Interaction of Human α-1-Proteinase Inhibitor with Neutrophil Myeloperoxidase†

N. R. Matheson, P. S. Wong, M. Schuyler, and J. Travis*

ABSTRACT: We have previously shown that α-1-proteinase inhibitor (α-1-PI) can be inactivated by myeloperoxidase in the presence of hydrogen peroxide and chloride ion [Matheson, N. R., Wong, P. S., & Travis, J. (1979) *Biochem. Biophys. Res. Commun. 88*, 402–409]. Further studies given here demonstrate that there is a direct dependence on the concentration of α-1-PI, which becomes saturating at a concentration of about 4.5 μM. There is also a dependence on the concentration of H_2O_2 to 95 μM, after which increasing concentrations become increasingly inhibitory. Chloride ion is required for myeloperoxidase oxidative action, but the cations Na^+, NH_4^+, or K^+ have little effect. $MgCl_2$ or $CaCl_2$ is twice as active as NaCl while other anions are ineffective. There is a very sharp pH optimum at pH 6.2, under physiological NaCl concentration, and approximately half the rate of inactivation of α-1-PI occurs at pH 5.9 or 6.5. At a higher concentration of NaCl (0.64 M), the optimum shifts slightly to pH 6.5. Sodium dodecyl sulfate gel electrophoresis indicates that α-1-PI oxidized by either myeloperoxidase or *N*-chlorosuccinimide is not different in size from native α-1-PI. When oxidized α-1-PI is incubated with porcine elastase, the α-1-PI is converted to a modified form of lower molecular weight. Amino acid sequence analysis confirms these results in that the sequence is different from that of native α-1-PI. Furthermore, the data clearly show that the two methionyl residues which are oxidized by chemical or enzymatic methods are eight residues apart and that the reactive-site methionine is one of these two. Thus, it is apparent that oxidative processes directly affect the inhibitory activity of α-1-PI by modification of the reactive-site methionine of this protein.

The development of pulmonary emphysema is currently believed to occur by uncontrolled proteolysis of lung tissue due to insufficient levels of plasma proteinase inhibitors (Laurell & Eriksson, 1963). The lung-degrading proteinases appear to be released, either by cell death or by cell leakage during phagocytosis, from polymorphonuclear leukocytes (specifically, neutrophils) sequestered in the lungs (Henson, 1972; Weissman et al., 1972).

Pulmonary emphysema occurs frequently in individuals with a genetic deficiency in the plasma levels of α-1-proteinase inhibitor (α-1-PI),[1] the inhibitor usually present in highest concentrations in plasma (about 130 mg/100 mL of plasma) (Heimburger et al., 1971). However, the disease also occurs in individuals with apparently normal levels of α-1-PI. For this reason, we have been investigating the structure and function of human α-1-PI in order to better understand its physiological role in regulating tissue proteolysis. We have recently discovered that the enzyme myeloperoxidase, present in very high concentrations in neutrophils, is able to cause the oxidation of two methionyl residues in α-1-PI, in the presence of hydrogen peroxide and chloride ion, thereby inactivating the inhibitor (Matheson et al., 1979). The oxidative inactivation of α-1-PI by myeloperoxidase has also been recently shown by others (Carp & Janoff, 1980). Since myeloperoxidase may be readily released from neutrophils, as is the case for proteinases, during phagocytosis or cell death, its oxidative inactivation of α-1-PI may indirectly result in enhancement of proteolytic destruction of lung tissue, even in individuals with genetically normal levels of inhibitor.

The aim of the present investigation was to further study the parameters of the myeloperoxidase-mediated oxidative inactivation of α-1-PI. We have also determined the location of both oxidized methionine residues in the α-1-PI molecule and examined the fate of the oxidized protein after either SucNCl oxidation (Johnson & Travis, 1979) or myeloperoxidase oxidation, during interaction with both pancreatic and neutrophil elastase.

Experimental Procedures

Materials

Outdated human plasma was obtained from the American Red Cross, Atlanta, GA. Human alveolar macrophages were obtained from the Veterans Administration Hospital, Cleveland, OH. The samples used contained between 1% and 3% polymorphonuclear leukocytes as contaminants. Human α-1-PI and human leukocyte elastase were prepared as previously described (Pannell et al., 1974; Baugh & Travis, 1976). Human leukocyte myeloperoxidase was purified to homogeneity as described in the preceding paper (Matheson et al., 1980). Porcine pancreatic elastase and *tert*-butoxycarbonyl-L-alanyl *p*-nitrophenyl ester were purchased from Sigma. SucNCl was obtained from Aldrich Chemical Co., and hydrogen peroxide (30%) was purchased from Baker Chemical Co.

Methods

Inactivation of α-1-PI by the Myeloperoxidase System. The inactivation reaction was performed as described previously (Matheson et al., 1979) except that the concentrations of each of the reaction mixture constituents were varied in order to study their effects on the reaction. The range of reactants used in the system was as follows: α-1-PI, 1.8–6.3 μM; hydrogen peroxide, 0.025–1.12 mM; NaCl, 0.08–1.28 M; pH 4.75–7.40. NaI concentration was varied from 1 to 20 mM. All other salts tested were in the same concentration range as for NaCl.

