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

Melissa M. Anderson,* Stanley L. Hazen,* Fong F. Hsu,* and Jay W. Heinecke*‡
*Department of Medicine and ‡Department of Molecular Biology and Pharmacology, Washington University School of Medicine, St. Louis, Missouri 63110

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$_2$O$_2$-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-hydroxylaldehyde which mediates protein cross-linking and formation of N$\alpha$-(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 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$_2^-$; 4), which then dismutates to form H$_2$O$_2$. Myeloperoxidase, a secreted heme protein, amplifies the toxic potential of H$_2$O$_2$ by producing reactive intermediates (5–10).

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

$$\text{H}_2\text{O}_2 + \text{Cl}^- + \text{H}^+ \rightarrow \text{HOCl} + \text{H}_2\text{O} \tag{1}$$

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 HOCI, and trace quantities of aldehydes have been detected in amino acids exposed to high concentrations of reagent HOCI 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$_2$O$_2$ 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$_2$O$_2$ and chloride, and reagent HOCI converts L-tyrosine into the aldehyde, strongly implicating HOCI in the reaction pathway. Aldehyde production accounts for $\sim$ 75% of H$_2$O$_2$ 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$_2$O$_2$-chloride system raises the possibility that other amino acids might similarly be converted to aldehydes by phagocyte-generated HOCI. 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 αβ-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-(carboxymethyl)lysine (27), an advanced glycation end (AGE) 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-H₂O₂-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 αβ-unsaturated aldehydes generated by activated phagocytes may play an important role in generating AGE products and damaging cellular targets.

Methods

Sodium phosphate and H₂O₂ 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 (Aₓₓₓₓ/Aₓₚₚ 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 hydrazide 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 mM of monochloramine were determined spectrophotometrically using ε₇₅₀ = 136.6 M⁻¹ cm⁻¹, ε₇₂₅ = 350 M⁻¹ cm⁻¹, and ε₅₂₅ = 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 H₂O₂ 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 H₂O₂ 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, H₂O₂, 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 HClO was substituted

1. Abbreviations used in this paper: AGE, advanced glycation end product; DTPA, diethylenetriamine pentaacetic acid; MBTH, 3-methyl-2-benzothiazolinone hydrazide hydrochloride; M⁻, molecular ion; NCI, negative-ion chemical ionization; PFB, pentafluorobenzyl. Generation of α-hydroxy and αβ-unsaturated Aldehydes by Myeloperoxidase 425
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$_2$O$_2$-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 from the enzymatic system (Fig. 2 A). 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.

Figure 1. Reverse phase HPLC analysis of the L-serine–derived aldehyde generated by the myeloperoxidase-H$_2$O$_2$-chloride system (A) and HOCl (B). (A) The complete system (Complete) consisted of L-serine (200 μM), myeloperoxidase (10 nM), H$_2$O$_2$ (200 μM), and NaCl (150 mM) in 1 ml Buffer A (50 mM phosphate, pH 7.0). Myeloperoxidase (MPO), chloride ion or H$_2$O$_2$ was omitted where indicated. The reaction was initiated by the addition of H$_2$O$_2$ 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.

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$_2$O$_2$ (200 μM), and NaCl (150 mM) in 1 ml Buffer A (50 mM phosphate, pH 7.0). Myeloperoxidase (MPO), chloride ion or H$_2$O$_2$ 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.
were each incubated with the complete myeloperoxidase-H$_2$O$_2$-chloride system for 2 h at 37°C. The retention times of the PFB-oxime derivatives with the greatest ion currents were observed at m/z 269, which is the calculated m/z of the molecular ion (M$^-$) of the PFB-oxime derivative of glycolaldehyde. A prominent ion characteristic of loss of HF from M$^-$ was observed at m/z 255 (Fig. 3A). Other ions consistent with the proposed structure were observed at m/z 217 (M$^-$ – HF – H$_2$O), 204 (M$^-$ – HF – CH$_2$OH), 196 (M$^-$ – N=CH-CH$_2$-OH – H), 178 (M$^-$ – HF – N=C-CH$_2$-OH), and 74 (M$^-$ – C$_3$F$_7$-CH$_3$).

The l-threonyl 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. 3B) and strongly resembled that of the glycolaldehyde derivative (Fig. 3A). A low abundance ion was observed at m/z 269, which is the calculated m/z of the M$^-$ 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$^-$ – HF), 231 (M$^-$ – HF – H$_2$O), 204 (M$^-$ – HF – CH(OH)CH$_3$), 196 (M$^-$ – N=CH-CH(OH)CH$_3$ – H), 178 (M$^-$ – HF – N=C-CH(OH)CH$_3$), and 88 (M$^-$ – C$_3$F$_7$-CH$_3$). Collectively, these results indicate that the myeloperoxidase-H$_2$O$_2$-chloride system converts l-threonyl into 2-hydroxypropanal.

