Purification and Characterization of Two Human Pancreatic Elastases†

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ABSTRACT: Two proteases with elastolytic activity (elastases 1 and 2) have been isolated from activated extracts of human pancreatic tissue. The purification procedure for both elastases included ammonium sulfate fractionation followed by ion-exchange chromatography on CM-Sephadex C-50. Elastase 1 was further purified by chromatography on DEAE-Sephadex A-50. The homogeneity of both enzymes was demonstrated by Sephadex G-75 gel filtration, analytical polyacrylamide disc gel electrophoresis at pH 2.3, 4.5, and 8.3, and sodium dodecyl sulfate–polyacrylamide gel electrophoresis at pH 8.3. Both enzymes hydrolyzed undyed elastin as well as Remazol brilliant blue elastin and Congo red elastin. Activities and kinetic parameters using several synthetic substrates are also reported. The enzymes were further characterized in terms of molecular weight, amino acid composition, and N-terminal and penultimate amino acid residues. Their inhibition by the human serum protease inhibitors α_2-macroglobulin and α_1-antitrypsin was also studied. Elastase 1 appears to be very similar to human protease E (Mallory, P. A., and Travis, J. (1975), *Biochemistry 14,* 722). Elastase 2 is distinct from all human pancreatic proteases which have been characterized to date.

Pancreatic elastase (pancreatopeptidase E, EC 3.4.21.11) is unique among proteolytic enzymes of animal tissue in that it rapidly hydrolyzes the scleroprotein elastin (Partridge and Davis, 1955). Because of its unique specificity in degrading elastin, pancreatic elastase has been implicated in emphysema (Kaplan et al., 1973; Loeven, 1972) and atherosclerosis (Balo and Banga, 1953) as well as in aging (Hall, 1964). Pancreatic elastase has also been shown to produce the vascular injury observed in acute pancreatitis (Geokas et al., 1968).

The major form of porcine pancreatic elastase has been well characterized (Shotton, 1970; Hartley and Shotton, 1971). The identification of human pancreatic elastase has been attempted in the past using esterase activity toward synthetic substrates which are specific for porcine elastase. Thus Mallory and Travis (1975) isolated a human pancreatic protease on the basis of esterase activity toward *N*-acetyl-L-alanyl-L-alanyl-L-alanine methyl ester [Ac(Ala)$_3$OMe[1]], which has been shown to be a specific substrate for the porcine enzyme (Gertler and Hofmann, 1970). However, this protease exhibited negligible activity against a variety of elastin substrates. Feinstein et al. (1974) also reported isolation of two human pancreatic "elastases" on the basis of esterase activity toward Ac-(Ala)$_3$OMe. However, these authors did not attempt to determine the elastolytic activity of these proteases. On the other hand, Trowbridge and Moon (1972) reported partial purification of human pancreatic elastase by salt fractionation of homogenized pancreatic tissue followed by adsorption of the enzyme on powdered human elastin, but criteria of purity and detailed characterization of this protease were not presented.

The purification of human pancreatic elastase in this laboratory was based on the assay of elastolytic activity using RBB elastin as substrate. This technique is somewhat less convenient than assays utilizing Ac(Ala)$_3$OMe, but it is the only approach which assures purification of proteases with specific elastolytic activity. Two distinct proteases have been isolated from extracts of human pancreatic tissue on the basis of their activity toward RBB elastin. This report describes the purification to homogeneity and characterization of these two proteases, designated human pancreatic elastases 1 and 2.

Materials and Methods

Materials. Human pancreatic tissue was obtained at autopsy from this hospital. Specimens with evidence of pancreatic disease were rejected. They were stored at −20 °C for up to 1 week before preparation of acetone powder (see Results). For long-term storage, pancreases were held at −70 °C.

Porcine pancreatic elastase (Code ESFF) and elastin (EL33J828) were obtained from Worthington Biochemical Corporation. Ac(Ala)$_3$OMe, t-Boc-Ala-NP, and Tos-PhCH$_2$Cl were obtained from Cyclo Chemical Company. Suc(Ala)$_3$NA was obtained from Bachem, Inc. PhCH$_2$SO$_2$F, BzArgOEt, BzTyrOEt, and AcTyrOEt were obtained from Sigma Chemical Company. Trasylol (a commercial preparation of trypsin inhibitor from bovine lung containing 10 000 Kallikrein inhibitor units per ml) was obtained from FBA Pharmaceutical Company.

Preparation of Elastase Substrates. RBB elastin (Rinderknecht et al., 1968a) and RBB hide (Rinderknecht et al., 1970) were prepared as described previously. Congo red elastin was prepared according to the method of Shotton (1970). A

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[1] Abbreviations used are: Ac(Ala)$_3$OMe, *N*-acetyl-L-alanyl-L-alanyl-L-alanine methyl ester; t-Boc-Ala-NP, *N-tert*-butyloxycarbonyl-L-alanine *p*-nitrophenyl ester; Suc(Ala)$_3$NA, succinyl-L-alanyl-L-alanyl-L-alanine-*p*-nitroanilide; AcTyrOEt, *N*-acetyl-L-tyrosine ethyl ester; TosArgOMe, *p*-toluenesulfonyl-L-arginine methyl ester; BzArgOEt, *N*-benzoyl-L-arginine ethyl ester; BzTyrOEt, *N*-benzoyl-L-tyrosine ethyl ester; PhCH$_2$SO$_2$F, phenylmethanesulfonyl fluoride; Tos-PhCH$_2$Cl, L-1-tosylamido-2-phenylethyl chloromethyl ketone; RBB elastin, Remazol brilliant blue stained elastin powder, RBB hide, Remazol brilliant blue stained hide powder; PhNCS, phenyl isothiocyanate; CM, carboxymethyl; DEAE, diethylaminoethyl; Tris, tris(hydroxymethyl)aminomethane; PMS-elastase, phenylmethanesulfonylelastase.

1 mg/ml solution of the resulting Congo red elastin gave an absorbance of 0.40 at 495 nm after complete hydrolysis with porcine elastase.

Assays of Elastolytic Activity. Since the elastolytic activity of porcine pancreatic elastase has been shown to vary with both the batch of elastin employed (Loeven, 1963) and the nature of the dye used for staining (Loeven, 1970), no absolute units of human pancreatic elastase activity are defined in this report. Instead the elastolytic activity of the human elastases is expressed in amounts relative to that of pure porcine elastase. One unit of activity is thus defined as the amount of elastin substrate hydrolyzed by 1.0 mg of porcine elastase, under the assay conditions given.

Protein concentration was determined by the method of Lowry et al. (1951) during purification, and by absorbance at 280 nm using $\epsilon_{280}^{1\%}$ 20.2 (Shotton, 1970) for purified elastases. Elastolytic activity was determined using RBB elastin as previously described (Rinderknecht et al., 1968a) with the following modifications: (1) a final volume of 2 ml of assay mixture containing 5 mg/ml RBB elastin was employed; (2) after incubation at 37 °C, the assay mixtures were centrifuged at 5000 rpm for 10 min at 0 °C and the absorbance of the supernatant was measured at 595 nm; and (3) enzyme assays during purification included 100 μl of Trasylol[2] in the incubation mixture. This level of Trasylol was shown to be sufficient to inhibit trypsin activity in the aliquots employed for determination of elastolytic activity in typical pancreatic extracts.

