Biochimica et Biophysica Acta, 612 (1980) 233—244

BBA 68924

PARTIAL PURIFICATION AND CHARACTERIZATION OF MOUSE PERITONEAL EXUDATIVE MACROPHAGE ELASTASE *

RICHARD R. WHITE **, DAVID NORBY ***, AARON JANOFF and ROSEMARIE DEARING

Department of Pathology, State University of New York, Stony Brook, NY 11794 (U.S.A.)

(Received June 21st, 1979)
(Revised manuscript received October 15th, 1979)

Key words: Elastase; α-2-Macroglobulin; α-1-proteinase inhibitor; (Mouse peritoneal macrophage)

Summary

Mouse peritoneal exudate macrophage elastase can be significantly purified with 60% recovery of the starting activity by affinity chromatography against SDS-treated α-elastin covalently linked to agarose beads. The enzyme has an apparent M_r of 26 500 based on SDS-acrylamide gel electrophoresis. Molecular sieving chromatography on Sephadex gel gives a M_r for macrophage elastase of 21 000—28 000. The enzyme is not inhibited by chloromethyl ketone inactivators specific for pancreatic and leukocyte elastase nor by phenylmethylsulfonyl fluoride. Macrophage elastase also does not bind to tritiated diisopropylphosphorofluoridate. The enzyme is inhibited by EDTA and thus appears to be a metallo-protease. Macrophage elastase is resistant to human α_1-proteinase inhibitor and to human and mouse α_2-macroglobulin. In view of its lack of susceptibility to these endogenous serum proteinase inhibitors, macrophage elastase may play an important role in physiological and pathological remodeling of connective tissues.

Introduction

Elastin is a functionally important connective tissue protein in the lung. Destruction or remodeling of this protein has been implicated in the pathogen-

* Part of this work was presented at the annual American Lung Association Meeting, Las Vegas, NV, 1979 (see Ref. 12).

** To whom reprint requests should be addressed.

*** Present address: Department of Orthopaedics, Case Western Reserve University, Cleveland, OH 44106, U.S.A.

esis of pulmonary emphysema [1—3]. Enzymes capable of degrading lung elastin have been described in several different types of cells. The two most thoroughly characterized elastases are those of the porcine pancrease [4] and the human polymorphonuclear neutrophilic leukocyte [5,6]. In addition, murine alveolar and peritoneal exudative macrophages have been shown to secrete an enzyme capable of hydrolysing particulate elastin [7,8]. The role of macrophage elastase in the remodeling of lung elastin in pulmonary emphysema is unclear. Studies on the participation of macrophage elastase in the physiological and pathological turnover of elastin would be greatly facilitated by the purification and characterization of this enzyme. Several preliminary reports have recently appeared describing the purification and partial characterization of an elastolytic enzyme from macrophages [9—12]. In these studies, purification was achieved by a combination of several chromatographic procedures. In the present paper, we describe a single step affinity chromatography procedure for the purification of elastase from the culture medium of mouse peritoneal exudative macrophages. In addition, the effects of various naturally occurring and synthetic inhibitors on macrophage elastase are described.

Materials and Methods

Materials. Amicon PM-10 diaflo membranes and chambers were purchased from Amicon Corporation (Lexington, MA). Agarose A-5M and rabbit anti-human α_2-macroglobulin antiserum were obtained from BioRad Laboratories (Rockville, NY). Rabbit anti-human α_1-proteinase inhibitor antiserum was purchased from Accurate Chemical and Scientific Corp. (Hicksville, NY). Sephadex G-50 superfine was purchased from Pharmacia Fine Chemicals (Piscataway, NJ). α-Elastin covalently linked to agarose beads, particulate bovine neck ligament elastin (<400 mesh), and porcine pancreatic elastase were purchased from Elastin Products (St. Louis, MO). Human polymorphonuclear neutrophil elastase was purifed as described before [6]. Bacto-agar and Dulbecco's modified Eagle's minimal medium were purchased from GIBCO (Grand Island, NY). Male DBA/2 mice were purchased from Jackson Laboratories (Bar Harbor, ME). The chloromethyl ketone inhibitors: Ac-$(Ala)_4$-CH_2Cl, Ac-$(Ala)_2$-Pro-Ala-CH_2Cl, Ac-Ala-Pro-Val-Ala-CH_2Cl, and MeOSucc-$(Ala)_2$-Pro-Val-CH_2Cl were gifts from Dr. J.C. Powers (Atlanta, GA). α_1-Proteinase inhibitor was purchased from Worthington Biochemical Corp. (Freehold, NJ). Thioglycolate medium was from Difco Laboratory (Detroit, MI). Disposable tissue culture plasticware was purchased from Fisher Chemical Company (St. Louis, MO). Soybean trypsin inhibitor, phenylmethylsulfonyl fluoride, lactalbumin hydrolysate, sodium dodecyl sulphate (SDS), EDTA, crosslinked bovine hemoglobin, succinyl-L-alanyl-L-alanyl-L-alanyl-*p*-nitroanilide, and all other reagents (unless otherwise specified) were purchased from Sigma Chemical Co. (St. Louis, MO). Tritiated diisopropylphosphorofluoridate was from New England Nuclear (Boston, MA).

