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Preliminary Communication

Isotope Effects in the Photosensitized Dimerization of Pyrimidines

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Many of the biological effects of ultraviolet radiation can be explained in terms of specific chemical and physical changes in the DNA. Correlations have been made between the survival of u. v. irradiated cells and the production of certain types of lesions in the DNA.[1] The pyrimidines in the DNA are the most sensitive receptors of u. v. photons. Both *in vitro* and *in vivo* irradiation of DNA with u. v. light of wavelenghs shorter than 300 nm yield cyclobutyl pyrimidine dimers. Pyrimidines can also be dimerized with wavelengths longer than 300 nm in the presence of carbonyl compounds by means of energy transfer.[2,3,4,5] This process is known as molecular photosensitization and yields exclusively dimers without concomitant photohydration. Molecular photosensitization is of fundamental biologic importance, as wavelengths longer than 300 nm are present in the sun spectrum on the earth's surface.

It is now known that the thymine dimer ($\widehat{TT}$) is not the only lesion introduced into the DNA molecule by u. v. light. The thymine dimer, however, is the most extensively studied photoproduct, and correlations have been demonstrated between the presence of this product following u. v. irradiation and the ensuing biological manifestations.[1]

Proposals concerning the reaction mechanisms of the sensitized photodimerization of pyrimidines have been suggested;[6,7] these include population of the triplet state of thymine or uracil from the triplet state of a suitable sensitizer. In our earlier work[7] it was proposed that the ketone-photosensitized dimerization of pyrimidines takes place through an intermediate formation of a complex between the donor (*e. g.* ketone molecule) and the acceptor (pyrimidine molecule), giving an unstable adduct of Type I (Fig. 1), which can react with another pyrimidine molecule in its ground state.

If this proposal is correct, then only a few out of many potential photosensitizers would be active in promoting pyrimidine dimerization. In order to explore the validity of this hypothesis, we investigated the ability of several classes of compounds, having triplet energies higher or similar to thymine (T) as photosensitizers in thymine dimer formation.[8] We also wanted to answer the question whether the energy transfer from the sensitizer to the substrate occurs through a simple physical mechanism (Hammond)[9] or whether it is

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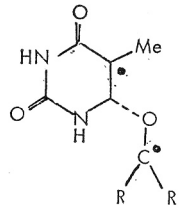
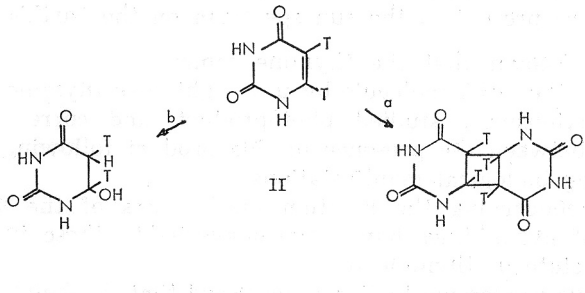


I

Fig. 1.

restricted by some complex-forming reaction (Schenck's relay mechanism).[10] This question is not only an academic one. In all biological systems that are exposed to light, it is of major importance whether a chromophore which absorbs light can transfer its surplus energy of excitation to another important biological macromolecule in an unrestricted way, or whether the energy transfer is somehow restricted by an intermediate complex formation between a donor and an acceptor. In view of this, we decided to perform some experiments with some double labeled (^{3}H, ^{14}C) pyrimidines in order to get a better understanding of the mechanism of this photosensitized reaction.

A few years ago, we showed[11] that double labeled (2-^{14}C; 5,6-^{3}H) uracil (U) (II) upon UV irradiation (254 nm) gave the dimer (UU) (III) with only a slight observable isotope effect (kIE = 0.970 with 10^7 erg/cm^2), whereas, the photohydrate (IV) was formed with a marked enrichment of tritium (Fig. 2).



IV II III

Fig. 2.

