STRUCTURE SPECIFICITY OF POLYDEOXYRIBONUCLEOTIDES FOR THE PHOTOREACTION WITH PSORALEN

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1. Introduction

Psoralen and other furocoumarins photoreact with pyrimidine bases of DNA, bot *in vitro* and *in vivo* [1–8], under irradiation at long wavelength ultraviolet light. A C_4-cycloaddition to the 5,6-double bond of pyrimidine bases occurs: furocoumarins can photoreact either with their 3,4- or 4′,5′-double bond, forming therefore two types of photoadducts. Moreover, behaving as bifunctional agents, they can form adducts, in which one furocoumarin molecule is linked with two pyrimidine bases; in this case an inter-stand cross-linking is formed in native DNA [9–11]. It has been shown by Cole [10] and Chandra et al. [12–14] that the formation of inter-strand crosslinking is responsible for the photodynamic damage to biological systems by psoralen. The intercalation of a furocoumarin molecule between the planes of two base pairs of native DNA is strongly affected by substitutions in the furocoumarin molecule.

At this point the question arises if the intercalation and the subsequent photoaddition of the furocoumarin molecules occur randomly along the DNA molecule, or, alternatively, if there are in DNA some specific regions which are preferred or prohibited for the furocoumarins. For the first approach to clarify this problem, we have studied the possibility of interaction between psoralen and two polydeoxyribonucleotides of known regular repeating sequences, that is poly(dA-dT) and poly(dA·dT).

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2. Materials and methods

Calf thymus DNA was purchased from Schwartz/Mainn, Orangeburg, New York. Its hypochromicity, determined according to Marmur and Doty [15], was more than 40%. Polydeoxyribonucleotides and triphosphates were supplied by Boehringer Mannheim, Tutzing, Germany. *E. coli* K12 cells (mid-log phase) were obtained from Miles Laboratories Ltd., England.

Psoralen was extracted from fig leaves and tritiated, as described earlier [16]; its specific activity was 5.4×10^6 dpm/μmole.

2.1. *Irradiation procedure*

Portions of 2 ml of aqueous solutions containing 0.01 M NaCl, 40 μg/ml of the polydeoxyribonucleotide, or native DNA and 3 μg/ml of labeled psoralen were irradiated into calibrated glass tubes immersed in a thermostatically controlled apparatus at 22°C, by means of two HPW 125 Philips lamps, which emit almost exclusively at 365 nm. The total radiation intensity incident on 2 ml of the solution corresponded to 2.9×10^{16} quanta/sec, determined by means of a chemical actinometer [17]. The polymers used in the RNA-polymerase assay system were irradiated in a similar manner, but contained 5 μg/ml of unlabelled psoralen; the total irradiation time was 8 min.

2.2. *Binding of [^{3}H]psoralen to polymers*

The various samples of the polynucleotides and of native DNA, after irradiation in the presence of labelled psoralen, were gel-filtered through a Sephadex G-25 column (1.5 × 21 cm). The elution of the column was carried out with 0.01 M NaCl and the rate flow was 15 drops per minute; fractions of 70 drops were collected, and absorbance and radioactivity was determined. Under these conditions the macromolecules containing linked psoralen were constantly eluted in the 5th fraction while the unbound psoralen appeared in fractions 20–23.

2.3. *RNA-polymerase reaction*

RNA-polymerase was isolated from *E. coli* K12 cells according to the procedure by Burgess [18] and stored in the buffer containing 50% glycerol at −20°C. The reaction mixture contained in 0.25 ml, 0.04 M Tris, pH 7.9; 0.01 M $MgCl_2$; 0.1 mM EDTA; 0.1 mM dithiothreitol; 0.15 M KCl, 0.15 mM UTP, CTP, GTP and [^{3}H]ATP (2 μCi) and 0.15 mg/ml of the template. The reaction was started with 15 μg enzyme protein and incubations were carried out for 20 min at 37°C. In case of synthetic polymers CTP and GTP were omitted from the reaction mixture.

2.4. *Radioactive measurements*

Radioactivity measurements were performed using a 3375 Packard liquid scintillation spectrometer, employing a dioxane, or toluene base scintillator. The apparatus efficiency in the experimental conditions used was in the range 25–37% for counting tritium.

3. Results and discussion

The formation of molecular complexes between psoralen and the two polydeoxyribonucleotides has been studied by means of the spectrophotometric methods [19]. The results obtained by determining the optical densities of aqueous solutions of psoralen alone and in the presence of polydeoxyribonucleotides (or, for a comparison, in the presence of native DNA) are reported in table 1.