Methods

Cell culture. Peritoneal exudative macrophages were collected from male DBA/2 mice, 9—15 weeks of age, by a modification of the method of Stewart

et al. [13]. Mice were injected intraperitoneally with 2.0 ml 3.0% thioglycolate medium. Inflammatory cells were harvested 4 days later by saline lavage. After harvesting, the cells were washed twice in saline, resuspended in Dulbecco's modified Eagle's minimal medium containing 15% fetal calf serum, and cultured at 37°C in 75-cm^2 culture flasks at a density of $10 \cdot 10^6$ cells/flask in a volume of 10 ml. All incubations were conducted in a humidified environment containing 10% CO_2 and 90% room air. At 2 h, and again at 24 h, the cultures were washed with Dulbecco's modified Eagle's minimal medium containing 15% fetal calf serum to remove non-adherent cells.

Preparation of conditioned medium. Conditioned medium was the source of the macrophage elastase, and was prepared as follows. 2-day old monolayer cultures of macrophages were placed in Dulbecco's modified Eagle's minimal medium containing 0.2% lactalbumin hydrolysate. Over the course of the next 2 weeks, the medium was collected at 2-day intervals, and replaced with an equal volume of fresh medium [8]. The pooled medium was clarified by centrifugation at $500 \times g$ for 15 min and then dialyzed in the cold against several changes of 50 mM Tris-maleate buffer (pH 7.6) containing 200 mM NaCl and 5 mM $CaCl_2$. After dialysis, the conditioned medium was concentrated 10—20-fold by ultrafiltration through a PM-10 Amicon Diaflo membrane under 50 lb/$inch^2$ of nitrogen and stored at 4°C.

Elastase assay. Elastase was routinely assayed using SDS-treated, particulate (<400 mesh) bovine ligament elastin (SDS/elastin, 1 : 4) suspended in agar [7,8].

Preparation of α-elastin column for affinity chromatography. A slurry of α-elastin (oxalic acid-solubilized elastin) covalently linked to agarose beads was mixed with SDS at a ratio of SDS/α-elastin (1 : 16, w/w) and stirred gently at 22°C. After 24 h, the SDS-treated beads were placed in a 0.5×35 cm glass column. After settling for 24 h at 4°C, the column was equilibrated with 50 mM Tris-maleate buffer (pH 7.6) containing 5 mM $CaCl_2$ and 200 mM NaCl.

Sephadex chromatography. Sephadex G-50 superfine was used to determine the molecular weight of macrophage elastase. A 1.0×20 cm column of gel was equilibrated at 4°C with 50 mM Tris-maleate buffer (pH 7.6) containing 5 mM $CaCl_2$ and 200 mM NaCl. Ovalbumin (M_r 44 000), soybean trypsin inhibitor (M_r 22 500) and cytochrome *c* (M_r 12 500) were dissolved in the same buffer and used as molecular weight standards. Protein content of each fraction was measured at 280 nm on a Uvicord I((LKB Inst., Hicksville, NY). Each fraction was assayed for elastase activity by the method described above.

Polyacrylamide gel electrophoresis. Polyacrylamide gel electrophoresis was performed by the method of Porzio and Pearson [14]. Each gel was pre-electrophoresed for 12 h at 1 mA per gel in 200 mM Tris-glycine buffer (pH 8.8) with or without 0.1% SDS. No sample or stacking gel were employed. For determination of molecular weight, cross-linked bovine hemoglobins of M_r 16 000, 32 000, 48 000 and 64 000 were dissolved in 0.025 M Tris buffer (pH 7.4) containing 0.2% SDS and were used as standards.

Human serum was electrophoresed in 5.0% polyacrylamide gels, pH 8.5, by the method of Davis [15].

Purification of α_2-macroglobulin. α_2-Macroglobulin was purified from the serum or plasma of DBA/2 mice and human volunteers by a modification of

the method of Roberts et al. [16]. Purity of the α_2-macroglobulin preparations was monitored by immunoelectrophoresis. Activity of the purified inhibitor was evaluated by measuring its capacity to preserve the hydrolytic activity of porcine pancreatic elastase towards succinyl-L-alanyl-L-alanyl-L-alanyl-*p*-nitroanilide, in the presence of excess α_1-proteinase inhibitor.

Inhibition experiments

Direct assays. Soybean trypsin inhibitor, α_1-proteinase inhibitor and α_2-macroglobulin were mixed with samples of partially purified macrophage elastase and preincubated at 22°C for 60 min prior to assaying for enzyme activity in elastin-agar gels as described above. The chloromethyl ketone inhibitors, Ac-$(Ala)_4$-CH_2Cl, Ac-Ala-Pro-Val-Ala-CH_2Cl and MeOSucc-$(Ala)_2$-Pro-Val-CH_2Cl, were dissolved in 50 mM Tris-maleate buffer (pH 7.6) containing 5 mM $CaCl_2$ and 200 mM NaCl. These inhibitors were then mixed with partially purified macrophage elastase and incubated at 22°C for 60 min prior to assaying for elastase activity. EDTA (disodium salt) was incorporated into the elastase assay plate at a concentration of 30 mM. Phenylmethylsulfonyl fluoride was dissolved in dimethylsulfoxide at a concentration of 120 mM, and was then diluted in Dulbecco's modified Eagle's minimal medium and lactalbumin hydrolysate containing macrophage elastase. Preincubation of phenylmethyl sulfonyl fluoride with elastase was for 60 min at 37°C. The inhibitor-enzyme solution was then dialysed overnight against 50 mM Tris-maleate buffer containing 200 mM NaCl and 5 mM $CaCl_2$. The diffusate was then assayed for elastase activity.

