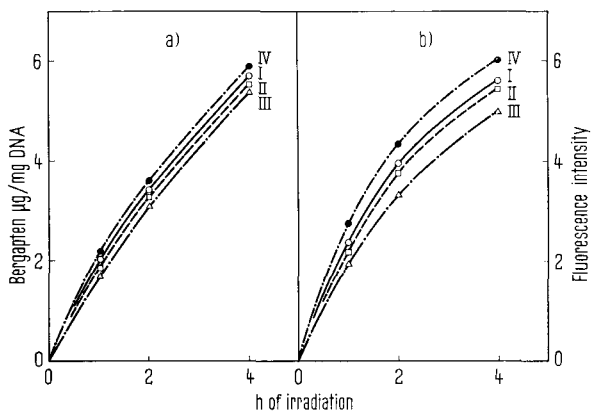
The Photoreaction Between DNA and the Skin-Photosensitizing Furocoumarins Studied Using Labelled Bergapten

In foregoing communications[1-3] we have pointed out the capacity of furocoumarins to bind in the dark with DNA, and we have reported some evidences of a photoreaction which occurs when a solution of DNA containing a skin-photosensitizing furocoumarin is irradiated at 3655 Å. These results were obtained in the course of our studies on the mechanism of the photosensitizing action of some furocoumarins[4].

The photoreaction between DNA and these substances has now been confirmed using a labelled skin-active furocoumarin, i.e. $O^{14}CH_3$ bergapten, or 5-methoxy-psoralen, that we prepared by methylation of the corresponding phenol (bergaptol or 5-hydroxy-psoralen) with $I^{14}CH_3$.

Four different samples of DNA were used in these experiments (see Figure). The photoreactions were studied by determining both the radioactivity (in a liquid scintillator counter, SELO, Milano) and the fluorescence (at 400 nm, using an Aminco-Bowman spectrophotofluorimeter; activating wavelength 330 nm).

Aqueous 0.1% solutions of DNA, containing 20 μg/ml of labelled bergapten, were irradiated at 3655 Å (Philips HPW 125 lamp at a distance of 16 cm; irradiation 0.98 mW/cm^2) and then sodium chloride was added to a concentration 1 M. The DNA was precipitated with 2 vol of ethyl alcohol, centrifuged, washed with 70% ethyl alcohol, and solubilized again in the same volume of water as in the initial stage. The obtained solutions were utilized for the determination of the fluorescence directly and the radioactivity, using 0.2 ml of the solutions with 3 ml of absolute ethyl alcohol and 3 ml of toluene solution of scintillator (the apparatus efficiency in these experimental conditions was 20%).



Photoreactions between bergapten-$O^{14}CH_3$ and DNA. Aqueous 0.1% solutions of DNA containing 20 μg/ml of labelled bergapten irradiated at 3655Å (0.98 mW/cm^2). In a) is reported the amount (μg) of bergapten which, after irradiation, is linked to 1 mg of DNA, as calculated on the basis of the radioactivity measurements. In b) is reported the intensity (arbitrary units) of the fluorescence assumed by DNA after irradiation. I, Calf thymus DNA, extracted with strong salt solution and deproteinized by saturation with sodium chloride[7]. P% = 7.32; N/P = 1.68; Tm[8] = 83.6°. II, Calf thymus DNA, extracted with the aid of sodium dodecilsulphate[9]. P% = 8.36; N/P = 1.665; Tm = 89°. III, Calf thymus DNA, highly polymerized (Mann Research Laboratories, New York). P% = 7.05; N/P = 1.73; Tm = 87°. IV, Salmon sperm DNA, highly polymerized (Mann Research Laboratories,New York). P% = 8.16; N/P = 1.69; Tm = 86°.

In these experiments, the four samples of DNA examined showed a very similar behaviour (see Figure). In the period of irradiation used (4 h)[5] the small samples of DNA irradiated, precipitated and solubilized again in water showed a gradual increase both of the radioactivity and the fluorescence with the increasing length of irradiation. On the other hand, radioactivity and fluorescence were not present without irradiation.

The quantum yield, determined in the first 2 h of irradiation on the basis of the amount of bergapten bound to DNA (calculated from the assumed radioactivity), was $5.2 \cdot 10^{-3}$.

The results obtained confirm the photoreaction between DNA and bergapten after irradiation at 3655 Å. Moreover they indicate that the binding between bergapten and DNA which takes place in the dark[1] is very weak and the 'complex' formed breaks up completely on precipitation of DNA with ethanol. On the other hand, after irradiation a more stable chemical linkage is formed between the furocoumarin and the macromolecule.

As we have previously found, the furocoumarins photoreact only with the pyrimidine derivatives. We therefore think that also in the DNA the reactive sites are the pyrimidine bases.

In the experimental conditions indicated above, we have calculated that the ratio between the molecules of bergapten linked to DNA and the nucleotides present in the same DNA is 1:154 after 2 h of irradiation.

Other more detailed results on this subject will be published elsewhere.

We think that this photoreaction between skin-photosensitizing furocoumarins and DNA could explain the mechanism of the biological photosensitizing effects of the furocoumarins so far observed, such as the skin-erythema, the DNA virus inactivation, and the lethal and mutagenic action on bacteria and on mammalian cells adapted to in vitro growth.

Riassunto. Ricerche eseguite con bergaptene-$O^{14}CH_3$ confermano che tra questa sostanza ed il DNA avviene una fotoreazione in seguito ad irradiazione a 3655 Å, che porta alla formazione di una stabile combinazione tra la furocumarina e lo stesso DNA.

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