Human Neutrophils Employ the Myeloperoxidase–Hydrogen Peroxide–Chloride System to Convert Hydroxy-amino Acids into Glycolaldehyde, 2-Hydroxypropanal, and Acrolein

A Mechanism for the Generation of Highly Reactive α-Hydroxy and α,β-Unsaturated Aldehydes by Phagocytes at Sites of Inflammation

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Abstract

Reactive aldehydes derived from reducing sugars and lipid peroxidation play a critical role in the formation of advanced glycation end (AGE) products and oxidative tissue damage. We have recently proposed another mechanism for aldehyde generation at sites of inflammation that involves myeloperoxidase, a heme enzyme secreted by activated phagocytes. We now demonstrate that human neutrophils employ the myeloperoxidase-H_2O_2-chloride system to produce α-hydroxy and α,β-unsaturated aldehydes from hydroxy-amino acids in high yield. Identities of the aldehydes were established using mass spectrometry and high performance liquid chromatography. Activated neutrophils converted L-serine to glycolaldehyde, an α-hydroxyaldehyde which mediates protein cross-linking and formation of N^{ϵ}-(carboxymethyl)lysine, an AGE product. L-Threonine was similarly oxidized to 2-hydroxypropanal and its dehydration product, acrolein, an extremely reactive α,β-unsaturated aldehyde which alkylates proteins and nucleic acids. Aldehyde generation required neutrophil activation and a free hydroxy-amino acid; it was inhibited by catalase and heme poisons, implicating H_2O_2 and myeloperoxidase in the cellular reaction. Aldehyde production by purified myeloperoxidase required H_2O_2 and chloride, and was mimicked by reagent hypochlorous acid (HOCl) in the absence of enzyme, suggesting that the reaction pathway involves a chlorinated intermediate. Collectively, these results indicate that the myeloperoxidase-H_2O_2-chloride system of phagocytes converts free hydroxy-amino acids into highly reactive α-hydroxy and α,β-unsaturated aldehydes. The generation of glycolaldehyde, 2-hydroxypropanal, and acrolein by activated phagocytes may thus play a role in AGE product formation and tissue damage at sites of inflammation. (*J. Clin. Invest.* 1997. 99:424–432.) Key words: advanced glycation end product • oxidation-reduction • atherosclerosis • diabetes mellitus • hypochlorous acid

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Introduction

Phagocytic white blood cells employ the myeloperoxidase-H_2O_2 system to generate reactive oxygen intermediates that kill invading bacteria, viruses, and tumor cells (1–6). Partially reduced oxygen species are also potentially damaging to cellular lipids, nucleic acids, and proteins (1–6); the production of such species by activated phagocytes has been implicated in the damage of normal tissues under pathological conditions (1–3). The initial pathway for oxidant generation involves a membrane-associated NADPH oxidase that reduces oxygen to superoxide anion ($O_2^{\cdot -}$; 4), which then dismutates to form H_2O_2. Myeloperoxidase, a secreted heme protein, amplifies the toxic potential of H_2O_2 by producing reactive intermediates (5–10).

The major product of myeloperoxidase at plasma concentrations of chloride ion is hypochlorous acid (HOCl; 7, 8):

$$H_2O_2 + Cl^- + H^+ \rightarrow HOCl + H_2O \quad (1)$$

This potent cytotoxin chlorinates electron rich substrates and oxidatively bleaches heme proteins, nucleotides and carotenoids (8–12). Indirect evidence suggests that reactive carbonyls form in proteins (13–15) and amino acids (16, 17) exposed to myeloperoxidase-generated HOCl, and trace quantities of aldehydes have been detected in amino acids exposed to high concentrations of reagent HOCl under strongly acidic conditions (18). Neither the reaction pathways nor the physiological relevance of the products generated in these reactions have been clearly established.

We have demonstrated that the myeloperoxidase-H_2O_2 system converts the phenolic amino acid L-tyrosine to several distinct oxidizing products (19–24). Tyrosyl radical is the major product formed in the absence of chloride (19–22). This long-lived, selectively reactive intermediate damages both proteins and lipids by reactions that are independent of free metal ions. At neutral pH in the presence of chloride ion, myeloperoxidase converts L-tyrosine into *p*-hydroxyphenylacetaldehyde, an amphipathic aldehyde (23). The enzymatic reaction requires H_2O_2 and chloride, and reagent HOCl converts L-tyrosine into the aldehyde, strongly implicating HOCl in the reaction pathway. Aldehyde production accounts for ~ 75% of H_2O_2 generation of optimally stimulated human neutrophils, indicating that the reaction is facile at physiological concentrations of L-tyrosine and chloride (23).

The near quantitative conversion of L-tyrosine to *p*-hydroxyphenylacetaldehyde by the myeloperoxidase-H_2O_2-chloride system raises the possibility that other amino acids might similarly be converted to aldehydes by phagocyte-generated HOCl. The predicted products of L-serine and L-threonine oxidation

by the proposed reaction pathway are glycolaldehyde (2-hydroxyethanal) and 2-hydroxypropanal; the latter species might then undergo dehydration to form acrolein (2-propenal). The α-hydroxy and α,β-unsaturated moieties would make these products of amino acid oxidation unusually reactive (25, 26). Indeed, both glycolaldehyde and acrolein form covalent adducts with proteins and nucleic acids (25–27). Glycolaldehyde has recently been shown to generate N^{ϵ}-(carboxymethyl)lysine (27), an advanced glycation end (AGE)[1] product (28). Reactive aldehydes derived from lipid peroxidation and AGE products have been implicated in protein cross-linking and the pathogenesis of vascular disease (25–27, 29–31).

In the current studies, we demonstrate that the myeloperoxidase-H_2O_2-chloride system of phagocytes converts L-serine into glycolaldehyde and L-threonine into 2-hydroxypropanal and acrolein. Our observations suggest that highly reactive α-hydroxy and α,β-unsaturated aldehydes generated by activated phagocytes may play an important role in generating AGE products and damaging cellular targets.

Methods

Sodium phosphate and H_2O_2 were obtained from Fisher Scientific Co. (Pittsburgh, PA). Boehringer Mannheim Biochemicals (Indianapolis, IN) provided crystalline catalase (from bovine liver; thymol-free). HPLC solvents were purchased from Baxter Healthcare Corp. (McGaw Park, IL). Glycolaldehyde was purchased from Fluka Chemical Co. (Ronkonkoma, NY). All other materials were purchased from Sigma Chemical Co. (St. Louis, MO) unless otherwise indicated.

