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Human α-1-Antichymotrypsin: Interaction with Chymotrypsin-Like Proteinases†

James Travis,* Jean Bowen, and Robert Baugh

ABSTRACT: The interaction of human plasma α-1-antichymotrypsin with serine proteinases from different tissues has been investigated. The protein was found to form stable complexes with pancreatic chymotrypsin, leukocyte cathepsin G, and mast cell chymotrypsin. No inhibition of pancreatic trypsin or leukocyte elastase could be demonstrated. With mixtures containing both α-1-antichymotrypsin and α-1-proteinase inhibitor, it was found that the former preferentially inactivated leukocyte cathepsin G, while the latter showed a strong preference for pancreatic chymotrypsin. However, leukocyte elastase was specifically inactivated by α-1-proteinase inhibitor even in 1:1 mixtures with chymotrypsin. All of these results taken together suggest that one of the primary functions of α-1-antichymotrypsin is to inactivate leukocyte cathepsin G, while α-1-proteinase inhibitor controls the activity of other serine proteinases, particularly leukocyte elastase.

The mechanism by which many of the plasma proteinase inhibitors function has received a great deal of attention in recent years due to their possible role or relationship with the disease states. In particular, intense studies on α-2-macroglobulin (α_2M),[1] α-1-proteinase inhibitor (α-1-PI), and antithrombin III (AT III) have been initiated due to their respective links with cystic fibrosis (Shapira et al., 1977), pulmonary emphysema (Laurell & Eriksson, 1963), and thrombotic episodes (Harpel & Rosenberg, 1976). However, very little, if any, attention has been given to the function of α-1-antichymotrypsin (α-1-Achy), a major early stage acute phase plasma protein (Aronsen et al., 1972) which tends to be localized in high concentration in bronchial fluid (Ryley & Brogan, 1972). When one considers that the granule fractions of human neutrophils and basophils contain chymotrypsin-like enzymes (Mounter & Atiyeh, 1960) which may be utilized during phagocytosis, a potential role of α-1-Achy in controlling the degradative function of these proteinases must also be envisioned. In addition, other types of cells, such as mast cells, also contain a high concentration of chymotrypsin-like enzymes (Yurt & Austen, 1978), the function of which, although unknown, must also be regulated.

In the previous paper in this series (Travis et al., 1978), we described the properties of α-1-Achy isolated from human plasma in three rapid, uncomplicated steps. We now present data which indicate that α-1-Achy is a controlling protein for the chymotrypsin-like activity present in phagocytic cells. These results conclusively support both our preliminary report (Baugh et al., 1976) as well as that of Ohlsson & Akesson (1976) in which it was suggested that α-1-Achy was a more effective inhibitor of leukocyte cathepsin G than α-1-PI.

Materials and Methods

Human leukocytes were obtained from healthy donors as well as from patients with chronic granulocytic leukemia. The isolation of both cells and granules has been previously described (Baugh & Travis, 1976).

Dog mastocytoma tissue was generously provided by Dr. C. Rawlings, College of Veterinary Medicine, University of Georgia. Partial purification of the chymotrypsin-like enzyme present in this tissue was obtained by extraction with 0.05 M Tris-HCl, 1.9 M NaCl, pH 8.0, followed by chromatography on DEAE-cellulose at pH 8.0 in 0.05 M Tris-HCl. The bound enzyme was subsequently eluted with 0.5 M NaCl by stepwise elution and found to be approximately 85% pure as judged by $NaDodSO_4$ gel electrophoresis. The properties of this enzyme will be described separately (P. Robertie & J. Travis, manuscript in preparation).

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[1] Abbreviations used: α_2M, α-2-macroglobulin; α-1-PI, α-1-proteinase inhibitor; AT III, antithrombin III; α-1-Achy, α-1-antichymotrypsin; $NaDodSO_4$, sodium dodecyl sulfate; Bz-L-Tyr-OEt, benzoyl-L-tyrosyl ethyl ester; Tos-PheCH_2Cl, tosyl-L-phenylalanyl chloromethyl ketone.

