Myeloperoxidase Catalyzed Bromination of Nucleic Acid Bases and Related Compounds

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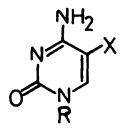
Synopsis. Oxidation of cytosine, uracil, adenine, cytidine, and 2′-deoxycytidine by myeloperoxidase in the presence of hydrogen peroxide and potassium bromide in sodium acetate buffer solution at pH 5.0 resulted in bromination of the substrates. A comparison of bromination of 1,3-dimethyluracil catalyzed by peroxidases such as myeloperoxidase, lactoperoxidase, chloroperoxidase, and horseradish peroxidase was also studied.

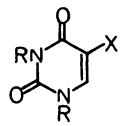
Myeloperoxidase (MPO) plays an important role for disinfection with H_2O_2 and halide ions in phagolysosome.[1] On the other hand, mutation[2] and carcinogenesis[3] are known to be caused by human phagocytes. Furthermore, halogenated nucleic acid bases and their deoxyribonucleosides such as 5-bromouracil (**2b**)[4] and 5-bromo-2′-deoxyuridine[5] have mutagenic activities. Therefore, MPO catalyzed halogenation of nucleic acids is of interest. Odajima[6] first reported the incorporation of chlorine into RNA and adenine (**3a**) by MPO in the presence of H_2O_2 and NaCl. However, structures of the chlorinated products were not thoroughly determined.[6] As part of the investigation of the enzymatic and non-enzymatic oxidation of nucleic acid bases,[7] MPO catalyzed halogenation of nucleic acid bases was studied.[8]

MPO catalyzed halogenation of nucleic acid bases was carried out in sodium acetate buffer solution at pH 5.0.[6] While oxidation of cytosine (**1a**), uracil (**2a**), and **3a** with H_2O_2 and NaCl in the presence of MPO gave only a trace amount of product, a similar treatment with KBr instead of NaCl gave brominated products in moderate yields. The results are shown in Table 1. Table 1 shows that the yields of brominated products from **1a**, **2a**, and **3a** decrease in that order. On the other hand, no reaction occurred when thymine was similarly treated. The reaction of guanine was not attempted because it was only slightly soluble in the buffer solution. Treatment of cytidine (**4a**) and 2′-deoxycytidine (**5a**) with H_2O_2 and KBr in the presence of MPO also gave 5-bromocytidine (**4b**) and 5-bromo-2′-deoxycytidine (**5b**), respectively. The compounds **1a**, **2a**, **3a**, **4a**, and **5a** were unreactive to H_2O_2 and KBr in the absence of MPO under similar conditions.

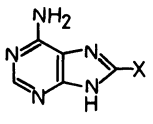
In an attempt to further elucidate the MPO catalyzed oxidation, a comparison of oxidation of 1,3-dimethyluracil (**6a**) by MPO, lactoperoxidase (LPO), chloroperoxidase (CPO), and horseradish peroxidase (HPO)



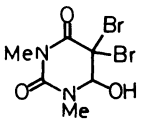
1a : R = H, X = H
1b : R = H, X = Br
4a : R = β-D-ribofuranosyl
X = H
4b : R = β-D-ribofuranosyl
X = Br
5a : R = 2-deoxy-β-D-ribofuranosyl
X = H
5b : R = 2-deoxy-β-D-ribofuranosyl
X = Br



2a : R = H, X = H
2b : R = H, X = Br
6a : R = Me, X = H
6b : R = Me, X = Br



3a : X = H
3b : X = Br



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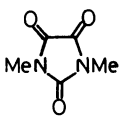

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Table 1. Peroxidase Catalyzed Bromination of Nucleic Acid Bases and the Related Compounds[a]

Substrate	Peroxidase	KBr	H_2O_2	Temp	Products (Isolated yield/%[b])
		mmol	mmol	°C	
1a	MPO (200 U)	2	2	25—27	**1b**(22)[c]
2a	MPO (200 U)	2	2	25—27	**2b**(4)[c]
3a	MPO (200 U)	2	2	25—27	**3b**(0.9)[c]
4a	MPO (200 U)	4	1	35—38	**4b**(4)[c]
5a	MPO (200 U)	4	1	35—38	**5b**(5)[c]
6a	MPO (200 U)	2	2	25—27	**6a**(88), **6b**(6)
6a	LPO (400 U)	2	2	25—27	**6a**(88), **6b**(4)
6a	CPO (250 U)	2	2	25—27	**6a**(61), **6b**(16), **7**(6), **8**(8)

a) Reaction conditions: substrate (1 mmol), for 1 h reaction. b) Yield based on substrate used. c) Yield of the recovered substrate was not determined.

was investigated. As shown in Table 1, MPO and LPO catalyzed reactions of **6a** yielded 5-bromo-1,3-dimethyluracil (**6b**) whereas CPO catalyzed oxidation gave not only **6b** but also 5,5-dibromo-6-hydroxy-1,3-dimethyl-5,6-dihydrouracil (**7**) and 1,3-dimethylparabanic acid (**8**). However, almost no reaction occurred when **6a** was similarly treated with HPO.