Isolation of myeloperoxidase. Myeloperoxidase was extracted with cetyltrimethylammonium bromide from human leukocytes obtained by leukopheresis. Solubilized myeloperoxidase was purified by lectin affinity chromatography and size-exclusion chromatography as described (21, 32). The enzyme (A_{430}/A_{280} ratio of 0.6) was dialyzed against distilled water and stored in 50% glycerol at −20°C. Enzyme concentration was determined spectrophotometrically (32).

Isolation of human neutrophils. Neutrophils were isolated by buoyant density centrifugation as previously described (23). The cells were washed twice by centrifugation with Medium A (magnesium-, calcium-, phenol-, and bicarbonate-free HBSS (GIBCO-BRL, Gaithersburg, MD) supplemented with 1 mM diethylenetriamine pentaacetic acid (DTPA, pH 7.2). Residual red blood cells were removed by hypotonic lysis at 4°C. Neutrophils were pelleted by centrifugation, resuspended in Medium A, and immediately used for experiments.

Glycolaldehyde production. Glycolaldehyde production was measured following derivatization with 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH; 33, 34). Reaction mixture (200 μl) was first incubated for 20 min at room temperature with 568 μl of sodium phosphate buffer (5 mM; pH 7.0), 25 μl HCl (6 N), and 132 μl MBTH (155 mM). The azine derivative was then reacted with 75 μl $FeCl_3$ (370 mM) and incubated for > 10 min at room temperature. The resulting blue-colored MBTH derivative was stable for at least 5 h. When the hydrogen ion concentration of the myeloperoxidase reaction mixture was varied (see Fig. 5), 200 mM glycine buffer (pH 4) was substituted for 5 mM sodium phosphate (pH 7.0) to maintain the final pH of the MBTH derivatization reaction at ≤ 4. Control experiments demonstrated that the MBTH assay was not influenced by other variations in reaction conditions (see Fig. 5). Aldehyde production was quantified spectrophotometrically by measuring the absorbance of the MBTH adduct at 598 nm and by comparison with a standard curve generated with authentic glycolaldehyde.

High performance liquid chromatography (HPLC). MBTH derivatives of aldehydes were analyzed by reverse phase HPLC on a C_{18} column (μPorasil, 5 μm resin, 4.6 mm × 250 mm; Beckman Instruments Inc., Fullerton, CA) at a flow rate of 0.7 ml/min. The MBTH derivative of the compounds generated by myeloperoxidase and neutrophils comigrated with derivatives of authentic glycolaldehyde and acrolein on two independent gradient systems and were baseline resolved from other low molecular weight aliphatic aldehydes. The first system (system A) used the C_{18} column equilibrated with solvent A (5% methanol, 0.1% trifluoroacetic acid, pH 2.5) followed by elution of derivatized aldehyde with a discontinuous gradient generated with solvent B (90% methanol, 0.1% trifluoroacetic acid, pH 2.5). The gradient was: 0% solvent B for 5 min, 0–60% solvent B over 5 min, and then 60–100% solvent B over 30 min. The second system (system B) used the C_{18} column equilibrated with 20 mM of sodium phosphate buffer (33). The MBTH derivatives were eluted from the column with a linear gradient of acetonitrile: 0% acetonitrile for 5 min, and then 0–100% acetonitrile over 60 min.

Amino acid derivatization. Aldehydes were derivatized with 0–(2, 3, 4, 5, 6–pentafluorobenzyl)hydroxylamine dissolved in pyridine (1:200, aldehyde: pentafluorobenzylhydroxylamine, mol/mol). The reaction mixture:pyridine solution (1:1, vol/vol) was incubated at 65°C for 1 h, dried under anhydrous N_2 at 40°C, resuspended in 0.25 ml cyclohexane, and washed with 0.25 ml 10% (vol/vol) $HClO_4$ in H_2O (35). The cyclohexane phase was collected and dried under N_2, and the derivatized material was subjected to analysis.

Instrumentation. Derivatized reaction products were analyzed on a 12-m HP-1 capillary column (Hewlett-Packard Co., Palo Alto, CA; 0.2-mm i.d., 0.33-mm file thickness) by gas chromatography-mass spectrometry (GC-MS) using a Hewlett-Packard 5988A mass spectrometer in the negative-ion chemical ionization (NCI) mode with methane as the reagent gas. Samples were injected in the splitless mode. The initial GC column temperature was 70°C for 1 min; the temperature was increased to 210°C at 60°C/min, and then to 250°C at 10°C/min. The injector, transfer line, and source temperatures were 250°C, 250°C, and 150°C, respectively. High resolution mass spectrometry was performed on the pentaflourobenzyl (PFB)-oxime derivative of aldehydes as previously described (23).

General procedures. Absorption spectra were obtained with a Beckman DU-7 spectrophotometer equipped with thermostatically controlled cells. The concentrations of H_2O_2, HOCl, and L-serine monochloramine were determined spectrophotometrically using ϵ_{240} = 43.6 M^{-1} cm^{-1}, ϵ_{292} = 350 M^{-1} cm^{-1}, and ϵ_{252} = 429 M^{-1} cm^{-1}, respectively (36–38).

Results

Myeloperoxidase converts L-serine into glycolaldehyde and L-threonine into acrolein and 2-hydroxypropanal in a reaction requiring H_2O_2 and chloride. Addition of purified myeloperoxidase to buffer A (50-mM sodium phosphate, pH 7.0) supplemented with 200 μM of L-serine, 150 mM NaCl, and 200 μM H_2O_2 generated a compound that reacted with MBTH, a derivatizing agent which forms a stable colored adduct with aldehydes. Following derivatization with MBTH, the compound comigrated with authentic glycolaldehyde on reverse phase HPLC employing two independent solvent systems (Methods). Aldehyde synthesis required chloride, H_2O_2, and myeloperoxidase (Fig. 1 *A*). There was no aldehyde formation by myeloperoxidase in the absence of serine. A compound with identical chromatographic behavior to glycolaldehyde was generated from L-serine when reagent HOCl was substituted

1. *Abbreviations used in this paper:* AGE, advanced glycation end product; DTPA, diethylenetriamine pentaacetic acid; MBTH, 3-methyl-2-benzothiazolinone hydrazone hydrochloride; M^{-}, molecular ion; NCI, negative-ion chemical ionization; PFB, pentafluorobenzyl.

