Neutrophil myeloperoxidase-mediated chloride-dependent inactivation of *Candida utilis* uricase

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Urate oxidase(uricase)from *Candida utilis*, an essential thiol enzyme, was examined for its sensitivity to myeloperoxidase, an oxidant present in neutrophils. Upon exposure to a system composed of myeloperoxidase, hydrogen peroxide and chloride at neutral or moderately alkaline pH, the uricase exhibited comparable activity to the untreated enzyme ; but upon exposure at moderately acidic pH, it exhibited less than 8.0 ± 3.0 (mean $\pm$ SE, $n=5$) % of the activity of the untreated enzyme. Thus the myeloperoxidase-H₂O₂-chloride system significantly inactivated uricase only at moderately acidic pH. This inactivation was prevented by the presence of N-acetylmethionine, a methionine analogue and a thioether compound, or glutathione, a thiol compound analogous to amino acid, indicating that it was due to the oxidation and damage of the methionine residue and/or the thiol group in uricase by the myeloperoxidase system, accompanying the loss of catalytic activity of the uricase.

Rec. 12/18/1997, Acc. 2/6/1998, pp 147~156

key words : myeloperoxidase, neutrophils, uricase,
N-acetylmethionine, glutathione

Introduction

At neutral pH, the oxidation system composed of myeloperoxidase, hydrogen peroxide and a halide, chloride or bromide, inactivates α_1 -proteinase inhibitor^{1~3)}, which has methionine at its reactive site and is the major plasma inhibitor of elastolytic activity. This system similarly inactivates chemotactic factor⁴⁾ and transferrin iron binding capacity⁵⁾, damages fibronectin⁶⁾ and denatures endothelial cell matrix heparan sulfate proteoglycan⁷⁾. It also inactivates *Clostridium difficile* cytotoxin⁸⁾. We have reported that the microbial metabolite aflatoxin B₁, a carcinogen, is oxygenated by the myeloperoxidase-hydrogen peroxide-chloride system at pH 5.0, with incorporation of one atom of oxygen per mole of aflatoxin B₁ molecule⁹⁾, and that benzo[a]pyrene¹⁰⁾, another

chemical carcinogen, and prostaglandin E₁¹¹⁾ are chlorinated by the myeloperoxidase-hydrogen peroxide-chloride system at pH 5.0. We have also reported that such enzymes as alkaline phosphatase, chymopapain, collagenase, lysozyme, papain, pepsin and trypsin were inactivated and the microbial metabolite antibiotics antimycins A₁, A₂ and A₃ were detoxicated by the system composed of myeloperoxidase, hydrogen peroxide and chloride at moderately acidic pHs of 5.0 to 6.0, and that myeloperoxidase could be replaced by neutrophils¹²⁾. Thereafter, Droždž and Naskalski reported that human serum albumin, α_1 -antiproteinase, lysozyme and human leukocyte ribonuclease were inactivated by the myeloperoxidase-hydrogen peroxide-chloride system¹³⁾ under the same moderately acidic pH conditions as in our studies^{9~12)}.

Myeloperoxidase is considered to work in the body's self-defence system in animals, and to be a unique enzyme in having two optimum pHs. It catalyzes peroxidation of phenolic hydrogen

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donors such as *o*-methoxyphenol in neutral or alkaline solution¹⁴⁾ and peroxidation of halides, chloride, bromide and iodide, at acidic to moderately acidic pH¹⁵⁾. The latter reaction results in the irreversible destruction of chemical compounds such as fuchsin basic¹⁶⁾, the cytotoxicity of cells such as B-16 melanoma tumor cells¹⁷⁾ and the hemolysis of mammalian erythrocytes such as horse, human and pig erythrocytes¹⁸⁾ in the presence of hydrogen peroxide and a halide, chloride and bromide, at moderately acidic pH. At acidic to moderately acidic pH, myeloperoxidase peroxidizes chloride, bromide, and iodide; lactoperoxidase and saliva peroxidase peroxidize bromide and iodide; and horseradish peroxidase peroxidizes iodide¹⁵⁾. There is, however, no report on the inactivation of oxygen oxidoreductase by this myeloperoxidase-hydrogen peroxide-halide system.

