FORMATION OF INTER-STRAND CROSS-LINKINGS ON DNA OF GUINEA PIG SKIN AFTER APPLICATION OF PSORALEN AND IRRADIATION AT 365 nm

F. Dall'AQUA, S. MARCIANI, D. VEDALDI and G. RODIGHIERO

Istituto di Chimica farmaceutica, Cattedra di Chimica farmaceutica applicata dell'Università di Padova, Via F. Marzolo 5, 35100 Padova, Italy

Received 8 September 1972

1. Introduction

It is well known that furocoumarins (psoralens) are able to bind *in vitro* to native DNA under irradiation at 365 nm [1–2]. A C_4-cyclo-addition to the 5,6-double bond of the pyrimidine bases takes place [3–5]. As furocoumarins have two photoreactive double bonds, they can give both monofunctional additions (involving either their 3,4- or 4′, 5′-double bonds) and bifunctional additions. In the last case inter-strand cross-linkings are formed in native DNA.

This photobinding ability appears to be tightly connected with the photosensitizing properties that furocoumarins exert on human and guinea-pig skin and on other biological substrates [6]. Recently, Pathak and Krämer [7] found that after application of labelled 4, 5′, 8-trimethyl-psoralen on guinea-pig skin and irradiation at 365 nm, the DNA extracted from the skin was radioactive. In the present study, we demonstrate that psoralen (the parent linear furocoumarin) not only photobinds to DNA of the guinea-pig skin, but also forms inter-strand cross-linkings, behaving therefore *in vivo* in a way strictly analogous as *in vitro*.

2. Materials and methods

Psoralen: the tritiated compound [8] was used, with specific activity 8.7×10^9 dpm/mmole.

Radioactivity determinations were carried out using a Packard 3375 Model liquid scintillation spectrometer, with a dioxane base scintillator (4 g POP, 0.075 g POPOP, 120 g naphthalene, dioxane up to 1000 ml).

Irradiation of guinea pig skin and extraction of DNA: the abdomens of three seven-months old guinea pigs (4 × 10 cm) were accurately depilated with scissors and uniformly treated with an alcoholic solution of [^{3}H] psoralen (50 μg/cm^2). The animals were then immobilised and their abdomens irradiated for 90 min at 365 nm using Philips HPW 125 lamps (1.25×10^{16} quanta/sec/cm^2). Soon after irradiation, the treated skin was removed under anaesthesia, minutely triturated with scissors and homogenized in the cold by means of a Wirtis Model 23 apparatus. Epidermal DNA was isolated following the method of Szybalska and Szybalski [9]; all dialysis and lyophilization steps, however, were avoided using an Amicon Diaflo SM 50 membrane.

Column chromatographic separations: the separation of double stranded DNA from single stranded nucleic acids (RNA or denatured DNA) was carried out by means of a hydroxylapatite chromatographic column (0.7 × 4 cm), using a linear gradient of 0.05–0.3 M phosphate buffer (pH 6.98) and collecting fractions of 3.5 ml.

3. Results and discussion

In a typical experiment, 0.966 mg of native DNA,

North-Holland Publishing Company – Amsterdam

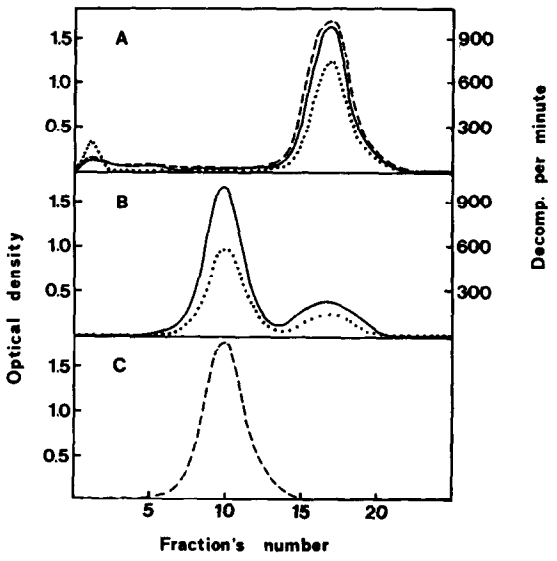

Fig. 1. Chromatographic behaviour on hydroxylapatite column. (——) O.D. of the fractions of DNA extracted from guinea pig skin after treatment with psoralen and irradiation at 365 nm; (·····) radioactivity of the same fractions. (– – – –) O.D. of the fractions of DNA extracted from untreated guinea pig skin. Part A) before heat denaturation; parts B and C) after heat denaturation and quenching in ice.

completely purified from RNA by means of hydroxylapatite column chromatography, has been isolated from guinea pig skin after irradiation in the presence of tritiated psoralen. Its total amount of radioactivity was 2,515 dpm, corresponding to a binding of one psoralen molecule every 9,700 nucleotides of the DNA (see fig. 1).

