PHOTOSENSITIZED SPLITTING OF PYRIMIDINE DIMERS BY INDOLE DERIVATIVES.

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SUMMARY : Excitation of tryptophan and 5-hydroxytryptophan at wavelengths longer than 290 and 310 nm, respectively, in the presence of pyrimidine dimers leads to a sensitized splitting of these dimers. The reaction is more efficient in frozen than in fluid aqueous solutions. The fluorescence of tryptophan and 5-hydroxytryptophan is quenched by thymine dimers. These results suggest that splitting occurs as a result of electron transfer from the excited indole derivative to the pyrimidine dimer.

Among photochemical damages induced in DNA by ultra-violet irradiation, pyrimidine dimers represent the main biological lesions [1]. These dimers can be monomerized either by irradiation at short wavelengths (240 nm) [2] or by irradiation in the presence of a photoreactivating enzyme at wavelengths longer than 310 nm where neither native DNA nor the dimers absorb light [3]. Irradiating at short wavelengths introduces other photochemical damages whereas the splitting of pyrimidine dimers by the photoreactivating enzyme appears to be the only reaction taking place [3]. Photosensitized splitting of pyrimidine dimers by simple chemical substances could shed some light on the mechanism of action of the photoreactivating enzyme [4][5]. Such photosensitizers could also be used to remove selectively this type of damage in U.V.-irradiated DNA [5][6]. The chemical nature of the chromophoric group of the photoreactivating enzyme which absorbs photoreactivating light has not been elucidated yet. It could be possible that only the complex between the enzyme and U.V.-irradiated DNA (but not the free enzyme) is able to absorb photoreactivating light.

We previously reported that tryptophan and other indole derivatives could form intermolecular complexes with the components of nucleic acids in frozen [7][8] as well as in fluid [9] aqueous solutions. These complexes absorb light at longer wavelengths than do the separated partners. This new absorption is due to a transfer of electronic

charge from the indole ring (electron donor) to the purine or pyrimidine ring (electron acceptor). The results reported here show that pyrimidine dimers are split in the presence of tryptophan or 5-hydroxytryptophan by irradiation at wavelengths where only the latter absorbs light. This splitting takes place either in fluid aqueous solution at room temperature or even more efficiently in frozen aqueous solutions. The fluorescence of tryptophan and 5-hydroxytryptophan is quenched by pyrimidine dimers, probably as a result of excited-state electron transfer.

Experimental :

Chemicals were purchased from either Calbiochem or Aldrich, and used without further purification. ^{14}C-labeled compounds were obtained from the C.E.A. (Saclay). Orotic acid dimer was prepared by irradiation of orotic acid in fluid aqueous solutions. Uracil dimer (cis-syn) was obtained by irradiation of uracil in frozen aqueous solutions. Cis-syn thymine dimer was a gift from Pr. WANG.

Fluid aqueous solutions containing pyrimidine dimers and 5-hydroxyindole derivatives were irradiated at 313 nm using a mercury HBO 500 W lamp and a Huet grating monochromator. For irradiation of frozen aqueous solutions either a glass filter or a liquid filter were used depending on the photosensitizer. Transmission curves are given in figure 2.

Aliquots of the irradiated solutions (usually 100 µl) were spotted on Whatman n° 1 paper and chromatographed with isopropanol, concentrated HCl, water (4/1/1). Radioactivity was assayed by liquid scintillation counting of strips from the chromatograms using a Beckman scintillation counter.

Absorption and fluorescence spectra were recorded with a Perkin-Elmer 402 and a Jobin-Yvon spectrophotometers, respectively.

Results :

In fluid aqueous solutions, irradiation at 313 nm of mixtures of orotic acid or uracil dimers with 5-hydroxyindole derivatives leads to a splitting of dimers as shown in figure 1. Only monomers are produced in this reaction as shown by chromatographic comparison with authentic samples of these monomers. We did not detect any other product derived from the pyrimidine ring. On figure 1, it can be seen that the percentage of split dimers (and the corresponding amount of monomers) increases with irradiation time.

The photosensitized splitting of pyrimidine dimers in frozen aqueous solutions can also be followed by absorbance measurements. The

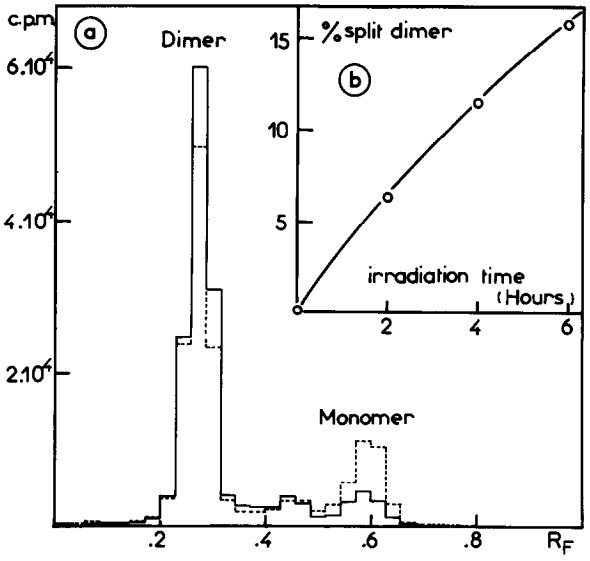

Figure 1 : a) Chromatographic analysis of a mixture of orotic acid dimer (3×10^{-4} M) and 5-hydroxytryptamine (10^{-3} M) before and after 6 hours irradiation at 313 nm. R_F values of dimer and monomer are 0.30 and 0.60 respectively.

b) Percentage of split dimers as a function of irradiation time for the same mixture as in a).

absorption spectrum of a mixture of 5-hydroxytryptophan (5×10^{-4}M) and cis-syn thymine dimer (5×10^{-4} M) changes upon irradiation at $\lambda > 310$ nm as shown in figure 2a. The absorbance of 5-hydroxytryptophan above 290 nm does not change indicating that this compound does not undergo any important photochemical reaction upon irradiation in frozen aqueous solutions. The difference spectrum between the unirradiated and the 30 min.-irradiated samples is identical to that of thymine. By chromatographic analysis of the 30 min.-irradiated sample a spot is obtained that has the same R_F as an authentic sample of thymine. This spot eluted with water gives an absorption spectrum identical to that of thymine both at pH 7 and at pH 13. Therefore, it is clear that thymine dimers are monomerized in a photosensitized reaction involving 5-hydroxytryptophan as the sensitizer.

