Mechanism-based inactivation of gastric peroxidase by mercaptomethylimidazole

Uday BANDYOPADHYAY, Dipak Kr. BHATTACHARYYA and Ranajit K. BANERJEE*

Department of Physiology, Indian Institute of Chemical Biology, 4 Raja S.C. Mullick Road, Calcutta 700032, India

The mechanism of inhibition of gastric peroxidase (GPO) activity by mercaptomethylimidazole (MMI), an inducer of gastric acid secretion, has been investigated. Incubation of purified GPO with MMI in the presence of H_2O_2 results in irreversible inactivation of the enzyme. No significant inactivation occurs in the absence of H_2O_2 or MMI, suggesting the involvement of peroxidase-catalysed oxidized MMI (MMI_{ox}) in the inactivation process. The inactivation follows pseudo-first-order kinetics consistent with a mechanism-based (suicide) mode. The pseudo-first-order kinetic constants at pH 8 are $k_1 = 111 \mu M$, $k_{inact} = 0.55 \text{ min}^{-1}$ and $t_{1/2} = 1.25 \text{ min}$, and the second-order rate constant is $0.53 \times 10^4 \text{ M}^{-1} \cdot \text{min}^{-1}$. Propylthiouracil also inactivates GPO activity in the same manner but its efficiency ($k_{inact}/k_1 = 0.46 \text{ mM}^{-1} \cdot \text{min}^{-1}$) is about 10 times lower than that

of MMI ($k_{inact}/k_1 = 5 \text{ mM}^{-1} \cdot \text{min}^{-1}$). The rate of inactivation with MMI shows pH-dependence with an inflection point at 7.3, indicating the involvement in the inactivation process of an ionizable group on the enzyme with a pK_a of 7.3. The enzyme is remarkably protected against inactivation by micromolar concentrations of electron donors such as iodide and bromide but not by chloride. Although GPO oxidizes MMI slowly, iodide stimulates it through enzymic generation of I^+ which is reduced back to I^- by MMI. Although MMI_{ox} is formed at a much higher rate in the presence of I^- , a constant concentration of I^- maintained via the reduction of I^+ by MMI, protects the active site of the enzyme against inactivation. We suggest that MMI inactivates catalytically active GPO by acting as a suicidal substrate.

INTRODUCTION

Gastric mucosa of mouse and rat has long been known to contain a highly active peroxidase (Banerjee and Datta, 1981; Banerjee et al., 1982; De and Banerjee, 1984). The enzyme from rat stomach has been purified to homogeneity and shown to be entirely different from thyroid and other extrathyroidal peroxidases (De and Banerjee 1986). We have further observed that mercaptomethylimidazole (MMI), an antithyroid drug of the thionamide group, induces acid secretion *in vivo* in mice (Bhattacharjee et al., 1989). This drug also stimulates gastric acid secretion both *in vivo* and *in vitro* in rat stomach with concomitant inhibition of the gastric peroxidase (GPO) activity (Bandyopadhyay et al., 1992). Thus, besides its antithyroid effect, this drug plays a new role in induction of acid secretion through its inhibitory effect on GPO activity (Banerjee et al., 1990; Bandyopadhyay et al., 1992). However, how MMI inhibits GPO activity is not yet known. The mechanism of inhibition of thyroid peroxidase (TPO) and lactoperoxidase (LPO) by thiocarbamides has been described (Taurog, 1976; Davidson et al., 1978; Edelhock et al., 1979; Engler et al., 1982; Ohtaki et al., 1982; Nakamura et al., 1984; Doerge, 1986b). A substantial body of experimental evidence suggests that modification of the iron porphyrin of TPO and LPO occurs concomitantly with the loss of enzyme activity (Engler et al., 1982; Ohtaki et al., 1982; Nakamura et al., 1984). Most antithyroid drugs, such as MMI, propylthiouracil (PTU) and thiourea, are thiol-containing compounds. The inhibitory action of thiol compounds on TPO-catalysed iodination is believed to be mediated through two independent reactions: the interaction of active iodine species with the thiol group (Thomas and Aune, 1977) and irreversible

