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FLUORESCENCE QUENCHING OF 9-AMINOACRIDINE BY ADENOSINE-5'-MONOPHOSPHATE AND GUANOSINE-5'-MONOPHOSPHATE

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The fluorescence properties (spectrum, lifetime and quantum yield) of 9-aminoacridine cation in aqueous solutions of nucleotides have been examined. It was found that the fluorescence of the dye is quenched by adenosine-5'-monophosphate and guanosine-5'-monophosphate. Quantitative analysis of the results shows that the quenching of fluorescence is caused by dynamic and static quenching processes.

The interaction of 9-aminoacridine cation (9-AAH$^+$) 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-AAH$^+$ bound to DNA is almost completely quenched. This phenomenon probably is the result of a specific interaction between the dye and DNA bases. It is possible that a similar interaction plays an important role in the biological actions of the dye.[1,3,4]

In the present study, the fluorescence properties of 9-AAH$^+$ in aqueous solutions of nucleotides have been investigated to elucidate the interaction between the dye and DNA bases.

The nucleotides, chromatographically pure, were obtained from Sigma Chemical Co.; the 5'-monophosphates of adenosine, guanosine, thymidine and cytidine are abbreviated as AMP, GMP, TMP and CMP, respectively. The free base of 9-AA hydrochloride (Tokyo Kasei) was repeatedly recrystallized from a methanol-water mixture, and then the methanol solution of the free base was twice passed through an alumina column. Any trace of impurity was not detected by thin-layer chromatogaphy on silica gel.

Absorption spectra were measured with a Shimadzu UV-200S spectrophotometer. 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. Fluorescence quantum yields were determined according to the method of Parker and Rees.[5] Fluorescence lifetimes were measured with a JASCO FL-10 phase fluorometer.[2] All the measurements were carried out in a 5mM phosphate buffer (pH 6.9); 9-AA exists as monovalent cation at this pH.

Typical absorption spectra are shown in Fig. 1 for the 9-AAH$^+$-GMP system; similar absorption changes were also observed with other nucleotides. The isosbestic points in the absorption spectra clearly indicate that a specific complex is formed between nucleotides and 9-AAH$^+$. By assuming an equilibrium like $A + Q \rightleftarrows AQ$, the dependence of absorption spectra on the concentration of nucleotides was analyzed with the aid of the Ketelaar method;[6] here, A and Q denote the dye and nucleotide mole-

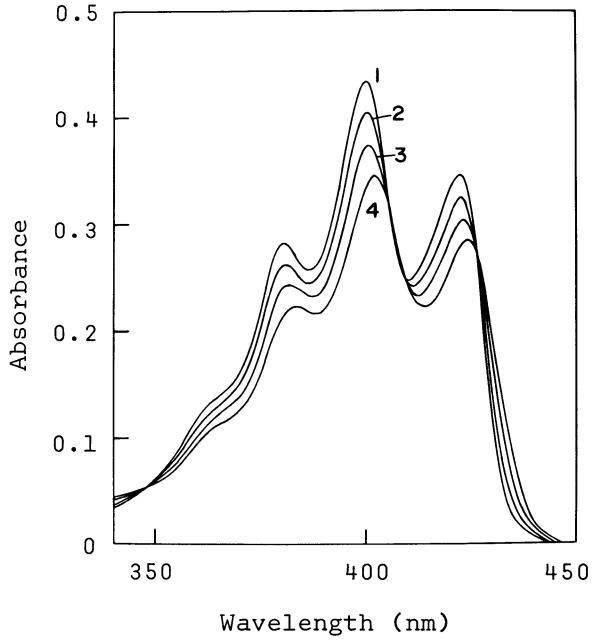

Fig. 1. Absorption spectra of 9-AAH$^+$-GMP system at 25°C. 9-AAH$^+$: 4.5 x 10^{-5} M. GMP: (1) 0, (2) 1.3 x 10^{-3} M, (3) 5.0 x 10^{-3} M, (4) 1.5 x 10^{-2} M.