Congo red elastin assays were performed as described by Shotton (1970) with a total assay volume of 1.0 ml. Elastolytic activity was determined by measuring the time required to achieve 50% digestion of Congo red elastin.

Hydrolysis of undyed elastin was monitored by measuring the increase in soluble amino groups produced from insoluble elastin after 30 min of incubation using fluorescamine (Udenfriend et al., 1972; Böhlen et al., 1973) which reacts specifically with primary amino groups to give a highly fluorescent product.

Powdered elastin was dried overnight in vacuo, ground with a mortar and pestle, and passed through a 140 mesh sieve. The resulting powder was suspended to 5 mg/ml in 50 mM triethanolamine hydrochloride (pH 8.8) containing 0.025% Triton X-100. The suspension was washed with several changes of the same buffer by decantation and/or centrifugation until the fluorescence, as determined below, was negligibly higher than that of a buffer blank.

Aliquots of elastase were mixed with 1.0 ml of elastin suspension and incubated for 30 min at 37 °C with intermittent gentle shaking. The tubes were then centrifuged at 0 °C for 10 min at 5000 rpm in the Sorvall HS-4 rotor. An aliquot of 0.8 ml of the resulting supernatant was carefully removed and added to 1.0 ml of 50 mM sodium phosphate (pH 8.0). Fluorescamine (0.5 ml of a solution of 0.3 mg/ml in acetone) was then rapidly added as the tubes were being agitated on a Vortex mixer. Fluorescence was measured about 15 min later with a Turner Model 430 spectrofluorimeter with excitation and emission set at 390 and 475 nm, respectively. Fluorescence of substrate incubated in the absence of enzyme was subtracted from each value. The fluorescence produced with porcine elastase, human elastase 1, or human elastase 2 was linear over the range examined, from approximately 0.05 to 0.25 μg per assay.

Assay of General Proteolytic Activity. Nonspecific protease activity was measured using RBB hide as previously described (Rinderknecht et al., 1970) in assay mixtures containing 5 mg/ml substrate with a total assay volume of 2 ml. After incubation assay mixtures were centrifuged at 5000 rpm for 10 min and the absorbance of the supernatant at 595 nm was recorded.

Assay of Esterase Activity. Hydrolysis of $Ac(Ala)_3OMe$ was determined according to the method of Gertler and Hofmann (1970) using a substrate concentration of 8.0 mM. Activity toward AcTyrOEt was measured according to the procedure described by Wilcox (1970) with a substrate concentration of 10 mM. Hydrolysis of BzTyrOEt was measured according to the procedure described by Walsh and Wilcox (1970) while activity toward BzArgOEt was determined according to the procedure described by Schwert and Tanaka (1955). Hydrolysis of TosArgOMe was measured as described by Walsh (1970). This method was also employed to measure tryptic activity in pancreas extracts.

Kinetic Measurements with $Suc(Ala)_3NA$. Hydrolysis of $Suc(Ala)_3NA$ was measured according to the method of Bieth et al. (1974). Three separate velocity measurements were made at each of seven substrate concentrations. For K_m studies, final enzyme concentrations were 0.18 μg/ml for porcine elastase, 2.5 μg/ml for elastase 1, and 8.5 μg/ml for elastase 2. Substrate concentrations of 0.1–10 mM were used for porcine elastase and elastase 1, while concentrations of 1–20 mM were employed for kinetic measurements with elastase 2. The assays were performed in 0.2 M Tris-HCl buffer (pH 8.0) at 25 °C. The data were plotted according to Eadie and Hofstee and the slope and intercept of the best-fitting straight line determined by least-squares analysis with an Olivetti Model 101 programmable calculator.

Kinetic Measurements with t-Boc-Ala-NP. Hydrolysis of t-Boc-Ala-NP was measured in 50 mM sodium phosphate (pH 6.5) at 25 °C according to a modification of the procedure of Visser and Blout (1972) by following the production of *p*-nitrophenol at 347.5 nm. Activity measurements were made at various substrate concentrations by using equal aliquots of appropriate dilutions of an 80 mM solution of t-Boc-Ala-NP in methanol, so that all measurements were made in 1% (v/v) methanol. Hydrolysis rates in the absence of enzyme were subtracted at each substrate concentration used. Enzyme concentrations employed were 0.44 μg/ml for porcine elastase, 1.08 μg/ml for human elastase 1, and 0.95 μg/ml for human elastase 2.

Amino Acid Composition. The amino acid compositions were derived from analyses of protein samples which had been hydrolyzed in 6 N HCl at 110 °C for 24 h. The values for serine and threonine are uncorrected. Half-cystine was determined after performic acid oxidation as cysteic acid by the method of Hirs (1967).

N-Terminal Sequence Determination. The N-terminal amino acid residues of each enzyme were determined by the dansylation procedure described by Weiner et al. (1972) on the modified enzymes as well as on performic acid oxidized elastase 1 and 2. Thus a 20-nmol sample of each enzyme was dissolved in 0.5 M $NaHCO_3$ (pH 9.8) containing 1% (w/v) sodium dodecyl sulfate and reacted with dansyl chloride in a solution containing 33% (v/v) acetone. Aliquots of dansylated protein were hydrolyzed for 4 h and for 24 h at 110 °C in 6 N HCl and the resulting dansyl amino acid derivatives were identified by the procedure of Gray (1972). The penultimate

[2] The protease inhibitor from bovine lung prepared commercially as Trasylol has been shown to possess the same amino acid sequence as Kunitz Pancreatic Trypsin Inhibitor (Kassell, B. (1970), *Methods Enzymol. 19*, 844).

amino acid residue was determined by performing one round of Edman degradation on elastase 1 or 2 in the same buffer using PhNCS, as described by Weiner et al. (1972), and identifying the new N-terminal amino acid residue by dansylation.

Analytical Polyacrylamide Disc Gel Electrophoresis. Proteins were subjected to polyacrylamide disc gel electrophoresis at pH 8.3 by the method of Davis (1964), at pH 4.5 by the method of Reisfeld et al. (1962), and at pH 2.3 as described by Brewer and Ashworth (1969). The stacking gel was omitted in the pH 8.3 system since its inclusion did not increase resolution. In some experiments, the resolving and stacking gels in the pH 2.3 system contained 4 M urea. In all cases, gels were stained with Coomassie brilliant blue and were destained by the method of Fairbanks et al. (1971).

Discontinuous Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis. Discontinuous sodium dodecyl sulfate gel electrophoresis was performed using a thin slab of acrylamide gel, according to the general procedures described by Studier (1972, 1973). Gel thickness was reduced to $\frac{1}{32}$ in., and a sample slot mold with 13 slots was employed. Samples were completely reduced and denatured by heating for 2 min in a boiling water bath in sample buffer containing 1% (w/v) sodium dodecyl sulfate and 1% (v/v) β-mercaptoethanol. The buffer compositions for samples and in the running gel, stacking gel, and electrophoresis buffer were as originally described by Laemmli (1970). The running gel contained 12.5% (w/v) acrylamide and 0.58% *N,N'*-methylenebisacrylamide. The gels were stained and destained as described above.