The aims of this study were to investigate whether *Candida utilis* uricase (EC 1. 7. 3. 3), which is a thiol enzyme oxygen oxidoreductase¹⁹⁾, and which has each twelve residue of methionine and cysteine, a sulfur containing amino acid, in one molecule²⁰⁾, is inactivated by exposure to the myeloperoxidase-hydrogen peroxide-chloride system in the same pH range as that where chloride, bromide and iodide are peroxidized by the myeloperoxidase system; (b) to investigate whether the reaction between the uricase and the myeloperoxidase system is affected by the presence of thio-compounds such as N-acetylmethionine, a methionine analogue and a thioether compound, or glutathione, a thiol compound analogous to amino acid; and (c) to propose the mechanism of the myeloperoxidase system-mediated reactions. Using an oxygen analysis method, we demonstrated that this inactivation does indeed occur.

Materials and methods

Myeloperoxidase (EC 1. 11. 1. 7) was prepared from leukocytes of normal pig peripheral blood²¹⁾. The myeloperoxidase concentration was calculated on the basis of extinction coefficient of $95 \text{ mM}^{-1} \text{ cm}^{-1}$ at 430 nm. The myeloperoxidase preparation typically exhibited an R. Z.

value ($A_{430\text{nm}}/A_{280\text{nm}}$) of 0.85 and was previously confirmed to give a single band on polyacrylamide gel electrophoresis. This preparation could oxidize $50.0 \mu\text{moles}$ of *o*-methoxyphenol per min per mg protein. The turnover number was calculated from the initial rate of increase in absorbance at 470 nm due to accumulation of oxidized *o*-methoxyphenol (extinction coefficient of $5.57 \text{ mM}^{-1} \text{ cm}^{-1}$ at 470 nm)²²⁾ in the reaction mixture (final volume of 2.5 ml) composed of 24 mM *o*-methoxyphenol, $50 \mu\text{M}$ hydrogen peroxide, 20 nM myeloperoxidase and 0.1 M phosphate buffer (pH 7.5) at 25°C. *Candida utilis* uricase (urate: oxygen oxidoreductase; EC 1. 7. 3. 3) purchased from TOYOKO (Osaka) was used without further purification. The stock uric acid solution (6 mM) was prepared in 8 mM lithium carbonate aqueous solution. Uric acid and lithium carbonate were purchased from Wako Pure Chemicals (Osaka). N-acetylmethionine, glutathione disulfide (oxidized form of glutathione) and glutathione (reduced form) were purchased from Wako (Osaka), Sigma (St. Louis) and P-L Biochemicals, Inc. (Milwaukee), respectively. Other reagents were obtained from commercial sources in the highest available states of purity. The buffers used were acetate (acetic acid/sodium acetate) for pH 4.0–5.8, phosphate (potassium phosphate, monobasic/sodium phosphate, dibasic) for pH 6.0–7.8 and borate (boric acid/sodium carbonate) for pH 8.0–9.0.

Exposure of uricase to the myeloperoxidase system was carried out as follows unless otherwise noted. The uricase (1.25 mg/ml : 6.63 units/mg) was incubated with 50 nM myeloperoxidase, 0.2 mM hydrogen peroxide and 35 mM sodium chloride in 0.1 M buffer in the range of pH 4.0 to 9.0 for 30 min at 25°C, then an equivolume of 15 mM aqueous sodium azide solution was added to stop the reaction catalyzed by myeloperoxidase. Sodium azide, an inhibitor of hematin peroxidase²³⁾, was confirmed to have no effect on the activity of uricase. Following the addition of azide, reaction mixtures were kept on ice and immediately assayed for uricase activity.