Reaction (b), therefore, proceeds with an inverse secondary kinetic isotope effect.[12] Since the reaction proceeds with a saturation of an ethylenic double bond (*i. e.*, $sp^2 \rightarrow sp^3$ hybridization) where the tritium is attached to an unsaturated carbon, such an effect was expected. In the dimer (III), on the other hand, the initial pyrimidine double bonds become a part of a cyclobutane ring, which is known to have hybridization intermediate between sp^2 and sp^3. This might explain why the isotope effect in the formation of the dimer (III) is markedly less than in the formation of the hydrate (IV). Using the same conditions, we could show that cytosine derivatives gave photohydrates too, even though the unstable photoproducts of cytosine derivatives could not be isolated.[13]

With this in mind, we, in the course of this work, irradiated double labeled (^{3}H, ^{14}C) uracil in the presence of acetone with $\lambda > 300$ nm. As seen

from Figure 3, the »direct« formation of the dimers with an energy transfer mechanism (Hammond) would yield (V) with an essentially unchanged isotope effect. Pathway (b), however, through a »Schenck-type intermediate«, would proceed with an inverse secondary kinetic isotopic effect which would be manifested in a different $^3H : ^{14}C$ ratio in the isolated product (V).[14] — No attempt to analyze the isomeric structure of the dimers has been performed in this work. It should be mentioned, however, that a mixture of stereo-isomers of V is formed under these conditions.[6]

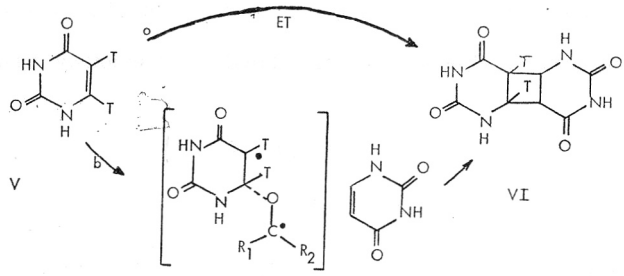


Fig. 3.

The results of this preliminary investigation are summarized in Table I. As can be seen, the $^3H : ^{14}C$ ratios in VI exceed that in V by about 14 and 20% respectively, depending on the u. v. dose used. The values of the kinetic isotope effects obtained also suggest that the Schenck relay mechanism (pathway b) may be favored over a direct energy transfer mechanism in this biologically significant photoreaction.

TABLE I

Yields and $^3H : ^{14}C$ ratios of the isolated uracil dimers ($\widehat{UU}$) formed by acetone sensitized photodimerization

U.v. dose[a] 10^7 erg/cm² *	$\widehat{UU}$ Yield %	$^3H : ^{14}C$ ratio[b] in VI	Isotope effect (kIE)
0.4	8	12.3 ± 0.2	0.813
1.0	14	11.6 ± 0.3	0.860

$^3H : ^{14}C$ ratio in the starting material (U) 10.1

* Measured with a black ray u. v. intensity meter (UV products, San Gabriel, Co.)

[b] Mean value from three separate irradiations.

EXPERIMENTAL

Ultraviolet irradiation was carried out with a 500 watt high pressure mercury lamp (Christie Electric Co., Los Angeles, Ca.). The samples were irradiated in an open quartz cuvette (1 cm light path) at room temperature in presence of air. The cuvette was placed directly behind a glass filter (window glass 7.5 × 2.5 × 0.2 cm) that trasmitted wavelengths greater than 300 nm.

The starting uracil-2-^{14}C; 5, 6-^{3}H was obtained by mixing uracil-2-^{14}C (3 mCi/mmol) with uracil-5, 6-^{3}H (4 Ci/mmol; both New England Nuclear Corp., Boston, Ma.). Ratio $^3H : ^{14}C$ in the starting material was 10 : 1. The ^{3}H-labeled uracil

contained the label uniformly distributed in positions 5 and 6. The location of this label was verified by preparing 5-Br-uracil-6-^{3}H from uracil-5, 6-^{3}H by bromination in CCl_4. [Uracil-2-^{14}C; 5, 6-^{3}H concentration 10^{-3} M in water; acetone conc. 10^{-4} M.] Aliquots of the irradiated solutions were spotted on an ion-exchange paper loaded with IRC-50 resin (grade WA-2; Reeve Angel, Clifton, N. J.). The chromatograms were developed using the solvent system of 0.1 M acetic acid adjusted to pH = 4.8 with ammonia[15]. The uracil spot was located by examining the paper chromatogram under u. v. The dried paper chromatograms were then sliced into 1 cm portions and eluted with 1 ml water in Packard vials; the radioactivity was measured after the addition of 10 ml Butler-dioxane based scintillation fluid. The double isotope counting was carried out by the method given by Bush[6]. R_f values for uracil and of uracil dimers under these experimental conditions were 0.50 and 0.65, respectively.

