FORMATION AND DECAY OF PEROXY RADICALS OF SOME PYRIMIDINE DERIVATIVES IN WATER

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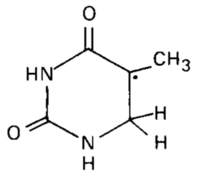
The transient formation of organic tetraoxides as intermediates in oxidation reactions has been postulated [1] and evidence for their existence is accumulating [2]. Tetraoxides have been invoked in mechanistic considerations of the autoxidation of hydrocarbons [3], in the radiolytic oxidation of cyclohexane [4] and in the free radical oxidation of alcohols in aqueous solutions [5]. We wish to present results which support the formation of the tetraoxides of some pyrimidine derivatives in aqueous solution. These are produced via the dimerization of the corresponding peroxy radicals. The factors which govern their formation and decay will be presented.

The technique of pulse radiolysis and kinetic absorption spectrophotometry was used [6] to produce the peroxy radicals, follow their decay and the concomitant formation of tetraoxides, and lastly the formation of final product. The radiolysis of water gives rise to the primary radicals e_{aq}^- (2.8), OH (2.8) OH (0.6), with the G-value (yields/100 eV) given in parenthesis. In the presence of N_2O, most of the hydrated electrons are converted to hydroxyl radicals: $e_{aq}^- + N_2O \rightarrow OH + OH^- + N_2$.

Since pyrimidine [4–6] and peroxy radicals [4,5] absorb at wavelength below ~ 300 nm, they were produced from dihydroderivatives instead of pyrimidines. The main reaction with dihydrothymine (TH_2) is H-atom abstraction

$$OH + TH_2 \rightarrow \cdot TH + H_2O \qquad (1)$$

with $k_1 = 2.5 \times 10^9\ M^{-1}\ s^{-1}$ (ref. [7]), to give two transient species:



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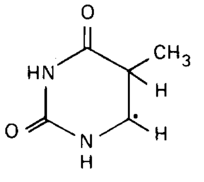

II

Radicals I and II have a broad absorption spectrum with $\lambda_{max} = 410$ nm, $\epsilon_{410} = 450\ M^{-1}\ cm^{-1}$ and a second band with $\lambda_{max} < 245$ nm and $\epsilon_{245} \sim 3000\ M^{-1}\ cm^{-1}$; and are similar to those of the ·UH radical [8]. In the absence of oxygen or other reactive additive, the ·TH radicals decay by second-order kinetics

$$2\ \cdot TH \rightarrow \text{products}\ k_2 = 8.0 \times 10^8\ M^{-1}\ s^{-1} \qquad (2)$$

At pH 6–7, thymine (T) is produced [9]. 50% of the ·TH radicals disproportionate to give T under pulse conditions. Another possible product is the dimer HT–TH, the absorption band of which is expected to be below 240 nm, like TH_2, since it is a 'saturated'

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molecule. More recently evidence was offered [10] for charge transfer between two ·TH radicals with the formation of thymine and thymine hydrate.

In the presence of oxygen (13 mM N_2O and 0.7 mM O_2 concentrations were chosen to prevent the formation of $\cdot O_2^-$ via $e^-_{aq} + O_2$), the ·TH radicals rapidly react with O_2 to form peroxy radicals.

$$\cdot TH + O_2 \rightarrow \cdot OOTH \quad k_3 = 1.3 \times 10^9\ M^{-1}\ s^{-1} \qquad (3)$$

The spectrum of ·OOTH is distinctly different from that of ·TH radicals, see fig. 1, with $\lambda_{max} = 240$ nm and $\epsilon_{240} = 1300\ M^{-1}\ cm^{-1}$. Such an absorption spectrum is characteristic of organic peroxy radicals (4–6).

