

RADIATION-INDUCED HYDROXYLATION OF THYMINE SENSITIZED
BY NITRO COMPOUNDS IN N₂O-SATURATED AQUEOUS SOLUTION

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Hydroxylation of thymine (1) to give thymine glycol (3) is remarkably promoted by the addition of nitro compounds (2a-f) in the γ -radiolysis of the N₂O-saturated aqueous solution, although overall decomposition of 1 is depressed to some extent. The G-value of 3 increases linearly with increasing the one-electron reduction potential of 2a-f.

Radiation-induced reactions of pyrimidine bases in aqueous solution have been extensively studied to elucidate the chemical mechanism of the DNA damage by ionizing radiation. It has been reported that pyrimidine bases undergo oxidation to give pyrimidine glycols and barbituric acid derivatives in relatively high yields on γ -ray irradiation in the presence of copper (II),¹⁾ ferricyanide,²⁾ tetranitromethane²⁾ and p-nitroacetophenone.³⁾ We now report that a variety of nitro compounds; 4-nitroimidazole (2a), 2-nitroimidazole (2b), 1-(2-nitro-1-imidazolyl)-3-methoxy-2-propanol (misonidazole, 2c), 1-methyl-2-nitroimidazole (2d), p-nitroacetophenone (2e) and 5-nitro-2-furoic acid (2f) are effective for promotion of the preferential oxidation of thymine (1) to give thymine glycol (3) in the γ -radiolysis of the N₂O-saturated aqueous solution. Particular emphasis is placed to the evidence that the efficiency of the nitro compounds increases with increasing their oxidizing abilities.

On ⁶⁰Co γ -ray irradiation (76 krad) of a deaerated aqueous solution (5 cm³) of thymine (1 mmol dm⁻³) buffered at pH 7.0±0.1 with phosphate (2 mmol dm⁻³), 1 decomposed with the G-value⁴⁾ for conversion, G(1)=1.81. Among the major products thymine glycol (3),^{5,6)} 5-hydroxy-5,6-dihydrothymine (4),⁷⁾ 6-hydroxy-5,6-dihydrothymine (5),⁷⁾ 5-hydroxymethyluracil (6), 5,6-dihydrothymine (7), 5-methylbarbituric acid (8)⁸⁾ and N¹-formyl-N²-pyruvylurea (9)⁹⁾ were detected by high-speed liquid

Table G-values of products in the γ -radiolysis of thymine (1)

| Product | Condition Additive E (A/A ⁻) ^a /mV | deaerated | | N ₂ O-saturated | | | | | |
|---|---|-------------------|-------------------|----------------------------|-----------|-----------|-----------|-----------|-----------|
| | | none | none | <u>2a</u> | <u>2b</u> | <u>2c</u> | <u>2d</u> | <u>2e</u> | <u>2f</u> |
| | | | | -527 | -418 | -398 | -390 | -353 | -317 |
| <u>3</u> thymine glycol (cis + trans) | | 0.13 | 0.28 | 0.66 | 0.85 | 1.76 | 1.47 | 2.10 | 1.95 |
| <u>4</u> 5-hydroxy-5,6- dihydrothymine | | 0.07 ^b | 0.18 ^b | 0 | 0 | 0 | 0 | 0 | 0 |
| <u>5</u> 6-hydroxy-5,6- dihydrothymine | | 0.08 | 0.12 | 0.03 | 0.02 | 0.07 | 0.03 | 0.07 | 0.04 |
| <u>6</u> hydroxymethyl- uracil | | 0.14 | 0.49 | 0.10 | 0.10 | 0.17 | 0.18 | 0.22 | 0.09 |
| <u>7</u> dihydrothymine | | 0.28 | 0.07 | 0 | 0 | 0.05 | 0.05 | 0.15 | 0.08 |
| <u>8</u> 5-methyl- barbituric acid | | 0.09 | 0.08 | 0.08 | 0.05 | 0.08 | 0.05 | 0.03 | 0 |
| <u>9</u> formylpyruvylurea | | 0.11 | 0.08 | 0.05 | 0.02 | 0.16 | 0.13 | 0.06 | 0.09 |
| G _{total} (<u>3-9</u>) | | 0.83 | 1.03 | 0.92 | 1.04 | 2.29 | 1.91 | 2.63 | 2.25 |
| G(unidentified) | | 0.97 | 2.09 | 0.79 | 1.55 | 0.68 | 0.28 | 0.07 | 0.31 |
| G(<u>1</u>) | | 1.81 | 3.17 | 1.71 | 2.59 | 2.97 | 2.19 | 2.70 | 2.56 |

