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CHEMOTACTIC FACTOR INACTIVATION BY MYELOPEROXIDASE-MEDIATED OXIDATION OF METHIONINE[1]

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The myeloperoxidase-H_2O_2-halide system of the polymorphonuclear leukocyte is capable of inactivating certain leukoattractants, including complement-derived and synthetic peptide agents. The biochemical basis for this phenomenon was explored. The peroxidase system readily inactivated methionine-containing chemoattractants (C5a, f-met-leu-phe, f-met-leu-phe-lys), whereas agents not containing methionine (f-norleu-leu-phe, f-phe-leu-phe-leu-phe) were unaffected. Exposure of f-met-leu-phe to the myeloperoxidase system also resulted in a marked decrease in the binding of the peptide to specific neutrophil membrane receptors, but peroxidase treatment of f-norleu-leu-phe had no effect on its receptor binding. The inactivation of f-met-leu-phe by the myeloperoxidase system was blocked by reducing agents (2-mercaptoethanol, ascorbic acid) and by free methionine, but not by methionine sulfoxide. Thin-layer chromatography of f-met-leu-[^{3}H]phe demonstrated that peroxidase treatment resulted in a shift to a more slowly migrating species with an R_F value corresponding to that of the peptide containing chemically oxidized methionine.

These results indicate that the inactivation of peptide chemoattractants by the myeloperoxidase system is a consequence of methionine oxidation and that this biochemical alteration decreases the affinity of the peptide for neutrophil membrane receptors. Since activated neutrophils secrete myeloperoxidase and H_2O_2, we suggest that the inactivation of inflammatory mediators by this enzyme system may constitute a control mechanism in the tissue response to injury. Other biologic mediators, especially those containing thioether groups, may also be affected by oxidants generated via the myeloperoxidase-catalyzed oxidation of halides by H_2O_2.

The enzymatic inactivation of humoral mediators very likely serves an important function in the maintenance of tissue homeostasis and in the response to injury. For example, modulation of the acute inflammatory response may involve the degradation of leukocyte chemoattractants by enzymes present in serum (1, 2) or in inflammatory cells (3–9). Within the azurophil granules of the polymorphonuclear leukocyte (PMN)[4] are several neutral proteases that can inactivate complement (C) system-derived (C5a) and synthetic peptide chemotactic factors by hydrolytic mechanisms (3–8). These PMN enzymes include chymotrypsin-like cationic protein (cathepsin G) (7, 8), elastase (7), and two other unidentified azurophil granule proteases (4). Furthermore, when PMN are stimulated by phagocytosis or by exposure to certain soluble membrane-perturbing agents (e.g., chemoattractants), lysosomal enzymes including chemotactic factor inactivators are secreted into the extracellular milieu (3–5, 7).

The existence of nonproteolytic mechanisms for the modification of inflammatory mediators is indicated by the recent report from our laboratory that the myeloperoxidase-H_2O_2-halide system of the PMN is capable of inactivating C5a as well as a synthetic peptide leukoattractant, *N*-formylmethionyl-leucyl-phenylalanine (f-met-leu-phe) (10). Myeloperoxidase (MPO) is present in the azurophil granules and in combination with the PMN metabolic product, H_2O_2, and a halide co-factor; this enzyme serves an important microbicidal function within phagocytic vacuoles (11–13). Activated PMN also secrete MPO and H_2O_2 into the extracellular fluid where they may exert biologic effects such as the lysis of mammalian cells (14, 15). We found that chemotactic factor inactivation by the MPO system was dependent on enzymatically active MPO, H_2O_2 or a peroxide-generating enzyme system, and a halide co-factor (10). The process was time dependent, reaching maximal levels within 1 to 5 min, and temperature dependent, occurring at 37°C, but not at 0°C. H_2O_2 alone was inactive even at concentrations as high as 10 mM, whereas in the presence of MPO and a halide, 0.1 to 10 μM H_2O_2 was effective. Bromide, iodide, and chloride were all active as oxidizable co-factors, although chloride was the most effective at physiologic concentrations.

In the current studies, we have addressed the molecular mechanism of the inactivation of chemotactic factors by the MPO-H_2O_2-halide system. The data indicate that loss of biologic activity is associated with oxidation of methionine, a chemical change that precludes effective binding of the chemoattractant to PMN membrane receptors.

