Human Leukocyte Granule Elastase: Rapid Isolation and Characterization†

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ABSTRACT: Human granulocytic elastases have been purified by a two-step procedure involving affinity chromatography of crude extracts of leukocytic granules on Sepharose–Trasylol, followed by ion-exchange chromatography on CM-cellulose to resolve the isoelastases. All of these enzymes were found to be glycoproteins with the carbohydrate content of the major form being composed essentially of only neutral sugars. The molecular weight of this form was found to be near 30 000 daltons with the other forms being slightly higher. Preliminary structural analyses indicate that all of the elastase isozymes have identical NH_2-terminal sequences suggesting that the differences in mobility of the four proteins are not due to different degrees of activation from a common zymogen but, more likely, from minor changes in carbohydrate content. Human granulocytic elastases are less active on ligament elastin than porcine pancreatic elastase, but both are inhibited by synthetic elastase active-site directed low molecular weight compounds (Tuhy, P. M., and Powers, J. C. (1975), *FEBS Lett. 50,* 359) as well as by plasma α-1-proteinase inhibitor (formerly called α-1-antitrypsin). In the latter case a stable complex with mol wt of 78 000 daltons is formed indicating the formation of a 1:1 complex.

The development of chronic obstructive lung disease has been proposed to occur in α-1-PI[1] deficient individuals by elastolytic degradation of lung alveoli (Janoff, 1972). The source of the elastin-degrading activity is believed to be from phagocytes, in particular polymorphonuclear leukocyte granules and alveolar macrophages. Both have been shown to contain enzymes which hydrolyze elastase-specific synthetic substrates (Janoff and Scherer, 1968; Janoff et al., 1971). In fact, in leukocyte granules, at least four such enzymes having different electrophoretic mobilities have been observed (Janoff, 1973; Ohlsson and Olsson, 1974).

Several laboratories have developed methods for the isolation of these isoelastases either as a mixture or as separate entities (Janoff, 1973; Ohlsson and Olsson, 1974; Schmidt and Havemann, 1974; Taylor and Crawford, 1975). Each of the procedures described, however, has had its drawbacks in that either the number and type of purification steps required resulted in a poor yield or the material isolated was not well characterized.

In our laboratory we have taken advantage of the weak inhibitory activity of the proteinase inhibitor, Trasylol (bovine Kunitz basic trypsin inhibitor), toward elastase (Janoff, 1972), to isolate granule elastases by a simple two-step procedure from leukocyte granule extracts. This report describes the techniques used for the purification of these proteins as well as some of the properties of the predominant form of the four isoenzymes present in leukocytes.

Experimental Section

Materials. Leukocytes were obtained from normal subjects by leukapheresis by courtesy of Dr. Ralph Vogler, Department of Haematology, Emory University, Atlanta, Ga. Z-Ala-NP and *t*-Boc-Ala-NP[1] were obtained from Cyclo Chemical Co. CM-cellulose-52 was purchased from Whatman Chemical Co. Sepharose 4B was a product of Pharmacia Fine Chemicals. Bovine elastin and Bz-L-Arg-OEt[1] were purchased from Schwarz-Mann. Orcein–elastin, congo red–elastin, Coomassie brilliant blue G-250, and heparin sulfate were obtained from Sigma Chemical Co. Trasylol was a gift of Dr. Ernst Truscheit, Bayer AG, Wuppertal, West Germany. Human α-1-PI was prepared by the method of Pannell et al. (1974).

Methods. Enzyme Assays. Elastase esterolytic activity was measured spectrophotometrically using Z-Ala-NP or *t*-Boc-Ala-NP (Geneste and Bender, 1969; Visser and Blout, 1972). The procedure followed was that described by Mallory and Travis (1975).

Protein concentration was determined spectrophotometrically by the method of Warburg and Christian (1942). For experiments involving purified elastase preparations, an experimentally determined extinction coefficient of 9.85 (1% solution, 280 nm) was used (Babul and Stellwagen, 1969).

Elastin digestion assays were performed by the method of Shotton (1970).