The mass spectrum of the other major l-threonyl oxidation product generated by myeloperoxidase was identical to that of authentic acrolein (Fig. 3C). Ions consistent with the proposed structure were observed at m/z 251 (M$^-$), 231 (M$^-$ – HF), 201 (M$^-$ – HF – NO), 181 (M$^-$ – O=N=CH-CH=CH$_2$), 167 (M$^-$ – CH$_2$-O-N=CH-CH=CH$_2$), and 70 (M$^-$ – C$_3$F$_7$-CH$_3$).

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-threonyl were identical to those of the complete myeloperoxidase-H$_2$O$_2$-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-threonyl 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$_2$O and L-serine, myeloperoxidase used over 80% of the H$_2$O$_2$ 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$_2$O$_2$ 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 reaction pathway.

Figure 3. Negative-ion chemical ionization mass spectra of glycolaldehyde (A), 2-hydroxypropanal (B), and acrolein (C) produced by the myeloperoxidase-H$_2$O$_2$-chloride system. L-Serine and L-threonyl were each incubated with the complete myeloperoxidase-H$_2$O$_2$-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 anti-isomers of each oxime derivative were baseline resolved on reconstructed ion chromatograms. The mass spectra of the isomers of the L-serine (A) and L-threonyl (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$_2$O$_2$-chloride system. The negative-ion chemical ionization (NCI) mass spectra of the PFB-oxime of the L-serine oxidation product (Fig. 3A) 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$^-$) of the PFB-oxime derivative of glycolaldehyde. A prominent ion characteristic of loss of HF from M$^-$ was observed at m/z 255 (Fig. 3A). Other ions consistent with the proposed structure were observed at m/z 217 (M$^-$ – HF – H$_2$O), 204 (M$^-$ – HF – CH$_2$OH), 196 (M$^-$ – N=CH-CH$_2$-OH – H), 178 (M$^-$ – HF – N=C-CH$_2$-OH), and 74 (M$^-$ – C$_3$F$_7$-CH$_3$).

Generation of α-Hydroxy and α,β-Unsaturated Aldehydes by Myeloperoxidase

427
action pathway. The enzymatic reaction reached completion within 90 min at 37°C with near-quantitative conversion of H₂O₂ into glycolaldehyde. Glycolaldehyde synthesis was optimal when the concentrations of L-serine and H₂O₂ were equal (Fig. 5). The decrease in glycolaldehyde production at higher concentrations of H₂O₂ 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 (HOCH₂CH₂[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 HOCl. UV difference spectroscopy revealed formation of a product with an absorbance optimum at 252 nm, consistent with HOCl.

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.

Figure 4. Progress curve of glycolaldehyde generation by myeloperoxidase. The reaction mixture consisted of L-serine (200 μM), myeloperoxidase (10 nM), H₂O₂ (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 37°C or 25°C. At 0°C monochloramine was formed quantitatively, as assessed by the monochloramine production (38). Under these conditions, the product with an absorbance optimum at 252 nm, consistent with HOCl. UV difference spectroscopy revealed formation of a product with an absorbance optimum at 252 nm, consistent with HOCl.

Figure 5. Reaction requirements for glycolaldehyde generation by myeloperoxidase. L-Serine was oxidized with the myeloperoxidase-H₂O₂-chloride system as described in the legend to Fig. 1. Conditions were varied by incubating the reaction mixture at the indicated pH (A), H₂O₂ 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 (10⁹/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.
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 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 (10⁶/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.

**Figure 9.** Detection of acrolein generated by activated human neutrophils by selected ion monitoring. L-Threonine (1 mM) was oxidized by human neutrophils (10⁶/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$_2$O$_2$ in the reaction. Superoxide dismutase (which facilitates dismutation of O$_2^-$ to H$_2$O$_2$ 500-fold at neutral pH; 4) increased the yield of the reaction (Fig. 7 A), perhaps by increasing the availability of H$_2$O$_2$. Alternatively, superoxide dismutase may have prevented inactivation of myeloperoxidase by O$_2^-$. 

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$_2$O$_2$-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$^+$), 231 (M$^+$ – HF) and 201 (M$^+$ – 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$_2$O$_2$-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 L-serine oxidation product generated by the myeloperoxidase-H$_2$O$_2$-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$_2$O$_2$ 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$_2$O$_2$. 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 HOCI 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 HOCI 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.

Acknowledgments

Gas chromatography-mass spectrometry experiments were performed at the Washington University School of Medicine Mass Spectrometry Resource. This research was supported by a Washington University/Monsanto Biomedical Research Agreement, and awards R01-DK-02456, and RR-00954 from the National Institutes of Health. M.M. Anderson was supported by the Lucille P. Markey Special Emphasis Program in Human Pathobiology. S.L. Hazen is a Howard Hughes Medical Institute Physician Postdoctoral Research Fellow. J.W. Heinecke is an Established Investigator of the American Heart Association.

References


Generation of α-Hydroxy and α,β-Unsaturated Aldehydes by Myeloperoxidase


