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Luminescence in DNA-Acridine Dye Complexes. II. Sensitized Delayed Fluorescence in the Complexes

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When frozen aqueous solutions of DNA-acridine dye complexes or nucleotide-dye mixtures were excited by ultraviolet light, a sensitized delayed fluorescence of the dye was observed. This delayed fluorescence was interpreted by means of a mechanism in which the excitation energy is transferred from the triplet state of the nucleotides to the first excited singlet state of the dye. The efficiency and decays of the sensitized delayed fluorescence revealed that the energy transfer depends greatly on the separation between the energy donor and acceptor and that donor-acceptor pairs spaced very closely are important in the energy transfer. In DNA-acridine dye complexes, the thymine triplet seems to play the most important role as an energy donor. The decrease in the delayed fluorescence on the denaturation of DNA indicates that the energy transfer is sensitive to the conformation of the macromolecule.

In the preceding paper,[1] the luminescence properties of acridine dyes upon the excitation of DNA-acridine dye complexes by visible light have been reported. According to Lerman's intercalation model,[2] in which the dye molecules are in close proximity with purine-pyrimidine base pairs in the double-stranded helix, the electronic excitation-energy transfer between DNA bases and dye molecules may be expected to occur efficiently. Recently, three types of energy transfer, singlet-singlet,[3] triplet-singlet,[4] and triplet-triplet,[5] have been shown to occur in the complexes; DNA bases served as the energy donors, and the bound dye, as the energy acceptor. In order to obtain further information on the energy transfer between DNA bases and dye molecules, our investigations have now been extended to the luminescence properties of the complexes with excitation in the region of DNA absorption. A sensitized delayed fluorescence was observed for various acridine dyes in frozen aqueous solutions of the complexes;[6,7] this was interpreted in terms of energy transfer from the triplet state of the DNA bases to the singlet state of the dye, as was first suggested by Isenberg *et al.*[4] Further, a sensitized delayed fluorescence due to the triplet-singlet energy transfer has also been observed in frozen aqueous solutions of the nucleoside-, nucleotide-, and polynucleotide-acridine dye systems.[8] This paper will present the details of our further investigation of the DNA-proflavine and nucleotide-proflavine systems.

Experimental

Materials. Calf thymus DNA (Type I) and yeast RNA (Type XI) were obtained from the Sigma Chemical Co. The nucleotides*1 were purchased from either Sigma or Miles Laboratories, Inc. These materials were generally used without further treatment. The proflavine was the same as that described in Part I.[1]

Measurements. The emission and absorption measurements were described in Part I.[1] The measurements were carried out at 77°K in frozen aqueous solutions (0.01M phosphate buffer, pH 7.0). No correction of the emission and excitation spectra was made. Unless otherwise stated, all the luminescence data presented

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2) L. S. Lerman, *J. Mol. Biol.*, **3**, 18 (1961); *Proc. Natl. Acad. Sci. U. S.*, **49**, 94 (1963).
3) G. Weill and M. Calvin, *Biopolymers*, **1**, 401 (1963).
4) I. Isenberg, R. B. Leslie, S. L. Baird, Jr., R. Rosenbluth and R. Bersohn, *Proc. Natl. Acad. Sci. U. S.*, **52**, 379 (1964).
5) W. C. Galley, *Biochemistry*, **6**, 1279 (1968).
6) Y. Kubota, Y. Fujisaki and M. Miura, *Kogyo Kagaku Zasshi*, **72**, 252 (1969).
7) Y. Kubota, Y. Fujisaki and M. Miura, This Bulletin, **42**, 853 (1969).
8) Y. Kubota, to be published.

*1 The 5′-monophosphates of adenosine, guanosine, cytidine, thymidine, and uridine are abbreviated as AMP, GMP, CMP, TMP, and UMP respectively.

in this paper were obtained by means of the excitation of the complexes or nucleotide-dye mixtures by 280 nm; the total nucleotide concentration was 5×10^{-3}M in phosphate units, and the proflavine concentration, 5×10^{-5}M. In general, the maximum intensity of the sensitized delayed fluorescence was obtained at a dye concentration of *ca.* 5×10^{-5}M.

Results and Discussion

Figure 1 shows the delayed emission spectra of the DNA-proflavine complex in a frozen aqueous solution. With an increase in the dye concentration, the DNA phosphorescence diminishes and two emissions appear; one, having a spectrum identical with the dye fluorescence, is identified as a delayed fluorescence of the dye, and the other, peaking at *ca.* 570 nm, as a phosphorescence of the dye.