† From the Department of Biochemistry, University of Georgia, Athens, Georgia 30602, and the Veterans Administration Hospital, Cleveland, Ohio 44106 (M.S.). *Received April 29, 1980; revised manuscript received August 15, 1980.* This research was supported in part by Grant HL-14778 from the National Institutes of Health and a grant from The Council for Tobacco Research-U.S.A. N.R.M. is a holder of a Young Investigatorship in Pulmonary Disease (HL-24524).

[1] Abbreviations used: α-1-PI, α-1-proteinase inhibitor; SucNCl, *N*-chlorosuccinimide; $NaDodSO_4$, sodium dodecyl sulfate.

0006-2960/81/0420-0331$01.00/0 © 1981 American Chemical Society

In some cases, citrated plasma was used in place of, or in addition to, α-1-PI. The plasma was tested in its native state, after boiling of a 1:3 dilution in 0.1 M sodium phosphate buffer, pH 7.0, followed by centrifugation, or after dialysis against 2 L of the phosphate buffer.

In experiments in which only plasma or treated plasma was used, the reaction mixtures were incubated at 22 °C and contained in 0.5 mL 0.37 mM hydrogen peroxide, 0.16 M NaCl, 0.2 M sodium phosphate buffer, pH 6.1, 0.04 mL of plasma containing 1.41 μM α-1-PI, and 3.5–6.9 nM purified myeloperoxidase. Aliquots were removed at 1-min intervals, added to catalase to stop the reaction, and assayed for residual α-1-PI inhibitory activity (Matheson et al., 1979). Where plasma and purified α-1-PI were utilized together, the reaction mixtures were the same except that purified α-1-PI (2.12 μM) was added as well and the myeloperoxidase concentration was 6.9 nM. The residual inhibitory activity of α-1-PI for each concentration of variable (or pH) was determined at least four times. This residual activity was converted into the percent of original α-1-PI inhibitory activity remaining per mL and plotted vs. time (usually 1–5 min). An average slope for each concentration or pH was drawn, and the percent of original α-1-PI inhibitory activity per min per mL was taken from it. This was usually converted into nmol of α-1-PI inactivated per min per mL (the rate of inactivation of α-1-PI) and plotted vs. the appropriate variable (α-1-PI, hydrogen peroxide, or salt concentrations, or pH).

Assays for Myeloperoxidase Activity in Macrophages. Human alveolar macrophages, obtained as a suspension in Hanks balanced salt solution, were pelleted by centrifugation at 800*g* for 10 min. The cells were homogenized in 3.0 mL of 0.2 M sodium phosphate, pH 6.1 by using a Potter–Elvehjem glass homogenizer. The homogenate was centrifuged for 10 min at 800*g* and the supernatant retained. The pellet was subsequently resuspended in 3.0 mL of buffer.

In order for peroxidase activity to be tested, the reaction mixtures contained in 0.5 mL 2.68 μM α-1-PI, 0.37 mM hydrogen peroxide, 0.15 M NaCl or 5 mM NaI, 0.2 M sodium phosphate, pH 6.1, and 0.05 mL of supernatant or resuspended pellet obtained by macrophage extraction. At 1-min intervals, aliquots were removed and added to a solution of catalase to stop the reaction. The remaining α-1-PI inhibitory activity was then measured (Matheson et al., 1979).

Oxidation of α-1-PI and Treatment of Oxidized α-1-PI with Elastase for Sequence Analysis. Samples of 11 mg of α-1-PI (208.3 nmol) were enzymatically oxidized by incubation at room temperature with 4.08 μg of myeloperoxidase (0.35 nmol), 0.37 mM hydrogen peroxide (final concentration), and 0.16 M NaCl (final concentration), at pH 6.2, until inhibitory activity toward porcine elastase had disappeared. Porcine elastase activity was measured by the ability of the enzyme to hydrolyze *tert*-butoxycarbonyl-L-alanyl *p*-nitrophenyl ester at 347.5 nm (Baugh & Travis, 1976) in both the presence and the absence of added inhibitor or inhibitor–myeloperoxidase reaction mixture. Samples of 11 mg of α-1-PI were chemically oxidized by incubation at room temperature with SucNCl at a ratio of SucNCl to α-1-PI of 20:1, at pH 8.8, until all of the inhibitory activity had disappeared (Johnson & Travis, 1979). The samples were dialyzed exhaustively against water and incubated with either porcine pancreatic elastase (0.23 mg, 5.2 nmol) in 0.2 M Tris-HCl, pH 7.6, or leukocyte elastase (1.52 mg, 135.6 nmol) in 0.2 M sodium phosphate buffer, pH 6.5. After 12 h at room temperature, the reaction mixture was passed through a Sephadex G-75 column (1.8 × 94 cm) equilibrated with 0.2 M acetic acid in order to remove any peptides from the modified inhibitor. In each case, a single protein peak, immunologically reactive against antiserum to α-1-PI, was obtained, and this protein was dialyzed against water and lyophilized.

