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RADIATION-INDUCED REDUCTIVE CONVERSION OF 5-BROMO-6-HYDROXYTHYMINE TO THYMINE PROMOTED BY TRANSITION METAL SALTS IN DEAERATED AQUEOUS SOLUTION

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The radiation-induced reduction of 5-bromo-6-hydroxythymine to produce thymine (2) in deaerated aqueous solution was remarkably promoted by the addition of lower-valent transition metal salts ($K_4Fe(CN)_6$ (3a), CuCl (3b), K_2PtCl_6 (3c), and $FeSO_4$ (3d)). It is suggested that the possible intermediate hydroxythymine-5-yl radical undergoes one-electron reduction by 3a-d to the corresponding anion which eliminates OH^- to produce 2.

5,6-Dihydrouracil and 5,6-dihydrothymine ($PyrH_2$) react with ·OH to give uracil and thymine, respectively, in almost quantitative yields in the presence of appropriate oxidants such as Cu^{2+}.[1] The reaction mechanism has been suggested to involve one-electron oxidation of dihydropyrimidine-5-yl and 6-yl radicals (PyrH·) produced by H-atom abstraction of the ·OH from the dihydropyrimidines. The so-formed dihydropyrimidine cations ($PyrH^+$) may generate the parent pyrimidines via proton elimination ($PyrH^+ \longrightarrow Pyr + H^+$). The oxidation of the dihydropyrimidinyl radicals has been characterized in detail by means of pulse radiolysis.[2] In contrast, quite few work concerning generation of the parent pyrimidines via reductive process has been performed despite being of radiation chemical and biological interests. Although reductive conversion of thymine glycol (5,6-dihydroxy-5,6-dihydrothymine) to thymine has been the only example,[3] the mechanism has not yet been clarified. We report here the finding that radiolytic reduction of 5-bromo-6-hydroxythymine (1) as a C_5-C_6 saturated halopyrimidine to produce thymine (2) in deaerated aqueous solution is remarkably promoted by the addition of lower-valent transition metal salts ($K_2Fe(CN)_6$ (3a), CuCl (3b), K_2PtCl_4(3c), and $FeSO_4$ (3d)).

Radiation-induced reduction of 1 (1 mM) was carried out with a ^{60}Co γ-ray source (380 Gy h^{-1}) in deaerated aqueous solution containing sodium formate (100 mM). The pH of the solution was adjusted to 3.0 ± 0.1 with phosphoric acid since 1 was fairly unstable in neutral solution to give thymine glycol almost quantitatively.

On irradiation up to 1.52 kGy, 1 decomposed with the G-value[4] for conversion, G(-1) = 5.3. Among the major radiolysis products, thymine 2 (G(2) = 0.4), 6-hydroxy-5,6-dihydrothymine (4) (G(4) = 1.0), and 5-methylbarbituric acid (5) (G(5) = 0.8) were confirmed by high performance liquid chromatography (HPLC, monitored with UV absorption at 210 nm) using authentic samples[5],[6] (Table 1). Irradiation of 1 in the presence of 3a-d (1 mM) with oxidation potentials of $E^0(M^{n+}/M^{(n+1)+}) > -0.77$ V (vs. NHE)[7] led to enormous increase in the yield of 2 up to 50 - 90 % (based on 1 decomposed) accompanied by the decreases of the 4 and 5 yields (Table 1).[8] In contrast, the metal salts with more negative $E^0(M^{n+}/M^{(n+1)+})$ values[7] ($VOSO_4$ (3e), Tl_2SO_4 (3f), $MnSO_4$ (3g), and $CoSO_4$ (3h)) little affected the formation of 2, 4 and 5, although the decomposition of 1 was depressed to some

Table 1. G-value for the decomposition of 5-bromo-6-hydroxythymine (1) (G(-1)) and those for the formation of radiolysis products: (2), thymine; (4), 6-hydroxy-5,6-dihydrothymine; (5), 5-methylbarbituric acid. Dose 0 - 1.52 kGy; pH 3.0.

