Regulation of Elastolysis of Insoluble Elastin by Human Leukocyte Elastase: Stimulation by Lysine-Rich Ligands, Anionic Detergents, and Ionic Strength†

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ABSTRACT: Human leukocyte elastase (HL elastase) activity is stimulated against insoluble elastin by the cationic, lysine-rich, heparin-neutralizing protein platelet factor 4 [PF_4; Lonky, S. A., & Wohl, H. (1981) *J. Clin. Invest. 67*, 817]. Stimulation of HL elastase is also induced by oligolysines of a polymer length greater than four and by lysine-rich histones. This stimulation of HL elastase elastolytic activity is dependent on polymer length and can be duplicated when lysine-rich ligands are preadsorbed onto the insoluble elastin. While the binding of lysine-rich ligands is electrostatic in nature, the binding of HL elastase to elastin remains near 90% of control values in 2.0 M NaCl. The electrostatic nature of HL elastase binding to substrate is seen in its competition for binding sites with lysine-rich ligands and in the moderate enhancement of HL elastase activity against elastin precoated with the anionic detergent sodium dodecyl sulfate. Although elastin precoated with lysine-rich ligands is a better substrate for HL elastase, it binds less enzyme than does native elastin. Thus, HL elastase elastolytic activity is not entirely dependent on HL elastase binding to elastin, and it appears that some HL elastase binding sites favor elastolysis while others do not. The data suggest that electrostatically governed sites on elastin are more likely to be nonproductive for HL elastase, while hydrophobic binding sites are more likely to be productive. Lysine-rich ligands, such as PF_4, preferentially bind to nonproductive sites, increasing the likelihood of productive HL elastase–elastin interactions. In addition, experiments performed under conditions in which PF_4 could not be adsorbed onto elastin demonstrate that free PF_4 stimulates HL elastase against insoluble elastin, but to a far less degree than under conditions when electrostatic binding of PF_4 is favored. These findings are considered in relationship to pancreatic elastase activity against elastin and with regard to the structural and functional relationships of elastin in the lung.

The granules of human polymorphonuclear (PMN)[1] leukocytes contain an elastolytic protease with enzymatic activity at neutral pH (Janoff & Scherer, 1968). The substrates attacked by this human leukocyte elastase (HL elastase) are similar to those degraded by porcine pancreatic elastase and include insoluble elastin (Taylor & Crawford, 1975), oxalic acid solublized elastin (Keller & Mandl, 1971), and the synthetic substrates *N*-succinyl-L-trialanine *p*-nitroanilide (Suc-Ala_3NA) and *N*-*t*-Boc-alanine *p*-nitrophenyl ester (Bieth & Wermuth, 1973; Visser & Blout, 1972). Interest in the regulation of HL elastase activity has been heightened by the fact that insoluble elastin is an important structural protein in many tissues and by experimental data implicating elastolysis in the pathogenesis of many diseases including pulmonary emphysema (Senior et al., 1977; Janoff et al., 1977). It has been suggested that some of the factors which regulate pancreatic elastase activity may regulate HL elastase activity as well (Kagan et al., 1977).

Several synthetic or naturally occurring ligands have been identified which can increase the hydrolysis of insoluble elastin by pancreatic elastase, including various alkyl sulfates and anionic fatty acids (Jordan et al., 1974). These hydrophobic amphiphiles enhance pancreatic elastase activity by binding to elastin and increasing the net anionic character of the substrate. Since pancreatic elastase is cationic, increased electrostatic attraction between enzyme and substrate occurs, resulting in enzyme stimulation. Consistent with the electrostatic nature of pancreatic elastase–insoluble elastin interactions, the adsorption of basic ligands such as poly(L-lysine) and poly(L-arginine) onto elastin results in pancreatic elastase inhibition (Gertler, 1971). In contrast to these data for pancreatic elastase, we have reported significant stimulation of HL elastase elastolytic activity by basic proteins such as platelet factor 4 (PF_4) and poly(L-lysine) (Lonky & Wohl, 1981), and others have reported stimulation of HL elastase by increasing ionic strength (Boudier et al., 1980; Lestinenne & Bieth, 1980).

The present studies further describe the electrostatic and nonelectrostatic nature of HL elastase–elastin interactions and provide evidence that lysine-rich ligands such as PF_4 stimulate HL elastase primarily by binding to the elastin substrate, thereby increasing productive elastolysis.

Experimental Procedures

Materials

Bovine ligamentum nuchae elastin (BLNE) prepared according to the method of Partridge et al. (1955), porcine pancreatic elastase, and the lysine-rich histone IIIs (M_r 21 500) were obtained from Sigma or Miles-Yeda; tetralysine was synthesized as described below and purified by CM-Sephadex chromatography. Poly(L-arginine hydrochloride) (M_r 13 900) was obtained from Research Plus. NaB^3H_4 (220 Ci/mol) was obtained from New England Nuclear Corp. Oxalic acid

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[1] Abbreviations: HL elastase, human leukocyte elastase; PF_4, platelet factor 4; PMN, polymorphonuclear; BLNE, bovine ligamentum nuchae elastin; $NaDodSO_4$, sodium dodecyl sulfate; PMSF, phenylmethanesulfonyl fluoride; TLC, thin-layer chromatography; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; NBA, *N*-*t*-Boc-alanine *p*-nitrophenyl ester; F-moc-E, 9-fluorenylmethyloxycarbonyl.

