PHOTOCHEMISTRY OF IODOURACIL. II

EFFECTS OF SULFUR COMPOUNDS, ETHANOL AND OXYGEN*

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The effect of several compounds on the photoproducts obtained from UV-irradiation of iodouracil (IU) has been investigated. A mechanism has been postulated based on these studies that may explain the specific protection afforded by cysteamine and cystamine on the survival of UV-irradiated T1 phage substituted with bromouracil or IU (Hotz, 1963; Rupp and Prusoff, 1964 and unpublished data).

The radiochromatograms shown in Fig. 1 indicate that uracil was the major photoproduct following irradiation of IU-2-C^{14} in ethanol or in aqueous solutions of cysteamine and mercaptoethanol. In the presence of cystamine, a large peak appeared near the origin which has been identified tentatively as the thioether, S-(5-uracilyl)cysteamine. (This peak was observed also when IU-2-C^{14} was irradiated in the presence of cysteamine and was presumably due to the presence of cystamine.) The UV absorption spectrum is similar to that of 5-uracilyl-disulfide (Bardos et al., 1955) and the UV absorption spectrum of the reaction product with dinitrofluorobenzene

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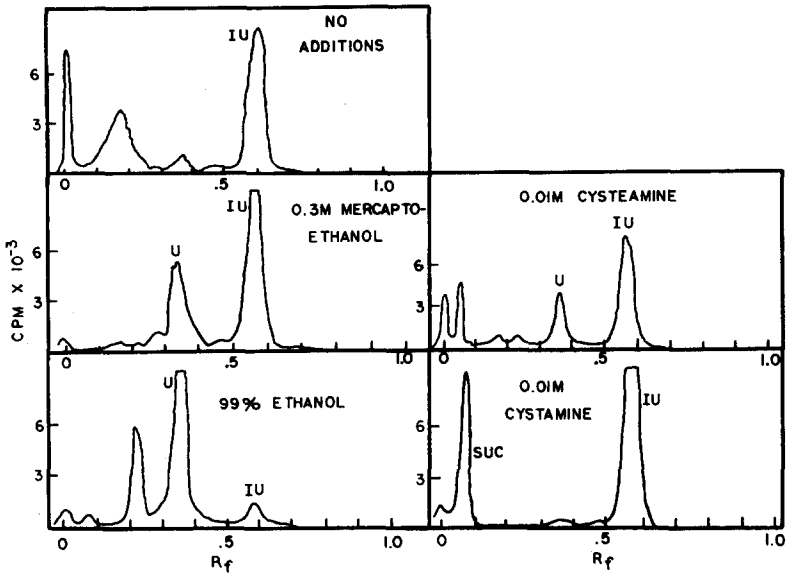

Fig. 1. Radiochromatograms of irradiated IU-2-C^{14}. Iodouracil (0.5 ml, 25-50 mμmoles, 3.5 mc/mmole) was irradiated for fifteen min at a distance of 5 cm from a GE 15W germicidal lamp. Sulfur compounds were present in aqueous solutions at the concentrations indicated. The entire reaction mixture was applied to a 5 x 4 cm area on a Whatman #1 paper strip and concentrated into a narrow band with H_2O as described by Wulff (1963). The chromatograms were developed by the descending technique with 86% aqueous butanol and scanned with a Packard radiochromatogram scanner. Peaks identified on the chromatograms have also been obtained by anion exchange chromatography. IU, iodouracil; U, uracil; SUC, S-(5-uracilyl) cysteamine.

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produced by the method described by Dubin (1960) indicated the presence of a primary amino group.

Studies in the preceding paper indicated that O_2 is involved in the photochemical degradation of IU in water. Since the formation of a thioether in the presence of cystamine presumably does not involve O_2, the opportunity was afforded to investigate the possible competition between O_2 and cystamine for a common intermediate (Fig. 2).