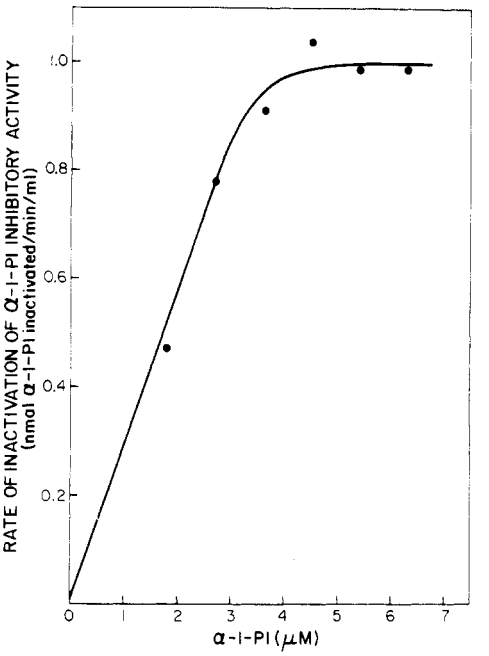

FIGURE 1: Effect of concentration of α-1-PI on the rate of inactivation by myeloperoxidase. α-1-PI (1.8–6.3 μM) was incubated at room temperature with 3.0 nM myeloperoxidase in the presence of 0.374 mM H_2O_2, 0.16 M NaCl, and 0.2 M sodium phosphate buffer, pH 6.1, and assayed as described previously (Matheson et al., 1979).

Sequence Analysis. The amino-terminal sequence of the modified inhibitor (6 mg each time) was performed as described previously (Johnson & Travis, 1979) and included the use of alkaline back-hydrolysis of the phenylthiohydantoins to determine whether methionine or methionine sulfoxide residues were present (Mendez & Lai, 1975).

Gel Electrophoresis. Native α-1-PI, α-1-PI complexes, oxidized α-1-PI, and modified α-1-PI were demonstrated by gel electrophoresis after treatment with 1% sodium dodecyl sulfate. Electrophoresis was run in an Ortec apparatus by using the procedure outlined in Ortec Life Science Note 13.

Results

Concentration Dependence of α-1-PI on Its Rate of Inactivation. The rate of loss of inhibitory activity of α-1-PI was found to depend on the initial concentration of this plasma protein. With increasing concentrations of α-1-PI, the rate increased linearly to about 4.5 μM under the conditions described in Figure 1. The rate then remained constant to 6.3 μM, the highest concentration examined. Concentrations of α-1-PI between 2.7 and 3.6 μM were utilized in most cases in the experiments described below.

Effect of Hydrogen Peroxide Concentration on the Rate of Inactivation of α-1-PI. At low concentrations of hydrogen peroxide (up to 95 μM), the rate of loss of α-1-PI inhibitory activity was found to increase linearly (Figure 2). However, as the concentration of hydrogen peroxide was further increased, the rate of inactivation progressively decreased. Apparently, at sufficiently high concentrations of hydrogen peroxide, the myeloperoxidase–H_2O_2–Cl^- reaction was inhibited. This effect has been previously noted (Zgliczynski et al., 1968).

When the NaCl concentration was lowered from 0.16 to 0.08 M, the optimum hydrogen peroxide concentration remained the same, but the rate of inactivation never became

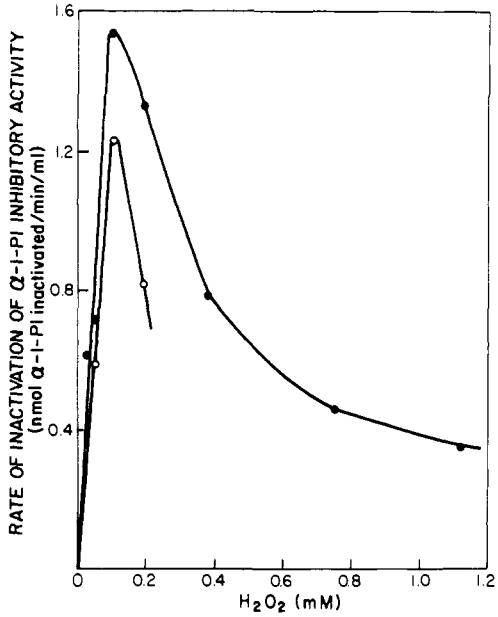

FIGURE 2: Effect of concentration of H_2O_2 on the rate of inactivation of α-1-PI by myeloperoxidase. α-1-PI (3.6 μM) was incubated with myeloperoxidase as described in the legend to Figure 1, except that the H_2O_2 concentration was varied from 0.025 to 1.12 mM. Rate of inactivation of α-1-PI with H_2O_2 concentration: (●) 0.16 M NaCl; (○) 0.08 M NaCl.

as rapid as with 0.16 M NaCl. This was contrary to the effect found by others (Sbarra et al., 1977), who reported that the rate of chlorination of diethanolamine by myeloperoxidase, hydrogen peroxide, and chloride ion increased with decreasing chloride concentration.