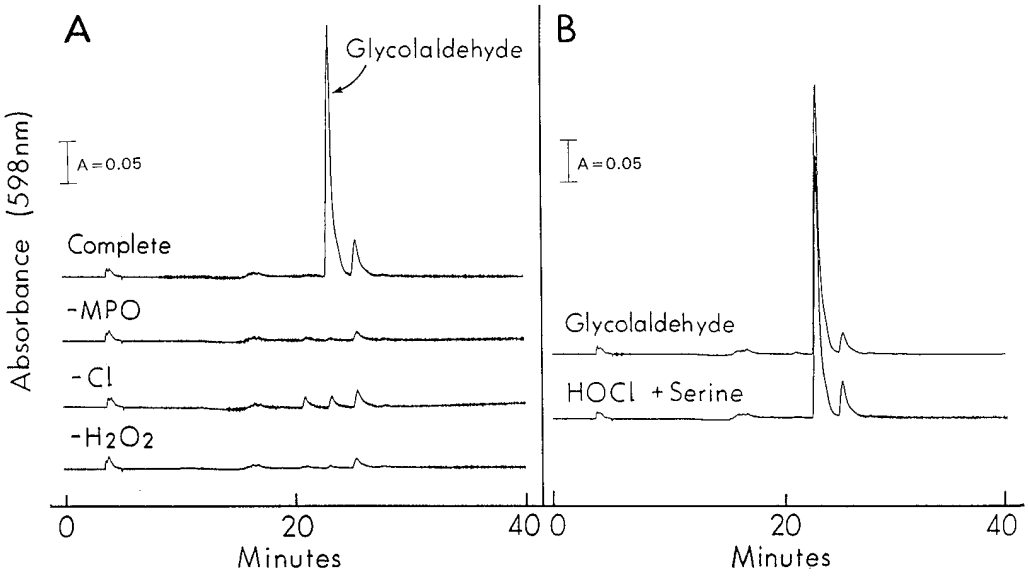

Figure 1. Reverse phase HPLC analysis of the L-serine–derived aldehyde generated by the myeloperoxidase-H_2O_2-chloride system (*A*) and HOCl (*B*). (*A*) The complete system (Complete) consisted of L-serine (200 μM), myeloperoxidase (10 nM), H_2O_2 (200 μM), and NaCl (150 mM) in 1 ml Buffer A (50 mM phosphate, pH 7.0). Myeloperoxidase (MPO), chloride ion or H_2O_2 was omitted where indicated. The reaction was initiated by the addition of H_2O_2 to the reaction mixture. After a 2-h incubation at 37°C, the reaction mixture was derivatized with MBTH and subjected to HPLC analysis on a C_{18} column using system A as described in Methods. (*B*) HPLC analysis of the MBTH derivatives of authentic glycolaldehyde and the product of the incubation of L-serine (200 μM) with HOCl (200 μM). All samples were diluted 1:5 in the derivatization procedure and the HPLC injection volume was 100 μl. Retention times (min) of authentic carbonyl compounds were: glyceraldehyde, 20.5; glyoxal, 21.4; glycolaldehyde, 22.9; acrolein, 23.5; formaldehyde, 24.5; glyoxylic acid, 24.9; and acetaldehyde, 27.1.

for the enzymatic system (Fig. 1 *B*), implicating HOCl generation by myeloperoxidase in the reaction pathway.

The reaction of myeloperoxidase with L-threonine produced two compounds that reacted with MBTH as monitored by reverse phase HPLC (Fig. 2 *A*). One compound comigrated with the MBTH derivative of authentic acrolein (the predicted dehydration product of 2-hydroxypropanal); the other was tentatively assigned the structure of the MBTH derivative of 2-hydroxypropanal. Generation of the aldehydes required enzyme, peroxide, and halogen (Fig. 2 *A*). The complete myeloperoxidase system failed to generate either aldehyde in the absence of threonine. Reaction of L-threonine with reagent HOCl generated compounds with identical retention times on HPLC analysis (Fig. 2 *B*). The early-eluting peak again comigrated with authentic acrolein. These results suggest that myeloperoxidase converts L-serine into glycolaldehyde and L-threonine into 2-hydroxypropanal and acrolein by a chloride- and peroxide-dependent reaction.

Mass spectrometric analysis of the amino acid oxidation products generated by myeloperoxidase. To confirm the structure of the aldehyde generated from L-serine by the myeloperoxidase-H_2O_2-chloride system, we first established the retention time and mass spectrum of the PFB-oxime derivative of authentic glycolaldehyde, and then analyzed the product formed

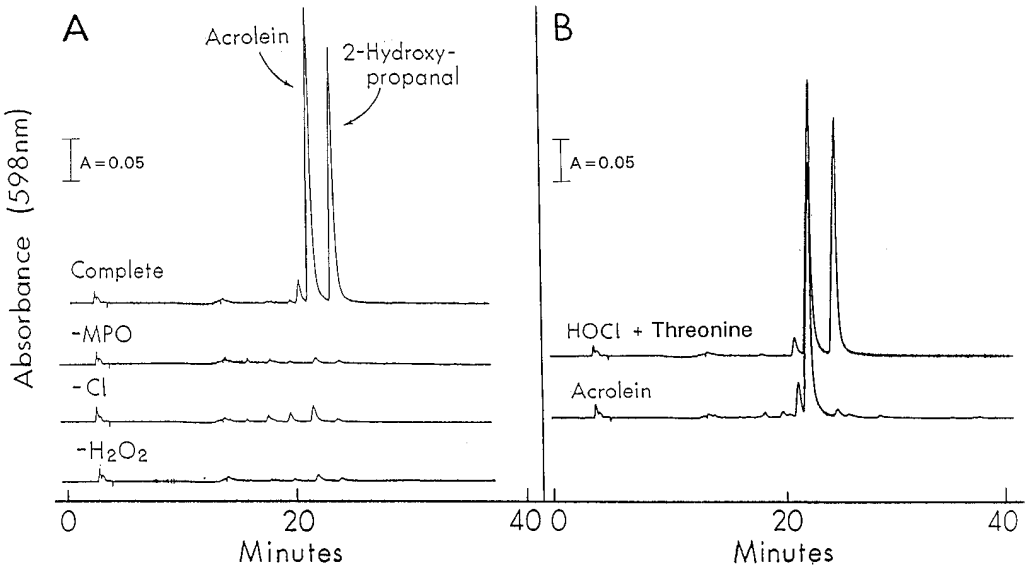

Figure 2. Reverse phase HPLC analysis of L-threonine-derived aldehydes generated by myeloperoxidase. (*A*) The complete system (Complete) consisted of L-threonine (200 μM), myeloperoxidase (10 nM), H_2O_2 (200 μM), and NaCl (150 mM) in 1 ml Buffer A (50 mM phosphate, pH 7.0). Myeloperoxidase (MPO), chloride ion or H_2O_2 was omitted where indicated. After a 2-h incubation at 37°C, the reaction mixture was derivatized with MBTH and subjected to HPLC analysis on a C_{18} column (system A) as described in Methods. The early eluting peak seen with the complete myeloperoxidase system comigrated with authentic acrolein and the late eluting peak was tentatively assigned to 2-hydroxypropanal. (*B*) HPLC analysis of the MBTH derivatives of authentic acrolein and the product of the incubation of L-threonine (200 μM) with HOCl (200 μM). All samples were diluted 1:5 in the derivatization procedure and the HPLC injection volume was 100 μl.

