Purification of Human Elastase[1] (36903)

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Elastase, pancreatopeptidase E, is a pancreatic enzyme unique in its ability to hydrolyze the scleroprotein, elastin. Since the initial report by Balo and Banga (1) on the elastolytic activity of pancreatic extract, a number of investigators have determined the chemical and biologic characteristics of porcine elastase. Lewis, Williams and Brink (2, 3) purified porcine elastase and determined some of its properties. It was found to possess proteolytic activity against a wide range of protein substrates. Brown, Kauffman and Hartley (4) reported on the primary structure of porcine elastase in 1967; they also noted that it had many residues common to bovine trypsin and chymotrypsin.

Although many studies have been conducted on the biologic properties of elastase, its precise functions and significance remain unclear. The presence of elastase in exocrine cells of the pancreas (5, 6) and in pancreatic juice (7) as well as its general proteolytic activity indicate a digestive function. Balo and Banga (8) and Loeven (9) have reported that the amount of elastase in the pancreas is reduced in arteriosclerotic individuals. Also, elastase has been found to reduce clotting time *in vitro* (10). Hall (11)has suggested that the aging of arterial tissue is characterized by the absence of elastase activity and further proposed that arterial changes were affected by changes in the balance between elastase and its inhibitor. Recently, Geokas (12) and Geokas *et al.* (13) presented evidence that elastase was involved in the production of vascular injury in acute pancreatitis.

Very little information on human elastase is available. The purpose of this study was to purify the human enzyme.

Experimental Procedure and Results. Preparation of elastin and orcein–elastin. Powdered elastin was prepared from human aortas by a modification of the method of Grant and Robbins (14). Aortas without gross evidence of arteriosclerosis were stripped free of fat and intima and cut into small pieces. These were stirred three times with acetone for 10 to 15 min, followed by one 15-min washing with ethyl ether and multiple washings with distilled water. The residue was suspended in 0.1 *N* NaOH and boiled. As the supernatant became yellow, the 0.1 *N* NaOH was replaced until further boiling yielded a colorless supernatant. The residue was then washed with distilled water until the pH was approximately 7. An aqueous suspension of the residue was heated to boiling and the elastin preparation was filtered at 60 to 70°. The residue was lyophilized and pulverized to a fine powder. The final product was used for the adsorptive purification of elastase and for the preparation of orcein–elastin substrate of the assay system.

Orcein–elastin was prepared by mixing 500 mg of human aortic elastin with 5 ml of a solution of 1% orcein in 70% ethanol to which 0.05 ml of concentrated HCl had been added. The mixture was allowed to stand at room temperature for 2.5 hr with occasional stirring and then centrifuged. The stained elastin was repeatedly washed with 70% ethanol and distilled water until the supernatant gave a reading of less than 0.005 OD at 590 μm. The orcein–elastin was then air-dried, pulverized, and stored at 4° until used.

Assay. Elastase activity was assayed by a modification of the photometric method of Sachar *et al.* (15). All assays were performed

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in duplicate, and appropriate reagent blanks were included.

The assay system consisted of 6 mg of the orcein–elastin substrate and 1 mg of the enzyme preparation dissolved in 1 ml of 0.05 *M* carbonate buffer, pH 8.8. The mixture was incubated in an H_2O bath at 37°, with occasional stirring. At the end of 1 hr, the reaction was stopped by adding 0.33 ml of 0.2 *M* phosphate buffer, pH 6. The tubes were centrifuged and the OD of the supernatant was read against a reagent blank on the Beckman D.U. The activity of the preparation was determined from a standard curve prepared by complete digestion of known amounts of orcein–elastin.

One elastase unit was defined as that amount of enzyme which would solubilize 1 mg of elastin in 1 hr.

Elastase activity was further confirmed using the method of Sbarra, Gilfillan and Bardawil (16). Enzyme samples were placed in wells in agar elastin plates; elastase activity was identified by zones of clearing around wells containing the enzyme. This method was also used for screening various fractions containing pigmented material which interfered with the spectrophotometric assay.

