

Candidacidal Activity of Myeloperoxidase: Mechanisms of Inhibitory Influence of Soluble Cell Wall Mannan

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We have previously demonstrated the ability of human neutrophil myeloperoxidase to bind to mannan isolated from *Candida albicans*. Mannan may therefore be a primary component of the yeast cell wall which provides for binding of myeloperoxidase, a requirement potentially important for the candidacidal activity of the enzyme. In this report, we describe experiments to consider the relationship of the mannan-binding activity of myeloperoxidase to its candidacidal activity and the possibility that free mannan may inhibit myeloperoxidase-mediated candidacidal activity. We observed that binding of myeloperoxidase to the target yeasts was required for killing of *C. albicans*. We also observed that addition of soluble mannan significantly reduced myeloperoxidase-mediated killing of the yeasts in a dose-dependent manner by antagonizing binding of myeloperoxidase. Soluble mannan was demonstrated to have a similar dose-dependent inhibitory effect on neutrophil-mediated candidacidal activity without influencing phagocytosis of the organism. On the basis of these observations, we speculate that mannan solubilized in plasma and tissue fluid may interfere with neutrophil-mediated host defense against *Candida* infection.

Candida yeast infections are common in compromised hosts (3, 5, 15). Once established, the yeasts may be able to escape elimination by inhibiting host defense functions. This possibility is supported by observations that both cell-mediated and neutrophil-mediated fungicidal activities may be depressed in patients with candidiasis (4, 9, 12, 18). One yeast-derived factor inhibiting cell-mediated immune function has been characterized as mannan solubilized from yeast cell walls (9).

Because of the potential significance of yeast-mediated modulation of the host defense system we have tested the effect of mannan on human neutrophil functions involved in the cellular response to microbial infection (21). We found that mannan caused a selective inhibition of extracellular accumulation of the lysosomal enzyme myeloperoxidase after a phagocytic stimulus. We subsequently demonstrated that this phenomenon was related to the ability of the enzyme to bind to neutrophil-associated mannan. The ability of myeloperoxidase to bind to mannan suggests that mannan may be a primary component of the yeast cell wall providing for attachment of the enzyme to target yeasts. Such binding of myeloperoxidase to bacteria has been shown to increase myeloperoxidase-mediated bactericidal activity (11, 19).

We have investigated the role of the mannan-

binding activity of myeloperoxidase in the candidacidal activity of the enzyme (13). Our results demonstrate that this binding capacity provides for attachment of the enzyme to the surface of target yeasts to increase the fungicidal activity of the enzyme. In addition, our results demonstrate that soluble mannan is inhibitory for both extra- and intracellular killing of *Candida* yeasts (14). We propose that this effect of mannan is due to interference with the binding of the enzyme to the yeasts.

MATERIALS AND METHODS

Isolation of neutrophils. Blood was drawn from healthy individuals into heparinized syringes (10 U/ml), and neutrophils were isolated by the method of Ferrante and Thong (7). The cell suspension medium used throughout this study was Dulbecco phosphate-buffered saline (PBS) (GIBCO Laboratories, Grand Island, N.Y.).

Isolation of myeloperoxidase. Myeloperoxidase was isolated from isolated human neutrophils by the method of Andrews and Krinsky (1). The isolated enzyme had an R.Z. value of 0.62. Myeloperoxidase activity was quantitated by the method of Baggiolini et al. (2).

Isolation of mannan. Mannan was isolated from *Candida albicans* 2252 by the procedure of Peat et al. (17).

Assay of myeloperoxidase-mediated candidacidal activity. *C. albicans* 2252 was inoculated into yeast nitrogen base minimal medium (8) and incubated over-

