Identification of New Products in the γ-Radiolysis of Deoxygenated Solutions of Uracil and the Effect of pH

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Summary A dramatic pH dependence on the yield of radiation-induced uracil destruction has been observed, and is attributed to the increased yield of a new radiolytic dimer in acid solution.

IN order to understand the mechanisms of radiation damage to nucleic acids, many experiments have been conducted on pyrimidines in dilute aqueous solution. The *G*(-U) values† for uracil in dilute, deoxygenated solutions vary from 1—2·7[1,2] and some of the products have been identified.[1,3,4] We now report two new observations concerning deoxygenated uracil solutions irradiated with ^{60}Co γ-rays. The first is the dependence of *G*(-U) on the pH of the solution during irradiation for pH values in the range pH 5—9, and the second is the detection of a dimeric product which has not been previously reported. It is the

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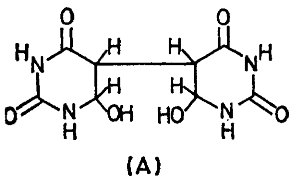
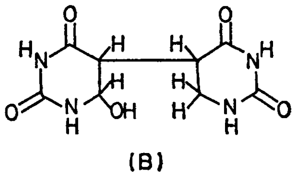
† G(-U) is the G-value for the destruction of uracil defined as the number of uracil molecule changed per 100 eV of absorbed energy in the solutions.

large increase in the yield of this dimeric product which is responsible for the large increase in G(-U) under acidic conditions.

We have obtained reproducible values of G(-U) by carefully deoxygenating and controlling the pH of the irradiated solutions. The water used was quadruply distilled, the last two stages in quartz. The oxygen was removed by bubbling the solutions with ultra-pure nitrogen for at least 30 min before irradiation. The pH of solutions of pH 6—8 was found to drift toward higher values during bubbling with N_2, possibly because of the removal of dissolved CO_2. By adjusting the pH with small amounts of NaOH or H_2SO_4 before bubbling and after about 15 min bubbling, the pH could be controlled to ±0·1 unit.



(A)



(B)

The values of G(-U) measured by loss of o.d.$_{260}$ or by chromatography of samples containing uracil-2-^{14}C, agree to within 10%. We have found that G(-U) is quite reproducible, but is strongly dependent on pH (Figure) and varies by a factor of 4 between pH 6—8. The G-values follow a typical ionization curve with $pK_a = 6·8$ and plateau values of 3·3 at pH 5 and 0·7 at pH 8·5. This pK_a of 6·8 does not correspond to the pK_a of uracil which is 9·4. There is no strong dependence on uracil concentration; the G-values for samples from 10^{-4} M—10^{-2} M were in the range 3·1—3·5 at pH 5.

Table. Radiolysis products of uracil

	R_u[a]	Identification	G(Product) pH 5	 pH 8·3
(I)	0—0·12	radiolytic dimer	2·4	0·4
(II)	0·2	*cis*-glycol +5-hydroxy-5,6-dihydrouracil	0·1	0·1
(III)	0·43	*trans*-glycol	0·3	0
(IV)	0·63	6-hydroxy-5,6-dihydrouracil	0·1	0·2
(V)	0·8	isobarbituric acid 5,6-dihydrouracil	0·4	0·1

[a] $R_u = R_f$ (product)/R_f (uracil).

For chemical characterization of the products, uracil solutions (10^{-2} M) containing uracil-2-^{14}C were irradiated at pH 5 and pH 8·3 and the products separated by repeated paper chromatography. The R_u values, G-values, and identifications of the products are shown in the Table. Products (II) to (V) have been seen in other studies[1,4] and will not be discussed further here. The properties of (I) which is the major product at both pH 5 and pH 8·5 do not correspond to any of the known radiolysis products, or uracil. Chemical tests have shown that it is not one of the alloxan family of compounds as suggested by Smith and Hays,[4] nor is it a cyclobutane photodimer because it does not revert to uracil when irradiated by 240 nm light.[5] The mass spectrum of (I) is extremely complex and shows peaks up to molecular weights of 242 and 258. On the basis of this and other evidence,[6] we have concluded that (I) is most likely a mixture of structures such as (A) and (B) which are joined by a single bond between the 5 and/or 6 positions of the pyrimidine rings.

The extremely low value of G(-U), (0·8), found at pH 8·5 cannot be accounted for by normal disproportionation reactions since the total G-value of the radicals in the solution is approximately 6. This low value can be explained by a mechanism recently proposed by Haysom *et al.*[7] in which two pyrimidine radicals (·R) react with each other by an electron transfer process [reaction (1)].

$$\begin{array}{l} \cdot R + \cdot R \longrightarrow R^+ + R^- \\ \qquad\qquad\quad \downarrow H_2O \ \downarrow H_2O \\ \qquad\qquad\quad \text{product or unsaturated} \\ \qquad\qquad\quad \text{pyrimidine} \end{array} \qquad (1)$$

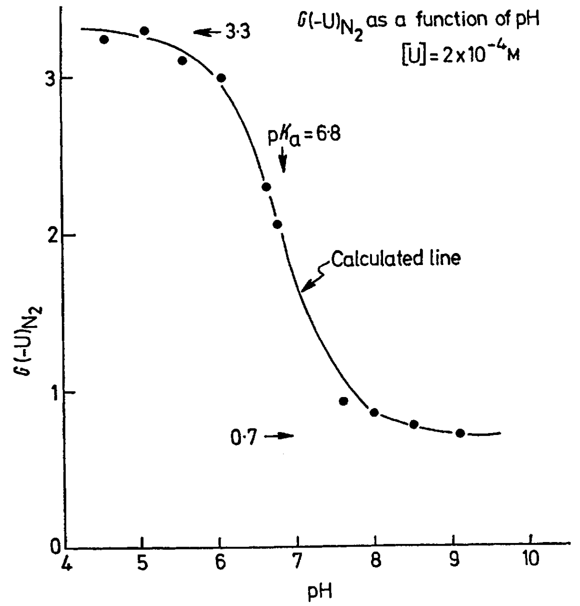


Figure. G(-U) measured by loss of o.d.$_{260}$ plotted as a function of pH. The line is a calculated ionization curve with plateau values of 3·3 and 0·7 and centre point at pH 6·8. [U] = 2 × 10^{-4} M. Dose range: 0—20 kRad.

The resulting ions react with water either to form products or to revert to the unsaturated pyrimidine. In the case of uracil where dimeric products are formed, we propose reaction (2). This explains how dimers and monomeric products arise. It also explains the pH dependence of the yields since the uracil radicals formed by reaction of e^-_{aq} and ·OH with uracil appear to ionize near pH 7.[8] Thus at pH 8·5 some of the radicals will be charged, and if they

$$\begin{array}{l} \qquad\quad\ \nearrow \text{Dimer} \\ \cdot R + \cdot R \\ \qquad\quad\ \searrow R^+ + R^- \\ \qquad\qquad \downarrow H_2O \ \downarrow H_2O \\ \qquad\qquad \text{products or} \\ \qquad\qquad \text{uracil} \end{array} \qquad (2)$$

undergo reaction (2) different products and yields would be expected. The mechanism explains how both the yields and nature of the products depend on the state of ionization of the transient uracil radicals. Preliminary experiments carried out in this laboratory have shown that the *G*-values for the destruction of a number of other pyrimidines and polynucleotides are also dependent on pH. This evidence implies that reaction (2) may be a general mechanism in the radiolysis of nucleic acids and may also be of general importance in radical–radical reactions.

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