Competition assays. In addition to direct inhibition assays, α_2-macroglobulin was also tested indirectly for its ability for form complexes with macrophage elastase (and other secreted macrophage proteases). Concentrated conditioned medium (100 µl) was mixed with 100 µl α_2-macroglobulin (30 µg). After 15 min incubation at 22°C, 50 µl porcine pancreatic elastase (0.5 µg) was added and the incubation continued for an additional 15 min. An excess of α_1-proteinase inhibitor (50 µg in 50 µl) was added to completely inhibit any free porcine pancreatic elastase present in the reaction mixture. After an additional 15 min incubation, the mixture was assayed for porcine pancreatic elastase by measuring the release of *p*-nitroaniline from succinyl-L-alanyl-L-alanyl-L-alanyl-*p*-nitroanilide at 410 nm. In the presence of excess α_1-proteinase inhibitor any release of *p*-nitroanilide is due to porcine pancreatic elastase present in complexes with α_2-macroglobulin [16—18]. Complex formation between porcine pancreatic elastase and α_2-macroglobulin alone was compared to that between porcine pancreatic elastase and α_2-macroglobulin preincubated with macrophage conditioned medium. If macrophage enzymes present in the conditioned medium were able to form complexes with α_2-macroglobulin, the subsequent ability of α_2-macroglobulin to form complexes with porcine pancreatic elastase would be diminished and this would be reflected in a decreased rate of hydrolysis of succinyl-L-alanyl-L-alanyl-L-alanyl-*p*-nitroanilide. Controls were included to demonstrate that, under identical conditions, trypsin (which is known to form complexes with α_2-macroglobulin) would diminish the capacity of α_2-macroglobulin to bind porcine pancreatic elastase. Finally, similar competition experiments were conducted to determine if

macrophage elastase (or any other enzyme in the conditioned medium) would form complexes with human α_1-proteinase inhibitor.

Serum assays. 15 µl human serum were fractionated by electrophoresis on 5% polyacrylamide tube gels at pH 8.5. After electrophoresis, the gel was frozen and sliced into 1-mm discs. Each disc was eluted overnight at 37° C in 100 µl Tris-maleate buffer (pH 7.6) containing 5 mM $CaCl_2$ and 200 mM NaCl. The eluate from each gel disc was mixed with an equal volume of concentrated conditioned medium from peritoneal macrophages or with 5 ng porcine pancreatic elastase. After 15 min incubation at 22° C the mixtures were assayed for elastase activity on SDS-elastin agar assay plates. Immunodiffusion analysis was also performed on each gel disc eluate with rabbit antiserum to human α_1-proteinase inhibitor or α_2-macroglobulin, to identify the gel fractions which contained these serum inhibitors.

Experiments with [^{3}H]diisopropylphosphorofluoridate. 10 µl of a solution of tritiated diisopropylphosphorofluoridate containing 0.25 mCi ^{3}H/0.046 mg were added to 1 ml of concentrated conditioned medium or partially purified macrophage elastase and incubated at 37° C for 24 h. The mixtures were then dialyzed extensively against 25 mM Tris buffer (pH 7.4) and subsequently electrophoresed at pH 8.8 on 10% polyacrylamide gels containing 0.1% SDS, according to the procedure of Porzio and Pearson [14]. After electrophoresis, the gels were frozen at —70° C and sliced into 1-mm sections, each of which was then eluted overnight at 37° C with 4.5 ml Econofluor containing 3.0% Protosol (New England Nuclear, Boston, MA). The eluates were counted on a Searle Mark II liquid scintillation counter (Des Plaines, IL).

Results

Purification

Typical results of affinity chromatography are shown in Fig. 1. Macrophage elastase does not bind to SDS-treated α-elastin. It is, instead, retarded during its passage through the column so that the bulk of enzyme activity elutes after the major protein peak. Collection and concentration of fractions 40—65 in Fig. 1 yielded a clear solution containing about 60% of the elastase activity of the concentrated conditioned medium applied to the column. Densitometry scanning of analytical SDS-acrylamide gel electrophoretograms of starting conditioned medium and partly purified enzyme revealed significant purification. Typical gel patterns are shown in Fig. 2A and B.

Although the partly purified macrophage elastase contained traces of other proteins (see Fig. 2B), the enzyme preparation was free of serine proteases. This was shown as follows. Crude macrophage-conditioned medium was incubated with [^{3}H]diisopropylphosphorofluoridate, dialyzed and subjected to polyacrylamide gel electrophoresis. Three prominent peaks of radioactivity were observed, presumably associated with serine proteases. When the partly purified macrophage elastase was identically treated, no radioactivity could be recovered from the gel.

