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ENZYMATIC INACTIVATION OF HUMAN ALPHA-1-PROTEINASE INHIBITOR BY NEUTROPHIL MYELOPEROXIDASE

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Summary: Human myeloperoxidase, in the presence of H_2O_2 and halide ion, can catalytically inactivate human alpha-1-proteinase inhibitor (α-1-PI), the major plasma inhibitor of elastolytic activity. The rate of inactivation is directly proportional to both the myeloperoxidase and α-1-PI concentrations and inversely proportional to the H_2O_2 concentration. Amino acid analysis of the oxidized α-1-PI reveals that the only modified amino acid is methionine, which is converted to the sulfoxide form during the course of the reaction. Significantly, this system has no effect on either α-2-macroglobulin (α_2M) or α-1-antichymotrypsin (α-1-Achy), two other important plasma proteinase inhibitors, nor can the system be replaced with horseradish peroxidase. Since it has been shown recently that methionine occupies part of the reactive site of α-1-PI, it is possible that the release of myeloperoxidase by leukocytes during phagocytosis inactivates this inhibitor through oxidation of this particular residue, thereby indirectly augmenting the proteolytic activity released by these same cells.

The development of pulmonary emphysema is currently believed to be due to uncontrolled proteolysis of lung tissue in the absence of sufficient quantities of endogenous proteinase inhibitors. This theory originally arose from the discovery that a) a genetic deficiency in plasma levels of α-1-proteinase inhibitor could be correlated with the early appearance of the disease (1,2) and b) emphysematous-like lesions could be produced in animal lungs by endotracheal administration of papain (3). The source of the lung-degrading proteinases appears to be polymorphonuclear leukocytes, specifically neutrophils, which become sequestered in the lung, particularly in individuals exposed to pollutants (e.g., cigarette smoke) (4,5). Proteinases from these cells are released by either cell death or alternatively by cell leakage during phagocytosis. A second potential source of proteinases is believed to be from alveolar macrophages (6) which also accumulate in large numbers in

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the lung. However, the evidence available indicates that they can only secrete trace quantities of proteolytic activity over a prolonged period in cell culture (7).

It is important to note that, although a genetic defect in α-1-PI production may result in the development of emphysema, this disease occurs frequently in individuals with apparently normal inhibitor levels. For this reason we have been investigating the structure and function of α-1-PI in order to better understand its physiological role in regulating tissue proteolysis. We have recently found that a methionine residue forms an integral part of the reactive site of α-1-PI (8) and that chemical oxidation of the inhibitor results in the modification of this residue and the concomitant inactivation of α-1-PI towards leukocyte elastase (9). The sensitivity of α-1-PI towards chemical oxidants led us to a detailed investigation of potential biological oxidants which might be responsible for the inactivation of this inibitor, thereby resulting in a lowered inhibitory capacity in the lung similar to the genetic abnormality. We have found that neutrophil myeloperoxidase can catalytically inactivate α-1-PI in the presence of H_2O_2 and chloride ion. This enzyme is present in large quantities in neutrophils and has been classically associated with the bactericidal activity of these cells during phagocytosis (10,11). However, like the proteinases in neutrophils, myeloperoxidase can be readily released into the tissues where the oxidative effect described below could indirectly result in enhancement of the proteolytic degradation of lung tissue, even in individuals with genetically normal levels of α-1-PI.

Materials and Methods

Human α-1-PI (12), α-1-antichymotrypsin (α-1-Achy) (13), leukocyte elastase (14), leukocyte cathepsin G (13), and α-2-macroglobulin (α_2M) (15) were prepared and assayed by methods developed in this laboratory. Human leukocyte myeloperoxidase was purified to homogeneity by chromatography on CM-cellulose of leukocyte granule extracts (P. Wong, N.R. Matheson, and J. Travis, manuscript

in preparation). Bovine trypsin, bovine α-chymotrypsin, porcine trypsin, and porcine elastase were obtained from Worthington Biochemicals.

