-2A spectrophotofluorometer, respectively. For fluorescence measurements, the excitation wavelength was set at one of isosbestic points. Fluorescence spectra were corrected for the unequal quantum response of the detector system. Transient fluorescence decay curves were measured with an Ortec time-resolved emission spectrophotometer.[3] The excitation light at 375 nm was obtained with an air-filled (0.5 atm) flash lamp and an interference filter (Japan Vacuum Optics). The emission was observed by an RCA 8850 photomultiplier tube through a grating monochromator (Applied Photophysics Ltd.), the bandwidths being 2–10 nm. Observed decay curves were analyzed by the methods of Laplace transformation[4] and nonlinear least-squares.[5] Both methods yielded very similar results. The fit between the observed and theoretical decay curves was evaluated by convolving the apparatus response function[6] with the decay parameters obtained by analysis and by inspection of the reduced χ^2, the weighed residuals, and the autocorrelation function of the residuals.[5,8]

All measurements were made in 0.005 M phosphate buffer (pH 6.9) at 25°C. The dye concentration was 1.0–1.3 x 10^{-5} M. The nucleotide concentration was varied in the 0–0.1 M range.

Absorption spectra of the dye-nucleotide systems exhibited several isosbestic points, indicating that a specific complex is formed between the dye and nucleotides.

The ground-state association constant K_g was determined by assuming that a 1:1 stoichiometric complex is exclusively formed (Table 1).[1,3] The presence of AMP or GMP markedly quenched the fluorescence of the dye. The progressive change of the 9AA fluorescence in the presence of AMP is shown in Fig. 1; similar change was also observed with the 10Me-9AA-AMP system. This change indicates that the fluorescence spectrum can be assigned to the superposition of emissions of both the free and the complexed dyes. On the other hand, the fluorescence and fluorescence-excitation spectra of the dye in the presence of GMP were identical with the corresponding spectra of the free dye.

Quenching data can be analyzed according to the following kinetic scheme:[9,10]



In this scheme, A and Q, respectively, are the dye and nucleotide molecules, I_a is the total light quanta absorbed by the solution, and $\delta=\{1+(\varepsilon'/\varepsilon)K_g[Q]\}^{-1}$ is the fraction of light absorbed by A; ε and ε' are the molar extinction coefficients of A and AQ at the excitation wavelength. From the proposed kinetic scheme the total fluorescence intensity at time t, $I_f(t)$, is given by:

$$I_f(t) = k_f[A^*] + k_f'[(AQ)^*] = A_1e^{-\lambda_1 t} + A_2e^{-\lambda_2 t} \quad (1)$$

where

$$\lambda_{1,2} = 1/2[(k_f + k_i + k_1[Q] + k_f' + k_i' + k_{-1}) \mp \{(k_f' + k_i' + k_{-1} - k_f - k_i - k_1[Q])^2 + 4k_1k_{-1}[Q]\}^{1/2}] \quad (2)$$

$$\lambda_1 + \lambda_2 = k_f + k_i + k_1[Q] + k_f' + k_i' + k_{-1} = 1/\tau_0 + 1/\tau_0' + k_{-1} + k_1[Q] \quad (3)$$

The following steady-state equation can also be derived:[10]

$$\frac{\phi}{\phi_0} = \frac{\delta + k_{-1}\tau_0' + R(1 - \delta + k_1\tau_0[Q])}{1 + k_{-1}\tau_0' + k_1\tau_0[Q]} \quad (4)$$

where ϕ is the apparent fluorescence quantum yield in the presence of quencher and $R=\phi_0'/\phi_0$ is the ratio of the quantum yield of the complex AQ to that of the free A.

