Reactions of Hydroxyalkyl Radicals with Uracil

Sudhindra N. Bhattacharyya* and Parikshit C. Mandal†

*Nuclear Chemistry Division, Saha Institute of Nuclear Physics, Sector-1, Block-'AF',
Bidhannagar, Calcutta 700 064, India*

The base degradation yields when aqueous solutions of uracil containing methyl, ethyl or t-butyl alcohol were γ-irradiated, were 2.0 ± 0.2, 4.2 ± 0.5 and 1.3 ± 0.1, respectively. These differences are attributed to differences in reactivity of CH_2OH (I), CH_3CHOH (II) and $CH_2(CH_3)_2COH$ (III) with uracil (U). III does not react with U whereas I and II react with U to form U^- and aldehyde.

To obtain an insight into the mechanism of the radiation-chemical degradation of DNA, many radiolytic studies with model compounds such as pyrimidine bases have been undertaken.[1,2] Recently[3] it has been established that the reducing species, e_{aq}^-, which is an important product of water radiolysis, inactivates the DNA molecule, and studies with individual pyrimidine bases have indicated that e_{aq}^- readily forms adducts with them, leading ultimately to their degradation.[1,4-6] Reactions of e_{aq}^- are usually studied in the presence of OH scavengers such as alcohols. Hence a study of the reactions of the hydroxyalkyl radicals that are formed as a result of the reaction of OH with an alcohol with pyrimidine bases is important in this context. The hydroxyalkyl radical is formed *via*

$$ROH + OH \rightarrow RO + H_2O \tag{1}$$

where ROH represents the alcohol and RO the hydroxyalkyl radical. In this paper we show that the methanol and ethanol radicals reduce uracil in deaerated solution, whereas no such reduction was discernible in case of radicals derived from t-butyl alcohol.

Experimental

Materials

Uracil (Merck) used was recrystallised three times from triply distilled water. [2-^{14}C]uracil (14 mCi dm^3 mmol^{-1}) was procured from BARC, Bombay. Other chemicals and solvents were of analytical-reagent grade. Deaeration was carried out by bubbling pure argon gas through each 5 cm^3 of experimental solution for 30 min. Pure N_2O was used in the investigation.

Irradiation

The irradiation source comprised a ^{60}Co gamma cell. The dose rate (1.92×10^{17} eV cm^{-3} min^{-1} or 30.7 Gy min^{-1}) was measured with the help of a Fricke dosimeter, taking $G(Fe^{3+}) = 15.6$; Fe^{3+} was determined spectrophotometrically at 305 nm.

Analysis

Uracil solutions containing various alcohols were irradiated in neutral solution (pH 5–6) without a buffer. No significant shift in pH was observed after irradiation in the dose range $(3-40) \times 10^{17}$ eV cm^{-3}, *i.e.* 48–640 Gy. The products of radiolysis were separated

by paper chromatography using n-butyl alcohol–water (86:14) mixtures as solvent and they were identified as described earlier.[7-11] The base degradation yield and the yields of the radiolytic products were determined from the relative activity in the area of the respective peaks in the active chromatogram. The degradation of the base was also determined by spectrophotometry from the loss of absorbance at 260 nm. The carbonyl compounds were determined spectrophotometrically by using DNPH reagent.[12] The extinction coefficient was determined with respect to formaldehyde only.

Results and Discussion

Both the degradation of uracil and the product formation in the case of radiolysis of uracil in the presence of alcohols were determined at different absorbed doses, and the respective G values were ascertained from plots of yield *vs.* dose, which were found to be linear. The observed G values are shown in tables 1 and 2.

Fig. 1 shows the effect of t-butyl alcohol concentration on the observed $G(-U)$ values under different conditions. It is evident from the figure that under argon-saturated conditions $G(-U)$ decreases with increasing $[Bu^tOH]$ until $[Bu^tOH] \approx 5 \times 10^{-3}$ dm^3 mol^{-1}, whence there is no further change in $G(-U)$ with increasing alcohol concentration. At this high concentration of alcohol the OH radicals are scavenged,[11] leaving only e_{aq}^-. Hence the observed plateau value of $G(-U)$ is due to e_{aq}^- only. This is further corroborated by the observation that $G(-U)$ becomes insignificant when radiolysis is carried out in the presence of t-butyl alcohol ($> 5 \times 10^{-3}$ mol dm^{-3}) in air- or N$_2$O-saturated solution.

