Myeloperoxidase-Mediated Oxidation of Methionine and Amino Acid Decarboxylation

MIN-FU TSAN

Divisions of Hematology and Nuclear Medicine, The Johns Hopkins Medical Institutions, Baltimore, Maryland 21205

Received 10 August 1981/Accepted 3 December 1981

The myeloperoxidase (MPO) system (MPO-H$_2$O$_2$-halide) is a potent antimicrobial system operative in neutrophils (9). The exact mechanism by which this system kills bacteria is not clear. Several possibilities have been proposed; these include MPO-mediated halogenation (8, 14), decarboxylation of amino acids with the generation of aldehydes (11, 13, 22), and MPO-mediated production of singlet oxygen ($^{1}$O$_2$) (9, 12). However, whether the MPO system produces $^{1}$O$_2$ remains controversial (6, 7).

Previous studies (17) from this laboratory have demonstrated that during phagocytosis, neutrophils oxidize methionine to methionine sulfoxide and that the oxidation of methionine is dependent on the MPO system. Evidence has also accumulated to indicate that MPO-mediated oxidation of methionine is responsible for the inactivation of $\alpha_1$-proteinase inhibitor ($\alpha_1$-antitrypsin) (2, 10), $\alpha$-formylmethionyl chemotactic peptides (3, 18), or C5a (3) by human neutrophils or by the MPO system. The MPO-mediated inactivation of $\alpha_1$-proteinase inhibitor may contribute to the pathogenesis of emphysema (2, 10). The MPO-mediated inactivation of chemotactic peptides may be a mechanism by which neutrophils modulate the inflammatory process (3, 18). If the MPO system also oxidizes methionine at the active site of bacterial enzymes, this may be one mechanism by which this system damages microorganisms.

In this study, the MPO-mediated oxidation of methionine and decarboxylation of alanine were compared. Evidence that the agent responsible for the MPO-mediated oxidation of methionine might play an important role in the killing of bacteria is also provided.

MATERIALS AND METHODS

Chemicals. L-[$U-^{14}$C]methionine and L-[$U-^{14}$C]alanine were obtained from New England Nuclear Corp., Boston, Mass. L-[$1-^{14}$C]Alanine was obtained from ICN Pharmaceuticals, Inc., Irvine, Calif. L-methionine-DL-sulfoxide and L-methionine sulfone were from Sigma Chemical Co., St. Louis, Mo. L-[$U-^{14}$C]methionine sulfoxide was obtained through chemical oxidation of L-[$U-^{14}$C]methionine by adding 0.1 ml of 3%
H₂O₂ to 5 μCi of l-[U-¹⁴C]methionine (293 mCi/mM) in a final volume of 0.5 ml in water. The mixture was incubated at 4°C for 3 h. It was then evaporated to dryness in a vacuum desiccator. The reaction product was dissolved in 5 ml of water. Using thin-layer chromatography (silica gel without fluorescence indicator; Eastman Kodak, Rochester, N.Y.) with three different solvent systems, we found that under this condition, all [¹⁴C]methionine was oxidized to [¹⁴C]methionine sulfoxide, and no [¹⁴C]methionine sulfone was obtained. The solvent systems used were tert-butanol:methylthylketone:water:ammonium hydroxide, 40:30:20:10; and n-butanol:glacial acetic acid:water, 50:25:25; and a lower-phase solvent from a mixture of phenol and water (5).

Isolation of MPO. Canine MPO was isolated as described previously (M. F. Tsan, J. Cell. Physiol., in press). Briefly, dogs were given an intramuscular injection of terpineine (1 ml) to induce leukocytosis. One week later, leukocytes were isolated from 2 liters of heparinized blood. MPO was then isolated from canine leukocytes through the end of step 6 (the step before crystallization) by the method of Agner (1). The final preparation exhibited a Reinheitszahl value (absorbancy at 430 nm/absorbancy at 280 nm) of 0.78. The activity of MPO was determined by the ortho-dianisidine method (20). One unit of peroxidase is the amount of enzyme which decomposes 1 μmol of H₂O₂ per min at 25°C.

