THE ROLE OF THE PHAGOCYTE IN HOST-PARASITE INTERACTIONS. XXIII. RELATION OF BACTERICIDAL ACTIVITY TO PEROXIDASE-ASSOCIATED DECARBOXYLATION AND DEAMINATION

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Peroxidase-mediated decarboxylation and deamination of appropriate substrates appear to be associated with bactericidal activity of the myeloperoxidase-H_2O_2-chloride antimicrobial system. Products resulting from these reactions, possibly aldehydes, may be the actual bactericidal agents.

Myeloperoxidase (MPO), H_2O_2 and halide ions act synergistically to kill bacteria (1,2), fungi (3) and viruses (4). The precise mechanism by which these agents function in killing microorganisms is presently unknown. The system functions best at an acid pH (1,2). Either iodide, bromide or chloride can satisfy the halide requirement (5). Since the concentration of chloride ion required for bactericidal activity is within the range of the chloride concentration of extracellular fluid within the phagocytic vacuole, chloride may be the participating halide. In a recent study it was reported that MPO, isolated from leukocytes of patients with chronic myelogenous leukemia, could in the presence of H_2O_2 and chloride, at pH 5.5., decarboxylate and deaminate several amino acids to aldehydes (6). It has been known, at least since 1894, that aldehydes are effective bactericidal agents (7). Amino acids and other substrates for peroxidase e.g. fatty acids (8), are found in

the phagocyte and in bacteria and could give rise to aldehydes by a MPO-mediated decarboxylation and deamination. It is tempting to suggest that the mechanism responsible for the antimicrobial activity of the MPO-H_2O_2-chloride system is its ability to deaminate and decarboxylate appropriate substrates to aldehydes.

This preliminary report presents evidence suggesting that deamination and decarboxylation of amino acids or other substrates may be the mechanism whereby the MPO-H_2O_2-chloride complex functions in killing bacteria.

Granules containing MPO were isolated from guinea pig peritoneal polymorphonuclear leukocytes (PMN) as described previously (2). The peroxidase activity was determined by guaiacol oxidation in phosphate buffer, pH 7.0, unless indicated otherwise (9). Escherichia coli was grown as previously described (10), harvested in the logarithmic phase, washed and suspended in Krebs-Ringer phosphate buffer (KRPB), pH 5.5. Bactericidal activity was determined by adding 2×10^4 E. coli to combinations of 0.1 μmoles of H_2O_2, 0.003 or 0.03 guaiacol units of peroxidase and 0.8 μmoles of taurine made up to a final volume of 2.0 ml with KRPB, pH 5.5, as indicated in individual experiments. After a 30 minute incubation at 37°C, aliquots were diluted and plated to obtain a viable cell count. The KRPB used contained sufficient chloride to satisfy the halide requirement for bactericidal activity. L-alanine decarboxylation activity was determined as described previously (11). Horseradish peroxidase, type II, (HPO) 110 Purpurogallin units/mg and crystalline taurine were obtained from Sigma Chemical Co., St. Louis, Missouri. H_2O_2 was purchased from Merck and Co., Inc., Rahway, New Jersey. All chemicals used in these studies were of reagent grade.

The enzymatic activities of MPO-containing granules and crystalline HPO have been measured for peroxidative activity in two different ways: guaiacol oxidation and L-alanine decarboxylation (9,11). At concentrations of

the two enzymes having equivalent guaiacol unit activity, L-alanine decarboxylation was evident only with MPO. HPO did not show significant decarboxylation activity even at 300% higher concentrations. This indicates that L-alanine and guaiacol are both substrates for MPO, whereas L-alanine under the given experimental conditions is not decarboxylated by HPO. We have previously shown that the MPO-containing granules also catalyze H_2O_2-dependent deamination (11). Further, it has been reported that a highly purified MPO catalyzed guaiacol oxidation as well as decarboxylation and deamination of several amino acids, including L-alanine (6).

TABLE 1

COMPARISON OF BACTERICIDAL ACTIVITY OF MYELOPEROXIDASE (MPO) AND HORSERADISH PEROXIDASE (HPO) ON ESCHERICHIA COLI[a]

Supplements	Viable cell counts (organisms/ml)
None	9.4×10^3
H_2O_2	9.2×10^3
MPO (0.003 units)	9.5×10^3
H_2O_2 + MPO (0.003 units)	$<3.5 \times 10^1$
HPO (0.003 units)	9.5×10^3
H_2O_2 + HPO (0.003 units)	1.0×10^4
HPO (0.03 units)	9.9×10^3
H_2O_2 + HPO (0.03 units)	1.0×10^4

[a] Reaction mixture contained E. coli, 2×10^4 organisms, Krebs-Ringer phosphate buffer, pH 5.5 to a final volume of 2.0 ml; and the supplements as indicated below: H_2O_2, 0.1 μmoles; MPO, 0.003 guaiacol units; HPO, 0.003 or 0.03 guaiacol units. Incubation period was 30 minutes.