Fig. 2 shows the variation of $G(-U)$ with MeOH concentration. A consideration of the rate-constant data[1, 13] indicates that at the high concentrations of methyl alcohol $[(2-100) \times 10^{-3}$ mol dm$^{-3}]$ 51–98% of the OH radicals will be scavenged, the result being the formation of CH$_2$OH radicals. The $G(-U)$ value at such high concentrations of MeOH is *ca.* 1.3. Similar results were also obtained with EtOH. The effect of uracil concentration on $G(-U)$ under conditions where OH radicals are expected to be scavenged by alcohols was also studied, and the results are shown in fig. 3. Note that the range of uracil concentration chosen is sufficient to attain steady-state conditions for its radiolysis. Note also that the steady values of $G(-U)$ (fig. 3) under such conditions were found to be 2.0 ± 0.2 for MeOH, 4.2 ± 0.5 for EtOH and 1.3 ± 0.1 for Bu^{t}OH. Under these conditions e_{aq}^- reacts with uracil, giving U$^-$ which subsequently protonates to UH:[1, 4-6, 14]

$$U + e_{aq}^- \rightarrow U^- \tag{2}$$

$$U^- + H^+ \rightarrow UH. \tag{3}$$

Hence these differences in $G(-U)$ values may be attributed to the differences in reactivity of hydroxyalkyl radicals (RO) with uracil:

$$RO + U \rightarrow products \tag{4}$$

or with the protonated electron adduct of uracil, UH:

$$RO + UH \rightarrow products. \tag{5}$$

In the presence of MeOH, however, when the radiolysis was carried out in N$_2$O-saturated solution, the same yield values as in deaerated solutions were also observed (fig. 2). Under these conditions e_{aq}^- is scavenged by N$_2$O and hence neither U$^-$ nor UH is formed from e_{aq}^-. Neglecting the small contribution of $[G(H) \approx 0.5]$ it may be assumed that the system comprises mostly uracil and RO. Hence it must be concluded that the major pathway for uracil degradation under these conditions is the reaction between RO and uracil [reaction (4)].

Now, depending upon its reactivity, the hydroxyalkyl radical (RO) might undergo

Table 1. Yields of carbonyl compounds and $G(-U)$ in the γ-radiolysis of uracil $(2 \times 10^{-3}$ mol dm$^{-3})$ in the presence of various alcohols

alcohol	[alcohol] /mol dm^{-3}	condition	$G(-U)^a$	$G(>C=O)$
MeOH	0.4	argon-saturated	2.0	1.5
MeOH	0.4	N$_2$O-saturated	2.0	3.6
EtOH	0.4	argon-saturated	4.5	2.4
EtOH	0.4	N$_2$O-saturated	4.5	5.0
Bu^{t}OH	0.4	argon-saturated	1.3	0.0
Bu^{t}OH	0.4	N$_2$O-saturated	0.2	0.0

^{a} Measured by loss of absorbance at 260 nm.

Table 2. Yields of various radiolytic products in the γ-radiolysis of uracil in the presence of alcohols at *ca.* pH 5.6

G(products)	condition			
	A		B	
G(dimer)	0.2	(20%)	0.2	(9%)
G(hydroxydihydrouracil)	0.2	(20%)	0.3	(13%)
G(dihydrouracil)	0.4	(40%)	1.6	(69%)
G(−uracil)	1.0	$(1.3)^a$	2.3	$(2.0)^a$