Irradiation of IU-2-C^{14} in air was performed in the presence of a concentration of cystamine that produced approximately equal

amounts of the thioether (cystamine product) and those photoproducts normally formed in the absence of cystamine (water product). When IU-2-C^{14} was irradiated in the same concentration of cystamine but in a N_2 atmosphere, the "cystamine product" was obtained in very high yield, whereas irradiation of the reaction mixture in O_2 resulted

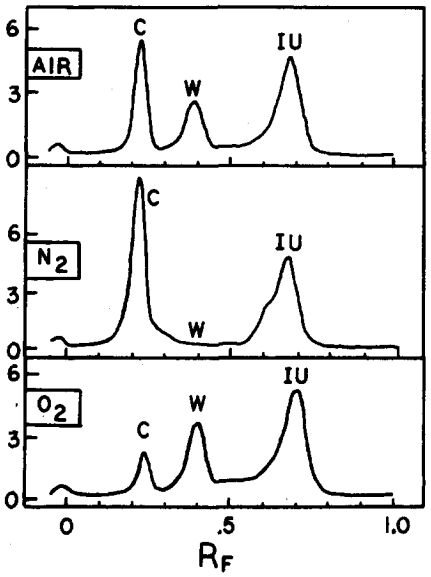

Fig. 2. The effect of oxygen, nitrogen and air on the photolysis of iodouracil-2-C^{14} in the presence of cystamine. A solution of IU-2-C^{14} (0.5×10^{-4} M) in the presence of cystamine (3×10^{-4} M) was irradiated in a 25 mm cylindrical cell (#600-IS, Scientific Cell Co., Forest Hills, N. Y.) with the optical surfaces horizontal. The appropriate gas was passed through the cell at a rate of 200 ml/min for 15 minutes before irradiation as well as during irradiation (15 min, 5 cm from a GE 15W germicidal lamp). The solution was stirred with a magnetic stirring bar. Following irradiation the entire reaction mixture was applied to Whatman #1 paper, concentrated at the origin as indicated in Fig. 1, and developed in the isopropanol-HCl system described by Wyatt (1951). In this system, the various products obtained following the irradiation of IU-2-C^{14} in water concentrate in one main area (R_f 0.40) termed W for "water product." The main photoproduct obtained following the irradiation of IU-2-C^{14} in the presence of a high concentration of cystamine is indicated by C for "cystamine product."

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in an increase in the proportion of "water product" to that of "cystamine product." These findings suggest a competition between cystamine and O_2 for a reactive intermediate.

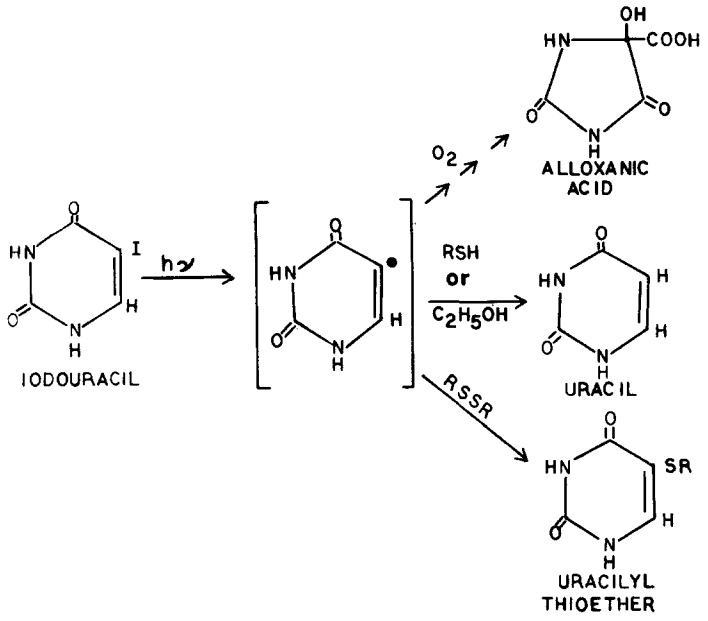

Fig. 3. Proposed scheme of the photolysis of iodouracil indicating the role of the uracil radical as a common intermediate in the formation of different products.

The formation of a uracil radical as a common intermediate is suggested (Fig. 3) from consideration of these products and the failure to find any photoproduct that contained both C^{14} and iodine (from IU-2-C^{14} or IU-I^{125}). This radical may be produced by a photolytic cleavage of the carbon-iodine bond. The formation of the various products can be explained by subsequent dark reactions of the uracil radical. Combination of the uracil radical with O_2 followed by subsequent reactions may lead to the product which is converted into alloxanic acid in base. The formation of uracil by irradiation of an aqueous solution of IU in the presence of mercaptans is readily explained since radicals are known to abstract hydrogen atoms very rapidly from these compounds. The formation of uracil by irradiation of IU in ethanol can also be explained by hydrogen abstraction. (The peak at R_f 0.21 (Fig. 1) is readily converted to uracil and may be the ethanol addition product of uracil.)