Inhibition Studies. Inhibition experiments were carried out by mixing a fixed quantity of enzyme (10^{-6} M) with varying amounts of α-1-PI in 0.05 M Tris-HCl, 0.05 M NaCl (pH 8.0). After incubation for 15 min at 25 °C, the mixtures were assayed for esterolytic activity. A molecular weight of 52 000 and an extinction coefficient of 5.3 (1% solution, 280 nm) was used in computing the α-1-PI concentration (Pannell et al., 1974).

Amino Terminal Sequence Analysis. The amino-terminal sequence of the predominant form of leukocyte granule elastase, designated E_4, as well as of mixtures of the four isoenzymes was determined using a Beckman Model 890C

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[1] Abbreviations used are: α-1-PI, α-1-proteinase inhibitor; Z-Ala-NP, carbobenzoxy-L-alanine *p*-nitrophenyl ester; *t*-Boc-Ala-NP, *tert*-butyloxycarbonyl-L-alanine *p*-nitrophenyl ester; Bz-L-Arg-OEt, *N*-benzoyl-L-arginine ethyl ester.

protein sequenator (Edman and Begg, 1967). The program followed was that of the manufacturer.

Preparation of Sepharose-Trasylol. Trasylol was coupled to Sepharose 4B by a modification of the procedure of March et al. (1974). In a typical experiment, 100 ml of washed Sepharose 4B gel was suspended in an equal volume of 2 M Na_2CO_3 and cooled to 15 °C. Twenty-five grams of cyanogen bromide, dissolved in 10 ml of acetonitrile, was added and the mixture stirred for 1–2 min. The activated gel was then washed quickly with 6 l. of cold distilled water. One liter of a cold solution of 0.1 M $NaHCO_3$ (pH 7.0) was added, followed by 200 mg of Trasylol, dissolved in 25 ml of the same buffer. The mixture was stirred overnight at 4 °C and the coupled product washed exhaustively with distilled water.

Other Analytical Techniques. Amino acid analysis, carbohydrate analysis, ultracentrifuge studies, and immunoelectrophoresis were performed as described elsewhere (Coan et al., 1971; Pannell et al., 1974). Vertical gel slab electrophoresis (Ortec) in an acid system (pH 4.3) was performed using a 7.5% acrylamide gel as described by the manufacturer. Dodecyl sulfate-acrylamide gel electrophoresis was adapted to the vertical gel slab electrophoresis system (Ortec) from a modification of the procedure of Laemmli (1970). The system consisted of a 9.0% separating gel (pH 8.8), a 3.0% stacking gel (pH 6.8), and a 7.5% well-forming gel (pH 6.8) using a six-slot well-former. All solutions contained 0.1% dodecyl sulfate. Gels were stained directly with Coomassie brilliant blue G-250 (Reisner et al., 1975).

Antisera to granule elastase were produced in white albino rabbits by multiple injections of the enzyme (1 mg) suspended in Freund's complete adjuvant. After 3 weeks, booster injections of enzyme (0.5 mg) were given and the animals bled 1 week later.

Results

Isolation of Human Leukocyte Elastase(s). I. Preparation of Leukocytes. Initially, fresh whole human blood was used as a starting material for the isolation of leukocytes. The procedure involved several tedious steps including dextran sedimentation, low-speed centrifugation, and lysis of contaminating red cells. Although this resulted in the isolation of a significant quantity of cells, it was found that preparations of leukocytes obtained by leukapheresis of normal, healthy donors could be handled much more rapidly.

Leukocyte preparations obtained by leukapheresis were suspended in 0.15 M KCl, and the mixture was centrifuged at 1000*g*. The resulting pellet was washed with cold distilled water for 1 min to lyse contaminating erythrocytes and 1.5 M KCl was added to bring the suspension to isotonic strength. The cells were recentrifuged, and the above lysis procedure was followed until no erythrocytes remained. Approximately 1.8×10^{10} cells could be routinely obtained by this procedure.

II. Preparation and Extraction of Leukocyte Granules. The granulocyte preparation described above was suspended in 500 ml of a solution containing 1 mg/ml of heparin sulfate in 0.2 M sucrose. The mixture was then stirred at 4 °C for 24 h during which cell lysis occurred. The suspension became extremely viscous during this treatment, due to the release of DNA, which made centrifugation very difficult. This was obviated by either adding external DNAse at room temperature or by warming the mixture to 37 °C in which case the viscosity was decreased by the action of endogenous DNAse.

