Photochemistry of the DNA-Acridine Dye Complexes

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Studies of the DNA-acridine dye complexes from the photochemical viewpoint are of great interest in relation to the strong mutagenic activity and photodynamic action of acridine dyes, since the interaction between π-electrons of DNA bases and those of dyes may be expected to occur according to Lerman's intercalation model.[1]

In this study, ESR measurements were made after the visible-light irradiation of the DNA-acridine dye complexes in various frozen solutions at 77°K. Figure 1 shows the results on the DNA-proflavine complex. The spectrum at $g=2$ is identified as being due to a thymine free radical by comparing with the results obtained after the γ-ray- or UV-irradiation of thymine and DNA[2,3] (*cf.* Fig. 2). A doublet signal with a splitting constant of 505 gauss is ascribed to the hydrogen atom. The results obtained were independent of the kind of acridine dyes (acridine orange, proflavine, and acriflavine). This phenomenon seems general in the DNA-acridine dye complexes.[4] It is very significant that the same results were obtained in the case of thymine and in that of DNA-acridine dye complexes, as may be seen in Figs. 1 and 2.

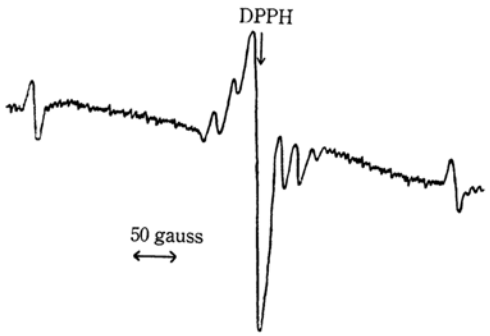

Fig. 1. ESR spectra after visible-light irradiation of DNA-proflavine complex in phosphate buffer (pH 7) for 4 hr at 77°K.

Irradiation source: 750 W tungsten lamp with a 1-cm filter of 10% $CuSO_4$ aqueous solution
Proflavine: 5×10^{-4} M
DNA: 2.5×10^{-2} M in phosphate unit

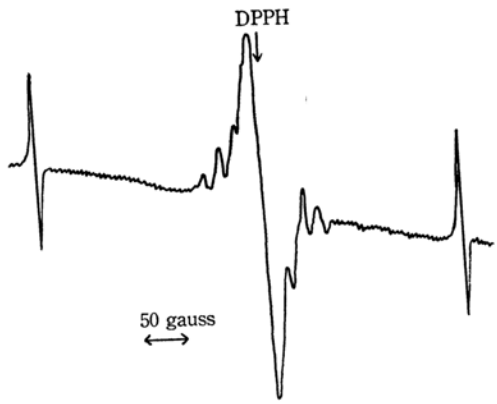

Fig. 2. ESR spectra after UV irradiation (2537 Å) of thymine for 1.5 hr at 77°K.

On the other hand, when a small amount (below 5% by volume) of an alcohol (methanol, ethanol, *etc.*) is added to an aqueous solution of the DNA-acridine dye complexes, or when a water-alcohol mixture (1 : 1 by volume) is used as the solvent, the radicals due to the photosensitized decomposition of alcohols are observed instead of the thymine free radical and the hydrogen atom. In the absence of DNA, however, acridine dyes only slightly or scarcely at all decompose alcohol molecules. In view of the bond energy of alcohols, it is probable that the excited triplet states of dyes with a higher energy by the two-photon process (triplet-triplet absorption) participate in the radical formation, and that, furthermore, the bound acridine dyes must be activated more than free dyes. This was confirmed by the fact that the rate of radical formation is nearly proportional to the square of the light intensity and that it increases remarkably with an increase in the ratio of nucleotide to dye. This activation seems likely to be related to the formation of the thymine free radical, to the photosensitized decomposition of alcohols, and also to the photodynamic and photomutagenic action of acridine dyes.

Further details will be published in the near future.

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3) J. Eisinger and R. G. Shulman, *Proc. Natl. Acad. Sic. U. S.*, **50**, 694 (1963).
4) Very recently Delmelle and Duchesne (*C. R. Acad. Sci., Paris, Ser. D*, **264**, 138 (1967)) have reported the formation of thymine free radical in the DNA-acriflavine system. However, they missed observation of hydrogn atom because they measured at 150°K.