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Sensitized Delayed Fluorescence in Frozen Aqueous Solutions of Dinucleotide- and Polyadenylic Acid-Proflavine Systems

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We have previously shown that the sensitized delayed fluorescence (SDF) in frozen aqueous solutions of DNA- and mononucleotide-acridine dye systems arises from the triplet-singlet energy transfer, in which excitation energy is transferred from the triplet state of the DNA base to the singlet state of the dye.[1] This phenomenon has also been observed in frozen aqueous solutions of dinucleotide- and polynucleotide-acridine systems. This paper will present the results on adenosine dinucleotide- and polyadenylic acid-proflavine systems.

Experimental

Adenosine monophosphate (AMP) and cytidine monophosphate (CMP) were purchased from the Sigma Chemical Co. Thymidine monophosphate (TMP), adenyl-3′: 5′-adenosine (ApA), adenyl-3′: 5′-cytidine (ApC), and polyadenylic acid (poly A) were purchased from Miles Laboratories, Inc. Proflavine (PF) was the same sample as has been described previously.[2]

The emission spectra were measured at 77°K in the same way as has been described previously.[2] The solvents used were a 0.01M phosphate buffer (pH 7.0) and an ethylene glycol-buffer mixture.[3] The concentration of nucleotide was 5×10^{-3}M (in base units); it was spectrophotometrically determined.[4,5] The concentration of PF was changed from 5×10^{-6} to 2×10^{-4}M. Unless otherwise stated, all the luminescence data presented in this paper were obtained by an excitation at 280 nm.

Results and Discussion

ApA- and Poly A-PF Systems. Figures 1 and 2 show the total and delayed emission spectra of the ApA- and poly A-PF systems respectively. In the

1) Y. Kubota, This Bulletin, **43**, 3126 (1970).

2) Y. Kubota, *ibid.*, **43**, 3121 (1970).

3) This solvent contains 1% of ethylene glycol by volume and will hereinafter be abbreviated as EGW.

4) M. M. Warshaw and I. Tinoco, Jr., *J. Mol. Biol.*, **13**, 54 (1965).

5) D. N. Holcomb and I. Tinoco, Jr., *Biopolymers*, **3**, 121 (1965).

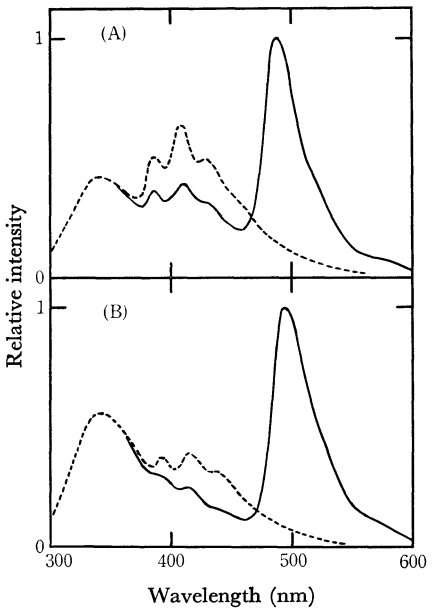

Fig. 1. Total emission spectra of (A) ApA-PF and (B) poly A-PF systems in frozen EGW solutions.
----: in the absence of PF
——: in the presence of PF
Nucleotide: 5×10^{-3}M in adenine residue. PF: 5×10^{-5}M.

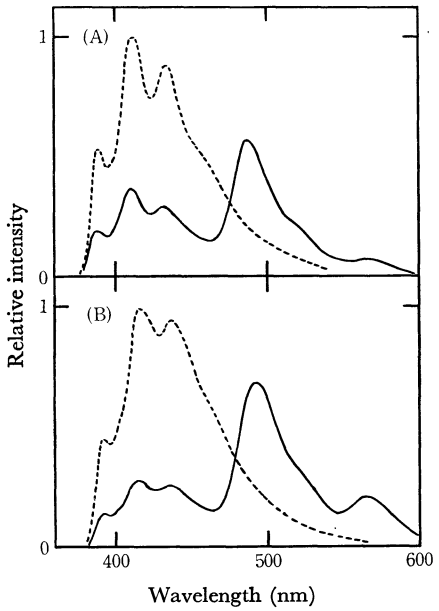

Fig. 2. Delayed emission spectra of (A) ApA-PF and (B) poly A-PF systems in frozen EGW solutions.
----: in the absence of PF
——: in the presence of PF
Nucleotide: 5×10^{-3}M in adenine residue. PF: 5×10^{-5}M.

absence of PF, the band centered at 340 nm is ascribed to the adenine fluorescence, and the emission at 390—500 nm, to the adenine phosphorescence.[6] In the presence of PF, the delayed emissions are composed of adenine phosphorescence, SDF (490 nm), and the phosphorescence (570 nm) of PF. It may be seen from Fig. 1 that the adenine phosphorescence is selectively quenched in the presence of PF. Further, the excitation spectrum of SDF is the same as that of the adenine phosphorescence. As has been interpreted previously in connection with mononucleotide-PF systems,[1] it may be concluded that this SDF is caused by the energy transfer from the triplet state of the adenine residue to the singlet state of PF.

Next, the phosphorescence efficiency of the adenine residue, $\phi_P{}^D$, can be estimated from the energy-transfer data. $\phi_P{}^D$ is given by the following equation:[7,8]

$$\phi_P{}^D = \frac{\Phi_P}{\Phi_{SDF}} \cdot \frac{\phi_{TR}}{1-\phi_{TR}} \Phi_F{}^A$$

where Φ_P is the quantum yield of the adenine phosphorescence in the presence of PF; Φ_{SDF}, the quantum yield of SDF; ϕ_{TR}, the efficiency of energy transfer,[9] and $\Phi_F{}^A$, the quantum yield of the PF fluorescence.[10] From our data, the $\phi_P{}^D$ values of AMP, ApA, and poly A were estimated to be 0.28, 0.22, and 0.16 respectively. These values are not extremely accurate because of some sources of error;[7] however, the value of AMP can be compared with the value of 0.3 calculated from the data of Guéron *et al.*[12] It may be considered, therefore, that the values presented here are reasonable and that the $\phi_P{}^D$ value of poly A is somewhat smaller than that of AMP.