MATERIALS AND METHODS

Chemoattractants. The chemotactic fragment of the fifth component of complement (C5a) was partially purified by Seph-

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[4] Abbreviations used in this paper: MPO, myeloperoxidase; α_1-PI, α_1-proteinase inhibitor; PMN, polymorphonuclear leukocyte.

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adex G-75 filtration of zymosan-activated human serum as previously described (16). The active product eluted with an apparent m.w. of 15,000 and was nearly completely inhibited by antibody to human C5, but not by antibody to human C3 (Meloy Laboratories, Springfield, Va.). *N*-formylmethionyl peptides were synthesized as described (17) by Drs. R. Freer and A. Day (University of Virginia) under NIH contract DE52477. All amino acids were of the L configuration. The lyophilized peptides were dissolved at 10 mM in dimethyl sulfoxide, and subsequent dilutions were made in distilled water.

Chemotaxis assays. Chemotactic activity was determined by two previously described methods, the ^{51}Cr assay (18) and the leading front assay (19). PMN were purified from normal human blood by dextran sedimentation and hypotonic lysis of erythrocytes. In the ^{51}Cr assay, PMN were labeled with $Na_2{}^{51}CrO_4$ (New England Nuclear Corp., Boston, Mass. 200 to 500 μCi/μgCr), washed, and suspended in Gey's medium (Microbiological Associates, Bethesda, Md.) at 2.3×10^6 granulocytes/ml (purity 80 to 90%). In the leading front assay, the labeling step was omitted, but washing and suspension in Gey's medium was the same. For the ^{51}Cr assay, chambers were incubated for 3 hr and chemotaxis was determined as the mean cpm of ^{51}Cr in the lower of two Micropore filters with mean pore diameter of 3 μm (Sartorius Membranfilter, Göttingen, West Germany). For the leading front assay, chambers were incubated for 1 hr and chemotaxis was determined as the mean depth of penetration in microns into the filter (3 μm pore diameter, Sartorius) of the leading two cells. Each sample was tested in four replicate chambers.

Exposure of chemotactic factors to the MPO system. The chemoattractants were incubated with the components of the MPO system as detailed in the legends to the figures and tables and as we have previously described (10). After this incubation, samples were assayed for chemotactic activity or for receptor binding (see below) or were subjected to thin-layer chromatography (see below). Samples to be tested for chemotactic activity were diluted to a total volume of 4 ml in iced gelatin-Veronal-buffered saline containing 70 μM Ca^{2+} and 0.5 mM Mg^{2+}, and aliquots of 0.8 ml were placed in the lower compartments of each of four chemotaxis chambers. Controls included samples without the chemoattractant (background) and samples with the chemoattractant, but none of the components of the MPO system (positive control). After determining chemotactic activity as either mean cpm (^{51}Cr assay) or mean micron migration (leading front assay), the data were expressed as percent inactivation as follows:

$$\text{Inactivation (\%)} = \frac{\text{Positive control} - \text{experimental sample}}{\text{Positive control} - \text{background}} \times 100$$

The mean background activity and positive control activities were similar to those described previously (10). As we have reported in detail (10), the inactivation of chemoattractants under these conditions requires enzymatically active MPO, H_2O_2, and a halide, and it is time and temperature dependent.

Receptor binding assay. Chemotactic peptides exposed to the MPO system were tested for binding to PMN membrane receptors by determining their capacity to inhibit the binding of a radiolabeled ligand, f-norleu-leu-[^{3}H]phe as previously reported (20). Rabbit peritoneal PMN (4.4×10^6 cells) were treated briefly with 0.1 mM tosyl-L-phenylalanyl chloromethane (TPCK; Calbiochem, Gaithersburg, Md.) and then incubated at 0°C for 1 hr in 2 ml of Gey's balanced salt solution containing 2.1 nM f-norleu-leu-[^{3}H]phe (125,000 dpm) (obtained from Drs. R. Freer and A. Day, University of Virginia, and New England Nuclear Corp., Boston, Mass.) and varying amounts of the peroxidase-treated peptides. The incubation was terminated by rapid filtration onto Whatman glass fiber filters (GF/B) attached to a low pressure chamber (Hoefer Scientific Instruments, San Francisco, Calif.). The filters were washed with two 7-ml portions of iced 0.02 M phosphate-buffered saline, pH 7.4, air-dried, placed in 10 ml of Aquasol (New England Nuclear Corp.), and counted in a liquid scintillation spectrometer. The nonspecific binding of the ligand, determined in samples containing a 1000-fold excess of unlabeled f-norleu-leu-phe, averaged 233 cpm of ^{3}H or approximately 10% of the total binding; this value was substracted from all experimental samples.