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solubilized BLNE- and elastin-agar plates (Alphasin) were purchased from Elastin Products. Sodium dodecyl sulfate was obtained from Pierce Chemical Co.

Methods

HL Elastase Purification. Polymorphonuclear leukocyte granules were collected as previously described (Lonky & Wohl, 1981). The granules were lysed by repeated freeze–thawing (5 times), and the supernatant obtained after centrifugation (27000*g* for 20 min at 4 °C) was stored at −20 °C. Purifications of crude lysosomal extract were performed by affinity chromatrography on a Sepharose–elastin column (1.6 × 20 cm) as described by Taylor & Crawford (1975). Fractions exhibiting activity against NBA (Visser & Blout, 1972) were pooled and concentrated to contain greater than 10 000 NBA esterase units/mL (1.20–1.41 mg of HL elastase/mL). Polyacrylamide gel electrophoresis using the method of Reisfeld et al. (1962) demonstrated the typical isoenzyme pattern, and $NaDodSO_4$–polyacrylamide gel electrophoresis of this enzyme revealed a single band with a molecular weight of 24 000–26 000. Inactivation of HL elastase with phenylmethanesulfonyl fluoride was performed as previously described (Taylor & Crawford, 1975), and PMSF-inactivated elastase exhibited no activity against [^{3}H]elastin.

PF_4 Purification. Purification of PF_4 was carried out by affinity chromatography by using a heparin–ε-aminocaproic acid–Sepharose affinity column as previously described (Levine & Wohl, 1976). PF_4 activiy in pooled fractions was measured by the method of Poplawski & Niewiarowski (1964) as modified by Levine & Wohl (1976). Heparin neutralizing activity ranged from 8 to 15 μg of PF_4/unig of heparin. $NaDodSO_4$–polyacrylamide gel electrophoresis of purified PF_4 revealed a molecular weight of 7800. Prior to use in experiments, PF_4 in column buffer was dialyzed against the experimental buffer.

Synthesis of Tetralysine. Solid phase synthesis of tetralysine was carried out by the method of Meienhofer et al. (1979) using α-F-Moc-E-*t*-Boc-L-lysine (Bachem) and a *p*-(benzyloxy)benzyl alcohol polystyrene resin. Cleavage of the finished peptide, after removal of the F-Moc protecting group, was accomplished by the addition of 4 N HCl in dioxane. The oligolysine was extracted from the resin–peptide mixture by alternate washes with H_2O and methanol until the washes were peptide free as determined by spot testing on silica gel TLC plates. The washes were pooled and lyophilized. Further purification was carried out by ion-exchange chromatography on CM-Sephadex, eluting with an NH_4HCO_3 gradient.

Tritiation of Insoluble Elastin. Bovine ligamentum nuchae elastin was tritiated according to the procedure of Lent et al. (1969) as modified by Blumenfeld & Gallup (1966). The specific activity of the [^{3}H]elastin obtained was 1.55×10^5 cpm/mg. [^{3}H]Elastin was suspended to 0.01% Triton X-100 and washed as previously described (Lonky & Wohl, 1981) to prevent clumping and to allow for reproducible pipetting. Tritonized [^{3}H]elastin (200 mg/mL) wasa stored in 0.02 M phosphate containing 0.15 M NaCl at pH 7.4 at 4 °C. Prior to use, [^{3}H]elastin was washed 3 times with the appropriate experimental buffer.

Iodination of Proteins. PF_4 and HL elastase were radioiodinated with ^{125}I according to the method of David & Reisfeld (1974). The specific activity of ^{125}I-labeled PF_4 was between 11 000 and 13 000 cpm/μg. ^{125}I-Labeled HL elastase demonstrated activity against elastin–agar plates which was similar to that of the nonradioiodinated enzyme. ^{125}I-Labeled PF_4 demonstrated antiheparin activity which was unchanged from the activity of the nonradioioinated protein.

Assay of Elastolytic Activity. All [^{3}H]elastin hydrolysis experiments and all binding studies were carried out in 1.5-mL Eppendorf centrifuge tubes which were pretreated with Surfacil to decrease protein sticking. Preliminary experiments revealed that such treatment had no effect on enzyme activity. The effects of basic proteins on HL elastase activity were determined by adding 100 μL of suspended [^{3}H]elastin (in 0.2 M Tris-HCl, pH 8.0) to 900 μL of 0.2 M Tris-HCl, pH 8.0, containing 5 μg of HL elastase and varying amounts of basic protein. Basic proteins and HL elastase had been allowed to preincubate at 25 °C for 10–30 min prior to addition of substrate. Incubation of [^{3}H]elastin with the test solutions, or with basic proteins or buffer alone, was carried out at 37 °C in a shaking water bath for 3 h. The reaction was terminated by cooling the tubes at 4 °C, and the tubes were centrifuged (4000 rpm for 8 min at 4 °C). A 400-μL sample of supernatant was pipetted into 20-mL screw-top scintillation vials containing 10 mL of Biofluor (New England Nuclear) and counted for radioactivity. Experiments with varying NaCl concentrations were carried out in an identical fashion, with both substrate suspensions and enzyme–protein mixtures containing the desired salt concentration. In experiments where various ligands were first adsorbed onto the substrate, the substrate–ligand complex was pipetted into test tubes containing HL elastase, and incubations were carried out as detailed above. All incubations were performed in triplicate, and the values reported are the means of three determinations.