MPO-mediated oxidation of methionine. Oxidation of methionine was performed as described previously (Tsan, in press). The complete system included MPO, 50 mM U/ml; H₂O₂, 0.1 mM; Cl⁻, 75 mM; and 0.1 μCi of [¹⁴C]methionine, 0.1 mM in a final volume of 1 ml in 0.02 M phosphate buffer, pH 6.0. H₂O₂ was added last to initiate the reaction. After being incubated for desired intervals at 37°C in a water bath with constant shaking, the test tubes were immersed in ice, and 0.1 ml of 50 mM methionine was added to prevent further oxidation of [¹⁴C]methionine. [¹⁴C]methionine and [¹⁴C]methionine sulfoxide were separated by using thin-layer chromatography and quantified with a liquid scintillation counter as described before (17). The results were expressed as the percentage of methionine oxidized to methionine sulfoxide. Control experiments were always done in the absence of the MPO system to assess the spontaneous oxidation of methionine, which was negligible.

MPO-mediated decarboxylation of amino acids. Measurement of [¹⁴C]carbon dioxide production was done as described previously (19). The complete system included MPO, 50 mM U/ml; H₂O₂, 0.1 mM; Cl⁻, 75 mM; and 0.1 mM [¹⁴C]-α-amino acid in a final volume of 1 ml in 0.02 M phosphate buffer, pH 6.0. The amounts of radioactivity used were 0.1 μCi for ¹⁴C uniformly labeled alanine, methionine, or methionine sulfoxide and 0.03 μCi for [¹⁴C]alanine. Similar results were obtained whether [U-¹⁴C]alanine or [¹⁴C]alanine was used. H₂O₂ was added last to initiate the reaction. After incubation for desired intervals at 37°C in a water bath with constant shaking, the reaction was stopped, and [¹⁴C]CO₂ was quantified as described previously (19). The results were expressed as the percentage of amino acid decarboxylated. Control experiments were always performed in the absence of the MPO system to assess the spontaneous release of [¹⁴C]O₂, which was negligible.

MPO-mediated bacterial killing. MPO-mediated bacterial killing was performed as described previously (19). The complete system included MPO, 50 mM U/ml; H₂O₂, 0.1 mM; Cl⁻, 75 mM; and 5 × 10⁵ Staphylococcus aureus (ATCC 25923) or 3 × 10⁵ Klebsiella pneumoniae (a clinical isolate from The Johns Hopkins Hospital) in a final volume of 1 ml in 0.02 M phosphate buffer, pH 6.0. H₂O₂ was added last to initiate the reaction. After being incubated for 1 h at 37°C in a water bath with constant shaking, serial dilutions were made, and the number of bacteria was determined by the pour plate technique as described previously (19).

Statistical analysis. Statistical differences were determined by using Student's t test for independent means (4).

RESULTS

MPO-mediated decarboxylation of alanine and methionine. Żeligczynski et al. (22) demonstrated that the MPO system decarboxylates a variety of amino acids. However, in previous studies (17; Tsan, in press) it was shown that methionine is oxidized to methionine sulfoxide by the MPO system. Therefore, the MPO-mediated decarboxylation of alanine and methionine was compared. As shown in Fig. 1a, 52% of alanine was decarboxylated after a 30-min incubation, whereas only 5% of methionine was decarboxylated. Increasing the concentration of H₂O₂ from 0.1 to 1 mM did not increase the decarboxylation of methionine by the MPO system (three experiments, data not shown). In contrast, under similar conditions, 83% of methionine was oxidized to methionine sulfoxide (Fig. 1b). The decarboxylation of alanine or methionine and the oxidation of methionine required the complete MPO system; elimination of MPO, H₂O₂, or Cl⁻ abol-

FIG. 1. MPO-mediated decarboxylation of alanine and methionine (a) and oxidation of methionine (b). The complete system included MPO, 50 mM U/ml; H₂O₂, 0.1 mM; Cl⁻, 75 mM; and 0.1 mM [¹⁴C]alanine or [¹⁴C]methionine in a final volume of 1 ml in 0.02 M phosphate buffer, pH 6.0. After being incubated for various intervals at 37°C, the production of [¹⁴C]CO₂ or oxidation of methionine to methionine sulfoxide was determined. Each point was the mean ± the standard error of the mean of three experiments.
ished the reactions completely (three experiments, data not shown).

