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PROMOTION EFFECT OF 2,2,6,6-TETRAMETHYLPIPERIDINE-1-OXYLS ON THE RADIOLYTIC HYDROXYLATION OF THYMINE IN DEAERATED AQUEOUS SOLUTION

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Remarkable promotion of the hydroxylation of thymine to give thymine glycol with almost complete depression of side reactions by 2,2,6,6-tetramethylpiperidine-1-oxyl (TMPO·) derivatives was observed in the γ-radiolyses of the N_2- and N_2O-saturated aqueous solutions. In the O_2-saturated solution, TMPO· depressed the thymine conversion close to the level under N_2 while promoted the formation of thymine glycol to some extent by decreasing side reactions.

Recently, we reported the redox-related mode of the radiosensitizing action of electron-affinic nitro compounds, *e. g.* 1-(2-nitro-1-imidazolyl)-3-methoxy-2-propanol (misonidazol), which enhance the radiation-induced hydroxylation of thymine to thymine glycol in N_2O-saturated aqueous solution.1) A good correlation was also demonstrated2) between the yield of thymine glycol in the sensitized chemical system and the radiation-induced inactivation of V-79-379A cells in the sensitized biological system3) under anoxic conditions.

On the other hand, the effective radiosensitization of anoxic V-79 Chinese hamster cells has been achieved by several derivatives of 2,2,6,6-tetramethylpiperidine-1-oxyl (TMPO·).4) In order to clarify the chemical mechanism of the radiosensitization by such stable radicals, we studied the effects of some TMPO· derivatives on the radiolyses of thymine in N_2-, N_2O-, and O_2-saturated aqueous solutions.

Typically, a solution of thymine (1.0 mmol dm^{-3}) in 5 dm^3 of saline (buffered at pH 7.0 with phosphate) containing TMPO·, 2,2,6,6-tetramethyl-4-piperidinol-1-oxyl (TMPN), or 2,2,6,6-tetramethyl-4-piperidone-1-oxyl (TAN) (0.5 mmol dm^{-3}) was γ-irradiated at a dose rate of 280 Gy h^{-1} and room temperature. The products were analyzed by HPLC (monitored with UV absorption at 210 nm) and identified by comparison with authentic samples.

γ-Irradiation of N_2-saturated aqueous solution of thymine in the absence of TMPO· derivatives gave a variety of products; i.e. thymine glycol (TG), 6-hydroxy-5,6-dihydrothymine (6-HDT), 5-hydroxymethyluracil (HMU), 5,6-dihydrothymine (DHT), 5-methylbarbituric acid (MBA), and N^1-formyl-N^2-pyruvylurea (FPU). The yields of these products were in the range 7-12%, while larger portion of the converted thymine (50%) has not yet been identified5) (Table 1).

Table 1. G-value of products in the γ-radiolysis of thymine in the presence of TMPO· derivatives

Product		N_2-saturated				N_2O-saturated				O_2-saturated	
		NONE	TMPO·	TMPN	TAN	NONE	TMPO·	TMPN	TAN	NONE	TMPO·
Thymine glycol	(TG)	0.08	0.84	1.00	0.68	0.33	1.91	1.97	1.28	1.27	1.00
6-Hydroxy-5,6-dihydrothymine	(6-HDT)	0.12	0.00	0.00	0.00	0.14	0.00	0.00	0.00	0.03	0.00
5-Hydroxymethyluracil	(HMU)	0.16	0.30	0.33	0.27	0.39	0.62	0.72	0.59	0.08	0.03
5,6-Dihydrothymine	(DHT)	0.35	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5-Methylbarbituric acid	(MBA)	0.08	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.28	0.23
N^1-Formyl-N^2-pyruvylurea	(FPU)	0.09	0.15	0.13	0.19	0.15	0.37	0.30	0.35	0.40	0.24
G(total)		0.88	1.29	1.46	1.14	1.27	2.90	2.99	2.22	2.06	1.50
G(unidentified)[a)]		0.87	0.04	0.05	0.06	2.10	0.13	0.11	0.08	0.50	0.16
G(-thymine)		1.75	1.33	1.51	1.20	3.37	3.03	3.10	2.30	2.56	1.66