The uricase activity was evaluated from the extent of reduction of molecular oxygen by the uricase, which was measured using an oxygen analyzer equipped with a YSI model 5331 oxygen electrode (Yellow Springs Instrument) and a recorder (Hitachi, type 056). The reaction chamber was an airtight glass cell containing 1 ml of the reaction solution under continuous stirring at $24 \pm 0.1^\circ\text{C}$. The concentration of dissolved oxygen in buffer solution (pH 7.0) at 24°C was calculated as $270 \mu\text{M}^{23)}$. The catalytic activity of the uricase was assayed by addition of 1 to $10 \mu\text{l}$ of uricase solution to 1 ml of the reaction solution containing 0.4 mM uric acid in 50 mM buffer at pH 8.5, the optimal pH of native uricase, and measuring the rate of reduction of molecular oxygen. Under this condition and when $6.25 \mu\text{g/ml}$ (6.63 units/mg) of uricase was used, the rate of O_2 reduction was $33 \mu\text{M O}_2/\text{min}$. The uricase used was previously confirmed to show a linear relationship between catalytic activity and concentration up to $25 \mu\text{g/ml}$ by two assay methods, the oxygen analysis used in the present study, and spectrophotometry²⁴⁾, in which the catalytic activity of the native uricase was determined by measuring the rate of decrease in absorbance at 294 nm accompanying the oxidation of urate at 24°C . The spectrophotometric analysis was carried out with a Hitachi spectrophotometer, type 200-20 or 557.

The effects of incubation time, the concentrations of myeloperoxidase, hydrogen peroxide, chloride, N-acetylmethionine and glutathione, and pH on the uricase were evaluated relative to the activity of untreated uricase as 100% activity, which was estimated from the rate of reduction of oxygen by $6.25 \mu\text{g/ml}$ (6.63 units/mg) in the presence of 0.4 mM urate in 0.05 M buffer solution at pH 8.5 at 24°C . The 100% activities of untreated uricase, as control, were respectively used those of the given amount of uricase, which was previously incubated in 0.1 M buffer solution at a given pH for 30 min or a given time at 25°C in the absence of other components such as myeloperoxidase, hydrogen peroxide, sodium chloride, N-acetylmethionine

Fig. 1 Effect of incubation time on the inactivation of uricase by the myeloperoxidase- H_2O_2 -chloride system at pH 5.5

Curve(a) : The mixture of 1.25 mg/ml uricase, 50 nM myeloperoxidase, 0.2 mM hydrogen peroxide and 35 mM sodium chloride in 0.1 M acetate buffer (pH 5.5) was incubated at 25°C . The reaction was started by the addition of myeloperoxidase to the reaction mixture. Portions of the mixture were withdrawn at the indicated times and mixed with an equivolume of 15 mM NaN_3 solution, and $10\text{-}\mu\text{l}$ portions of these mixtures were assayed for uricase activity at pH 8.5, as described in Materials and methods.

Curve(b) : A control : the experiment was performed under the same conditions with those of experiment (a), except the myeloperoxidase was absent. Hydrogen peroxide was added at zero incubation time.

and glutathione, and was added a myeloperoxidase inhibitor azide. This incubation was confirmed to have no effect on the activity of uricase.

Results

When uricase was exposed to the system composed of myeloperoxidase, hydrogen peroxide and chloride for 30 min in 0.1 M buffer solution at 25°C , the enzyme exposed at pH 5.5 lost over 90% of the catalytic activity compared with the untreated enzyme (Fig. 1, curve a), while that exposed at pH 8.5 had comparable catalytic activity to the untreated enzyme. In control systems from which myeloperoxidase

Table 1 The catalytic activity of uricase exposed to a system composed of myeloperoxidase, hydrogen peroxide and chloride in comparison with that of the untreated uricase, at pH 5.5 and 8.5

Uricase	Activity(%)	
	At pH 5.5	At pH 8.5
Untreated	100	100
Exposed to MPO+H ₂ O ₂ +Cl ⁻ system	8.0±3.0	99.0±2.0
Exposed to H ₂ O ₂ +Cl ⁻ system	99.0±3.0	99.0±2.5
Exposed to MPO+Cl ⁻ system	99.0±3.0	99.0±2.0
Exposed to MPO+H ₂ O ₂ system	98.0±2.5	98.0±3.0
Exposed to heated MPO+H ₂ O ₂ +Cl ⁻ system	99.0±2.0	99.0±2.5
Heated	0	0

The conditions were the same as those in Fig. 1, except that the uricase was incubated in 0.1 M buffer solution at a given pH in each reaction system for 30 min at 25°C. The activity values at pH 5.5 and 8.5 both are expressed as the means±standard error of the determinations on the five preparations. MPO, myeloperoxidase.