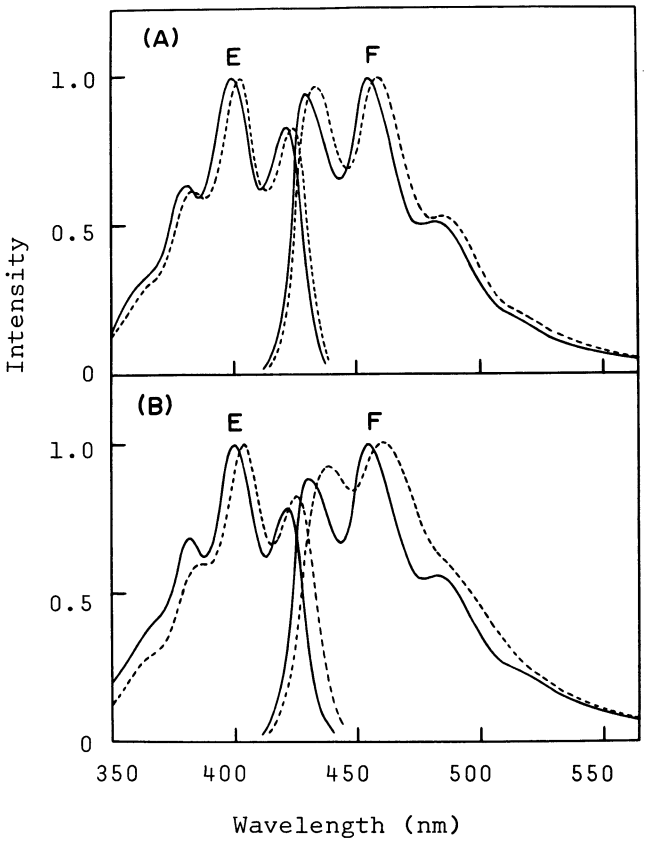

Fig. 2. Normalized fluorescence quantum (F) and FE (E) spectra of 9-AAH$^+$ in the presence of nucleotides at 25°C.
(A) —— 9-AAH$^+$ alone (4.0 x 10^{-6} M),
--- TMP (5.1 x 10^{-2} M).
(B) —— GMP (5.2 x 10^{-2} M),
--- AMP (4.0 x 10^{-2} M).

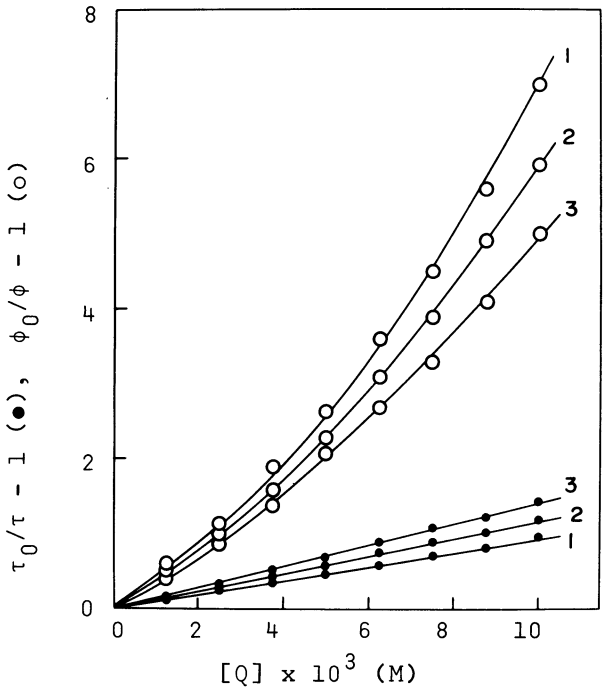

Fig. 3. Stern-Volmer plots for 9-AAH$^+$-GMP system at (1) 20°C, (2) 30°C and (3) 40°C. 9-AAH$^+$: 4.5 x 10^{-6} M. The excitation and emission wavelengths were 405 and 430 nm.