inactivation of the enzyme (Taurog, 1976; Davidson et al., 1978). In the case of LPO, the irreversible inactivation caused by these compounds is accompanied by a change in the optical absorption spectrum of the enzyme (Michot et al., 1979; Pommier and Cahnmann, 1979; Edelhock et al., 1979). It is suggested that the irreversible inactivation of LPO and TPO by MMI results from the accelerated conversion of compound I into compound II by MMI and interaction of MMI with compound II to form a catalytically inactive MMI adduct (Ohtaki et al., 1982). It has been shown that LPO compound II reacts with MMI to form a typical sulphaem-containing haemoprotein (Nakamura et al., 1984) as do haemoglobin, myoglobin and catalase (Nicholls, 1961) when they react with sulphide. Nakamura et al. (1984) called this adduct sulphactoperoxidase. Doerge (1986a,b) suggested the involvement of enzymic sulphoxidation of thiocarbamide by LPO resulting in mechanism-based inhibition. Using radiolabelled MMI and PTU, firm binding of these drugs with concomitant inactivation of the enzyme was shown to occur on their addition to oxidized TPO only. However, neither binding nor inactivation occurs on addition of these drugs to native TPO (Engler et al., 1982).

So far most of the work reported on TPO and LPO has been carried out with a view to understanding the mechanism of action of the antithyroid drugs of the thionamide group. Here we present a novel finding that MMI, a new secretion-stimulating compound (Bhattacharyya et al., 1989; Bandyopadhyay et al., 1992), also inhibits the activity of GPO. Kinetic evidence has been provided to show that MMI inactivates GPO irreversibly by acting as a suicidal substrate following mechanism-based kinetics, and an ionizable group on the enzyme with pK_a 7.3 greatly enhances the rate of inactivation. Evidence is also

Abbreviations used: MMI, mercaptomethylimidazole; MMI_{ox} , oxidized MMI; PTU, propylthiouracil; GPO, gastric peroxidase; TPO, thyroid peroxidase; LPO, lactoperoxidase.

* To whom all correspondence should be addressed.

presented that the enzyme is protected against inactivation in the presence of electron donors such as iodide or bromide and not by chloride.

EXPERIMENTAL

Materials

MMI, 1-methylimidazole, 2-mercaptoimidazole, imidazole, PTU, thiourea and BSA were obtained from Sigma. ICI was purchased from Aldrich and Na^{125}I was obtained from the Bhabha Atomic Research Centre, Trombay, India. All other chemicals used were of analytical grade.

Inactivation of GPO by MMI

GPO was purified from rat stomach as described previously (De and Banerjee 1986). All kinetic measurements were made in a Pye-Unicam SP8-100 u.v./visible recording spectrophotometer at $25 \pm 1^\circ\text{C}$. To measure the peroxidase activity, iodide oxidation was monitored by following the formation of I_3^- at 353 nm (Alexander, 1962) as described previously (Banerjee et al., 1986). The rate of inactivation of GPO by MMI was measured by incubation of GPO ($0.05 \mu\text{M}$) in the presence of H_2O_2 ($100 \mu\text{M}$) and MMI ($8\text{--}60 \mu\text{M}$) in a final volume of $30 \mu\text{l}$ containing 50 mM Tris/HCl buffer, pH 8. At various time intervals after addition of the inactivator and H_2O_2 , the incubation mixture was transferred to a cuvette containing 1 ml of assay mixture containing 50 mM sodium acetate buffer (pH 5.2), 1.7 mM KI and 0.27 mM H_2O_2 . Stoichiometric measurements of H_2O_2 were made by adding an excess of the drug ($60 \mu\text{M}$) to a mixture of

GPO ($0.1 \mu\text{M}$) and various concentrations of H_2O_2 ($0\text{--}420 \mu\text{M}$) in 50 mM Tris/HCl buffer, pH 8. After a 5 min incubation, a portion was added to the assay mixture to determine the enzyme activity. The effect of pH on the inactivation by MMI in the presence of H_2O_2 was determined by incubating the enzyme in 50 mM sodium phosphate or Tris/HCl buffers of pH values $6\text{--}9.5$. Analogue studies were carried out in the same incubation mixture as mentioned above except that MMI was replaced by equimolar concentrations of the analogue. During the course of the study of substrate protection against inactivation, electron donor was added to the incubation mixture containing the enzyme before the addition of MMI and H_2O_2 .