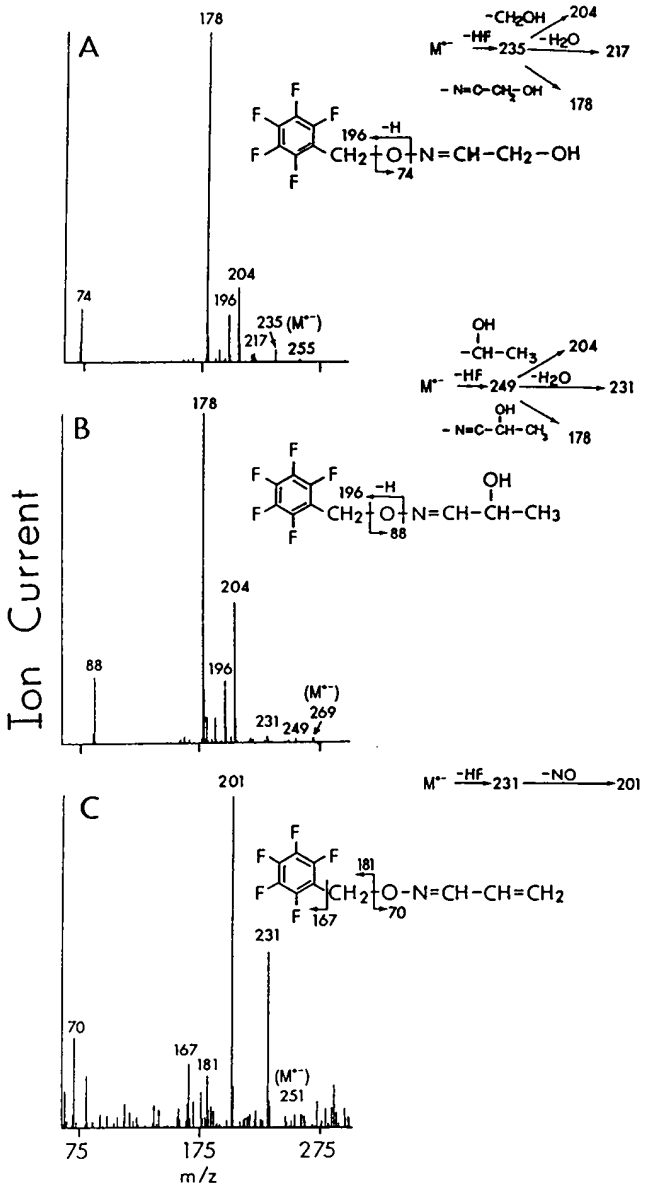

Figure 3. Negative-ion chemical ionization mass spectra of glycolaldehyde (*A*), 2-hydroxypropanal (*B*), and acrolein (*C*) produced by the myeloperoxidase-H_2O_2-chloride system. L-Serine and L-threonine were each incubated with the complete myeloperoxidase-H_2O_2-chloride system for 2 h at 37°C as described in the legend to Fig. 1. Reaction mixtures were derivatized with PFB hydroxylamine in pyridine and subjected to NCI GC-MS analysis as described in Methods. The anticipated syn- and antiisomers of each oxime derivative were baseline resolved on reconstructed ion chromatograms. The mass spectra of the isomers of the L-serine (*A*) and L-threonine (*B*, *C*) oxidation products with the greatest ion currents are shown. Insets represent the structures and proposed fragmentation patterns of the PFB-oximes of glycolaldehyde (*A*), 2-hydroxypropanal (*B*), and acrolein (*C*). The proposed fragmentation pathways were verified by tandem mass spectrometry and high resolution mass spectrometry (Hsu, Hazen, and Heinecke, unpublished observation). Ions at m/z 178 and 201 arise through the formation of a six-membered ring intermediate formed upon loss of HF, and subsequent loss of the indicated species. The retention times of the PFB-oxime derivatives with the greatest ion currents for glycolaldehyde, 2-hydroxypropanal, and acrolein were 4.68, 4.79, and 4.52 min, respectively, under the GC conditions described in Methods.

by the myeloperoxidase-H_2O_2-chloride system. The negative-ion chemical ionization (NCI) mass spectra of the PFB-oxime of the L-serine oxidation product (Fig. 3 *A*) and authentic glycolaldehyde were essentially identical. The mass spectrum of the L-serine oxidation product exhibited a low abundance ion at mass-to-charge ratio (m/z) 255, which is the calculated m/z of the molecular ion ($M^{\cdot -}$) of the PFB-oxime derivative of glycolaldehyde. A prominent ion characteristic of loss of HF from $M^{\cdot -}$ was observed at m/z 235 (Fig. 3 *A*). Other ions consonant with the proposed structure were observed at m/z 217 ($M^{\cdot -} - HF - H_2O$), 204 ($M^{\cdot -} - HF - CH_2OH$), 196 ($M^{\cdot -} - N{=}CH\text{-}CH_2\text{-}OH - H$), 178 ($M^{\cdot -} - HF - N{=}C\text{-}CH_2\text{-}OH$), and 74 ($M^{\cdot -} - C_6F_5\text{-}CH_2$).