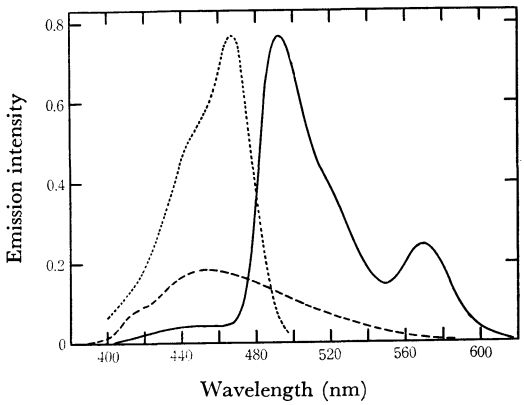

Fig. 1. Delayed emission spectra of DNA (----) and the DNA-proflavine complex (———) and absorption spectrum of the complex (--------). The scale of ordinates is the same on Figs. 1 and 5.

As is shown in Fig. 2, the excitation spectrum of the delayed fluorescence is not similar to that of the dye fluorescence or the phosphorescence, but it is identical with that of the DNA phosphorescence. Therefore, it may be concluded that the excitation of the DNA phosphorescence is responsible for the production of the delayed fluorescence; hereafter, this will be called a *sensitized delayed fluorescence.* The intensity of the sensitized delayed fluorescence is proportional to the intensity of the exciting light. Furthermore, the decay of the DNA phosphorescence is remarkably affected by the addition of the dye; it becomes faster than in the absence of the dye (see Fig. 3). This means that an additional pathway is introduced for the decay of the DNA triplet. In view of these results, it is reasonable to assume that the sensitized delayed fluorescence arises from the triplet-singlet energy transfer,[4] in which the

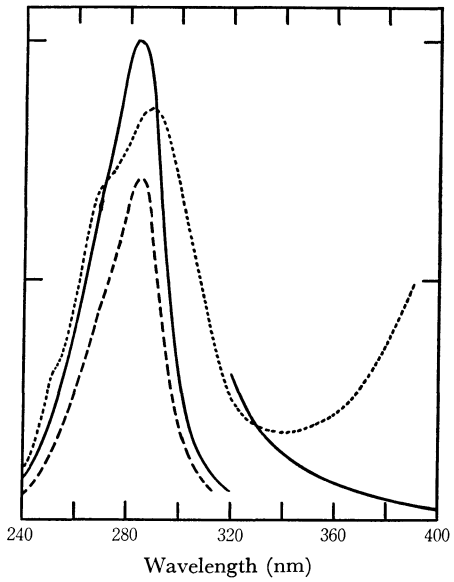

Fig. 2. Excitation spectra of delayed emissions of the DNA-proflavine complex.
----- DNA phosphorescence
——— Sensitized delayed fluoresncne
-------- Proflavine fluoresence or phosphorescence

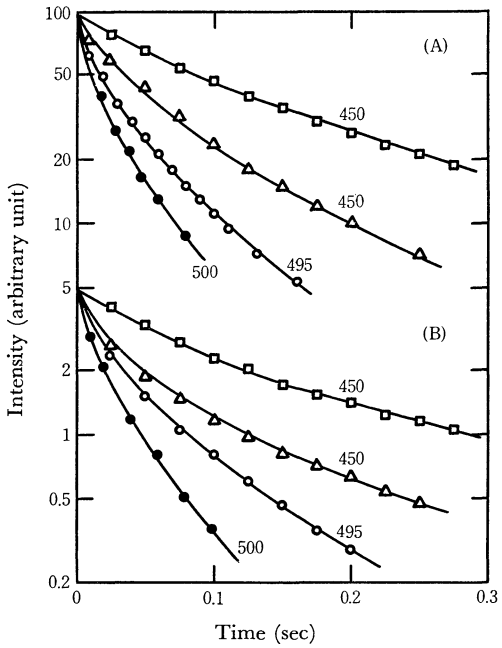

Fig. 3. The decay curves of the delayed emissions of (A) native DNA-proflavine and (B) denatured DNA-proflavine complexes. Each figure represents the wavelength at which the decay was measured.
DNA phosphorescence in the absence (□) and in the presence (△) of proflavine (5×10^{-5}M), sensitized delayed fluorescence of proflavine (○: 5×10^{-5}M, ●: 10^{-4}M)

DNA bases are the triplet donors and the bound dye is the singlet acceptor.

