

LIGHT-INDUCED FREE RADICALS IN DNA-ACRIDINE COMPLEXES STUDIED BY ESR

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1. Introduction

When frozen solutions of complexes of DNA and certain organic dyes such as acridines are illuminated, paramagnetism can be observed [1,2]. As the mutagenic and growth-inhibiting activities of these dyes are enhanced by the presence of light [3–6], it is a reasonable hypothesis that the paramagnetic species formed under illumination are involved in these increased activities. Indeed, a rough parallelism has been pointed out between the induction of free radicals in bacteriophages exposed to various dyes and light and both the rate of phage inactivation and the occurrence of mutations [7,8]. Energy transfer from the dyes to the DNA has been proposed as the mechanism for the formation of the free radicals [2].

Acridines have shown to bind in at least two different modes to DNA [8,9]. At low dye concentration a monomeric binding form is observed, while at high concentration an associated binding form predominates. The biologically active form is apparently the monomeric one [10].

In the present study we have investigated the radical yield in DNA-acridine complexes as a function of the density of dye molecules on the DNA chain (expressed as the P/D ratio). We have found a correlation between radical formation and the monomeric binding form. Furthermore an attempt was made to determine to what extent the radicals are preferentially formed in regions of specific base composition by comparing qualitatively and quantitatively the results obtained with DNA and poly dAT. Although not conclusive,

our results may imply that mainly the A-T base pairs are involved in the radical formation.

2. Materials and methods

Experiments were performed with acridine orange (AO) or proflavine (PF) as the dye moiety. *Acridine orange* (3,6-dimethylamino-acridinium-chlorion) was precipitated as the free base, purified from concomitant $ZnCl_2$ by chromatography on Al_2O_3 with chloroform as solvent [11], and finally transformed into the hydrochloride. *Proflavine* (3,6-aminoacridine) was obtained as the hemi-sulphate (British Drug House Co). DNA was a sample of highly polymerized calf thymus DNA from Worthington Chemical Co. (Batch 7LB). *poly dAT* was a generous gift of Dr. T.Jovin, Max-Planck-Institut für Physikalische Chemie, Göttingen, Germany. The samples were dissolved in 1.5 mM NaCl – 0.15 mM Na-citrate, pH 7.

ESR measurements were made at 77°K with a Varian X-band spectrometer, Model V-4500, equipped with 100 kc field modulation and a 12-in. magnet. ESR spectra were obtained on an xy-recorder and on punched paper tape after digitizing in a CAT computer. The microwave bridge was modified, enabling us to work at microwave power low enough (< 30 uW) to avoid saturation in the samples investigated.

The samples, in 3 mm quartz tubes, were generally illuminated at 77°K directly in the microwave cavity (Varian Multipurpose Cavity, Model V-4531) by an Osram 1600 W Xenon lamp, focused on the sample

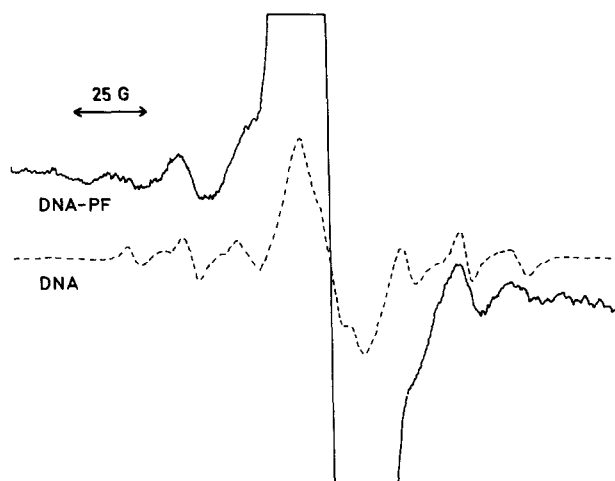


Fig. 1. The wing structure of an ESR spectrum of illuminated DNA-PF complex of P/D = 80 and PF concentration = 2.5×10^{-4} M, compared with a spectrum of slightly annealed γ -irradiated pure DNA. Temperature = 77°K.

by a simple lens system. Optical filters in 1 cm glass cuvettes were used to screen off the undesired parts of the lamp spectrum: H₂O for absorption of the infrared radiation, and a solution of anthracene in toluene (circa 0.1 g/l) for absorption of ultraviolet radiation below 380 nm.

Double integrations of the ESR spectra were performed on an IBM 360-75 computer.

3. Results

Illumination of DNA-PF and DNA-AO frozen solutions gave ESR spectra consisting of a singlet line with a linewidth of about 20 G, in accordance with the results reported by Delmelle et al. [1,2]. The growth of the ESR signal was approximately exponential. Illuminated dyes alone gave relatively weak signals of about the same linewidth, whereas pure DNA gave no detectable signal.

In the wings of the ESR spectra a weak sub-structure is observed (fig. 1, cf. also ref. [1]), which with high probability originates from a free radical normally formed upon γ -, X-ray, or UV-irradiation of DNA by hydrogen addition to the thymine base. However, little is known about the sites of the unpaired electrons that give rise to the main unresolved ESR signal.

The ESR spectra from illuminated poly dAT-PF samples are qualitatively about the same as those from the DNA-PF samples (fig. 2). The wing substructure is, however, somewhat more pronounced in the spectra from the poly dAT samples.

Series of quantitative measurements were made on samples with constant dye concentrations but with varying P/D ratios for the complexes DNA-AO and DNA-PF. In each series all samples were put into matched quartz tubes, illuminated for 6 min and investigated during the same day. Double integral values of the recorded first derivative ESR lines were taken as relative measures of the concentration of free radicals.