Peroxidase activity measured by radioliodide incorporation into BSA

Peroxidase-catalysed radioiodide organification of protein was measured as described previously (Taurog, 1976). The incubation mixture contained in a final volume of 1 ml : 50 mM Tris/HCl, pH 8, $100 \mu\text{M}$ Na^{125}I (10^6 c.p.m.), 1 mg of BSA, $0.01 \mu\text{M}$ GPO and $100 \mu\text{M}$ H_2O_2 added last to start the reaction. It was incubated for 30 min at $25 \pm 1^\circ\text{C}$ and the reaction was stopped by the addition of ice-cold trichloroacetic acid (5% final concentration). The reaction mixture was immediately vortexed and placed into an ice-bath for 10 min followed by centrifugation in an Eppendorf microfuge to obtain the pellet. It was washed with $3 \times 2 \text{ ml}$ of cold 5% trichloroacetic acid containing 1 mM KI and 1 mM MMI and counted for radioactivity in a γ -ray solid-scintillation counter. Peroxidase activity is expressed as nmol of I^- incorporated into BSA/min per mg of enzyme protein.

Figure 1 Kinetics of the inactivation of GPO by MMI in the presence of $100 \mu\text{M}$ H_2O_2

(a) Calculation of the pseudo-first-order rate constant of inactivation for iodide oxidation. GPO ($0.05 \mu\text{M}$) was incubated with different concentrations of MMI in the presence of $100 \mu\text{M}$ H_2O_2 at $25 \pm 1^\circ\text{C}$ in a final volume of 1 ml containing 50 mM Tris/HCl buffer, pH 8. The data were plotted as described in the text. The concentrations (μM) of MMI used are indicated in parentheses. Inset (b) Determination of second-order rate constant of inactivation of iodide oxidation by GPO. The slopes of the straight lines obtained in (a) were plotted against concentration of MMI. The slope of this line indicates the second-order rate constant of inactivation, which is $0.53 \times 10^4 \text{ min}^{-1} \cdot \text{M}^{-1}$. Inset (c) Kinetics of mechanism-based inactivation of GPO by MMI. The times required for half-time of inactivation at each concentration of MMI obtained from the straight lines of (a) were plotted against their corresponding reciprocal MMI concentrations. Kinetic constants ($t_{1/2}$) were calculated from the y-axis intercept and k_1 from the x-axis intercept of the straight line. k_{inact} was calculated by dividing the first-order rate constant (0.69) by the $t_{1/2}$ value (Jung and Metcalf, 1975). Data are means $\pm$ S.E.M. ($n = 3$).

Oxidation of MMI

Oxidation of MMI was monitored in a Shimadzu UV-2201 u.v./visible recording spectrophotometer by following the rate of disappearance of its absorbance at 250 nm (Taurog, 1976) at $25 \pm 1^\circ\text{C}$. The incubation mixture contained in a final volume of 1 ml: 50 mM Tris/HCl, pH 8, 10 μM MMI and 0.02 μM GPO in the presence or absence of various concentrations of iodide (50–500 μM). Non-enzymic oxidation of MMI by I^+ was also monitored under identical conditions in the absence of enzyme and iodide but after stepwise addition of ICl (final concentration of 10 μM ICl added at a time).