Elastin plate experiments were carried out as previously described (Senior et al., 1971), and plates were incubated at 37 °C for 24 h.

Binding Studies. Binding studies of ligands to insoluble elastin were performed as follows: 2 mg of either native BLNE or BLNE which had been reduced with nonlabeled $NaBH_4$ was suspended in 1.0 mL of distilled H_2O and incubated with the desired concentration of ligand for 30 min at 37 °C in a shaking water bath. Each sample was then quickly centrifuged, and aliquots of the supernatant were assayed for the concentration of unbound ligand. For polyamino acids, the supernatant solutions were hydrolyzed in vacuo in 6 N HCl at 110 °C for 18 h, and the amount of polyamino acid was estimated on a Durrum D-500 amino acid analyzer. The concentration of histone IIIs in the binding supernatants was determined by the method of Lowry et al. (1951). The binding of ^{125}I-labeled PF_4 to BLNE was determined as follows: 2–4 mg of elastin substrate, or substrate coated with ligand, was suspended in 1.0 mL of 0.02 M phosphate buffer (pH 7.4) and incubated with the desired concentration of ^{125}I-labeled PF_4. Following the 30-min incubation period (at 37 °C), the tubes were centrifuged (4000 rpm × 10 min) and washed once with buffer, and the pellet was transferred to a 12 × 75 mm glass centrifuge tube for counting in a scintillation counter. After being counted, the pellets were washed with water, lyophilized, and weighed. For each ligand, control binding solutions (without BLNE) were prepared to determine the initial concentrations. To test the effect of NaCl on ligand binding, substrate was suspended in distilled H_2O containing varying amounts of salt, and the ligands were added.

When BLNE precoated with various ligands was used as a substrate for HL elastase, the adsorption of ligand to substrate was performed as outlined above. Following the 30-min incubation at 37 °C, the samples were centrifuged and the pellets washed twice in distilled H_2O. The remaining substrate–ligand pellet was then suspended in the appropriate experimental buffer, and aliquots were pipetted into Eppendorf tubes. Unless noted in the text, no unbound ligand was present

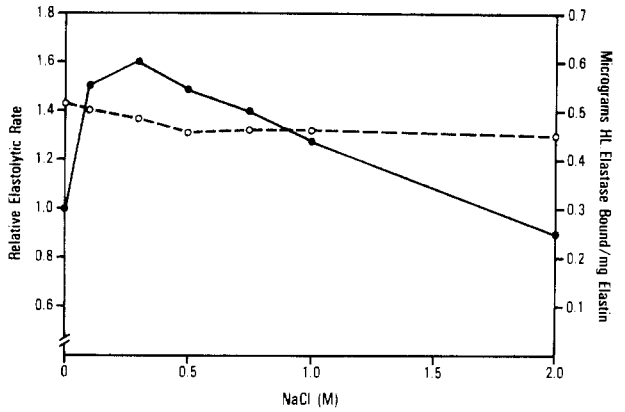

FIGURE 1: Effect of varying ionic strength on the elastolytic activity of 5 μg (0.17 nmol) of HL elastase against [^{3}H]elastin (●) and on the binding of 5 μg of HL elastase to reduced, nontritiated elastin (○). Hydrolyses and binding expirements were done in 0.2 M Tris, pH 8.0, containing various NaCl concentrations. Five micrograms of HL elastase in buffer alone resulted in the release of 10.6×10^3 cpm/mg of elastin after 3 h.

in experiments using substrate–ligand complexes.

Binding of ^{125}I-labeled HL elastase to elastin and the isolation of elastin–enzyme sedimentable complexes were performed as previously described for pancreatic elastase (Kagan et al., 1972). Briefly, 2–4 mg of elastin, or elastin precoated with ligand, was suspended in 1.0 mL of 0.2 M Tris-HCl (pH 8.0), incubated with 5 μg of ^{125}I-labeled HL elastase for 2 min at 4 °C, and mixed by inversion. The tubes were immediately centrifuged (4000 rpm × 8 min at 4 °C), and the pellet was washed twice with Tris buffer. The pellet was resuspended in Tris buffer and transferred to a 12 × 75 mm glass centrifuge tube for counting. After being counted, the elastin was washed with water, lyophilized, and weighed. Control solutions of ^{125}I-labeled HL elastase were pipetted into binding tubes without substrate and counted to determined the initial counts per milliliter of unbound HL elastases.

Results

Effects of Ionic Strength on HL Elastase Binding Elastolysis. Studies by Hall & Czerkowski (1961) have shown that for pancreatic elastase the initial stage of elastin solubilization involves the electrostatic attraction between enzyme and substrate and the formation of an insoluble elastin–enzyme complex. However, data from Boudier et al. (1980) suggest that electrostatic forces play a less important role in regulating HL elastase–elastin interactions, with enzyme activity increasing against both insoluble elastin and Suc-Ala_3NA as ionic strength increases. Using [^{3}H]elastin as a substrate, we also found that ionic strength has a positive effect on elastolysis (Figure 1) and that there is a peak effect seen between 0.3 and 0.45 M NaCl. Simultaneous ^{125}I-labeled elastase binding studies (Figure 1) demonstrate that increasing ionic strength has little effect on the binding of HL elastase to elastin, with only a 10% drop in binding measured at 2.0 M NaCl. Thus, it appears that the binding of HL elastase to insoluble elastin is not solely governed by electrostatic forces. Given the strongly nonpolar, hydrophobic nature of elastin, it is likely that hydrophobic forces play an important role in the binding of HL elastase to elastin. These data support the hypothesis of Lestinenne & Bieth (1980), who have postulated that the stimulation of HL elastase by ionic strength is due to conformational changes in the enzyme and not changes in enzyme binding.