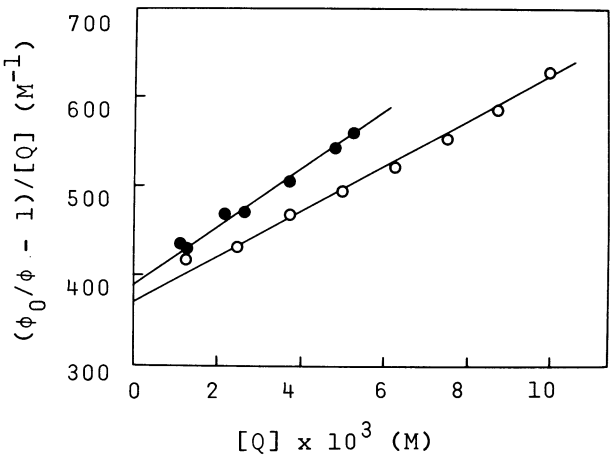

Fig. 4. The function of $(\phi_0/\phi - 1)/[Q]$ for 9-AAH$^+$-AMP (●) and 9-AAH$^+$-GMP (o) systems at 25°C.

Table 1. Fluorescence lifetimes and quantum yields of 9-AAH$^+$ in the presence of nucleotides at 25°C

Nucleotide	Concn. (M x 10^2)	τ (ns)	ϕ/ϕ_0
AMP	4.0	2.5	0.01_7
	2.5	3.7	0.03_6
GMP	5.2	2.9	0.02_6
	2.2	5.5	0.05
TMP	4.5	19.5	1.10
	2.1	18.9	1.08
CMP	4.3	19.6	1.10
	2.0	18.9	1.08

The concentration of 9-AAH$^+$ was 3.9 x 10^{-6} M.
The lifetime of 9-AAH$^+$ was 17.5 ns.

Table 2. Quenching results

Nucleotide	Temp. (°C)	$K^{a)}$ (M^{-1})	$k_1\tau_0{}^{a)}$ (M^{-1})	$k_1\tau_0{}^{b)}$ (M^{-1})	τ_0 (ns)	k_1 x 10^{-9} ($M^{-1}s^{-1}$)
GMP	20	300	90	95	17.6	5.4
	25	260	103	106	17.5	6.1
	30	220	117	117	17.5	6.7
	35	190	125	127	17.4	7.3
	40	160	138	143	17.4	8.2
AMP	25	330	132	144	17.5	8.2

a) Obtained by using Eq. 1. b) Obtained by using Eq. 2.

cules, respectively. The association constants at 25°C were found to be 250 M^{-1} for GMP, 300 M^{-1} for AMP, and about 50 M^{-1} for TMP and CMP. These values are comparable to those for the acridine orange-nucleoside[7] and proflavine-nucleotide[8,9] systems.

The presence of nucleotides markedly affected the fluorescence properties of 9-AAH$^+$. Table 1 summarizes the fluorescence lifetimes (τ) and relative fluorescence quantum yields (ϕ/ϕ_0) of 9-AAH$^+$ in the presence of an excess of nucleotides; here, ϕ_0 and ϕ are the quantum yields of 9-AAH$^+$ in the absence and presence of nucleotides. As is clearly seen in Table 1, AMP and GMP cause marked decreases in both the lifetime and the quantum yield, whereas TMP and CMP result in slight increases in both quantities. Thus, the fluorescence and FE spectra of 9-AAH$^+$ in the presence of an excess of nucleotides were examined to obtain some information on the fluorescing species. The fluorescence spectra of the 9-AAH$^+$-AMP system are strongly dependent on the concentration of AMP. They are almost identical with that of the dye alone when

[AMP] is below ca. 7 x 10^{-3} M; however, a red shift and a broadening of the fluorescence band become pronounced above this concentration (Fig. 2). All the FE spectra were the same as the corresponding absorption spectra except for the 9-AAH^+-GMP system. Figure 2 shows that the fluorescence and FE spectra of the 9-AAH^+-GMP system are superimposable on the corresponding spectra of the dye alone. These findings suggest that AMP and GMP very strongly quench the fluorescence of 9-AAH^+ and AMP is responsible for a weak fluorescence of the 9-AAH^+-AMP system.