The phenomenon of the sensitized delayed fluorescence was also found in the cases of denatured DNA and RNA. In both cases, the intensity of the sensitized delayed fluorescence greatly decreased with a decrease in the P/D value, the ratio of the number of nucleotides to that of dye molecules. Figure 4 shows that the ratio of the intensity of the

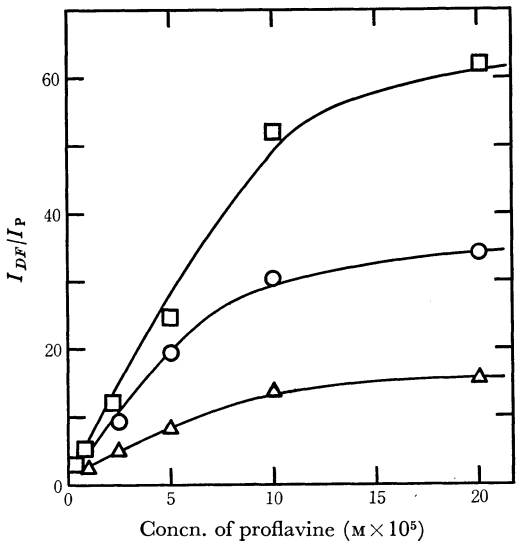

Fig. 4. The ratio of the intensity of sensitized delayed fluorescence to that of donor phosphorescence (I_{DF}/I_P) *vs.* dye concentration. Both intensities are corrected for the spectral sensitivity of the detector.
□, native DNA-proflavine; ○, denatured DNA-proflavine △, RNA-proflavine

sensitized delayed fluorescence to that of the donor phosphorescence (I_{DF}/I_P) increases with an increase in the dye concentration, and then reaches a limiting value. In the case of denatured DNA or RNA, this value is smaller than that in the native DNA. In other words, the energy in the denatured DNA cannot be efficiently transferred to the bound dye in spite of a considerable enhancement of the DNA phosphorescence on denaturation.[9] This means that the energy transfer is sensitive to the conformation of the macromolecular complex; that is, the double helical structure is essential for the energy transfer to occur efficiently.

Next, our investigations were extended to the luminescence properties of the nucleotide-dye systems in order to ascertain how the DNA constituent bases contribute to the energy transfer. Some typical results are shown in Fig. 5. The sensitized delayed fluorescence was also observed in frozen

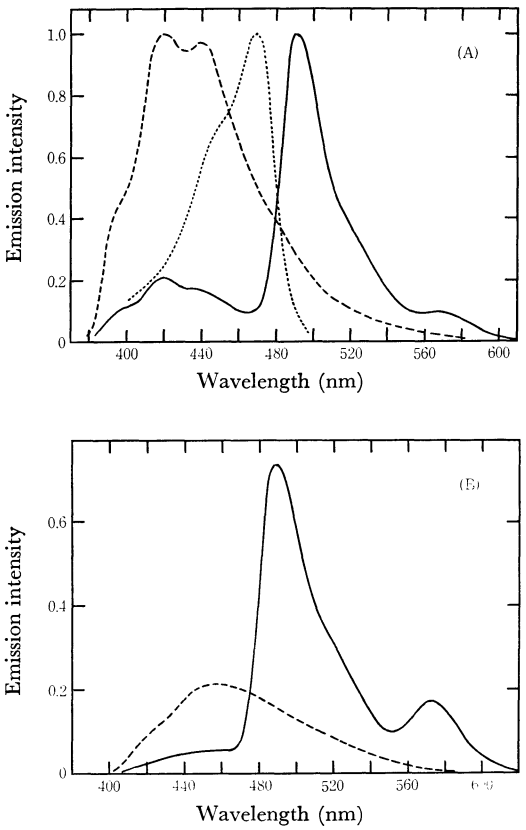

Fig. 5. Delayed emission spectra of (A) AMP-proflavine and (B) TMP-proflavine systems.
------ Nucleotide phosphorescence in the absence of proflavine
——— Delayed emissions in the presence of proflavine
-------- Absorption spectrum of proflavine in the presence of nucleotide

aqueous solutions of nucleotide-proflavine systems. This delayed fluorescence can also be interpreted in terms of the triplet-singlet energy transfer on the basis of the following observations: (1) the excitation spectrum of the sensitized delayed fluorescence is identical with the phosphorescence-excitation spectrum of each nucleotide, (2) the intensity of the sensitized delayed fluorescence varies linearly with the intensity of the exciting light, (3) the addition of the dye greatly quenches the phosphorescence of nucleotide, but has little effect on the fluorescence, and (4) the initial decay of the nucleotide phosphorescence becomes rapid upon the addition of the dye (see Fig. 6) and becomes more and more faster as the dye concentration increases. Furthermore, it is expected that the transfer proceeds with a high probability, since the dye absorption well overlaps with the phosphorescence spectrum of each nucleotide (Fig. 5).[10]

