Fluorescent and non fluorescent C₄-cycloadducts in the photoreaction at 365 nm between psoralen-³H and DNA

F. BORDIN, L. MUSAJO, and R. BEVILACQUA
Istituto di Chimica Farmaceutica of University of Padua


In the present paper we have studied quantitatively the formation of these two classes of photoadducts, by using uniformly tritium-labelled psoralen (4.5·10⁶ dpm/mM) and native DNA.

Methods

The conditions of experiment are very close to those which we have previously followed.

Aqueous solutions (1.5 ml) at 0.15% of DNA (extracted from salmon sperm, highly polymerised, Calbiochem, Los Angeles; P = 8.21%, N/P = 1.78) containing 20 μg/ml of psoralen-³H have been irradiated at 22 °C (HPW 125 Philips lamps, 365 nm; intensity of irradiation 2.92·10¹⁵ quanta/cm²/sec); after irradiation sodium chloride was added up to a concentration of 1 M. DNA was precipitated with two volumes of ethanol, centrifuged, washed with 70% ethanol and air dried. The samples were then hydrolysed with 0.5 ml of 5 N hydrochloric acid (60° at 100 °C).

In these conditions the pyrimidine bases involved in the cycloaddition are freed quantitatively, because of the higher sensitivity to acids of the glucoside linkage of 5,6-dihydropyrimidines. After hydrolysing the entire radioactivity linked to DNA has been proved liable to dialysis. By chromatography only photoadducts with pyrimidine bases have been found and none with nucleosides or nucleotides.

1) The hydrochloric acid does not modify the fluorescent photoadduct psoralen-thymine as was proved by examination of u.v. and fluorescence spectra determined on a standard crystalline substance, while the photocompound psoralen-cytosine is subject to deamina-

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tion, as was already known yielding the corresponding adduct psoralen-uracil. The behaviour of these substances permits therefore easy identification and their quantitative recovery.

2) The instability of non fluorescent photoadducts however does not affect their determination. The radioactivity of the furocoumarin moiety of the molecule has been proved regainable also after heating with 5 N hydrochloric acid.

A non fluorescent photoadduct between psoralen and thymine had already been isolated by mild hydrolysis with 0.4 N hydrochloric acid from DNA irradiated in the presence of psoralen: in paper chromatography (Schleicher and Schüll 2043-b, water as solvent) it showed \( R_F 0.80 \).

After hydrolysis of DNA irradiated in the presence of psoralen-3H by heating at 100° for 60' with 5 N hydrochloric acid, no radioactivity was detectable at \( R_F 0.80 \); but on the other hand, a radioactive non fluorescent spot was formed at \( R_F 0.34 \).

We have ascertained that, when a sample of pure non fluorescent photoadduct obtained by irradiation of psoralen-3H and thymine which had an \( R_F 0.80 \) in paper chromatography (solvent water) was heated at 100° for 60' with 5 N hydrochloric acid, by successive paper chromatography no radioactive spot was obtained at \( R_F 0.80 \), but the entire radioactivity of the labelled photoadduct was recovered at \( R_F 0.34 \).

Therefore we have assumed that the radioactivity measurements of this decomposition product can provide useful data for the determination of non fluorescent photoadduct.

The DNA hydrolysates, obtained as indicated above, were further placed on Schleicher and Schüll 2043-b chromatographic paper, and developed with water.

In the chromatograms at 365 nm a spot was visible with violet fluorescence at \( R_F 0.60 \) due to the psoralen-thymine and psoralen-uracil photoadducts (the last resulting, as above mentioned, from the deamination of psoralen-cytosine). The chromatograms have been cut into uniform strips which have been quantitatively eluted with absolute ethanol; 3 ml of these solutions mixed with 3 ml of scintillating solution (5 g of 2,5 diphenyloxazol and 0.5 g of 2.2'-p-phenyl-bis-5 phenyloxazol in 1000 ml of toluene) were used for the radioactivity measurements with a liquid scintillation counter (SELO-Milano). Corresponding quantities were used for the determination of the fluorescence of the eluates (\( \lambda \) fluoresc.400 nm, \( \lambda \) exc. 330 nm) using an Aminco-Bowman spectrophotofluorimeter, with an XY Aminco recorder.

**Results**

Fig. 1 shows the results obtained in one of these experiments, as follows:

a) At \( R_F 0.60 \) corresponding to fluorescent photoadducts psoralen-thymine and psoralen-uracil, both radioactivity and fluorescence maxima have been obtained. Even after purification by means of a new chromatographic separation (solvent: n-butanol-acetic acid-water 4:1:5) the value of the radioactivity of these fluorescent substances has remained nearly unchanged.

b) At \( R_F 0.34 \) the presence of a radioactive band is evident, while the fluorescence is nearly absent.

This band shows the same \( R_F \) value as the substance yielded by heating non-fluorescent psoralen-thymine photoadduct with 5 N hydrochloric acid, as above mentioned.
c) The whole of these results has therefore made it possible for us to determine the contribution by each of the two classes of cycloadducts to the photo- reaction between psoralen-3H and DNA. A series of checks carried out shows that these measurements may be effected with a maximum error of about ± 5 per cent.

Fig. 2 shows the formation of the two classes of photoadducts at increasing periods of irradiation, expressed as a percent of the total radioactivity of each DNA and psoralen-3H solution sample, and the ratio between the two values obtained at different times of irradiation.

This series of experiments has been repeated three times with identical results. From the data obtained it is evident that in the photoreaction at 365 nm between psoralen-3H and DNA the fluorescent photoadducts form in a larger quantity than the non fluorescent ones; their ratio is about 3:1 with small differences with increasing length of irradiation.

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Über die Wirkung von Antikörpern auf die ATPase-Aktivität und den aktiven Na-K-Transport von E. coli und Menschen-Erythrozyten

R. AVERDUNK *, TH. GÜNTHER **, F. DORN ** und U. ZIMMERMANN ***


Antiseren gegen eine ATPase-Präparation und gegen Protoplasten aus E. coli hemmen die ATPase-Aktivität zu 80 Prozent.

Die pH-Abhängigkeit, das Substratoptimum und die Substratspezifität werden durch Antikörper gegen ATPase nicht beeinflußt.

Der aktive K-Transport, der **K-Turnover, die Atmung und der Glucose-Verbrauch werden bei E. coli durch Antiserum nicht verändert.

Antikörper gegen die Membran-ATPase von Menschen-Erythrozyten hemmen die Na-K-abhängige und -unabhängige ATPase zu jeweils 90 Prozent.


Methoden

Das Züchten der Bakterien und die Präparation der ATPase aus E. coli wurden bereits früher beschrieben6,7.

Zur Präparation der Erythrozyten-ATPase wurde frisch entnommenes mit Na-Citrat ungerinnbar gemachtes Blut verwendet. Die Erythrozyten wurden zweimal