The formation of ·OOTH (and ·OOUH) radicals in oxygenated solutions was also confirmed using the *p*-benzoquinone (BQ) method [11], which discriminates between various free radicals on the basis of electron transfer reactions:

$$\cdot TH + BQ \rightarrow T + BQ^- + H^+ \quad k_4 = 4.0 \times 10^9\ M^{-1}\ s^{-1} \qquad (4)$$

$$\cdot O_2^- + BQ \rightarrow O_2 + BQ^- \quad k_5 = 9.0 \times 10^8\ M^{-1}\ s^{-1} \qquad (5)$$

$$\cdot OOTH + BQ \rightarrow \text{no (or very slow) reaction} \qquad (6)$$

The second-order decay of ·OOTH was monitored at 270 nm, and a new longer-lived intermediate was observed. The absorption spectrum of this intermediate, see fig. 1, has a $\lambda_{max} = 260$ nm and $\epsilon_{260} = 1300\ M^{-1}\ cm^{-1}$. It decays by a first-order process to give an intense absorption with $\lambda_{max} = 265$ nm as a final permanent product. The spectrum of this product appears to be identical with that of thymine, see fig. 1. Based on these observations, it is suggested that the intermediate is a tetraoxide produced according to reaction (7).

$$2\,\cdot OOTH \rightarrow HTOOOOTH \quad k_7 = 6.0 \times 10^6\ M^{-1}\ s^{-1} \qquad (7)$$

The decay of the tetraoxide was monitored at 265 nm through the formation of thymine.

$$HTOOOOTH \rightarrow \text{thymine + products} \qquad (8)$$

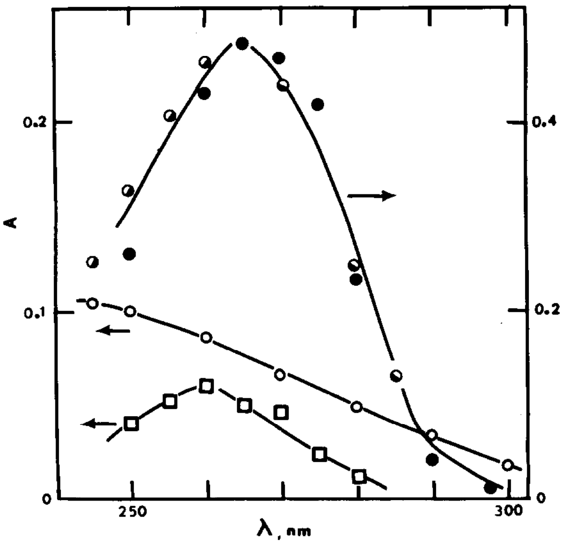

Fig. 1. Transient absorption spectra produced from the reaction of OH radicals with 1 mM dihydrothymine (TH_2) in presence of 13 mM N_2O and 0.7 mM O_2 at 6.0: (a) peroxy radical ·OOTH, ○ (A read at 5 μsec); (b) tetraoxide HTOOOOTH, □ (A read at 3.0 msec); (c) final product, ● (A read at 0.3 sec); (d) final product, ◐, after X-ray steady-state radiolysis, and (e) full line is the ground state absorption spectrum of thymine at pH 6.0. Dose/pulse ~ 8 *k*rads.

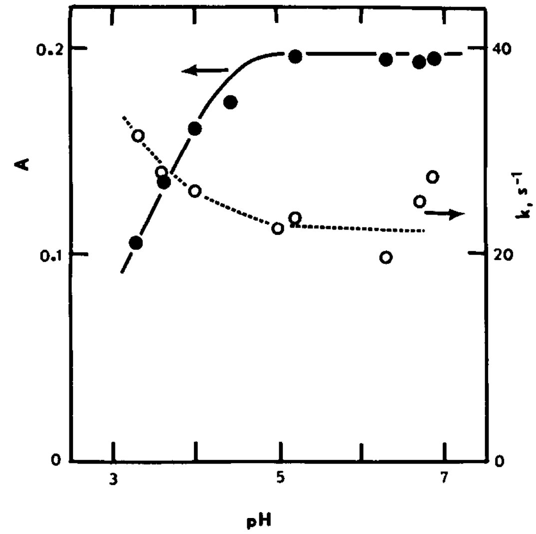

Fig. 2. Dependence upon pH of the first-order decay rate constant of HTOOOOTH in water, ○, (under conditions given in fig. 1); and relative yield of thymine as a function of pH, ●.