The L-threonine oxidation products generated by myeloperoxidase were also subjected to derivatization and GC-MS analysis (Fig. 3, *B* and *C*). The mass spectrum of the derivatized product tentatively designated 2-hydroxypropanal was consistent with the proposed structure (Fig. 3 *B*) and strongly resembled that of the glycolaldehyde derivative (Fig. 3 *A*). A low abundance ion was observed at m/z 269, which is the calculated m/z of the $M^{\cdot -}$ of the PFB-oxime derivative of 2-hydroxypropanal. Additional ions in the mass spectrum consistent with the proposed structure were observed at m/z 249 ($M^{\cdot -} - HF$), 231 ($M^{\cdot -} - HF - H_2O$), 204 ($M^{\cdot -} - HF - CH(OH)CH_3$), 196 ($M^{\cdot -} - N{=}CH\text{-}CH(OH)CH_3 - H$), 178 ($M^{\cdot -} - HF - N{=}C\text{-}CH(OH)CH_3$), and 88 ($M^{\cdot -} - C_6F_5\text{-}CH_2$). Collectively, these results indicate that the myeloperoxidase-H_2O_2-chloride system converts L-threonine into 2-hydroxypropanal.

The mass spectrum of the other major L-threonine oxidation product generated by myeloperoxidase was identical to that of authentic acrolein (Fig. 3 *C*). Ions consistent with the proposed structure were observed at m/z 251 ($M^{\cdot -}$), 231 ($M^{\cdot -} - HF$), 201 ($M^{\cdot -} - HF - NO$), 181 ($M^{\cdot -} - O\text{-}N{=}CH\text{-}CH{=}CH_2$), 167 ($M^{\cdot -} - CH_2\text{-}O\text{-}N{=}CH\text{-}CH{=}CH_2$), and 70 ($M^{\cdot -} - C_6F_5\text{-}CH_2$).

The GC retention times and mass spectra of the PFB-oximes of the oxidation products formed by HOCl-mediated oxidation of L-serine and L-threonine were identical to those of the complete myeloperoxidase-H_2O_2-Cl^- system. Moreover, the retention times and mass spectra of the aldehydes designated as glycolaldehyde and acrolein generated by myeloperoxidase and HOCl were identical to those of authentic derivatized glycolaldehyde and acrolein (data not shown). These results confirm that glycolaldehyde and acrolein are the products, respectively, of L-serine and L-threonine oxidation by myeloperoxidase, and strongly suggest that the reaction pathway is mediated by enzymatically generated HOCl.

Glycolaldehyde is the major product of L-serine oxidation by myeloperoxidase. In the presence of equimolar quantities of H_2O_2 and L-serine, myeloperoxidase used over 80% of the H_2O_2 in the reaction mixture to generate glycolaldehyde (Figs. 4 and 5). Aldehyde formation was optimal at neutral pH and physiological concentrations of L-serine and chloride (Fig. 5). It was inhibited by the addition of the H_2O_2 scavenger catalase (400 nM), as well as by the heme poisons sodium azide (5 mM), sodium cyanide (1 mM), or 3-amino-1,2,4-triazole (10 mM), consistent with a peroxidase-dependent reaction. Heat inactivation of myeloperoxidase (100°C for 5 min) also blocked glycolaldehyde formation.

The progress curve for the myeloperoxidase-catalyzed formation of glycolaldehyde from L-serine exhibited a lag phase (Fig. 4), suggesting that an intermediate was involved in the re-

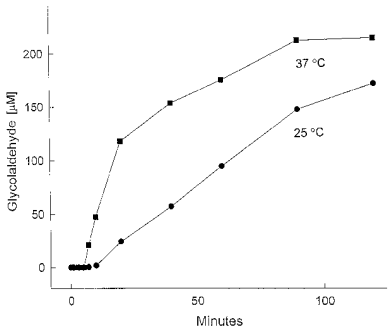

Figure 4. Progress curve of glycolaldehyde generation by myeloperoxidase. The reaction mixture consisted of L-serine (200 μM), myeloperoxidase (10 nM), H_2O_2 (200 μM), and NaCl (150 mM) in Buffer A (50 mM phosphate, pH 7.0). The reaction was carried out for the indicated times at either 25°C or 37°C. Aliquots of the reaction mixture were derivatized with MBTH and subjected to spectrophotometric analysis as described in Methods.

action pathway. The enzymatic reaction reached completion within 90 min at 37°C with near-quantitative conversion of H_2O_2 into glycolaldehyde. Glycolaldehyde synthesis was optimal when the concentrations of L-serine and H_2O_2 were equal (Fig. 5). The decrease in glycolaldehyde production at higher concentrations of H_2O_2 may reflect oxidative inactivation of the enzyme, substrate inhibition, or further oxidation of glycolaldehyde. Glycolaldehyde production by myeloperoxidase required chloride and was optimal at plasma concentrations of halide (Fig. 5).

Serine monochloramine is an intermediate in the formation of glycolaldehyde from L-serine. The lag phase for enzymatic generation of glycolaldehyde formation, together with the ability of reagent HOCl to substitute for myeloperoxidase, suggested that a chlorinated intermediate was involved in glycolaldehyde generation. A candidate intermediate is serine monochloramine ($HOCH_2CH[COOH]NHCl$), which should be formed by the reaction of L-serine with HOCl. To test the potential role of monochloramine formation in the synthesis of glycolaldehyde, L-serine was reacted at 0°C with an equimolar quantity of HOCl. UV difference spectroscopy revealed formation of a product with an absorbance optimum at 252 nm, consistent with monochloramine production (38). Under these conditions, the monochloramine was formed quantitatively, as assessed by the change in absorbance at 252 nm (38). At 0°C the intermediate was stable; however, as the temperature of the solution was

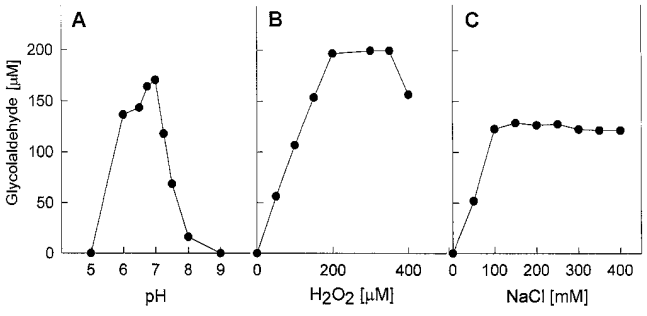

Figure 5. Reaction requirements for glycolaldehyde generation by myeloperoxidase. L-Serine was oxidized with the myeloperoxidase-H_2O_2-chloride system as described in the legend to Fig. 1. Conditions were varied by incubating the reaction mixture at the indicated pH (*A*), H_2O_2 concentration (*B*), and chloride ion concentration (*C*). Glycolaldehyde production was measured after a 2-h incubation at 37°C by derivatization of the reaction mixture with MBTH and monitoring of the change in absorbance at 598 nm as described in Methods.