Phenyl radicals readily abstract hydrogen atoms from organic solvents such as ethanol and do not abstract hydrogen atoms from water, but rather persist in the latter until they react with some other molecule more susceptible to attack (Waters, 1959). The thioether, obtained in the presence of cystamine, is probably formed by the attack of the uracil radical on one of the disulfide sulfur atoms in the manner described for the S_H2 (bimolecular homolytic substitution) reaction of phenyl radicals and aliphatic disulfides (Pryor and Platt, 1963; Pryor and Guard, 1964).

The photochemistry of IU reported here has been examined in the hope that IU incorporated into DNA may behave similarly. UV survival studies of T1 phage substituted with IU can be reasonably interpreted in terms of the mechanism of photolysis of iodouracil presented here. Reaction of a macromolecular uracil radical with oxygen is suggested to be a lethal event if it occurs in a critical portion of the DNA molecule. However, if the macromolecular uracil radical were to react by hydrogen abstraction to form uracil, we suggest that the coding should remain unaltered and that this type of conversion would not constitute a lethal event. The protective effects of cysteamine and mercaptoethanol can be explained on this basis. Although uracil in DNA would be expected to form dimers with neighboring thymine or uracil residues, this reaction is not considered to be important in the phage survival studies since such an event would require a minimum of two hits in a very small region of the DNA polymer and therefore would not be expected to occur before a large number of other single hits had occurred along the DNA molecule.

The formation of the thioether, S-(5-uracilyl)cysteamine, as the major product of the irradiation of IU in the presence of cystamine is of interest in relation to its possible occurrence at the macromolecular level. We have found that cystamine protects T1 phage

substituted with IU as effectively as does cysteamine (Rupp and Prusoff, unpublished data). We suggest, for a working hypothesis, that the thioether is formed in the phage DNA and codes like thymine or uracil because the coding side of the pyrimidine has been retained. The formation of the thioether provides a mechanism in which protein and DNA may become covalently linked through attack of a photochemically produced DNA radical on a protein disulfide link. Crosslinking of protein and DNA has been observed (Smith, 1962b; Alexander and Moroson, 1962) but the chemical nature of the crosslink was not determined.

The mechanism of photolysis of iodouracil presented here may explain the results obtained with bromouracil in two species of bacteria where the same photoproducts were obtained but in different proportions (Smith, 1964; Wacker et al., 1961). These results may reflect different intracellular concentrations or availability of mercaptans, disulfides or other compounds that may react with the postulated uracil radical.

Although the mechanism described here for the photochemical reaction of iodouracil is compatible with the UV survival of IU substituted phage, its final acceptance or rejection must await further chemical studies at the macromolecular level.

In DNA, the uracil radical may conceivably react with adjoining DNA bases by routes which may not be observed in our model experiments. Smith (1962a) has suggested that dimers derived from bromouracil may arise by a mechanism comparable to the formation of biphenyl when iodobenzene is irradiated in benzene, a process almost certainly involving the phenyl radical (Wolf and Kharasch, 1961; Blair et al., 1959, 1960). A dimer formed in this manner would have only one bond linking the two rings in contrast to the two bonds that form the cyclobutane ring of the known thymine and uracil dimers. A dimer of this type (single bond) would be consistent with the kinetics of

phage inactivation since it would be formed from IU in a one hit process in contrast to the situation discussed previously where one hit would convert IU to uracil and a second hit to a dimer. Another possibility is suggested by the studies of Blair et al. (1959, 1960) where phenyl radicals, produced by UV irradiation of iodobenzene, abstracted hydrogen atoms from other iodobenzene molecules to form benzene. If a uracil radical in DNA were to react with a neighboring base in a similar manner, the uracil radical would be converted to uracil and the neighboring base would be left as a radical subject to further reactions that may result in a lethal alteration.

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