Thus, methionine was preferentially oxidized to methionine sulfoxide by the MPO system. It is possible that the thioether group of methionine prevents its carboxylic group from being decarboxylated. To test this possibility, methionine was oxidized to methionine sulfoxide by incubating methionine with the MPO system for 30 min. Another sample of H₂O₂ (0.1 ml, 1 mM) was then added to the reaction mixtures, and ⁴⁰C₂₀₂ production was determined. The addition of the second sample of H₂O₂ caused a marked output of ⁴⁰C₂₀₂ (Fig. 2a). Decarboxylation of methionine sulfoxide by the MPO system was further confirmed by using [¹⁴C]methionine sulfoxide. Addition of the MPO system to [¹⁴C]methionine sulfoxide caused a marked ¹⁴C₂₀₂ output (Fig. 2b). Chromatographic analysis revealed that a new product was formed, presumably the decarboxylated product of methionine sulfoxide (Fig. 3). No methionine sulfoxide was noted, suggesting that methionine sulfoxide was not oxidized to methionine sulfone by the MPO system.

To determine whether the agent responsible for the MPO-mediated decarboxylation of alanine is the same as that for the oxidation of methionine, I studied the effect of methionine on the MPO-mediated decarboxylation of alanine and the effect of alanine on the MPO-mediated oxidation of methionine. Methionine markedly inhibited the decarboxylation of alanine; at the same concentration as alanine (0.1 mM), methionine inhibited the decarboxylation of alanine completely (Fig. 4a). In contrast, alanine at a concentration of 20 mM, which was 200 times

that of methionine, had no effect on the MPO-mediated oxidation of methionine (Fig. 4b). It should be noted that methionine also markedly inhibited the MPO-mediated decarboxylation of methionine sulfoxide (68% inhibition at 0.1 mM methionine and 100% inhibition at 1 mM methionine, mean of two experiments, methionine sulfoxide concentration was 0.1 mM).

![FIG. 2. MPO-mediated decarboxylation of methionine (a) and methionine sulfoxide (b).](image)

![FIG. 4. Effect of methionine on the MPO-mediated decarboxylation of alanine (a).](image)
all of these amino acids and taurine markedly inhibited the MPO-mediated decarboxylation of alanine. Previous studies (17; Tsan, in press) demonstrated that these amino acids have no effect on the peroxidase activity of MPO.

**MPO-mediated bacterial killing.** The MPO-mediated amino acid decarboxylation has been proposed as a possible bacterial killing mechanism (11, 13, 22). It has been shown that MPO-mediated oxidation of methionine is responsible for the inactivation of α1-proteinase inhibitor (2, 10) or methionine-containing chemotactic peptides (3, 18) by human neutrophils or the MPO system. Thus, if the MPO system also oxidizes methionine at the active site of bacterial enzymes, this may be one mechanism by which this system damages microorganisms. As shown in Table 2, NaN₃, methionine, and tryptophan, which inhibited the MPO-mediated oxidation of methionine (see Table 1), also inhibited the killing of *S. aureus* or *K. pneumoniae* by the MPO system; whereas histidine, alanine, and glycine, which did not inhibit the oxidation of methionine, had less or no effect on the killing of these two bacteria by the MPO system.

**DISCUSSION**

In this study, the MPO-mediated oxidation of methionine and the MPO-mediated decarboxylation of amino acids were compared. Since previous work on the MPO-mediated decarboxylation of amino acids has been primarily done with alanine (11, 15, 16, 22) it was also used in this study. Both MPO-mediated oxidation of methionine and decarboxylation of alanine have an acid pH optimum (15; Tsan, in press). Consequently, the experiments were carried out at pH 6. As demonstrated in this study, the MPO system decarboxylated 50% of alanine, whereas

---

**Table 1. Effect of various inhibitors on the MPO-mediated oxidation of methionine and decarboxylation of alanine**

<table>
<thead>
<tr>
<th>Inhibitor (mM)</th>
<th>Methionine oxidation (no. of expt)</th>
<th>Alanine decarboxylation (no. of expt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaN (0.1)</td>
<td>5.1 ± 1.9 (4)</td>
<td>3.9 ± 1.4 (3)</td>
</tr>
<tr>
<td>Methionine (1)</td>
<td>12.2 ± 0.6 (4)</td>
<td>0.0 ± 0.0 (3)</td>
</tr>
<tr>
<td>Alanine (1)</td>
<td>111.5 ± 6.9 (5)</td>
<td>10.5 ± 1.3 (3)</td>
</tr>
<tr>
<td>Tryptophan (1)</td>
<td>7.5 ± 1.7 (5)</td>
<td>0.6 ± 0.2 (3)</td>
</tr>
<tr>
<td>Histidine (1)</td>
<td>131.6 ± 13.2 (5)</td>
<td>1.7 ± 0.5 (3)</td>
</tr>
<tr>
<td>Glycine (1)</td>
<td>105.8 ± 3.2 (5)</td>
<td>15.1 ± 1.3 (3)</td>
</tr>
<tr>
<td>Taurine (1)</td>
<td>100.3 ± 4.4 (4)</td>
<td>3.3 ± 0.6 (3)</td>
</tr>
</tbody>
</table>

