5-Ethyldeoxyuridine, a Thymidine Analog: Photochemical Transformation

Abstract. *Irradiation at 254 millimicrons transforms 5-ethyldeoxyuridine to deoxyuridine by way of photohydration of the 5,6 bond and elimination of ethanol. At wavelengths to the red side of 265 millimicrons, photodimerization is the principal reaction, with a pronounced oxygen effect. The results are related to the photochemistry of thymidine and of bacteriophages containing incorporated 5-ethyluracil in place of thymine.*

It has been shown that 5-ethyluracil (EtU) can substitute for thymine in bacterial DNA (*1*), and that 5-ethyldeoxyuridine (EtUdR) is readily incorporated into phage DNA (*2*). A preliminary study of the photochemistry of phage T_3, in which 65 percent of the thymine residues had been replaced by 5-EtU, revealed that the sensitivity to radiation (at 254 mμ), as well as subsequent photo- and dark-reactivation, was practically identical with that for the normal phage. Therefore of interest was examination of the photochemical behavior of ethyluracil and its glycosides.

Irradiation of a neutral aqueous solution of EtUdR at 254 mμ leads to gradual disappearance of the principal absorption maximum at 267 mμ, with a quantum yield of about 4×10^{-3} compared to 21×10^{-3} for deoxyuridine (UdR). After irradiation, from 70 to 80 percent of the loss in absorption can be restored by acidification, alkalization, or heating at neutral *p*H (Fig. 1). This fact is reminiscent of the simple dark-reversible photohydration of UdR and other uracil glycosides (*3*).

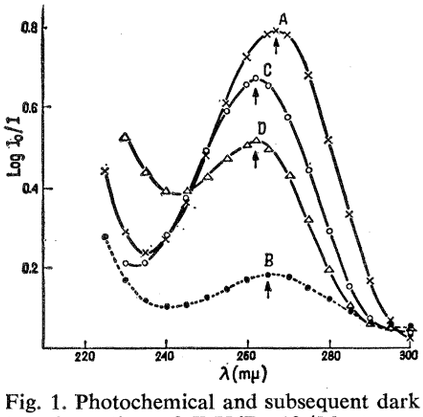
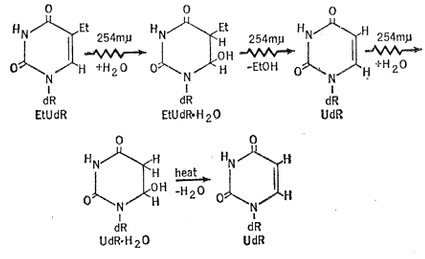


Fig. 1. Photochemical and subsequent dark transformation of EtUdR, $10^{-4}M$ at neutral *p*H. (*A*) Absorption spectrum before irradiation; (*B*) spectrum after 180-minute irradiation at 254 mμ; (*C*) spectrum of irradiated solution after heating for 2 hours at 100°C; (*D*) spectrum of heated irradiated solution at *p*H 12. Arrows point to absorption maxima of spectra.

Closer examination demonstrated that the photochemical reaction must be more complex. The λ_{max} of the dark-reverted absorption spectrum was at 262 mμ, and the radiation sensitivity of the reverted product was increased by a factor of 4 to 5—that is, it was similar to that for UdR.

Thin-layer chromatography of the irradiated EtUdR solution, before and after dark "reversal," showed that in fact the "reverted" product was UdR, and that the irradiated solution contained hydrated UdR and UdR (70 and 30 percent respectively); with MN-300 cellulose and water-saturated butanol the R_F values for EtUdR, UdR, and UdR·H_2O were 0.77, 0.48, and 0.27, respectively. The UdR was further identified by elution and absorption spectroscopy at neutral and alkaline *p*H; the UdR·H_2O, by elution, dark reversal to UdR, and identification of the latter. The light and dark reactions undergone by EtUdR may therefore be as follows:



Attempts to isolate EtUdR·H_2O were unsuccessful, probably because of its high sensitivity to radiation, but the photochemical liberation of ethanol was confirmed directly by use of an enzymic procedure (*4*).