If the contribution of the complex AQ to the fluorescence emission is negligible in all circumstances, that is, $\phi_0' \ll \phi_0$ and $k_{-1}\tau_0' \ll 1$, Eqs. 1 and 4 reduce to Eqs. 5 and 6, respectively.

$$I_f(t) = Ae^{-(k_f + k_i + k_1[Q])t} = Ae^{-t/\tau},\ 1/\tau = 1/\tau_0 + k_1[Q] \quad (5)$$

$$\phi/\phi_0 = \delta/(1 + k_1\tau_0[Q]) \quad (6)$$

Figure 2 shows the ϕ_0/ϕ vs. [Q] plots for the 9AA-AMP and 9AA-GMP systems. The values of $R=\phi_0'/\phi_0$ were found to be 0 and 0.06 for the dye-GMP and dye-AMP systems, respectively, by extrapolating the apparent quantum yields to infinite nucleotide concentration. To obtain steady-state quenching parameters, nonlinear regression analysis[8] was performed by employing Eq. 6 for the dye-GMP and Eq. 4 for the dye-AMP system, in which R was taken as the known parameter. The results obtained by analyses are summarized in Table 1. The fluorescence lifetime of the dye, τ_0, was measured in the absence of quencher. The fluorescence lifetime of the complexed dye (τ_0') was determined from the fluorescence decay curve of the dye in the presence of an excess of AMP (0.05-0.1 M), where the contribution of the free dye was very small. As expected from the proposed scheme, we experimentally observed a single-exponential fluorescence decay for

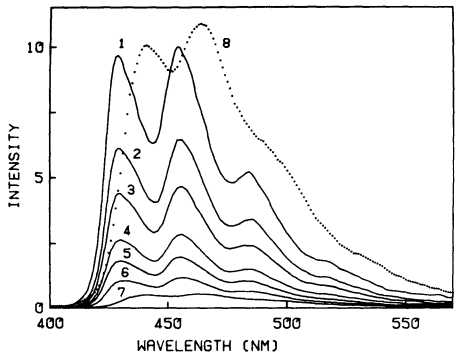


Fig. 1. Fluorescence spectra of the 9AA-AMP system. The excitation wavelength was 405 nm. The spectra were corrected for distortions caused by scattering.
AMP: (1) 0 M, (2) 0.001 M, (3) 0.002 M, (4) 0.004 M, (5) 0.006 M, (6) 0.01 M, (7) 0.1 M, (8) 0.1 M (x 20).

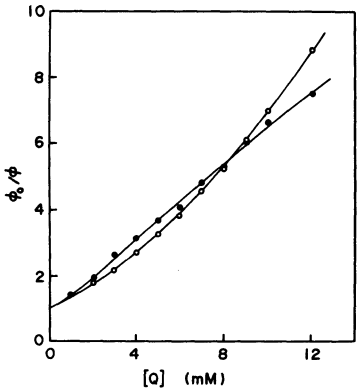


Fig. 2. Best fit between experimental and calculated (solid line) data for the change in the 9AA fluorescence yield with increasing quencher concentration.
●: AMP; o: GMP.

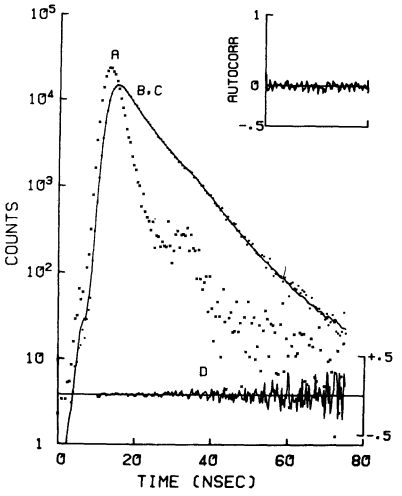


Fig. 3. Fluorescence decay curve obtained in the 9AA-AMP (0.012 M) system. The emission wavelength was 460 nm. A: Apparatus response function. B: Observed decay curve. C: Calculated decay curve (smooth curve). D: Weighed residuals. The inset is the autocorrelation function of the residuals. Parameters obtained: τ_1=7.61 ns, τ_2=1.53 ns, A_1=0.132, A_2=0.207, and X^2=1.32.