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Human pancreatic trypsin, chymotrypsin, leukocyte elastase, α-1-PI, and α-1-Achy were obtained by procedures originating from this laboratory (Travis & Roberts, 1969; Coan et al., 1971; Baugh & Travis, 1976; Pannell et al., 1974; Travis et al., 1978).

Techniques utilized for the assay of human trypsin and human leukocyte elastase esterase activity were as previously described (Travis & Roberts, 1969; Baugh & Travis, 1976). Cathepsin G, mast cell chymotrypsin, and human pancreatic chymotrypsin esterase activities were monitored with Bz-L-Tyr-OEt (Travis et al., 1978). An extinction coefficient of 6.64 ($E^{1\%}_{280nm}$) determined experimentally (Babul & Stellwagen, 1969) was utilized in measuring the protein concentration of cathepsin G in assays with the purified protein.

Measurements of the comparative inhibitory activities of α-1-PI and α-1-Achy in mixtures utilized the trypsin specificity shown only by the former inhibitor. The mixture of inhibitors was first incubated with a specific proteinase for 5 min in 0.05 M Tris, 0.05 M NaCl, pH 8.0, at room temperature. Residual trypsin inhibitory activity due to α-1-PI was measured by addition of a slight excess of a known concentration of Tos-Phe CH_2Cl-treated porcine trypsin (Miles), incubation for 2 min, and assay for loss of trypsin esterase activity. Residual chymotrypsin activity due to active α-1-Achy was then determined by the addition of an excess of a known concentration of bovine α-chymotrypsin (Worthington), incubation for 2 min, and assay for loss of chymotrypsin esterase activity. Since all of the inhibitory activity due to free α-1-PI in incubation mixtures was effectively removed by the addition of the trypsin, all residual inhibitory activity toward chymotrypsin was attributed to the presence of free α-1-Achy. In all cases the rates of hydrolysis of substrates were found to be linear and control experiments indicated no dissociation of complexes with either α-1-PI or α-1-Achy in the time intervals utilized.

Results

Purification and Properties of Human Leukocyte Cathepsin G. The isolation of cathepsin G essentially followed the procedure utilized for the preparation of leukocyte elastase (Baugh & Travis, 1976). This included heparin lysis of leukocytes to release intact granules and extraction of the granules with 0.2 M sodium acetate buffer, pH 4.5, 0.8 M NaCl. After each 25-mL extraction, the mixture was centrifuged at 30 000*g* for 10 min and the yellow green extract retained. It was routinely found that a total of three extractions of the leukocyte granule pellet was required to solubilize all of the elastase and cathepsin G.

After adjustment to pH 8.0 by the dropwise addition of 1 M Tris the leukocyte granule extract (76 mL) was applied to a column of Sepharose–trasylol (3.5 × 15 cm) equilibrated with 0.05 M Tris-HCl, 0.8 M NaCl, pH 8.0. The column was then washed with the same buffer until the A_{280nm} was less than 0.020. Both elastase and cathepsin G were desorbed from the column by elution with 0.05 M sodium acetate, 0.8 M NaCl, pH 4.5.

The mixture obtained by affinity chromagraphy was dialyzed vs. 0.05 M sodium acetate, 0.15 M NaCl, pH 5.5, and applied to a column of CM-cellulose (2.1 × 10 cm) equilibrated with the same buffer. Stepwise elution of all of the elastase isozymes was accomplished by washing the column with 0.05 M sodium acetate, 0.45 M NaCl, pH 5.5. Cathepsin G was eluted by a second wash with 0.05 M sodium acetate, 0.8 M NaCl, pH 5.5. When a gradient from 0.45 M NaCl to 0.8 M NaCl was utilized, partial separation of cathepsin G isozymes could be obtained.

From 10^{11} cells it was possible to obtain about 35 mg of elastase and 25 mg of cathepsin G. However, fractionation of granulocytes from one patient with a high basophil count (15%) resulted in the isolation of cathepsin G in almost twice the normal yield compared with elastase, indicating that basophils contain a high concentration of this enzyme but very little, if any, elastase. Acid gel electrophoresis of protein fractions at each stage of the purification of each enzyme(s) is shown in Figure 1. Cathepsin G was recovered in 75% yield with a 20-fold purification from granule extracts by this procedure. No elastase esterase activity or cross-reaction with antisera to leukocyte elastase by immunodiffusion could be detected.