Role of Electrostatic Forces in HL Elastase Activity: Effects of NaDodSO$_4$. Kagan et al. (1972) extended the initial studies of Hall & Czerkowski (1961) and demonstrated that for pancreatic elastase the formation of an enzyme–substrate complex and the rate of initial elastolysis are dependent on charge complimentarity between the anionic elastin substrate and the cationic enzyme. Since HL elastase is also cationic, it has been postulated (Stone et al., 1977) that its activity would be increased against NaDodSO$_4$–elastin, a more anionic substrate. In experiments where 2 mg of elastin was preincubated with various concentrations of NaDodSO$_4$ (from 0 to 0.5% NaDodSO$_4$) and then exposed to 5 μg of HL elastase (in 0.05 M NH_4HCO_3, pH 8.4), enhancement of elastolytic activity was seen. Prior to addition of enzyme, NaDodSO$_4$-treated elastin was washed twice with distilled water, suspended in buffer, and transferred to an Eppendorf tube. The optimum NaDodSO$_4$ concentration for preincubation is 0.1%, which is similar to values previously described for pancreatic elastase (Kagan et al., 1972). However, the amount of stimulation of HL elastase is less than that described for pancreatic elastase against NaDodSO$_4$–elastin, HL elastase activity being increased only 80% above control values while similar NaDodSO$_4$ treatment of elastin leads to a 3.5–6-fold increase in pancreatic elastase activity. Thus, electrostatic attraction between HL elastase and elastin does influence enzyme attraction and subsequent activity, but to a smaller degree than that seen with pancreatic elastase. That this stimulation of HL elastase is due to the interaction of NaDodSO$_4$ with substrate and not enzyme was demonstrated by the results of experiments in which HL elastase (5 μg) was incubated with various concentrations of NaDodSO$_4$ prior to addition of substrate. NaDodSO$_4$ inactivated HL elastase when the two were mixed. A similar result has been demonstrated for pancreatic elastase (Visser & Blout, 1972; Kagan et al., 1972) and attributed to enzyme conformational changes induced by the detergent.

Effects of Basic Proteins on Elastolysis. [^{3}H]Elastin (2 mg) was added to mixtures of HL elastase (5 μg) and varying amounts of basic proteins in 2 M Tris-HCl (pH 8.0). The release of ^{3}H after 3 h was measured, corrected for background release of ^{3}H, and compared with the cpm released by 5 μg of HL elastase alone. The results of these experiments are shown in Figure 2 and demonstrate that PF_4, the lysine-rich histone IIIs, and poly(L-lysines) of varying chain lengths stimulate HL elastase activity against [^{3}H]elastin. A minimum chain length of eight residues appears to be necessary for poly(L-lysines) to stimulate HL elastase, since tetralysine does not stimulate the enzyme, and the degree of stimulation is dependent on the size of the basic ligand tested. The longer the lysine polymer length (above 4), the greater the stimulation of enzyme activity. Despite the fact that PF_4 has a cluster of only four lysines near its carboxy terminus (Hermodson et al., 1977), it demonstrates activity comparable to a lysine polymer length of 4000–6000. Histone IIIs demonstrates activity comparable to a lysine polymer length of 3400. Poly(L-arginine) (M_r 13 800) resulted in moderate inhibition of HL elastase activity, demonstrating that not all basic ligands stimulate HL elastase elastolytic activity.

Similar results were found in assays done on elastin–agar plates. In elastin plate assays, either adding mixtures of enzyme and ligand or preinfusing ligands and then adding enzyme to the well resulted in HLE stimulation or, in the case of polyarginine, inhibition.

Studies of Lysine-Rich Ligand Binding to Elastin. Data regarding the adsorption of lysine-rich ligands onto reduced BLNE are shown in the left panel of Figure 3. The binding of each ligand is 10–15% greater when native BLNE is used,

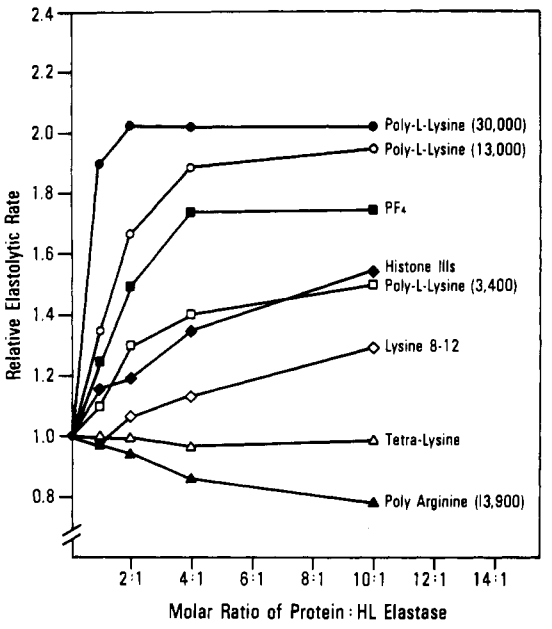

FIGURE 2: Effect of various basic ligands on the elastolytic activity of 5 μg (0.17 nmol) of HL elastase. Ligands and enzyme are mixed and incubated at 25 °C for 30 min in 0.2 M Tris, pH 8.0, prior to addition of [^{3}H]elastin.