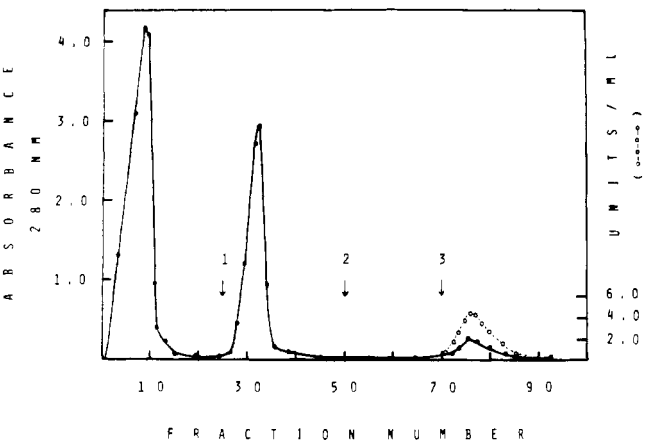

FIGURE 1. Chromatography of human leukocyte granule extracts on Sepharose-Trasylol. The column (3.5 × 15 cm) was equilibrated with 0.05 M Tris-HCl–0.05 M NaCl (pH 8.0). After sample application (50 ml of dialyzed granule extract), the column was washed with starting buffer until the A_{280nm} was less than 0.020, followed by 0.05 M Tris-HCl, 0.4 M NaCl (1), and 0.05 M sodium phosphate–0.4 M NaCl (pH 6.5) (2). Granulocytic elastase was eluted with 0.05 M sodium acetate–0.4 M NaCl (pH 5.0) (3). (●) A_{280nm}; (○) elastase esterase activity (1 unit = $\Delta A_{347.5nm}$ of 1.00/min).

In order to isolate the leukocyte granules, the cell lysate was first centrifuged at 1000*g* for 10 min to remove small quantities of unlysed cells and cell debris. The supernatant was then re-centrifuged at 30 000*g* for 30 min to pellet the granules. The granules were suspended in 0.34 M sucrose, and the process of low and high speed centrifugation was repeated. Finally, the high speed pellet was frozen at −20 °C until needed.

Leukocyte granule extracts were prepared by homogenization of granule pellets in 0.2 M sodium acetate (pH 4.0) using a Potter-Elvejhem glass homogenizer. The granule extract was centrifuged at 30 000*g* for 10 min and the pellet re-homogenized until enzyme was no longer solubilized. Three extractions were usually found to be sufficient.

III. Sepharose-Trasylol Chromatography. Leukocyte granule extracts were adjusted to pH 8.0 by one of two procedures. The extracts were either adjusted directly by the dropwise addition of 2 M Tris (unbuffered) or by dialysis against 0.05 M Tris-HCl, 0.05 M NaCl (pH 8.0). In the latter method a precipitate, containing most of the granule chymotrypsins, was obtained. With the former procedure these enzymes were completely retained in the extracts.

In either case the extract, at pH 8.0, was applied to a column of Sepharose-Trasylol equilibrated with 0.05 M Tris-HCl, 0.05 M NaCl (pH 8.0). The column was washed with this buffer to remove unbound protein and also with 0.05 M sodium phosphate, 0.4 M NaCl (pH 6.5), to remove traces of other adsorbed proteins. The elastase(s) and chymotrypsin(s) remained bound to the column. Elution of the elastases was accomplished by developing the affinity column with 0.05 M sodium acetate, 0.4 M NaCl (pH 5.0) (Figure 1). Chymotrypsins were eluted with 0.05 M sodium acetate, 0.4 M NaCl (pH 4.5).

The chymotrypsin fractions were free of elastase but the elastase fractions always contained small amounts of chymotrypsin if the original extracts were not dialyzed. The specific activity of the elastases eluted from this column were 20.0 if no chymotrypsins were present and varied from 12.0 to 16.0 if contamination with those enzymes occurred.