The partially purified enzyme preparations contained about half of the original elastase activity found in the starting conditioned medium before concentration. The ratio of specific activity of purified enzyme to specific activity

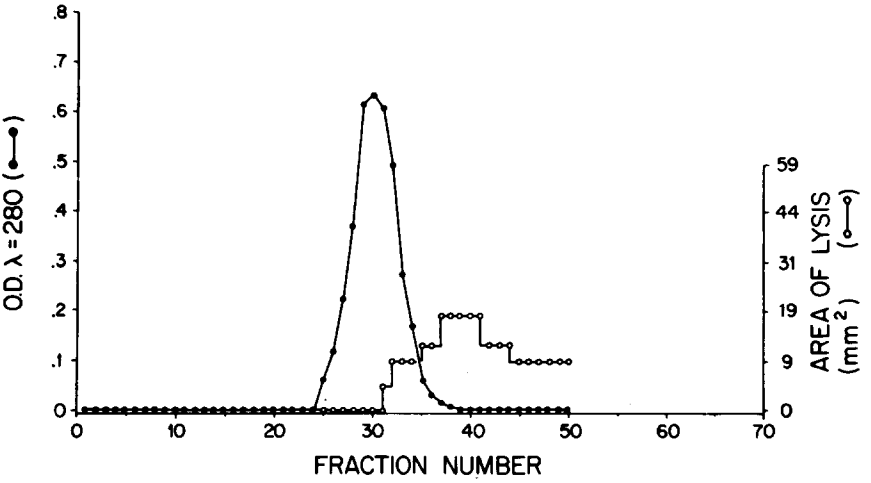

Fig. 1. Affinity chromatography of peritoneal exudative macrophage conditioned medium on SDS-treated α-elastin covalently linked to agarose beads. The closed circles represent protein concentration in the column effluent and the open circles correspond to elastase activity of the effluent fractions in the SDS-elastin agar plate assay. Measurements of protein and enzyme activity were not performed beyond fraction 50 in this experiment. Fractions 40—65 were pooled for further study of macrophage elastase.

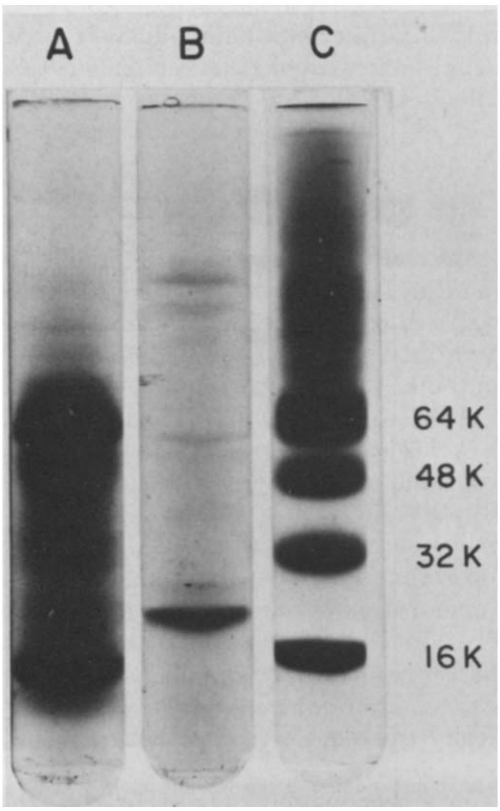

Fig. 2. 0.1% SDS/10% polyacrylamide gels, stained with Coomassie blue 250G. The samples were electrophoresed at 1 mA/gel at pH 8.8 in 200 mM Tris-glycine buffer containing 0.1% SDS. (a), crude conditioned medium; (b), partially purified macrophage elastase fractions; (c), crosslinked-hemoglobin molecular weight marker proteins.

of starting conditioned medium showed that a 1300-fold enrichment of elastase was obtained by the affinity chromatography procedure. Activity of the purified enzyme was retained for up to 6 months during storage at 4°C.

Determination of molecular weight

In SDS-acrylamide gel electrophoresis the partially purified elastase migrated with a mobility between that of M_r 16 000 and M_r 32 000 cross-linked bovine hemoglobins (Fig. 2, B and C. A molecular ratio of 26 500 was calculated for macrophage elastase, based on its mobility in SDS-acrylamide gels.

During molecular-sieving chromatography in Sephadex G-50, macrophage elastase eluted from the column between ovalbumin and cytochrome *c*, and just ahead of soybean trypsin inhibitor. On the basis of its elution volume in Sephadex, the M_r of macrophage elastase lies between 21 000 and 28 000. This indicates that none of the trace components present in the purified enzyme preparation, except for the one migrating just behind the main band (Fig. 2B), could be the macrophage elastase since these trace components all have $M_r > 32\,000$ (compare gels B and C in Fig. 2).