Molecular Weight Determinations with Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis. The discontinuous sodium dodecyl sulfate-polyacrylamide gel electrophoresis procedure was used to determine the molecular weights of human elastases 1 and 2 following the general procedures of Weber and Osborn (1969). The proteins employed as standards were ovalbumin, pepsinogen, glyceraldehyde-3-phosphate dehydrogenase, porcine carboxypeptidase B, chymotrypsinogen, myoglobin, and α-chymotrypsin treated with diisopropyl fluorophosphate.

Molecular Weight Determination by Sephadex G-75 Gel Filtration. Elastase 1 or 2 was applied to a 2.0 × 96 cm column of Sephadex G-75 equilibrated with 10 mM sodium phosphate buffer (pH 6.5) in a 2-ml sample which contained ovalbumin, carboxypeptidase B, chymotrypsinogen A, and cytochrome *c* as molecular weight standards. The molecular weights of elastase 1 and 2 were determined by plotting the log of the molecular weight vs. elution volume (Whitaker, 1963).

Inhibition with $PhCH_2SO_2F$. $PhCH_2SO_2F$ was dissolved in isopropyl alcohol to a concentration of 8 mg/ml. A 10-μl aliquot of $PhCH_2SO_2F$ solution was added to 1 mg of elastase 1 or 2 in 1 ml of 50 mM Tris-HCl (pH 8.0) at 0 °C. After 15 min a second aliquot of 10 μl of $PhCH_2SO_2F$ solution was added. The product of inactivation, PMS-elastase, was purified by gel filtration on a 1.2 × 45 cm column of Sephadex G-75 equilibrated with 10 mM sodium phosphate buffer (pH 6.5).

Inhibition with Tos-$PhCH_2Cl$. A 1-ml solution of elastase 2 (0.1 mg/ml) was incubated in Tris-HCl (pH 7.0) with 10 μl of Tos-$PhCH_2Cl$ (4 mg/ml in methanol) at 37 °C. A second 1-ml sample of elastase 2 was incubated under the same conditions in the absence of Tos-$PhCH_2Cl$ as a control. Solutions of human chymotrypsin II (0.1 mg/ml) were also incubated under the same conditions. Aliquots of elastase 2 and chymotrypsin II were removed at 15-min intervals for determination of enzymatic activity remaining with $Suc(Ala)_3NA$ and BzTyrOEt, respectively. Elastolytic activity and esterase activity toward AcTyrOEt were also determined with aliquots of untreated and Tos-$PhCH_2Cl$ treated elastase 2 after 1.5 h of incubation at 37 °C.

Inhibition with Human α_2-Macroglobulin. α_2-Macroglobulin was purified from human plasma by a combination of Sephadex G-150 and G-200 gel filtration and DEAE-cellulose ion-exchange chromatography. Inhibition of proteolytic activity toward RBB hide was determined using enzyme concentrations of 0.65 μg/ml for elastases 1 and 2 and 0.27 μg/ml for porcine elastase. The concentration of α_2-macroglobulin was 0–30 μg/ml for the human elastases and 0–15 μg/ml for porcine elastase. The enzymes were incubated for 30 min at 0 °C with varying amounts of α_2-macroglobulin in a total volume of 150 μl of 20 mM Tris-HCl buffer (pH 6.8) containing 2 mM $CaCl_2$ prior to addition of RBB hide for assay.

Inhibition with Human α_1-Antitrypsin. Human α_1-antitrypsin was purified from plasma by the method of Pannell et al. (1974). Inhibition of elastases 1 and 2 and porcine elastase was measured using RBB hide as substrate. The concentrations of human elastases 1 and 2 and porcine elastase employed in the assays were 0.68, 0.73, and 0.29 μg/ml, respectively. Inhibition of the elastases over an α_1-antitrypsin range of 0–10 μg/ml was investigated.

The inhibitor-enzyme ratios at complete inhibition were determined for both α_1-antitrypsin and α_2-macroglobulin by extrapolating the linear portion of the dose-response curve for inhibition as described by Laskowski and Sealock (1971). In all cases the linear part of the inhibition curve extended to 75% inhibition or more. The time dependence of inhibition was also examined, at approximately 50% inhibition, in order to confirm completeness of the reaction.

Human anionic trypsin and chymotrypsins I and II were purified according to the procedure described by Feinstein et al. (1974). The enzymes were judged to be homogeneous by the criterion of discontinuous sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis.

Results

Purification of Human Elastase 1 and Elastase 2

All purification steps were carried out at 0 °C, except where noted. Elastase activity with RBB elastin as substrate was determined as described in Materials and Methods.

1. Preparation of Acetone Powder. Acetone powder from a single pancreas was obtained by extracting the diced, semi-frozen tissue with 500 ml of chilled (−20 °C) acetone for 1 min in a Waring blender. The suspension was then centrifuged for 10 min at 6000 rpm in the GSA rotor of the Sorvall RC-2B centrifuge. The supernatant was then discarded. This procedure was repeated twice more with acetone, once with 1:1 acetone-ether, and once with ether. The residue was then dried in a vacuum desiccator by water aspiration for 1 h, followed by evacuation overnight with a mechanical vacuum pump. The dry powder was then ground briefly with a mortar and pestle and stored in a sealed jar at −20 °C. Activity of powders was assessed within 1 week of preparation.

2. Preparation and Activation of Initial Extract. Earlier results obtained in this laboratory by assay of samples of individual pancreases for tryptic activity after 6–8 h autoactivation indicated that the amount of trypsin per pancreas varied by as much as 1000-fold, depending probably on the physiological state of the pancreas at the time of death. Trypsin and elastase activities were therefore determined by the procedure given below on 0.5-g samples of the acetone powder of each

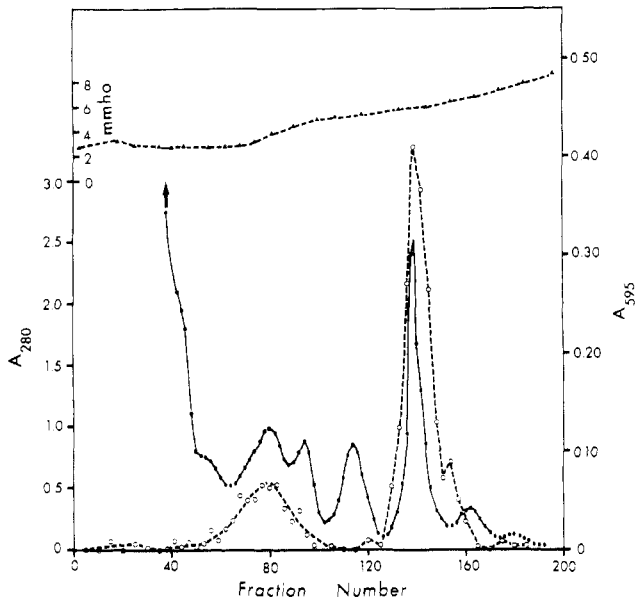

FIGURE 1: Chromatography of partially purified elastase from activated human pancreatic tissue extract on CM-Sephadex C-50. Elastolytic activity was determined on 3-μl aliquots of the fractions indicated as described in Materials and Methods. Fractions of 10 ml were collected. (●—●) A_{280}; (○ - - - ○) A_{595} (elastolytic activity); (▲ - - - ▲) conductivity.