As the decrease of the absorbing property of psoralen is correlated with the formation of a molecular complex, we can see that the complex with poly d(A−T) is formed even to a greater extent than with DNA,

Table 1

Absorbance at 330 nm of aqueous solutions of psoralen (10 μg/ml) alone or in the presence of poly d(A·T), poly d(A−T) and of calf thymus native DNA (500 μg/ml).

Polydeoxyribonucleotides	*A* at 330 nm	Δ *A* %
None	0.215	–
Poly d(A·T)	0.208	3.25
Calf-thymus DNA (native)	0.194	9.76
Poly d(A−T)	0.180	16.27

while with poly d(A·T) it takes place to a very reduced extent. The binding of psoralen to the polydeoxyribonucleotides under irradiation in aqueous solution at 365 nm is reported in fig. 1. The much higher rate of the photoreaction with poly d(A−T) than with poly d(A·T) is evident.

This so different behaviour both in the formation of the complex and in the photoreaction is very probably due to the different conformation of the two polydeoxyribonucleotides. In fact Langridge [20] has demonstrated by X-ray diffraction methods that

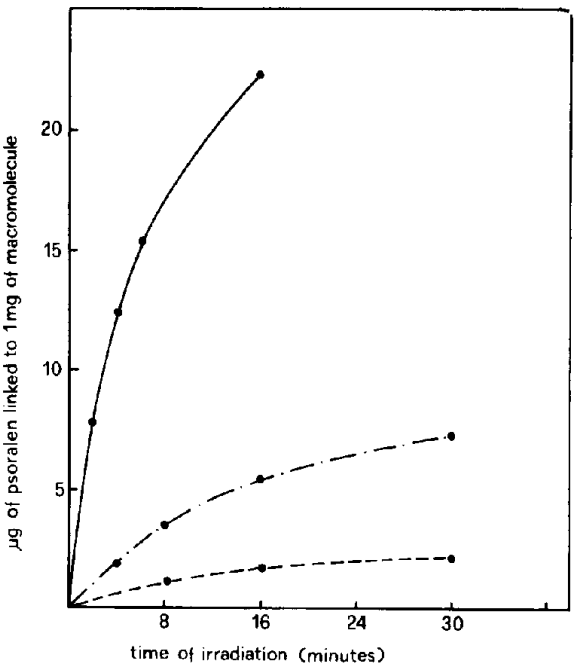

Fig. 1. Photoreactivity (365 nm) of psoralen with poly d(A−T) ●—●—●; poly d(A·T) ●– –●– –●; and native calf-thymus DNA (●———●).

Table 2
Template activities of DNA and polydeoxyribonucleotides after psoralen treatment in the DNA-dependent RNA polymerase reaction.

System	[^{3}H]AMP incorporation into RNA (% of control) in the presence of			
	Denatured DNA	Native DNA	Poly d(A−T)	Poly d(A·T)
Controls (without psoralen)	100	100	100	100
+Psoralen (without irradiation)	96	78	67	94
+Psoralen and light (365 nm)	63	56	6	92

For experimental details see Materials and methods.

poly d(A−T) has a conformation practically identical to that of native DNA, while poly d(A·T), like those polydeoxyribonucleotides which are also base-paired but contain only pyrimidines on one strand and only purines on the other, gives X-ray diffraction patterns which differ from those of native DNA, indicating a different conformation [21].

We have also examined the formation of cross-linkings between the two strands of each of the polydeoxyribonucleotides by determining their absorbance–temperature profiles before and after irradiation in the presence of psoralen. These studies have shown a very high amount of cross-linking of psoralen in poly d(A−T), whereas no cross-linking occured with the polymer poly d(A·T). The results of these studies will be published in detail elsewhere [22].

In earlier studies [23], examining various furocoumarin derivatives, a correlation was found between the ability of various derivatives to bind to native DNA and the provoked decrease of the template efficiency of the same DNA in RNA-polymerase reaction. As reported in table 2, the irradiation at 365 nm of the two polydeoxyribonucleotides in the presence of psoralen has a very different effect on their template activities in the RNA–polymerase reaction. In fact, while the activity of poly d(A·T) is pracically not inhibited, the activity of poly d(A−T) is rather completely lost. The activities of native calf-thymus DNA and of denatured DNA, irradiated and tested under similar experimental conditions, are decreased to an intermediate degree. These results are very closely correlated with those of the photochemical interaction.

4. Conclusion

The results now obtained clearly show that really the structure and the conformation of the polydeoxyribonucleotide chains may have an influence on the possibility of psoralen to intercalate in DNA and to photoreact with the pyrimidine bases. In particular, we can say that those regions of DNA in which the base sequence is analogous, to that of poly d(A·T) are 'prohibited' for the photoreaction of psoralen. By contrast, a situation analogous to that of poly d(A−T) appears to be highly favourable for the photobinding of psoralen and for the formation of cross-linkings.

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