RESULTS

Inactivation of GPO by MMI

Preincubation of GPO with increasing concentrations of MMI in the presence of fixed H_2O_2 concentration results in concentration- and time-dependent irreversible inactivation of the enzyme following pseudo-first-order kinetics (Figure 1a). When K_{obs} , obtained from the slope of each line was plotted against MMI concentration, a straight line (Figure 1b inset) was obtained from which a second-order rate constant was calculated to be $0.53 \times 10^4 \text{ M}^{-1} \cdot \text{min}^{-1}$ at 25°C . The half-life of the enzyme inactivation ($t_{1/2}$) at each MMI concentration, when plotted against the reciprocal of MMI concentration, yields a straight line (Figure 1c inset). From this plot, the affinity and kinetic constants were calculated and the values of k_i , k_{inact} , and $t_{1/2}$ were found to be 111 μM , 0.55 min^{-1} and 1.25 min respectively.

PTU, another well-known thionamide, also inhibits GPO irreversibly in the presence of H_2O_2 in a time- and concentration-dependent manner. This inactivation, like that caused by MMI, follows pseudo-first-order kinetics (Figure 2a) from which the second-order rate constant of $0.37 \times 10^3 \text{ M}^{-1} \cdot \text{min}^{-1}$ can be calculated from the plot shown in Figure 2(b inset). The k_i , k_{inact} ,

Figure 2 Kinetics of inactivation of GPO by PTU

Experimental details and calculations were the same as in Figure 1 except that PTU (50–300 μM) was used instead of MMI for the inactivation of GPO. Data are means $\pm$ S.E.M. ($n = 3$).

Figure 3 Dependence of GPO inactivation on the stoichiometric amount of MMI and H_2O_2

The activity remaining (%) is shown as a function of $\text{H}_2\text{O}_2/\text{MMI}$ ratio in the preincubation mixture. Experimental details are provided in the Experimental section.

and $t_{1/2}$ (as calculated from the plot shown in Figure 2(c inset) were found to be 500 μM , 0.23 min^{-1} and 3 min respectively. The result shows that PTU is less potent than MMI in inactivating GPO. Efficiency of inactivation (k_{inact}/k_i) was calculated to be $5 \text{ mM}^{-1} \cdot \text{min}^{-1}$ for MMI and $0.46 \text{ mM}^{-1} \cdot \text{min}^{-1}$ for PTU, indicating that MMI is at least 10 times more potent than PTU. If $t_{1/2}$ or the minimum time for half-inactivation under saturating concentration of inhibitor is considered, MMI was also found to be more potent than PTU. Thiourea also inactivates GPO but is even less effective than PTU (results not shown).

The stoichiometric dependence of inactivation on H_2O_2 concentration is shown in Figure 3. Two equivalents of H_2O_2 with respect to MMI are required for 50% inhibition, and 4 equivalents are needed for 80% inactivation. Control experiments show no significant inactivation of the enzyme when preincubated with MMI or H_2O_2 alone. No catalytic activity could be recovered by either dilution of the reaction mixture or removing MMI and H_2O_2 by Sephadex G-25 chromatography in a spin column.

pH-dependence of GPO inactivation by MMI

The inactivation of GPO by MMI is dependent on the pH of the preincubation medium (Figure 4a). The rate of inactivation is lower at pH values below 7 whereas it increases above pH 7 attaining a maximum at pH 8.5–9. The pH-dependence of inactivation may reflect the requirement of an ionizable group on the enzyme for loss of catalytic activity with MMI. The pK_a value of any ionizable group was therefore calculated from the kinetic data, which may be expressed as follows:

$$K_{\text{obs}} = \frac{(K_{\text{obs}})_{\text{max}}}{1 + \frac{[\text{H}^+]}{K_a}} \quad (1)$$

which in linear form may be expressed as

$$K_{\text{obs}} (\text{H}^+) = K_a (K_{\text{obs}})_{\text{max}} - K_a K_{\text{obs}} \quad (2)$$

where K_a is the dissociation constant of the reacting group and $(K_{\text{obs}})_{\text{max}}$ is the pseudo-first-order rate constant of the unprotonated reacting group (Takeuchi et al., 1986). The pseudo-first-order rate constant was determined and plotted against pH (Figure 4a). The open circles indicate experimental data and the solid line indicates the theoretical curve. From a plot of $K_{\text{obs}} (\text{H}^+)$ against K_{obs} (values taken from Figure 4a), a straight line is obtained, the slope of which gives rise to a pK_a value of 7.3 (Figure 4b). It thus appears that ionization of a group on the