Preparation of elastase. Human pancreata obtained at autopsy and stored at —26° were minced and homogenized in 4 vol of 0.1 *M* acetate buffer, pH 4.5, at 4°. The homogenate was strained through gauze and centrifuged at 5000 rpm at 4° for 30 min. The supernatant was brought to 45% saturation with ammonium sulfate and again centrifuged at 5000 rpm at 4° for 15 min. The precipitate was then washed three times with 45% saturated ammonium sulfate solution in 0.1 *M* acetate buffer, and dialyzed against multiple changes of distilled H_2O at 4° until the dialysate demonstrated a negative reaction when tested with Nessler's reagent. The preparation was then lyophilized.

The lyophilized extract was suspended to 1% in 0.1 *M* acetate buffer (pH 4.7), containing 0.1 *M* NaCl, stirred at 25° for 1 hr, and centrifuged at 2000 rpm for 1 hr. The residue was discarded. Pulverized elastin (20 mg/ml) was added to the supernatant, stirred for 1 hr at room temperature, and centrifuged at 9000 rpm for 10 min. The precipitate was then washed twice with buffered saline, pH 4.7. The washed precipitate was suspended in 0.2 *N* acetic acid, in a vol equal to the supernatant to which elastin had been added, stirred for 1 hr at room temperature, and centrifuged at 9000 rpm for 10 min. The supernatant was brought to pH 4.7 with 4 *N* NaOH and then to 50% saturation with ammonium sulfate and refrigerated at 4° for 18 hr. The mixture was centrifuged at 4000 rpm for 1 hr at 4°, suspended in 0.05 *M* carbonate buffer (pH 8.8), dialyzed against multiple changes of distilled H_2O at 4°, and then lyophilized. The elastase preparations were further purified by repeating the above procedures.

The protein content of the elastase preparations was determined by the method of Lowry *et al.* (17), and elastase activity per mg of protein was ascertained.

The crude extracts, *i.e.*, those obtained in the initial extraction with 4 vol of 0.1 *M* acetate buffer (pH 4.5) at 4°, showed no elastolytic activity. This was attributed to the low concentration of elastase and the presence of inhibitors in the extracts. The precipitates which were obtained by 45% saturation with $(NH_4)_2SO_4$ and dialyzed against cold distilled water consistently showed elastolytic activity. The specific activity varied greatly in individual pancreata, ranging from a minimum of 1 unit/mg to a maximum of 13 units of elastase activity/mg.

When the 45% ammonium sulfate fractions were adsorbed on powdered elastin and then eluted, there was a significant increase in activity, with some variation in individual pancreata. The elastase activities ranged from 9 to 19 units/mg. The yield varied from 5 to 10 mg/100 g of pancreas. Repeating the procedure of adsorption and elution from powdered elastin of the less active preparations, *e.g.*, 9 units/mg. usually resulted in increase in activity, whereas similar treatment of the best preparations did not produce a significant increase in activity. The most active preparations contained 19 units/mg. (Crystalline porcine elastase,[2] fur-

[2] Worthington Biochemical Corp., Freehold, NJ.

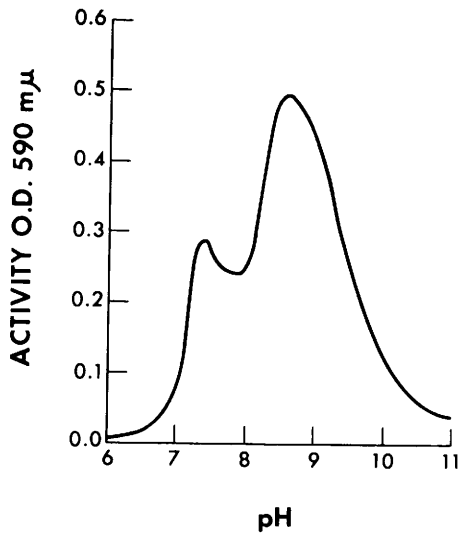

FIG. 1. Effect of pH on activity of human elastase (1 mg/ml). Substrate: human orcein–elastin.

ther purified with powdered elastin, had an activity of 35 units/mg in our assay system.) When purified human elastase was electrophoresed on agarose (18) in 0.05 *M* barbital buffer at pH 8.8, 150 V, 20 mA, and stained with 0.2% amidoschwarz, only a single band was present.