Amino acid analysis of native and oxidized proteins was performed by acid hydrolysis followed by separation and quantitation on a Beckman Model 119CL amino acid analyzer. Methionine and tryptophan were analyzed separately, the former after cyanogen bromide treatment and acid hydrolysis (16) and the latter by differences in fluorescence after excitation at 290 nm and emission at 340 nm

Results and Discussion

The effect of human neutrophil myeloperoxidase, in conjunction with H_2O_2 and Cl^-, on the inhibitory acitivity of α-1-PI was determined as described in

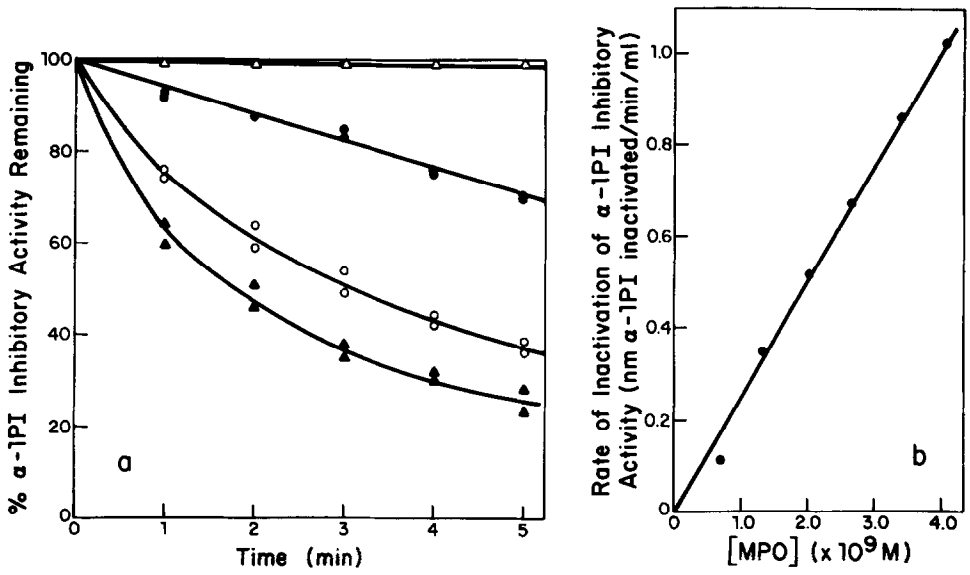

Figure 1. Dependence of the inactivation of α-1-PI by myeloperoxidase on time and myeloperoxidase concentration. Myeloperoxidase (10^{-9} M) was incubated at room temperature with α-1-PI (2.68×10^{-6} M) in the presence of 3.74×10^{-4} M H_2O_2, 0.16M NaCl, and 0.2M sodium phosphate, pH 6.1. At one min intervals an aliquot was removed and added to a solution of catalase (final concentration 3.6×10^{-7} M) which was sufficient to destroy the H_2O_2 in seconds and thus stop the reaction. To each sample porcine pancreatic elastase was added (final concentration 1.75×10^{-6} M) and the mixture incubated for three min (final concentration of α-1-PI 1.61×10^{-6} M). The residual elastase activity was then determined by spectrophotometric assay (15). The loss of α-1-PI inhibitory activity toward elastase was determined by comparison to controls of elastase alone and of elastase and native α-1-PI. a), Percent loss of α-1-PI inhibitory activity with time: ●, 0.68×10^{-9} M myeloperoxidase; ○, 2.70×10^{-9} M myeloperoxidase; ▲, 4.06×10^{-9} M myeloperoxidase; △, 4.06×10^{-9} M myeloperoxidase, no H_2O_2 or 3.74×10^{-4} M H_2O_2, no myeloperoxidase, or 9.04×10^{-7} M horseradish peroxidase. b), Dependence of rate of inactivation of α-1-PI on myeloperoxidase concentration.

the legend to Figure 1. The loss of inhibitory activity proceeded with time (Figure 1A) and required all three added components. Elimination of Cl^- reduced the rate of inactivation to approximately one-twentieth of that found in the presence of 0.16M NaCl (not shown) while replacement of myeloperoxidase with horseradish peroxidase caused no inhibition of α-1-PI at all. Finally, as shown in Figure 1B, the rate of loss of inhibitory activity was dependent on the concentration of myeloperoxidase.