This result confirms what was previously reported by Pathak and Krämer [7] on the ability of 4,5′,8-trimethyl-psoralen to photobind to DNA also *in vivo*.

To investigate on the existence of cross-linkings in this DNA, we have examined its behaviour on hydroxylapatite column chromatography before and after heat-denaturation. Native DNA was eluted as a single component, as shown in fig. 1, part A; its behaviour was identical to that of a sample of DNA extracted from untreated guinea pig skin.

However, after heating the aqueous solutions in a boiling water bath for 5 min, then quenching in ice and keeping there for 20 min, while the control DNA had the behaviour shown in fig. 1, part C (it was eluted at a lower ionic strength, being completely in the single stranded state), the DNA extracted from the irradiated skin had the behaviour shown in fig. 1, part B: while the main part was eluted at the ionic strength corresponding to the single stranded DNA, a small fraction (about 22%) is eluted at the gradient value corresponding to the double stranded DNA. Therefore, in DNA extracted from the treated guinea pig skin is present a fraction which is able to renature after heat denaturation. It is known that renaturing ability is a characteristic property of cross-linked DNA, and was clearly evidenced also in our preceding studies [10–12].

For a confirmation, we have brought together the eluted fractions corresponding to double helix DNA and, after desalting, we have determined the optical density–temperature profiles by increasing and then by decreasing the temperature. The results showed that, by cooling, a rather complete renaturation occurs, after the initial heat denaturation (fig. 2).

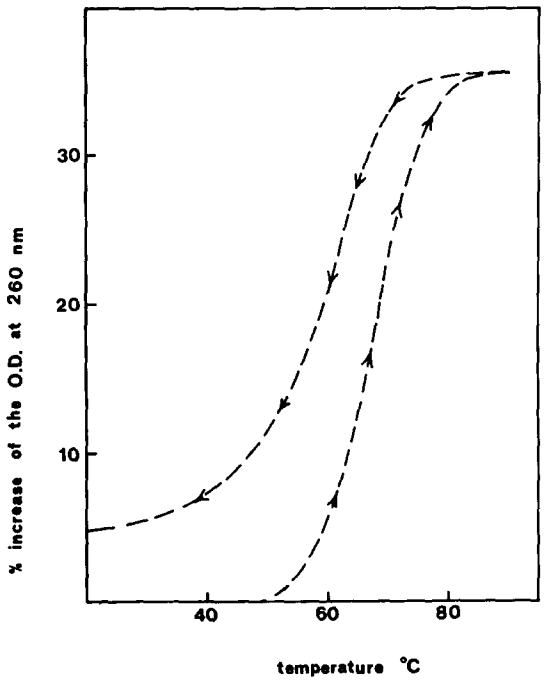

Fig. 2. Optical density variations of the aqueous solution of the double stranded fraction isolated from heat-denatured DNA (see fig. 1, part A) by increasing and subsequently by decreasing the temperature.

These results clearly show that psoralen not only is able to photobind to DNA in guinea pig skin, but it forms also cross-linkings. Very probably cross-linking formation plays a role in determining the effects which follow irradiation of the skin in the presence of furocoumarins.

References

[1] L. Musajo, G. Rodighiero and F. Dall'Acqua, Experientia (Basel) 21 (1965) 24.
[2] L. Musajo, G. Rodighiero, A. Breccia, F. Dall'Acqua and G. Malesani, Photochem. Photobiol. 5 (1966) 739.
[3] C.H. Krauch, D.M. Krämer and A. Waker, Photochem. Photobiol. 6 (1967) 341.
[4] L. Musajo, F. Bordin, G. Caporale, S. Marciani and G. Rigatti, Photochem. Photobiol. 6 (1967) 711.
[5] L. Musajo, F. Bordin and R. Bevilacqua, Photochem. Photobiol. 6 (1967) 927.
[6] G. Rodighiero, L. Musajo, F. Dall'Acqua, S. Marciani, G. Caporale and L. Ciavatta, Biochim. Biophys. Acta 217 (1970) 40.
[7] M.A. Pathak and D.M. Krämer, Biochim. Biophys. Acta 195 (1969) 197.
[8] S. Marciani, F. Dall'Acqua and C. Colombini, Ann. Chim. Rome 59 (1969) 1067.
[9] E.H. Szybalska and W. Szybalski, Proc. Natl. Acad. Sci. U.S. 48 (1962) 2026.
[10] F. Dall'Acqua, S. Marciani and G. Rodighiero, FEBS Letters 9 (1970) 121.
[11] F. Dall'Acqua, S. Marciani, L. Ciavatta and G. Rodighiero, Z. Naturforsch. 26b (1971) 561.
[12] S. Marciani, M. Terbojevic and F. Dall'Acqua, Z. Naturforsch. 27b (1972) 197.