As mentioned above, decarboxylation and deamination of different amino acids (6) or decarboxylation of fatty acids (8) results in aldehyde formation. Since the bactericidal nature of aldehydes is well established, experiments were designed to explore the possible relationship of aldehyde formation resulting from these reactions to bactericidal activity. The two enzymes, MPO and HPO, in concentrations having equivalent guaiacol unit activity, were examined for bactericidal activity (Table 1). Significant bactericidal activity was noted with MPO. In contrast, the equivalent concentration of HPO, as well as a 10 fold higher concentration, did not result in bactericidal activity. This indicates that not all peroxidative activity is related to bactericidal activity. However, that peroxidation which results in decarboxylation of amino acids, in this case, L-alanine (11) does parallel bactericidal activity.

Taurine has been shown to be a competitive inhibitor of MPO catalyzed amino acid decarboxylation and deamination (6). In our hands taurine inhibited both decarboxylation and deamination activities of MPO-containing granules (11). To determine whether taurine was a general inhibitor of MPO, the effect of taurine on guaiacol oxidation by MPO-containing granules was examined. The guaiacol oxidation at pH 5.5 was not inhibited in the presence of 4×10^{-4} M taurine. In an effort to further relate the various types of peroxidase activity to bactericidal activity, taurine was added to the bactericidal system at a concentration which inhibited L-alanine decarboxylation and deamination (6,11), but not guaiacol oxidation. Data in Table 2 indicate that bactericidal activity is inhibited in the presence of taurine. Moreover, this concentration of taurine inhibits decarboxylation activity but not guaiacol oxidation of MPO-containing granules. This suggests that MPO-mediated decarboxylation is associated with bactericidal action. MPO-mediated deamination and decarboxylation occur at a one to one ratio (6). This finding, combined with our previous observation

TABLE 2

A COMPARISON OF GUAIACOL OXIDATION, 1-^{14}C-L-ALANINE DECARBOXYLATION AND BACTERICIDAL ACTIVITY OF MYELOPEROXIDASE (MPO)-CONTAINING GRANULES IN THE ABSENCE AND PRESENCE OF 4 X 10^{-4} M TAURINE

Taurine 4 x 10^{-4} M	Bactericidal activity % killed[a]	Decarboxylation mμmoles $^{14}CO_2$[b]	Guaiacol oxidation guaiacol units per 10^8 cells
-	99.95	282	0.299
+	27.0	127	0.297

[a] Complete bactericidal system contained, in a final volume of 2 ml, E. coli, H_2O_2 and MPO in concentrations indicated in Table 1.

[b] Reaction mixture contained 0.03 guaiacol units of MPO-containing granules; 0.3 μmoles H_2O_2; 5.4 μmoles 1-^{14}C-L-alanine (specific activity 0.055 μc/μmole); 900 μmoles NaCl; and 0.06 M phosphate buffer, pH 5.5, to a final volume of 3 ml. Taurine, when present, was added at a concentration of 1.2 μmoles. $^{14}CO_2$ was trapped in 20% KOH in a center well and counted in a liquid scintillation counter. Incubation period was 30 minutes at 37°C.

[c] Reaction mixture for guaiacol oxidation contained 18.0 μmoles phosphate buffer, pH 5.5; 50 μmoles guaiacol; 750 μmoles sucrose; and MPO-containing granules in a total volume of 3.0 ml. 0.1 μmole H_2O_2 was added last to start the reaction in the sample cuvette. Taurine, when present, was added in a concentration of 1.2 μmoles. The activity was calculated as described previously (9).

that taurine inhibits H_2O_2-dependent deamination of MPO-containing granules (11), suggests that MPO-dependent deamination, as well as decarboxylation, is involved in the bactericidal activity.

The above and previously reported (11) data suggest that H_2O_2-dependent decarboxylation and deamination by MPO-containing granules in a chloride medium are related to bactericidal activity of the MPO-H_2O_2-chloride bactericidal system against the test organism used. Possible sources of amino acid or fatty acid substrates for these reactions may be the bacterial cell surface or possibly the phagocytic granule. It is possible that aldehydes formed from decarboxylation and deamination of appropriate substrates may be the agents responsible for bactericidal activity in the MPO-H_2O_2-chloride system.

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