Effect of Chloride and Other Ions on Myeloperoxidase Inactivation of α-1-PI. The dependence of myeloperoxidase activity on the presence of halide ion, as noted by others (Zglicynski et al., 1968; Klebanoff, 1968), suggested that it would also be required for the inactivation of α-1-PI. Indeed, as shown in Figure 3, halide ion was required, and there was no loss of α-1-PI inhibitory activity in its absence. Furthermore, nonhalide salts such as Na_2SO_4 or magnesium acetate could not replace halides in the myeloperoxidase system. The counterions for halide salts, for the most part, had little effect. With either NaCl or NH_4Cl, essentially the same rate of inactivation of α-1-PI inhibitory activity was observed, with a maximum rate of 0.96 M salt. With KCl, the maximum was somewhat lower and broader while with KBr the maximum was somewhat higher but also at 0.96 M. As the concentration of salt was increased further, there was a decrease in the rate of inactivation of α-1-PI. The decrease was due to the chloride ion concentration and not due to ionic strength. When experiments were performed with 0.96 M NaCl and Na_2SO_4 in sufficient amounts to bring the ionic strength to that of 1.28 and 1.60 M NaCl, the rates of inactivation of α-1-PI were the same as if only 0.96 M NaCl was present.

A similar effect, inhibition by high salt concentration of the oxidation of cysteine to cysteic acid by the myeloperoxidase system, was observed by Zgliczynski et al. (1968). This group demonstrated that their pH optimum shifted to higher pH values as the NaCl concentration was increased. They concluded that the inhibition of this reaction at higher concentrations of chloride ion may have been due to alteration of the pH optimum. That explanation may have validity in this situation as well. At 0.16 M NaCl, the pH optimum for the inactivation of α-1-PI was 6.2. At 0.64 M NaCl, the pH optimum had shifted to pH 6.5. The effect of higher concentrations of NaCl on the pH optimum was not determined

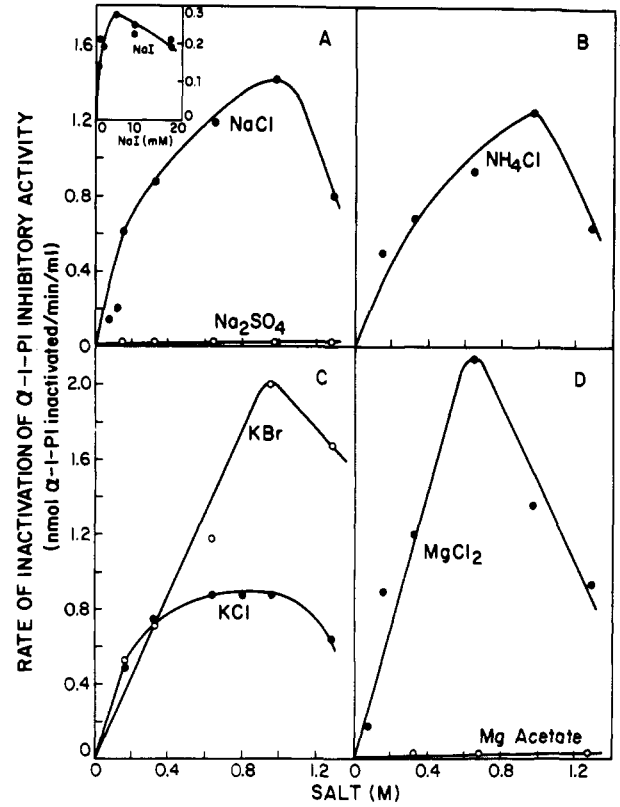

FIGURE 3: Effect of salts on the rate of inactivaton of α-1-PI by myeloperoxidase. α-1-PI (2.7 μM) was incubated with 3.0 nM myeloperoxidase in the presence of 0.374 nM H_2O_2, 0.2 M sodium phosphate, pH 6.1, and various salts. Concentration of NaI varied from 0 to 20 mM. Concentration of all other salts used varied from 0 to 1.28 M. (A) (●) NaCl; (○) Na_2SO_4; inset, NaI. (B) (●) NH_4Cl. (C) (●) KCl; (○) KBr. (D) (●) $MgCl_2$; (○) magnesium acetate.

in the present study but probably would have also caused the pH optimum to shift further. Thus, since the salt effect experiments were performed at pH 6.2, the optimum pH for the physiological salt concentration, perhaps the rate of inactivation of α-1-PI only appeared to decrease at a very high salt concentration.

Effect of pH on Myeloperoxidase-Mediated Inactivation of α-1-PI. The rate of inactivation of α-1-PI by myeloperoxidase was found to have a pH optimum of 6.2 in the presence of 0.16 M NaCl (Figure 4). However, within ±0.3 pH units of this value, the rate of inactivation was reduced by half. This pH optimum is relatively high when compared to that of pH 5.3 for the oxidation of α-amino acids (Zgliczynski et al., 1968). At higher concentrations (0.64 M), the optimum pH for α-1-PI inactivation was slightly shifted to pH 6.5, which agrees with the upward shift found for amino acid oxidation under the same conditions.