(A) 2×10^{-3} mol dm^{-3} uracil in the presence of 0.4 mol dm^{-3} Bu^{t}OH in argon-saturated solution. (B) 2×10^{-3} mol dm^{-3} uracil in the presence of 0.4 mol dm^{-3} MeOH in argon-saturated solution. ^{a} Obtained from the loss of absorbance at 260 nm.
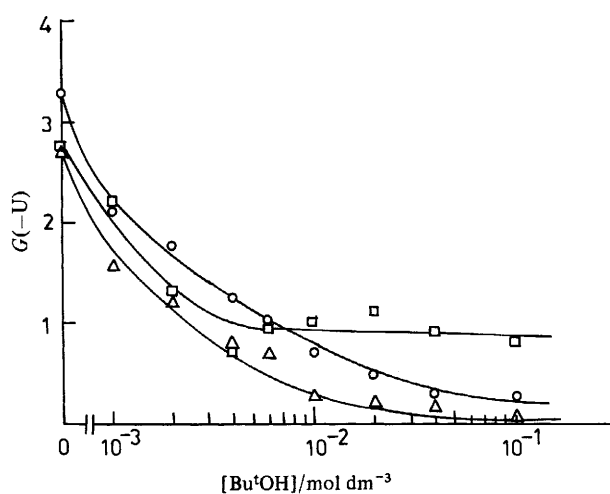
Fig. 1. Effect of t-butyl alcohol concentration on $G(-U)$: $\bigcirc$, N$_2$O-saturated solution; $\square$, argon-saturated solution; $\triangle$, aerated solution; pH 5.6.
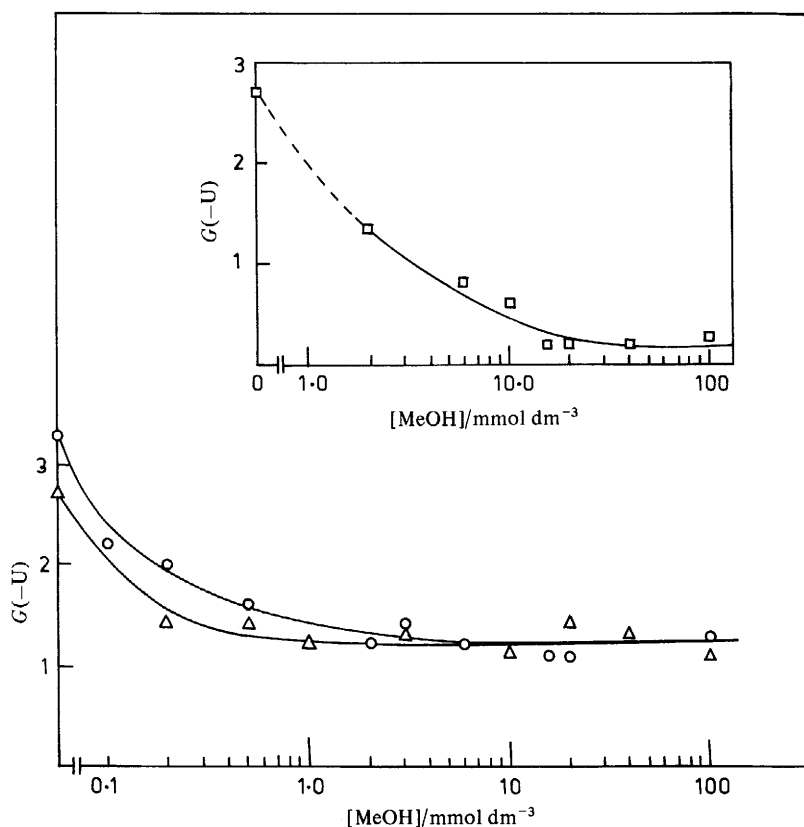
Fig. 2. Effect of methyl alcohol concentration on the radiolytic decomposition yield of uracil: □, aerated solution; ○, N_2O-saturated solution; △, argon-saturated solution; $[U] = 2 \times 10^{-4}$ mol dm^{-3}, *ca.* pH 5.5.
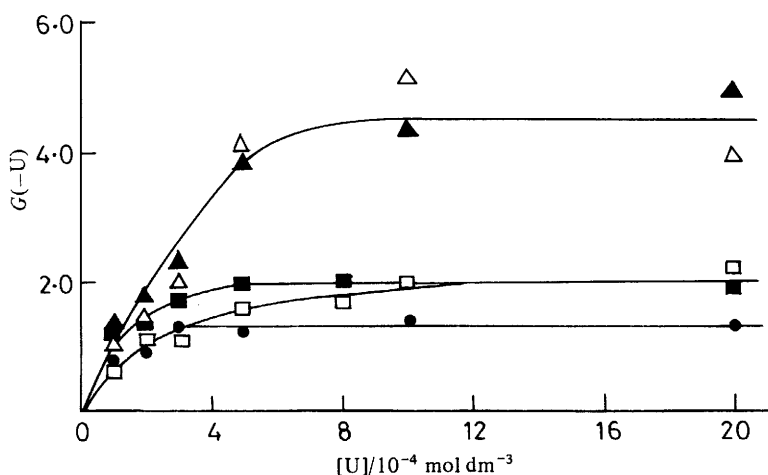