was omitted, the catalytic activity of the uricase was scarcely reduced compared with that of the untreated enzyme (Fig. 1, curve b). In control systems from which hydrogen peroxide or chloride was omitted, the activity was again almost the same as that of the untreated enzyme. The action of uricase was shown to be related to its enzymic activity by the fact that the dissolved oxygen level in the reaction solution was unchanged when uricase was replaced by the heated uricase (at 100°C for 60 sec). The myeloperoxidase requirement was also shown to be related to its enzymic activity by the fact that the oxygen level was not reduced in the reaction mixture containing myeloperoxidase that had been inactivated by heating (at 85°C for 2 min). These experiments were repeated five times, and the results were treated statistically and are summarized in Table 1. On exposure to a system composed of myeloperoxidase, hydrogen peroxide and chloride at pH 8.5, or to the control systems, the uricase exhibited comparable activity to the untreated enzyme; but upon exposure at pH 5.5, it exhibited less than 8.0±3.0 (mean±SE, n=5) % of the activity of the untreated enzyme. The loss of uricase activity also increased as the concentration of myeloperoxidase, hydrogen peroxide or chloride in the system was increased (Fig. 2-4). The effect of myeloperoxidase, hydrogen peroxide

and chloride was increased up to over 90% of loss of uricase activity, and it was reached a plateau approximately at 50 nM of myeloperoxidase, 0.07 mM hydrogen peroxide and 25 mM chloride, respectively.

The effect of pH on the inactivation of uricase caused by the system composed of myeloperoxidase, hydrogen peroxide and chloride was examined by measuring the uricase activity after incubation of at different pH values in the range of pH 4.0 to 9.0 in the presence or absence of the myeloperoxidase system (Fig. 5). The results show that the catalytic activity of uricase was substantially abolished by incubation in the presence of the myeloperoxidase system at around pH 5.5, but not at neutral to moderately alkaline pH, under which conditions it was comparable to that of uricase (untreated uricase) incubated in the absence of the myeloperoxidase system. At pH 5.5, the untreated uricase showed 100% activity (curve b) and the uricase exposed to the myeloperoxidase system showed almost 0% activity (curve a). In the acidic region below pH 5.5, the activity of untreated uricase fell steeply (curve b). These results indicate that the loss of catalytic activity of uricase in the presence of myeloperoxidase system at moderately acidic pH was mostly due to the abolishment of uricase activity by the myeloperoxidase sys-

Fig. 2 Effect of myeloperoxidase concentration on the inactivation of uricase by the myeloperoxidase- H_2O_2 -chloride system at pH 5.5

Mixtures of 1.25 mg/ml uricase, 0.2 mM hydrogen peroxide, 35 mM sodium chloride and various concentrations of myeloperoxidase in 0.1 M acetate buffer (pH 5.5) were incubated for 30 min at 25°C. The reaction was started by the last addition of myeloperoxidase to the mixture. After the incubation, portions of each mixture were mixed with an equivolume of 15 mM NaN_3 solution, then 10- μl portions of these mixtures were assayed for uricase activity at pH 8.5, as described in Materials and methods.

Fig. 3 Effect of hydrogen peroxide concentration on the inactivation of uricase by the myeloperoxidase- H_2O_2 -chloride system at pH 5.5

Mixtures of 1.25 mg/ml uricase, 50 nM myeloperoxidase, 35 mM sodium chloride and various concentrations of hydrogen peroxide in 0.1 M acetate buffer (pH 5.5) were incubated for 30 min at 25°C. The reaction was started by the addition of myeloperoxidase to the mixture. After the incubation, portions of each mixture were mixed with an equivolume of 15 mM NaN_3 solution, then 10- μl portions of these mixtures were assayed for uricase activity at pH 8.5, as described in Materials and methods.

tem, that the myeloperoxidase system attacks uricase at moderately acidic pH, resulting in the inactivation of the uricase, and that the effect of pH observed here can be thought to reflect the pH behaviour of the myeloperoxidase system itself.