ApC-PF Systems. Recently, triplet-triplet energy transfer between nucleotide molecules has been found to occur in dinucleotides or in mixtures of nucleotide molecules.[13-15] In order to obtain some information on the relation between this energy transfer and SDF, we also examined the ApC-PF, (AMP+CMP)-PF, and (AMP+TMP)-PF systems. In the case of ApC or mixtures of mononucleotides, the phosphorescence intensity of adenine lowers remarkably compared with that of AMP alone, while the phosphorescence intensity of cytosine or thymine increases; this phenomenon may be ascribed to the occurrence of the triplet-triplet energy transfer. In parallel with this phenomenon, the decay of SDF in the case of ApC or mixtures becomes faster than that in the case of AMP alone (Fig. 3). As may be seen from Fig. 3, all the decays of SDF deviate from single exponentials. It is, therefore, impossible to determine meaningful rate constants as reciprocals of the decay times of SDF. For convenience,

6) T. Montenay-Garestier, C. Hélene, and A. M. Michelson, *Biochim. Biophys. Acta*, **182**, 342 (1969).

7) R. G. Bennett, R. P. Schwenker, and R. E. Kellogg, *J. Chem. Phys.*, **41**, 3040 (1964).

8) W. C. Galley and L. Stryer, *Biochemistry*, **8**, 1831 (1969).

9) ϕ_{TR} can be estimated from the measurements of the ratio of the phosphorescence to the fluorescence of adenine in the presence of PF, (I_P/I_F), and in the absence of PF, $(I_P/I_F)_0$:

$$\phi_{TR} = 1 - \frac{(I_P/I_F)}{(I_P/I_F)_0}$$

10) $\Phi_F{}^A$ in nucleotide solutions was determined to be 0.37 by using 9-aminoacridine as the standard of the quantum yield.[11]

11) G. Weber and F. W. J. Teale, *Trans. Faraday Soc.*, **53**, 646 (1957).

12) M. Guéron, J. Eisinger, and R. G. Shulman, *J. Chem. Phys.*, **47**, 4077 (1967).

13) C. Hélene, *Biochem. Biophys. Res. Commun.*, **22**, 237 (1966).

14) C. Hélene, P. Douzou, and A. M. Michelson, *Proc. Natl. Acad. Sci. U. S.*, **55**, 376 (1916).

15) M. Guéron, R. G. Shulman, and J. Eisinger, *ibid.*, **56**, 814 (1966).

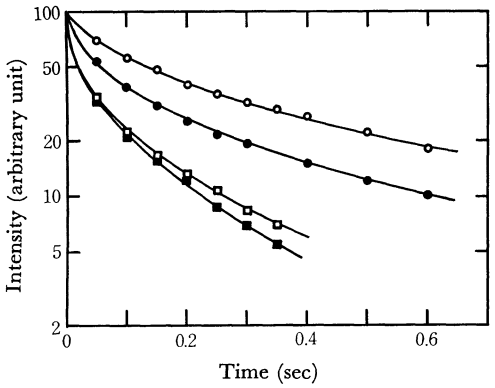

Fig. 3. Decay curves of SDF.
○: AMP-PF ■: CMP-PF ●: (AMP+CMP)-PF
□: ApC-PF Total nucleotide: 5×10^{-3}M in base unit
PF: 5×10^{-5}M

we adopt the half-life, $\tau_{1/2}$, as a qualitative measure of rate constants. As may be noticed from Table 1, the $\tau_{1/2}$ values in the cases of the mononucleotide-PF systems depend on the phosphorescence lifetimes of nucleotides, τ_P, and are about one tenth of τ_P. Consequently, the $\tau_{1/2}$ values will tell us which nucleotide is important as the energy donor in the case of ApC or mixtures. The $\tau_{1/2}$ value in the case of ApC or mixtures is comparable to that in the case of CMP or TMP (Table 1). This indicates that the triplet state of CMP or TMP plays an important role; that is, first, a base-to-base energy transfer produces the triplet state of CMP or TMP, and next, the triplet energy is transferred to the singlet state of PF. This

Table 1. Decays of nucleotide phosphorescence (τ_P) and SDF ($\tau_{1/2}$) in frozen aqueous solutions

Nucleotide	τ_P (sec)		$\tau_{1/2}$ (msec)
AMP	2.32		135[a]
CMP	0.40		30[a]
TMP	0.20		20[a]
AMP+CMP (1:1)	non-exponential		
	fast (0.4)	60%	60
	slow (2.2)	40%	
AMP+TMP (1:1)	non-exponential		
	fast (0.2)	90%	24
	slow (1.8)	10%	
ApC	non-exponential		
	fast (0.4)	85%	35
	slow (2.3)	15%	
ApA	2.28		42
Poly A	non-exponential		
	fast (0.5)	25%	38
	slow (2.2)	75%	

Total nucleotide: 5×10^{-3} M in base units. PF: 5×10^{-5} M.
a) Ref. 1.

conclusion confirms our previous interpretation of SDF in DNA-acridine complexes.[1] On the other hand, the $\tau_{1/2}$ values in the cases of ApA and poly A are much smaller than that in the case of AMP; this phenomenon probably results from the difference in conformation between the monomer and the polymers of adenylic acid.[4,5]

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