*The experiments were performed in 1 ml of phosphate buffer (pH 6.0) containing MPO, 50 mM/m; H₂O₂, 0.1 mM; Cl⁻, 75 mM; and methionine, 0.1 mM or alanine, 0.1 mM. The incubation time was 15 min at 37°C for methionine oxidation and 30 min at 37°C for alanine decarboxylation. The control value for the MPO-mediated oxidation of methionine was 77.2 ± 9.7% (7) of methionine oxidized to methionine sulfoxide and 42.6 ± 4.6% for alanine decarboxylation (3). The numbers in parentheses indicate the number of experiments.

**Table 2. Effect of various inhibitors on the MPO-mediated bacterial killing**

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th><em>S. aureus</em> × 10⁻⁶ (no. of expt)</th>
<th><em>K. pneumoniae</em> × 10⁻⁷ (no. of expt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>5.51 ± 0.64 (9)</td>
<td>3.17 ± 0.29 (7)</td>
</tr>
<tr>
<td>Complete system (MPO-H₂O₂-Cl⁻)</td>
<td>0.00000 ± 0.00000 (9)</td>
<td>0.00000 ± 0.00000 (7)</td>
</tr>
<tr>
<td>MPO omitted</td>
<td>5.66 ± 1.52 (5)</td>
<td>2.10 ± 0.32 (3)</td>
</tr>
<tr>
<td>H₂O₂ omitted</td>
<td>6.12 ± 1.33 (4)</td>
<td>1.80 ± 0.15 (3)</td>
</tr>
<tr>
<td>+ 0.1 mM NaN₃</td>
<td>2.79 ± 0.56 (4)</td>
<td>2.70 ± 0.56 (3)</td>
</tr>
<tr>
<td>+ 1 mM methionine</td>
<td>5.98 ± 1.06 (5)</td>
<td>2.70 ± 0.56 (3)</td>
</tr>
<tr>
<td>+ 1 mM alanine</td>
<td>0.00000 ± 0.00000 (3)</td>
<td>0.00000 ± 0.00000 (4)</td>
</tr>
<tr>
<td>+ 1 mM tryptophan</td>
<td>3.60 ± 0.61 (4)</td>
<td>2.33 ± 0.22 (3)</td>
</tr>
<tr>
<td>+ 1 mM histidine</td>
<td>0.014 ± 0.008 (4)</td>
<td>0.00003 ± 0.00003 (3)</td>
</tr>
<tr>
<td>+ 1 mM glycine</td>
<td>0.003 ± 0.003 (3)</td>
<td>0.00000 ± 0.00000 (3)</td>
</tr>
</tbody>
</table>

*The bacteria (*S. aureus*, 5 × 10⁶ or *K. pneumoniae*, 3 × 10⁷) were incubated in the presence or absence of the MPO system (MPO, 50 mM/m; H₂O₂, 0.1 mM; Cl⁻, 75 mM) in 1 ml of phosphate buffer (pH 6.0) for 60 min at 37°C. Additions were as indicated in the table. After incubation, the viable bacterial count was determined by the pour plate technique. The numbers in parentheses indicate the number of experiments.*
under the same experimental conditions, it only
decarboxylated 5% of methionine. In contrast, it
oxidized 80% of methionine to methionine sul-
oxide. Once methionine was oxidized to methio-
nine sulfoxide, it was decarboxylated by the
MPO system. Thus, the thioether group of methi-
one prevented its carboxylic group from
being decarboxylated by the MPO system. Methi-
onine at the same concentration as alanine
completely inhibited the decarboxylation of alan-
ine. In contrast, alanine at a concentration 200
times that of methionine had no effect on the
MPO-mediated oxidation of methionine.
Furthermore, histidine stimulated the oxidation
of methionine, whereas it markedly inhibited
the decarboxylation of alanine. The decarboxyla-
tion of alanine is thought to be mediated by OCI−
produced by the MPO-H2O2-Cl− system (21).
Although OCI− can oxidize methionine to methio-
nine sulfoxide, our previous study (Tsán, in press)
has shown that the MPO-mediated oxidation of
methionine is not mediated by OCI−. Thus, the
agent responsible for the oxidation of methione
is different from the agent responsi-
ble for the decarboxylation of alanine by the
MPO system. This is consistent with the previ-
ous observation that the MPO-H2O2-I− system
does not decarboxylate alanine (11), whereas it
is able to oxidize methionine to methionine
sulfoxide (Tsán, in press).