The abolishment of catalytic activity of uricase caused by exposure to the myeloperoxidase-hydrogen peroxide-chloride system was completely prevented by the presence of N-acetylmethionine and also prevented over 86% by the presence of glutathione (Fig. 6, Table 2). From these results, it was concluded that the inactivation of uricase by the myeloperoxidase-hydrogen peroxide-chloride system was probably due to the attack by this system on the methionine residue and/or the thiol group of the uricase, resulting in damage of the catalytic site.

Discussion

The finding in the present study that the system composed of myeloperoxidase, hydrogen peroxide and chloride, inactivated uricase at moderately acidic pH was consistent with earlier findings that myeloperoxidase catalyzes peroxidation of the chloride¹⁵⁾, resulting in the destruction of target substances such as fuchsin basic¹⁶⁾, oxygenation of aflatoxin B₁⁹⁾, chlorination of benzo[a]pyrene¹⁰⁾ and prostaglandin E₁¹¹⁾, inactivation of antimycins and hydrolases¹²⁾, cytolysis of living cells such as B-16 melanoma tumor cells¹⁷⁾ and hemolysis of mammalian erythrocytes¹⁸⁾ in the presence of hydrogen peroxide and chloride. This finding suggested that the peroxidation of the halide is the rate-limiting step in these processes. It is also worth noting that the optimal pHs in the moderately acidic range of pH 5.0 to 6.0 observed in the present study and in our previous

Fig. 4 Effect of chloride concentration on the inactivation of uricase by the myeloperoxidase H_2O_2 -chloride system at pH 5.5

Mixtures of 1.25 mg/ml uricase, 50 nM myeloperoxidase, 0.2 mM hydrogen peroxide and various concentrations of sodium chloride in 0.1 M acetate buffer (pH 5.5) were incubated for 30 min at 25°C. The reaction was started by the addition of myeloperoxidase to the mixture. After the incubation, portions of each mixture were mixed with an equivolume of 15 mM NaN_3 solution, then 10- μ l portions of these mixtures were assayed for uricase activity at pH 8.5, as described in Materials and methods.

studies^{9-12,17,18}) lie within the limits of the pH changes, from neutral or slightly acidic to moderately acidic (pH 4.7 to 5.2), which occur in cytoplasm of leukocytes during phagocytosis, as reported by Pavlov and Solov'ev²⁵). This parallelism suggests that myeloperoxidase localized in phagolytic leukocytes, namely, neutrophils, naturally acts at acidic pH in the presence of a halide ion, probably chloride.

The protective effect of N-acetylmethionine and glutathione on the uricase against attack by the myeloperoxidase system indicates that the target of attack is thioether and/or thiol, sulfhydryl, residues in the uricase, which probably functions to express the catalytic activity of the uricase. This protective effect probably reflects that N-acetylmethionine and glutathione in place of the thioether and/or the thiol residues in the uricase scavenged the oxidants,

Fig. 5 Effect of pH on the inactivation of uricase by the myeloperoxidase- H_2O_2 -chloride system.

Curve (a) : Mixtures of 1.25 mg/ml uricase, 50 nM myeloperoxidase, 0.2 mM hydrogen peroxide and 35 mM sodium chloride in 0.1 M buffer solutions at various pHs were incubated for 30 min at 25°C. The reaction was started by the addition of myeloperoxidase to the mixture. After the incubation, portions of each mixture were mixed with an equivolume of 15 mM NaN_3 solution, then 10- μ l portions of these mixtures were assayed for uricase activity at pH 8.5 as described in Materials and methods.

Curve (b) : Untreated uricase : solutions of 1.25 mg/ml native uricase in 0.1 M buffer at the given pH were incubated at 25°C for 30 min, then a portion of each solution was mixed with an equivolume of 15 mM NaN_3 solution, and 10- μ l portions of these mixtures were assayed for uricase activity at pH 8.5, as (a).

which would consist of the products of chloride peroxidized by myeloperoxidase and/or their by-products, as described below. Inactivation of the uricase is probably the result of oxidative damage by the myeloperoxidase system, involving oxidation of methionine residue to chlorothioether, methionine sulfoxide and methionine sulfone and/or oxidation of SH residue to chlorothioether, sulphinic acid and sulphonic acid. The thioether and thiol groups in the uricase might essentially be susceptible to the myeloperoxidase system, and those are probably located at a position where those are

