STRUCTURAL SPECIFICITY FOR THE PHOToinACTIVATION OF NUCLEIC ACIDS BY FUROCOUMARINS

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1. Introduction

Furocoumarins constitute a well known group of naturally occurring and synthetic substances which, when added to several biological systems and irradiated at long wavelengths of ultraviolet light, produce various interesting effects, which are not observed without furocoumarins or irradiation. We recall erythema on human or guinea-pig skin [1-5], death of bacteria [6-8], formation of mutants in Sarcina lutea cultures [9] and of giant cells in mammalian cells adapted to in vitro growth [10], inactivation of DNA viruses [11], destruction of the tumor-transmitting capacity of the Ehrlich ascites tumor cells [12] and disorders in the development of sea-urchin eggs fertilized by sperm irradiated in the presence of furocoumarins [13].

These biological effects are very probably due to a photoreaction between nucleic acids and furocoumarins, which has been investigated recently by Musajo, Rodighiero et al. [14, 15] and by Krauch, Krämer and Wacker [16]. The damage done to nucleic acids by irradiation at 365 nm in the presence of furocoumarins (linkage of a furocoumarin molecule to a pyrimidine base forming a C₄-cyclo-adduct) is of a different nature to that produced by irradiation in the presence of dyes [17, 18] (oxidation of guanine moieties) or in the presence of ketones [19-21] (thymine or uracil dimer formation).

Both in producing their biological effects and in photoreacting with nucleic acids furocoumarins show well defined structural requirements [3, 22].

In the present study several furocoumarins (fig. 1) were examined in order to correlate their structural requirements for their binding to DNA and to ribosomal RNA and their ability to photoinactivate DNA (template efficiency in the RNA polymerase reaction) and ribosomes.

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Fig. 1.
2. Materials and methods

Nucleoside triphosphates, phosphoenol pyruvate-K (PEP) and PEP-kinase were obtained from Böhringer Mannheim, Tutzing, Germany; radioactively labeled triphosphates from New England Nuclear Corp., Dreieichenhain, Frankfurt; DNA of herring sperms from F.Mack, Illertissen, Germany; DNA from calf thymus from Mann Research Laboratories, New York; polynucleotides from Miles Chemical Co., Elkhart, Indiana, U.S.A. and ribosomal RNA of yeast from Calbiochem, Luzern, Switzerland.

2.1. Furocoumarins

Psoralen (m.p. 164°) was extracted from the leaves of Ficus carica and bergapten (5-methoxy-psoralen; m.p. 190°) from bergamot oil. Xanthotoxol (8-hydroxy-psoralen) was prepared by demethylation of xanthotoxin (8-methoxy-psoralen) [23]. 8-Methyl-psoralen and 4,5',8-trimethyl-psoralen were synthetic products [24]. Labeled furocoumarins: bergapten-O\(^{14}\)CH\(_3\) was prepared by methylation of 5-hydroxy-psoralen with \(^{14}\)CH\(_3\) (specific activity 8.5 \times 10^8 dpm/mmole) [25]. Psoralen, 8-methyl-psoralen, 4,5',8-trimethyl-psoralen and xanthotoxol were tritiated compounds [26], prepared by the Wilzbach method; specific activities: psoralen-\(^3\)H 4.5 \times 10^8 dpm/mmole; 8-methyl-psoralen-\(^3\)H 2.8 \times 10^9 dpm/mmole; trimethyl-psoralen-\(^3\)H 4.23 \times 10^{10} dpm/mmole, xanthotoxol-\(^3\)H 7.4 \times 10^9 dpm/mmole.

2.2. Irradiation of DNA and RNA in the presence of furocoumarins

Small quantities of alcoholic solutions of the furocoumarins were added to aqueous 0.1% solutions of native calf thymus DNA or ribosomal RNA to produce final concentrations of 10 \(\mu\)g/ml for DNA and 4 \(\mu\)g/ml for RNA. These solutions were irradiated at 365 nm and 22°, in calibrated glass tubes, using a device already described [27].

After irradiation, solid sodium chloride was added to the solutions to a final concentration of 1 M. The nucleic acid was then precipitated by addition of \(4\) ml of absolute ethyl alcohol; the precipitate was removed by centrifugation, washed with \(2\) ml of ethyl alcohol-water (80:20) and dissolved in \(2\) ml of water. The radioactivity of the solutions thus obtained was determined with a dioxan scintillator in the usual manner.

2.3. Template efficiency of DNA in RNA polymerase reaction

DNA-dependent RNA polymerase from E. coli K12 cell was isolated by the method of Burgess [28]; enzyme obtained after DEAE-cellulose chromatography was used in our system. The template efficiency of DNA in the RNA polymerase reaction was measured as described earlier [29].

2.4. Protein synthesis in vitro

Ribosomes and 105,000 g supernatant were prepared as described before [29]. Amino acid incorporation was measured by the paper-disc technique of Mans and Novelli [30]. The reaction mixture of Nirenberg and Matthaei [31] was used, except that UTP and CTP were omitted.

3. Results and discussion

The binding capacity of a group of furocoumarins (8-methyl-psoralen, 4,5',8-trimethyl-psoralen, psoralen, xanthotoxin, bergapten, xanthotoxol) to native calf-thymus DNA and to yeast-ribosomal RNA under irradiation at 365 nm is shown in table 1. These studies were carried out by irradiating under standard conditions aqueous solutions of the nucleic acids and labelled furocoumarins. After irradiation DNA or RNA was precipitated and the bound radioactivity was measured. From these data the amounts of furocoumarins linked to nucleic acid were calculated.