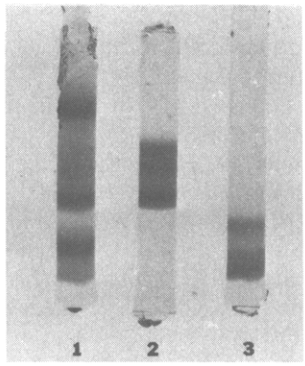

FIGURE 1: Acid disc gel electrophoresis of human leukocyte granule extract and purified fractions. Electrophoresis was at pH 4.3 in a standard 7.5% gel. Direction of migration is from anode (top) to cathode (bottom). (1) Crude extract (200 μg); (2) elastase isozymes from CM-cellulose chromatography (150 μg); (3) cathepsin G isozymes from CM-cellulose chromatography (150 μg).

The molecular weights of the isozymes of cathepsin G, as determined from $NaDodSO_4$ gel electrophoresis, were found to range from 24 000 to 26 000. The difference in size and electrophoretic mobility is probably not attributable to carbohydrate content as in the case of leukocyte elastase (Baugh & Travis, 1976) since only traces of hexosamine and neutral suger were detectable in analyses of the enzyme. It is also not due to different degrees of activation of a zymogen either since no ambiguity was found in the sequence of the first ten residues of a mixture of the isoenzymes. This sequence was found to be Ile-Ile-Gly-Gly-Arg-Glu-Ser-Arg-Pro-His.

Specificity of α-1-Antichymotrypsin. In order to determine the specificity of α-1-Achy, a number of different serine proteinases were incubated with this inhibitor for 5 min in 0.05 M Tris-HCl, 0.05 M NaCl, pH 8.0 at room temperature. An inhibitor:enzyme molar ratio of 4:1 was chosen and samples of each mixture were subjected to both $NaDodSO_4$ gel electrophoresis and analysis for enzymatic activity. It was found (Figure 2) that complex formation could only be detected when α-1-Achy was incubated with human leukocyte cathepsin G, human pancreatic chymotrypsin, or the partially purified chymotrypsin-like enzyme isolated from canine mastocytoma tissue. No complexes were detectable with either human leukocyte elastase or human cationic trypsin. These data were in complete agreement with enzyme assays of the incubation mixtures in that no inhibition of elastase or tryptic activity could be detected, while the total abolition of leukocyte cathepsin G, pancreatic chymotrypsin, and mast cell chymotrypsin activities was noted in the presence of α-1-Achy.

Preincubation of α-1-Achy (1 mg/mL) at pH 3.0 for 5 min prior to incubation at pH 8.0 resulted in a loss of 85% of the inhibitory activity toward the three chymotrypsin-like enzymes. However, in the reverse experiment no release of enzyme activity could be demonstrated after acidification of

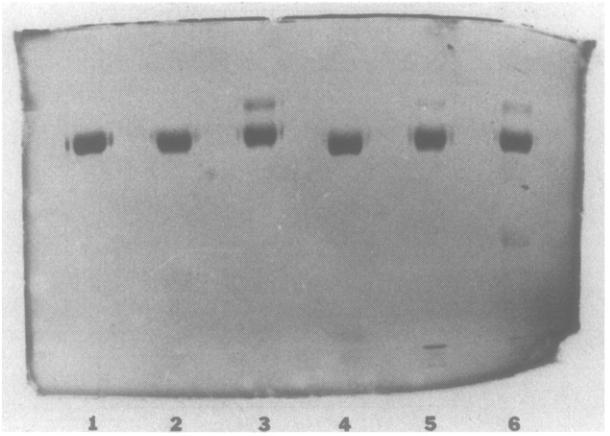

FIGURE 2: Specificity of human α-1-Achy with serine proteinases. Samples of α-1-Achy (50 μg) were incubated with 5 μg of proteinase in 0.05 M Tris-HCl, 0.05 M NaCl, pH 8.0, for 5 min at room temperature. The solution was made 1% in $NaDodSO_4$, boiled for 2 min, and subjected to $NaDodSO_4$ gel electrophoresis in a 9% separating gel. (1) α-1-Achy; (2) α-1-Achy + human trypsin; (3) α-1-Achy + human chymotrypsin; (4) α-1-Achy + leukocyte elastase; (5) α-1-Achy + leukocyte cathepsin G; (6) α-1-Achy + mast cell chymotrypsin.