Effect of Enzymatic Oxidation of α-1-PI on Interaction with Elastolytic Enzymes. It has been previously shown that while native α-1-PI forms distinct sodium dodecyl sulfate stable complexes with elastolytic enzymes (Johnson & Travis, 1979) the chemically oxidized inhibitor complexes either very slowly (leukocyte elastase) or not at all (porcine elastase). As shown in Figure 5, the same effect occurs with myeloperoxidase-modified α-1-PI. In this experiment, it can be seen that both native (lane 1) and enzymatically oxidized α-1-PI (lane 3) have the same molecular weight (52 000), the only real difference being that the oxidized protein has two modified methionine residues (Matheson et al., 1979). However, when native inhibitor is incubated with either porcine elastase (lane 4) or leukocyte elastase (not shown), a sodium dodecyl suflfate stable complex is formed while with enzymatically oxidized inhibitor

Table I: Comparative Amino Acid Sequence at the Reactive Center of Native and Enzymatically Oxidized α-1-PI

	P_8 P_7 P_6 P_5 P_4 P_3 P_2 P_1	P_1' P_2' P_3' P_4' P_5' P_6'
α-1-PI (native)[a]	Leu-Glu-Ala-Ile-Pro-Met	Ser-Ile-Pro-Pro-Glu-Val
α-1-PI (oxidized)[b]	Met(O)-Phe-Leu-Glu-Ala-Ile-Pro-Met(O)	Ser-Ile-Pro-Pro-Glu-Val

[a] From papain digest of native α-1-PI (Johnson & Travis, 1978). [b] From elastase digest of oxidized α-1-PI. Repetitive yield from Ile(5)–Ile(9) was 96%.

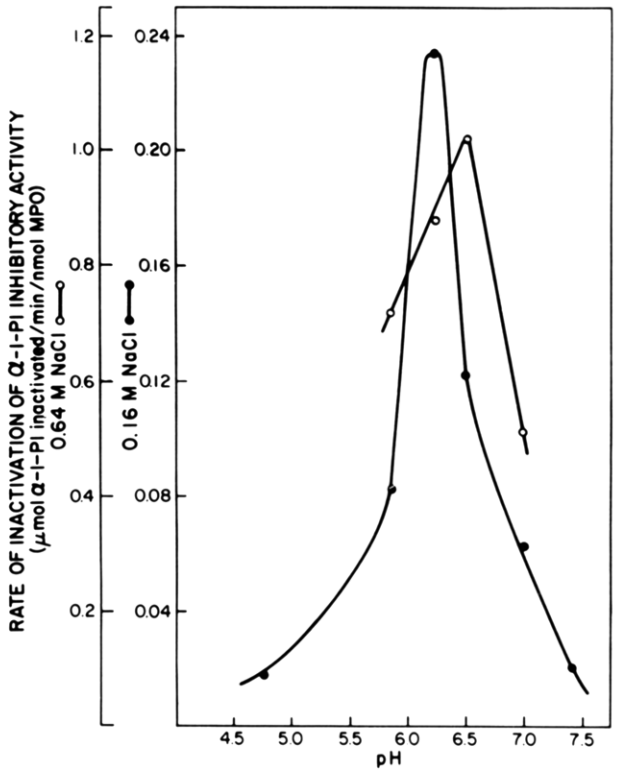

FIGURE 4: Effect on pH on the rate of inactivation of α-1-PI by myeloperoxidase. α-1-PI was incubated with myeloperoxidase as described in the legend of Figure 1, except that the pH was varied from 4.75 to 7.40 and the concentration of α-1-PI was 2.6 μM.

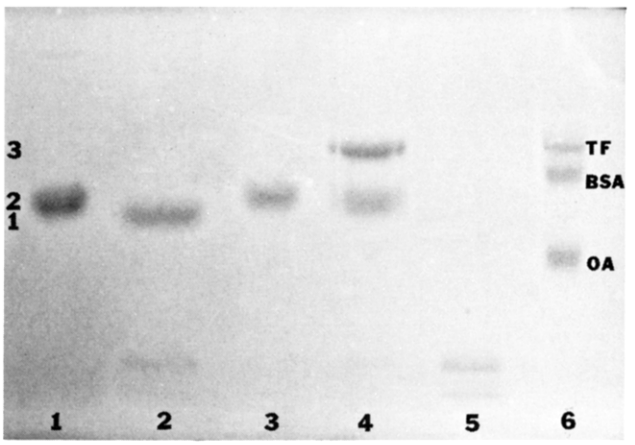

FIGURE 5: $NaDodSO_4$ gel electrophoresis of interaction products formed between native of oxidized α-1-PI and porcine elastase. Elastase (30 μg) was mixed with either native α-1-PI (40 μg) or oxidized α-1-PI (40 μg) which had been formed by treatment with either myeloperoxidase (2 μg) or a 20-fold excess of SucNCl, as described under methods. The mixtures were made in 1% in $NaDodSO_4$, boiled for 5 min, and subjected to gel electrophoresis. (1) Oxidized α-1-PI; (2) oxidized α-1-PI + porcine elastase; (3) native α-1-PI; (4) native α-1-PI + porcine elastase; (5) porcine elastase; (6) standard protein samples: transferrin (TF) 76 000; bovine serum albumin (BSA) 68 000; ovalbumin (OA) 43 000. The gel was stained with Coomassie Brilliant Blue G. Direction of migration is from top to bottom. (Band 1) Modified α-1-PI; (band 2) native or oxidized α-1-PI; (band 3) α-1-PI-porcine elastase complex.

conversion to a modified form (lane 2) of molecular weight 46 000 occurs. These results are in complete agreement with those reported previously for the chemically oxidized inhibitor (Johnson & Travis, 1979) and indicate that modification of the reactive-site methionine must have occurred by myeloperoxidase action.