It should be noted that a phase fluorometer gives the true lifetime only when the decay is a single exponential one. Then, the fluorescence decay curves were examined by single photon counting method; the results[10] showed that the decay law followed a single exponential function when [AMP] is below 7 x 10^{-3} M and [GMP] is below 2 x 10^{-2} M. For the other systems, where no fluorescence quenching occurs, the emission was a sum of the contributions from free and complexed dye. At a sufficiently high concentration of nucleotides, however, the emission mainly resulted from the dye complexed with nucleotides.

Stern-Volmer plots are shown in Fig. 3 for the 9-AAH^+-GMP system. The data of fluorescence quenching cover a concentration range of nucleotides where the emission obeys a single exponential decay. A plot of τ_0/τ against the concentration of GMP ([Q]) satisfies the requirements of the Stern-Volmer law, while a plot of ϕ_0/ϕ against [Q] does not; it is evident that the data deviate greatly from linearity at a higher concentration range of GMP. To explain the equation which fits the experimental data, we assume the dynamic and static quenching processes which obey the following scheme.[11,12]

$$\begin{array}{lcl} \text{dyanmic:} & A^* + Q \overset{k_1}{\rightleftharpoons} (AQ)^* & \longrightarrow \text{quenching} \\ & \uparrow\downarrow \qquad\quad \uparrow & \\ \text{static:} & A + Q \overset{K}{\rightleftharpoons} AQ & \end{array}$$

In view of findings mentioned above, it seems reasonable to conclude that (AQ)* is non-fluorescent or very weakly fluorescent in the present systems. Therefore, we obtain the following equations under photostationary conditions.[11,12]

$$(\phi_0/\phi - 1)/[Q] = K' + k_1\tau_0 + K'k_1\tau_0[Q] \qquad (1)$$

$$\tau_0/\tau - 1 = k_1\tau_0[Q] \qquad (2)$$

where

$$K' = K(\varepsilon_{AQ}/\varepsilon_A) \qquad (3)$$

and where K=[AQ]/[A][Q] is the molar association constant of the unexcited complex, and ε_A and ε_{AQ} are the molar extinction coefficients of A and AQ at the excitation wavelength.

It is predicted from Eq. 1 that the plots of $(\phi_0/\phi - 1)/[Q]$ against [Q] should give a straight line. Figure 4 shows that actual plots for the 9-AAH^+-AMP and 9-AAH^+-GMP systems at 25°C fit well into a linear relationship. The K and $k_1\tau_0$ values were determined to be 260 M^{-1} and 103 M^{-1} for the 9-AAH^+-GMP system, and 330 M^{-1} and 132 M^{-1} for the 9-AAH^+-AMP system, respectively. Each K value is in good agreement with the value obtained from the absorption spectra, and each $k_1\tau_0$ value also coincides well with the value obtained from a linear realtion between $(\tau_0/\tau - 1)$ and [Q]. The results of fluorescence quenching are summarized in Table 2.

Table 2 also lists the k_1 values calculated by using τ_0 values. These values are of the order of 5–8 x 10^9 $M^{-1}s^{-1}$, showing that the quenching process is a diffusion-controlled one.[13] According to the Einstein-Smoluchowski diffusion theory,[13] the diffusion-controlled bimolecular rate constant k_1 for the ideal case of large spherical molecules depends only on the temperature and the viscosity of the solvent,

$$k_1 = 8RT/3000\eta = A\exp(-\Delta E/RT) \quad (4)$$

where R is the gas constant, T the absolute temperature and η the viscosity of the solvent in poises. The Arrhenius plot of log k_1 against 1/T for the 9-AAH^+-GMP system gave 3.9 kcal mol^{-1} for the activation energy (ΔE) of the diffusion-controlled reaction. This value is approximately equal to 4.3 kcal mol^{-1} calculated by using Eq. 4 in the case of water.