Studies with inhibitors

A variety of low molecular weight inhibitors were tested for their effects on macrophage elastase in the elastin-agar assay system and the results are shown in Table I. In addition, a variety of naturally-occurring inhibitors were tested for their effects on macrophage elastase in the same assay system and the

TABLE I

INHIBITION OF VARIOUS ELASTOLYTIC ENZYMES BY SYNTHETIC AND NATURALLY-OCCURRING INHIBITORS USING THE SDS-ELASTIN AGAR ASSAY

Inhibitor-enzyme mixtures were preincubated for 60 min at 22°C prior to assaying. The concentrations shown are the final concentrations. The enzyme-inhibitor mixtures employing phenylmethylsulfonyl fluoride were incubated for 1 h at 37°C and were then extensively dialyzed against 50 mM Tris-maleate plus 200 mM NaCl and 5 mM $CaCl_2$ prior to assaying. Serum albumin (rabbit) was twice recrystallized (Sigma Chemical Co., St. Louis, Mo). +, complete inhibition; ±, partial inhibition; —, no inhibition.

Inhibitor	Final inhibitor concentration	Porcine pancreatic elastase	Human leukocyte elastase	Mouse macrophage elastase
Ac-$(Ala)_4$-CH_2CL	5 mM	+	+	—
Ac-$(Ala)_2$-Pro-Ala-CH_2CL	5 mM	+	+	—
Ac-Ala-Pro-Val-Ala-CH_2CL	5 mM	+	+	—
MeOSucc-$(Ala)_2$-Pro-Val-CH_2CL	10 mM	+	+	—
Phenylmethylsulfonyl fluoride	40 mM	+	+	—
EDTA (Disodium salt)	30 mM	—	±	+
Soybean trypsin inhibitor	8 mg/ml	—	+	—
α_1-proteinase inhibitor (human)	50 μg/ml	+	+	—
α_2-macroglobulin (human)	1.25 mg/ml	+	+	—
(mouse)	1.25 mg/ml	+	+	—
whole serum (human)				
normal (PiMM)	5% (v/v)	+	+	+
α_1-proteinase inhibitor-deficient (PiZ)	5% (v/v)	+	+	+
serum albumin	30 μg/ml	—	—	±
	60 μg/ml	—	—	±
	3.0 mg/ml	—	—	+

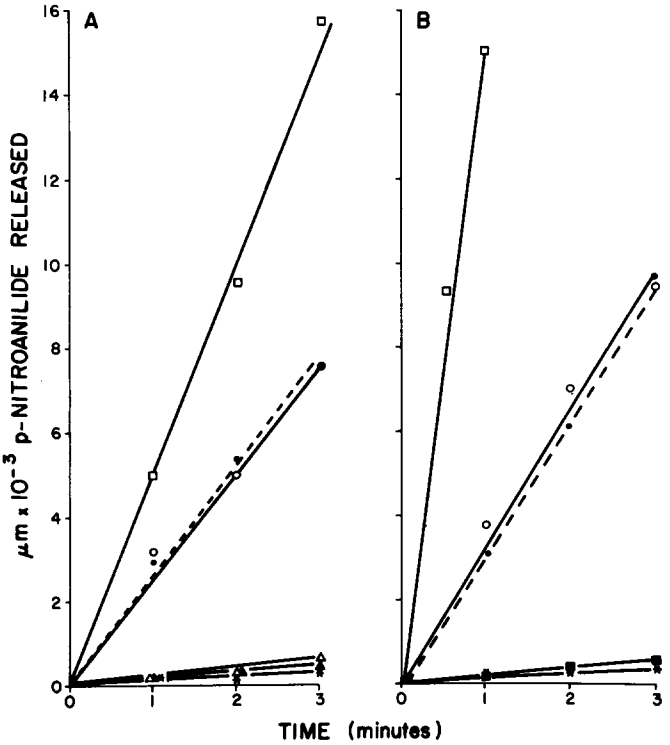

Fig. 3. Analysis of the binding of macrophage elastase to α_1-proteinase inhibitor or α_2-macroglobulin. Panel A: The effects of preincubation with macrophage conditioned medium on the porcine pancreatic elastase binding-capacity of mouse α_2-macroglobulin. Residual porcine pancreatic elastase activity was measured using succinyl-L-alanyl-L-alanyl-L-alanyl-*p*-nitroanilide as substrate in 0.2 M Tris buffer (pH 8.0) at 22° C. (*), substrate hydrolysis in the absence of all other reagents; (▲), substrate plus α_1-proteinase inhibitor; (△), substrate plus α_1-proteinase inhibitor plus α_2-macroglobulin; (■), substrate plus porcine pancreatic elastase; (○), α_2-macroglobulin plus porcine pancreatic elastase plus α_1-proteinase inhibitor plus substrate; (●), conditioned medium plus α_2-macroglobulin plus porcine pancreatic elastase plus α_1-proteinase inhibitor plus substrate. Sequence and amounts of reagents added, incubation times and principles of the assay method are described in greater detail under Methods. Panel B: The effect of preincubation with macrophage conditioned medium on the porcine pancreatic elastase binding-capacity of human α_1-proteinase inhibitor (50 μg). (*), substrate hydrolysis in the absence of all other reagents; (■), substrate plus conditioned medium; (□), substrate plus porcine pancreatic elastase; (○), α_1-proteinase inhibitor plus porcine pancreatic elastase plus substrate; (●), conditioned medium plus α_1-proteinase inhibitor plus porcine pancreatic elastase plus substrate. Concentration of all reactants (except α_1-proteinase inhibitor) as in Panel A.

