CHEMISTRY LETTERS, pp. 1441-1444, 1983. © The Chemical Society of Japan 1983

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

Sei-ichi NISHIMOTO, Hiroshi IDE, Kikumi NAKAMICHI, Nobuaki OTSUKI, and Tsutomu KAGIYA*
Department of Hydrocarbon Chemistry, Faculty of Engineering,
Kyoto University, Sakyo-ku, Kyoto 606

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 $CO_2^{\bar{\cdot}}$ and H^+ at pH 3 [reaction (3)], since the reported pK_a value of $\cdot CO_2H$ is 1.6.[10]

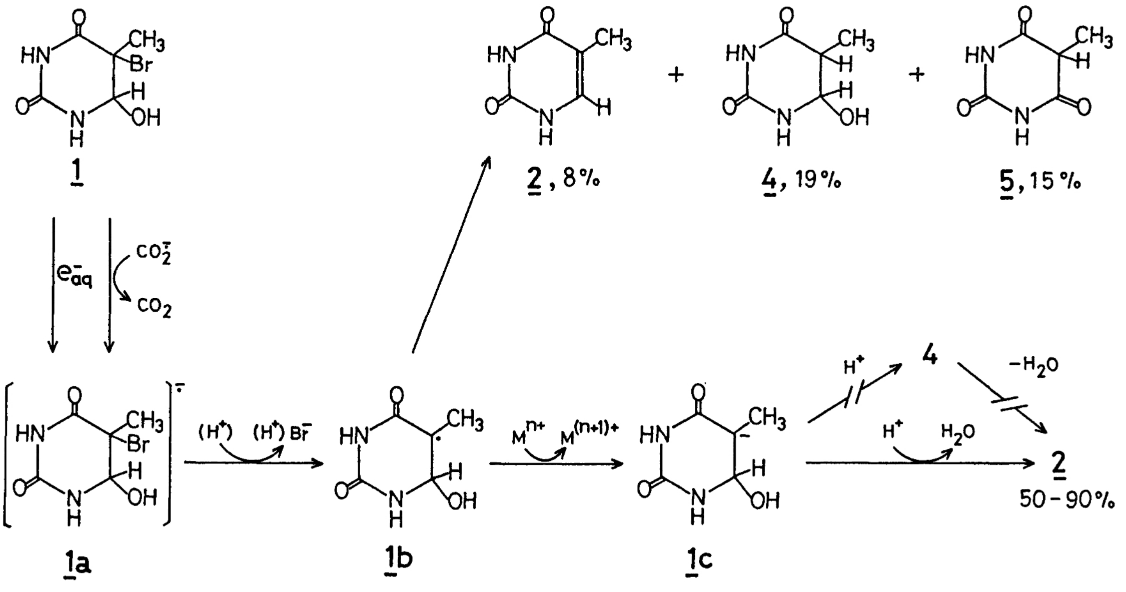
$$H_2O \longrightarrow \cdot OH\ (\text{G-value} = 2.7),\ H\cdot\ (0.55),\ e_{aq}^-\ (2.7) \qquad (1)$$

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

$$\cdot CO_2H \longrightarrow CO_2^{\bar{\cdot}} + H^+ \qquad (3)$$

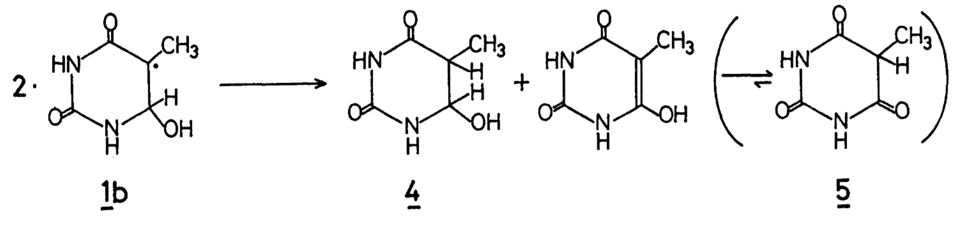
The reaction between e_{aq}^- and H^+ possibly occurs to give H· which should be converted to $CO_2^{\bar{\cdot}}$ as in reactions (2) and (3). Accordingly, the active species responsible for the reaction of 1 are virtually limited to such reducing species as e_{aq}^- and $CO_2^{\bar{\cdot}}$ ($G(e_{aq}^-) + G(CO_2^{\bar{\cdot}}) \sim 6$) 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 $CO_2^{\bar{\cdot}}$ (-1.3 V[12], < -0.64 V[13] vs. SCE in water), it is also probable that $CO_2^{\bar{\cdot}}$ 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 ($G(e_{aq}^-) + G(CO_2^{\bar{\cdot}}) \sim 6$) 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.

