

## Contribution of Monofunctional Adducts formed by Furocoumarins with DNA to the Inhibition of Nucleic Acids Synthesis

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In addition to the bifunctional adducts (cross-linkages), that furocoumarins on radiation at 365 nm form in DNA, monofunctional adducts also proved able to inhibit the nucleic acid synthesis in Ehrlich ascites tumor cells.

The photosensitizing properties that furocoumarins (psoralens) exert on various biological systems<sup>1–5</sup> are attributed to a photochemical addition to DNA<sup>6</sup>, which occurs under irradiation by long wavelength ultraviolet light (365 nm). That is to say, furocoumarins photoreact with the pyrimidine bases of DNA and can form both monofunctional<sup>7, 8</sup> and bifunctional<sup>9</sup> cycloadducts; in the latter case inter-strand cross-linkings take place. The greatest importance for the biological consequences due to the photosensitization process has been ascribed to the bifunctional adducts. In fact, in some instances (killing of bacteria<sup>10</sup>, skin-photosensitization<sup>11</sup>) a parallelism has been found between the formation of cross-linkings in DNA and the biological effect produced. Recently, nevertheless, using angelicin, a furocoumarin unable to form inter-strand cross-linkages, we demonstrated that it might also be photobiologically active<sup>12</sup>.

Therefore, with the aim of evaluating the contribution given by the different adducts in the production of the biological effects, we have studied the DNA and RNA synthesis in Ehrlich ascites tumor cells after irradiation in the presence of several furocoumarins which were known to have very different abilities of photobinding to native DNA<sup>13</sup> and of producing cross-linkages<sup>9</sup>. Thereafter we compared these properties with those now observed regarding their inhibiting effect on DNA and RNA synthesis.

The experimental methods used were the same as previously described<sup>14</sup>. The Ehrlich tumor cells were suspended in balanced saline solution containing the furocoumarin ( $2 \times 10^7$  cells and  $3.5 \mu\text{g}$

of substance per ml). 5 ml of cell suspension were poured into Petri dishes, 5 cm in diameter, placed on crushed ice, and irradiated with a Philips HPW 125 lamp, emitting mainly at 365 nm. According to the photosensitizing potency of the furocoumarin studied, the irradiations were performed by placing the lamp at two different distances, 20 cm for bergapten, psoralen, 5',8-dimethylpsoralen and 45 cm for 8-methylpsoralen, 4,8-dimethylpsoralen, 4,5',8-trimethylpsoralen, 4,4',8-trimethylpsoralen, with an incident radiation on the whole solution of  $2.02 \times 10^{16}$  and  $3.5 \times 10^{15}$  quanta $\cdot\text{sec}^{-1}$  respectively. The irradiated cells, washed with ice cold Hank's solution, were incubated at 37 °C for 30 min in the same medium ( $4 \times 10^7$  cells/ml) in the presence of a tritiated nucleoside ( $3 \mu\text{Ci/ml}$  of [<sup>3</sup>H]-thymidine, 5 Ci/mM, or of [<sup>3</sup>H]uridine, 4.6 Ci/mM; from the Radiochemical Centre, Amersham, England). Then, the nucleic acids were extracted by the hot 10% sodium chloride method<sup>14</sup> and their specific activity determined using a modified Bray's solution<sup>15</sup> with a liquid scintillation spectrometer Packard Model 3375 and carrying out the diphenylamine<sup>16</sup> or orcinol<sup>17</sup> reaction.

For each furocoumarin the experiment was carried out as follows: a. six samples of cell suspension containing the furocoumarin were irradiated for increasing times; b. samples of cell suspension without furocoumarin were irradiated for the same times; c. a sample of cell suspension containing the furocoumarin was kept in the dark.

Irradiation in the absence of the drug, as well as incubation in the dark, was ineffective for every furocoumarin studied; inhibition of nucleic acids synthesis occurred only by irradiating in the presence of a furocoumarin, as already described<sup>14</sup>.

On the basis of the inhibition observed by increasing the irradiation time and using the probit analysis, we calculated the  $D_{50}$ , *i. e.*, the ultraviolet radiation dose that, in the presence of the furocoumarin studied, yields a 50% inhibition.

The results obtained are shown in Table I. On the basis of these, we can divide the substances into three groups: a first, comprehending 4,4',8-trimethylpsoralen, 4,5',8-trimethylpsoralen and 4,8-dimethylpsoralen, showed a very high capacity of inhibiting the nucleic acid synthesis. A second one, comprehending 8-methylpsoralen and 5',8-dimethylpsoralen, exerted a lower activity; in the third group we find psoralen and bergapten (5-methoxy-psoralen), with the lowest activity.

As can be seen from the other data reported in Table I for a comparison, this picture is not in agreement with the ability to form cross-linkages of the same compounds. By contrast, it shows a better

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Table I. Inhibition of DNA and RNA synthesis in Ehrlich ascites tumor cells by irradiation at 365 nm in the presence of 3.5  $\mu\text{g/ml}$  of a photosensitizing furocoumarin, compared with cross-linkage formation and photoreactivity. Specific activities of the controls were around  $50 \times 10^4$  dpm/mg for DNA and  $45 \times 10^4$  dpm/mg for RNA; dark incubation with the drugs or irradiation in their absence were both ineffective.  $D_{50}$  is the ultraviolet radiation dose that, in the presence of the furocoumarin studied, yields at 50% inhibition. Relative activity indicates the fold activity with respect to psoralen.

Furocoumarins	Inhibition of the Synthesis					
	$D_{50}$ quanta $\times 10^{-18}$	DNA Relative activity (psoralen = 1)	$D_{50}$ quanta $\times 10^{-18}$	RNA Relative activity (psoralen = 1)	Relative cross-linkage formation in DNA (psoralen = 1)	Relative photoreactivity with DNA (psoralen = 1)
4,4',8-trimethylpsoralen	0.31	31.6	0.34	41	1.93	5.17
4,5',8-trimethylpsoralen	0.50	19.6	0.54	26	1.8	7.10
4,8-dimethylpsoralen	0.65	15.0	0.45	31	1.7	5.89
8-methylpsoralen	1.04	9.4	1.55	9	3.1	3.73
5,8-dimethylpsoralen	1.82	5.4	11.2	1.2	2.2	4.66
psoralen	9.8	1	14.0	1	1	1
bergapten	20	0.5	29.9	0.47	0.55	0.33

correlation with the entire photoreactivity of the various furocoumarins\*. This is related to the amount of the substance which is capable of linking covalently to the macromolecule by irradiation,

without distinguishing between monofunctional and bifunctional adducts.

Therefore, the di- and tri-methylpsoralens of the first group, having a high photoreactivity but a small capacity of forming cross-linkages, evidently form mainly monofunctional adducts. The high activity now shown by these compounds demonstrates that the template activity of DNA is also strongly affected by monofunctional adducts, other than by the difunctional ones.

\* This picture is different from those which have been obtained studying other biological effects, such as the killing of bacteria<sup>10</sup> and skin-photosensitization<sup>11</sup>. It may be that repair processes are involved in determining this difference.

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