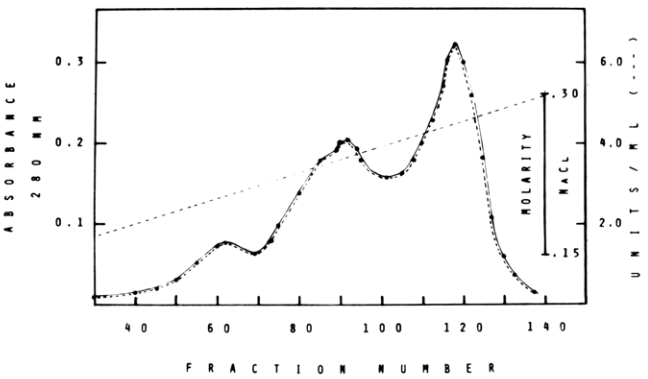

FIGURE 2. CM-cellulose chromatography of human leukocyte granule elastase isozymes. The column (2.9 × 13 cm) was equilibrated with 0.02 M sodium acetate, 0.15 M NaCl (pH 5.5), and eluted with a linear gradient (400 ml) to 0.3 M NaCl as indicated: (●—●) A_{280nm}; (●- - -●) elastase esterase activity (1 unit = $\Delta A_{347.5nm}$ of 1.00/min).

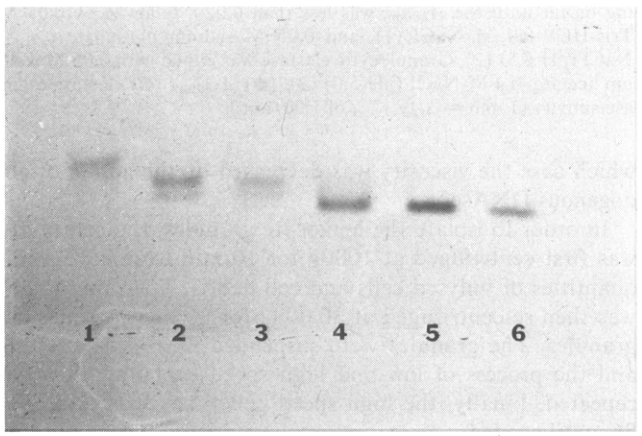

FIGURE 3. Gel slab electrophoresis of human leukocyte granule elastase fractions eluted from CM-cellulose. Running pH was 4.3. Direction of migration is from anode (top) to cathode (bottom): (1) pool (fractions 40–60) (100 μg); (2) pool (fractions 61–80) (100 μg); (3) pool (fractions 81–100) (50 μg); (4) pool (fractions 101–115) (50 μg); (5) pool (fractions 116–125) (50 μg); (6) pool (fractions 126–140) (50 μg).

As will be described subsequently, the elastase isozyme preparations obtained after this single step would appear to be sufficiently pure for most biochemical studies.

IV. CM-Cellulose Chromatography. In order to compare the properties of the individual isozymes of leukocytic elastase, further separation was performed by ion-exchange chromatography on CM-cellulose. The elastase isozymes were dialyzed against 0.02 M sodium acetate, 0.15 M NaCl (pH 5.5), and applied to a column of CM-cellulose equilibrated in the same buffer. All protein was adsorbed to the column and washing with equilibration buffer did not elute any proteins. The mixture of elastase isozymes was separated into individual components by using a linear gradient from 0.02 M sodium acetate, 0.15 M NaCl (pH 5.5), to 0.02 M sodium acetate, 0.3 M NaCl (pH 5.5) (Figure 2). When the gradient was completed, the column was washed with the high salt buffer to elute the last traces of the major elastase component. Subsequent washing with 0.02 M sodium acetate, 1 M NaCl (pH 5.5), eluted leukocyte chymotrypsins if present in the initially applied material. These latter enzymes will be described in a separate communication.

Figure 3 indicates the separation obtained by CM-cellulose using acid gel slab electrophoresis. The components isolated have been given the numbers E_1 through E_4 in order of their elution from CM-cellulose and also their rate of mobility during gel electrophoresis. Gel electrophoresis of various fractions at each step of the purification is shown in Figure 4.