The van't Hoff plot of log K against 1/T for the 9-AAH^+-GMP system gave $\Delta H°$=-5.1 kcal mol^{-1} and $\Delta S°$=-6.0 cal deg^{-1} mol^{-1} for the enthalpy and entropy of ground-state complex formation, respectively. These values are comparable to those for the proflavine-GMP system.[8]

In the case of proflavine which is also strongly mutagenic, only GMP quenched the fluorescence of the dye; other nucleotides resulted in the fluorescence enhancement.[8,9] Therefore, the results of the 9-AAH^+-AMP, 9-AAH^+-GMP and proflavine-GMP systems imply that the quenching of fluorescence may result from a specific interaction between dye and nucleotide molecules.

Recently, a charge-transfer mechanism has been successful in explaining the quenching of fluorescence.[14] The molecular orbital calculations[15–19] show that acridines have good electron-acceptor ability and the lower ionization potential of guanine would result in a donor character of guanine towards the dye. However, the results of 9-AAH^+ are somewhat surprising in view of the fact that AMP, which is a poorer electron donor than GMP,[18,19] quenches the fluorescence of 9-AAH^+. The higher reduction potential of 9-AAH^+ relative to proflavine indicates that 9-AAH^+ has better electron-acceptor ability.[20] On the other hand, fluorescence quenching was not observed in nucleotide solutions when one or both hydrogens of the primary amino groups of the acridine ring were substituted by alkyl groups which cause the donation of electrons to nitrogen atoms.[2,21] Further, the values of $\Delta H°$ and $\Delta S°$ for the 9-AAH^+-GMP system are to be expected for charge transfer and for hydrogen bonding interactions. In view of these findings, it seems likely that, in addition to charge transfer interactions, hydrogen bonding interactions are responsible for the quenching of fluorescence of 9-AAH^+.

Schreiber and Daune[4] found a correlation between mutagenicity and fluorescence quenching of acridine dyes when intercalated close to a GC pair; they attributed frameshift mutations to the modification of guanine residues. The present study shows that 9-AAH^+ may modify the electronic state of both adenine and guanine residues; it is possible that such a modification is related to the strong mutagenic activity of 9-AAH^+.

References

1) A. Albert, "The Acridines," 2nd ed., Arnold, London (1965), p. 493.
2) Y. Kubota, Chem. Lett., 1973, 293.
3) A. Orgel and S. Brenner, J. Mol. Biol., 3, 762 (1961).
4) J. P. Schreiber and M. P. Daune, J. Mol. Biol., 38, 487 (1974).
5) C. A. Parker and W. T. Rees, Analyst, 85, 587 (1960).
6) J. A. A. Ketelaar, C. V. de Stolpe, A. Goudsmit, and W. Dzcubas, Rec. Trav. Chim., 71, 1104 (1952).
7) G. Tomita, Biophysik, 4, 118 (1967).
8) S. Georghiou, Photochem. Photobiol., 22, 103 (1975); ibid., 24, 417 (1976).
9) Y. Kubota, to be published.
10) Y. Kubota and R. F. Steiner, unpublished results.
11) A. Weller, "Progress in Reaction Kinetics," Vol. 1, ed. by G. Porter, Pergamon Press, London (1961), p. 187.
12) J. B. Birks, "Photophysics of Aromatic Molecules," Wiley-Interscience, New York (1970), p. 441.
13) C. A. Parker, "Photoluminescence of Solutions," Elsevier, Amsterdam (1968), p. 74.
14) N. Mataga and T. Kubota, "Molecular Interactions and Electronic Spectra," Marcel Dekker, New York (1970), p. 411.
15) B. Pullman, C. R. Acad. Sci. Paris, 255, 3255 (1962).
16) J. A. Singer and W. P. Purcell, J. Med. Chem., 10, 754 (1967).
17) N. E. Sharpless and C. L. Greenblatt, Expl. Parasitol., 24, 216 (1969).
18) B. Pullman and A. Pullman, "Quantum Biochemistry," Interscience, New York (1963), p. 185.
19) C. Nagata, A. Imamura, and H. Fujita, Adv. Biophys., 4, 1 (1973).
20) B. Breyer, G. S. Buchanan, and H. Duewell, J. Chem. Soc., 1944, 360.
21) Y. Kubota, unpublished results.

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