results are also given in Table I. Soybean trypsin inhibitor, at a concentration as high as 8 mg/ml, was ineffective at inhibiting macrophage elastase, although, at this concentration it completely inhibited human leukocyte elastase. Human α_1-proteinase inhibitor and human and mouse α_2-macroglobulin at concentrations which completely inhibited human leukocyte elastase and porcine pancreatic elastase also failed to inhibit the macrophage enzyme.

Inhibition of macrophage elastase by α_1-proteinase inhibitor and α_2-macroglobulin was also studied by observing whether the conditioned medium from peritoneal exudative macrophages could compete with porcine pancreatic elastase for binding to the two serum inhibitors (Fig. 3). It can be seen in Panel A of the figure that preincubation of 30 μg mouse α_2-macroglobulin with

100 μl concentrated conditioned medium from macrophages did not result in a measurable reduction in the capacity of α_2-macroglobulin to form complexes with porcine pancreatic elastase, upon subsequent addition of the latter enzyme (compare curves ○———○ and ●———● in panel A of Fig. 3). Under identical conditions, 5 μg trypsin preincubated with 30 μg mouse α_2-macroglobulin caused 100% reduction in the capacity of the inhibitor to subse-

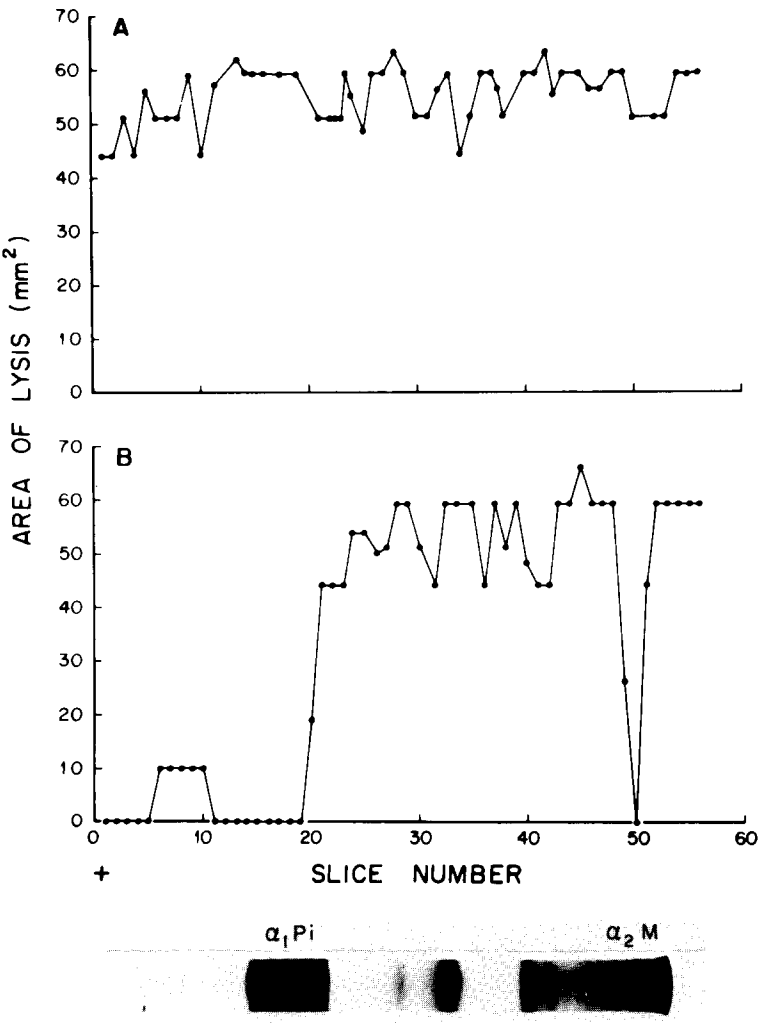

Fig. 4. Top: Elastase inhibitory capacity of electrophoretically fractionated human serum. Whole human serum was electrophoresed on a 5% polyacrylamide gel at pH 8.5. The gel was then frozen, sliced and the slices eluted in 50 mM Tris-maleate buffer (pH 7.6) containing 5 mM $CaCl_2$ and 200 mM NaCl. Each point shown represents the elastase inhibitor activity of the eluate from a single slice. Panel A: inhibition pattern for macrophage elastase. Panel B: inhibition pattern for porcine pancreatic elastase. Bottom: Typical electrophoretic banding pattern of whole human serum electrophoresed on a 5% polyacrylamide gel and stained with Coomassie blue 250G. Regions of the gel containing α_1-proteinase inhibitor and α_2-macroglobulin were identified by immunodiffusion against specific antisera to these inhibitors.

quently form complexes with porcine pancreatic elastase. Similar results were obtained in experiments with human α_1-proteinase inhibitor as the inhibitor (Panel B, Fig. 3).