Thin layer chromatography. Chemotactic peptides were dissolved in ethanol (50 to 100 μl) and spotted on plastic sheets coated with silica gel (Eastman Kodak Co., Rochester, N. Y.). ^{3}H-labeled peptides were used in the experiments with the MPO system, and approximately 1.5×10^6 cpm of ^{3}H were applied to each silica gel sheet. The f-met-leu-[^{3}H]phe (40 to 60 Ci/mmole) was obtained from New England Nuclear Corp., and the f-norleu-leu-[^{3}H]phe was as described above. Nonradioactive standards consisted of 0.05 to 0.1 μmoles of f-met-leu-phe and the same material after oxidation for 1 hour at 25°C in performic acid (nine volumes formic acid plus one volume 30% H_2O_2 kept at 0°C for 3 to 4 hr before use). The chromatograms were developed for 6 to 8 hr with a mobile phase consisting of n-butanol, acetic acid, and water in a 4:1:1 ratio by volume. Sheets were air-dried and cut into strips. Radiolabeled peptides were detected with a chromatograph scanner (Packard Instrument Co., Downers Grove, Ill.), and nonlabeled peptides were visualized with either a peptide reagent (tolidine) or a thioether reagent (iodoplatinate). These procedures represent minor modifications of those used previously (9, 21).

Special materials. MPO was prepared from canine PMN by the method of Agner (22) through the end of step 6. Peroxidase activity was determined before each experiment by the o-dianisidine method (23); 1 unit of activity is the amount causing the utilization of 1 μmole of substrate/min at 25°C (24). H_2O_2 (30% solution) and L-ascorbic acid were from Fisher Scientific Co., Fair Lawn, N. J. L-methionine, DL-methionine sulfoxide, 2-mercaptoethanol, and glucose oxidase (type V, 1460 units/ml, 200 units/mg) were obtained from Sigma Chemical Co., St. Louis, Mo. One unit of glucose oxidase catalyzes the oxidation of 1 μmole of glucose/min at 35°C, pH 5.1.

Statistics. Standard error (S.E.) was used as an estimate of variance, and means were compared with Student *t*-test (two-tailed).

RESULTS

Inactivation of chemoattractants by the MPO system. Our previous finding that the MPO-H_2O_2-halide system can inactivate certain chemoattractants (10) is amplified by the data presented in Table I. There is a marked loss of activity of C5a, f-met-leu-phe, and f-met-leu-phe-lys on exposure to the components of the MPO system. In sharp contrast, no significant inactivation of f-norleu-leu-phe or f-phe-leu-phe-leu-phe occurred with identical MPO system treatment. The concentrations of MPO, H_2O_2, and chloride employed in these studies were each approximately 10-fold in excess of the minimal amounts required to inactivate C5a and f-met-leu-phe (10), suggesting that the resistance of f-norleu-leu-phe and f-phe-leu-phe-leu-phe was not simply due to minor differences in sensitivity among these various peptides. Nonetheless, we examined the effect of increasing the concentration of MPO system com-

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ponents, using alternative halide co-factors, and substituting a peroxide-generating enzyme system, glucose plus glucose oxidase, for reagent H_2O_2 (Table II). Nearly complete inactivation of f-met-leu-phe was observed in all instances. Both f-norleu-leu-phe and f-phe-leu-phe-leu-phe remained resistant to the MPO system. Slight, although significant, inactivation of the pentapeptide (5 to 16%) was detected with high MPO and H_2O_2 concentrations. In every case, the inactivation of f-met-leu-phe was significantly greater than that of other peptides ($p < 0.01$ for all comparisons).