MPO-mediated decarboxylation of amino acids has
been suggested as one mechanism by
which the MPO system kills microorganisms.
Zgliczynski et al. (21) propose that in the
presence of MPO and H2O2, Cl− is oxidized to
hypochlorous acid. Hypochlorous acid reacts
with amino acids to form chloramine, which in
turn decomposes spontaneously to ammonia,
CO2, Cl−, and a corresponding aldehyde. The bac-
teria are killed either by the formation of
soluble aldehydes with microbicidal activity (11,
15, 16) or by the formation of structural alde-
hydes on the surfaces of the organisms (13).
However, it has been shown that free aldehydes
are either not toxic enough (11) or are not
produced in sufficient quantity (9) to account for
the observed bacterial killing. Thus, if the MPO
system kills bacteria by the decarboxylation of
amino acids, it would presumably be through
the disruption of amino acid-containing macromol-
eules (13).

Recently, the MPO system has been found to
inactivate a number of enzymes or bioactive
peptides through the oxidation of methionine to
methionine sulfoxide. These include α1-protein-
ase inhibitor (2, 10), C5a (3) and n-formylmethio-
nyl synthetic chemotactic peptides (3, 18). In
the cases of MPO-mediated oxidation and inacti-
vation of α1-proteinase inhibitor (2) or n-formyl-
methionylleucylphenylalanine (18), no decar-
boxylation of amino acids has been noted. Thus,
in the presence of methionine, decarboxylation
of amino acids is unlikely to occur.

In this study, it was demonstrated that there
was a better correlation between MPO-mediated
oxidation of methionine and bacterial killing
than between MPO-mediated decarboxylation of
alanine and bacterial killing. Methionine and
tryptophan, which inhibited MPO-mediated oxida-
tion of methionine, inhibited the MPO-mediated
oxidation killing of S. aureus and K. pneumoniae;
whereas histidine, alanine, and glycine did not
inhibit the MPO-mediated oxidation of methio-
nine and had less or no effect on the killing of
these two microorganisms. In contrast, all of
these amino acids inhibited the decarboxylation
of alanine. Paul et al. (11) have shown that
glycine and alanine inhibit the killing of Esche-
richia coli by the MPO system. However, al-
though the final concentrations of alanine and
glycine in their study are the same as those in
mine, they used only 106 E. coli, whereas I used
5 × 106 S. aureus and 3 × 107 K. pneumoniae.
Increasing the concentration of these amino
acids to 100 mM, e.g., 100-fold higher, alanine,
but not glycine, completely inhibited the killing of
S. aureus or K. pneumoniae by the MPO
system (three experiments, data not shown).
Thus, there is competition between bacterial
components and these amino acids for the
agent(s) responsible for the MPO-mediated bac-
terial killing. In addition, there is also some
species difference.

The results presented in this study suggest
that the agent(s) responsible for the oxidation of
methionine may play an important role in the
bacterial killing. The agent (or agents) responsi-
ble for the MPO-mediated oxidation of methio-
nine is not clear. However, my previous study
(Tsán, in press) suggests that it is neither O2 nor
hypochlorite. Further studies are necessary to
clarify this point.

ACKNOWLEDGMENTS

This work was supported by Public Health Service Re-
search grants AI-13004 and GM-10548 from the Na-
tional Institutes of Health. The author is the recipient of a Research
Career Development Award (AI-00194) from the National
Institute of Allergy and Infectious Diseases.
The author is grateful to Berlina Newman and Rebecca C.
Denison for their technical assistance.

LITERATURE CITED

Scand. 12:89-94.
inflammation. Phagocyte-derived oxidants suppress the
elastase-inhibitory capacity of alpha-proteinase inhibitor
3. Clark, R. A., S. Stoe, K. Venkatasubramanian, and E.
Schißmann. 1980. Chemotactic factor inactivation by
myeloperoxidase-mediated oxidation of methionine. J.
4. Croxton, F. E. 1953. Elementary statistics with applica-
MYELOPEROXIDASE-MEDIATED OXIDATIONS


