INTER-STRAND CROSS-LINKAGES OCCURRING IN THE PHOTOREACTION BETWEEN PSORALEN AND DNA

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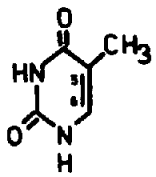
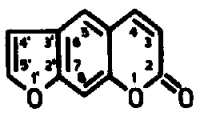
1. Introduction

The various biological photosensitizing properties exerted by some furocoumarins or psoralens when irradiated with long wavelength ultraviolet light [1–3], appear to be explained by the photoreaction of these compounds with nucleic acids [14]. In this photoreaction, a C_4-cyclo-addition of furocoumarins to the 5,6-double bond of pyrimidine bases (thymine, cytosine, uracil) takes place. Furocoumarins can photoreact either with their 4′,5′- [5] or with their 3,4-double bond [6, 7]; consequently, two types of photoadducts



thymine



psoralen

are known, both deriving from one molecule of furocoumarin and one pyridimidine base; they have been obtained by irradiation of psoralen and simple pyrimidine bases as well as by hydrolysis of DNA after irradiation in the presence of psoralen [8].

The present paper gives evidence that psoralen added to a solution of native DNA and irradiated at 365 nm can react both with the 3,4- and 4′,5′-double bond, forming photoadducts deriving from one molecule of psoralen and two pyrimidine bases. Behaving therefore as a bifunctional reagent, psoralen can form cross-linkages between the two strands of DNA.

2. Materials and methods

Calf-thymus DNA highly polymerized (Mann Research Laboratories, New York), having a hypochromicity higher than 37% was used. ^{3}H-Psoralen and ^{3}H-4′,5′-dihydropsoralen were prepared in this laboratory [9]; specific activities: 4.5×10^8 and 7.11×10^9 dpm/mmole respectively.

For determining the fluorescence, 0.5 ml of the samples was diluted with 2 ml of 0.1 M phosphate buffer, pH 7.0, and examined in an Aminco Bowman spectrophotofluorimeter.

The radioactivity measurements were performed using a LS-150 Beckman liquid scintillation spectrometer: 0.2 ml of the samples was diluted with 1 ml of water and added to 10 ml of dioxane base scintillator (PPO 4 g, POPOP 0.075 g, naphthalene 120 g, dioxane up to 1000 ml).

For irradiation, 2 ml of the samples was placed in glass calibrated tubes 1.2 cm in diameter, immersed in a cell with glass walls, in which thermostatically controlled water (22°) circulated [10]. The irradiation was made with two HPW 125 Philips lamps, which emit almost exclusively at 365 nm, placed on both sides of the cell at a distance of 3.5 cm. The total incident radiation on the 2 ml of the solutions was equivalent to 2.9×10^{16} quanta/sec.

3. Results and discussion

A first indication that furocoumarins can photoreact both with the 3,4- and 4′,5′-double bond was found by studying the fluorescence of DNA after irradiation in the presence of furocoumarins.

In previous papers, it has been reported [5–7] that photoadducts furocoumarin-pyrimidine base in which the 4′,5′-double bond is involved, have a brilliant violet fluorescence when observed at 365 nm light, while photoadducts in which the 3,4-double bond of furocoumarin is involved are not fluorescent.

We have also reported that DNA irradiated at 365 nm in aqueous solution in the presence of a labelled furocouramin, and then precipitated with ethanol, showed both radioactivity and violet fluorescence [10, 11]. While radioactivity increased by increasing the period of irradiation, fluorescence in contrast showed a peculair behaviour; generally it increased in the first period of irradiation, but then it remained almost constant or decreased.

This behaviour has not hitherto been explained. We have thus performed the following experiment: ten samples of 2 ml of a 0.05% aqueous solution of DNA containing 2 mM NaCl and 10 μg/ml of ^{3}H-psoralen were irradiated for 10 min. From the collected samples, DNA was precipitated with ethanol as described in previous papers [10, 11]; excess of psoralen was thus removed. On the basis of radioactivity measurements, this DNA contained covalently bound 4.02 μg of psoralen per mg of DNA, corresponding to 1 molecule of psoralen for every 136 nucleotides (P atoms). This DNA-psoralen combination was dissolved in water and samples of this solution were reirradiated at 365 nm for different times. After irradiation, DNA of each sample was reprecipitated, redissolved in water and both its radioactivity and its fluorescence were measured.