H_2O_2 was found to be one of the products. The values of k_8 are pH dependent, see fig. 2, and appear to be acid–base catalyzed. Due to the instability of dihydrothymine above pH 7, the kinetics at higher pH values were not investigated. Reaction (8) probably involves a few steps, with the elimination of the first thymine as the rate-determining step.

The yield of thymine produced in reaction (8) is not stoichiometric, since only ~ 67% of the ·OOTH radical give T at pH > 5.0. In the corresponding experiments with dihydrouracil, a 100% yield of uracil was observed, i.e. one tetraoxide gives two uracil molecules. Lower thymine yield is observed because of the presence of the C_5-CH_3 group hence (a) some of the OH radicals attack the $-CH_3$ group, (b) stereochemical considerations are introduced.

The formation of thymine in the γ-radiolysis of oxygenated solutions of dihydrothymine was unequivocally proved [9]. Pulse radiolysis of that same system shows similar results except that the yield of thymine is lower (40% efficiency) than in N_2O/O_2 mixtures. This is due to the reaction of $\cdot O_2^-$ with ·OOTH radicals to produce a hydroperoxide [12].

$$\cdot O_2{}^- + \cdot OOTH \xrightarrow{H^+} HTOOH + O_2 \quad k_9{\sim}k_7 \quad (9)$$

The yield of thymine in N_2O/O_2 mixtures was found to be pH-dependent below pH ~ 5.0, see fig. 2. The drop in yield in acid solution seems to parallel the general acid–base changes observed in the kinetics of formation of T. The mechanism is not clear; the participation of the N_1-H has, however, been eliminated since 1-methyl-dihydrothymine behaves very similarly as TH_2. It is possibly associated with the acidic properties of the H atoms in C_5 and C_6 positions.

In conclusion we wish to stress the possibility of formation of tetroxides in radiation biology and physiology, and whenever chemical and biochemical reactions occur via free radical and/or electron transfer processes in the presence of oxygen. The formation of 'mixed' tetraoxides RO_4R' could also lead to different kinetics and final products. Further elucidation of the formation and decay of tetraoxides is desirable.

Acknowledgements

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References

[1] Bartlett, P. D. and Guaraldi, G. (1967) J. Am. Chem. Soc. 89, 4799.

[2] Swern, D., ed. (1970) Organic Peroxides, Wiley, New York.

[3] Russell, G. A. (1957) J. Am. Chem. Soc. 79, 3871.

[4] Simic, M. and Hayon, E. (1971) J. Phys. Chem. 75, 1677.

[5] Stockhausen, K., Henglein, A. and Beck, G. (1969) Ber. Bunsenges Physik. Chem. 73, 567.

[6] Simic, M., Neta, P. and Hayon, E. (1969) J. Phys. Chem. 73, 3794;
Keene, J. P., Black, E. D. and Hayon, E. (1969) Rev. Sci. Instrum. 40, 1199.

[7] Greenstock, C. L., Hunt, J. W. and Ng, M. (1969) Trans. Faraday Soc. 65, 3279.

[8] Hayon, E. and Simic, M. (1973) J. Am. Chem. Soc. 95, 1029.

[9] Snipes, W. and Bernhard, W. (1968) Radiation Res. 33, 162.

[10] Haysom, H. R., Phillips, J. M. and Scholes, G. (1972) JCS Chem. Comm. 1082.

[11] Simic, M. and Hayon, E. (1973) Biochem. Biophys. Res. Comm. 50, 364.

[12] Scholes, G. (1963) Progress in Biophysics 13, 59.