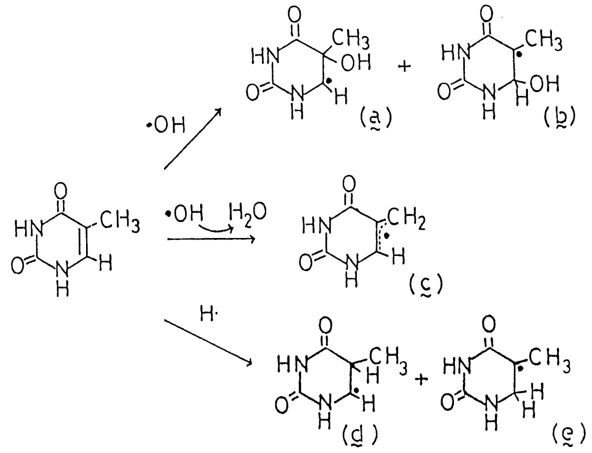
a) Calculated values based on material balance, G(unidentified)= G(-thymine)- G(total).

Although the G-value of thymine conversion (G(-T)) decreased by 14-31%, the presence of the TMPO· derivatives under N_2 enhanced remarkably the yields of TG (8.5-12.5 times), HMU (1.7-2.1 times), and FPU (1.4-2.1 times), depressing almost completely the formation of the 6-HDT, DHT, MBA, and unidentified products.

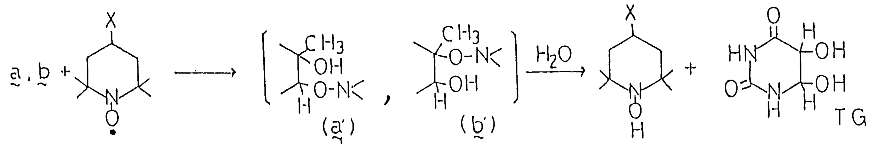
Both the decreased thymine conversion and the inhibited formation of 6-HDT, DHT, and MBA[6)] may be attributed to efficient scavenge of a primary active species of H· (G=0.55) by TMPO·. It is paticularly important to note the good material balance that the sum of the yields of TG, HMU, and FPU is nearly (95-97%) equal to the converted thymine (Table 1). Thus, the TMPO· derivatives could inhibit the reaction paths to produce a major amount of unidentified products as well as 6-HDT and MBA, leading to alternative products of TG, HMU, and FPU.

Similar effects of the TMPO· derivatives were also observed in the radiolysis of N_2O-saturated aqueous thymine solution (Table 1). The yields of hydroxylated products such as TG, 6-HDT, and HMU in the absence of TMPO· derivatives under N_2O were 1.8-2.5-times larger than those observed under N_2. This is the result of 2-times increase in the generation of ·OH (G=5.4) which arises from reactions of e_{aq}^- and H· with N_2O to form ·OH.[1)] In the presence of TMPO· derivatives the thymine conversion decreased by 8-32%, while increases were observed in the yields of TG (3.9-6.0 times), HMU (1.5-1.8 times), and FPU (2.0-2.5 times). The formations of DHT and 6-HDT as well as unidentified products were inhibited almost quantitavely. In the N_2O-saturated aqueous system, the main active species ·OH (G=5.4) is considerd to react with thymine, producing intermediate hydroxythymyl (a, b) and allyl-type (c) radicals. Similarly, the addition of H· (G=0.55) to thymine leads to hydrothymyl radicals (d, e). Such a reaction should fail to occur in the presence of TMPO· derivatives because of the efficient scavenge of the H· (reactions (1)-(3)).

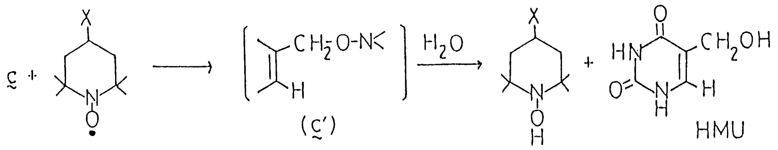
The intermediate radicals a-c, which are potential precursors to produce hydroxylated thymine derivatives, would be labile to partly self-decompose into unidentified products in the absence of TMPO· derivatives. In accord with the good material balance described above, however, the TMPO· derivatives can react efficiently with the a-c, thereby leading to the remarkable decrease in the G-value of unidentified compounds to < 0.13 and the enhanced formations of TG, HMU, and FPU.