Amino Acid Sequence Analysis of the Reactive Center of Chemically and Enzymatically Oxidized α-1-PI. In order to determine whether the reactive-site methionine of α-1-PI had been modified by either SucNCl or myeloperoxidase treatment, an examination of the amino-terminal sequence of the modified inhibitor, obtained after incubation of oxidized protein with porcine pancreatic elastase, was undertaken. The choice of enzymes utilized was made on the basis that chemically oxidized α-1-PI has no measurable association rate constant for porcine elastase (Beatty et al., 1980) while the leukocyte enzyme has a reduced k_{assoc} (1×10^4 vs. 3×10^7 for native inhibitor). Isolation of modified, oxidized inhibitor was readily attained by gel filtration, and the major protein peak obtained appeared homogeneous by sodium dodecyl sulfate gel electrophoresis.

The results obtained are given in Table I and clearly indicate that cleavage of oxidized α-1-PI by porcine elastase occurs at an X–Met(O) bond, eight residues on the amino-terminal side of the reactive-site methionine. The data, furthermore, show that both methionyl residues have been modified by either oxidative treatment. Since our previous results indicate that only two of the eight methionyl residues are modified, only these two residues must have been affected.

Table II: Effect of Myeloperoxidase Concentration on Human Plasma α-1-PI Activity[a]

plasma (mL)	myeloperoxidase (nM)	α-1-PI inactivated (nmol min^{-1} mL^{-1})
0.04	3.5	0.04 ± 0.005
0.04	5.2	0.15 ± 0.01
0.04	6.9	0.44 ± 0.02

[a] Reaction mixtures containing α-1-PI, myeloperoxidase, and/or treated or untreated plasma were prepared as described under Methods.

Effect of Myeloperoxidase on Human Plasma. In order to determine whether the myeloperoxidase system could affect the inhibitory activity of human plasma, the enzyme, in the presence of hydrogen peroxide and Cl^-, was mixed with plasma and the loss of endogenous α-1-PI inhibitory activity monitored. It was found that loss of α-1-PI inhibitory did occur, and that this effect was dependent on the concentration of myeloperoxidase added (Table II). Omission of enzyme or hydrogen peroxide resulted in the abolishment of an effect on plasma α-1-PI.

When purified α-1-PI was added to the myeloperoxidase–plasma reaction mixture, the rate of inactivation of the combined purified and endogenous α-1-PI was slower than with purified inhibitor alone. Furthermore, preincubation of myeloperoxidase and plasma for increased lengths of time prior to addition of hydrogen peroxide and α-1-PI caused a further

Table III: Effect of Plasma Concentration on Myeloperoxidase Activity[a]

purified α-1-PI (μM)	plasma (mL)	time (min)	α-1-PI inactivated [nmol min^{-1} (nmol of enzyme)$^{-1}$]	myeloperoxidase activity (%)
2.12		0	269 ± 15	
2.12	0.04	0	158 ± 12	100
2.12	0.04	1	107 ± 9	99
2.12	0.04	2.5	77 ± 6	84
2.12	0.04	5	46 ± 2	80
2.12	0.04	10	31 ± 3	70
2.12	0.04 (dialyzed)	5	44 ± 4	80
2.12	0.04 (boiled)	5	268 ± 19	

[a] Reaction mixtures containing α-1-PI, myeloperoxidase, and/or treated or untreated plasma were prepared as described under Methods.

time-dependent decrease in the rate of inactivation of α-1-PI (Table III). Myeloperoxidase activity was also decreased after incubation with plasma, but it cannot be determined whether this was sufficient to entirely account for the large effect on α-1-PI inactivation. Dialysis of the plasma before addition to the reaction mixture had no effect on the rate of loss of α-1-PI inhibitory activity. Thus, it is apparent that small molecules play no significant role in the above reactions. Boiling of the plasma prior to addition to the reaction mixture caused the plasma effect to disappear, and the α-1-PI was inactivated at the same rate as a sample to which plasma had not been added. These results indicate that a large molecule, probably protein, is responsible for the effect of plasma on the rate of α-1-PI inactivation. However, the mechanism by which this is occurring remains unknown. One possibility to be considered is that the effect of plasma on the rate of inactivation of α-1-PI by the myeloperoxidase system is due to a nonspecific effect of large amounts of protein. However, a large part of the decrease in rate depends on time of preincubation of myeloperoxidase with plasma. A nonspecific effect would not be expected to be time dependent. Also, the boiled plasma was centrifuged to remove the coagulated protein. Some protein would still be soluble and present in the supernatant. Nevertheless, the net result was no effect on the purified system. Thus, it is likely that whatever was present in plasma which decreased the rate of inactivation of α-1-PI was specific for one of the reactants or intermediates and was inactivated by boiling.