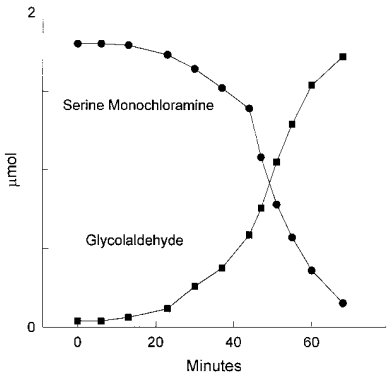

Figure 6. Progress curves of glycolaldehyde formation and L-serine monochloramine decomposition. L-Serine monochloramine was synthesized by the addition of HOCl (2 μmol) to 50 mM phosphate buffer (1 ml, pH 7.0) supplemented with L-serine (2 μmol) at 0°C in a quartz spectrophotometer cuvette. The cuvette was then gradually warmed to 45°C. Monochloramine decomposition and glycolaldehyde formation were quantified by the change in absorbance at 252 nm and by reaction with MBTH, respectively, as described in Methods. Control experiments demonstrated that the L-serine monochloramine was stable at 0°C and failed to react with MBTH.

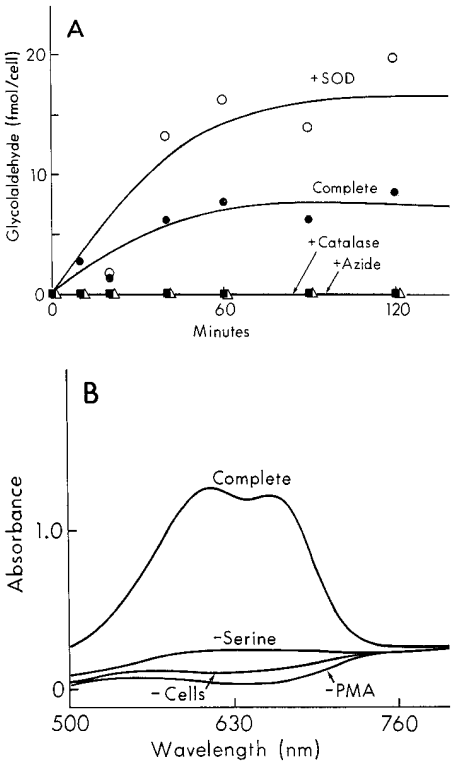

Figure 7. Progress curve (*A*) and reaction requirements (*B*) for glycolaldehyde production by activated human neutrophils. Freshly harvested human neutrophils (10^6/ml) were incubated at 37°C in Medium A supplemented with 1 mM L-serine. Cells were stimulated with 200 nM phorbol myristate acetate and maintained in suspension by intermittent inversion. Where indicated, SOD (170 nM), catalase (400 nM), or sodium azide (5 mM) were present. The reaction was stopped at the indicated times (*A*) or after 90 min (*B*) by removal of neutrophils by centrifugation (10 min × 8,000 *g* at 4°C). (*A*) The concentration of glycolaldehyde in the supernatant was immediately determined using MBTH as described in Methods. (*B*) Absorption spectra of the supernatant of the cells incubated under the indicated conditions and then derivatized with MBTH. The absorption spectrum of the complete system is identical to that of the MBTH derivative of authentic glycolaldehyde.

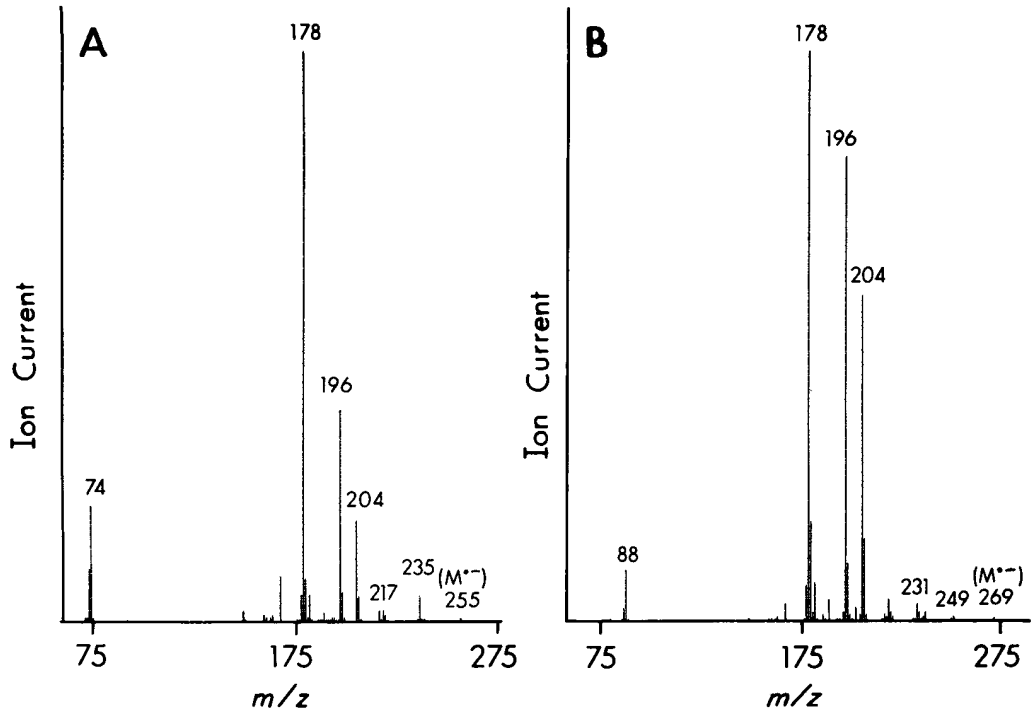

Figure 8. Negative-ion chemical ionization mass spectra of the PFB-oximes of glycolaldehyde (*A*) and 2-hydroxypropanal (*B*) produced by activated human neutrophils. (*A*) L-Serine (1 mM) or (*B*) L-threonine (1 mM) was incubated for 90 min with phorbol ester-stimulated human neutrophils (10^6/ml) in Medium A supplemented with superoxide dismutase (170 nM) as described in the legend to Fig. 7. The supernatant of the incubation medium was subjected to derivatization with PFB hydroxylamine and GC-MS analysis as described in Methods.

gradually increased to 45°C, serine monochloramine decomposed forming glycolaldehyde as detected by MBTH derivatization. Near quantitative conversion of serine monochloramine to glycolaldehyde was observed at each time point (Fig. 6). These results strongly suggest that serine monochloramine is an intermediate in the formation of glycolaldehyde by myeloperoxidase.