Although many reports concerning halogenation of nucleic acid bases and nucleosides have been published,[9] it was found that **1a**, **2a**, **3a**, **4a**, and **5a** were reacted with H_2O_2 and KBr in the presence of MPO to yield the corresponding brominated products. The results are of interest in connection with the mechanism of mutation caused by phagocytes.

Experimental

MPO from human leukocytes (200 U/vial) was purchased from The Green Cross Corporation (Osaka). LPO from bovine milk and CPO from Caldariomyces fumago were purchased from Sigma Chemical Co. (St. Leuis, USA).

MPO Catalyzed Bromination of Nucleic Acid Bases 1a, 2a, and 3a. A solution of nucleic acid base (1 mmol), MPO (200 U), and KBr (2 mmol) in 0.2 mol dm^{-3} sodium acetate buffer solution at pH 5.0 (110 ml) was stirred. The reaction was initiated by addition of H_2O_2 and incubated at 25—27 °C. Hydrogen peroxide (0.4 mmol) in the sodium acetate buffer solution (2 ml) was added into the solution at 10 min intervals. After 60 min, catalase was added to remove any remaining H_2O_2. The reaction mixture was evaporated to give a white solid mass. The residue was extracted with a large amount of methanol. Droplet countercurrent chromatography (Tokyo Rikakikai, DCC-300-G2) was used for separation of the extract. The separation with $CHCl_3$–MeOH–H_2O (5:5:3) by descending method resulted in the isolation of the brominated products. The isolation of **1a**, **2a**, and **3a** was not attempted. The structures of the brominated products were confirmed by comparison of ^1H NMR spectra (JEOL PMX60A spectrometer) and thin-layer chromatography (TLC) (E. Merck, Silica gel 60 sheet F_{254}) with the authentic samples obtained commercially.

Bromination of 4a and 5a. A solution of **4a** and **5a** (1 mmol), MPO (200 U), and KBr (4 mmol) in the sodium acetate buffer solution described before (110 ml) was stirred at 35—38 °C. The reaction was started by adding H_2O_2 (0.5 mmol) in the buffer solution (5 ml). After 30 min, H_2O_2 (0.5 mmol) in the buffer solution (5 ml) was further added into the solution containing MPO. After 60 min, catalase was added. The reaction mixture was evaporated and extracted with a large amount of methanol. The extract was evaporated and chromatographed on silica gel using a mixture of $CHCl_3$ and MeOH (8:2) to yield the brominated products. The products **4b** and **5b** were further purified by liquid chromatography with low pressure pump (Fuji-gel packed column RQ-2 ODS silica gel 24 mmϕ×360 mm, mobile phase: a mixture of water and acetonitrile (10:1), detected at 250 nm). The structures of the products were confirmed by comparison of ^1H NMR spectra and TLC with the authentic samples obtained commercially. The isolation of **4a** and **5a** was not attempted.

Peroxidase Catalyzed Oxidation of 6a. Reaction of **6a** with H_2O_2 and KBr in the presence of MPO was carried out according to the manner described for the MPO catalyzed bromination of **1a**, **2a**, and **3a**. Under similar conditions, LPO catalyzed bromination in 0.25 mol dm^{-3} potassium phosphate buffer solution at pH 6.0 and CPO catalyzed oxidation in 0.25 mol dm^{-3} potassium phosphate buffer solution at pH 3.0 of **6a** were achieved. The reaction mixture was evaporated and extracted with a large amount of $CHCl_3$. The extract was evaporated and chromatographed on silica-gel TLC (Wako Pure Chemical, Wakogel B-5F) using ethyl acetate or a mixture of ethyl acetate and hexane (2:1, v/v) to give **6a**, **6b**, **7**, and **8**. The structures of **6b**,[10] **7**,[10] and **8**[11] were confirmed by comparison of ^1H NMR spectra and TLC with the samples prepared according to the published procedures.

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