Although macrophage elastase appeared not to form inhibitory complexes with α_1-proteinase inhibitor or α_2-macroglobulin, whole human serum (either normal or α_1-proteinase inhibitor-deficient) was capable of suppressing the elastolytic activity of macrophage culture fluids in the elastin-agar assay (Table I). Two possible explanations for this apparent discrepancy were tested further. First, since another serum inhibitor(s) besides α_1-proteinase inhibitor and α_2-macroglobulin might be responsible for the suppression of macrophage elastase observed with whole serum, whole human serum was fractionated by polyacrylamide gel electrophoresis and all serum fractions were then tested for inhibition of both the macrophage and the porcine pancreatic elastase in elastin-agar assays. The results are given in Fig. 4. Inhibitory activity against porcine pancreatic elastase was observed in two serum fractions and could be correlated with the presence of α_1-proteinase inhibitor or α_2-macroglobulin in these fractions, as determined by immunodiffusion against their respective antisera. No fraction of the serum, however, gave inhibition of macrophage elastase, including fractions containing α_1-proteinase inhibitor or α_2-macroglobulin (Fig. 4).

A second possibility was that the inhibition of macrophage elastase by whole serum was due to nonspecific effects of high concentrations of protein in the elastin-agar assay. When rabbit serum albumin was substituted for human serum over a wide range of protein concentrations, it was found that 3 mg/ml albumin completely suppressed the activity of macrophage elastase in elastin-agar assays (partial inhibition occurred at concentrations as low as 30 μg/ml). Thus, suppression by 5% whole human serum (protein concentration, 3 mg/ml) may have been due to a similar nonspecific effect of serum proteins.

Discussion

The foregoing results show that mouse peritoneal exudate macrophage elastase can be conveniently purified by a one-step affinity chromatography procedure employing SDS-treated α-elastin covalently linked to agarose beads. Treatment of the affinity ligand with SDS improved recovery to 60% of starting activity with a high degree of purification. Concentrated conditioned medium of macrophage monolayer cultures provides a convenient starting source of enzyme, since these cells continuously secrete elastase in culture over a period of several weeks [7,8] and do not require serum in the culture medium for secretion to occur. Thus, a major source of contaminating proteins can be avoided at the outset.

As shown in Table I, the macrophage enzyme was not inhibited by any of the chloromethyl ketone inhibitors of porcine pancreatic elastase and human leukocyte elastase, even after 60 min preincubation. The inhibition of macrophage elastase by EDTA and the lack of inhibition by phenylmethylsulfonyl fluoride support the conclusion that macrophage elastase is a metallo-protease, as was recently suggested by others [7,9—11].

Of particular interest is the apparent lack of inhibition of macrophage

elastase by α_2-macroglobulin of either mouse or human origins or by human α_1-proteinase inhibitor. It was previously reported that α_1-proteinase inhibitor is a poor inhibitor of murine macrophage elastase [8], however, the limitations of the radial diffusion assay employed in the previous study made interpretation of the results difficult. In the present study, this question has been re-examined employing another assay system. Since the saturation or enzymatic modification of either α_2-macroglobulin or α_1-proteinase inhibitor by a protease prevents the subsequent binding of any additional proteases [19—23], competition experiments were utilized to answer this question. The results of these competition experiments (Fig. 3) support the conclusion that macrophage elastase does not bind to either inhibitor (moreover, since the experiments were performed with crude conditioned medium, it may be that none of the extracellular proteases present in the macrophage culture fluids form complexes with these inhibitors). Although some other endopeptidases of narrow substrate specificity are similarly unaffected by α_1-proteinase inhibitor and α_2-macroglobulin [24—27], this is not the case for other mammalian elastases. Thus the foregoing results demonstrate a rather unique property for macrophage elastase.

Despite the fact that neither α_1-proteinase inhibitor or α_2-macroglobulin inhibited macrophage elastase, sera of either the PiZ or PiMM phenotypes (at concentrations as low as 5%) completely inhibited this enzyme in elastin-agar assays (Table I). Studies with electrophoretically-fractionated whole human serum appeared to rule out another serum inhibitor as the responsible agent (Fig. 4). While it is possible that serum inhibitors were inactivated during electrophoresis or by freezing and thawing of the gel, these procedures did not destroy the functional capacity of serum α_1-proteinase inhibitor or α_2-macroglobulin to inhibit porcine pancreatic elastase (Fig. 4). Our experiments with albumin (Table I) suggest that nonspecific effects of serum proteins may have been responsible for the suppression of macrophage elastase activity by whole serum in the elastin-agar assay.

Elastolytic enzymes have been implicated in the pathogenesis of emphysema [2,28,29]. Since the numbers of alveolar macrophages are increased in heavy cigarette smokers [30—32], and the increased concentrations of these cells occur in areas of the lung in which emphysema frequently develops [32], macrophages have been considered to be a potential source of lung-damaging enzymes in this disease. Despite the low substrate affinity of macrophage elastase for elastin [7], the relative insensitivity of this enzyme to endogenous serum inhibitors could result in a prolonged retention of macrophage elastase in an active form in lungs under pathological conditions. Macrophages have also been implicated in normal connective tissue turnover, as occurs during bone resorption [33] and wound healing. Again, the relative insensitivity of macrophage elastase and perhaps other macrophage neutral proteases to endogenous serum inhibitors could provide a mechanism for physiological connective tissue remodeling in the presence of serum.