Activated human phagocytes employ myeloperoxidase to generate glycolaldehyde, 2-hydroxypropanal and acrolein. Human neutrophils activated with phorbol myristate acetate in a balanced salt solution converted L-serine into glycolaldehyde, as determined by co-chromatography with authentic glycolaldehyde standard on two different reverse-phase HPLC sys-

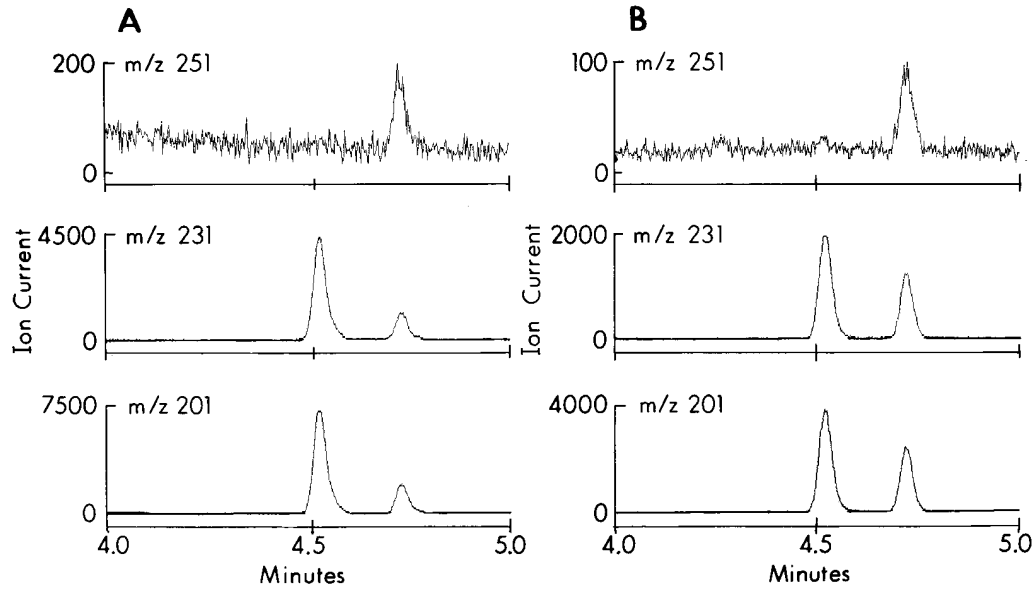

Figure 9. Detection of acrolein generated by activated human neutrophils by selected ion monitoring. L-Threonine (1 mM) was oxidized by human neutrophils (10^6/ml) activated with phorbol ester (200 nM) as described in the legend to Fig. 8. The supernatant of the cells (*A*) or authentic acrolein (*B*) was derivatized with PFB hydroxylamine and then analyzed by NCI GC-MS in the selected ion monitoring mode. The presence of two peaks is consistent with resolution of the syn- and anti-isomers of the PFB-oxime of the aldehyde.

tems (Methods). The progress curve for glycolaldehyde formation by neutrophils was linear initially and then reached a plateau by 90 min (Fig. 7 *A*). Glycolaldehyde formation required activation of the cells with phorbol myristate acetate (Fig. 7 *B*); aldehyde formation was also dependent upon L-serine (Fig. 7 *B*), and it was inhibited by catalase and heme poisons (Fig. 7 *A*), implicating myeloperoxidase and H_2O_2 in the reaction. Superoxide dismutase (which facilitates dismutation of $O_2^{\cdot-}$ to H_2O_2 500-fold at neutral pH; 4) increased the yield of the reaction (Fig. 7 *A*), perhaps by increasing the availability of H_2O_2. Alternatively, superoxide dismutase may have prevented inactivation of myeloperoxidase by $O_2^{\cdot-}$ (39, 40).

Glycolaldehyde is amphipathic and is likely to partition into cellular lipids. In addition, the aldehyde also reacts with free amino groups (25–27). The overall yield of glycolaldehyde measured in the cellular experiments (Fig. 7) is thus likely to represent a lower limit for the actual amount of aldehyde generated.

Incubation of human neutrophils with phorbol myristate acetate and L-threonine resulted in production of two MBTH-reactive aldehydes. The HPLC retention times of these products were identical to those observed with acrolein and 2-hydroxypropanal produced by either the myeloperoxidase/L-threonine or HOCl/L-threonine systems (Fig. 2). As with glycolaldehyde generation (Fig. 7), cell-mediated formation of acrolein and 2-hydroxypropanal was sensitive to inhibition by either heme poisons or catalase, and was augmented in the presence of superoxide dismutase (data not shown).

To confirm the structures of the aldehydes generated by activated neutrophils, their PFB-oximes were subjected to GC-MS analysis. The GC retention times and mass spectra of the derivatives of the L-serine oxidation product generated by neutrophils (Fig. 8 *A*), authentic glycolaldehyde, and the L-serine oxidation product formed by myeloperoxidase (Fig. 3 *A*) were essentially identical. Two major oxidation products were present in L-threonine exposed to activated neutrophils. The mass spectrum of the PFB-oxime of one of the oxidation products (Fig. 8 *B*) was indistinguishable from that of derivatized 2-hydroxypropanal generated by myeloperoxidase (compare Figs. 3 *B* and 8 *B*).

To confirm that acrolein was generated by activated neutrophils, selected ion monitoring was performed on the PFB-oximes of the L-threonine oxidation products. Authentic acrolein demonstrated two peaks with retention times of 4.5 and 4.7 min (Fig. 9 *B*), presumably representing the syn- and anti-isomers of the derivatized aldehyde. Ions with the same retention times and relative abundance were present in the PFB-oximes of L-threonine exposed to activated human neutrophils (Fig. 9 *A*), the complete myeloperoxidase-H_2O_2-chloride system (data not shown), and reagent HOCl (data not shown). Note that the ions observed by selected ion monitoring at m/z 251 ($M^{\cdot-}$), 231 ($M^{\cdot-}$ − HF) and 201 ($M^{\cdot-}$ − HF − NO) are present in the full-scan mass spectrum of the PFB-oxime of acrolein generated by myeloperoxidase (Fig. 3 *C*).