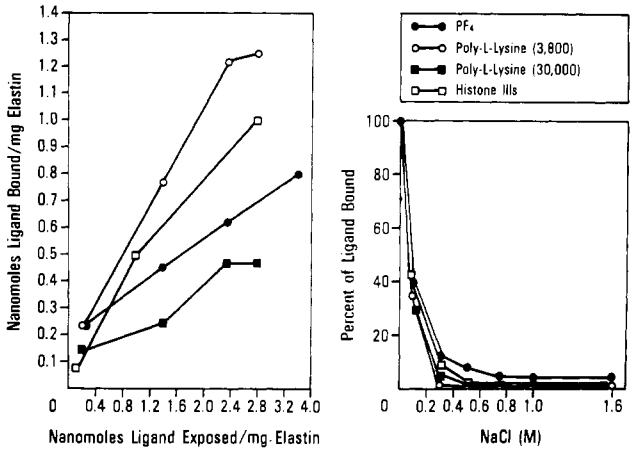

FIGURE 3: (Left panel) Amount of lysine-rich ligand bound to reduced elastin as ligand concentration is increased; (right panel) effect of increasing ionic strength on lysine-rich ligand binding. Experimental conditions are as described under Methods.

explaining why these experiments show 20% less binding of poly(L-lysine) to elastin than was found when Gertler (1971) used native BLNE as the substrate. The experiment detailed in the right panel of Figure 3 shows that each ligand binds to elastin via electrostatic forces, since binding is significantly diminished by NaCl concentrations of 0.1 M and virtually eliminated at salt concentrations of 0.3 M and above. In a separate experiment, BLNE was incubated with various poly(L-lysines) and then washed before being exposed to ^{125}I-labeled PF_4. The preadsorption of poly(L-lysine) to the insoluble substrate results in a marked drop in ^{125}I-labeled PF_4 binding (Figure 4), indicating that these lysine-rich ligands compete for binding sites on elastin. The longer chain length poly(L-lysine) results in a greater loss of PF_4 binding sites, indicating that multiple lysine residues are capable of attaching to the substrate.

Effects of Prebound Ligand on HL Elastase Binding and Activity. We hypothesized that the binding of lysine-rich ligands to elastin might be important for the observed stimulation of HL elastase and conducted studies in which ligands

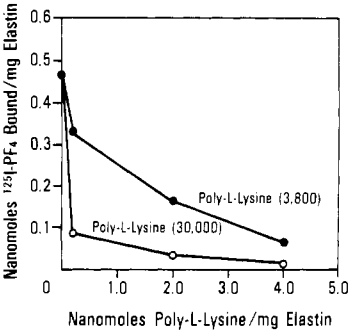

FIGURE 4: Binding of ^{125}I-labeled PF_4 to reduced elastin after elastin has been exposed to the concentrations of poly(L-lysine) indicated on the abscissa. Poly(L-lysines) were bound in H_2O, and ^{125}I-labeled PF_4 binding was performed in 0.02 M phosphate (pH 7.2).

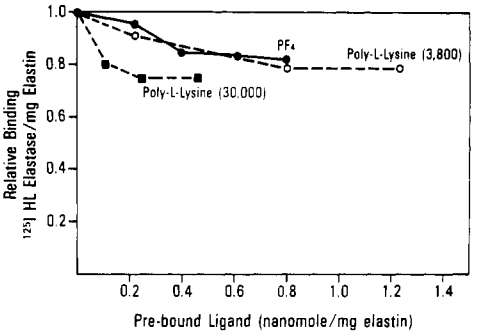

FIGURE 5: Relative binding of 5 μg of ^{125}I-labeled HL elastase to reduced elastin which has been precoated with lysine-rich ligands. HL elastase binding was performed in 0.2 M Tris, pH 8.0, at 4 °C for 2 min as described under Methods. Untreated reduced elastin binds 0.52 μg of ^{125}I-labeled HL elastase/mg.