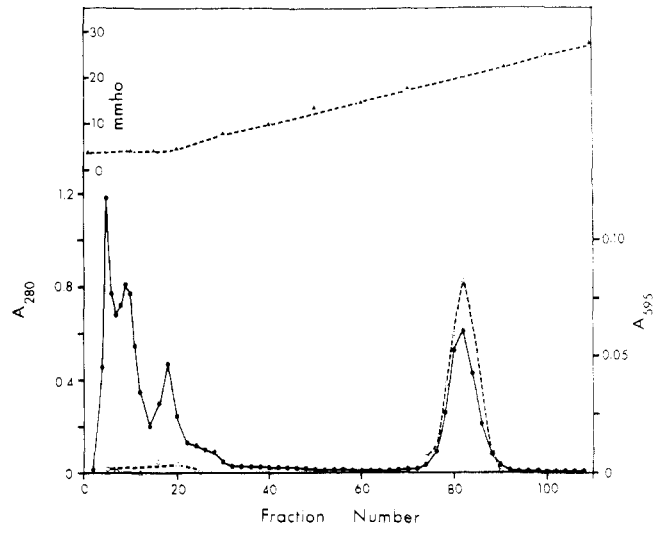

FIGURE 2: Chromatography of partially purified human elastase 1 on DEAE-Sephadex A-50. Elastolytic activity was determined on 4-μl aliquots of the fractions indicated as described in Materials and Methods. Fractions of 11.5 ml were collected. (●—●) A_{280}; (○ - - - ○) A_{595} (elastolytic activity); (▲ - - - ▲) conductivity.

pancreas in order to select pancreases containing sufficient elastase activity for purification.

Preparation and activation of the crude extract were performed as follows. The acetone powder was suspended in 20 ml of 10 mM $CaCl_2$ per g of dry weight by homogenization for 60 s at medium speed in a Virtis "45" homogenizer. After adjustment of pH to 8.0 with 1 M Tris base, the suspension was stirred at 4 °C in order to allow autoactivation to occur. Samples of 0.5 ml were withdrawn at approximately 1-h intervals and assayed for tryptic activity as described in Materials and Methods. Elastase activity was found to increase roughly in parallel with tryptic activity, but it was not measured as often. The extraction procedure was continued until two successive measurements of tryptic activity were found to be the same, indicating full activation. Full activation usually occurred after 6–8 h and for highly active pancreases would yield 100–200 units per ml of tryptic activity (0.5–1 mg/ml). At this time the suspension was centrifuged for 15 min at 12 000 rpm in the GSA rotor to yield the crude extract.

3. Acid Precipitation Step. The stirred crude extract was slowly adjusted to pH 5.1 by dropwise addition of 6% acetic acid. The adjusted extract was stirred for 5 min and centrifuged at 16 500 rpm for 10 min. The precipitate was discarded and the clear supernatant was then subjected to ammonium sulfate precipitation.

4. Ammonium Sulfate Precipitation. The pH 5.1 supernatant was slowly adjusted to 55% saturation by addition of finely powdered ammonium sulfate. After stirring for 10 min, the suspension was centrifuged at 15 000 rpm for 10 min. The precipitate was then resuspended in 50 mM sodium phosphate (pH 6.5) and dialyzed vs. two changes of this buffer.

5. CM-Sephadex Chromatography. The dialyzed material from ammonium sulfate precipitation was applied to a 2.0 × 20 cm column of CM-Sephadex C-50 equilibrated in 50 mM sodium phosphate (pH 6.5). Approximately 600 ml of this buffer was employed to wash the column, followed by a linear gradient from 50 to 200 mM sodium phosphate (pH 6.5) in a total volume of 1500 ml. The elution profile is shown in Figure 1. The elastase activity appeared in two peaks, designated elastase 1 and elastase 2. Fractions containing elastase 1 were pooled, concentrated by ultrafiltration with an Amicon PM-10 membrane, dialyzed vs. 50 mM sodium phosphate (pH 6.5), and applied to DEAE-Sephadex as described in the next section. Fractions containing elastase 2 were pooled, concentrated by ultrafiltration, and applied to a Sephadex G-75 column.

6. DEAE-Sephadex Chromatography of Elastase 1. Elastase 1 from the CM-Sephadex column was applied to a 2.0 × 17 cm column of DEAE-Sephadex A-50 equilibrated with 50 mM sodium phosphate (pH 6.5). The column was washed with 200 ml of the same buffer followed by a linear gradient from 0 to 0.4 M sodium chloride in 50 mM sodium phosphate (pH 6.5) in a total volume of 1.2 l. The elution profile is shown in Figure 2. Fractions containing elastase activity were pooled, concentrated by ultrafiltration, and applied to a Sephadex G-75 column.

Sephadex G-75 Gel Filtration of Elastase 1 or 2. In separate experiments, a 5-ml aliquot of concentrated elastase 1 or 2 was applied to a 2.0 × 96 cm Sephadex G-75 column equilibrated in 10 mM sodium phosphate (pH 6.5). In each case a homogeneous peak of protein and superimposable elastase activity was observed, accompanied by a variable amount of inactive, aggregated material which eluted at the void volume. In some experiments the specific activity of either elastase 1 or elastase 2 was unchanged after Sephadex G-75 gel filtration, while in other experiments the specific activity of the enzymes was slightly higher after gel filtration. The purification of the two elastases is shown in Table I. An 8.5-fold purification of elastase 1 and an 11.5-fold purification of elastase 2 were achieved.

Criteria of Homogeneity and Molecular Weight Studies.

Human elastase 1 was eluted from DEAE-Sephadex as a symmetrical peak. Additional evidence of homogeneity of this preparation was obtained by subjecting it to gel filtration on Sephadex G-75. The preparation eluted as a single peak with constant specific activity. The molecular weight of elastase 1 was estimated by gel filtration on Sephadex G-75 to be 33 000. Analytical disc gel electrophoresis of PMS-elastase 1, prepared as described in Materials and Methods, in a 10% acrylamide

TABLE I: Purification of Human Elastase 1 and Elastase 2 from Pancreas Powder.[a]

Fraction	Vol (ml)	Elastase (Units/ml)	Elastase (Total Units)	Protein (mg/ml)	Spec Act. (Units/mg)	Fold Purification	% Yield
Crude extract	195	0.215	41.9	13.0	0.0165	1.0	100
pH 5 supernatant	196	0.193	37.8	8.0	0.024	1.45	90
55% ammonium sulfate pellet predialysis	62	0.58	35.9	18.0	0.032	1.94	86
55% ammonium sulfate pellet postdialysis	160	0.134	21.4	7.3	0.018	1.09	51
Elastase 1 from CM-Sephadex	220	0.017	3.74	0.40	0.042	2.54	8.9
Elastase 1 from DEAE-Sephadex	149	0.016	2.38	0.14	0.114	6.9	5.7
Elastase 1 from Sephadex G-75	59	0.035	2.06	0.25	0.140	8.5	4.9
Elastase 2 from CM-Sephadex	132	0.098	12.9	0.51	0.192	11.6	31

[a] One unit of elastase activity is equal to the amount of product produced by 1.0 mg of porcine elastase as measured by hydrolysis of RBB elastin.