Figure 4 (a) pH-dependence of the inactivation rate of GPO with MMI and (b) experimentally obtained pseudo-first-order rate constants plotted according to eqn. (1)

(a) GPO ($0.05 \mu\text{M}$) was incubated with $30 \mu\text{M}$ MMI in 50 mM Tris/HCl buffer (pH 6–9.5). The pseudo-first-order rate constants obtained were plotted against pH ($\circ$). The curve obtained (—) is a theoretical one. (b) the pK_a value obtained from the slope was 7.3.

enzyme with pK_a 7.3 greatly enhances the rate of inactivation of GPO.

SH group of MMI is essential for inactivation

In order to discover which group of MMI (2-mercapto-1-methylimidazole) is involved in the inactivation of GPO, the enzyme was preincubated with various MMI analogues in the absence or presence of H_2O_2 to check the loss of activity (results not shown). Only 2-mercaptoimidazole is equally as active as 2-mercapto-1-methylimidazole whereas 1-methylimidazole, which is devoid of an SH group, is ineffective. Only imidazole has no effect. However, inactivation occurs only when the enzyme is preincubated with the former two compounds in the presence of H_2O_2 . Evidence is presented below (Figure 6a) that the enzyme slowly catalyses the oxidation of MMI by H_2O_2 . Presumably the compound produced by oxidation of the SH group of MMI by the catalytically active GPO– H_2O_2 complex is responsible for concomitant inactivation of the active enzyme.

Protection of GPO against MMI inactivation in the presence of electron donors

The kinetic data presented so far indicate that GPO is inactivated irreversibly by MMI which acts as a suicidal substrate for

oxidized GPO. However, inactivation could be prevented if the enzyme was preincubated with MMI in the presence of another electron donor such as iodide. Figure 5(a) shows the effect of increasing the concentration of iodide on the kinetics of GPO inactivation. Whereas $30 \mu\text{M}$ MMI alone could inhibit 80 % activity after 5 min, it caused 60 % inactivation in the presence of $30 \mu\text{M}$ iodide and 30 % in the presence of $100 \mu\text{M}$ iodide. Bromide also protects the enzyme against inactivation but requires slightly higher concentrations than iodide (Figure 5b). The protection by iodide or bromide is more effective during the initial (1 min) time of inactivation. However, chloride shows no protection under identical conditions.

Oxidation of MMI by GPO in the presence or absence of iodide

While the mechanism of protection by iodide was being studied, MMI oxidation by GPO was studied in the absence and presence of iodide (Figure 6a). In absence of iodide, the rate of oxidation of MMI is low. No oxidation, however, occurs in the absence of enzyme or H_2O_2 (results not shown) or in the presence of N_3^- , indicating that catalytically active GPO is capable of oxidizing MMI. However, the rate is low because the active enzyme is concomitantly inactivated by the generation of MMI_{ox} in the first few catalytic cycles. In the presence of iodide, the rate of oxidation of MMI increases significantly as a function of iodide concentration and is completely blocked in the presence of N_3^- , indicating that increased MMI oxidation by I^- is catalysed by peroxidase. The inset (Figure 6b) further shows that this enzyme-catalysed iodide-dependent MMI oxidation is a saturable process with respect to iodide concentration. It is interesting to note that, although MMI is oxidized at a much higher rate by iodide, the oxidation product could not inactivate the enzyme, whereas the latter is inactivated easily when MMI is oxidized at a lower rate in the absence of iodide. This is possible when the active site of the catalytically active GPO is protected by iodide from interaction with MMI_{ox} , which otherwise would form an irreversible complex for suicidal inactivation of the active enzyme.