Fig. 6 Effects of N-acetylmethionine and glutathione on the inactivation of uricase by the myeloperoxidase-H₂O₂-chloride system at pH 5.5. Mixtures of 1.25 mg/ml uricase, 50 nM myeloperoxidase, 0.2 mM hydrogen peroxide, 35 mM sodium chloride and various concentrations of N-acetylmethionine (A) or glutathione (B) in 0.1 M acetate buffer (pH 5.5) were incubated for 30 min at 25°C. The reaction was started by the addition of myeloperoxidase to the mixture. After the incubation, portions of each mixture were mixed with an equivalent volume of 15 mM NaN₃ solution, then 10-μl portions of these mixtures were assayed for uricase activity at pH 8.5, as described in Materials and methods.

Table 2 Protective effect of thio-compounds on the inactivation of uricase by the myeloperoxidase system

Thio-compound	Protected uricase activity (%)
N-acetylmethionine (0.1 mM)	100±0
Glutathione (1 mM)	86.8±0.5

The conditions were the same as those in Fig. 6, except that the concentrations of N-acetylmethionine and glutathione were 0.1 mM and 1 mM, respectively. The uricase activity values protected by N-acetylmethionine and glutathione are expressed as the means±standard error of the determinations of five preparations.

residue and/or the cleavage of disulfide bond in the uricase, because this inactivation was prevented approximately 30% when glutathione was replaced by glutathione disulfide (1mM) under the same conditions as in Table 2. The finding that N-acetylmethionine completely protected the uricase from the attack by the myeloperoxidase-hydrogen peroxide-chloride system at one tenth to sixth the concentration of glutathione suggests that methionine residue is more susceptible to this system than thiol group in uricase (Fig. 6, Table 2). The oxidation of sulfhydryl residue in protein by the myeloperoxidase system has been reported in the case of bovine serum albumin and β-lactoglobulin^{26,27}. The effect of thiol compound analogous to amino acid, such as glutathione, in protecting the thiol group of protein from oxidative attack by the myeloperoxidase system, has similarly been reported in the case of α₁-proteinase inhibitor²⁸. On the other hand, methionine-containing chemotactic factor (C5a) was readily inactivated by a system of myeloperoxidase, hydrogen peroxide and chloride, bromide or iodide as a result of the oxidation of methionine by this system⁴. The -SH of N-acetylcysteine and the -SS- group of cystine were both oxidized to cysteic acid by the myeloperoxidase system²⁹. Thus the halide-dependent myeloperoxidase-mediated oxidation system has a high reactivity with thio-groups, whether in proteins or free amino acids.

readily attacked, for example, on the surface of the enzyme protein. Furthermore, the inactivation of uricase by the myeloperoxidase system might be involved in the oxidation of disulfide

between the intermediate myeloperoxidase compounds I and/or II and the halide, which are shown with dotted lines in Fig. 7, probably take place only at acidic pH¹⁵⁾. These reactions appears to be regulated by the change of pH in the environment, with the result that the reactions themselves regulate the overall events caused by the myeloperoxidase-mediated reactions. This is probably due to the fact that the configuration of the hydrogen peroxide-peroxidase compound I and/or II as reaction intermediates³¹⁾ may be altered by the synergistic effect of the drop in basicity, i. e., acidification, of the reaction solution, and the presence of halide ion, resulting in a change in their affinity for the substrate, which is either a hydrogen donor or a halide ion. Thus the proposed mechanism in Fig. 7 allows interpretation of the reactions between first substrate, hydrogen peroxide, and hydrogen donors or halides catalyzed by myeloperoxidase and the subsequent events such as halogenation, oxygenation, destruction, inactivation and cytolysis mediated by the myeloperoxidase system in the presence of hydrogen peroxide and halides. This mechanism can also explain the processes of the similar events caused by lactoperoxidase and saliva peroxidase, which are hematin enzymes similar to myeloperoxidase, considered originally to work in the body's self-defense system in animals, and which also constitute a potent oxidation system together with hydrogen peroxide and the halides bromide and iodide (but not chloride) or the pseudohalide thiocyanate¹⁶⁻¹⁸⁾.