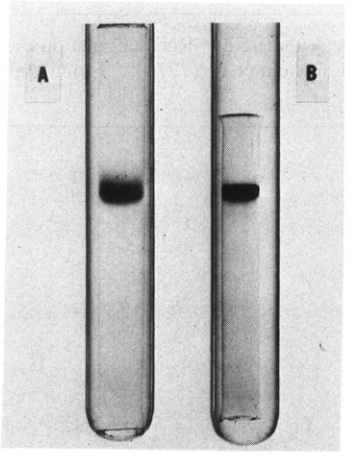

FIGURE 3: Gel electrophoresis of purified human elastases. (A) Electrophoresis of PMS-elastase 2 in a 15% acrylamide gel at pH 4.5. Migration was from top to bottom (anode to cathode). (B) Electrophoresis of PMS-elastase 1 in a 10% acrylamide gel at pH 8.3. Migration was from top to bottom (cathode to anode). Gels were run as described in Materials and Methods.

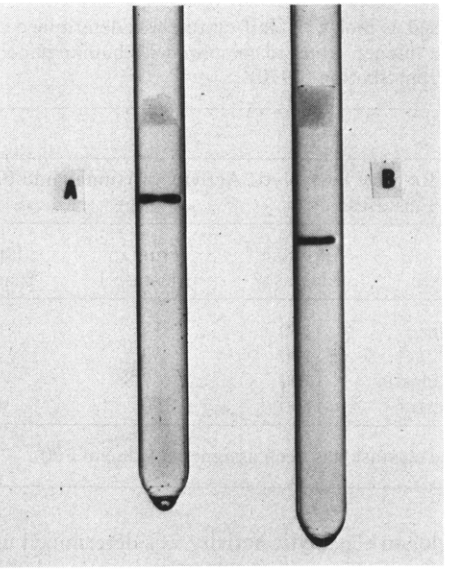

FIGURE 4: Gel electrophoresis of purified human elastases at pH 2.3. (A) Electrophoresis of PMS-elastase 1 in a 7.5% gel. (B) Electrophoresis of PMS-elastase 2 in a 7.5% gel containing 4 M urea. Migration was from top to bottom (anode to cathode). Gels were run as described in Materials and Methods.

gel at pH 8.3, showed only a single band with no minor contaminants (Figure 3). Elastase 1 did not enter a 10% gel during pH 4.5 electrophoresis; however, no other protein bands were observed. Analytical disc gel electrophoresis of PMS-elastase 1 in a 7.5% gel at pH 2.3 revealed a single band with no minor contaminants (Figure 4). Addition of 4 M urea to the resolving and stacking gels did not affect the observed electrophoretic pattern.

Human elastase 2 was eluted from the CM-Sephadex column as a symmetrical peak. Sephadex G-75 gel filtration of this material showed a symmetrical peak with constant specific activity. The molecular weight of elastase 2 was estimated by Sephadex G-75 gel filtration to be 26 600. Analytical disc gel electrophoresis of PMS-elastase 2 in a 15% acrylamide gel at pH 4.5 revealed a single component with no contaminants (Figure 3). PMS-elastase 2 did not enter a 10% acrylamide gel during electrophoresis at pH 8.3; however, no other protein bands were observed. Analytical disc gel electrophoresis of PMS-elastase 2 at pH 2.3 in a 7.5% acrylamide gel in the absence of urea revealed a variable amount of aggregated material at the interface of the resolving and stacking gels as well as a single migrating band. Addition of 4 M urea to both the stacking gel and the running gel converted the aggregated material to a single band with no minor contaminants (Figure 4).

Discontinuous sodium dodecyl sulfate–polyacrylamide gel electrophoresis of elastases 1 and 2 at pH 8.3 revealed a single major band for each enzyme and several minor bands of low molecular weight. Since these were thought to arise from autolysis, samples of elastase 1 and 2 were inactivated with $PhCH_2SO_2F$ followed by gel filtration on Sephadex G-75 (see Materials and Methods). When PMS-elastase 1 was subjected to discontinuous sodium dodecyl sulfate–polyacrylamide gel electrophoresis, a single component was detected (Figure 5) with a molecular weight estimated to be 29 300 using reference proteins of known molecular weight, as described in Materials and Methods. Discontinuous sodium dodecyl sulfate gel electrophoresis of PMS-elastase 2 revealed one component (Figure 5) with a molecular weight of 25 000 estimated by using proteins of known molecular weight as standards.

Human elastases 1 and 2 were stable when stored at −20 °C at pH 6.5 for a period of several months. The effect of NaCl

TABLE II: Amino Acid Composition of Human Elastases.[a]

Residue	Human Elastase 1	Human Elastase 2	Human[c,d] "Elastase 1"	Human[c,d] "Elastase 2"	Human[c,e] Protease E	Porcine[c,f] Elastase
Asp	10.2	11.9	10.5	12.6	10.0	10.3
Thr	5.4	4.8	5.9	5.3	5.8	8.2
Ser	8.5	11.6	6.7	11.0	8.5	9.4
Glu	8.7	6.0	8.8	5.7	8.6	8.2
Pro	7.0	5.6	6.7	5.7	6.1	3.0
Gly	11.7	11.5	10.9	9.8	12.4	10.7
Ala	7.1	7.1	6.7	6.5	7.0	7.3
Cys/2[b]	3.4	3.8	6.7	5.7	5.4	3.4
Val	8.9	9.1	10.1	8.9	8.9	11.6
Met	0.4	0.5		0.8	0.3	0.8
Ile	5.2	4.8	4.2	4.5	4.8	4.3
Leu	7.5	8.2	7.1	7.7	7.2	7.7
Tyr	3.4	4.5	2.9	4.9	2.7	4.7
Phe	3.1	1.0	2.5	1.2	2.5	1.3
Lys	3.2	3.1	3.4	3.7	3.3	1.3
His	2.2	2.3	2.5	2.4	2.6	2.6
Arg	4.2	4.2	4.2	3.3	3.5	5.2

[a] Expressed as mol %. [b] Half-cystine was determined after performic acid oxidation as cysteic acid. [c] Recalculated on the basis of mol % from closest integer values to compare with human pancreatic elastase 1 and 2. [d] From Feinstein et al. (1974). [e] From Mallory and Travis (1975). [f] From Shotton (1970).

TABLE III: Relative Proteolytic Activity of Human and Porcine Pancreatic Elastases.

Substrate	Porcine Elastase[a]	Human Elastase 1	Human Elastase 2
RBB elastin	100	15	18
RBB hide	100	32	34
Congo red elastin	100	2.8	7.6
Undyed elastin	100	50	42

[a] Porcine elastase has been assigned a value of 100.