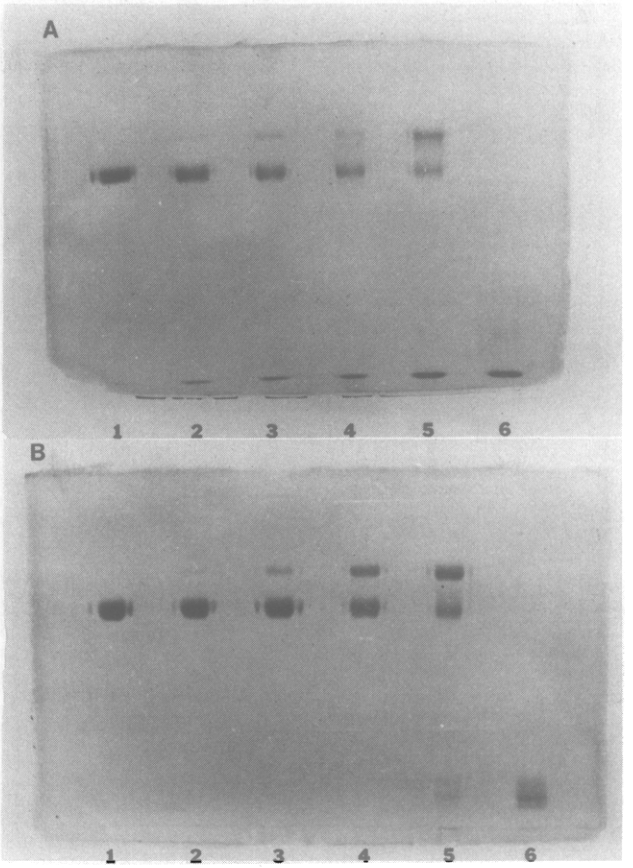

FIGURE 3: Interaction of α-1-Achy with human chymotrypsin and leukocyte cathepsin G. Samples of α-1-Achy (20 μg) and varying molar ratios of enzyme were incubated for 5 min at pH 8.0. The solution was then subjected to $NaDodSO_4$ gel electrophoresis as described in Figure 2. Molar ratios of α-1-Achy to enzyme are as follows: (1) α-1-Achy alone; (2) 1:0.1; (3) 1:0.2; (4) 1:0.5; (5) 1:1; (6) enzyme alone. (A) Human pancreatic chymotrypsin; (B) human leukocyte cathepsin G.

complexes formed at pH 8.0 between native inhibitor and enzyme.

Interaction of α-1-Achy with Human Chymotrypsin and Human Leukocyte Cathepsin G. When α-1-Achy was mixed with increasing concentrations of either human chymotrypsin or cathepsin G and the results were assessed by $NaDodSO_4$ gel electrophoresis, the formation of a high molecular weight complex could be readily observed (Figures 3A and 3B). In either case it was found that the complex had a molecular weight near 90 000, indicative of the formation of a 1:1 complex. Only at concentrations of enzyme higher than molecular equivalence with the inhibitor (Figures 3A and 3B, slot 5) could extra components be detected indicating that proteolysis of complexes had occurred. At no time were we able to demonstrate the complete conversion of native α-1-Achy into a $NaDodSO_4$-stable complex. The results observed probably represent an equilibrium between $NaDodSO_4$-stable and $NaDodSO_4$-labile forms of the complex since identical experiments using alkaline gel electrophoresis revealed complete conversion to a new component of slower mobility than native α-1-Achy.