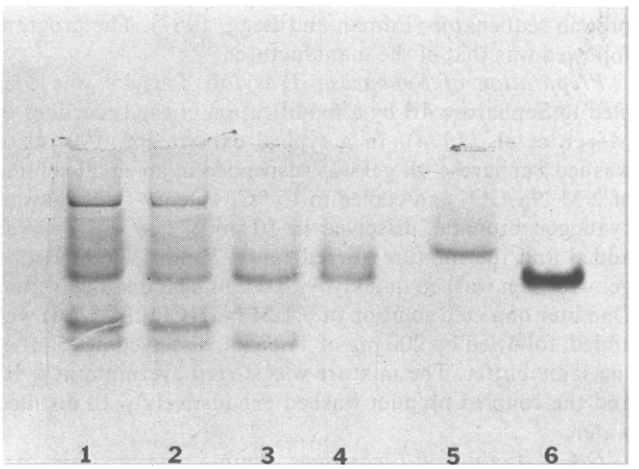

FIGURE 4. Gel slab electrophoresis of human leukocyte granule extract fractions at each stage of the purification of human granulocyte elastase(s). Patterns were stained with 1% Coomassie brilliant blue G-250 in 3% perchloric acid. Electrophoresis was at pH 4.3 in a standard 7.5% gel. Direction of migration is from anode (top) to cathode (bottom): (1) crude extract (200 μg); (2) dialyzed crude extract (200 μg); (3) elastase fraction from Sepharose–Trasylol using undialyzed crude extract (200 μg); (4) elastase fraction from Sepharose–Trasylol using dialyzed crude extract (200 μg); (5) pooled elastase fractions (40–60) from CM-cellulose (Figure 2) (50 μg); (6) pooled elastase fractions (116–125) from CM-cellulose (Figure 2) (100 μg).

Although this step gives no increase in specific activity if granule chymotrypsins are absent from the applied samples, it does serve to, at least partially, resolve the elastase isozymes from each other, in particular E_1 and E_4. The latter represents over 60% of the total elastase mixture and was used for most of the subsequent studies described. The purification scheme for the isolation of these enzymes from 1.8×10^{10} cells is given in Table I and indicates essentially complete recovery of the elastase isozyme mixture after the affinity chromatography step. Some losses are taken during the CM-cellulose chromatographic step.

Molecular Weight. The molecular weight of E_4 was determined by high-speed equilibrium ultracentrifugation using a rotor speed of 40 000 rpm. The molecular weight calculated from these data was found to be 29 500 when a partial specific volume of 0.718, as calculated from amino acid and carbohydrate composition, was used.

Dodecyl sulfate gel electrophoresis of E_4 was performed after incubation in dodecyl sulfate with or without β-mercaptoethanol. In either case a single component was identified with a molecular weight of 28 900 as determined using appropriate protein standards. These results substantiate the fact that no cleavages in the polypeptide chain due to autolysis have occurred during the purification process.

Amino Acid and Carbohydrate Composition. The amino acid composition of E_4 is presented in Table II, together with those reported for porcine elastase (Shotton, 1970) and human protease E (Mallory and Travis, 1975). The number of residues of each amino acid present was based on an assumed value of 20 residues of leucine per mole of protein. A molecular weight of 24 500 was calculated from

Table I: Purification of Human Leukocyte Elastase.

Fractionation Step	Total Protein (mg)[a]	Total Activity (units)[b]	Recovery (%)	Specific Activity (units/mg)	Purification
1. Crude extract	320	415	100	1.3	1.0
2. Sepharose–Trasylol column	21	420	101	20	16
3. CM-cellulose column					
Total	13	260	63	20	16
E4	6.6	132	32	20	

[a] Based on absorbance at 280 nm. [b] Based on esterase activity toward Z-Ala-NP. 1 unit = $\Delta A_{347.5nm}$ of 1.00/min.

these data but no contribution due to carbohydrate content is included.