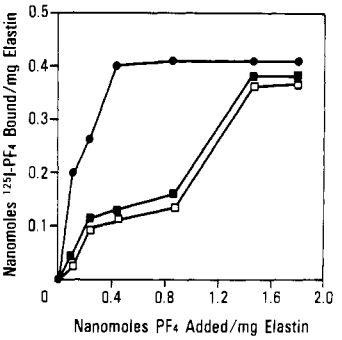

FIGURE 6: Binding of ^{125}I-labeled PF_4 to reduced elastin when ^{125}I-labeled PF_4 is exposed to elastin (●) or when ^{125}I-labeled PF_4 is mixed with 0.2 nmol of active (■) or PMSF-inactivated (□) HL elastase. The amount of ^{125}I-labeled PF_4 in the binding mixtures is shown on the abscissa. Binding was at 37 °C for 30 min in 0.2 M Tris in 0.1 M NaCl, pH 8.0.

were prebound to the substrate. When elastin is precoated with lysine-rich ligands and then exposed to ^{125}I-labeled elastase, HL elastase binding is decreased (Figure 5). Binding of HL elastase to elastin precoated with poly(L-lysine) (M_r 30000) is diminished 25%, and for the lower molecular weight poly(L-lysine) (M_r 13000), PF_4, and histone IIIs (not shown), enzyme binding is diminished 15%. Further evidence of competition between HL elastase and lysine-rich ligands for binding sites on elastin comes from experiments in which 2 mg of insoluble elastin substrate is suspended in solutions of 0.2 M Tris, pH 8.0, containing ^{125}I-labeled PF_4 alone or ^{125}I-labeled PF_4 mixed with 5.0 μg of either active or PMSF-inactivated HL elastase (Figure 6). It can be seen that either active or PMSF-inactivated HL elastase results in a decrease in ^{125}I-labeled PF binding to the substrate. This decrease in PF_4 binding to BLNE in the presence of HL

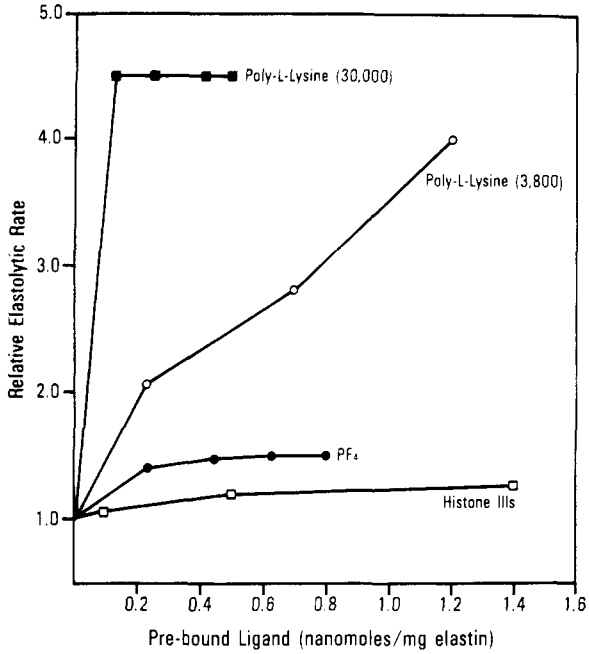

FIGURE 7: Effect of prebound lysine-rich ligands on the suitability of [^{3}H]elastin as substrate for 5 μg of elastase. Binding of ligands was performed in H_2O, and hydrolysis was performed in 0.2 M Tris, pH 8.0 at 37 °C, for 3 h. 5 μg (0.17 nmol) of HL elastase results in the release of 1.4×10^3 cpm/mg of elastin in 3 h.

elastase is seen until a 10-fold molar excess of PF_4 is present in the binding mixture.

When elastin which has been precoated with lysine-rich ligands is exposed to HL elastase (in 0.2 M Tris, pH 8.0) and incubated at 37 °C for 3 h, a significant enhancement of elastolysis is seen (Figure 7). HL elastase activity is increased to a greater extent when elastin is precoated with lysine-rich ligands with larger molecular weights and longer chain lengths. Thus, the adsorption of 0.2 nmol of poly(L-lysine) (M_r 30000) increased enzyme activity to 4.5 times control values, while the same amount of prebound poly(L-lysine) (M_r 3800) results in only a 2-fold stimulation. Prebound PF_4 results in a 1.5 times increase in HL elastase activity, less than is seen when PF_4 and elastase are mixed and then exposed to elastin substrate (Figure 2).

Effect of Ionic Strength on Stimulation of HL Elastase by Lysine-Rich Ligands. The data thus far described suggest that the stimulation of HL elastase activity against insoluble elastin by lysine-rich ligands can be accounted for by the binding of ligand to elastin. To determine whether this mechanism applies to conditions in which enzyme and ligand are first mixed together and then exposed to elastin, we conducted experiments in which ligand and enzyme were mixed (at various molar ratios of ligand to enzyme) in 0.2 M Tris (pH 8.0) with varying concentrations of NaCl for 30 min at 25 °C prior to the addition of tritiated substrate. Following substrate addition, the mixtures were incubated at 37 °C for 3 h, and the radioactivity released was measured. Control tubes, containing HL elastase and [^{3}H]elastin at various salt concentrations, were also run, and the effect of ligand addition was compared with these controls. Data for poly(L-lysines) and histone IIIs were similar, and a representative curve for poly(L-lysine) (M_r 30000) is shown in Figure 8. In buffer alone, poly(L-lysine) results in a 2-fold stimulation of HL elastase. As the NaCl concentration is increased, this stimulation of HL elastase by poly(L-lysine) (M_r 30000) is abolished. At concentrations of NaCl greater than 0.3 M, poly(L-lysines) and histone IIIs fail to stimulate HL elastase against insoluble elastin. Since we