Effect of pH on activity. The effect of pH on the enzymatic activity of human elastase was determined. Elastase, 1 mg/ml in 5.0×10^{-2} *M* carbonate, ranging from pH 6.0 to 11.0, was incubated with orcein–elastin for 1 hr. The pH of the mixture was determined before and after incubation. The same range of pH values was used with 0.1 *M* borate buffer, and the activity of elastase determined. The maximum activity of elastase was between 8.6 and 8.9, with a sharp drop in activity in the more alkaline range (Fig. 1). In the lower range, elastase activity began at pH 7.4, with a low shoulder extending to pH 8.2. A similar curve was obtained with 0.1 *M* borate buffer.

Effect of trypsin. Activation of proelastase by trypsin has been reported by Grant and Robbins (19). In order to ascertain the possible presence of proelastase, a preparation with an activity of 9 units/mg was treated with trypsin to activate any proelastase present. Trypsin (1.0 and 0.5 mg/ml) was added to 0.05 *M* bicarbonate buffer (pH 8.8), containing 1.0 mg/ml elastase, and incubated for 3 hr at 37°. This solution was then tested for elastase activity in the orcein–elastin assay system described above. Elastase, not pretreated, and trypsin alone were also tested for their elastolytic activities. Pretreatment of human elastase with trypsin did not increase the elastolytic activity of the preparation (Table I). The activities of the elastase preparations, with or without trypsin, were essentially the same. Trypsin alone had no elastolytic activity.

Discussion. Elastase activity was present in the highly purified preparations of all of the pancreata examined, the amount of activity varying widely in individual specimens. In this study, we found no correlation between specific disease states and the elastase content of the pancreas. The pancreata containing the most activity were obtained from individuals undergoing prolonged fasting prior to death, suggesting that elastase was stored as a zymogen and that the lack of secretory stimulation led to an increase in elastase concentration. This indicates that correlations between elastase content of human pancreas and specific disease states may be unreliable unless the prandial state of the individual is taken into consideration.

Grant and Robbins (19) reported the presence of elastase as a zymogen in the porcine pancreas and in canine pancreatic juice. They observed that trypsin or a duodenal extract was necessary for the activation of the enzyme and also found that the elastolytic effect of the duodenal extract was abolished if soybean and pancreatic trypsin inhibitors were present from the beginning of the activation period. If these inhibitors were

TABLE I. Effect of Trypsin on Human Elastase Activity.

Elastase (mg/ml)	Trypsin (mg/ml)	Activity (U/mg)	Activity (%)
1.0	0	9.5	100
1.0	0.5	9.2	97
1.0	1.0	9.0	95
0	1.0	0.1	1
0	0.5	0.1	1
0	0	0.1	1

present only during the assay, however, no inhibition was noted. In our study, when human elastase was assayed with trypsin added, no enhancement of activity was apparent. This indicates that no proelastase was present in the purified elastase preparations and also that activation of the proenzyme occurred either before or during the extraction procedure.

Summary. Elastase was obtained from human pancreas by extraction with sodium acetate buffer, pH 4.5, fractionation with ammonium sulfate, and adsorption on powdered elastin. The enzyme was eluted from elastin with dilute acetic acid and precipitated with ammonium sulfate. An orcein–elastin substrate was used for assay of the enzymatic activity. Maximum activity occurred at pH 8.6 to 8.9. Proelastase was not present in the purified preparations, *i.e.*, there was no enhancement of enzymatic activity upon treatment with trypsin.

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