THE ROLE OF pH IN THE CHEMILUMINESCENT RESPONSE OF THE MYELOPEROXIDASE-HALIDE-HOOH ANTIMICROBIAL SYSTEM

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SUMMARY: Myeloperoxidase extracted from human polymorphonuclear (PMN) leukocytes yields a chemiluminescence in the presence of halide cofactor and HOOH. This enzyme has an established pH optimum for microbicidal activity. The effects of varying H^+ concentration on the myeloperoxidase system was investigated using the criterion of chemiluminescence, and a pH optimum was established with either Cl^- or Br^- as cofactor. This optimum was in agreement with that established for antimicrobial action. The electronegative character of the various halides and the effect of various H^+ concentrations are considered with respect to halide oxidation, and the role of the oxidized halide-HOOH reaction in the generation of singlet multiplicity molecular oxygen is discussed.

I. INTRODUCTION

Decrease in the intravascular pH of the phagolysosome of the phagocytizing PMN leukocytes is a well-documented phenomenon (1,2,3). This increase in hydrogen ion (H^+) concentration is necessary for activation of the acid optimum enzymes present in the primary (A-type) granules of the PMN leukocyte (4). With regard to microbicidal activity, probably the one most important enzyme of this group is myeloperoxidase (MPO). This heme-containing peroxidase accounts for approximately 5% of the dry weight of PMN leukocytes (5), and has a pH optimum for microbicidal activity reported as approximately 5.0 (3,4,6).

Evidence for the generation of electronically excited

Abbreviations are: MPO, myeloperoxidase; PMN, polymorphonuclear; CL, chemiluminescence; $^1O_2$, excited singlet multiplicity molecular oxygen; X^-, either chloride, bromide, or iodide.

molecules in the microbicidal action of the MPO-X^--HOOH system has been presented, and the role of particular halides and halide concentrations in this phenomenon has been investigated (7,8). Because the microbicidal pH optimum has been established, the association of chemiluminescence and pH was investigated in an attempt to clarify the mechanism of MPO-X^--HOOH-mediated electronic excitation and its relationship to the antimicrobial activity of this system.

II. MATERIALS AND METHODS

Human PMN leukocytes were obtained from peripheral blood by venipuncture. The PMN leukocytes were then harvested and purified, and myeloperoxidase was extracted as previously described (7,8). Peroxidase activity was assayed using the o-dianizidine method described by Klebanoff (6).

Chemiluminescence was monitored by photomultiplication methods using a Packard Scintillation Spectrometer Model 3320 operated in the out-of-coincidence mode. Measurement of CL by this method reflected the disintegration of electronically excited molecules, and did not involve the use of radioactive materials or phosphors (8,9).

The desired concentration of halide and enzyme was added to the counting vials and the system was adjusted to the desired pH using 0.1 M acetate buffer or 0.1 M phosphate buffer. After allowing for mixing (10 - 15 minutes), the reaction was initiated by addition of HOOH using a microliter syringe.

All chemicals were of reagent grade and water was glass-distilled. A Beckman Zeromatic pH meter was used for pH (H^+ activity) determinations.

III. RESULTS

Figure 1 represents the temporal traces of CL obtained

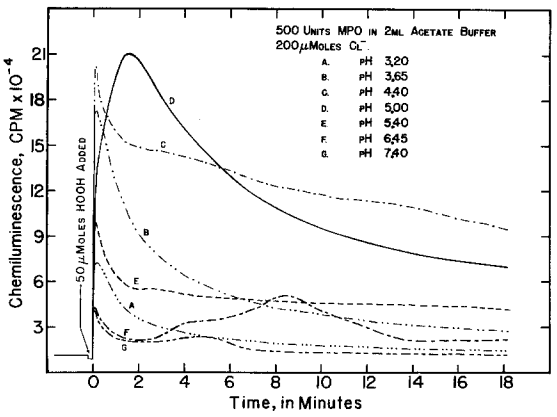


Figure 1. Comparison of continuous temporal traces of chemiluminescent intensity from the MPO-Cl^--HOOH system over a range of pH, 3.2 to 7.4.

from the MPO-Cl^--HOOH microbicidal system over a range of pH from 3.2 to 7.4. The maximum CL activity correlated with the established pH for microbicidal activity, 4.4 to 5.0 (6). Note that each velocity curve has a characteristic pattern for the particular pH, and that change in shape is dependent on change in pH. This kinetic parameter would be missed if only numerical integrals of CL activities were presented. This is especially apparent in Figure 2, where the MPO-Br^--HOOH system was investigated. Figure 3 represents the pattern of temporal traces of CL from the MPO-I^--HOOH system.

