Delayed Fluorescence of the DNA-Acridine Dye Complexes in a Frozen Aqueous Solution

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In order to obtain further information regarding the energy transfer between the DNA base-dye molecules and between dye-dye molecules in the DNA-acridine dye complexes, the luminescence of the complexes was measured under various conditions by using a Hitachi MPF-2A fluorescence spectrophotometer with an attachment for phosphorescence measurements.[1]

Figures 1 and 2 show typical results obtained for the DNA-proflavine complexes. In the presence of proflavine, the DNA phosphorescence with a peak about 460 mμ diminishes and two new peaks appear; the emissions around 580 mμ and 495 mμ may be ascribed, respectively, to the phosphorescence (P) and delayed fluorescence (DF) of the dye bound to DNA.

The DF observed at a high P/D[*1] value seems to be due to the excitation energy transfer from the DNA triplet state to the dye singlet state, as was first suggested by Isenberg *et al.*,[2] in view of the facts that its excitation spectrum is identical with that of the DNA phosphorescence, that the absorption spectra of the complexes overlap to a great extent with the phosphorescence spectrum of DNA, and that the intensity of DF varies proportionally to the intensities of P of DNA and the dye. The intensity of DF decreases with a decrease in the P/D value; this decrease is especially remarkable in the case of denatured DNA.

On the other hand, the DF at a low P/D value appears to be a result of the triplet-triplet annihilation of the dyes, for its excitation spectrum is identical with that of the P of the dye and the intensity of DF is proportinal to the square of the intensity of the P of the dye.[3,4] This type of DF, newly observed in the DNA-dye complexes, becomes remarkable with a decrease in the P/D value. The DF at neither high nor low P/D values decays exponentially. The values of the half-life of DF, an order of 10—60 msec, depend on the P/D value and on the kind of acridine dye. A similar phenomenon of DF was also observed in the cases of acridine orange, acridine yellow, and acriflavine, except that DF due to the photoionization of the dye was additionally observed in the DNA-acriflavine complexes.[5] Further study of the mechanism of DF is in progress.

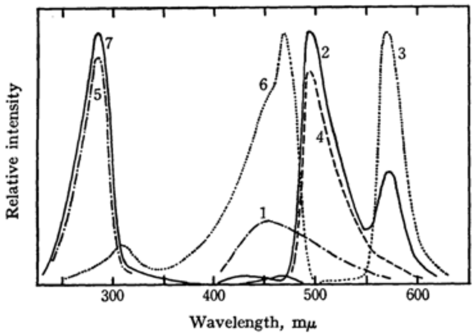

Fig. 1. Excitation (ex.) and emission (em.) spectra of DNA-proflavine complex at 77°K.
Solvent: 0.01 м phosphate buffer + 0.01 м NaCl (pH 7)
DNA: 10^{-2} м in phosphate unit
Proflavine: 10^{-4} м
(1) DNA phosphorescence; long-lived em. spectra of complex excited at (2) 285 mμ and (3) 470 mμ; (4) total em. spectrum of complex; ex. spectra of (5) DNA phosphorescence, (6) phosphorescence of complex, and (7) delayed fluorescence of complx

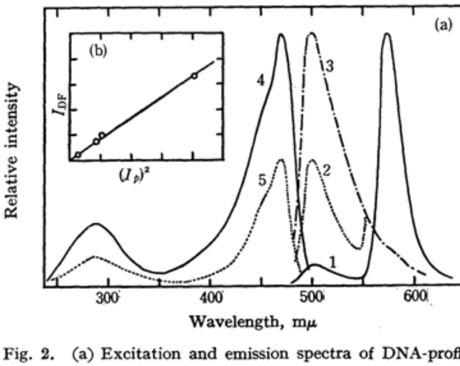

Fig. 2. (a) Excitation and emission spectra of DNA-proflavine complex at 77°K.
DNA: 2.5 × 10^{-4} м in phosphate unit Proflavine: 10^{-4} м
(1) Long-lived em. spectrum of complex excited at 470 mμ; (2) a sextuple sensitivity of (1); (3) total em. spectrum of complex;ex. spectra of (4) phosphorescence and (5) delayed fluorescence of complex
(b) A relationship between I_P and I_{DF}.
I_P : the intensity of phosphorescence at 580 mμ
I_{DF}: the intensity of delayed fluorescence at 500 mμ

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[*1] The ratio of the number of nucleotides to that of dye molecules.

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