The foregoing reaction sequence was also exhibited by the α-anomer of EtUdR, EtUR, and 1-methyl- and 1,3-dimethyl-EtU, but with different quantum yields. The reaction rates were independent of concentration over the range 10^{-3} to $10^{-4}M$ and exhibited no oxygen effect.

Attention was then directed to the effect of irradiation at longer wavelengths, the radiation source being a high-pressure mercury lamp with a Jena WG-7 filter to cut off at 265 to 270 mμ. Under these conditions irradiation likewise led to disappearance of the characteristic absorption maximum, but the photoproduct isolated by thin-layer chromatography was stable to heat at neutral *p*H and to acid and alkali

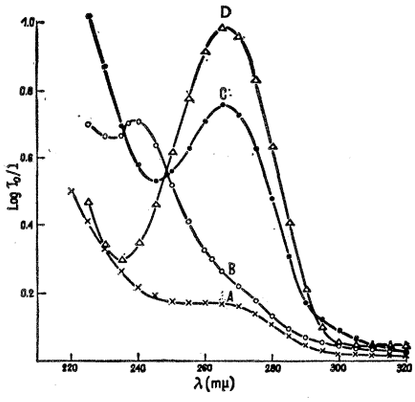


Fig. 2. Photoproduct resulting from irradiation, at wavelengths to the red side of 265 mμ, of a $10^{-3}M$ neutral aqueous solution of EtUdR. (*A*) Absorption spectrum of photoproduct at neutral *p*H (the shoulder at 260 mμ indicates the presence of a small amount of initial product); (*B*) spectrum at *p*H 13; (*C*) spectrum after irradiation at 254 mμ; (*D*) spectrum of solution in *C* after neutralization.

at room temperature. At neutral *p*H it exhibited only end absorption in the ultraviolet, but at alkaline *p*H it possessed an absorption maximum at 240 mμ, as for uracil and thymine photodimers at *p*H 13 (*5*); in fact, irradiation of the isolated photoproduct, in either neutral or alkaline medium at 254 mμ, led to the reappearance of EtUdR, which was confirmed spectroscopically (Fig. 2) and by thin-layer chromatography. Thus it appears that the principal product of irradiation of EtUdR at $\lambda > 265$ mμ is the photodimer.

This finding was further confirmed by comparison of the properties of the photoproduct with those of the photodimer of EtUdR, isolated from an irradiated frozen aqueous solution of the latter, and by the observation that the photochemical reaction at $\lambda > 265$ mμ was extremely sensitive to the presence of oxygen—even more so than the aqueous-solution photodimerization of orotic acid (*6*)—and suggestive of oxygen quenching of a triplet-state intermediate.

Although our irradiation system did not enable absolute estimation of the quantum yield for the dimerization reaction, qualitative measurements indicated that the value is in the neighborhood of 0.02—not less than that for thymine residues in DNA. Comparison of the rates of photodimerization of EtUdR and thymidine in ice at 254 mμ showed only a 15-percent difference between the two. (By contrast, EtU dimerizes much less readily than thymine under these conditions.)

It is also worth noting that the photodimerization reaction at $\lambda > 265$ mμ was accompanied by formation of only traces of UdR—that is, photohydration was practically absent under these conditions. This, together with the absence of an O_2 effect at 254 mμ, and the pronounced influence of O_2 at $\lambda > 265$ mμ, suggests that the difference in photochemical reactions may be due to formation of different excited states, at least in aqueous medium. In a polynucleotide chain, where the EtU residues are suitably oriented for dimerization, both photohydration (with concomitant reversal to hydrated uracil) and dimerization would be expected as competing reactions, and results of preliminary experiments with synthetic polynucleotides are in agreement with this idea. The values for the quantum yields of the two reactions are also qualitatively in agreement with the unchanged sensitivity to radiation of phage T_3 containing incorporated EtU.

These results are of additional interest in relation to the aqueous-solution photochemistry of thymidine. The relative radiation resistance of thymine in aqueous medium at $\lambda = 254$ mμ has been variously interpreted in terms of formation of the photohydrate, which is unstable because of its rapid reversion to the parent compound (*3*, *7*, *8*). However, Nofre and Ogier (*9*), who reported the chemical synthesis of hydrated thymine, claimed that its half-life at neutral *p*H and room temperature is of the order of several hours, apparently in contradiction to the above hypothesis (*9*).