Effects of the MPO system on binding of chemotactic peptides to PMN receptors. We next attempted to determine whether the inactivation of chemoattractants was associated with alterations in their interaction with specific receptors on the PMN cell membrane (20, 25). These experiments were designed to help decide between two alternative possibilities: 1) loss of activity was a consequence of decreased affinity for receptors or 2) receptor binding was normal and some subsequent step in the locomotion process was impaired. By comparing an MPO-sensitive peptide, f-met-leu-phe, with an MPO-resistant agent, f-norleu-leu-phe, we also hoped to gain further insight into the mechanism of inactivation. The experiments evaluated the binding of MPO-treated and control peptides by determining their ability to specifically block the binding to rabbit PMN of f-norleu-leu-[^{3}H]phe. Both control peptides were able to compete very effectively with the radiolabeled ligand for binding sites (Fig. 1). The data clearly indicate a major decrease in receptor binding of MPO-treated f-met-leu-phe. In contrast, there was no effect of the MPO system on the binding affinity of f-norleu-leu-phe.

TABLE I

Effect of the MPO system on various chemoattractants[a]

Chemoattractant	Inactivation
	%
C5a	94.4 ± 1.8 (17)[b, c]
f-met-leu-phe	98.3 ± 4.4 (15)[c]
f-met-leu-phe-lys	75.6 ± 10.0 (6)[c]
f-norleu-leu-phe	0.1 ± 9.2 (7)
f-phe-leu-phe-leu-phe	10.9 ± 8.0 (10)

[a] The reaction mixture contained 0.8 ml of C5a, 50 pmoles of f-met-leu-phe, f-met-leu-phe-lys, or f-norleu-leu-phe or 0.5 nmoles of f-phe-leu-phe-leu-phe. With C5a, the reaction was performed in 1.0 ml of 40 mM sodium phosphate buffer, pH 7.0 and with the oligopeptides 0.5 ml of 20 mM sodium phosphate buffer, pH 7.0. The MPO system components employed were as follows: MPO—16 mU/ml; H_2O_2—10 μM; NaCl—0.1 M. Samples were incubated at 37°C for 15 min. The ^{51}Cr assay was used for C5a and f-met-leu-phe-lys, whereas the leading front assay was used for f-met-leu-phe, f-norleu-leu-phe, and f-phe-leu-phe-leu-phe.

[b] Mean ± S.E. (number of experiments).

[c] $p < 0.001$ *vs* control; others not significant.

Involvement of methionine oxidation in MPO-mediated inactivation of chemoattractants. Attention was directed to structural properties of chemoattractants that might determine their sensitivity or resistance to inactivation by the peroxidase system. One obvious difference between the group of three MPO-sensitive agents and the two resistant peptides (Table I) is the presence of methionine in the former and its absence from the latter. It should be noted that recent data delineating the primary structure of human C5a indicate the presence of a single methionine residue near the carboxy terminus at position 69 (26). The data on receptor binding also suggest methionine involvement in that only the methionine-containing peptide displayed decreased binding after exposure to the MPO system (Fig. 1). Since the thioether group of methionine might be expected to be particularly susceptible to oxidation, we hypothesized that inactivation of chemotactic peptides was a consequence of MPO-mediated methionine oxidation. This was tested by examining the ability of free methionine as well as nonspecific reducing agents to block the inactivation of chemotactic peptides by the MPO system. As illustrated in Figure 2 (left panel), methionine displayed dose-related inhibition of f-met-leu-phe inactivation. Under the conditions employed, the concentration of methionine required to decrease inactivation to 50% was 4 μM. In contrast, oxidized methionine failed to inhibit significantly at concentrations as high as 0.1 mM, although slight inhibition was observed at 1 mM. When the MPO concentration was increased 4-fold, there was approximately a one-*log* increase in the amount of methionine required to inhibit peptide inactivation (50% inactivation at 60 μM methionine, data not shown). Ascorbic acid and 2-mercaptoethanol both blocked MPO-mediated inactivation of f-met-leu-phe in a dose-related fashion, with 60 μM and 80 μM, respectively, required for a decrease to 50% inactivation (Fig. 2, right panel).

Chromatographic analysis of MPO-treated chemotactic peptides. The behavior of f-met-leu-phe and oxidized f-met-leu-phe in the thin layer chromatographic system was first characterized by using chemical detection methods (Fig. 3, lower panel). The nonoxidized peptide was easily detected with both peptide and thioether reagents and had an R_F value of 0.85. The chemically oxidized f-met-leu-phe displayed a distinctly slower rate of migration (R_F 0.77) and was detectable with the

TABLE II

Effect of variations in the MPO system on inactivation of formylated peptide chemoattractants[a]