The finding that oxidative inactivation of uricase caused by an oxidant of neutrophils, myeloperoxidase, in a moderately acidic pH range within the limits of the pH changes, from neutral or slightly acidic to moderately acidic, which occurs in cytoplasm of leukocytes during phagocytosis²⁵⁾, suggests the possibility that the myeloperoxidase system modulates a steady amount of enzyme protein and/or a steady state of catalytic activity of each enzyme in the lysosomes of neutrophils that participate in the inflammatory response. The protective effect

of N-acetylmethionine and glutathione on target from the attack by myeloperoxidase system also suggests the possibility that methionine and glutathione modulate a steady amount of oxidant such as free halide radical, halide cation, molecular halide and hypohalite, which would be produced by the myeloperoxidase system. Therefore, it appears that methionine and glutathione are clinically applicable as a therapeutic antioxidant in leukocytoses such as inflammatory diseases and granulocytic leukemia. This study provides an insight into the function, mechanism, and biological significance of the myeloperoxidase system in neutrophils as an important self-defense system in animals.

References

- 1) Matheson NR, Wong PS, Travis J : Enzymatic inactivation of human α_1 -proteinase inhibitor by neutrophil myeloperoxidase. *Biochem. Biophys. Res. Commun.* 88 : 402-409, 1979.
- 2) Clark RA, Stone PJ, Hag AE, Calore JD, Franzblau C : Myeloperoxidase-catalyzed inactivation of α_1 -protease inhibitor by human neutrophils. *J. Biol. Chem.* 256 : 3348-3353, 1981.
- 3) Zaslow MC, Clark RA, Stone PJ, Calore J, Snider GL, Franzblau C : Myeloperoxidase-induced inactivation of α_1 -antiprotease in hamsters. *J. Lab. Clin. Med.* 105 : 178-184, 1985.
- 4) Clark RA, Szot S, Venkatasubramanian K, Schiffmann E : Chemotactic factor inactivation by myeloperoxidase-mediated oxidation of methionine. *J. Immunol.* 124 : 2020-2026, 1980.
- 5) Clark RA, Pearson DW : Inactivation of transferrin iron binding capacity the neutrophil myeloperoxidase system. *J. Biol. Chem.* 264 : 9420-9427, 1989.
- 6) Vissers MCM, Winterbourn CC : Oxidative damage to fibronectin. I. The effects of the neutrophil myeloperoxidase system and HOCl. *Arch. Biochem. Biophys.* 285 : 53-59, 1991.
- 7) Klebanoff SJ, Kinsella MG, Wight TN : Degradation of endothelial cell matrix heparan sulfate proteoglycan by elastase and the myeloperoxidase- H_2O_2 -chloride system. *Am. J. Pathol.* 143 : 907-917, 1993.
- 8) Ooi W, Levine HG, LaMont JT, Clark RA : Inactivation of *Clostridium difficile* cytotoxin by the neutrophil myeloperoxidase system. *J. Infect. Dis.* 149 : 215-219, 1984.
- 9) Odajima T : Oxidative destruction of the microbial metabolite aflatoxin by the myeloperoxidase-hydrogen peroxide-chloride system. *Arch. Oral Biol.* 26 : 339-340, 1981.
- 10) Odajima T, Onishi M, Sato N, Kashiwabara M,