Myeloperoxidase Activity in Human Alveolar Macrophages. There has been considerable controversy with regard to the possibility that macrophages may contain myeloperoxidase (Van Furth et al., 1970; Paul et al., 1973; Romeo et al., 1973; Nathan et al., 1979). We have found that purified α-1-PI can be inactivated by extracts of human alveolar macrophages. In agreement with one group (Paul et al., 1973), we found that inactivation occurred only in the presence of I^- and not with Cl^- (Table IV). If I^- or hydrogen peroxide were omitted, no inactivation of α-1-PI occurred. Some macrophage preparations which were utilized contained 6–20% contamination with polymorphonuclear leukocytes. When these preparations were tested, α-1-PI could be inactivated in the presence of both I^- and Cl^- (not shown). Obviously, the myeloperoxidase activity of the leukocytes in these more heavily contaminated preparations was now detectable. Since the lowest leukocyte contamination where a chloride effect could be detected was not very different from that where no chloride effect could be detected, it is unlikely there was any nonspecific protein effect which inhibited the chloride-dependent system but not the iodide-dependent system. Both the supernatant and the resuspended pellet in the macrophage extracts were found to exhibit activity in the guaiacol assay. Thus, human alveolar macrophages do contain peroxidase activity which, in the presence of hydrogen peroxide and I^-, can inactivate α-1-PI. This is obviously a different enzyme from myeloperoxidase since it cannot function when Cl^- is the source of halide ion.

Table IV: Inactivation of α-1-PI by Macrophage Peroxidase[a]

system	α-1-PI inhibitory activity remaining (%)
supernatant + Cl^- or I^-	100
pellet + Cl^- or I^-	100
supernatant + H_2O_2	100
pellet + H_2O_2	100
supernatant + H_2O_2 + Cl^-	100
supernatant + H_2O_2 + I^-	15
pellet + H_2O_2 + Cl^-	100
pellet + H_2O_2 + I^-	26

[a] Homogenates of human alveolar macrophages were prepared and the supernatant and pellet fractions tested for α-1-PI-inhibiting activity as described under Methods. Approximately 8×10^6 macrophages were utilized in preparing homogenates.

Discussion

In many ways, the factors which are either influenced or required for the inactivation of α-1-PI by myeloperoxidase are similar to those previously found for other myeloperoxidase reactions (Zgliczynski et al., 1968; Klebanoff, 1968). However, there are some major differences. For example, the pH optimum for the oxidation of α-1-PI is 6.2, compared to 5.3 for the oxidation of α-amino acids and 5.0–5.5 for the chlorination of taurine (Naskalski, 1977). The pH inside the phagocytic vacuoles of polymorphonuclear leukocytes has been reported to be 6.0 (Jensen & Bainton, 1973) and also 4.0 (Mandell, 1970). Certainly, the pH is acidic. As the myeloperoxidase and other components of the granules are released extracellularly during phagocytosis, the microenvironment must begin to rise toward the plasma pH of 7.4. Thus, although the myeloperoxidase system has an optimum rate of inactivation of α-1-pI at pH 6.2, in vitro, it is also capable of inactivating α-1-PI at less acidic pH values, albeit at decreased rates.

Hydrogen peroxide is required for the inactivation of α-1-PI by myeloperoxidase, and this is present in adequate amounts inside the cells. Therefore, during phagocytosis or cell death, this substrate would also be expected to be released into the external milieu surrounding the cell (Root et al., 1975). Oxidation of α-1-PI by myeloperoxidase also requires halide ion, in common with other myeloperoxidase oxidation reactions. Chloride ion is certainly present in sufficient amounts in vivo to satisfy this requirement.

We have previously shown that when α-1-PI is oxidized enzymatically by myeloperoxidase only two methionyl residues are modified (Matheson et al., 1979). Such an effect was also noted when α-1-PI was oxidized chemically with SucNCl (Johnson & Travis, 1979). From the sequence results reported here, it seems obvious that the methionyl residues oxidized by either method are the same. Earlier (Johnson & Travis, 1979), we showed that in chemically oxidized α-1-PI the reactive-site methionine was oxidized, but the P_{16}' methionyl residue was not, thus accounting for only one of the oxidized residues. We have found that in either enzymatically or chemically oxidized α-1-PI the reactive-site methionine and the P_8 methionine were

both oxidized, in agreement with the two residues reported from amino acid analysis. Oxidation of the reactive-site methionine also accounts for the inactivation of α-1-PI by myeloperoxidase.

The data reported both here and previously (Johnson & Travis, 1979) support the proposal that either chemical or biological oxidation of α-1-PI inactivates the protein by conversion of two methionyl residues to the sulfoxide derivatives. That the affected methionyl residues are the same in either case is confirmed by the following results: (1) neither oxidized protein can form a sodium dodecyl sulfate stable complex with porcine elastase; (2) porcine elastase causes conversion of either oxidized protein to a modified form of slightly lower molecular weight; (3) the amino-terminal sequence of the modified, oxidized inhibitor is the same in both cases, and two methionyl sulfoxide residues are present in the first eight residues.

We have tried to investigate the interaction of oxidized α-1-PI with neutrophil elastase. However, the results were difficult to interpret quantitatively because the enzyme could either form a complex with the oxidized protein or, alternatively, convert it into a modified form. In fact, we have found that with α-1-PI oxidized by either procedure products were observed by amino terminal sequence analysis which indicated not only cleavage as in the case of porcine elastase at an X–Met(O) bond but also hydrolysis of the reactive-site Met(O)–Ser bond, presumably due to complex turnover (N. R. Matheson, P. S. Wong, M. Schuyler, and J. Travis, unpublished). However, a complete interpretation of the results could not be established.