The results in fig. 1 show that while radioactivity remained constant, fluorescence decreased rapidly, especially in the first period of irradiation. These results can be explained assuming that, in the first irradiation, a number of psoralen molecules link themselves to the pyrimidine bases forming fluorescent 4′,5′-photoadducts. By reirradiation, these psoralen molecules photoreact again with other pyrimidine bases engaging also their 3,4-double bond. In such a way, photoadducts are formed containing one psoralen molecule and two pyrimidine bases. Although this new type of photoadduct has not yet isolated, we can as-

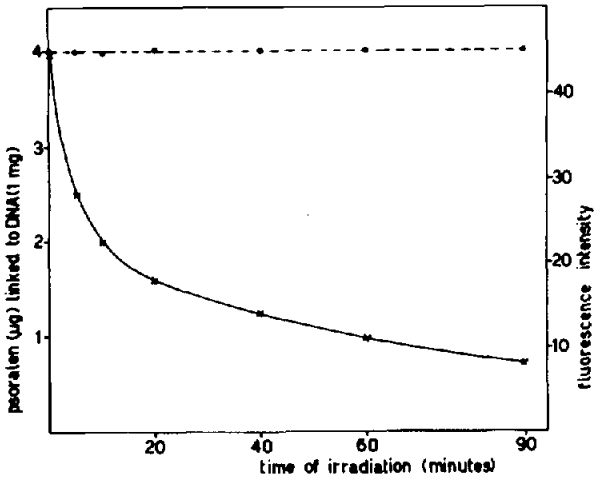


Fig. 1. Results obtained by irradiating at 365 nm a photoadduct DNA-^{3}H-psoralen: - - - - - amount of ^{3}H-psoralen linked to DNA (μg/mg DNA); —— fluorescence intensity (activating wavelength: 330 nm; maximum fluorescent wavelength: 400 nm).

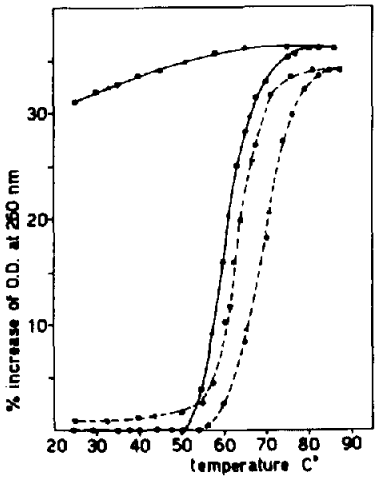


Fig. 2. Optical densities of aqueous 2 mM NaCl solutions of DNA 0.05% containing 10 μg/ml of psoralen by increasing and subsequently by decreasing the temperature: —— non-irradiated solution; - - - -solution irradiated 10 min at 365 nm.

sume that it is not fluorescent on the basis of the properties of the 3,4-photoadducts [6,]7.

Moreover, it is known that when a furocoumarin is added to an aqueous solution of DNA, a molecular complex is formed (without any irradiation) in which very weak bonds are involved [12]. Various properties of these complexes suggest than an intercalation of the planar furocoumarine molecules takes place between the planes formed by the bases in double helix DNA structure. Therefore it appeared possible that the two pyrimidine bases linked to the psoralen molecule belong to two different strands of DNA; in such a way cross-linkages can be formed between the two strands.

To investigate this possibility, we have examined the denaturation and the renaturation capacity of DNA before and after irradiation in the presence of psoralen, operating in the experimental conditions used for the determination of T_m. We previously reported [13] that the T_m value of DNA increased after irradiation in the presence of furocoumarins. We have determined the optical density of an aqueous 2 mM NaCl solution of DNA at various increasing temperatures (CF_4 Optica spectrophotometer with quartz cuvettes having an optical path of 1 mm) according to Marmur and Doty [14]. After the temperature was reached at which the optical density remained constant, we gradually decreased the temperature of the solution, again determining the optical density. The results obtained with non-irradiated DNA and with DNA irridiated in the presence of psoralen are shown in fig. 2. Non-irradiated DNA underwent denaturation with increasing temperature and when the temperature was decreased, it renatured only to a small degree. In contrast, DNA irradiated in the presence of psoralen underwent denaturation at a higher temperature (higher T_m value) and with decreasing temperature, gradually underwent almost complete renaturation.

To explain this different behaviour, we may assume that in non-irradiated DNA, after denaturation the two strands can easily separate and therefore renaturation does not easily occur; after irradiation, when some cross-linkages are present, the two strands can swell by heating and therefore partial denaturation also occurs but the two strands cannot completely separate and therefore, by decreasing the temperature, renaturation can occur easily.

In conclusion, these results show that psoralen, irradiated at 365 nm, can photoreact both with the 4′,5′- and the 3,4-double bond, thus behaving as a bifunctional reagent. When it photoreacts with native DNA, cross-linkages are possible between the two strands of the macromolecule.

In addition, previous results obtained with light-scattering measurements [15], which showed no evident changes in molecular weight and in DNA conformation after irradiation in the presence of psoralen, indicate that no cross-linkages occur between two separate double-stranded DNA molecules.

Acknowledgement

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