Peroxidase System			Inactivation		
MPO	H_2O_2	Halide	f-mlp	f-nllp	f-plplp
mUnits/ml	*μM*	*mM*		%	
16	10	Cl^- 100	98.3 ± 4.4 (15)[b, c]	0.1 ± 9.2 (7)	10.9 ± 8.0 (10)
16	50	Cl^- 100		−13.8 ± 7.8 (2)	16.0 ± 5.6 (10)[d]
32	50	Cl^- 100		10.2 ± 8.9 (7)	5.3 ± 2.1 (7)[d]
16	10	I^- 1.0	80.9 ± 5.1 (7)[c]	0.5 ± 17.8 (3)	
16	10	Br^- 0.1	87.8 ± 6.1 (4)[c]	19.4 ± 10.4 (4)	
16	G.O. 0.14 units	Cl^- 100	73.6 ± 3.0 (2)[d]	11.2 ± 9.0 (5)	

[a] The reaction mixture was as described in Table I except that the components of the MPO system were varied as noted. G.O. indicates the use of glucose oxidase (plus 10 mM glucose) as a source of H_2O_2. The leading front assay was used in all instances except the effect of the I^- system on f-met-leu-phe that was done with the ^{51}Cr assay.

[b] Mean ± S.E. (number of experiments).

[c] $p < 0.001$ *vs* control.

[d] $p < 0.05$ *vs* control.

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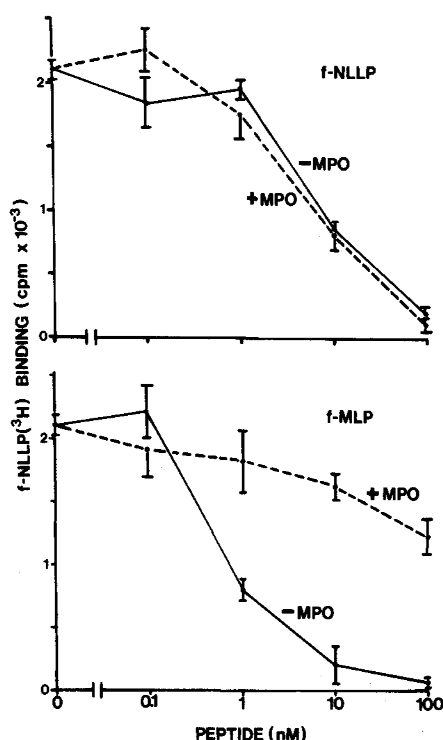

Figure 1. Effect of the MPO system on the binding of chemotactic peptides to PMN receptors. The peptides were first exposed to the MPO system as follows: reaction mixtures contained 20 nmoles of f-met-leu-phe or f-norleu-leu-phe, 20 mM sodium phosphate buffer pH 7.0 and 0.1 M NaCl in a volume of 0.5 ml; these control samples (●——●) were compared with samples that were identical except for the addition of MPO—16 munits/ml and H_2O_2—10 μM (●-----●). After incubation at 37°C for 15 min, the samples were placed in an ice bath, diluted 1/1 in glacial acetic acid, frozen, and lyophilized. The peptides were dissolved in Gey's balanced salt solution and tested in varying concentrations for their ability to compete with the radiolabeled ligand f-norleu-leu-[^{3}H]phe for binding to rabbit PMN as described in *Methods*. Control samples containing the complete MPO system, but no peptide, had no effect on binding. The data presented are the means (±S.E.) of triplicate samples. Control f-met-leu-phe caused marked inhibition of ligand binding at 1 nM or greater (lower panel). MPO-treated f-met-leu-phe was significantly less effective in blocking ligand binding ($p < 0.01$ for 1, 10 or 100 nM peptide). In contrast, f-norleu-leu-phe caused marked inhibition of ligand binding whether or not it had been exposed to the MPO system (*upper panel*).

peptide reagent, but not with the thioether reagent. The latter finding indicates that the methionine residue was the site of oxidation. By using these compounds as standards, the chromatographic behavior of control and MPO-treated chemotactic peptides was studied by using radiolabeled peptides (Fig. 3, upper panels). Control f-met-leu-[^{3}H]phe had an R_F value identical to the chemically detected f-met-leu-phe standard, whereas the MPO-treated labeled peptide showed a shift to a more slowly migrating peak corresponding to the chemically oxidized standard. A rapidly migrating (R_F 0.91) radiolabeled impurity was consistently detected in the commercially obtained f-met-leu-[^{3}H]phe. This unidentified component was not observed in the MPO-treated peptide. When any of the three components of the MPO system—MPO, H_2O_2, or chloride—was omitted, the shift to the slower peak did not occur, indicating a requirement for the complete peroxidase system. The position of f-norleu-leu-[^{3}H]phe in the chromatogram was identical for the control and MPO-treated peptide. These data demonstrate that MPO-mediated inactivation of chemotactic peptides is associated with oxidation of methionine.