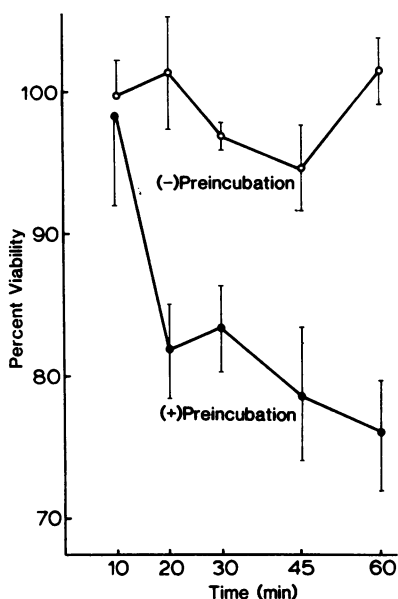


FIG. 1. Time course of myeloperoxidase-mediated fungicidal activity. The data are presented as percent viability of yeasts relative to the number of yeasts in a control inoculum (mean \pm standard deviation). Each point summarizes the results of six independent experiments, each conducted in duplicate. (-)Preincubation, Myeloperoxidase added to target yeasts simultaneously with hydrogen peroxide; (+)preincubation, myeloperoxidase preincubated with target yeasts at 4°C for 30 min before addition of hydrogen peroxide. ($P < 0.01$ for viability after 60 min of incubation.)

night at 37°C. The yeasts were then washed three times in sterile PBS. The yeast suspension was adjusted to a final optical density of 1.0 at 660 nm (absorbance value of 1.0 at 660 nm equals 2.0×10^7 yeast cells per ml) in PBS. A 0.1-ml sample of yeast cells was incubated with 1.1 mU of myeloperoxidase at 4°C for 30 min. After preincubation, hydrogen peroxide was added to give a concentration of 0.02 mM in a final reaction volume of 1.0 ml. All reactants were diluted in PBS to give a final chloride ion concentration of 0.14 M. The reaction mixture was incubated at 37°C on a rotating table. Myeloperoxidase-mediated killing was stopped by transferring the reaction tubes to ice water. Yeast viability was determined by pour-plate methodology, using yeast extract-peptone-glucose agar (8). Candidacidal activity was tested in the presence or absence of mannan. Control experiments verified that the observed candidacidal activity was generated by the myeloperoxidase-hydrogen peroxide-halide antimicrobial system (10). Myeloperoxidase and its two cofactors, hydrogen peroxide and chloride ion, were required for candidacidal activity of the enzyme. Inhibitors of myeloperoxidase catalytic activity (0.1 mM azide, 0.1 mM cyanide, and 1 mM L-methionine) effectively inhibited the killing of the yeasts.

Assay of neutrophil-mediated candidacidal activity. Overnight cultures of *C. albicans* 2252 were opsonized by incubation in freshly prepared human serum at 37°C

for 30 min (20). After three washes, the yeasts were suspended in PBS to a final optical density of 2.0 at 660 nm. A 0.5-ml inoculum of opsonized yeasts was incubated with 5×10^6 human neutrophils. The approximate 4-to-1 ratio of yeasts to neutrophils provides for optimal phagocytosis and killing of *Candida* yeasts (6). The reaction mixture was incubated at 37°C on a rotating table. Phagocytosis was stopped after 90 min by placing the reaction tubes in ice water. The mixture was then sonicated for 5 s to disrupt the neutrophils, releasing ingested yeasts to allow for assaying the viability of a combination of ingested and noningested yeasts. Candidacidal activity was compared in the presence or absence of solubilized yeast mannan. Inhibition of candidacidal activity by azide, cyanide, and catalase characterized neutrophil-mediated candidacidal activity as being dependent upon myeloperoxidase (10).

RESULTS

Candidacidal activity of yeast-bound and free myeloperoxidase. Data in Fig. 1 compare the candidacidal activity of yeast-bound and free myeloperoxidase. Yeasts were preincubated with or without myeloperoxidase for 30 min at 4°C. Hydrogen peroxide alone or myeloperoxidase and hydrogen peroxide were then added to the respective reaction mixtures. Concentrations of myeloperoxidase and its cofactors in these experiments were chosen such that candidacidal activity would be enzyme limited. The