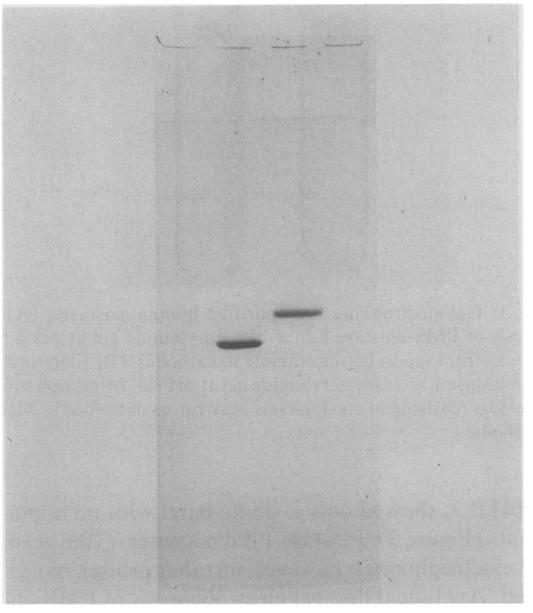

FIGURE 5: Discontinuous sodium dodecyl sulfate–polyacrylamide gel electrophoresis of purified human elastases. (Left) PMS-elastase 1. (Right) PMS-elastase 2. Migration was from top to bottom (cathode to anode). Gels were run as described in Materials and Methods.

and Trasylol on elastolytic activity was determined using RBB elastin in 50 mM Tris-HCl (pH 8.8) containing 0.025% Triton X-100. Concentrations of NaCl from 25 to 250 mM did not greatly affect elastase 1 except for slight inhibition above 150 mM. The activity of elastase 2 appeared to be enhanced in 25–250 mM NaCl, approaching approximately twice the activity observed in the absence of added salt.

Trasylol in concentrations of 50–500 Kallikrein inhibitor units/ml of assay solution did not affect the activity of human elastase 1 or elastase 2 but enhanced the elastolytic activity of porcine pancreatic elastase, approaching a twofold increase in activity at the highest level of inhibitor employed.

Amino Acid Composition. The mole percentage amino acid compositions of human elastases 1 and 2 are presented in Table II. The amino acid compositions of human "elastase 1" and "elastase 2" isolated by Feinstein et al. (1974) and the human "protease E" isolated by Mallory and Travis (1975), as well as of porcine elastase (Shotton, 1970), were recalculated in terms of mole percent from published nearest integer values and are also presented in Table II.

Activity toward Protein Substrates. The activities of human elastases 1 and 2 compared with that of porcine elastase against RBB elastin, Congo red elastin, and undyed elastin, as well as toward RBB hide are shown in Table III. Although the elastolytic activities of elastases 1 and 2 are lower than those of porcine elastase, the relative activities of the three enzymes are dependent on the substrate employed, the activities of the human elastases against undyed elastin being relatively higher than toward RBB elastin or Congo Red elastin. The human elastases were equally active against RBB hide, which we have employed previously for the determination of general proteolytic activity (Rinderknecht et al., 1968b). This substrate was used for assays of inhibition of purified human elastases by human serum protease inhibitors since α_2-macroglobulin inhibition can only be measured using high molecular weight substrates. RBB hide was preferable to RBB elastin for these assays since the linear range of absorbance at 595 nm (product formation) vs. enzyme concentration extended to much higher optical density values.

Substrate Specificity and Kinetic Parameters. Human elastases 1 and 2 were tested against three synthetic substrates

TABLE IV: Kinetic Parameters of Elastases toward $Suc(Ala)_3NA$ and t-Boc-Ala-NP.

Enzyme	K_m (mM) for $Suc(Ala)_3NA$	k_{cat} (s^{-1}) for $Suc(Ala)_3NA$	k_{cat}/K_m (mM^{-1}/s^{-1}) for $Suc(Ala)_3NA$	k_{cat}/K_m (mM^{-1}/s^{-1}) for t-Boc-Ala-NP
Porcine	1.7	19	11	11.5
Human elastase 1	1.4	0.55	0.39	3.2
Human elastase 2	13	0.11	0.008	0.92

which have been used previously for porcine elastase. For each substrate, the activity of porcine elastase was determined and used as a standard for comparison.

$Suc(Ala)_3NA$ has been used previously by Bieth et al. (1974) in a specific assay of porcine elastase which can be easily performed in a spectrophotometer. The kinetic parameters K_m and k_{cat} determined in this laboratory for porcine elastase and human elastases 1 and 2 are presented in Table IV. Porcine elastase hydrolyzes this substrate much more rapidly than either human enzyme. The lower activity of elastase 1 is primarily due to a lower k_{cat}, whereas elastase 2 also exhibits a large K_m, thus rendering it nearly inactive.

Kinetic parameters of porcine elastase and human elastases 1 and 2 were also determined for hydrolysis of t-Boc-Ala-NP. Visser and Blout (1972) determined a K_m of 0.3 mM for this substrate with porcine elastase. However, the procedure described by these authors included the use of varying concentrations of methanol or acetonitrile in the assay mixture. Other authors have shown that organic solvents substantially influenced the kinetic parameters of porcine elastase in the hydrolysis of certain low molecular weight substrates (Bieth et al., 1974; Bieth and Wermuth, 1973; Geneste and Bender, 1969). Thus a constant concentration of methanol was used in our experiments. In 5% (v/v) methanol the K_m for hydrolysis of t-Boc-Ala-NP by porcine elastase was much too large to determine. In 1% methanol, K_m's in the range of 1–3 mM were observed for all three elastases. However, the data obtained did not permit accurate kinetic analysis since, due to solubility limitations, the highest substrate concentration employed was 0.4–0.5 mM. The ratio k_{cat}/K_m obtained at low substrate concentrations is presented in Table IV. Again porcine elastase was relatively more active than the human elastases by this ratio of kinetic parameters, the ratio being approximately 11:3:1 for porcine elastase–human elastase 1–human elastase 2, respectively.

Human elastases 1 and 2 both hydrolyzed $Ac(Ala)_3OMe$ at rates less than that of porcine elastase, the relative rates being 1.0:0.78:0.12 for porcine elastase–elastase 1–elastase 2. Activities toward two chymotrypsin substrates (AcTyrOEt and BzTyrOEt) were determined. The relative rates of hydrolysis of AcTyrOEt were 0.04 and 0.35 for elastase 1 and 2 as compared with bovine α-chymotrypsin. Elastase 1 showed no activity toward BzTyrOEt, while the activity of elastase 2 was 0.02% of that of human chymotrypsin II. Neither elastase 1 nor elastase 2 hydrolyzed the trypsin substrates TosArgOMe or BzArgOEt at a measurable rate.

Inhibition by Serum Protease Inhibitors. Human elastases 1 and 2 and porcine elastase were tested for inhibition of hydrolysis of RBB hide by the human serum protease inhibitors α_1-antitrypsin and α_2-macroglobulin. Experiments measuring extent of inhibition vs. inhibitor concentration were performed as described in Materials and Methods. One milligram of purified α_2-macroglobulin inhibited 54 μg of elastase 1, 52 μg of elastase 2, and 60 μg of porcine elastase, while 0.22 mg of elastase 1, 0.28 mg of elastase 2, and 0.21 mg of porcine elastase were inhibited by 1 mg of α_1-antitrypsin. All three elastases were completely inhibited by each inhibitor at higher ratios of inhibitor to protease.