As described earlier (Johnson & Travis, 1976) we have found that dissociation of complexes of α-1-PI with pancreatic trypsin, chymotrypsin, and leukocyte elastase may be obtained by incubation with nucleophilic agents such as hydroxylamine and benzamidine. The resulting modified inhibitor had a lower molecular weight than the native protein as well as a new amino-terminal sequence homologous with the P_1'–P_4' reactive site of other proteinase inhibitors including the elastase binding site of the garden bean inhibitor (Wilson & Laskowski, 1974) and to the trypsin binding site of the lima bean inhibitor (Stevens et al., 1974). When complexes made from α-1-Achy (200 nmol) and cathepsin G (150 nmol) were incubated in 1.0 M benzamidine, 0.05 M Tris-HCl, pH 8.0 at 40 °C for 24 h, dissociation occurred. However, no apparent decrease in the molecular weight of the α-1-Achy obtained from this complex could be detected by $NaDodSO_4$ gel electrophoresis. When this protein was subsequently reisolated by Sephadex G-75 chromatography (Johnson & Travis, 1976) and subjected to sequence analysis, arginine could still be detected at the amino terminus, indicating that some native α-1-Achy was present. However, serine was also found to be present as a new amino terminus. Difficulties in quantitating these two residues have made it impossible to determine the relative amounts of what would appear to be a mixture of native and post-complex inhibitor. However, it would appear that, as in the case of α-1-PI, peptide bond cleavage did occur upon complex dissociation with strong nucleophiles (Johnson & Travis, 1976). Experiments are in progress to determine further sequences at what would appear to be the reactive center of the inhibitor.

Interaction of α-1-Achy and α-1-PI with Human Chymotrypsin and Human Leukocyte Cathepsin G. In order to determine a specific functional role for α-1-Achy in the regulation of proteolysis, this inhibitor was mixed in various proportions with α-1-PI in 0.05 M Tris-HCl, 0.05 M NaCl, pH 8.0 at room temperature for 5 min and the competition of each inhibitor for chymotrypsin and cathepsin G tested by $NaDodSO_4$ gel electrophoresis. The time chosen was found to be sufficient for complex formation to be complete with either inhibitor alone. In fact, control experiments indicated that inhibition was complete within 30 s of mixing.

It can be readily seen (Figure 4A, slots 2 and 4) that both inhibitors formed $NaDodSO_4$-stable complexes of differing molecular weight with human chymotrypsin (74 000 for the α-1-PI complex and 88 000 for the α-1-Achy complex). However, in all cases staining of α-1-Achy and its complexes was not as intense as with α-1-PI. We assume this to be due to interference by the large carbohydrate moiety in the α-1-Achy protein. In mixtures, very little complex of chymotrypsin with α-1-Achy was noted, even at α-1-Achy:α-1-PI molar ratios of