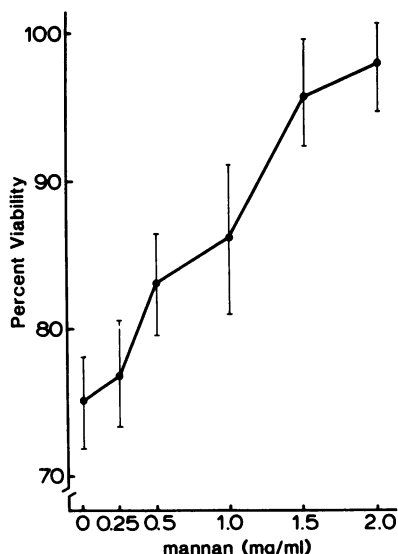


FIG. 2. Influence of *C. albicans* mannan on myeloperoxidase-mediated candidacidal activity. The data are presented as percent viability of *C. albicans* relative to the number of yeasts in a control inoculum (mean \pm standard deviation). Each point summarizes the results of six independent experiments, each conducted in duplicate. ($P < 0.01$ for percent viability with 2 mg versus 0 mg of mannan per ml.)

yeast suspensions were incubated at 37°C for an additional 10 to 60 min before plating. Preincubation with myeloperoxidase provided for 25% killing of target yeasts after a 60-min incubation with hydrogen peroxide. In contrast, simultaneous addition of myeloperoxidase and hydrogen peroxide resulted in no killing of yeasts over the 60-min incubation period. These results suggest that killing of *Candida* yeasts by myeloperoxidase-mediated mechanisms is most effective when the enzyme is adsorbed to the target yeasts.

Influence of soluble mannan on myeloperoxidase-mediated candidacidal activity. On the basis of our observation that myeloperoxidase binds to mannan without affecting the catalytic activity of the enzyme (21), we next considered the possibility that mannan isolated from *Candida* yeast might inhibit myeloperoxidase-mediated candidacidal activity by interfering with binding of the enzyme to target yeasts. To test this possibility, we preincubated yeasts with myeloperoxidase in the presence of various amounts of soluble mannan, before addition of hydrogen peroxide. Results summarized in Fig. 2 demonstrate a dose-dependent inhibitory influence of soluble mannan on myeloperoxidase-mediated candidacidal activity. Under the assay conditions, 0.5 mg of mannan per ml reduced killing from 24 to 15% and 2.0 mg of mannan per ml totally inhibited killing of the yeasts ($P < 0.01$).

Influence of solubilized mannan on binding of myeloperoxidase to *C. albicans*. On the basis of our observation that myeloperoxidase must be bound to target yeasts to provide for effective killing of the organisms, we considered whether

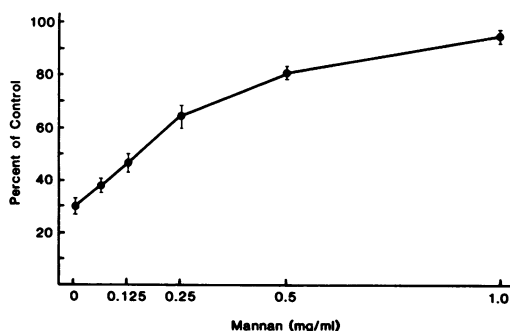


FIG. 3. Influence of solubilized *C. albicans* mannan on myeloperoxidase-yeast interaction. The data are presented as percentage of myeloperoxidase activity present in supernatant medium relative to the amount added to the reaction mixture (mean \pm standard deviation). Each point summarizes the results of a minimum of two independent experiments, each conducted in duplicate. ($P < 0.02$ for amount of myeloperoxidase activity in supernatant medium with 2 mg versus 0 mg of mannan per ml.)

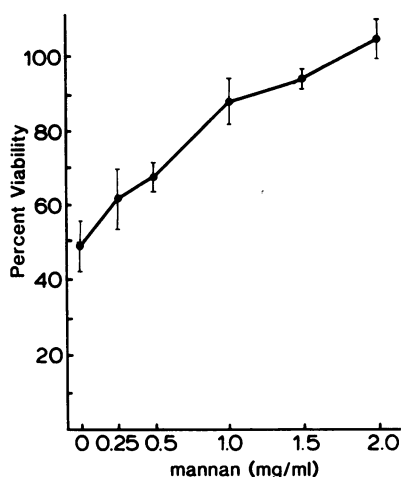


FIG. 4. Influence of *C. albicans* mannan on neutrophil-mediated candidacidal activity. Data are represented as percent viability of *C. albicans* related to the number of yeasts in a control inoculum (mean \pm standard deviation). Each point summarizes the results of three experiments, each conducted in duplicate. ($P < 0.01$ for percent viability at 2 mg versus 0 mg of mannan per ml.)