When carbohydrate analysis of E_4 was performed, a total of 22.8% sugar content was found. Nearly all (21.2%) was found to be neutral sugar with only traces of sialic acid and *N*-acetylhexosamine being detectable. The contribution of these components to the molecular weight of E_4 is on the order of 6000 daltons, indicating that a summation of the molecular weights by amino acid and carbohydrate analysis is very close to that calculated from the ultracentrifuge and dodecyl sulfate gel electrophoresis data.

Amino-Terminal Sequence Analysis. In order to determine whether the formation of the four forms of elastase present in granule extracts might represent artifacts due to autolytic cleavage or different degrees of activation in the case of the existence of zymogen precursors, the mixture of all four proteins as well as E_4 was subjected to sequential analysis. The results for each are given below together with the amino-terminal sequence of porcine pancreatic elastase (Shotton, 1970).

E_4	Ile	Val	Gly	Gly	Thr	Leu	Ala
Mixture	Ile	Val	Gly	Gly	Thr	Leu	Ala
Porcine elastase	Val	Val	Gly	Gly	Thr	Gly	Ala

The sequences, at least through the first seven residues, of all four proteins were found to be identical, with no indication of the presence of other amino acids during the analyses due to microheterogeneity. These results would suggest that the differences in electrophoretic mobility of the four proteins are probably due to differences in carbohydrate content or, more remotely, to differences at the carboxyl terminal of each elastase. A further possibility, of course, is that differences in amide content in each protein could account for mobility differences during electrophoresis. A comparison of the sequence of these enzymes with that of porcine elastase shows a striking similarity between the two, especially when it is considered that one enzyme is from human leukocyte granules and the other is from porcine pancreas. No immunological cross reaction with either porcine elastase or human protease E (Mallory and Travis, 1975) could, however, be demonstrated using antisera to human leukocyte granule elastase (E_4).

Elastolytic Activity. To test for elastolytic activity, crude leukocyte granule extracts as well as E_4 were incubated with congo red–elastin, orcein–elastin, and powdered bovine elastin. For comparison, porcine pancreatic elastase was also tested. Although all three substrates were readily digested by the three different enzyme preparations when equal moles of the two elastase samples and an equivalent amount of crude extract (elastase esterase activity) were added, the rate of digestion by porcine elastase was 2.5 (undyed elastin), 4.2 (orcein–elastin), and 8.8 (congo red–elastin) times faster than E_4. Furthermore, crude extracts were twice as active as E_4, indicating the presence of other proteases, probably acting synergistically.

Table II: Amino Acid Composition of Mammalian Elastases.

Amino Acid	Residues/Molecule: Human Leukocyte Elastase (E_4)[a]	Human Protease E[b]	Porcine Elastase[c]
Lysine	1	9	3
Histidine	4	7	6
Arginine	22	10	12
Aspartic acid	24	28	24
Threonine	7	16	19
Serine	13	24	22
Glutamic acid	18	24	19
Proline	10	17	7
Glycine	28	35	25
Alanine	24	20	17
Half-cystine	6	15	18
Valine	25	25	27
Methionine	2	1	2
Isoleucine	11	14	10
Leucine	20	21	18
Tyrosine	3	8	11
Phenylalanine	9	7	3
Tryptophan[d]	2	9	7

[a] This paper. [b] Mallory and Travis (1975). [c] Shotton (1970). [d] Determined spectrophotometrically (Goodwin and Morton, 1946).

Interaction of Leukocyte Granule Elastase with Human α-1-Proteinase Inhibitor. When varying amounts of either the elastase isozyme mixture or E_4 were mixed with human α-1-proteinase inhibitor and the samples examined by dodecyl sulfate gel electrophoresis, a single new component of molecular weight 78 000 could be identified (Figure 5). This indicated the formation of a 1:1 complex and was confirmed by direct measurement of inhibition of elastase esterase activity by the addition of stoichiometric quantities of enzyme with inhibitor. The slightly lower molecular weight of this complex than that which might be expected of 82 000 may be due to cleavage of a fragment from α-1-PI during the interaction with elastase. Similar results have been noted during the interaction of α-1-PI with porcine trypsin (Johnson and Travis, 1975). The band migrating just ahead of α-1-PI is probably modified inhibitor and the band just ahead of the 78 000 molecular weight complex appearing with increasing enzyme concentration is complex from which a small molecular weight peptide has been proteolytically cleaved.