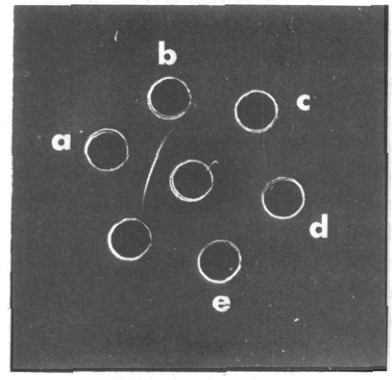

FIGURE 6: Immunodiffusion of human proteases with antiserum to elastase 2. Center well contains undiluted antiserum to purified elastase 2. (a) PMS-elastase 2 (0.35 mg/ml); (b) PMS-human chymotrypsin II (0.35 mg/ml); (c) elastase 1 (0.25 mg/ml); (d) human chymotrypsin I (0.25 mg/ml); (e) human anionic trypsin (0.20 mg/ml).

Effect of Inhibitors on Elastase 2. $PhCH_2SO_2F$ treatment of elastase 2 abolished both specific elastolytic activity as well as activity toward $Suc(Ala)_3NA$. On the other hand, incubation of elastase 2 with Tos-$PhCH_2Cl$ for a period four times that necessary for complete inhibition of chymotrypsin II did not affect the elastolytic activity of elastase 2 toward RBB elastin or the ability of the enzyme to hydrolyze AcTyrOEt or $Suc(Ala)_3NA$.

N-Terminal Amino Acid Residues. The N-terminal residue of both human elastases was shown to be valine using the method for dansylation of proteins in sodium dodecyl sulfate described by Weiner et al. (1972). After 4 h of hydrolysis, the N-terminal residues of both elastase 1 and elastase 2 were identified to be a mixture of valine and a valine peptide. After 24 h of hydrolysis, the N-terminal dansylvaline spot was very pronounced, while the spot attributed to the dansylvaline peptide was greatly reduced in both proteins. The penultimate residues of elastases 1 and 2 were both shown to be leucine by identification of the dansyl derivative of the new N-terminal amino acid residue after one round of Edman degradation performed as described in Materials and Methods.

Immunological Studies. Immunodiffusion experiments revealed a strong precipitin line between elastase 2 and specific antiserum produced against purified elastase 2, as shown in Figure 6. Furthermore, neither elastase 1, human chymotrypsin I or II, or human anionic trypsin gave a visible precipitin line with antiserum to elastase 2.

Discussion

Recent communications have demonstrated the presence of several proteases in human pancreatic tissue extracts which

possessed activity toward $Ac(Ala)_3OMe$, a substrate hydrolyzed specifically by porcine pancreatic elastase (Feinstein et al., 1974; Mallory and Travis, 1975). However, purification of one protease which hydrolyzed $Ac(Ala)_3OMe$ yielded an enzyme with negligible activity against undyed elastin, Congo red elastin, or orcein elastin (Mallory and Travis, 1975).

In this laboratory, RBB elastin has been employed to assay elastolytic activity in human pancreatic tissue extracts. It has previously been shown that RBB elastin is a specific substrate for porcine elastase, although trypsin and chymotrypsin enhance its elastolytic activity (Rinderknecht et al., 1970). Trasylol was added to assay mixtures in order to limit potentiation of elastolytic activity by trypsin and chymotrypsin. Since it has been shown that human chymotrypsin II is not inhibited by Trasylol (Feinstein et al., 1974), the apparent elastolytic activity of pancreatic extracts in early stages of purification may represent elastase activity potentiated to some degree by chymotrypsin. Thus it is possible that losses of elastase activity in the first few steps of purification are at least partially due to separation of elastase from chymotrypsin and/or trypsin. It was observed that fractionation with 55% ammonium sulfate leaves considerable amounts of trypsin and chymotrypsin in the supernatant, and that dialysis vs. sodium phosphate causes rapid loss of tryptic activity, since human trypsin requires calcium for stability (Travis and Roberts, 1969; Mallory and Travis, 1973).

Isolation of proteases with elastolytic activity from human pancreatic extracts was facilitated by screening acetone powders of individual pancreases for tryptic activity. Approximately one in four pancreases obtained at autopsy in this hospital was rich in trypsin. Furthermore, we have observed that the level of elastase activity, as measured by hydrolysis of RBB elastin, parallels that of trypsin. In those pancreases selected for isolation of elastase, the total amount of elastolytic activity approached total tryptic activity on a weight basis, suggesting that human elastase is one of the major proteases in normal human pancreas.

Separation of human elastases 1 and 2 was readily accomplished by ion-exchange chromatography since the two enzymes were eluted at quite different salt concentrations from CM-Sephadex C-50, elastase 1 being anionic relative to elastase 2 (Figure 1). The anionic nature of human elastase 1 was further demonstrated by the observation that elastase 1 adhered strongly to DEAE-Sephadex at pH 6.5 (Figure 2). This difference in ionic properties was confirmed by polyacrylamide gel electrophoresis experiments, in which elastase 1 migrated as a single band in pH 8.3 gels but did not enter a pH 4.5 gel, while elastase 2 migrated as a single band in pH 4.5 gels but did not enter a pH 8.3 gel. The molecular weights of elastases 1 and 2 were determined to be 29 300 and 25 000, respectively, by discontinuous sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Sephadex G-75 gel filtration experiments revealed apparent molecular weights of 33 000 for elastase 1 and 26 500 for elastase 2. Thus, the two enzymes clearly differ in molecular weight as well as ionic properties. Human elastase 1, which is an anionic protein, hydrolyzes elastin substrates at rates similar to those of human elastase 2, a cationic protein. These results indicate that the suggestion of Gertler (1971a,b), that only cationic proteases are capable of digesting elastin, is perhaps an oversimplification.

The homogeneity of each of the two human elastases was demonstrated by the following criteria. Elastases 1 and 2 were eluted as symmetrical peaks from DEAE-Sephadex and CM-Sephadex, respectively, and both enzymes were eluted from Sephadex G-75 as symmetrical peaks with constant specific activity. Further indications of homogeneity of elastases 1 and 2 were their migration as single bands on polyacrylamide gel electrophoresis at pH 2.3, 4.5, and 8.3 and on discontinuous sodium dodecyl sulfate–polyacrylamide gel electrophoresis after treatment with $PhCH_2SO_2F$ and removal of autolysis products by gel filtration.

Human elastases 1 and 2 both hydrolyze undyed elastin and RBB hide at rates lower than that of porcine elastase. Both human enzymes are less active toward RBB elastin, but nevertheless this substrate is hydrolyzed at a significant rate (Table III). On the other hand, Congo red elastin is hydrolyzed at a greatly reduced rate by the human elastases, when compared with that of the porcine enzyme. The susceptibility of dyed elastin to hydrolysis by porcine elastase has been shown to depend on the nature of the dye and the procedure employed for staining (Loeven, 1970). It would appear that the elastolytic activities of the two human enzymes may be reduced to a greater extent than the activity of porcine elastase when assayed with the dyed elastin substrates employed in this study.