solubilized mannan might prevent binding of the enzyme to target yeasts. Binding of enzyme to yeasts was assessed indirectly by measurement of myeloperoxidase remaining free in solution after incubation with yeasts in the presence or absence of soluble mannan. Data summarized in Fig. 3 illustrate an inhibitory effect of solubilized mannan on binding of myeloperoxidase to the yeasts. In the absence of mannan, 30% of the enzyme became yeast bound. Mannan at concentrations ≥ 1 mg/ml completely inhibited binding of the enzyme to yeasts.

Effect of mannan on neutrophil-mediated candidacidal activity. Data in Fig. 4 illustrate that mannan has a dose-dependent inhibitory effect on neutrophil-mediated candidacidal activity. In the absence of mannan, neutrophils caused an average 50% decrease in yeast viability. In the presence of 1 mg of mannan per ml, the viability of target yeasts was increased to 94%. We have previously reported that mannan does not impair phagocytosis of serum-opsonized yeasts (21).

DISCUSSION

Experiments described in this report demonstrate that binding of myeloperoxidase to *Candida* yeasts is essential for enzyme-mediated fungicidal activity. They also demonstrate that cell wall mannan polysaccharide isolated from *C. albicans* can inhibit killing of *Candida* yeasts by both isolated myeloperoxidase and neutrophils. We suggest that this phenomenon may be due to

mannan-mediated inhibition of binding of myeloperoxidase to the target yeasts.

A greater killing influence of target-bound myeloperoxidase versus free enzyme has been previously demonstrated with *Escherichia coli* as the target microorganism (19), but this phenomenon has not been demonstrated for *Candida* yeasts. That bound enzyme would be more effective than free enzyme in killing microorganisms is not surprising. Binding of myeloperoxidase to microorganisms would result in generation of hypochlorous acid in close physical proximity to target components of the cells, increasing the possibility that components of the microorganism would become substrate for this highly reactive product of myeloperoxidase activity.

The mechanism of binding of myeloperoxidase to *Candida* yeasts appears to involve interaction of the enzyme with mannan, a polysaccharide representing 30 to 50% of the dry weight of the fungal cell wall (16). This interaction is evidenced by the ability of myeloperoxidase to bind to isolated mannan (21), most likely involving electrostatic interaction between the cationic enzyme and the anionic polysaccharide (C. D. Wright, G. R. Gray, and R. D. Nelson, Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, D74, p. 59). Soluble mannan may therefore interfere with myeloperoxidase-mediated killing of *Candida* yeasts by antagonizing binding of the enzyme to the intact yeasts. Arguments that inhibition of myeloperoxidase-mediated killing of the yeasts by mannan is due to inhibition of the catalytic activity of the enzyme or to scavenging of the catalytic products of the enzyme are not valid based upon our observation that mannan does not interfere with measurement of myeloperoxidase activity (21). The unimpaired catalytic activity of myeloperoxidase bound to mannan would also be expected since bound enzyme effectively killed the yeasts in these experiments. Whether soluble mannan added to suspensions of neutrophils and yeasts inhibits cell-mediated fungicidal activity by a similar mechanism is not certain at this time. However, it is likely that the mechanism does involve myeloperoxidase, because of the known contribution of lysosomal myeloperoxidase to neutrophil-mediated killing of *Candida* yeasts (13, 14).

Of what significance are these observations to understanding the pathogenesis of chronic infection involving *Candida* yeasts? It is tempting to speculate that mannan may interfere with both extra- and intracellular killing of yeasts in vivo. One could argue that the concentration of soluble mannan in patient plasma is not sufficient to produce this effect. Mannan concentrations in patient serum have been measured at over 100 µg/ml (9). However, since such levels of plasma

mannan cannot accurately reflect the levels of mannan present in fluids at sites of fungal invasion, it seems reasonable that mannan may accumulate to concentrations sufficient to inhibit killing of *Candida* yeasts at the local, if not at the systemic, level.

ACKNOWLEDGMENTS

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