Fig. 3. Effect of increasing concentration of uracil on $G(-U)$ in the γ-radiolysis of uracil in the presence of methyl, ethyl and t-butyl alcohol at pH 5.6: ▲, in the presence of 0.4 mol dm^{-3} EtOH in argon-saturated solution; △, in the presence of 0.4 mol dm^{-3} EtOH in N_2O-saturated solution; ■, in the presence of 0.4 mol dm^{-3} MeOH in argon-saturated solution; □, in the presence of 0.4 mol dm^{-3} MeOH in N_2O-saturated solution; ●, in the presence of 0.4 mol dm^{-3} Bu^{t}OH in argon-saturated solution.

reaction with uracil to form an alcohol adduct, as observed by Kamal and Garrison[15] and Brown *et al.*[16]:

$$CH_2OH + U \rightarrow U\!-\!CH_2OH \tag{6}$$

or undergo oxidation by uracil, giving U^- and carbonyl compound:[17]

$$CH_2OH + U \rightarrow U^- + CH_2O + H^+. \tag{7}$$
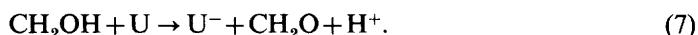
The formation of carbonyl compounds in the presence of MeOH and EtOH (table 1) indicates the occurrence of reaction (7). This is further supported by the fact that G(carbonyl) yields increased appreciably in N_2O-saturated solution, whereas there was no change in $G(-U)$. However, an insignificant value of $G(-U)$ and the absence of any carbonyl compounds in N_2O-saturated solution in the presence of Bu^{t}OH are indicative of the fact that the t-butanol radical does not reduce uracil to U^-. The observation is in agreement with the redox behaviour[18] of $\cdot CH_2(CH_3)_2COH$ compared with those of $\cdot CH_2OH$ and CH_3CHOH radicals, since the former is known to be oxidizing and the latter two are reducing.[19, 20] The observed data are therefore consistent with the major path for radiolytic degradation of uracil in the presence of alcohols involving the formation of U^- by the reaction of uracil with e_{aq}^- or with a hydroxyalkyl radical. U^- is then protonated to UH [reaction (3)], which can then undergo the following reactions:

$$2UH \longrightarrow UH^+ + UH^- \tag{8}$$

$$UH^+ + UH^- \longrightarrow dimer \tag{9}$$

$$UH^+ \xrightarrow{\text{H}_2\text{O}} UHOH \tag{10a}$$

$$UH^+ \xrightarrow{\text{H}_2\text{O}} U + H_3O^+ \tag{10b}$$

$$UH^- \xrightarrow{\text{H}_2\text{O}} UH_2. \tag{11}$$

This scheme is consistent with the observation that dihydrouracil is the major product (table 2). However, the increased yield of dihydrouracil (69%) in the presence of MeOH compared with that (40%) in the presence of Bu^{t}OH cannot be explained by means of the above scheme. In addition to the above-mentioned radical reactions there might be some other pathway leading to dihydrouracil from UH in the presence of reducing hydroxyalkyl radicals.