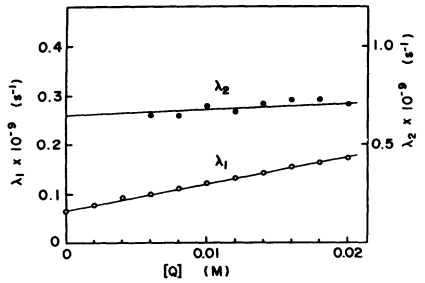


Fig. 4. Plots of the decay parameters associated with $A_1e^{-\lambda_1 t} + A_2e^{-\lambda_2 t}$ for 9AA quenched by AMP.

the dye-GMP system and a double-exponential fluorescence decay for the dye-AMP system. A typical decay curve obtained in the 9AA-AMP system is shown in Fig. 3. Figure 4 indicates plots of the decay parameters associated with $A_1e^{-\lambda_1 t} + A_2e^{-\lambda_2 t}$ for 9AA quenched by AMP. By employing Eqs. 3 and 5 for the dye-AMP and dye-GMP systems, respectively, the values of k_1 were determined (Table 1). Quenching behaviors of 10Me-9AA were very similar to those of 9AA.

Finally, the excited-state association constant K_e was estimated from K_g and from the spectral shift due to the complex formation, using the approximate equation[11]

$$\log K_e = \log K_g + (0.625/T)\Delta\nu \qquad (7)$$

where $\Delta\nu$ is the average of the spectral shifts in cm^{-1} between the maxima of the absorption and of the fluorescence spectra of the complex and those of the free dye.

A set of parameters for fluorescence quenching are summarized in Table 1. The

Table 1. Quenching parameters at 25°C

System	K_g (M^{-1})		K_e (M^{-1})		τ_0 (ns)	τ_0' (ns)	ϕ_0	ϕ_0'/ϕ_0	$k_1 \times 10^{-9}$ ($M^{-1}s^{-1}$)		$k_{-1} \times 10^{-6}$ (s^{-1})
	(1)	(2)	(3)	(4)					(2)	(5)	(2)
9AA-AMP	320	340	3100	2960	15.8	1.4_5	0.96	0.06	8.1_6	8.0	2.7_6
9AA-GMP	260	255	——	——	15.8	——	0.96	0	6.0_1	5.4_4	——
10Me-9AA-AMP	340	370	830	760	16.2	1.5_5	0.91	0.06	8.7_5	8.2	$11._5$
10Me-9AA-GMP	250	235	——	——	16.2	——	0.91	0	6.6_3	5.6_2	——

(1) Determined from absorption spectra. (2) Obtained from steady-state experiments, using Eqs. 4 and 6 for the dye-AMP and dye-GMP systems, respectively. (3) Determined using Eq. 7. (4) Determined using the relation $K_e=k_1/k_{-1}$. (5) Obtained from transient experiments, using Eqs. 3 and 5 for the dye-AMP and dye-GMP systems, respectively.

magnitude of the value of k_1 suggests that the quenching process is a diffusion-controlled one. Preliminary experiments revealed that the Arrhenius plot of log k_1 vs. 1/T gives 4.0–4.2 kcal mol^{-1} for the activation energy of the quenching reaction. This value is to be expected for the diffusion-controlled reaction. The rate constant k_1 obtained from steady-state experiments is found to be slightly larger than that obtained from transient experiments. This discrepancy may be due to transient effects associated with diffusion.[12] The rate constant for complex dissociation (k_{-1}) is extremely smaller than that for complex formation (k_1). Furthermore, the finding $K_e >> K_g$ suggests that the excitation of the dye results in strengthening the dye-nucleotide interaction. This may be the result of the change of the electronic charge distribution in the excited state which enhances the reactivity of the dye.[10] Interestingly quenching results of the non-mutagenic dye 10Me-9AA are very similar to those of the mutagenic dye 9AA. Perhaps a small change in the dye structure may be important in the biological activity of the dye.[3] More detailed studies will be published elsewhere.

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