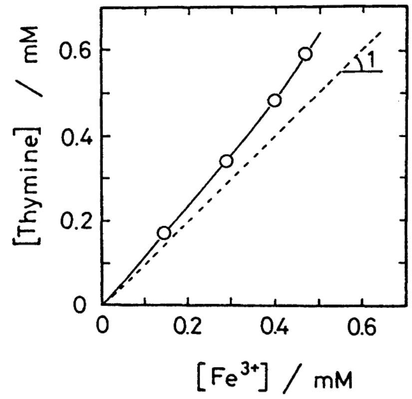


Fig. 1. Relationship between the product concentrations [Thymine] and [Fe^{3+}].

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.

References

1) H. R. Haysom, J. M. Phillips, and G. Scholes, J. Chem. Soc., Chem. Commun., 1972, 1082.
2) A. J. Swallow, "Reactions of Free Radicals Produced from Organic Compounds in Aqueous Solution by Means of Radiation," in "Progress in Reaction Kinetics," Oxford, Pergamon (1978), Vol. 9, pp. 314-315.
3) T. Hayashi and M. Namiki, Int. J. Radiat. Biol., 25, 235 (1974).
4) The number of molecules produced or changed per 100 eV of energy absorbed by the solution.
5) C. Nofre, A. Cier, R. Chapurlat, and J. M. Mareschi, Bull. Soc. Chim. Fr., 1965, 332.
6) B. Doumas and H. G. Biggs, J. Biol. Chem., 237, 2306 (1962).
7) "Kagakubinran Kisohen II," 2nd ed, ed by the Japan Chemical Society, Maruzen, Tokyo (1975), pp. 1204-1206.
8) The characteristic $E^0(M^{(n+1)+}/M^{n+})$ dependence of the enhanced formation of 2 may be accounted for by the Marcus theory (R. A. Marcus, J. Chem. Phys. 24, 966 (1956)). Thus, the reaction rate of electron transfer from metal salts to 1b (see Scheme 1) is expected to increase with decreasing $E^0(M^{(n+1)+}/M^{n+})$ and thereby increasing exothermicity until it reaches a maximum. However, further increase in exothermicity would diminish the rate.
9) "Dissociation Constants of Organic Acids in Aqueous Solution," ed by G. Kortur, W. Vogel, and K. Andrusson, Butterworths, London (1961).
10) G. V. Buxton and R. M. Sellers, J. Chem. Soc., Faraday Trans. 1, 66, 555 (1973).
11) The crrent-potential curve was measured at a scan rate of 0.5 V s^{-1} for an Ar-purged solution of 1 (10 mM) in acetonitrile containing 0.78 M $NaClO_4$ as a supporting electrolyte. The reduction potential was evaluated from the half-peak potential in an irreversible voltammogram obtained. In view of usual solvent effect on the reduction potential, the corresponding value in water would be more positive than -0.47 in acetonitrile.
12) D. J. Schiffrin, Faraday Discuss. Chem. Soc., 56, 75 (1974).
13) E. Papaconstantinou, Anal. Chem., 47, 1592 (1975).
14) G. E. Adams, "Current Topics in Radiation Research," ed by M. Ebert and A. Howard, Amsterdam, North-Holland (1967), Vol. III, pp. 35-93; J. Zimbrick, J. F. Ward, and L. S. Myers, Jr., Int. J. Radiat. Biol., 16, 505 (1965).
15) A. Henglein, W. Schnabel, and J. Wendenburg, "Einfuhrung in die Strahlenchemie," Verlag Chemie GmbH, Weinheim/Bergstr. (1969), Chap. 2.
16) S. Fujita and S. Steenken, J. Am. Chem. Soc., 103, 2540 (1981).

(Received July 2, 1983)