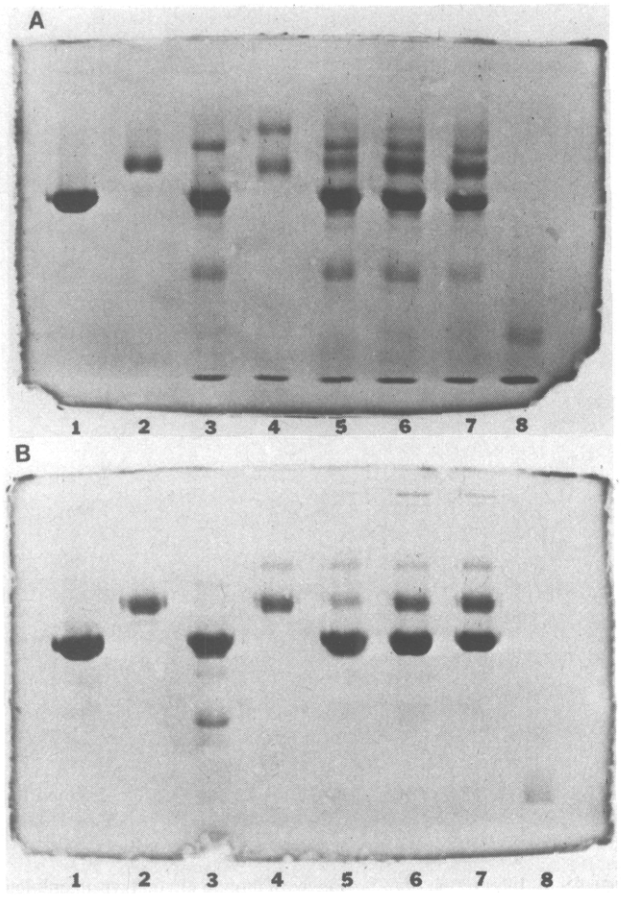

FIGURE 4: Competition of α-1-Achy and α-1-PI for human chymotrypsin and leukocyte cathepsin G. Inhibitors were mixed in varying proportions at pH 8.0 and a standard quantity of enzyme added. Samples were then subjected to $NaDodSO_4$ gel electrophoresis as given in Figure 2. (1) α-1-PI (20 μg); (2) α-1-Achy (20 μg); (3) α-1-PI (20 μg) + enzyme (5 μg); (4) α-1-Achy (20 μg) + enzyme (5 μg); (5) α-1-Achy (5 μg) + α-1-PI (20 μg) + enzyme (5 μg); (6) α-1-Achy (10 μg) + α-1-PI (20 μg) + enzyme (5 μg); (7) α-1-Achy (20 μg) + α-1-PI (20 μg) + enzyme (5 μg); (8) enzyme (5 μg). (A) Human chymotrypsin; (B) human leukocyte cathepsin G.

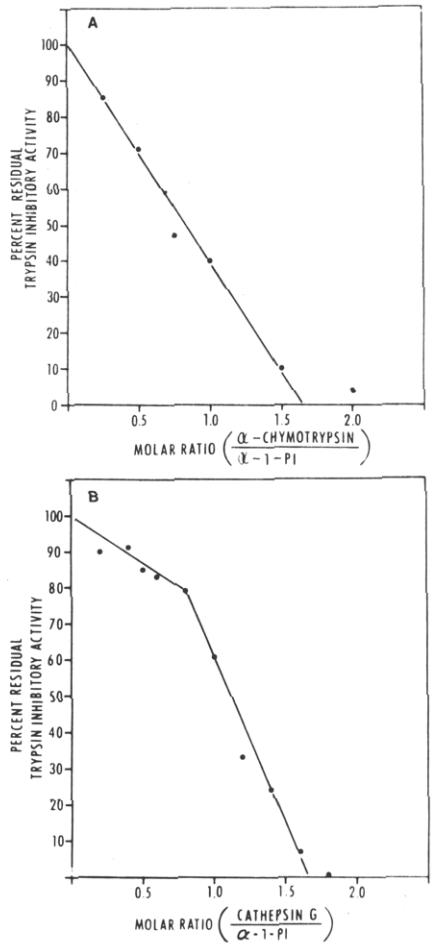

FIGURE 5: Competition of α-1-PI and α-1-Achy for human chymotrypsin and human leukocyte cathepsin G. Equimolar quantities of α-1-PI (52 μg) and α-1-Achy (68 μg) were mixed in 0.05 M Tris-HCl, 0.05 M NaCl, pH 8.0. Increasing concentrations of enzyme were added and after 5 min aliquots removed and residual trypsin inhibitory activity due to α-1-PI was measured. (A) α-1-PI + α-1-Achy + human chymotrypsin; (B) α-1-PI + α-1-Achy + human leukocyte cathepsin G.

1:1. Thus, it would appear that the pancreatic enzyme is predominantly controlled by α-1-PI. However, some complex breakdown with this inhibitor must also be occurring as judged by the consistent presence of a lower molecular weight species (34 000). Human α-1-Achy:chymotrypsin complexes appear to be more stable since no large breakdown products could be visualized.

In contrast to the above results, when mixtures of α-1-Achy and α-1-PI were incubated with cathepsin G, little α-1-PI complex could be demonstrated. Rather, an apparently rapid breakdown of any complex formed seemed to have occurred (Figure 4B, slot 3). However, α-1-Achy:cathepsin G complexes were very stable and must have been formed instantaneously even in the presence of a large excess of α-1-PI since few breakdown products due to α-1-PI:cathepsin G complexes could be detected. In fact, in mixtures of the two inhibitors with cathepsin G, only complexes between α-1-Achy and this enzyme were easily detectable, even at high α-1-PI:α-1-Achy molar ratios (Figure 4B, slot 4). The results strongly suggest that α-1-Achy controls the function of this enzyme, relative to α-1-PI.