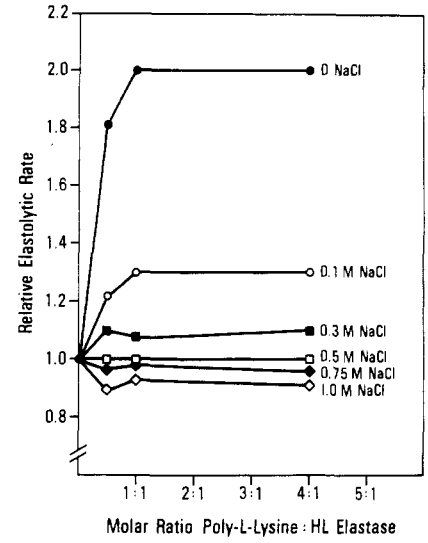

FIGURE 8: Effect of increasing ionic strength on the stimulation of HL elastase by poly(L-lysine) (M_r 30000). Poly(L-lysine) and 5 μg (0.17 nmol) of HL elastase were preincubated at the molar ratios indicated for 30 min at 25 °C in 0.2 M Tris with varying concentrations of NaCl and incubated with [^{3}H]elastin for 3 h at 37 °C. The relative elastolytic rate compares the HL elastase–ligand mixture with HL elastase alone at the same buffer/salt condition.

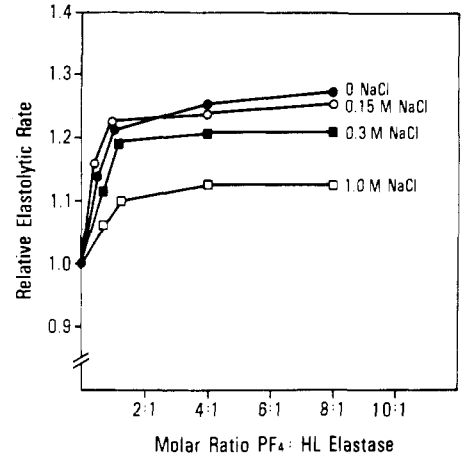

FIGURE 9: Effect of increasing ionic strength on the stimulation of HL elastase by PF_4. PF_4 and HL elastase were preincubated at the molar ratios indicated for 30 min at 25 °C in 0.2 M Tris with varying concentrations of NaCl and incubated with [^{3}H]elastin for 3 h at 37 °C.

have found that these ligands do not bind to elastin at salt concentrations above 0.3 M (right panel of Figure 3), it is reasonable to conclude that the binding of these lysine-rich ligands to insoluble elastin is a requirement for HL elastase stimulation and that in the absence of such binding no stimulation of enzyme occurs.

Data obtained when various molar ratios of PF_4 to HL elastase are exposed to [^{3}H]elastin in the presence of increasing NaCl concentrations are shown in Figure 9. In contrast to poly(L-lysines) and histones IIIs, PF_4 is still capable of stimulating HL elastase at salt concentrations as high as 1.0 M. Since we have shown that essentially no PF_4 is bound to elastin at this salt concentration (right panel of Figure 3), this stimulation is independent of PF_4 binding to elastin. The degree of HL elastase stimulation effected by nonadsorbed PF_4 is significantly less than the stimulation of HL elastase seen when PF_4 can be adsorbed onto the substrate (Figure 2). Thus, the majority of HL elastase stimulation by PF_4 occurs because PF_4 is adsorbed onto elastin, similar to poly(L-lysines) and histone IIIs. The stimulation of HL elastase by nonadsorbed

PF_4 is best explained by proposing a direct interaction between HL elastase and PF_4.

Discussion

Elastin is a nonpolar, cross-linked, highly insoluble protein which is resistant to attack by mammalian enzymes. This resistance to proteolysis is an important feature of this structural protein since elastin imparts unique physical properties to a number of organs, including the lungs (Hance & Crystal, 1975). Elastases which can attack elastin are found in both the pancreas and the polymorphonuclear leukocyte, and both enzymes are capable of inducing experimental lesions in mammalian lungs (Senior et al., 1977). The present study demonstrates that elastase from human leukocytes is stimulated when elastin is precoated with lysine-rich ligands, such as PF_4, certain histones, and poly(L-lysines). For each of these cationic ligands, HL elastase stimulation is dependent on ligand–substrate interactions, and for PF_4, there is evidence that some direct ligand–enzyme interaction occurs as well. In addition, HL elastase is stimulated by increasing ionic strength and by precoating the elastin substrate with the anionic detergent sodium dodecyl sulfate.

The ability of an enzyme to cleave elastin is dependent on the adsorption of that enzyme on to the substrate, and the nature of elastase adsorption to elastin is sufficiently strong to allow for the isolation of enzyme–substrate complexes by centrifugation. It was pointed out by Hall & Czerkowski (1961) that many cationic molecules, including multiple enzymes, can bind to elastin but that enzymatic cleavage of elastin will result only if the adsorbed molecule possesses the proper side-chain specificity to be an elastase. In keeping with this hypothesis, cationic molcules such as pancreatic proelastase (Gertler, 1971), tosyl elastase (Kagan et al., 1977), and the lysine-rich ligands studied in these experiments will all bind to elastin without effecting elastolysis. In addition to having the proper side-chain specificity, the adsorbed enzyme must be bound to a susceptible region of the substrate molecule, i.e., a region of elastin which can be cleaved by an elastolytic enzyme. For pancreatic elastase, Kagan et al. (1977) have referred to such regions as productive regions, since the adsorption of elastase to such a region results in a productive scission and the recycling of enzyme for further elastolysis. When elastase is bound to regions of elastin which are not susceptible to scission, enzyme–substrate complexes are formed which are nonproductive and permanent.