The various temporal traces of CL activities obtained at different H^+ concentrations bear a striking resemblance to the temporal traces of CL obtained when the pH is held constant and the concentrations of the different halides are varied (7). These similarities most probably reflect a Nernst type relationship between the H^+ activity and halide activity, and therefore express the electrochemical parameters involved in halide oxidation.

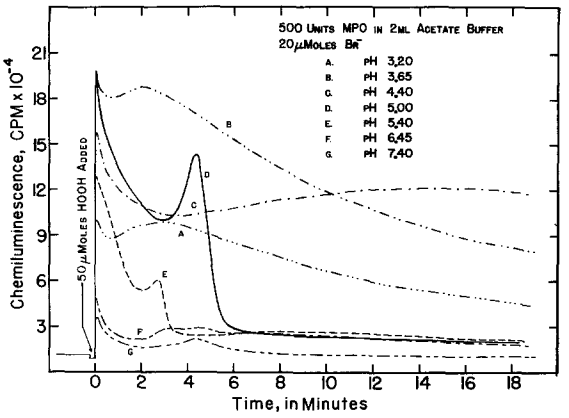


Figure 2. Comparison of continuous temporal traces of chemiluminescent intensity from the MPO-Br^--HOOH system over a range of pH, 3.2 to 7.4.

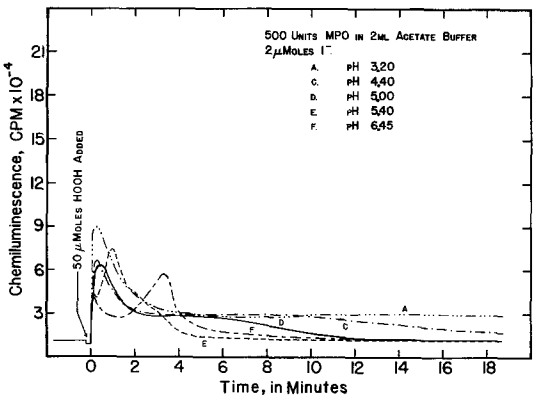


Figure 3. Comparison of continuous temporal traces of chemiluminescent intensity from the MPO-I^--HOOH system over a range of pH, 3.2 to 7.4.

In order to compare more directly the relationship between pH and halide, a plot of pH against integral of CL for the various halides is given in Figure 4.

IV. DISCUSSION

The antimicrobial activity of the PMN leukocyte is linked to metabolic alterations involving the mobilization of reducing potential, increased nonmitochondrial consumption of O_2, and

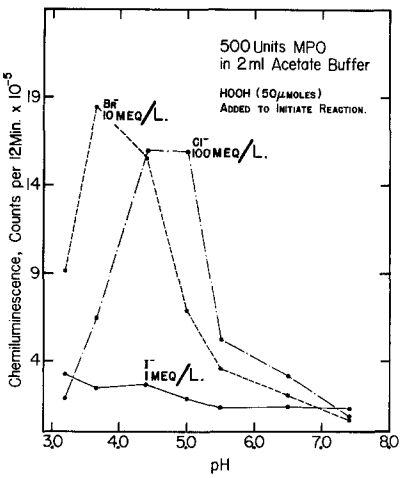


Figure 4. Plot of pH against integral of chemiluminescence obtained from the various MPO-X^--HOOH systems.

