

Reactions of 1-Methyl-2-Mercaptoimidazole with Hypochlorous Acid and Superoxide

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SUMMARY: Reactions of thioureylene antithyroid drugs (1-methyl-2-mercaptoimidazole and carbimazole) with hypochlorous acid (HOCl) and superoxide were followed optically and products were analyzed by mass spectrometry. 1-Methyl-2-mercaptoimidazole (MMI) and carbimazole reacted rapidly with HOCl with a rate constant of 1×10^7 and $7 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$, respectively. The characteristic spectrum assigned to MMI disulfide appeared immediately after addition of HOCl, followed by a slow conversion to a final spectrum. The conversion was dependent upon the ratio of HOCl to MMI and both antithyroid drugs uptake 3 moles HOCl for complete conversion. A similar sequence of spectral changes was also observed when the HOCl was replaced by myeloperoxidase (MPO)/ $\text{H}_2\text{O}_2/\text{Cl}^-$ system. The final oxidation product of MMI and carbimazole with HOCl and superoxide was 1-methylimidazole.

The inhibitory effect of thioureylene antithyroid compounds on peroxidase-catalyzed iodination has been investigated in detail (1,2). Final reaction products of MMI and carbimazole after the incubation with iodinating systems have been analyzed by HPLC and GC-mass spectrometry. The end products have been confirmed to be 1-methylimidazole and sulfate/sulfite (2).

Antioxidant and anti-inflammatory activity of MMI have been reported (3). Oxidative stress to cells is initiated by the generation of superoxide. Oxidative stress is also started by the formation of hypohalous acids (4). Hypohalous acid generation is catalyzed by MPO and eosinophil peroxidase in the presence of H_2O_2 and halides (5). We have observed that MMI rapidly reacted with HOCl and consumed 3 moles HOCl and have compared the reaction of MMI with HOCl and that with superoxide.

MATERIALS AND METHODS

MPO from human leukocytes, xanthine oxidase from bovine milk, xanthine, MMI, GSH, were purchased from Sigma-Aldrich Japan (Tokyo, Japan). Carbimazole was obtained from Tokyokasei (Tokyo, Japan). 1-Methylimidazole was obtained from Nacalai Tesque (Kyoto, Japan). Sodium hypochlorite (NaOCl) was obtained from Wako Pure Chemical Industry (Osaka, Japan). Reactions were carried out at 25°C, in 0.1M phosphate buffer, pH 7.0. Spectrophotometric measurements were performed with a Shimadzu MPS-2000 spectrophotometer. Desorption chemical ionization mass spectrometry was performed with a JEOL JMS-SX102QQ.

RESULTS

Reaction of MMI with HOCl was followed in the uv region ranging from 360 to 210 nm. Spectral changes to the final spectrum with time were dependent upon the ratio of HOCl added to fixed concentration of MMI (Fig. 1). When the ratio is slightly lower than 1, the new spectrum with a broad peak around 290-310 nm appeared immediately, followed by a slow conversion to MMI (Fig. 1A). The transiently formed spectrum was identical with that of MMI disulfide (2,7). If the molar ratio was raised above 3, MMI disulfide was then converted to a new spectrum (Fig. 1C). The spectral changes after the reaction of MMI with HOCl were completely inhibited when the reaction was started in the presence of equimolar concentration of GSH and HOCl (Fig. 1D). MPO catalyzes the oxidation of Cl^- at the expense of H_2O_2 , producing HOCl. Therefore, MMI was treated with MPO/ $\text{H}_2\text{O}_2/\text{Cl}^-$ system. MMI disulfide was formed transiently and was converted to the final spectrum with time. Carbimazole has an absorption peak at 291 nm, which was diminished by the addition of 2 molar excess of HOCl over carbimazole.

Final reaction products were, therefore, analyzed by DCI mass spectrometry. Reaction mixtures after a reaction of MMI with a limited amount of HOCl were subjected to mass spectrometry. MMI

and 1-methylimidazole gave the expected signals at 114 (m/z) and 82 (m/z), respectively. Increasing the HOCl concentration at constant MMI concentration gave a progressive decrease in the MMI peak. When the mole ratio of HOCl to MMI is 4, a complete loss of MMI was observed. Treatment of MMI with MPO/ $\text{H}_2\text{O}_2/\text{Cl}^-$ system was also performed. After 1 h, the reaction mixture gave one product peak at 82 (m/z) corresponding to 1-methylimidazole. 1-methylimidazole has an absorption peak at 210 nm. MMI sulfinate should be formed before the formation of 1-methylimidazole and sulfate (2). The final absorption spectrum that has a peak at 220 nm could be a MMI sulfinate (Fig. 1C). MMI sulfinate would be, then, degraded into 1-methylimidazole and sulfate during an ionization process. Addition of 3 moles HOCl to carbimazole resulted in the complete loss of carbimazole ($m/z = 186$) and MMI ($m/z = 114$). Present results indicate that carbimazole was converted to 1-methylimidazole through MMI.