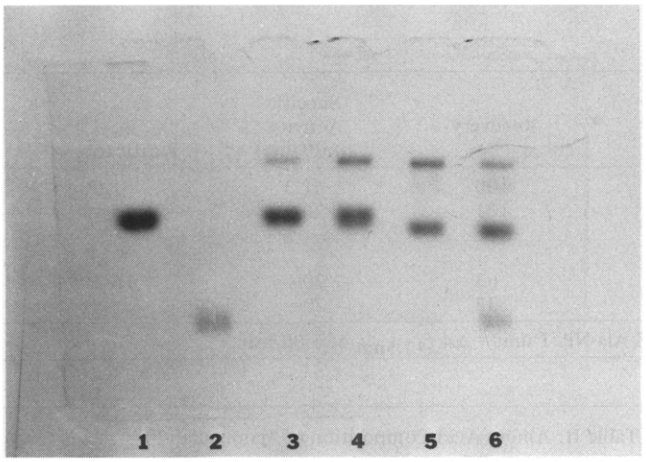

FIGURE 5. Gel electrophoresis of human leukocyte granule elastase α-1-PI complexes. Samples of enzyme and inhibitor were incubated for 15 min at pH 8.0. Twenty micrograms of inhibitor and varying molar ratios of enzyme were incubated with β-mercaptoethanol–dodecyl sulfate and subjected to vertical slab acrylamide electrophoresis. Molar ratios of α-1-PI to E_4 are indicated: (1) α-1-PI; (2) E_4; (3) 1:0.25; (4) 1:0.5; (5) 1:1; (6) 1:2. Marker proteins used to calibrate the molecular weight of the complex were bovine serum albumin, yeast enolase, carbonic anhydrase, and trypsinogen.

Discussion

Our laboratory has developed a simple two-step procedure for the separation of the isoenzymes of human granulocyte elastase using affinity chromatography on Sepharose–Trasylol and ion-exchange chromatography on CM-cellulose. The first step separates the elastases and chymotrypsin-like enzymes from all other proteins present in the crude granule extract due to their specific interaction with Trasylol. The second step not only separates the elastases from the chymotrypsin-like enzymes but also resolves the elastase isozyme mixture into simpler mixtures or single entities. This purification procedure takes only 2 days to accomplish from the extraction of crude material to the isolation of separate forms of elastase. The chymotrypsin-like enzymes can also be separated by a similar procedure and are currently being characterized.

The use of Sepharose–Trasylol as an affinity column may seem unusual for the isolation of proteases for two reasons. First, it is a "lysine active site" inhibitor (Laskowski and Sealock, 1971) and one might expect this residue to interact with activated Sepharose during the preparation of the affinity column. However, this does not apparently occur under our condition of coupling since we have previously shown that both human pancreatic trypsin and chymotrypsin are readily adsorbed on such an adduct (Johnson and Travis, 1974). Second, Trasylol has only a weak inhibitory activity toward granule elastase (Janoff, 1972). It might, therefore, not be expected to bind this enzyme. In fact, it does not interact strongly with elastase as shown by the ready dissociation of the complex at pH 5.0. Pancreatic trypsin, in contrast, does not dissociate from Sepharose–Trasylol columns until pH 2.5, indicating much stronger binding. It would seem, therefore, that even weak interactions between inhibitors and enzymes may make their isolation amenable by affinity chromatography. It should be noted, also, that the leukocyte granule chymotrypsins have a stronger interaction with Sepharose–Trasylol than those of the elastases. This has been shown to interfere with the capacity for elastase binding during purification. If elastase is to be isolated, therefore, it is recommended that the leukocyte granule extract be dialyzed to precipitate the chymotrypsins and thus decrease the subsequent interference in the binding of elastases to Sepharose–Trasylol.