Oxidation of iodide by GPO

As iodide oxidation at pH 8 occurs at a rate too low to be detected spectrophotometrically with the micromolar concentrations of iodide and nanomolar concentrations of GPO used here, the more sensitive peroxidase-catalysed radioiodide organification of BSA was studied to obtain evidence of possible

Figure 5 Protection of GPO against MMI inactivation by electron donors

Various concentrations (μM ; indicated in parentheses) of KI (a) or KBr (b) were added to the incubation mixture containing GPO ($0.05 \mu\text{M}$) before the addition of MMI ($30 \mu\text{M}$) and H_2O_2 ($100 \mu\text{M}$) in 50 mM Tris/HCl buffer, pH 8. After different time intervals, the remaining peroxidase activity was monitored as described in the Experimental section.

Figure 6 Effect of iodide on MMI oxidation

(a) MMI oxidation was monitored at 250 nm in a final volume of 1 ml containing 50 mM Tris/HCl buffer, pH 8, GPO (0.05 μM), H_2O_2 (100 μM) and MMI (10 μM) in the presence or absence of various concentrations of iodide as indicated or N_3^- (100 μM). Inset (b) Initial rate of MMI oxidation was plotted against various concentrations of iodide used.

Figure 7 Non-enzymic oxidation of MMI by ICI

Details are described in the Experimental section. (a) Effect of additions of 10 μM ICI, indicated by arrows, on the absorbance of MMI at 250 nm. (b) Corresponding reduction of I^+ to I^- by MMI. A, 10 μM MMI; B, 10 μM MMI + 10 μM ICI; C, B + 10 μM ICI; D, C + 10 μM ICI.

oxidation of I^- to I^+ (Taurog, 1976). The result (not shown) indicated that GPO can oxidize I^- and incorporates 55 nmol of I^- into BSA/min per mg of enzyme. This iodinating activity is dependent on the concentration of enzyme and H_2O_2 and is sensitive to N_3^- , an inhibitor of peroxidase. The data indicate that I^+ is produced from GPO-catalysed I^- oxidation at pH 8, a pH at which I^- protects the enzyme against MMI inactivation.

Non-enzymic oxidation of MMI by I^+

In order to investigate whether I^+ can oxidize MMI, a fixed amount of ICI was added to the reaction mixture containing only

MMI. Figure 7(a) shows that addition of 10 μM ICI causes an immediate decrease in absorbance of MMI at 250 nm indicating MMI oxidation: 20 μM ICI oxidizes 50 % of the MMI and 50 μM causes 83 % oxidation. Figure 7(b) shows that oxidation of MMI by I^+ is associated with the loss of MMI absorbance at 250 nm with the appearance of a hump at around 268 nm where MMI_{ox} absorbs (Taurog, 1976). The concomitant increase in the appearance of a peak at 226 nm (Awtrey and Connick, 1951) is due to reduction of I^+ to I^- by MMI. It thus appears that I^- is primarily oxidized by GPO to I^+ and this I^+ or the enzyme-bound I^+ ($E-I^+$) oxidizes MMI more effectively to form MMI_{ox} and I^- . Thus, although more MMI is oxidized, I^+ is recycled back to I^- to maintain a suitable concentration at the active site of the catalytically active GPO which is thus protected from interaction with MMI_{ox} .

DISCUSSION

MMI, a potent antithyroid drug of the thionamide group, irreversibly inactivates TPO and LPO; the mechanism has been extensively studied (Engler et al., 1982; Yamazaki, 1971; Ohtaki et al., 1982, 1985; Nakamura et al., 1984; Doerge, 1986a,b). It also inhibits the GPO activity with a concomitant increase in gastric acid secretion (Bandyopadhyay et al., 1992). Kinetic evidence shows that MMI inactivates GPO by acting as a suicidal substrate similarly to the mechanism for LPO (Doerge, 1986b). Catalytic turnover is also essential, indicating that the active oxidized enzyme is inactivated by the oxidation product of MMI (MMI_{ox}). Analogue studies indicate that the SH group of MMI is required for inactivation. Chemically, the SH group of MMI is highly susceptible to oxidation. Doerge (1986a,b) showed that the reactive intermediate formed by sulphoxidation of MMI by LPO compound II irreversibly inactivates LPO by interacting with its prosthetic haem group. Whether GPO is inactivated by a similar mechanism remains to be investigated.