Myeloperoxidase, with H_2O_2 and Cl^-, was also able to catalyze the inactivation of human leukocyte cathepsin G (13), bovine pancreatic trypsin, and porcine pancreatic trypsin. The relative rates of inactivation of various proteinases and proteinase inhibitors by this enzyme system is illustrated in Table 1. The rate of loss of α-1-PI activity is approximately 18-fold faster than the other proteinases affected. Significantly, several enzymes,

TABLE 1: RELATIVE EFFECT OF MYELOPEROXIDASE ON VARIOUS PROTEINASES AND PROTEINASE INHIBITORS

Protein	moles inactivated per min per mole myeloperoxidase
Human Alpha-1-Proteinase Inhibitor	254
Porcine Pancreatic Trypsin	16
Human Neutrophil Cathepsin G	14
Bovine Pancreatic Trypsin	14
Human Neutrophil Elastase	0
Porcine Pancreatic Elastase	0
Bovine Pancreatic Chymotrypsin	0
Human Alpha-1-Antichymotrypsin	0
Human Alpha-2-Macroglobulin	0

Myeloperoxidase (1.0×10^{-8}M) was incubated with each protein (2.0×10^{-6}M) at room temperature and the mixture treated subsequently as described in Figure 1. The α-1-antichymotrypsin activity was measured against bovine α-chymotrypsin (13) and α-2-macroglobulin with porcine pancreatic trypsin (15).

TABLE 2: EFFECT OF MYELOPEROXIDASE ON THE METHIONINE AND TRYPTOPHAN RESIDUES OF α-1-PI, CATHEPSIN G, AND TRYPSIN

Protein	Methionine	Tryptophan
Native α-1-PI	7	2
Oxidized α-1-PI	5	2
Native Cathepsin G	4	2
Oxidized Cathepsin G	4	0
Native Trypsin	2	4
Oxidized Trypsin	2	1

α-1-PI, cathepsin G, and trypsin were completely inactivated by myeloperoxidase using the conditions described in Figure 1. The myeloperoxidase was separated from α-1-PI by chromatography on CM-cellulose at pH 6.1 in 0.2M sodium phosphate, in which case the inhibitor passed directly through the column. Oxidized cathepsin G and oxidized trypsin were purified by passage through a column of sepharose-trasylol at pH 8.0 in 0.05M Tris-HCl, 1.0M NaCl, in which case the myeloperoxidase was unbound. The modified proteinases were then eluted with 0.05M sodium acetate buffer, pH 4.2, 1.0M NaCl (14).

including leukocyte elastase (15), as well as several proteinase inhibitors were not affected by myeloperoxidase treatment.

In order to determine which amino acids may have been modified during the inactivation of α-1-PI, cathepsin G, bovine trypsin, and porcine trypsin, amino acid analysis was performed on each protein before and after myeloperoxidase treatment. Only methionine residues were oxidized in α-1-PI (Table 2) with no loss in other oxidizable amino acids (tryptophan, histidine, and tyrosine). In contrast, only tryptophan residues were destroyed in the proteolytic enzymes examined. Studies with a chemical oxidant specific for methionine, N-chlorosuccinimide, have indicated that only two (of a total of seven) methionyl residues in α-1-PI are oxidized, including the reactive site methionine (8,9) with a parallel loss in inhibitory activity as oxidation of these residues proceeds. We believe that enzymatic oxidation of α-1-PI by myeloperoxidase most likely destroys the reactive site methionine as well. This assumption is based on the indirect evidence given in Figure 2 which exactly parallels the chemical oxidation data (9). The results given here show that short-time