In both complexes the double integral values increase as a function of the P/D ratio in the range investigated (fig. 3). The radical yield is significantly higher in the PF samples than in the AO ones.

An attempt was also made to compare the amount of free radicals formed in poly dAT-PF and DNA-PF samples investigated in parallel. Although the experimental errors are considerable, the radical yield in the dAT samples seems somewhat larger than in the DNA samples in the P/D range investigated (fig. 4).

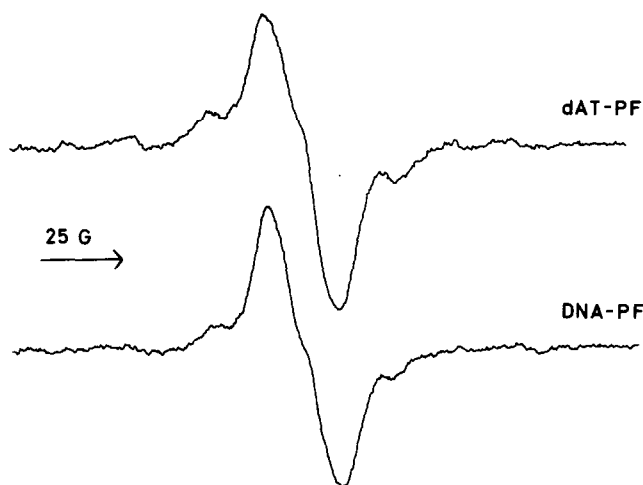


Fig. 2. ESR spectra of illuminated poly dAT-PF and DNA-PF complexes. In both samples $P/D = 20$, PF concentration = 1.0×10^{-4} M and temperature = 77°K .

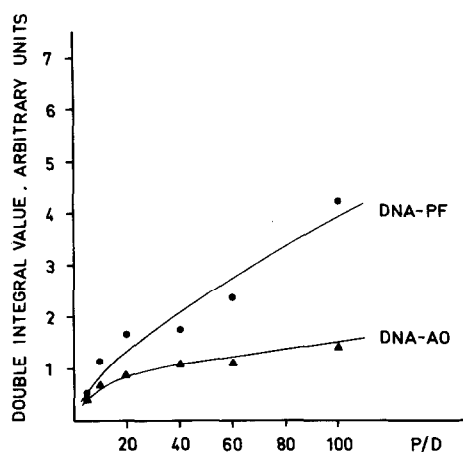


Fig. 3. Double integral values of ESR spectra of illuminated DNA-PF and DNA-AO complexes as functions of P/D . In all samples the dye concentration = 2.0×10^{-4} M and temperature = 77°K .

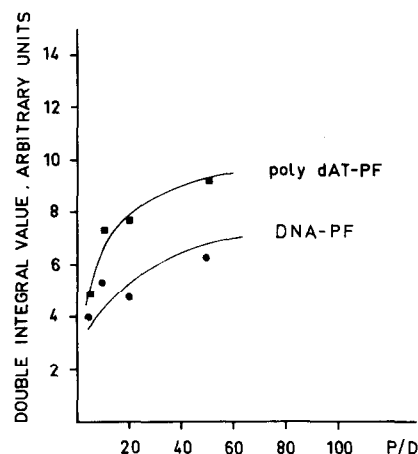


Fig. 4. Double integral values of ESR spectra of illuminated poly dAT-PF and parallel DNA-PF complexes as functions of P/D . PF concentration = 5×10^{-5} M, except for the $P/D = 50$ samples, where PF concentration = 2×10^{-5} M. The two $P/D = 50$ values have been corrected to 5×10^{-5} M PF concentration using a standard curve obtained with varying dye concentration. Temperature = 77°K .

4. Discussion

The increase in the light-induced radical formation with P/D (fig. 4) indicates that the monomeric dye binding form, which is particularly prominent at the higher P/D ratios used, is the cause of a high yield of free radicals. This monomeric binding form might not

simply depend on the dye concentration on the DNA chain. It might also represent a condition where the dye molecules are bound at particular sites, which by their steric arrangement (e.g. intercalation) may prevent interaction between neighbouring dye molecules.

Furthermore, the increased radical yield in poly dAT complexes compared with DNA complexes implies that these particular sites are present in both nucleic acids, but are more frequent in poly dAT.

Although no definite evidence exists at present, these results are consistent with a model, which assumes that the special binding sites involved in the formation of free radicals upon illumination are mainly situated in the A-T rich regions of DNA, and that the radicals formed are associated with these base pairs. This concept is also supported by data, which show that acriflavine (which is closely related to PF) preferentially binds to the A-T rich regions of DNA [11], and investigations [12,13], which have been interpreted in terms of a preferential intercalation of dyes between subsequent A-T base pairs.

If intercalation of the dyes is a necessary condition for the radical formation in the DNA-dye complexes cannot be conclusively deduced from our data. However, the result that AO, in which bulky methyl groups attached to the planar ring structure might render intercalation more difficult, could point into this direction.

In conclusion, the data presented here indicate that photosensibilization of DNA by acridines results in the formation of free radicals, which seem to originate at particular binding sites in DNA (possibly in the A-T rich regions). The formation and further reactions of these free radicals might lead to changes in the electronic structure of certain bases (e.g. thymine) and thus in the base-pairing properties. This obviously could be one reason for the mutations induced by acridines in the presence of light.

Acknowledgement

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