a: one-electron reduction potential relative to normal hydrogen electrode (NHE) evaluated by means of pulse radiolysis.¹³⁻¹⁵⁾ b: estimated value.

phase chromatography (monitored with uv absorption at 210 nm) (Table). These products (the total G-value is 0.83) were confirmed by comparison with authentic samples either prepared according to the reported method⁵⁻⁹⁾ or obtained commercially. A number of the other products (G-value=0.98) have not yet been characterized.

Identical products were obtained by the radiolysis under N₂O-saturated conditions but the yield of the respective product differed significantly from the corresponding yield in the deaerated system (Table). As can be seen from the Table, the G-values of hydroxylated thymine derivatives 3, 4, 5 and 6 increase by a factor of 2-3 whereas that of hydrogenated thymine 7 falls to 1/4 in the presence of N₂O.

It has been well established that primary intermediate species involved in the radiolysis of aqueous solution are hydroxyl radical ($\cdot\text{OH}$), hydrated electron (e_{aq}^-) and hydrogen atom ($\cdot\text{H}$); $G(\cdot\text{OH})=2.7$, $G(e_{\text{aq}}^-)=2.7$, $G(\cdot\text{H})=0.55$ at pH 7. Since e_{aq}^- is converted efficiently into $\cdot\text{OH}$ via the reaction 1; $\text{N}_2\text{O} + e_{\text{aq}}^- \longrightarrow \cdot\text{OH} + \text{OH}^- + \text{N}_2$ (reaction 1), the increases in the yields of 3, 4, 5 and 6 in the N₂O saturated aqueous solution can be correlated directly with the increase in the $G(\cdot\text{OH})$ from 2.7 to 5.4. It is thus suggested that under the conditions of the $\cdot\text{OH}$ generation 1 undergoes both addition across its C5-C6 double bond to give 5- and 6-hydroxythymyl radicals (1a, b) and hydrogen abstraction from its 5-methyl group to give the allyl radical (1c) (Scheme). As illustrated in the Scheme, 3, 4 and 5 are possibly derived from the common intermediates 1a, b (Path I and III) and 6 from 1c (Path V).^{10,11)}

The efficient conversion of e_{aq}^- (reaction 1) to $\cdot\text{OH}$ also accounts for lowering the G-value of 7, because the formation of 7 via reduction of 1 by e_{aq}^- ¹²⁾ may become

negligible. Furthermore, a small amount of $\underline{7}$ produced in the N_2O -saturated aqueous solution may be attributed to the addition of $\cdot H$ to $\underline{1}$.¹⁰⁾

It should be noted that reactions of $\underline{1}$ due to $\cdot OH$ under N_2O -saturated conditions are remarkably affected by the addition of a small amount (0.1 mmol dm^{-3}) of $\underline{2a-f}$ although the $G(-1)$ value decreases to some extent. Table shows a general feature for the effect of $\underline{2a-f}$ that the formation of glycol $\underline{3}$ is promoted by a factor of 2.7-7.5 along with the decreases in the yields of $\underline{4,5}$ and unidentified products.

Furthermore, the value of $G(\underline{3})$ increases linearly with increasing one-electron reduction potential $E(A/A^{\cdot-})$ of $\underline{2a-f}$ as shown in the Figure. This linear correlation suggests that the ability of $\underline{2a-f}$ as an oxidizing agent determines the preferential formation of $\underline{3}$. Since $\underline{1a}$ and $\underline{1b}$ are possible intermediates leading to $\underline{3,4}$ and $\underline{5}$, it is most likely that $\underline{1a}$ and $\underline{1b}$ undergo preferentially one-electron oxidation by $\underline{2a-f}$ to give the corresponding carbonium ions $\underline{1a'}$ and $\underline{1b'}$, respectively (Path II). The carbonium ions thus formed may undergo solvolytic substitution to give $\underline{3}$. The ready formation of $\underline{1a',b'}$ is responsible for the increase in $G(\underline{3})$ value.

