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Oxidation of Thymines with Hydrogen Peroxide in Hydrochloric Acid

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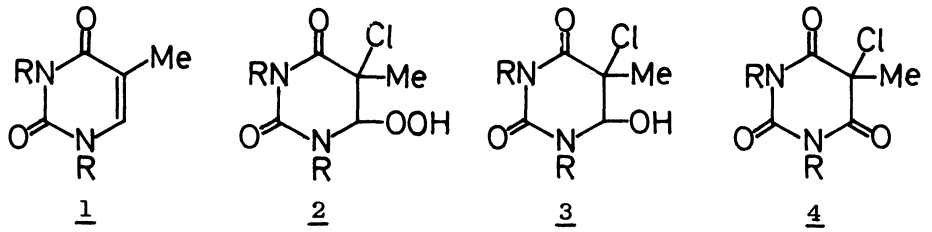
Oxidation of thymine and 1,3-dimethylthymine with hydrogen peroxide in hydrochloric acid (pH 1.0-2.2) at 35-38 °C gave the corresponding trans-5-chloro-6-hydroperoxy-5,6-dihydrothymines. The formation of the hydroperoxythymines was dependent on pH of the aqueous solutions. Reaction of the hydroperoxythymine with amino acids and nucleic acids related compounds in deuterium oxide was investigated by NMR spectroscopy.

In recent years, much attention has been focused into mutation caused by phagocytes because phagocytic cells produce active oxygen species containing H_2O_2 during respiration burst.[1] In the circulating polymorphonuclear leukocytes, myeloperoxidase serves an important role for bacterial killing. Hydrogen peroxide and chloride ion are required for the myeloperoxidase oxidative action. Therefore, oxidation of nucleic acids and their related compounds with H_2O_2 and Cl^- or with HOCl is of interest. Although oxidations of nucleic acid bases with HOCl and NaOCl were known,[2] little attention has been paid to the oxidation with H_2O_2 and Cl^-. During the course of an investigation on oxidation of nucleic acid bases with peroxides,[3] we found the formation of trans-5-chloro-6-hydroperoxy-5,6-dihydrothymines (2) by treatment of thymines (1) with H_2O_2 in HCl (pH 1.0-2.2).

Although thymine (1a) was unreactive to H_2O_2 in water, treatment of 1a with H_2O_2 in HCl gave the oxidation products. A solution of 1a (1 mmol) in the presence of H_2O_2 (4 mmol) in 0.1 mol dm^{-3} HCl (50 ml) was heated at 35-38 °C for 24 h. The reaction mixture was evaporated to give a white solid mass and it was triturated with acetone. Recovered 1a (0.43 mmol) was removed as the solid by filtration. The filtrate was chromatographed on silica gel tlc developed with a mixture of ethyl acetate and hexane (3:1) to give trans-5-chloro-6-hydroperoxy-5,6-dihydrothymine (2a)[4] (0.25 mmol), trans-5-chloro-6-hydroxy-5,6-dihydrothymine (3a)[3,5] (0.11 mmol), 5-chloro-5-methylbarbituric acid (4a) (0.06 mmol), and recovered 1a (0.13 mmol). On the other hand, treatment of 1a with H_2O_2 in 1.0 mol dm^{-3} HCl gave 3a (80%) and 4a (7%) but none of 2a was obtained. The compound 2a was thoroughly reduced to 3a[5] in dimethylsulfoxide within 90 min. Consequently, the stereochemistry of 2a was assigned as trans-configuration.

Almost no reaction occurred on treatment of 1a with H_2O_2 in the presence of NaCl in water (pH 7.0) at 35-38 °C for 24 h, whereas the treatment in 0.1 mol dm^{-3} HCl (pH 1.0) gave 2a, 3a, and 4a. Therefore, a relation between pH of the

aqueous solutions and the oxidation of 1a with H_2O_2 was investigated by using Clark and Lubs buffer solutions of pH 1.0-2.2 (solutions of HCl and KCl). The results are shown in Fig. 1. In order to elucidate the relation, 1,3-dimethylthymine (1b) was used as a model compound. The oxidation of 1b with H_2O_2 in Clark and Lubs solutions of pH 1.0-2.2 also gave trans-5-chloro-6-hydroperoxy-1,3-dimethyl-5,6-dihydrothymine (2b), trans-5-chloro-6-hydroxy-1,3-dimethyl-5,6-dihydrothymine (3b),[3] and 5-chloro-1,3,5-trimethylbarbituric acid (4b). The results, shown in Fig. 1, suggested that the formation of 2a and 2b and the conversion of 1b were dependent on the pH of Clark and Lubs solutions. Further, time course of the oxidation of 1b in the solution of pH 1.0 (Fig. 2) showed that the formation of 2b was almost completed within 24 h.