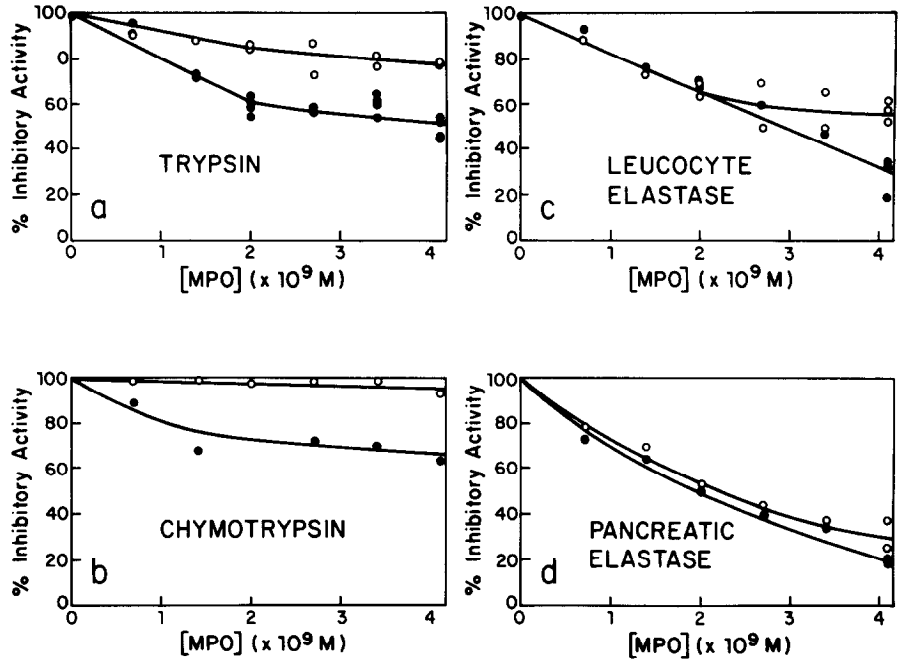

Figure 2. Effect of time of preincubation of myeloperoxidase treated α-1-PI with various proteinases. α-1-PI (final concentration 1.61 x 10^{-6}M) was incubated with myeloperoxidase for five min using the conditions described in Figure 1. The oxidized inhibitor was then mixed with either bovine pancreatic trypsin (1.66 x 10^{-6}M), bovine pancreatic chymotrypsin (1.71 x 10^{-6}M), human neutrophil elastase (1.75 x 10^{-6}M), or porcine pancreatic elastase (1.75 x 10^{-6}M). The proteinase was then assayed with an appropriate substrate. ●, 15 second preincubation; 0, 30 min preincubation.

preincubation of oxidized α-1-PI with trypsin, chymotrypsin, pancreatic elastase or leukocyte elastase (15 sec) yields a decrease in inhibitory activity compared to controls. However, after 30 min preincubation, substantial inhibitory activity towards trypsin and chymotrypsin is noted. With the leukocyte enzyme a slight increase in inhibitory activity can be seen but little effect occurs with the porcine pancreatic elastase preparation. All of these interactions of enzymatically oxidized α-1-PI with proteinases are similar to those found when N-chlorosuccinimide oxidized α-1-PI was incubated under identical conditions with the same enzymes (9), indicating that myeloperoxidase is probably oxidizing the reactive site methionine of this inhibitor. Curiously, with either chemical or biological oxidation two methionyl residues are oxidized. As yet, we have not been able to identify the location of the

second methionine, although we believe that it is very near to the reactive site methionine described elsewhere (8).

In summary, we have found that myeloperoxidase, a major enzyme present in human neutrophils, can inactivate human α-1-PI under in vitro conditions. These results suggest an alternative oxidative mechanism for the depletion of α-1-PI in tissues of individuals with genetically normal levels of this inhibitor in their plasma. Since neutrophils contain both the proteolytic enzymes and myeloperoxidase, the sequestering of these cells in the lung vasculature due to the presence of foreign materials could not only enhance the oxidation of a primary defense component of the lung but also lead to increased proteolysis of lung tissue and the development of pulmonary emphysema. The role of macrophages in the onset of lung disease is currently unknown. They can elicit elastolytic enzymes (7) in culture but the importance of this process cannot as yet be assessed. With regard to the production of oxidases there is no doubt that this does occur, although there is some debate whether an enzyme equivalent to myeloperoxidase exists in these cells in the resting versus the activated state (17). We are currently examining macrophage extracts in order to determine whether their role in the development of emphysema may, like the neutrophils, be a "biological accident" in which the phagocytic process inadvertently results in the inactivation of proteinase inhibitors, leaving lung tissue without sufficient defense against degradation by phagocytic proteinases from these same cells. Preliminary experiments suggest that macrophages, indeed, have this capability to inactivate α-1-PI.

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