For comparison, table 1 also shows the data relative to the skin-photosensitizing activities of the same substances. The results have been obtained [3, 22] by Musajo, Rodigheiro et al. by placing on human or guinea-pig skin constant amounts of the reactive compounds and by determining the minimum irradiation (365 nm) time necessary for the production of erythema.

As is apparent, a good correlation exists between the skin-photosensitizing activity of various furocoumarins and their binding capacity to DNA; however, the agreement with the binding capacity to RNA is less pronounced.
Table 1
Skin-photosensitizing activity of some furocoumarins and their photo-binding capacity to native calf-thymus DNA and to yeast ribosomal RNA.

<table>
<thead>
<tr>
<th>Furocoumarins</th>
<th>Skin-photosensitizing activity: minimum irradiation time required to produce erythema on guinea-pig skin* (min)</th>
<th>Percentage of furocoumarins‡ linked to DNA after irradiation** for (min)</th>
<th>Percentage of furocoumarins‡ linked to RNA after irradiation** for (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>8-Methyl-psoralen</td>
<td>5</td>
<td>47</td>
<td>66</td>
</tr>
<tr>
<td>4,5',8-Trimethyl-psoralen</td>
<td>10</td>
<td>93.5</td>
<td>96</td>
</tr>
<tr>
<td>Psoralen</td>
<td>27</td>
<td>18</td>
<td>29.5</td>
</tr>
<tr>
<td>Xanthotoxin</td>
<td>38</td>
<td>11.5</td>
<td>20.5</td>
</tr>
<tr>
<td>Bergapten</td>
<td>44</td>
<td>6.5</td>
<td>12.5</td>
</tr>
<tr>
<td>Xanthotoxol</td>
<td>inactive</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Test conditions: 2.5 µg of substance per cm² of guinea-pig skin; irradiation with an Osram HWA 500 W lamp (strongly emitting at 365 nm and in the visible region) at 45 cm from the skin. The appearance of erythema was checked after 24 hr.

** 2 ml of an aqueous solution containing 0.1% of DNA or RNA and 10 µg/ml (4 µg/ml in the case of RNA) of a furocoumarin was irradiated at 22°C with 2 Philips HPW 125 lamps (365 nm; 2.9 × 10¹⁶ quanta/sec).

† Referred to amount initially present.

Table 2
Photosensitized inhibition of the template activity of DNA by various furocoumarins.

<table>
<thead>
<tr>
<th>Furocoumarins used</th>
<th>¹⁴C-AMP incorporation (% of the control without furocoumarins)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control:</td>
<td></td>
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<tr>
<td>without furocoumarins</td>
<td>100</td>
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<tr>
<td>without DNA</td>
<td>2.7</td>
</tr>
<tr>
<td>4,5',8-Trimethyl-psoralen</td>
<td>15.7</td>
</tr>
<tr>
<td>8-Methyl-psoralen</td>
<td>29.4</td>
</tr>
<tr>
<td>Psoralen</td>
<td>45.9</td>
</tr>
<tr>
<td>Bergapten</td>
<td>86.9</td>
</tr>
<tr>
<td>Xanthotoxol</td>
<td>94.0</td>
</tr>
</tbody>
</table>

A 0.1% solution of DNA was irradiated for 45 min at 365 nm in a grating monochromator, supplied by Bausch and Lomb, Rochester, U.S.A. The concentration of furocoumarin in the DNA solutions was 10 µg/ml. All irradiations were carried out in 1 cm quartz cuvettes. The energy at the wavelength used was 3.5 × 10⁶ ergs/cm² min.

Table 3
Effect of furocoumarins on the activity of ribosomes.

<table>
<thead>
<tr>
<th>Furocoumarins used</th>
<th>Phenylalanine incorporation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control:</td>
<td>cpm/mg riboprotein % activity</td>
</tr>
<tr>
<td>without furocoumarin</td>
<td>2739</td>
</tr>
<tr>
<td>without ribosomes</td>
<td>25</td>
</tr>
<tr>
<td>Psoralen</td>
<td>1644</td>
</tr>
<tr>
<td>8-Methyl-psoralen</td>
<td>1667</td>
</tr>
<tr>
<td>Bergapten</td>
<td>1598</td>
</tr>
</tbody>
</table>
experiments the incorporation of $^{14}$C-AMP into RNA was measured. Under the conditions used there was an inhibition of the template activity of DNA. The results are shown in table 2. Highest inhibition was observed for trimethyl-psoralen, followed by 8-methyl-psoralen, psoralen, bergapten and xanthotoxol. The degree of this inhibition is very close to the photobinding capacity of the various substances to DNA and also to the skin-photosensitizing activity (table 1). The results now obtained with psoralen and xanthotoxol are quite similar to those obtained previously by Chandra and Wacker [32].

Additional experiments were designed to study the structural specificity of furcocoumarins with regard to the inactivation of RNA. We examined the inactivation of ribosomes after irradiation in the presence of various furcocoumarins in a cell-free protein synthesis system. Ribosomes are nucleoprotein particles composed of proteins and ribosomal RNA. The manner in which the protein part and the ribosomal RNA influence the functional activity of ribosomes is not yet well understood. Photoreactions which modify only RNA may, therefore, be employed to study this problem.

*E. coli* ribosomes were suspended in the buffer [31]. The suspension which contained 8–10 mg protein/ml was irradiated at 365 nm in the presence of various furcocoumarins. After irradiation ribosomal activity was tested by measuring the incorporation of $^{14}$C-phenylalanine into macromolecules in the presence of poly U. As follows from table 3, the three furcocoumarins psoralen, 8-methyl-psoralen and bergapten inhibit the activity of ribosomes to the same extent. This observation does not correspond well to the binding capacity of the furcocoumarins to ribosomal RNA (see table 1). This very interesting aspect must be more widely investigated.

References