Partition of Human Chymotrypsin and Human Cathepsin G between α-1-PI and α-1-Achy. As shown above, both cathepsin G and chymotrypsin form 1:1 molar complexes with either α-1-Achy or α-1-PI. However, there is obviously some preference of α-1-PI for chymotrypsin and α-1-Achy for cathepsin G. In order to quantitate the degree of interaction of each inhibitor with the two enzymes, increasing quantities of each enzyme were added separately to an equimolar mixture of α-1-PI and α-1-Achy. After 5 min the residual trypsin inhibitory activity due to free α-1-PI was measured.

As can be seen in Figure 5A, chymotrypsin was preferentially bound to α-1-PI, with 62% of the enzyme being inactivated by this inhibitor in an equimolar mixture of all three proteins. A similar result was obtained by Ohlsson (1971) using ^{125}I-labeled bovine α-chymotrypsin added to human serum and analyzing the data by antigen–antibody crossed-gel electrophoresis.

With cathepsin G a result opposite to that seen with chymotrypsin was noted (Figure 5B) with at least 68% of the enzyme being complexed by α-1-Achy. Furthermore, a definite breakpoint at a molar ratio of cathepsin G:α-1-PI of 0.8:1 can be seen, indicating that at this relative concentration most of the enzyme has been bound to α-1-Achy.

Interaction of Various Proteases with α-1-PI. In order to determine the relative affinities of proteolytic enzymes for α-1-PI, equimolar mixtures of various pairs of proteinases and α-1-PI were incubated together, and the inhibition of each protease was determined by measuring residual esterase activity. The results are presented in Table I and readily show that α-1-PI has a greater affinity for chymotrypsin than for

TABLE I: Affinity of Various Proteinases for α-1-PI.[a]

mixture	% inhibition trypsin (T)	chymotrypsin (C)	leukocyte elastase (LE)	cathepsin G (CAT G)	porcine elastase (PE)
T + C	30	70			
C + LE		50	50		
C + CAT G		80		20	
LE + CAT G			100	0	
T + CAT G	80			20	
C + PE		75			25

[a] Pairs of proteinases (5 μg each) were mixed together at pH 8.0 and α-1-PI (10 μg) was added. After 5 min the residual esterase activity of each enzyme was determined.

trypsin, a result which confirms the data of both Ohlsson (1971) and Aubry & Bieth (1977). α-1-PI also preferentially binds chymotrypsin as opposed to porcine elastase and human leukocyte cathepsin G. Equal partitioning of chymotrypsin and leukocyte elastase with α-1-PI was noted. α-1-PI exclusively bound leukocyte elastase in the presence of cathepsin G, further indicating that α-1-PI is not the primary inhibitor of cathepsin G. A similar observation has been made by Feinstein & Janoff (1975a) using crude granule extracts and purified α-1-PI. Experiments performed by incubating trypsin and cathepsin G gave anomalous results which indicated that both proteinases were 80% inhibited by an equimolar concentration of α-1-PI. However, when trypsin and cathepsin G were incubated together at equimolar concentrations, cathepsin G was inactivated by 60%. Thus, there must be proteolytic degradation of cathepsin G by trypsin even in the incubation mixtures with inhibitor.

Discussion

During the inflammatory process, polymorphonuclear leukocytes release their granule contents, including elastase and cathepsin G, into the extracellular medium. Tissue damage will result when the extracellular release from these cells exceeds the ability of proteinase inhibitors to neutralize the effects of these hydrolytic enzymes (Cline, 1975). Local consumption of proteinase inhibitors has been seen after infections and acute inflammations, i.e., with an accumulation of granulocytes (Ohlsson, 1974).

α-1-PI and α-1-Achy, together with α_2M and inter-α-trypsin inhibitor, constitute the primary defense mechanism against leukocyte granule proteases excreted either during phagocytosis of "foreign" substances or upon death of the cell. Most studies have focused attention upon α-1-PI and α_2M as the primary inhibitors of leukocyte granule proteases, and Ohlsson (1974) has shown that, of the two inhibitors, α-1-PI is the primary inhibitor of human pancreatic chymotrypsin, human leukocyte elastase, bovine α-chymotrypsin, and porcine trypsin, whereas α_2M is the major inhibitor of human leukocyte cathepsin G and porcine elastase.