The decreases in the yields of $\underline{4}$ and $\underline{5}$ occurring in the presence of $\underline{2a-f}$ are consistent with the efficient conversion of $\underline{1a,b}$ to $\underline{1a',b'}$.

Since the formation of unidentified products is depressed by the addition of $\underline{2a-f}$, it may be possible to predict that these products are derived from radical inter-

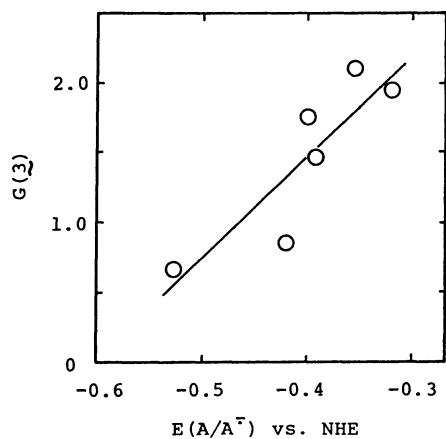
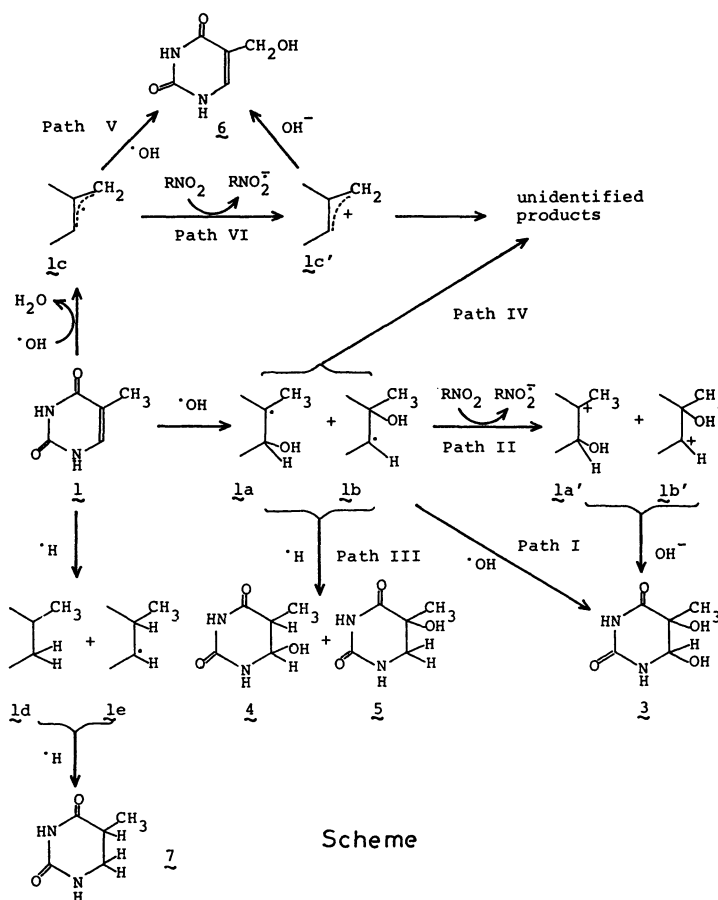


Figure Correlation between $G(\underline{3})$ and $E(A/A^{\cdot-})$.



mediates $\underline{1a}$ and $\underline{1b}$ (Path IV).

The formation of $\underline{6}$ was also depressed to less than 1/2 in the presence of $\underline{2a-f}$ (Table). It seems likely that electron transfer from $\underline{1c}$ to $\underline{2a-f}$ occurs to give a carbonium ion $\underline{1c'}$ (Path VI) which partly decomposes in competition with its conversion to $\underline{6}$.

Further study on the detailed mechanism of the sensitization by nitro compounds will be reported in a subsequent paper.

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(Received April 9, 1982)