The oxidative action of neutrophil myeloperoxidase on α-1-PI may be important in the regulation of the inhibitory activity of this protein. It has been reported that in model peptide chloro methyl ketones and nitroanilide substrates, similar in structure to the reactive site of α-1-PI, replacement of methionine by valine results in both increased rates of inhibition or hydrolysis, respectively, by neutrophil and porcine elastase (Nakajima et al., 1979). Thus, it would have seemed more efficient for a valyl residue to have been placed in the P_1 position of the reactive center of α-1-PI. however, with tissue remodelling a continuous process, some elastolytic activity would always be required, and this might best be accomplished by controlling α-1-PI activity through oxidative processes. Whether this in fact does play a role in controlling normal elastin turnover remains to be proven.

In the disease state, it is almost certain that oxidative processes play a role in decontrolling tissue degradation. In individuals exposed to external pollutants, it is well-known that both polymorphonuclear leukocytes and macrophages become sequestered in the lung. The former certainly have both oxidative (myeloperoxidase) and hydrolytic (elastase and cathepsin G) enzymes while the latter are known to release superoxide anion (Weiss et al., 1978). Thus, the potential for oxidation of plasma α-1-PI, which has penetrated into lung tissue, and for the uncontrolled hydrolysis of lung elastin is present under these conditions.

The demonstration of peroxidase activity in macrophages places further importance on the role of these cells in the development of emphysema. Much attention had been given to the fact that these cells can produce a weak elastolytic activity which might be deleterious to the elastin of the lung. However, in comparison to neutrophils, the quantity of enzyme produced by macrophages should be considered trivial, and greater attention must be paid to their potential as oxidizing agents. In fact, it may well be that the oxidation of α-1-PI by macrophages is more important than that provided by neutrophils since their numbers tend to increase dramatically during emphysema development.

References

Baugh, R., & Travis, J. (1976) *Biochemistry 15*, 836.

Beatty, K., Bieth, J., & Travis, J. (1980) *J. Biol. Chem. 255*, 3931.

Carp, H., & Janoff, A. (1980) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 39*, 565a.

Heimburger, N., Haupt, H., & Schwick, H. (1971) *Proc. Int. Res. Conf. Proteinase Inhibitors 1*, 1–21.

Henson, P. M. (1972) *Am. J. Pathol. 68*, 593.

Jensen, M. S., & Bainton, D. F. (1973) *J. Cell Biol. 56*, 379.

Johnson, D., & Travis, J. (1978) *J. Biol. Chem. 253*, 7142.

Johnson, D., & Travis, J. (1979) *J. Biol. Chem. 254*, 4022.

Klebanoff, S. J. (1968) *J. Bacteriol. 95*, 2131.

Laurell, C. B., & Eriksson, S. (1963) *Scand. J. Clin. Lab. Invest. 15*, 132.

Mandell, G. L. (1970) *Proc. Soc. Exp. Biol. Med. 134*, 447.

Matheson, N. R., Wong, P. S., & Travis, J. (1979) *Biochem. Biophys. Res. Commun. 88*, 402.

Matheson, N. R., Wong, P. S., & Travis, J. (1980) *Biochemistry*, preceding paper in this issue.

Mendez, E., & Lai, C. Y. (1975) *Anal. Biochem. 68*, 47.

Nakajima, K., Powers, J. C., Ashe, B. M., & Zimmerman, M. (1979) *J. Biol. Chem. 254*, 4027.

Naskalski, J. W. (1977) *Biochim. Biophys. Acta 485*, 291.

Nathan, C. F., Silverstein, S. C., Brukner, L. H., & Cohn, Z. A. (1979) *J. Exp. Med. 149*, 100.

Pannell, R., Johnson, D., & Travis, J. (1974) *Biochemistry 13*, 5439.

Paul, B. B., Strauss, R. R., Selvaraj, R. J., & Sbarra, A. J. (1973) *Science (Washington, D.C.) 181*, 849.

Romeo, D., Cramer, R., Marzi, T., Soranzo, M. R., Zabucchi, G., & Rossi, F. (1973) *J. Reticuloendothel. Soc. 13*, 399.

Root, R. K., Metcalf, J., Ohsino, N., & Chance, B. (1975) *J. Clin. Invest. 55*, 945.

Sbarra, A. J., Selvaraj, R. J., Paul, B. B., Poskitt, P. K. F., Mitchell, G. W., Louis, F., & Asbell, M. A. (1977) in *The Granuloycyte: Function and Clinical Utilization* (Greenwalt, T. J., & Jamieson, G., Eds.) p 38, Liss, New York.

Van Furth, R., Hirsh, J. G., & Fedorko, M. E. (1970) *J. Exp. Med. 132*, 794.

Weiss, S. J., King, G. W., & Lo Buglio, A. F. (1978) *Am. J. Hematol. 4*, 1.

Weissman, G., Zurier, R. B., & Hoffstein, S. (1972) *Am. J. Pathol. 68*, 539.

Zgliczynski, J. M., Stelmaszynska, T., Ostrowski, W., Naskalski, J., & Sznajd, J. (1968) *Eur. J. Biochem. 4*, 540.