The kinetic isotope effects were calculated as previously described[11] by the equation:

$$\frac{1}{(\mathrm{kIE})} = \frac{\log\left(1 - f\,\frac{R_p}{R_s}\right)}{\log\,(1-f)}$$

R_p = ratio $^{3}H:^{14}C$ in the product $\hat{(UU)}$; R_s = ratio $^{3}H:^{14}C$ in the starting material (U); f = reaction yield.

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REFERENCES

1. For reviews see: A. Wacker, *Molecular Mechanisms of Radiation Effects, Progr. Nucl. Acid Res.* **1** (1963) 369; K. C. Smith and P. Hanawalt, *Molecular Photobiology,* Academic Press New York 1969; J. G. Burr, *Advan. Photochem.* **6** (1968) 193; A. J. Varghese, in: A. C. Giese (Ed.), *Photo-* 207; C. Hélène and M. Charlier, *Biochimie* **53** (1971) 1175.
2. I. von Wilucki, H. Mathaus, and C. H. Krauch, ,*Photochem and physiology, Photochem. and Photobiol.* **6** (1967) 497.
3. D. Elad, C. Krüger and G. M. J. Schmidt, *Photochem. and Photobiol.* **6** (1967) 495.
4. A. A. Lamola and T. Yamane, *Proc. Nat. Acad. Sci U. S.* **58** (1967) 443.
5. C. H. Krauch, D. M. Krämer, P. Chandra, P. Mildner, H. Feller, and A. Wacker, *Angew. Chem.* **79** (1967) 944.
6. A. A. Lamola, *Photochem. and Photobiol.* **7** (1968) 619.
7. A. Kornhauser, J. N. Herak, and N. Trinajstić, *Chem. Commun.* (1968) 1108.
8. A. Kornhauser and M. A. Pathak, *Z. Naturforsch.* **27h** (1972) 550.
9. G. S. Hammond, N. J. Turro, and P. A. Leermakers, *J. Phys. Chem.* **66** (1962) 1144.
10. G. O. Schenck, *Naturwissenschaften* **35** (1948) 28; G. O. Schenk, *Z. Electrochem.* **64** (1960) 997; G. O. Schenck and R. Steinmetz, *Bull. Soc. Chim. Belg.* **71** (1962) 781.
11. A. Wacker, A. Kornhauser and L. Träger, *Z. Naturforsch.* **20b** (1965) 1043.
12. For a recent review see: D. E. Sunko and S. Borčić in: C. J. Collins and N. S. Bowman (Eds.), *Isotope Effects in Chemical Reactions,* Van Nostrand Reinhold Co., New York 1970.

13. A. Wacker, H. Dellweg, L. Träger, A. Kornhauser, E. Lodemann, G. Türck, R. Selzer, P. Chandra, and M. Ishimoto, *Photochem. and Photobiol.* **3** (1964) 369.
14. A. Kornhauser and J. B. Burnett, *Progress in Photobiology* in: G. O. Schenck (Ed.), *VI International Congress in Photobiology,* Bochum 1972. Paper No. 013.
15. R. D. Brown and C. E. Holt, *Anal. Biochem.* **20** (1967) 358.
16. E. T. Bush, *Anal. Chem.* **36** (1964) 1082.

SAŽETAK

Izotopni efekti kod fotosenzibilizirane dimerizacije pirimidina

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Primjenom sekundarnih izotopnih efekata, pokazano je da fotosenzibilizirana dimerizacija pirimidina teče pretežno preko Schenckova predajnog mehanizma.

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