Similar experiments performed with orotic acid and uracil dimers lead to the same conclusion. Chromatographic analysis with labeled dimers shows that split dimers are recovered as monomers. The amount of monomer formed is identical to that determined from absorbance measu-

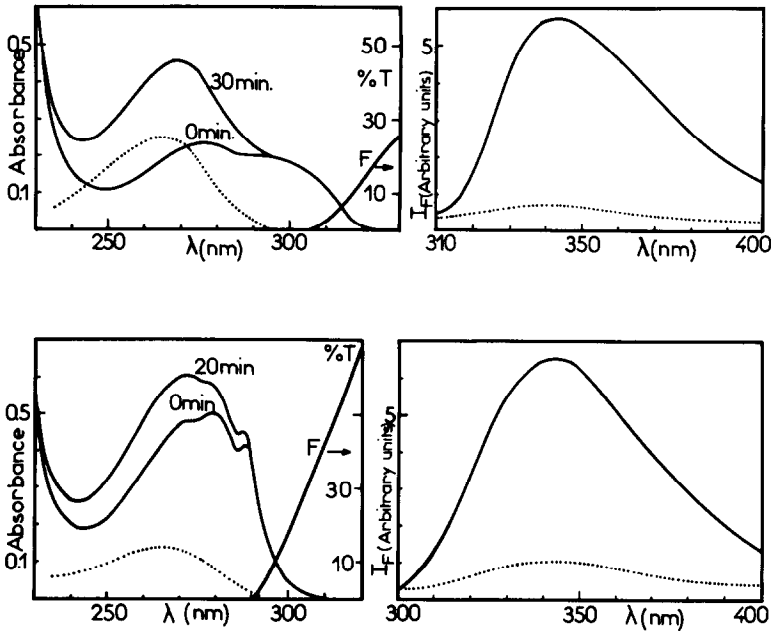

Figure 2 : **Upper part** : **Absorption spectra (left) of an aqueous mixture of 5-hydroxytryptophan and cis-syn thymine dimer (5×10^{-4} M each) before and after 30 minute-irradiation at 77 °K through filter F. Dotted line is the difference between these two spectra.**

Fluorescence spectra at 77 °K of frozen aqueous solutions (5×10^{-4} M) of 5-hydroxytryptophan in the absence (full line) and the presence (dotted line) of 5×10^{-4} M thymine dimer.

Lower part : **Same as above but 5-hydroxytryptophan is replaced by tryptophan and irradiation time was 20 minutes.**

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rements. If 5-hydroxytryptophan is replaced by tryptophan and irradiation carried out at $\lambda > 290$ nm, thymine dimers are still monomerized as shown by absorbance and chromatographic analysis (figure 2b).

To obtain some information on the mechanism of this photosensitized splitting, we measured the fluorescence spectrum of tryptophan and 5-hydroxytryptophan in the absence and the presence of thymine dimers. Tryptophan and 5-hydroxytryptophan at a concentration of 5×10^{-4} M excited at 290 nm emit a fluorescence of high quantum yield (λ_{max} = 342 and 348 nm, respectively). In the presence of an equimolar concentration of thymine dimers, the fluorescence of both compounds is completely

quenched (figure 2). This indicates a strong interaction between excited indole derivatives and thymine dimers.

Discussion :

Upon freezing aqueous solutions, the formation of ice crystals forces solute molecules into solid aggregates. Photodimerization of pyrimidine derivatives can be observed in such aggregates [10]. We already reported that the formation of mixed aggregates of tryptophan and nucleosides leads to the formation of intermolecular complexes [7] [8] [9]. In these complexes, transfer of electronic charge can take place from tryptophan to the base moiety of the nucleoside, pyrimidines being better electron acceptors than purines. The fluorescence quantum yield of these electron donor-acceptor complexes is very low when compared to that of the separated components [8]. Here, we observe a total quenching of the fluorescence of tryptophan and 5-hydroxytryptophan in the mixed aggregates obtained by freezing equimolar mixtures of these compounds and pyrimidine dimers. Indole derivatives are good electron donors [7] [8]. The electron-donor ability of the indole ring is increased by substitution of electron-donating groups (such as the 5-hydroxyl group). It is thus very likely that the quenching of fluorescence and the photosensitized splitting of pyrimidine dimers are both related to electron transfer processes. Since tryptophan and 5-hydroxytryptophan do not appear to be photochemically transformed during the splitting reaction in ice, one has to assume that the electron has to be given back to the indole derivative, for example in some ground-state process.

In fluid aqueous solutions, it is difficult to propose any mechanism since sensitizers themselves undergo photochemical reactions of their own. However it has been shown that photoejection of electrons could occur when indole derivatives are excited in aqueous solutions [11] [12]. The splitting of pyrimidine dimers could therefore result from their reaction with solvated electrons. Flash-photolysis experiments are undertaken to help understand the mechanism of the photosensitized splitting of pyrimidine dimers in fluid aqueous solutions.

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