1 2 3 4

a: R = H
b: R = Me

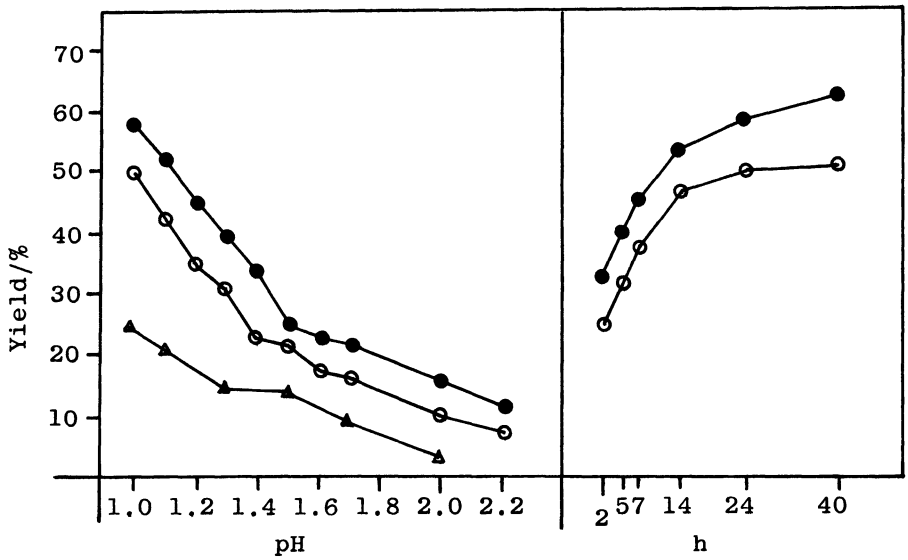


Fig. 1. Relation between changes of pH and the oxidation of thymines with H_2O_2. Conditions: 1a or 1b (1 mmol), H_2O_2 (4 mmol), Clark and Lubs buffer solutions of pH 1.0-2.2 (50 ml), at 35-38 °C, 24 h reaction.
● Conversion of 1b (%).
○ Formation of 2b (%).
△ Formation of 2a (%).
Yield (%) was the isolated one based on thymines used.

Fig. 2. Time course of the oxidation of 1,3-dimethylthymine with H_2O_2 in the solution of pH 1.0. Conditions: 1b (1 mmol), H_2O_2 (4 mmol), Clark and Lubs solution of pH 1.0 (50 ml), at 35-38 °C.
● Conversion of 1b (%).
○ Formation of 2b (%).
Yield (%) was the isolated one based on 1b used.

Irradiation of aqueous aerated solutions of 1a with X-ray gives a mixture of hydroperoxythymines[6] together with the other oxidized products.[7] The hydroperoxythymines are known to have mutagenic activities.[8] Furthermore, it was felt that the damage of 1a with H_2O_2 in HCl (pH 1.0-2.2) was of importance in connection with the fact that Cl^- is found abundantly in living organism and gastric juice is HCl (pH 1.0-1.5) solution containing digestive enzymes.

Hydroperoxythymines 2a and 2b were stable in D_2O and CD_3COCD_3 at room temperature for more than 3 days. On the other hand, heating of 2b in water at 90 °C for 7 h gave 4b (90%) without side reaction, while heating of 2b in 0.1 mol dm^{-3} HCl under similar conditions gave 3b (56%) and 4b (44%). Further, treatment of 3a with H_2O_2 in water at room temperature for 24 h gave 4a (15%) without side reaction.