Further, it has been observed (table 1) that the $G(-U)$ and $G(>C\!\!=\!\!O)$ yields are relatively higher in the presence of EtOH compared with those in the presence of MeOH. These differences in yield can only be accounted for if it is assumed that hydroxyethyl radicals are much more reducing than hydroxymethyl radicals. However, this assumption is not improbable. This is supported by the fact that the tendency for electron transfer from the hydroxyethyl radical is much higher than that from the hydroxymethyl radical.[21]

Note that in our product analysis we could not detect any alcohol adduct, as was reported earlier.[15, 16] However, from kinetic studies in an aerated solution of uracil there is some indirect evidence for the formation of such products. In the presence of oxygen no product is expected to be formed from e_{aq}^-,[22] and base degradation is achieved mainly through the reactions

$$U + OH \rightarrow UOH \tag{12}$$

$$UOH \xrightarrow{\text{O}_2} products. \tag{13}$$

However, in the presence of alcohols which compete for OH [reaction (1)] degradation yields would follow the relationship

$$\frac{G(-U)_0}{G(-U)} = 1 + \frac{k_1}{k_{12}} \frac{[ROH]}{[U]} \qquad (14)$$

where $G(-U)_0$ is the degradation yield in the absence of alcohol, $G(-U)$ is the degradation yield in the presence of alcohol, $[U]$ is the concentration of uracil, $[ROH]$ is the concentration of alcohol and k_{12} and k_1 are the reaction rate constants for reactions (12) and (1), respectively. However, the hydroxyalkyl radicals might undergo reaction (4), as discussed, or undergo the reaction

$$RO + O_2 \rightarrow products. \qquad (15)$$

Thus, considering reactions (1), (4), (12) and (15) one finds[23]

$$Q = \frac{G(-U)_0}{G(-U)} = \frac{\left(1 + \dfrac{k_1}{k_{12}} \dfrac{[ROH]}{[U]}\right)\left(1 + \dfrac{k_{15}}{k_4} \dfrac{[O_2]}{[U]}\right)}{1 + \dfrac{k_1}{k_{12}} \dfrac{[ROH]}{[U]} + \dfrac{k_{15}}{k_4} \dfrac{[O_2]}{[U]}}. \qquad (16)$$

In the limiting case, when the alcohol concentration is sufficiently high to scavenge the OH radicals completely, the remaining uracil degradation depends only on reaction (4). Under these conditions eqn (16) should take the form

$$Q_\infty = 1 + \frac{k_{15}}{k_4} \frac{[O_2]}{[U]}. \qquad (17)$$

Q_∞ is 12.9 ± 1.2 and 16 ± 1.7 for MeOH and EtOH, respectively, from which k_{15}/k_4 is estimated to be 10.9 ± 2.4 and 11.1 ± 1.3, respectively.

Eqn (16) can be rearranged to give

$$Q = 1 + \frac{k_1}{k_{12}} \frac{[ROH]}{[U]}\left(1 - \frac{Q}{Q_\alpha}\right) \qquad (18)$$

where Q_∞ is independent of $[ROH]$.

If Q is plotted against $([ROH]/[U])(1 - Q/Q_\infty)$, a straight line is expected with slope k_1/k_{12}. The results are plotted in fig. 4. From the plots the values k_1/k_{12} have been determined to be 0.11 for MeOH and 0.4 for EtOH. It follows that the ratio of the rate constants for the reactions of OH with MeOH and EtOH is $0.11/0.4 = 0.28$, which is compatible with earlier results.[13]

t-Butyl alcohol, however, shows no such effect. Fig. 1 shows the effect of increasing concentration of Bu^tOH on the $G(-U)$ values and the plot of $G(-U)_0/G(-U)$ against $[Bu^tOH]/[U]$ ratio is shown in fig. 5. The observed results have been shown to yield a straight line, from which $k_1/k_{12} = 0.08$, close to the literature value.[13] The observed straight-line course of the kinetic plot as shown in fig. 5 points to the conclusion that the radical RO formed in case of Bu^tOH does not undergo any processes of the type envisaged in reaction (4).