During the inflammatory process, in which polymorphonuclear leukocytes play a prominent role, the levels of both α-1-Achy and α-1-PI rise rapidly. These inhibitors thereby become of greatest importance since the molecular weight of α_2M precludes it from penetrating the extravascular space. Normally, the molar concentration of α-1-PI is four times that of α-1-Achy, but during the acute phase the α-1-Achy concentration rises rapidly up to four times its usual level and, within 8 h, whereas the α-1-PI concentration rises more slowly doubling in concentration within 3 days (Laurell, 1972). Thus, α-1-Achy would appear to be the most important proteinase inhibitor in the initial stages of the inflammatory process.

Although no specific physiological role for this protein has been discovered, Ryley & Brogan (1972) found that α-1-Achy was selectively concentrated in the bronchial lumen in patients with chronic bronchitis. They noted that the relative concentration of α-1-Achy in bronchial secretions was significantly higher than for other plasma proteins except IgA and must, therefore, have an important role in the protection of the lung against chymotrypsin-like enzymes. α-1-Achy has also been shown to rise in concentration after trauma (surgical, asceptic necrosis, myocardial infarction), and acute bacterial infections (Brogan et al., 1971).

Cathepsin G has been isolated as a mixture of isoenzymes in several laboratories (Ohlsson & Venge, 1974; Schmidt & Havemann, 1974; Rindler-Ludwig & Braunsteiner, 1975; Feinstein & Janoff, 1975b; Starkey & Barrett, 1976b). However, its physiological function has never been clearly elucidated. Whereas leukocyte elastase can degrade structural proteins including elastin and collagen (Starkey & Barrett, 1976a), a similar function had not been reported for cathepsin G until quite recently when Roughley (1977) and Roughley & Barrett (1977) demonstrated the breakdown of proteoglycan by both leukocyte elastase and cathepsin G. Our own investigations (Reilly & Travis, 1978) indicate that cathepsin G can also degrade elastin at about 10% the rate of leukocyte elastase and that this degradation is abolished by preincubation with α-1-Achy. Whether elastin proteolysis by cathepsin G can be enhanced by other factors or is important in physiological systems remains to be established.

The results of the interaction studies of α-1-PI and α-1-Achy with either trypsin, chymotrypsin, elastase, or cathepsin G indicate that α-1-PI preferentially interacts with elastase, chymotrypsin, and trypsin while α-1-Achy specifically inhibits chymotrypsin-like enzymes. Since only in extreme cases, such as pancreatitis or carcinoma of the pancreas, would one expect any significant pancreatic trypsin or chymotrypsin to be present in plasma, it is doubtful that the true physiological function of either inhibitor is to control the activity of these enzymes. One probable function of these proteins is in the control of bacterial or fungal proteinases released during invasion by microorganisms such as occurs in diseases such as farmer's lung. However, it would be more logical to conclude that α-1-PI and α-1-Achy were specifically developed to control, respectively, the proteolytic activities of leukocyte elastase (α-1-PI) and leukocyte cathepsin G (α-1-Achy). In certain tissues such as the lung, augmentation of this control could be supplied by other endogenous inhibitors present in that tissue (Tegner & Ohlsson, 1977).

Cathepsin G has been reported to have a number of unusual functions. For example, it can act as an antibacterial agent (Lehrer et al., 1973) and a promoter of phagocytosis (Odeberg & Ohlsson, 1975). The enzyme also has the ability to generate chemotactic activity from serum and C_3 and C_5 complement proteins, resulting in the production of biologically active products involved in cytolysis, chemotaxis, immune adherence, phagocytic enhancement, and histamine release (Ohlsson & Venge, 1975). These products provide a means by which neutrophils can perpetuate or augment their own presence once they have migrated into sites containing phagocytizable material. Recently, cell-detaching activity was demonstrated for granule proteases using HeLa cells and human newborn fibroblasts. Studies indicate that this activity is probably produced by cathepsin G (Taubman & Cogan, 1975). Presumably, the rapid increase in production of α-1-Achy in the acute phase is to ensure tight control over these activities in order to

maintain homeostasis. If this is correct, α-1-Achy may have a far more important physiological role than any of the other plasma inhibitors studied to date.

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