It has been reported that MMI reacts with superoxide and acts as an antioxidant. The rate constant for the reaction of MMI and carbimazole with superoxide is estimated to be 1.3×10^3 and <10

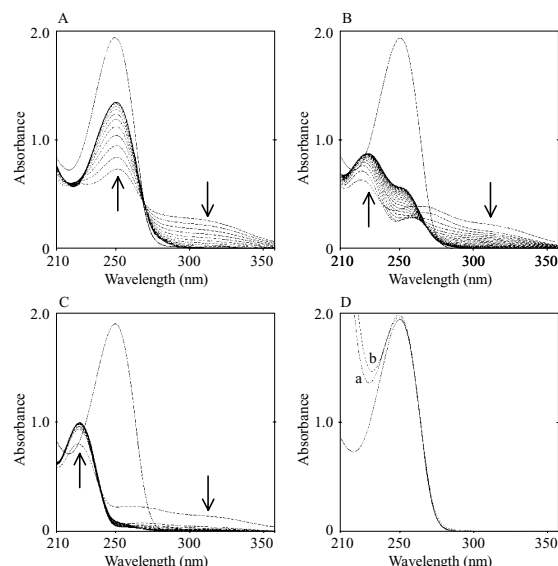


Fig. 1. Effects of HOCl concentrations upon the spectral changes of 1-methyl-2-mercaptoimidazole.

Concentrations were 120 μM 1-methyl-2-mercaptoimidazole and 105 (A), 315 (B) or 420 (C, D) μM HOCl in the absence or presence of 500 μM GSH (D). In D, spectra were obtained in the presence of GSH (a) and both in the presence of GSH and HOCl (b). Reactions were carried out in 0.1 M phosphate (pH7.0) and started by the addition of HOCl. Spectra were obtained after addition of HOCl at an interval of 2 min.

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Table 1. Second-order rate constants of MMI and carbimazole for the reactions with HOCl and superoxides, and the amounts of consumed HOCl by MMI and carbimazole

	Rate constants (M ⁻¹ s ⁻¹)		Consumed HOCl ³⁾ (mole ratio)
	HOCl ¹⁾	Superoxide ²⁾	
MMI	1.0 × 10 ⁷	1.3 × 10 ³	3
Carbimazole	7 × 10 ⁶	< 10	3
1-Methylimidazole	< 10 ⁴	< 10	
Glutathione	≥ 10 ⁷ ⁴⁾	8 × 10 ² ⁵⁾	4 ⁶⁾

^{1,2)}: Rate constants were estimated from the competition kinetics.

³⁾: Consumed HOCl for the complete disappearance of MMI and carbimazole.

⁴⁾: (Ref. 6)

⁵⁾: Shishido, N. et al. (2001) Arch. Biochem. Biophys., 387, 216-222.

⁶⁾: (Ref. 8)

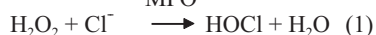
M⁻¹s⁻¹, respectively (Table 1). One-electron oxidation of sulfhydryl compounds by superoxide leads to the formation of disulfide and oxygen insertion. To confirm these products, MMI and carbimazole were treated with superoxide generating system. 1-Methylimidazole was detected with a decrease of MMI or carbimazole after incubation (data not shown).

DISCUSSION

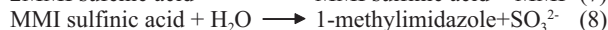
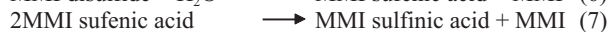
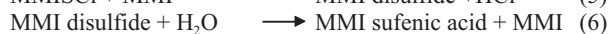
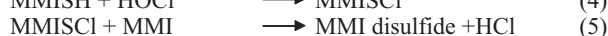
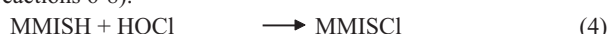
Estimated rate constants for the reaction of HOCl with MMI and carbimazole are slower than that with GSH (Table 1). The result is compatible with the observation that the spectral change was inhibited when the reaction was performed in the presence of equimolar concentration of MMI and GSH (Fig. 1D)

MPO reacts rapidly with H₂O₂ to form compound I, which catalyzes two-electron oxidations of Cl⁻, generating HOCl (reaction 1). When the reaction is performed in the presence of excess GSH, chlorination of GSH proceeds predominantly until H₂O₂ is exhausted (reaction 2). Resulting GSCl reacts with GSH to form GSSG (reaction 3). In these reactions, halides (I⁻, Br⁻, Cl⁻) play the role of effective mediator in peroxidase-catalyzed oxidation of GSH.

MPO



MMI disulfide is unstable and is spontaneously hydrolyzed to MMI and MMI sulfenic acid (reaction 6) (2). Consecutive spontaneous conversion of MMI sulfenic acid to 1-methylimidazole and free sulfenic acid through MMI sulfenic acid has been suggested (reactions 6-8).



GSH consumes totally 4 moles of HOCl (8). GSH contains one sulfhydryl group and a primary amino group. The sulfhydryl group and primary amino group of GSH consume 3 moles HOCl and one mole HOCl, respectively. Present results are compatible with the assumption that each MMI or carbimazole molecule takes up 3 moles HOCl (Table 1). When HOCl is in excess over MMI, reactions 4-8 could be accelerated as suggested in the case of GSH. Figure 1C supports the assumption that conversion of MMI to final product is accelerated in the presence of 4-fold mole excess of HOCl to MMI.

Superoxide generation in cells causes the formation of more harmful reactive oxygen species, which relates to oxidative stress to the cells. Rate constant for the reaction of superoxide with MMI was slightly faster than that with GSH. One-electron oxidation of the sulfhydryl group of MMI with superoxide also gave 1-methylimidazole (data not shown). Hypochlorous acids produced by MPO during the respiratory burst of stimulated neutrophils show a bactericidal activity and cytotoxicity (4,5). HOCl-induced cytotoxicity is protected by GSH and ascorbate through their scavenging activity (4). The present results show that MMI and carbimazole scavenge neutrophil-derived reactive oxygen species.

We can conclude that chlorination or one-electron oxidation of sulfhydryl group of MMI leads to the formation of 1-methylimidazole.

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