DISCUSSION

We have demonstrated that the MPO-H_2O_2-halide system of the neutrophil destroys the biologic activity of certain leukocyte chemotactic agents. Nearly complete inactivation of C5a, f-met-leu-phe, and f-met-leu-phe-lys was observed. MPO-treated f-met-leu-phe lost its ability to bind to intact PMN, indicating that chemotactic inactivation was a consequence of decreased affinity for membrane receptors. In contrast to these results, the MPO system failed to inactivate two other attractants, f-norleu-leu-phe and f-phe-leu-phe-leu-phe, and had no effect on receptor binding of f-norleu-leu-phe. These observations point to the importance of methionine in chemotactic factor inactivation since the MPO-susceptible agents all contain methionine, whereas the MPO-resistant peptides do not.

The MPO system generates highly reactive intermediates that are potent oxidizing agents (11–13). Since the thioether group of methionine is particularly susceptible to oxidation (27), it seems likely that such a reaction might account for the inactivation of methionine-containing chemoattractants. Experimental evidence for methionine oxidation in the present study was obtained by demonstrating an inhibitory effect on chemotactic factor inactivation of such reducing agents as 2-mercaptoethanol and ascorbic acid and of free methionine but not oxidized methionine. The most likely explanation for these results is that methionine competes with the chemotactic peptide for oxidants generated by the MPO system.

The final evidence supporting methionine oxidation was obtained from thin layer chromatographic analyses of MPO-treated peptides. The exposure of f-met-leu-phe to the complete MPO system resulted in a shift in its rate of migration, with the new R_F value corresponding to that of chemically oxidized f-met-leu-phe. This shift was not observed when any of the components of the MPO system were omitted. Furthermore, f-norleu-leu-phe displayed the same R_F value whether or not it

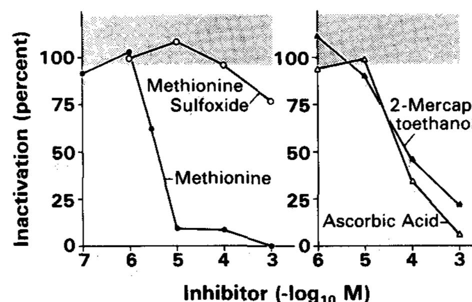

Figure 2. Inhibition of MPO-mediated inactivation of f-met-leu-phe by methionine, 2-mercaptoethanol, and ascorbic acid. The reaction mixture was as described in Table I for f-met-leu-phe except that the MPO concentration was 4 munits/ml and varying concentrations of L-methionine (●) and DL-methionine sulfoxide (○) (*left panel*), or 2-mercaptoethanol (▲) and L-ascorbic acid (△) (*right panel*) were added as indicated. Chemotaxis was determined by the leading front assay. The stippled area indicates the amount of inactivation observed in control samples containing the MPO system without inhibitors (mean ± S.D.). The data points are the means of four to seven experiments. Significance levels of differences from control are as follows: methionine—$p < 0.005$ for 3×10^{-6} M and $p < 0.001$ for 10^{-5} M or higher; methionine sulfoxide—$p < 0.02$ for 10^{-3} M; 2-mercaptoethanol—$p < 0.05$ for 10^{-5} M and $p < 0.001$ for 10^{-4} M or higher; ascorbic acid—$p < 0.001$ for 10^{-4} M or higher.