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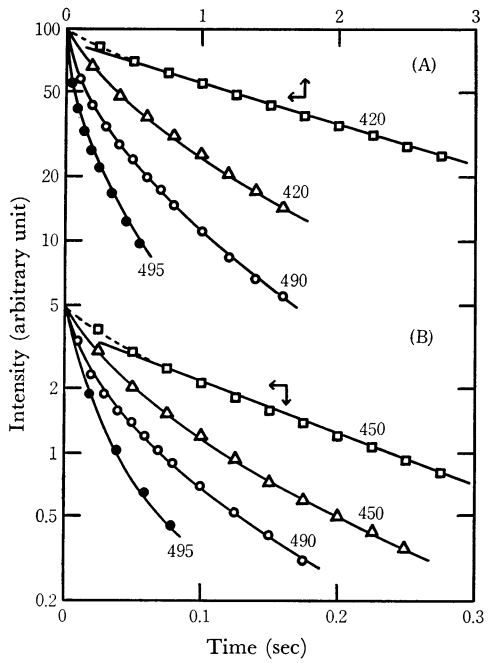

Fig. 6. The decay curves of delayed emissions from (A) AMP-proflavine and (B) TMP-proflavine systems. Each figure represents the wavelength at which the decay was meausred.
Nucleotide phosphorescence in the absence (□) and in the presence (△) of proflavine (5×10^{-5}M), sensitized delayed fluorescence of proflavine (○: 5×10^{-5}M, ●: 10^{-4}M)

As Figs. 3 and 6 show, the decays of both the nucleotide phosphorescence in the presence of the dye and the sensitized delayed fluorescence deviate remarkably from simple exponentials; they decay rapidly at the first stage, and then gradually become slower. The rapid decay at the first stage reflects the strong interaction between the energy donor and acceptor, located very close together. Further, in the frozen aqueous solutions, relatively low dye concentrations (below 5×10^{-6}M) are needed to observe the sensitized delayed fluorescence. On the other hand, the addition of alcohols before freezing the nucleotide-dye solutions resulted in a marked decrease in the sensitized delayed fluorescence despite the observation of a considerable enhancement in the nucleotide phosphorescence. However, the DNA-proflavine complexes exhibited a considerable sensitized delayed fluorescence, even in an alcohol-water mixed solvent. In the freezing of an aqueous solution, nucleotide molecules are considered to be excluded from the ice crystal and to form aggregates.[11] Proflavine also forms aggregates in the absence of a nucleotide, while it exists as the monomeric species in its presence (Fig. 5). Therefore, it seems likely that dye and nucleotide molecules form the stacked aggregates in which dye molecules are placed between nucleotide molecules by the face-to-face arrangement as well as at room temperature.[12,13] The decrease in the sensitized delayed fluorescence upon the addition of alcohols may be ascribed to the destruction of the aggregates. In conclusion, these results suggest that (1) the energy transfer depends greatly on the separation between the energy donor and acceptor; (2) most of donor and acceptor molecules are not distributed uniformly, but are spaced very closely; (3) the energy transfer takes place with a high efficiency between such molecules, and (4) the intercalation is most favorable for the efficient transfer.

The decay behavior of sensitized delayed fluorescence was independent of the kind of acridine dye (proflavine, acridine orange, acriflavine, *etc.*); that is, it appears that the rate constant of energy transfer is almost the same for these dyes. A meaningful transfer rate cannot be obtained because of the non-exponentiality of the decay, but the half-life ($\tau_{1/2}$) will serve as a qualitative measure of the transfer rate. In Table 1, the $\tau_{1/2}$ values are summarized, together with the lifetimes of the nucleotide phosphorescence (τ_P). It may be seen from Table 1 that $\tau_{1/2}$, which is dependent on τ_P, is about one tenth of τ_P, and that $\tau_{1/2}$ in the case of DNA is almost the same as that in the case of TMP. The phosphorescence spectrum of DNA resembles that of TMP. Its decay is composed of two exponen-

Table 1. Decays of nucleotide phosphorescence and sensitized delayed fluorescence of proflavine

Nucleotide	τ_P (sec)	$\tau_{1/2}$ (msec)
AMP	2.30*	135
GMP	1.15*	110
CMP	0.4*	30
UMP	0.3*	25
TMP	0.2*	20
native DNA	non-exponential	
	{fast (0.18) 95% slow (1.6) 5%	18
denatured DNA	non-exponential	
	{fast (0.18) 90% slow (1.7) 10%	23
RNA	non-exponential	34

Nucleotide: 5×10^{-3}M in phosphate unit
Proflavine: 5×10^{-5}M
* Slightly deviates from the exponentiality at the initial stage.

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tials; the major component is the fast one, which has the same lifetime as that of TMP. Therefore, the triplet in DNA appears to reside mainly at the thymine residue; this is consistent with the recent observations by the optical and ESR measurements.[14,15] This phenomenon seems to be achieved by energy transfer to thymine from other bases; this is possible, since thymine has the lowest triplet.[11,16] Hence, the data of the sensitized delayed fluorescence in the complexes can tentatively be interpreted as follows: first, the base-to-base energy transfer produced the thymine triplet, and then a part of the excitation energy is efficiently transferred to the dye placed closest to it.

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