Iodide, a putative biological substrate for peroxidase, protects GPO against inactivation. Oxidation of MMI by oxidized GPO is slow but increases in the presence of I^- . It is paradoxical that I^- , instead of favouring GPO inactivation by increased formation of MMI_{ox} , protects it. We have shown that I^- is oxidized by GPO, and I^+ non-enzymically oxidizes MMI to form I^- . Assuming that mechanism-based inactivators are poor substrates (Palfreyman et al., 1987), I^- would be oxidized by GPO at a faster rate than MMI. The enzyme- I^+ complex would thus oxidize unchanged MMI at a higher rate to form MMI_{ox} , I^- and native enzyme. This explains enzyme-catalysed iodide-induced MMI oxidation by GPO similar to that reported for TPO (Taurog, 1976) and LPO (Edelhoch et al., 1979). As a constant concentration of I^- is maintained near the active site of GPO, it is no longer susceptible to inactivation by MMI_{ox} . Most of the peroxidase substrates interact at or near the δ -mesocarbon of the haem edge for oxidation (Ortiz de Montellano, 1987; Ator and Ortiz de Montellano, 1987; Ator et al., 1987). Iodide, for example, binds at a site almost equidistant from peripheral haem 1- and 8-methyl groups in horseradish peroxidase (Sakurada et al., 1987b). If MMI interacts at the haem edge of GPO for oxidation, the reactive oxidation product may modify the electron-rich sites of the metalloporphyrin to cause irreversible inactivation.

Inactivation of GPO by MMI is controlled by an ionizable group on the enzyme with pK_a 7.3. The deprotonated enzyme is more susceptible to inactivation than the protonated form. Ignoring negligible ionization of MMI at pH 8 [pK_a of thiolimidazole is 10.8 (Stowell, 1961)], we suggest that ionization of a group on the enzyme with pK_a 7.3 greatly enhances the rate of inactivation. Presumably, the reactivity of GPO with MMI_{ox} is

controlled by this ionizable residue at the haem periphery. In several peroxidases, a distal histidine residue of pK_a 6–6.8 controls the catalysis (Poulos and Kraut, 1980; Finzel et al., 1984; Bosshard et al., 1984; Blanke and Hager, 1990; Bhattacharyya et al., 1992; Sakurada et al., 1987a; Modi et al., 1989a,b). Although a pK_a of 7.3 is rather high for a histidine residue unless its environment is perturbed, its possible role in GPO inactivation by MMI remains to be investigated. pH-dependent inactivation of GPO after modification with the histidine-specific reagent, diethyl pyrocarbonate, should shed more light on this. Nevertheless, the involvement of other amino acid functional groups with pK_a 7.3 cannot be excluded.

MMI reversibly inhibits chloroperoxidase activity as a competitive substrate (Morris and Hager, 1966). It acts as a mechanism-based inactivator for GPO (this study), TPO (Engler et al., 1982) and LPO (Doerge, 1986b) but not for prostaglandin hydroperoxidase or horseradish peroxidase (Petry and Eling, 1987). This diversity of MMI action is presumably due to differential geometry of the apoprotein around the same haem molecule of these enzymes. MMI may thus act as a suitable probe to obtain more information on the active-site topology of these peroxidases. However, we now suggest that MMI induces gastric acid secretion by irreversibly inactivating its peroxidase (Bandyopadhyay et al., 1992) by acting as a suicidal substrate. The possible involvement of increased endogenous H_2O_2 concentration in acid secretion is currently being investigated.

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