The present studies indicate that HL elastase and lysine-rich ligands compete for electrostatically governed binding sites on elastin and that HL elastase is still capable of binding productively to elastin when these sites are occupied by lysine-rich ligands. These results suggest that electrostatically governed sites are nonproductive for HL elastase and that lysine-rich ligands bind preferentially to these sites. Enhancement of elastolysis by these lysine-rich ligands can be explained by the increased likelihood of a productive interaction between HL elastase and elastin when nonproductive sites are occupied by basic ligands. Such enhancement of elastolysis could occur in the absence of increased enzyme binding, as was found in these studies. Support for this hypothesis comes from recent work by Yasutake & Powers (1981) demonstrating that HL elastase prefers to cleave elastin at regions which are close to the desmosine and isodesmosine cross-links. Such areas are rich in the nonpolar amino acid alanine (Foster et al., 1974), and hydrophobic binding would be favored at such sites. Since HL elastase is capable of cleaving elastase at alanine-rich regions (Zimmerman & Ashe, 1977), these cross-linked regions meet the requirement to be a productive site. In addition, such cross-linked regions are likely to be influenced by the positive charge of the pyridinium ring of desmosine and isodesmosine and would not be suitable binding sites for lysine-rich, cationic ligands which bind electrostatically. Indeed, pancreatic elastase does not bind well to these cross-linked regions (Yasutake & Powers, 1981).

In addition to the direct effect which results from the binding of lysine-rich ligands to HL elastase nonproductive sites on elastin, it is likely that the binding of these ligands results in conformational changes in elastin which also increase productive HL elastase binding. Support for this hypothesis comes from work by Kagan et al. (1972), who have shown that the adsorption of anionic detergents onto elastin results in conformational changes favorable for elastolysis, and by Kagan et al. (1977), who have suggested that similar conformational changes occur when tosyl elastase is bound to elastin. In these studies, the failure of poly(L-arginine) to stimulate elastolysis is likely the result of unfavorable conformational changes in the elastin substrate. It is of interest that Boudier et al. (1981) have recently described enhancement of HL elastase activity by cathepsin G. This stimulation is only seen against the native substrate, elastin, and not against synthetic substrates. Since cathepsin G is cationic, this selective activity against elastin might be due to the electrostatic binding of cathepsin G to the substrate, resulting in conformational changes favorable for elastolysis.

It is noteworthy that poly(L-lysine) of 8–12 residues in length is capable of stimulating HL elastase against elastin, while tetralysine does not have this capability. This suggests that there is a minimal number of electrostatic contacts which must form between the ligand and elastin in order to alter the binding profile of HL elastase and results in stimulation. With regard to PF_4, the cationic nature of this protein is dependent on its lysine-rich carboxy terminus (Hermodson et al., 1977) which contains the sequence (...Lys^{61}-Lys-Ile-Ile-Lys^{65}-Lys...), yet PF_4 can express the activity of a much longer lysine polymer. Preliminary circular dichroism data from our laboratory indicate that PF_4 possesses a helical structure, and it is likely that such a helix permits the remainder of the PF_4 molecule to interact with the substrate and confer HL elastase stimulatory activity.

The present experiments also show that free (nonadsorbed) PF_4 has the ability to enhance HL elastase activity against insoluble elastin. Since we have been unable to isolate a PF_4–HL elastase complex by polyacrylamide gel electrophoresis (Lonky & Wohl, 1981), we suspect that the association of PF_4 and HL elastase is one of loose binding, perhaps hydrophobic in nature. Lestinenne & Bieth (1980) have demonstrated that in addition to its scissile substrate binding site, HL elastase possesses a regulatory site which can bind additional substrate, fatty acid, alcohols, or organic molecules. This regulatory site binding is hydrophobic in nature and results in HL elastase stimulation against Suc-Ala_3NA. It is likely that free PF_4, or PF_4 fragments formed after cleavage by HL elastase (Lonky & Wohl, 1981), interacts with this HL elastase regulatory site and results in an enzyme conformational change and stimulation.

In summary, these studies demonstrate that HL elastase activity against insoluble elastin can be increased by anionic detergents such as $NaDodSO_4$, by increasing ionic strength, or by lysine-rich ligands and that stimulation by $NaDodSO_4$ or lysine-rich ligands is dependent on the adsorption of these molecules to elastin. It is reasonable to hypothesize that cationic molecules such as PF_4 or cathepsin G can attach to elastic fibers in vivo and result in enhanced elastolysis by

endogenously released HL elastase. We have recently shown that PF_4 is capable of increasing lung elastic tissue destruction of HL elastase, and data from our laboratory (Lonky et al., 1981) and the laboratories of others (Schneider et al., 1980) have demonstrated accumulation of platelets in acutely injured lungs. It is possible that these platelets can be activated to secrete PF_4, resulting in PF_4–elastin complex formation and a substrate more readily attacked by HL elastase at a later time.

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Registry No. Elastase, 9004-06-2; PF_4, 37270-94-3; $NaDodSO_4$, 151-21-3; poly(L-lysine), 25104-18-1; poly(L-lysine) (SRU), 38000-06-5.

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