- Ichida T, Takamatsu T, Yuki S : Halogenation of benzo[a]pyrene by myeloperoxidase. Jpn. J. Oral Biol. 27 : 282-290, 1985.
- 11) Onishi M, Odajima T : On the product of prostaglandin E₁ oxidized by the myeloperoxidase-H₂O₂-chloride system. Jpn. J. Oral Biol. 27 : 291-298, 1985.
 - 12) Odajima T : Study on the inactivation of antibiotics and hydrolases by myeloperoxidase. Jpn. J. Oral Biol. 27 : 1216-1227, 1985.
 - 13) Drożdż R, Naskalski JW : Inactivation and denaturation of some proteins by enzyme system : myeloperoxidase, chloride and hydrogen peroxide. Folia Histochem. Cytobiol. 31 : 71-75, 1993.
 - 14) Odajima T, Yamazaki I : Myeloperoxidase of the leukocyte of normal blood. V. The spectral conversion of myeloperoxidase to a cytochrome oxidase like derivative. Biochim. Biophys. Acta 284 : 368-374, 1972.
 - 15) Onishi M, Nakamura S, Odajima T : A direct observation of the peroxidase catalyzed oxidation of halide and thiocyanate ions. Jpn. J. Oral Biol. 30 : 334-339, 1988.
 - 16) Onishi M, Nakamura S, Odajima T : A chemical discriminating method of myeloperoxidase, lactoperoxidase, saliva peroxidase and horseradish peroxidase. Jpn. J. Oral Biol. 30 : 340-346, 1988.
 - 17) Odajima T, Onishi M, Hayama E, Motoji N, Momose Y, Shigematsu A : Cytolysis of B-16 melanoma tumor cells mediated by the myeloperoxidase and lactoperoxidase systems. Biol. Chem. 377 : 689-693, 1996.
 - 18) Odajima T, Onishi M : Hemolysis of mammalian erythrocytes mediated by the myeloperoxidase, lactoperoxidase and saliva peroxidase systems. Jpn. J. Oral Biol. 39 : 304-313, 1997.
 - 19) Itaya K, Fukumoto J, Yamamoto T : Studies on urate oxidase of *Candida utilis*. Part II. Some physical and chemical properties of the purified enzyme. Agr. Biol. Chem. 35 : 813-821, 1971.
 - 20) Nishimura H, Yoshida K, Yokota Y, Matsushima A, Inada Y : Physicochemical properties and states of sulfhydryl groups of uricase from *Candida utilis*. J. Biochem. 91 : 41-48, 1982.
 - 21) Odajima T : Myeloperoxidase of the leukocyte of normal blood. Nature of the prosthetic group of myeloperoxidase. J. Biochem. 87 : 379-391, 1980.
 - 22) Hosoya T, Morrison M : The isolation and purification of thyroid peroxidase. J. Biol. Chem. 242 : 2828-2836, 1967.
 - 23) Estabrook RW : Observations on the antimycin A inhibition of biological oxidations. I. Stoichiometry and pH effects. Biochim. Biophys. Acta 60 : 236-248, 1962.
 - 24) Praetorius E : Uric acid. In Methods of Enzymatic Analysis. (ed. Bergmeyer HU), Academic Press, New York, London, 1963, p 500-501.
 - 25) Pavlov EP, Solov'ev VN : pH changes of cytoplasm in phagocytosis of microorganisms stained with indicator dyes. Byull. Eksp. Biol. Med. 63 : 78-81, 1967.
 - 26) Thomas EL, Aune TM : Peroxidase-catalyzed oxidation of protein sulfhydryls mediated by iodine. Biochemistry 16 : 3581-3586, 1977.
 - 27) Aune TM, Thomas EL : Oxidation of protein sulfhydryls by products of peroxidase-catalyzed oxidation of thiocyanate ion. Biochemistry 17 : 1005-1010, 1978.
 - 28) Gressier B, Lebegue S, Gosset P, Brunet C, Luyckx M, Dine T, Cazin M, Cazin JC, Wallaert B : Protective role of glutathione on α_1 -proteinase inhibitor inactivation by the myeloperoxidase system. Hypothetic study for therapeutic strategy in the management of smokers' emphysema. Fundam. Clin. Pharmacol. 8 : 518-524, 1994.
 - 29) Drożdż R, Naskalski JW, Sznajd J : Oxidation of amino acids and peptides in reaction with myeloperoxidase, chloride and hydrogen peroxide. Biochim. Biophys. Acta 957 : 47-52, 1988.
 - 30) Odajima T : Myeloperoxidase and active oxygen. Metab. Dis. 14 : 39-50, 1977.
 - 31) Odajima T, Yamazaki I : Myeloperoxidase of the leukocyte of normal blood. I. Reaction of myeloperoxidase with hydrogen peroxide. Biochim. Biophys. Acta 206 : 71-77, 1970.
 - 32) Odajima T, Onishi M : Formation of adrenochrome from epinephrine by myeloperoxidase via a free radical : Its biological significance. Jpn. J. Oral Biol. 39 : 297-303, 1997.