Additive		$E^0(M^{(n+1)+}/M^{n+})$[a]/V	G(-1)	G(Product) 2	4	5
3a	$K_2Fe(CN)_6$	0.36	4.0	2.0	trace	trace
3b	CuCl	0.54	3.4	2.7	trace	trace
3c	K_2PtCl_4	(0.68)[b]	6.0	4.2	trace	trace
3d	$FeSO_4$	0.77	4.8	4.3	trace	trace
3e	$VOSO_4$	1.00	4.5	0.5	0.6	0.9
3f	Tl_2SO_4	(1.25)[b]	4.9	0.5	0.9	1.0
3g	$MnSO_4$	1.51	5.2	0.4	0.6	0.8
3h	$CoSO_4$	1.81	5.0	0.5	1.0	0.7
	none	—	5.3	0.4	1.0	0.8

a) Standard electrode potential vs. NHE for $M^{(n+1)+} + e^- \rightleftharpoons M^{n+}$ in aqueous solution, in which M^{n+} represents (3a) $Fe(CN)_6^{4-}$, (3b) CuCl, (3d) Fe^{2+}, (3e) VO^{2+}, (3g) Mn^{2+}, or (3h) Co^{2+}
b) The values of $E^0(M^{(n+2)+}/M^{n+})$ are given for M^{n+}: (3c) $PtCl_4^{2-}$ and (3f) Tl^+

extent with 3e-f (Table 1).[8] The dark conversion of 1 to 2 in each reaction system was negligible (only trace amount of 2 was yielded for 2 days). It is also noted that intensities of the HPLC elution bands of unidentified products were remarkably reduced in the presence of 3a-d.

It is well known that ·OH and H· produced by radiolysis of water are converted to $\cdot CO_2H$ by the reactions with HCO_2H (pK_a = 3.75)[9] [reaction (2)]. The so-formed $\cdot CO_2H$ dissociates into CO_2^- and H^+ at pH 3 [reaction (3)], since the reported pK_a value of $\cdot CO_2H$ is 1.6.[10]

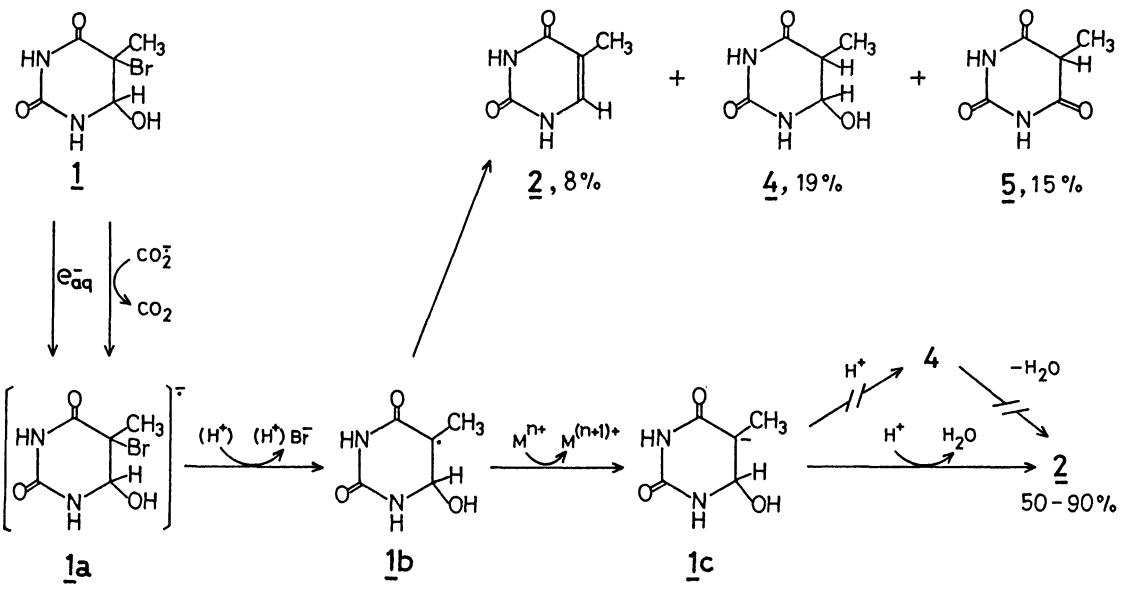
$$H_2O \longrightarrow \cdot OH\ (\text{G-value} = 2.7),\ H\cdot\ (0.55),\ e_{aq}^-\ (2.7) \qquad (1)$$