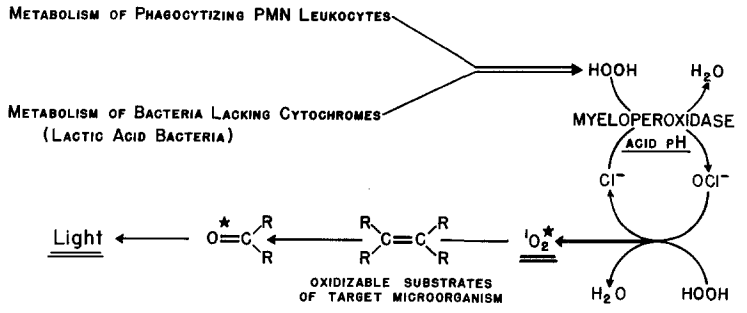
generation of HOOH (10,11,12). This "respiratory burst" can be correlated with the morphological phenomenon of "degranulation." In the degranulation process there is fusion of the primary and secondary granules of the PMN leukocyte with a vacuole containing the phagocytized organism (4). A temporal relationship has been established between the degranulation process and the pH of the phagocytic vacuole. The phagolysosome has been reported to become acid within 4 minutes after phagocytosis (3). This increase in H^+ concentration is consistent with the acid requirements of the enzymatic components of the primary granules.

Myeloperoxidase requires HOOH, a halide cofactor, and a pH of approximately 5.0 for microbicidal activity, and it has been suggested that this activity is linked with the oxidation of the halide. In 1958 Agner reported that MPO catalyzed the peroxidative oxidation of Cl^-, and further reported the necessity for Cl^- as a cofactor in certain MPO-mediated oxidations (13). More recently the MPO-Cl^--HOOH system has been reported to catalyze the deamination and decarboxylation of amino acids,

and it has been proposed that the Cl^- cofactor is oxidized to the OCl^-, capable of reacting with amino acids to form chloramines (14,15). These chloramines can decompose to yield NH_3, CO_2, Cl^-, and aldehyde. The resulting aldehyde has been proposed as an antimicrobial agent (16); however, when exogenous amino acids are added to the system, a competitive inhibition of killing resulted (17).

Separation of MPO from the target bacteria by a dialysis membrane abolished microbicidal activity, but added OCl^- freely traversed the membrane to kill the organism (12). If OCl^- is produced from Cl^- oxidation by HOOH, and if OCl^- further reacts with a second HOOH to generate a microbicidal species of short half-life, then these apparent discrepancies can be reconciled. Singlet multiplicity molecular oxygen ($^1O_2$) is generated in numerous reactions involving oxidized chloride (and bromide) derivatives and HOOH (18). The participation of $^1O_2$ in microbicidal activity thus explains the impossibility of chemical isolation of the microbicidal factor. This electronically excited species has a half-life dependent on the physical environment in which it is generated, and if substrate oxidation does not occur, $^1O_2$ will relax to its ground state, triplet multiplicity molecular oxygen (O_2).

The production of $^1O_2$ by the MPO-X^--HOOH system also explains the relationship between microbicidal activity of the PMN leukocyte and the generation of CL. The antimicrobial potential of the generated $^1O_2$ is related to its reactivity. The $^1O_2$ species is a good electrophile capable of reacting with regions of high electron density on the substrate molecule. The $n\pi^*$ electronically excited carbonyl products of such $^1O_2$-mediated oxidation can relax to ground state by emitting



MECHANISM OF MICROBICIDAL ACTION OF THE MYELOPEROXIDASE-Cl^--HOOH SYSTEM.

Figure 5. Schematic representation of the proposed mechanism of MPO-Cl^--HOOH antimicrobial action.

photons. Figure 5 is a schematic description of the proposed mechanism of MPO activity.

In summary, the microbicidal function of the MPO-X^--HOOH system is considered to revolve around the oxidation of a halide cofactor. This oxidation is dependent on electrochemical parameters such as halide concentration (activity) and pH (H^+ activity). Modification of either parameter will result in differences in ease of oxidizability for the particular halide in question. Once oxidized, the halide can either react directly with the substrate, as is the case with I^-, or may further react with a second molecule of HOOH to generate $^1O_2$.

Recent research by Klebanoff (19) and Krinsky (20) further supports the proposition that $^1O_2$ is involved in the antimicrobial activity of the PMN leukocyte.

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