From the above discussion it is evident that even in the presence of oxygen, the hydroxyethyl or hydroxymethyl radicals react with uracil to give products *via* reaction (4). In the previous section it was argued that in a deaerated medium the radiolytic products are formed mainly from U^- formed in reaction (7) and/or from reaction between e_{aq}^- and uracil. However, in the presence of oxygen U^- is known to revert back to uracil:[22]
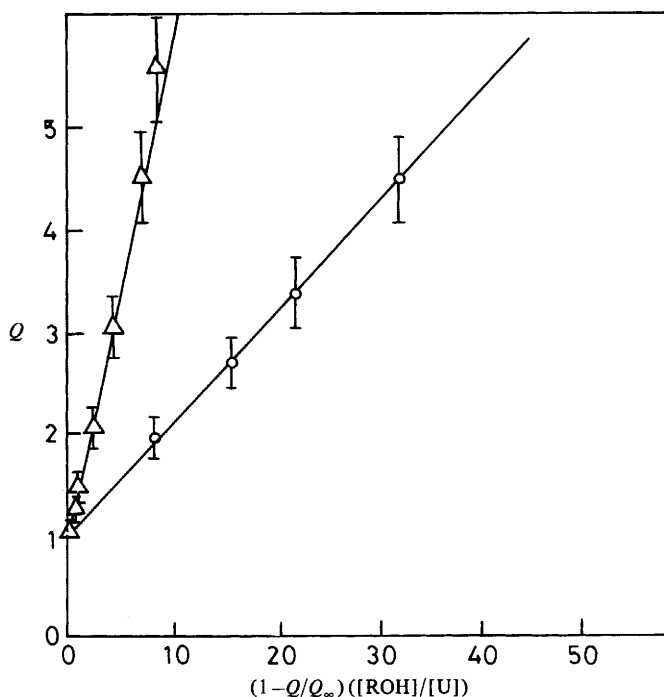
$$U^- + O_2 \rightarrow U + O_2^- \qquad (19)$$

$$UH + O_2 \rightarrow U(O_2)H \xrightarrow{-H^+} U(O_2)^- \rightarrow U + O_2^-. \qquad (20)$$
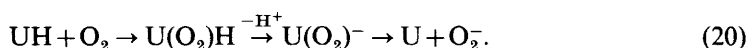
Fig. 4. Plot of $(1 - Q/Q_\infty)([ROH]/[U])$ against Q for methyl ($\bigcirc$) and ethyl ($\triangle$) alcohol. $[U] = 2 \times 10^{-4}$ mol dm^{-3}, *ca.* pH 5.6.
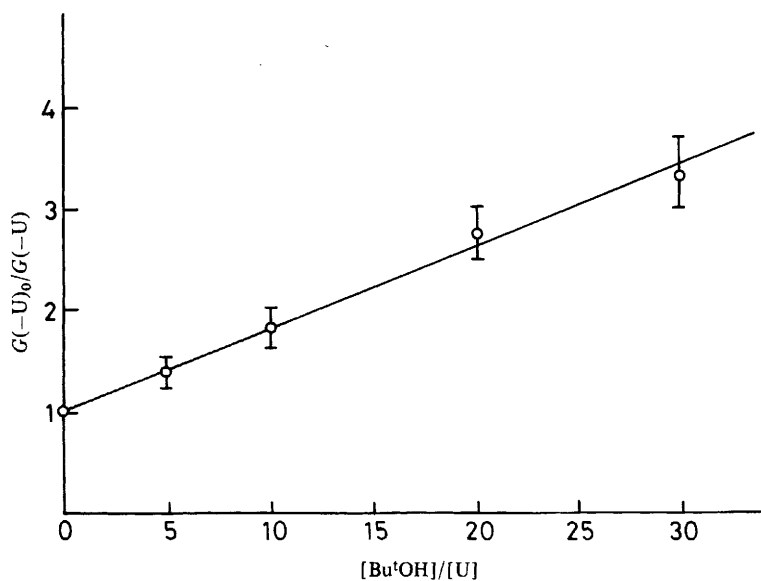

Fig. 5. Dependence of $G(-U)_0/G(-U)$ on $[Bu^tOH]/[U]$ in neutral aerated solution. $[U] = 2 \times 10^{-4}$ mol dm^{-3}.

Hence the product formed under the condition might be some adduct of alcohol radicals with uracil as was observed earlier by other workers.[15, 16] Failure to detect such products under our experimental conditions might be due to the fact that, owing to the small yield it could not be well separated from the uracil peak when developed in the paper chromatogram.

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