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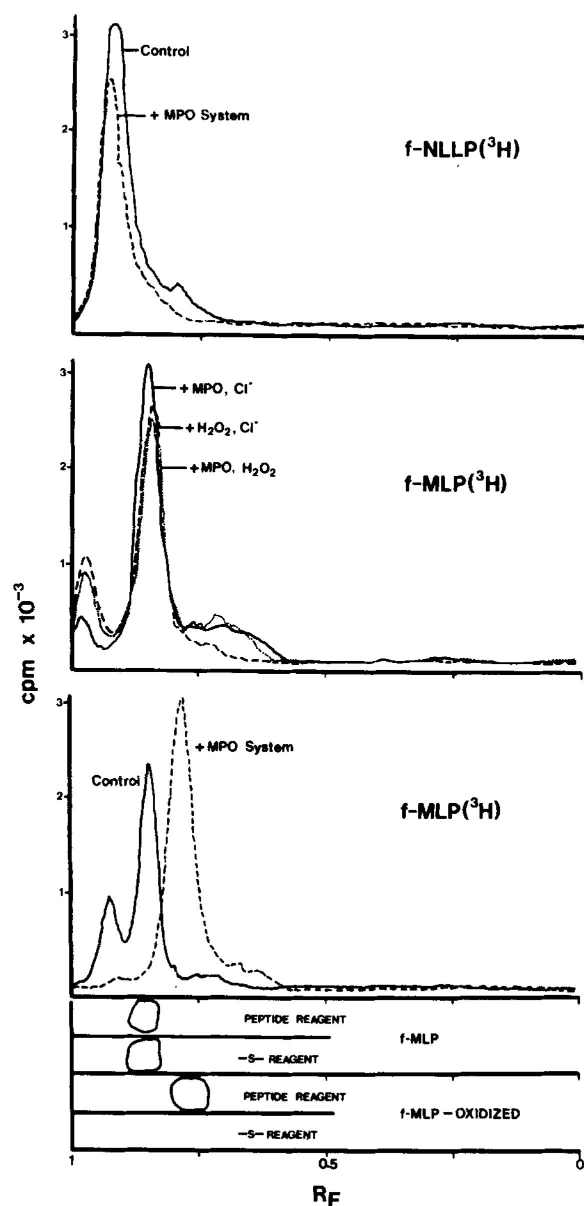

Figure 3. Thin layer chromatography of MPO-treated chemotactic peptides. The direction of migration is from right to left. The lower portion of the figure illustrates the chemical detection of the standards, f-met-leu-phe and performic acid-oxidized f-met-leu-phe. The f-met-leu-phe is detected by both the peptide reagent and the thioether reagent, whereas the oxidized compound is only detected with the peptide reagent, indicating oxidation of the thioether group. The three upper panels illustrate the chromatographic properties of chemotactic peptides exposed to the MPO system as follows: reaction mixtures contained 1.5×10^6 cpm of f-met-leu-[^{3}H]phe or 1.2×10^6 cpm of f-norleu-leu-[^{3}H]phe, 10 pmoles of unlabeled carrier f-met-leu-phe or f-norleu-leu-phe, respectively, and 20 mM sodium phosphate buffer pH 7.0 in a volume of 0.1 ml. These control samples were compared with samples containing the components of the MPO system: MPO—16 munits/ml, H_2O_2 10 μM, NaCl 10 mM. After incubation at 37°C for 15 min, the samples were placed in an ice bath, diluted 1/1 in glacial acetic acid, frozen, and lyophilized. The peptides were dissolved in ethanol and chromatographed as described in *Methods*. Exposure of f-met-leu-phe to the complete MPO system resulted in a shift to a more slowly migrating form whose position coincided with that of chemically oxidized f-met-leu-phe. A shift was not seen when any of the components of the MPO system were omitted or when f-norleu-leu-phe was treated with the complete MPO system.

was exposed to the MPO system. The identity of the oxidized f-met peptide is not known with certainty, but it is presumably either the sulfoxide $\left(O = S\begin{smallmatrix}C\\C\end{smallmatrix}\right)$ or sulfone $\left(\begin{smallmatrix}O\\O\end{smallmatrix}S\begin{smallmatrix}C\\C\end{smallmatrix}\right)$ derivative. Schiffmann *et al.* (21) have reported that neither of these oxidized forms of certain f-met peptide chemoattractants retains any biologic activity. Thus, our data clearly indicate that the inactivation of methionine-containing oligopeptide chemoattractants by the MPO-H_2O_2-halide system is a direct consequence of methionine oxidation. Whether a similar reaction accounts for the inactivation of C5a is not known, although the presence of a single methionine near the biologically important carboxy terminus of the molecule (26) makes this a distinct possibility. The answer to this question must await studies with highly purified C5a.

An additional implication of our observations is that methionine, and more specifically its thioether group, is involved in the binding of peptide chemoattractants to PMN membrane receptors. This is also supported by the observation that optimal binding occurs with f-met-leu-phe, whereas substitution of norleucine for methionine results in a compound, f-norleu-leu-phe, that has about 5% of the binding of the methionine-containing peptide (28).