In spite of the fact that much of hydroperoxides such as hydroperoxythymines[8] and fatty acid hydroperoxides[9] are mutagens, behavier of hydroperoxides in vivo is not appreciably known. On the other hand, 2a was clearly distinguishable from 3a and 4a in ^{1}H-NMR spectra.[4] These observations led us to investigate reaction of 2a with amino acids and nucleic acids related compounds in D_2O by NMR spectroscopy.

Some of amino acids such as glycine, alanine, tyrosine, serine, proline, glutamic acid, and glutamine were unreactive to 2a in D_2O for 24 h. On the other hand, decomposition of 2a was observed on treating with basic amino acids such as lysine, arginine, and histidine. Furthermore, treatment of 2a with amino acids containing sulfur such as methionine and cysteine immediately resulted in reduction to 3a. The reaction of 2a with methionine gave 3a and methionine sulfoxide, whereas reaction of 2a with methionine sulfoxide eventually resulted in formation of ca. 50% of 3a and methionine sulfone after 3 days. When two solutions of 2a and cysteine in D_2O were mixed, cystine was precipitated and 3a was formed, although oxidations of methionine and cysteine by hydroperoxides are well known. Treatment of 2a with cytosine and with cytidine also gave 3a and an unidentified product, while 2a was unreactive to adenine, adenosine, and thymidine.

The further investigation concerning oxidation of nucleic acid bases with H_2O_2 in HCl is now in progress.

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4) All new compounds were fully characterized by ^{1}H-NMR, IR, and mass spectroscopy and by elemetal analyses. The spectral data are given below.

2a: Mp 166-168 °C: NMR(D_2O) δ 1.94(s, 3H), 5.33(s, 1H); (CD_3COCD_3) δ 1.85(s, 3H), 5.23(d, 1H, J=4.5 Hz), 7.82(broad, d, 1H, J=4.5 Hz), 9.52(broad, 1H), 11.48(s, 1H); (CD_3SOCD_3) δ 1.80(s, 3H), 4.95(d, 1H, J=4.5 Hz), 8.40(broad, d, 1H, J=4.5 Hz), 10.30(broad, 1H), 11.94(s, 1H): IR(Nujol) 3340, 3250, 3200, 3050, 1710-1670(broad) cm^{-1}: mass spectrum, m/e(relative intensity) 196(0.4), 195(0.2), 193(0.2), 176(10), 163(26), 161(26), 133(12), 118(15), 92(36), 90 (100).

2b: Mp 147-149 °C: NMR(D_2O) δ 1.88(s, 3H), 3.16(s, 6H), 5.37(s, 1H); ($CDCl_3$) δ 1.91(s, 3H), 3.16(s, 3H), 3.18(s, 3H), 4.94(s, 1H), 10.02(s, 1H); (CD_3COCD_3) δ 1.86(s, 3H), 3.12(s, 3H), 3.14(s, 3H), 5.24(s, 1H), 11.34(s, 1H); (CD_3SOCD_3) δ 1.84(s, 3H), 3.10(s, 6H), 5.15(s, 1H), 12.10(s, 1H): IR(Nujol) 3210, 1715, 1660-1640(broad) cm^{-1}: mass spectrum(relative intensity) 223(1), 221(2), 206(4), 191(33), 189(100), 134(28), 132(86), 106(23), 104(70).

3a[3,5]: NMR(D_2O) δ 1.78(s, 3H), 5.11(s, 1H).

3b[3]: NMR(D_2O) δ 1.78(s, 3H), 3.15(s, 3H), 3.17(s, 3H), 5.01(s, 1H).

4a: Mp 198-200 °C: NMR(D_2O) δ 1.95(s, 3H); (CD_3SOCD_3) δ 1.90(s, 3H), 11.23(broad, 2H): IR(Nujol) 3190, 3080, 1710-1660(broad) cm^{-1}: mass spectrum(relative intensity) 178(5), 177(1), 176(14), 133(12), 92(34), 90(100).

4b: Mp 102-104 °C: NMR(D_2O) δ 2.00(s, 3H), 3.35(s, 6H); ($CDCl_3$) δ 2.00(s, 3H), 3.35(s, 6H): IR(Nujol) 1680-1670(broad) cm^{-1}: mass spectrum(relative intensity) 206(10), 205(3), 204(27), 147(22), 92(34), 90(100).

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