Preliminary trials with a view to demonstrating the possible photohydration and successive photoelimination of methanol from irradiated thymidine have so far been unsuccessful. On the other hand we have found that about 20 percent of the product (or products) of irradiation of thymidine in solution at $\lambda = 254$ mμ can be dark-reverted to one having an absorption maximum at about 265 mμ, but the nature of this product has not yet been elucidated.

Experiments with other 5-alkyl uracil nucleosides have shown some similarity in behavior to EtUdR, one of the final products being UdR. Further studies of these photochemical reactions should shed additional light on the photochemistry of thymine nucleosides and of thymine residues in DNA (*10*).

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9. C. Nofre and M.-H. Ogier [*Compt. Rend. Acad. Sci. Paris* **263**, 1401 (1966)] claim to have shown that their synthetic 5,6-dihydro-6-hydroxythymine is the *trans* isomer. It is of course possible, if photohydration of thymine does occur, that the *cis* isomer is formed preferentially and that this is the more labile.
10. We thank Maria Zylonis for technical assistance, and the World Health Organization, the International Atomic Energy Agency, and the Wellcome Trust for support.

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High-Resolution Nuclear Magnetic Resonance Spectra of Selectively Deuterated Staphylococcal Nuclease

Abstract. *An analog of staphylococcal nuclease has been prepared in which all amino acids, except the six following, are fully deuterated: tryptophan; methionine; tyrosine, in ring positions 2 and 6; histidine, in ring position 2; aspartic acid and asparagine, β-methylene; and glutamic acid and glutamine, γ-methylene. The analog has a much simpler high-resolution nuclear magnetic resonance spectrum than the fully protonated enzyme. The effects of calcium ion and of the inhibitor 3′,5′-thymidine diphosphate on the spectrum of the analog were readily detected.*

High-resolution nuclear magnetic resonance (NMR) spectra of proteins have thus far yielded rather little information because of the extensive overlap (envelopes) of the resonance lines of the constituent amino acids. Only the very few lines outside these envelopes —the histidine C2-H (protons on the carbon in the 2-position) resonances, which can be seen downfield from the region of the aromatic spectrum (*1*, *2*) and the high-field peaks seen in some proteins upfield from the methyl region (*3*, *4*)—have proved amenable to detailed interpretation. Nevertheless, these few studies do demonstrate that unique information is provided by NMR spectra of proteins. To take advantage of this information, procedures must be devised for simplification of the spectra.

In principle, the NMR spectral envelope of proteins can be simplified (i) by increasing the strength of the applied field, thereby increasing the separation between component lines, or (ii) by reducing the number of proton resonance lines through selective deuteration of the protein. In practice, however, the best available spectrometer (220 Mhz) does not give sufficient resolution to fully decipher the information contained in the spectrum (*3*). The chemical shifts of numerous amino acid peaks so overlap that many of the relatively broad peaks in proteins would not be separated even at frequencies of 1000 Mhz or more. Furthermore, the task of assigning the multitude of peaks would still remain. Jardetzky has indicated (*5*) that selective deuteration of proteins provides the method of choice in protein NMR spectroscopy. We now report the experimental realization of this proposal. Similar experiments have also been performed by Crespi, Rosenberg, and Katz (*6*).

The criteria for selecting staphylococcal nuclease as the object of our studies have been outlined (*5*). The nuclease and its selectively deuterated analog have been isolated from a pure culture of the Foggi strain of *Staphylococcus aureus* by a modification of the method of Fuchs, Cuatrecasas, and Anfinsen (*7*). In the preparation of the normal, protonated nuclease, the culture was grown on a complex medium containing casamino acids and yeast hydrolyzate. A chemically defined, synthetic medium containing a mixture of deuterated and protonated amino acids was used in the preparation of the selectively deuterated nuclease (*8*). The deuterated amino acid mixture used to prepare the selectively deuterated enzyme was obtained by the hydrolysis of protein isolated from algae (*Scenedesmus obliquus*) grown in 99 percent D_2O by Merck Sharp & Dohme of Canada. A portion of the mixture was separated by ion-exchange column chromatography, and the isotopic composition of the individual amino acids was determined by proton magnetic resonance spectroscopy. Proton resonance lines were un-