The toxicity of the MPO system to microorganisms is a consequence of both halogenation and oxidation reactions (11, 13). Halogenation involves the covalent binding of the halide co-factor, for example, iodide, to protein acceptors with the formation of such products as iodotyrosines, iodohistidines, and sulfenyl iodides (13). Chlorination and bromination have also been demonstrated (13). Oxidants formed by the MPO system may include halogens, hypohalous acids, chloramines, aldehydes, and singlet molecular oxygen (11, 13). One or more of these reactive intermediates is very likely responsible for the methionine oxidation observed in the current study. The potential involvement of singlet oxygen is supported by its demonstrated role in the oxidation of methionine or *N*-formylated methionine by singlet oxygen generating photoactivated dyes (27), the final oxidation product being methionine sulfoxide. A recent preliminary report described the oxidation of methionine to methionine sulfoxide by phagocytosing PMN and suggested the involvement of singlet oxygen in this phenomenon (29). It should be noted that the current evidence for the formation of singlet oxygen by intact PMN or by the isolated MPO system is not necessarily conclusive, in part because of a lack of specificity of the available scavenging agents (30–32). We have found that inactivation of f-met-leu-phe by the MPO system is blocked by diazobicyclo-octane and histidine (data not shown), agents considered to be singlet oxygen scavengers, but the apparent lack of specificity of these agents (31, 32) precludes a definite statement about singlet oxygen involvement in the observed methionine oxidation.

At least two other examples of mediator inactivation by peroxidases have been described. Henderson and Kaliner (33) recently demonstrated the inactivation of slow-reacting substance of anaphylaxis (SRS-A) by peroxidases from either horseradish or rat mast cell granules, especially in the presence of exogenous H_2O_2. Coupled with preliminary evidence that slow-reacting substance is a thioether fatty acid (34), these observations suggest that thioether oxidation may be responsible for inactivation. Matheson *et al.* (35) have reported that exposure of human α_1-proteinase inhibitor (α_1-PI) to purified myeloperoxidase, H_2O_2, and a halide resulted in a loss of its elastase-inhibitory activity. We have also observed this phe-

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nomenon and have documented that the loss of elastase inhibitory capacity is a consequence of the failure of MPO-treated α_1-PI to bind the enzyme (Clark, R., P. Stone, and C. Franzblau; manuscript in preparation). Carp and Janoff (36) found a partial suppression of elastase inhibition of α_1-PI by activated human PMN; this effect was partially blocked by azide and catalase, suggesting involvement of MPO and H_2O_2, although inhibition by SOD and mannitol raised the possibility of the participation of superoxide and hydroxyl radical as well. It is of interest that structural studies of several proteinase inhibitors, including α_1-PI, indicate the presence of a methionine residue at or near the elastase binding site and demonstrate that chemical oxidation of this methionine is associated with a loss of inhibitory activity (37–40). In the work of Matheson *et al.* (35), it appeared that α_1-PI inactivation by the MPO system was a result of the oxidation of the critical methionine.

The potential physiologic significance of the present observations is underscored by the recent demonstration in our laboratory that intact human PMN can inactivate methionine-containing chemoattractants by a mechanism dependent on the MPO-H_2O_2-halide system (Clark, R.; manuscript in preparation). In these studies, phorbol myristate acetate-stimulated human PMN have been shown to inactivate f-met-leu-phe and C5a, but not f-norleu-leu-phe or f-phe-leu-phe-leu-phe. Inactivation requires chloride and is inhibited by azide, catalase, and methionine, but not by heated catalase or oxidized methionine. Experiments with PMN from patients with chronic granulomatous disease and hereditary MPO deficiency indicate specific requirements for H_2O_2 and MPO, respectively.

We suggest that the secretion of MPO and H_2O_2 by activated PMN may serve an important modulating function in the tissue response to injury by causing the inactivation of certain inflammatory mediators. Under our experimental conditions with purified components, this process is a consequence of oxidation of the thioether linkage of methionine by oxidants formed via the MPO-catalayzed oxidation of chloride by H_2O_2. Interpretation of our studies in the light of reports from other investigators raises the possibility that peroxidase-mediated oxidation of thioethers may constitute a general mechanism for modifying the properties of diverse biologic mediators.

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