In view of well-known scavenging ability of TMPO· derivatives toward various radical species,[7)] it is most likely that radical combinations of the intermediates a-b with the TMPO· derivatives occur to form the corresponding adducts[8)] which undergo hydrolysis[9)] into TG (*via* a and b) and HMU (*via* c) as follows:

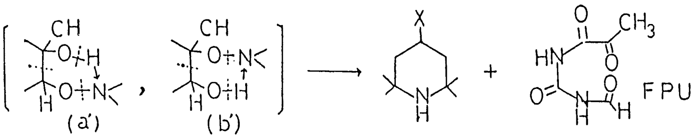


(4)



(5)

The self-decomposition of the adducts a and b by intramolecular hydrogen transfer (reaction (6)) may account for the increased yield of FPU.



(6)

The activity of the TMPO· derivatives for the selective hydroxylation of thymine (T) to TG (S_{TG}= G(TG)/G(-T)) and HMU (S_{HMU}= G(HMU)/G(-T)) was little affected by the change in substituent (X) at C-4 position of the piperidine ring, as shown in Table 2.

Table 2. Selectivities of TG (S_{TG}) and HMU (S_{HMU}) with TMPO· derivatives

	Atmosphere	TMPO	TMPN	TAN
S_{TG}	N_2	0.63	0.66	0.57
	N_2O	0.63	0.64	0.56
S_{HMU}	N_2	0.23	0.22	0.23
	N_2O	0.20	0.22	0.26
S_{TG+HMU}	N_2	0.86	0.88	0.80
	N_2O	0.83	0.87	0.82

Similar promotion effect on the radiolysis of thymine was also observed using O_2. Thus, the irradiation under O_2 without TMPO· led to 1.5-times increase in thymine conversion and higher yields of TG (16 times), MBA (3.5 times), and FPU (4.4 times) compared with the N_2-saturated system. The selectivity of the TG formation based on the thymine conversion was evaluated as 50%. In contrast, the remarkable depression of the conversions to unidentified products, 6-HDT, and HMU and the complete inhibition of the DHT formation were observed. This characteristic sensitization effect of O_2 is accounted for in terms of the preferential reactions with H· and e_{aq}^- to form less active species HOO· and O_2^- and with the intermediate hydroxythymyl radicals to produce TG.

The presence of TMPO· in O_2-saturated solution was found to bring about lowerings of the thymine conversion (35%) close to the level in N_2-saturated solution and of the formation of TG (21%), MBA (18%), FPU (40%), and unidentified products (68%). The decrease in thymine conversion may result from the scavenging action of TMPO· toward several fragment radicals which would be generated by side reactions in the system to initiate successive oxidation of thymine. It is however noted that the selectivity of TG formation was further enhanced from 0.5 to 0.6 in the presence of TMPO· under O_2, whereas those of HMU and FPU formations were virtually invariant. These results show that the TMPO· can attack hydroxythymyl radicals a and b, in competition with the larger amount of O_2, to produce TG in a manner similar to under anoxic conditions.

Further details on the characteristic role of TMPO· and related compounds in the radiolysis of thymine will be reported in a future paper.

References

1) T. Wada, H. Ide, S. Nishimoto, and T. Kagiya, Chem. Lett., 1982, 1041.

2) T. Kagiya, S. Nishimoto, H. Ide, and K. Nakamichi, Radiat. Phys. Chem., in press.

3) G.E. Adams, R. Flockhart, C.E. Smithen, I.J. Stratford, P. Wardman, and M.E. Watts, Radiat. Res., 67, 9 (1976).

4) B.C. Millar, T.C. Jenkins, and E.M. Fielden, Radiat. Res., 71, 516 (1977).

5) The product distribution under N_2 is substantially the same as that determined previously under deaerated conditions (see Ref. 1).

6) Disproportionations of hydrothymyl radicals (d, e) with each other and with 6-hydroxythymyl radical (b) are responsible for the formations of DHT and 6-HDT, respectively. Subsequent dehydrogenation of the 6-HDT by ·OH and/or H· is presumed to give MBA.

7) M.B. Neiman, "Aging and Stabilization of Polymer," Consultants Bureau, New York (1965), p. 33.

8) J. Cadet, M. Guttin-Lombard, and R. Teoule, Int. J. Radiat. Biol., 30, 1 (1976).

9) E.G. Rozansev, "Free Nitroxyl Radicals," Plenum Press, New York (1970), p. 96.

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