$$\cdot OH(H\cdot) + HCO_2H \longrightarrow \cdot CO_2H + H_2O(H_2) \qquad (2)$$

$$\cdot CO_2H \longrightarrow CO_2^- + H^+ \qquad (3)$$

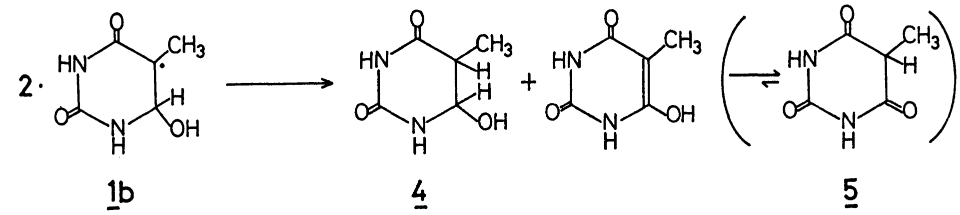
The reaction between e_{aq}^- and H^+ possibly occurs to give H· which should be converted to CO_2^- as in reactions (2) and (3). Accordingly, the active species responsible for the reaction of 1 are virtually limited to such reducing species as e_{aq}^- and CO_2^- ($G(e_{aq}^-) + G(CO_2^-) \sim 6$) under the present experimental conditions without 3a-h.

The radiation-induced conversion of 1 to 2 promoted by 3a-d may be rationalized as in Scheme 1. The one-electron reduction of 1 by e_{aq}^- occurs to produce the radical anion intermediate (1a). From the one-electron reduction potentials of 1 (-0.47 V vs. SCE evaluated in acetonitrile by cyclic voltammetry)[11] and CO_2^- (-1.3 V[12], < -0.64 V[13] vs. SCE in water), it is also probable that CO_2^- can reduce 1 to 1a. These are in accord with the fact that the G(-1)-value (5.3) is very close to that of total reducing species ($G(e_{aq}^-) + G(CO_2^-) \sim 6$) in the reaction without 3a-h. The efficient conversion of 1 by reducing species suggests that the radical anion 1a is liable to give 6-hydroxythymine-5-yl radical (1b) via elimination of Br^- (or HBr after protonation). In connection with the reductive debromination, it is known that 5-bromouracil reacts with e_{aq}^- by dissociative electron capture



Scheme 1.

to give uracilyl radical and bromide ion.[14] The major products 4 and 5 with approximately the same yields in the absence of 3a-h can be derived from disproportionation between the radicals 1b as follows.

 (4)

The UV spectral change revealed that Fe^{3+} (the absorption at 304 nm in 0.4 M H_2SO_4 with ε 2201 M^{-1} cm^{-1})[15] is produced on irradiation of an aqueous solution of 1 with 3d. Figure 1 shows a linear relationship between the product concentrations [2] and [Fe^{3+}], indicating that enhanced conversion of 1 to 2 (90 %) accompanies one-electron oxidation of Fe^{2+} to Fe^{3+}. It is reasonable to presume that the radical intermediate 1b undergoes one-electron reduction by 3a-d to give hydroxythymine anion (1c) (Scheme 1). Recently, Fujita et al. have suggested from the pulse radiolysis study that 1b, which is produced by ·OH addition to the pyrimidine ring-C_6 of thymine, oxidizes N,N,N',N'-tetramethyl-p-phenylenediamine.[16] Although 5-hydroxythymine-6-yl radical is produced simultaneously with 1b in the reported radiolysis system, their conclusion is in accord with the present result. The so-formed anion 1c may lead to 2 via the subsequent elimination of OH^-. The almost complete inhibition of the formation of 4 and 5 [reaction (4)] shows that the reduction of 1b to 1c by 3a-d is a highly efficient pathway. With 3e-f, however, such a reduction pathway should be minor because of their less reducing abilities as predicted by the oxidation potentials.

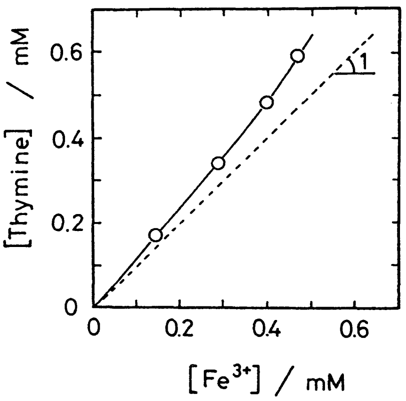


Fig. 1. Relationship between the product concentrations [Thymine] and [Fe^{3+}].

The possibility of the formation of 2 via dehydration of 4 was substantially ruled out by the evidence that 4 was very stable even at pH 3 and had half-life time of ca. 26 h (24 °C). It follows that the protonation of 1c to give 4 is not involved because yields of the 4 are negligible with 3a-d. Furthermore, in view of the selective formation of 2 via 1c, it seems likely that the disproportionation of 1b in the absence of 3a-d leads, besides the reaction (4), to 1c along with hydroxythymine cation by electron transfer.

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