Elastase 2, which appears to be the major elastase in human pancreas, is much less active toward $Ac(Ala)_3OMe$, $Suc(Ala)_3NA$, or t-Boc-Ala-NP than porcine elastase. It is therefore not surprising that previous attempts to isolate human pancreatic elastase on the basis of activity against substrates designed for the porcine enzyme were unsuccessful. It is of interest to note that human elastase 2 does hydrolyze the chymotrypsin substrate AcTyrOEt. Although porcine elastase does not hydrolyze this substrate (Shotton, 1970), a second porcine elastase with high activity toward AcTyrOEt has recently been reported (Ardelt, 1974; Uram and Lamy, 1969).

Since elastase 2 hydrolyzes AcTyrOEt and the purification of elastase 2 is somewhat similar to that of chymotrypsin II described by Coan et al. (1971), these two proteases were compared in detail. Elastase 2 does not hydrolyze BzTyrOEt, a substrate which is rapidly hydrolyzed by chymotrypsin II. Furthermore, $Tos\text{-}PhCH_2Cl$ (a potent, irreversible inhibitor of chymotrypsin II) has no effect on either the elastolytic activity or esterase activity of elastase 2. Human chymotrypsin II does not cross-react with antiserum produced against elastase 2, and the two enzymes are resolved in sodium dodecyl sulfate–polyacrylamide gel electrophoresis.[3] Finally, the published amino acid composition of chymotrypsin II (Coan et al., 1971) differs significantly from that of elastase 2. These results demonstrate that elastase 2 and human chymotrypsin II are unique proteases from human pancreas.

The human serum protease inhibitors α_1-antitrypsin and α_2-macroglobulin completely inhibited the general proteolytic activity of both human elastases. Furthermore, both α_1-antitrypsin and α_2-macroglobulin inhibited approximately equal amounts of human elastase 1 or 2 or porcine elastase. This observation suggests that the lower activity of the human elastases as compared with that of the porcine enzyme toward both high and low molecular weight substrates is due to differences in kinetic parameters and not to inactive protein which is purified along with active enzyme. It has been shown for both α_1-antitrypsin (Cohen, 1973) and α_2-macroglobulin (Barrett and Starkey, 1973) that a protease must be active in order to bind to these inhibitors. Thus if appreciable amounts of inactive enzyme were present in the purified elastase preparations, this would be reflected in the amount of enzyme (determined as protein by absorbance at 280 nm) bound per mg of inhibitor.

The N-terminal amino acid sequences of human elastases

[3] Unpublished observations in this laboratory.

1 and 2 were both determined to be Val-Leu, while that of porcine elastase is Val-Val (Hartley and Shotton, 1970). Porcine elastase is thought to be derived from proelastase by tryptic activation of the proenzyme (Uram and Lamy, 1969). Bovine trypsin, chymotrypsin, and thrombin and porcine elastase have all been shown to possess a similar activation site, and all four enzymes possess N-terminal sequences beginning with two hydrophobic, aliphatic amino acid residues (Hartley and Shotton, 1970). The fact that human elastases 1 and 2 possess two aliphatic residues at their respective N-termini suggests that they are activated by a mechanism similar to that of the previously studied serine proteases.

The amino acid compositions of elastases 1 and 2 are both similar to that of porcine elastase. Furthermore, the amino acid compositions of "elastases 1 and 2" isolated by Feinstein et al. (1974) are similar to human elastases 1 and 2, respectively, except for cysteine content. Porcine elastase contains eight half-cystine residues in the form of four disulfide bonds which are apparently an invariant structural feature of the serine proteases (Hartley and Shotton, 1970). The percent composition of half-cysteine obtained for each human elastase agrees well with that calculated for porcine elastase (Table II), indicating that these human proteases are probably similar in overall structure to the serine proteases previously studied.

In summary, human pancreatic elastase 1 is an anionic protease of 29 000 molecular weight with activity toward undyed elastin, RBB elastin, RBB hide, $Ac(Ala)_3OMe$, and t-Boc-Ala-NP. This enzyme slowly hydrolyzes Congo red elastin and $Suc(Ala)_3NA$ and is essentially inactive against AcTyrOEt, BzTyrOEt, BzArgOEt, and TosArgOMe. Human pancreatic elastase 2 is a cationic protease of 25 000 molecular weight which hydrolyzes undyed elastin, RBB elastin, RBB hide, and AcTyrOEt. This enzyme also hydrolyzes Congo red elastin, $Ac(Ala)_3OMe$, $Suc(Ala)_3NA$, and t-Boc-Ala-NP slowly and has negligible activity toward BzTyrOEt, BzArgOEt, and TosArgOMe. These results suggest that elastases 1 and 2 are clearly different from the previously described human pancreatic trypsins (Travis and Roberts, 1969; Mallory and Travis, 1973) and chymotrypsins (Coan et al., 1971; Coan and Travis, 1972).

Human elastase 2 is clearly different from the elastase-like protease (protease E) isolated by Mallory and Travis (1975). Elastase 1 appears to be similar to protease E in terms of amino acid composition, molecular weight, and ionic character. Both enzymes hydrolyze $Ac(Ala)_3OMe$, $Suc(Ala)_3NA$, and t-Boc-Ala-NP and are inactive toward TosArgOMe and AcTyrOEt. However, elastase 1 hydrolyzes undyed elastin as well as Congo red elastin and RBB elastin, while Mallory and Travis (1975) reported that protease E does not hydrolyze undyed elastin, Congo red elastin, or orcein elastin. In view of the many similarities between the two preparations, it is probable that elastase 1 and protease E are the same enzyme. The discrepancy with respect to elastolytic activity might be related to the variability in susceptibility of various lots of both undyed and dyed elastin to hydrolysis by human as well as porcine elastases as observed in this laboratory, and reported for porcine elastase by Loeven (1963, 1970).

The proteases isolated by Feinstein et al. (1974) and by Trowbridge and Moon (1972) were not sufficiently characterized to permit detailed comparison with the two human elastases described in this report. In fact, the two elastases isolated from activated human pancreatic tissue differ substantially from each other in both physical properties and substrate specificity. Neither of the human enzymes is closely comparable to porcine elastase if both substrate specificity and physical properties are taken into account. These findings suggest that further study of the human pancreatic elastases is necessary in order to form the basis for further exploration of the physiological role of these proteases in man.

Acknowledgments

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CORRECTIONS

Molecular Forms of Acetylcholinesterase from *Torpedo californica:* Their Relationship to Synaptic Membranes, by Jamson S. Lwebuga-Mukasa, Shelley Lappi, and Palmer Taylor,* Volume 15, No. 7, 1425–1434 (1976).

In Figure 2 the notations (a) and (b) have been reversed. (a) should denote the upper panel while (b) denotes the lower panel.

The Resolution of *Ascaris* Cuticle Collagen into Three Chain Types, by Herbert J. Evans, Catherine E. Sullivan, and Karl A. Piez,* Volume 15, No. 7, 1435–1439 (1976).

In the abstract, second column, lines 1 and 2 should read: ". . . 27 residue % glycine, 36 residue % proline, and 17 residues of . . .".