The purification procedure described here is much more convenient than that described by others (Janoff, 1973; Ohlsson and Olsson, 1974; Schmidt and Havemann, 1974; Taylor and Crawford, 1975). The steps are fast and reproducible and, if all one wants is the mixture of elastases, their isolation can be carried out in a few hours with excellent recovery of activity. There is no requirement for either preparative electrophoresis (Ohlsson and Olsson, 1974) or for the use of denaturing conditions (Taylor and Crawford, 1975) in the isolation procedure. Of most importance is the fact that the Sepharose–Trasylol can be quickly reequilibrated for further use. The preparation obtained is at least three times as active as those reported by Janoff (1973) and nearly twice as active as that reported by Taylor and Crawford (1975). No comparison can be made with the results of Ohlsson and Olsson (1974) or Schmidt and Havemann (1974) because of either differences in the assay procedure or insufficient data.

The molecular weight and chemical properties of leukocyte granule elastase compare favorably with those of Schmidt and Havemann (1974) (27 000), Ohlsson and Olsson (1974) (33 000), but differ considerably from those of Taylor and Crawford (1975) (22 000). The autolytic cleavages reported by the latter authors in their elastase preparations as well as the denaturing conditions used in the isolation make an interpretation of such a large discrepancy in molecular weight difficult to explain on an analytical basis.

The reasons for differences in electrophoretic mobility of the four isoelastases remain unclear. Taylor and Crawford (1975) have recently suggested that there is an increased carbohydrate content associated with decreased mobility of the four different enzymes. These results are incompatible with their own data or with those reported here in that it would suggest that all the enzymes have similar molecular weights with E_1 having nearly four times the carbohydrate content of E_4. Some of the elastase isozymes would, therefore, have to have much smaller protein moieties than others in order to account for such results.

Our own results suggest that the elastase isozymes consist of polypeptide chains of identical molecular weight with carbohydrate side chains differing only slightly in composition. In fact, the low sialic acid content in E_4 might be expected since it is the most basic protein and small increases in this sugar moiety in the other elastases might easily account for their reduced mobility. Alternatively, microheterogeneity in certain areas in the polypeptide chain could account for charge differences. It is clear, however, from the sequence data that differences are not due to different sites of cleavage during activation of a latent zymogen in leukocyte granules. It is highly unlikely that cleavages at the carboxyl-terminal region of the molecule occur.

The interaction of α-1-PI with leukocyte elastase as shown by both dodecyl sulfate gel electrophoresis and elastase esterase inhibition studies strongly suggests the formation of a 1:1 complex. Our previous data (Johnson and Travis, 1974) had suggested a 1:2 complex of this inhibitor with porcine trypsin. However, we now find only a 1:1 complex. These previous results had not sufficiently taken into account the theoretical esterase activity of 100% active trypsin preparations, now believed to be about 21 000 Bz-L-Arg-OEt units/mg of porcine trypsin at 25 °C. With these data

a 1:1 complex for the interaction of α-1-PI with trypsin is readily established through inhibition studies and correlates well with the results presented here for elastase interaction. The molecular weight of the elastase–α-1-PI complex is considerably higher than that reported by Taylor and Crawford of 67 000 (1975), and the difference is probably due to internal peptide bond cleavages in their preparation, as well as the fact that, after mercaptoethanol–dodecyl sulfate treatment, at least a small portion of their α-1-PI–elastase complex would have been dissociated. Electrophoresis after dodecyl sulfate treatment in the absence of mercaptoethanol would probably have given a molecular weight nearer that reported here. However, the results of such experiments were not reported by the authors.

Leukocyte granule elastase is a true elastase in that it can readily digest elastin. However, the enzyme does not digest this substrate as readily as porcine pancreatic elastase. This may not be the case for elastin from tissue sources other than neck ligament. Curiously, the presence of other proteins in crude granule extracts enhances elastin digestion, probably due to the presence of leukocyte chymotrypsin acting synergistically with elastase.

The question of the possible role of elastase(s) in lung digestion and the concomitant onset of emphysema has yet to be clearly answered. Fortunately, the development of a method for purifying leukocyte elastases, in particular E_4, has allowed us to raise a strong, monospecific antiserum to these enzymes. Experiments to detect elastase activity in emphysematous lung tissue by the indirect fluorescent antibody technique are in progress and should reveal whether leukocyte granule elastases play a significant role in lung proteolysis.

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