Acknowledgements

This work was partially supported by N.I.H. grant HL-14262 and by grant 1143 from the Council for Tobacco Research — U.S.A. Inc. R.R.W. is the recipient of N.I.H. Young Investigators grant HL-24186.

References

1 Johanson, W.G. and Pierce, A.K. (1972) J. Clin. Invest. 51, 288—293
2 Kaplan, P.D., Kuhn, C. and Pierce, J.A. (1973) J. Lab. Clin. Med. 82, 349—356
3 Pump, K.K. (1974) Chest 65, 431—436
4 Shotton, D.M. (1970) in Methods in Enzymology, (Perlmann, G.E. and Lorand, L., ed.), Vol. 19, pp. 113—140, Academic Press, New York
5 Baugh, R.J. and Travis, J. (1976) Biochemistry 15, 836—841
6 Feinstein, G. and Janoff, A. (1975) Biochim. Biophys. Acta 403, 493—505
7 Werb, Z. and Gordon, S. (1975) J. Exp. Med. 142, 361—377
8 White, R., Lin, H-S. and Kuhn, C. (1977) J. Exp. Med. 146, 802—808
9 DeCremoux, H., Hornebeck, W., Jaurand, M.-C., Bignon, J. and Robert, L. (1978) J. Pathol. 125, 171—177
10 Banda, M. and Werb, Z. (1979) Fed. Proc. 38, 1335 (Abstr.)
11 Hinman, L.M., Stevens, C.A., Matthay, R.A. and Gee, J.B.L. (1979) Fed. Proc. 38, 1436 (Abstr.)
12 White, R., Dearing, R., and Janoff, A. (1979) Am. Rev. Resp. Dis. 119, (4, Part 2) 369 (Abstr.)
13 Stewart, C.C., Lin, H. and Alders, C. (1975) J. Exp. Med. 141, 1114—1132
14 Porzio, M.A. and Pearson, A.M. (1977) Biochim. Biophys. Acta 490, 27—34
15 Davis, B.J. (1964) Ann. N.Y. Acad. Sci. 121, 404—427
16 Roberts, R.C., Riesen, W.A. and Hall, P.K. (1974) in Bayer Symposium V, Protease Inhibitors (Frits, H., Tschesche, H., Greene, L.J. and Truscheit, E., eds.), pp. 63—71, Springer Verlag, New York
17 Starkey, P.M. and Barrett, A.J. (1977) in Proteases in Mammalian Cells and Tissues (Barrett, A.J., ed.), pp. 663—696, North-Holland, Amsterdam
18 Nakamura, S., Ogata, H., Takeo, K., Kuwahara, A. and Suzuno, R. (1975) Hoppe-Seyler's Z. Physiol. Chem. 356, 677—692
19 Cohen, A.B. (1975) Biochim. Biophys. Acta 391, 193—200
20 Bloom, J.W. and Hunter, M.J. (1978) J. Biol. Chem. 253, 547—559
21 Johnson, D.A. and Travis, J. (1976) Biochem. Biophys. Res. Commun. 72, 33—39
22 Barrett, A.J. and Starkey, P.M. (1973) Biochem. J. 133, 709—724
23 Werb, Z., Burleigh, M.C., Barrett, A.J. and Starkey, P.M. (1974) Biochem. J. 139, 359—368
24 Cohen, A.B. (1976) in The Biochemical Basis of Pulmonary Function (Crystal, R.G., ed.), pp. 313—360, Marcel Dekker, New York
25 Rimon, A., Shamash, Y. and Shapiro, B. (1966) J. Biol. Chem. 241, 5102—5107
26 Harpel, P.C. (1973) J.Exp. Med. 138, 508—521
27 Scharpe, S., Eid, M., Cooreman, W. and Lauwers, A. (1976) Biochem. J. 153, 505—507
28 Janoff, A., Sloan, B., Weinbaum, G., Damiano, V., Sandhaus, R.A., Elias, J. and Kimbel, P. (1977) Am. Rev. Resp. Dis. 115, 461—478
29 Senior, R.M., Tegner, H., Kuhn, C., Ohlsson, K., Starcher, B.C. and Pierce, J.A. (1977) Am. Rev. Resp. Dis. 116, 469—475
30 Rodriquez, R.J., White, R.R., Senior, R.M. and Levine, E.A. (1977) Science 198, 313—314
31 Harris, J.O., Oslen, G.N., Castle, J.R. and Maloney, A.S. (1975) Am. Rev. Resp. Dis. 111, 579—586
32 Niewoehner, D.E., Kleinerman, J. and Rice, D.B. (1974) N. Engl. J.Med. 291, 755—758
33 Kahn, A.J., Stewart, C.C. and Teitelbaum, S.L. (1978) Science 199, 988—990