Discussion

Multiple lines of evidence indicate that activated human neutrophils employ the myeloperoxidase-H_2O_2–chloride system to convert hydroxy-amino acids into highly reactive α-hydroxy and α,β-unsaturated aldehydes. (*a*) The retention times on HPLC and gas chromatography, and the mass spectrum of the

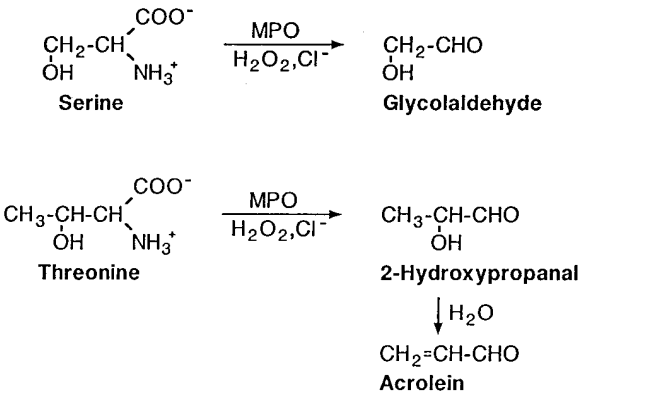

Figure 10. Proposed reaction pathway for the generation of α-hydroxy and α,β-unsaturated aldehydes from hydroxy-amino acids by myeloperoxidase (MPO).

L-serine oxidation product generated by the myeloperoxidase-H_2O_2-chloride system were indistinguishable from those of authentic glycolaldehyde. (*b*) The products of L-threonine oxidation by myeloperoxidase were identified similarly as 2-hydroxypropanal and acrolein. (*c*) Stimulation of human neutrophils with phorbol ester resulted in generation of glycolaldehyde from L-serine, as well as 2-hydroxypropanal and acrolein from L-threonine, as assessed by the migration of the compounds on two different reverse phase HPLC systems. (*d*) Heme poisons and the peroxide scavenger catalase inhibited aldehyde generation by activated phagocytes, implicating myeloperoxidase and H_2O_2 in the reaction. (*e*) The retention time on gas chromatography and the mass spectrum of the L-serine oxidation product generated by activated neutrophils were identical to those of authentic glycolaldehyde. (*f*) The products of L-threonine oxidation by activated neutrophils were likewise identified as 2-hydroxypropanal and acrolein by GC-MS analysis. Collectively, these results demonstrate that activated phagocytes generate glycolaldehyde from L-serine, and 2-hydroxypropanal and acrolein from L-threonine (Fig. 10).

Aldehyde synthesis by myeloperoxidase required a free hydroxy-amino acid, chloride ion, and H_2O_2. Moreover, in the absence of the enzymatic system, reagent HOCl converted L-serine and L-threonine into their respective aldehyde products, strongly implicating HOCl (or enzyme-bound hypochlorite; 41) in the reaction. The pathway for aldehyde synthesis is likely to involve the initial chlorination of the α-amino group of the amino acid. Reagent HOCl rapidly chlorinates amino groups (10, 11, 38), and studies employing UV difference spectroscopy identified a reaction intermediate with spectral features consistent with L-serine monochloramine when the amino acid was mixed with equimolar quantities of HOCl. The chloramine was stable at 0°C; however, it rapidly decomposed upon warming with production of glycolaldehyde in near quantitative yield. These observations suggest that serine monochloramine is an intermediate in the conversion of L-serine into glycolaldehyde by HOCl.

α-Hydroxyaldehydes are highly reactive molecules that rapidly form Schiff base adducts with protein amino residues (25–27). Reagent glycolaldehyde forms Amadori products and cross-links proteins in vitro (27). Glycolaldehyde and 2-hydroxypropanal generated by activated phagocytes may execute simi-

lar reactions in vivo. α-Hydroxyaldehydes also readily undergo dehydration to yield the corresponding α,β-unsaturated aldehydes. This class of compounds alkylates thiols, amino groups, and histidyl residues (25, 26). Similar Michael addition products derived from the lipid peroxidation product, 4-hydroxy-2-nonenal, have been detected in vivo with monoclonal antibodies (42). Acrolein, the most electrophilic α,β-unsaturated aldehyde known, reacts 100-fold more rapidly with thiols than 4-hydroxy-2-nonenal (25, 26). Acrolein reduces the colony-forming efficiency of mammalian cells, forms cyclic adducts with nucleosides in vitro, and is a potent mutagen, suggesting that reactive aldehydes generated by myeloperoxidase may also damage DNA.

L-Serine and L-threonine are both present in concentrations up to 200 μM in plasma (43) suggesting that hydroxy-amino acids may be substrates for oxidation by myeloperoxidase in vivo. Myeloperoxidase is a component of human atherosclerotic lesions (44) and the patterns of immunostaining for the enzyme and for HOCl modified proteins (45) are remarkably similar to those reported for oxidation-specific epitopes in rabbit atherosclerotic lesions (42). A wealth of evidence indicates that LDL, the major carrier of cholesterol in humans, must be oxidized to trigger the pathological events of atherosclerosis (31). LDL isolated from human aortic tissue exhibits immunoreactivity with polyclonal antibodies specific for acrolein-modified proteins (46). The results of the current study provide a plausible mechanism for acrolein generation in vivo. These observations suggest that myeloperoxidase may be a catalyst for LDL oxidation in vivo, and that acrolein generated by myeloperoxidase may play a role in converting LDL into an atherogenic particle.

The high yield of the L-serine and L-threonine oxidation products suggests that other free amino acids may be substrates for oxidation by myeloperoxidase. The total concentration of free amino acids in plasma is ~ 4 mM (43), suggesting that reactive aldehydes derived from amino acids may be major products of phagocyte activation in vivo. Other likely targets for oxidation by myeloperoxidase-generated HOCl include plasma antioxidants and protein amino groups. The mass spectrometric quantification of the levels of glycolaldehyde, 2-hydroxypropanal, and acrolein in inflammatory tissue should provide a powerful tool for investigating the role of hydroxy-amino acids as substrates for oxidation in vivo by myeloperoxidase.

α-Hydroxy and α,β-unsaturated aldehydes are highly reactive species that have been implicated in disorders ranging from ischemia-reperfusion injury to DNA damage and aging (25–31, 47–49). Because the generation of reactive aldehydes by myeloperoxidase is nearly quantitative at plasma concentrations of L-serine, L-threonine, and chloride, phagocyte-mediated formation of these products may be of central importance in promoting tissue injury at sites of inflammation.

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