Biochimica et Biophysica Acta, 612 (1980) 262—267

BBA 68928

A NOVEL METHOD OF ISOLATION AND SOME CHARACTERISTIC PROPERTIES OF HUMAN PANCREATIC ELASTASES

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(Received June 25th, 1979)

Key words: Sawdust column; Elastase; (Human pancreas)

Summary

One component of elastases of human pancreatic juice and pancreatic extract was obtained in a highly purified state by chromatography on a column of sawdust. The elastase obtained after repeated adsorption chromatography with NaCl-containing buffers was almost homogeneous by gel filtration and polyacrylamide gel electrophoresis. This elastase showed relatively high elastolytic activity, but relatively low hydrolytic activity towards succinyl trialanine *p*-nitroanilide, as compared with another component of pancreatic juice elastase (which was not absorbed onto sawdust). Both elastases isolated were alkaline earth metal-dependent enzymes.

Lewis et al. [1] first reported the isolation and some properties of human pancreatic elastase. Since that time, several papers have been published on purification of the enzyme [2—4], although the methods were rather complicated and laborious. We observed that porcine pancreatic elastase was precipitated by adding a dilute solution of lignin and, on addition of NaCl, the precipitate was dissolved to release the enzyme activity. This fact led us to try chromatography using a column of sawdust (a naturally occurring lignin derivative) to isolate human pancreatic elastase. The chromatography was effective for isolating one of the components of pancreatic juice elastase.

The present paper describes this method of isolation and some properties of elastase from human pancreatic tissue and juice by chromatography using a column of sawdust.

Materials and Methods

Sawdust (derived mainly from North American cedar) was sifted to particle size between 16 and 32 mesh after drying, and was suspended in 5 vols. (w/v) 2 M NaOH at 20°C for 4 h with gentle stirring, after removing air bubbles under reduced pressure. The sawdust was extensively washed with water, then suspended in 5 vols. (w/v) 2 M HCl overnight, and finally washed with water. The treated sawdust was placed in a column and equilibrated with $5 \cdot 10^{-3}$ M Tris-HCl/$5 \cdot 10^{-3}$ M calcium acetate buffer pH 7.6.

The human pancreatic juice was collected from patients suffering from periampullary neoplasm of the duodenum and, therefore, pancreaticoduodenectomy was performed as reported previously [5]. The juice was dialyzed against Tris-HCl/calcium acetate buffer and centrifuged at 18 000 × *g* for 30 min to remove flocculent material.

Human pancreatic tissue obtained on autopsy and confirmed as normal, histologically, was coarsely disrupted in a Waring Blendor, followed by washing with cold acetone and then with cold diethylether to remove lipid material. The treated tissue was dried in an air current and blended with a suitable amount of $1 \cdot 10^{-2}$ M Tris-HCl/$2 \cdot 10^{-3}$ M $CaCl_2$ buffer pH 7.6. The supernatant obtained by centrifuging this mixture at 18 000 × *g* for 30 min was used as the human pancreatic tissue extract.

Elastase was assayed by the method of Largman et al. [2], but using undyed human aortic elastin prepared by the method of Seifter et al. [6] and fluorescamine for determining liberated amino groups [7]. 1 ml enzyme and 1 ml 5% suspension of aortic elastin in $5 \cdot 10^{-2}$ M triethanolamine-HCl buffer (pH 8.3)/0.025% Triton X-100, was incubated at 37°C for 1 h with gentle stirring. The mixture was centrifuged at 0°C for a few minutes, 0.2 ml supernatant was taken into 2.5 ml $5 \cdot 10^{-2}$ M potassium phosphate buffer, pH 8.0, and 0.5 ml 0.3% fluorescamine/acetone was added. After the solution was left at room temperature for 30 min, the developed fluorescence was measured on a Hitachi Fluorescence Spectrophotometer Model 203 with excitation and emission set at 390 and 475 nm, respectively. 1 unit of elastase activity was defined as the enzyme amount which increased fluorescence intensity equivalent to 60 nmol DL-Leu-DL-Gly-DL-Phe as the standard in 1 h.

Esterase activity was also determined. Succinyl-L-Ala-L-Ala-L-Ala *p*-nitroanilide ($Suc(Ala)_3pNA$) was used as substrate and 20 µl 0.125 M substrate in *N*-methylpyrrolidone was added to 2.5 ml enzyme in 0.05 M Tris-HCl buffer, pH 8.3, at 40°C and the developed color was measured at 410 nm [8]. 1 unit of esterase activity was defined as the enzyme amount that liberated 1 nmol *p*-nitroanilide in 1 min.

Concentration of protein was determined approximately by estimating absorbance at 280 nm and the specific activity was calculated as enzyme activity/$E^{1\,cm}_{280\,nm}$.

Elastin of bovine neck ligament, $Suc(Ala)_3pNA$, DL-Leu-DL-Gly-DL-Phe, and fluorescamine were purchased from Sigma Chemical Co., Protein Research Foundation (Osaka), Nutritional Biochemicals and F. Hoffman-LaRoche and Co., respectively. Other chemicals were of pure grade commercially available.

Results

Purification of human pancreatic elastase

The elastolytic activity of human pancreatic juice was separated into two fractions on the sawdust column: one in the non-adsorbed fraction (fraction E1) and the other (fraction E2) in the eluate with 0.5 M NaCl in $5 \cdot 10^{-3}$ M Tris-HCl/$5 \cdot 10^{-3}$ M calcium acetate buffer. The elastolytic activity of fraction E1 was not adsorbed, even by the use of a new sawdust column. On the other hand, the elastolytic activity of fraction E2 was adsorbed again on the column after the fraction was desalted and eluted with 0.5 M NaCl. In the rechromatography, the elastolytic and esterase activities and protein concentration were in perfect parallel in the elution with NaCl.

A similar result was obtained from pancreatic tissue extract by sawdust chromatography. Changes in specific activity and recovered activity of the enzyme during purification are summarized in Table I.

Purity of human pancreatic juice elastase E2

The pancreatic juice elastase fraction E2 was concentrated in a membrane filter under nitrogen gas and then subjected to gel filtration using Sephadex G-75. The enzyme activity was eluted in a symmetrical peak, and paralleled the protein peak. A small amount of non-elastase protein, however, appeared later, and this had a molecular weight much less than the elastase. It was noteworthy, however, that the elastase fraction recovered after gel filtration by Sephadex G-75 showed no increase in the specific activity. The molecular weight of fraction E2 elastase estimate by the gel chromatography test was $2.5 \cdot 10^4$, (approximately the same as chymotrypsinogen). This preparation was homogeneous by analytical polyacrylamide gel electrophoresis at pH 4.3 and the protein band moved toward the cathode. The isoelectric point of the elastase was estimated to be 9.5.

TABLE I

SUMMARY OF PURIFICATION OF THE HUMAN PANCREATIC ELASTASES BY CHROMATOGRAPHY ON A SAWDUST COLUMN

	Volume (ml)	Total elastolytic activity (units)	Total protein ($E^{cm}_{280\ nm}$)	Specific activity (units/$E^{cm}_{280\ nm}$)	Recovery (%)
Pancreatic juice					
Juice applied	40	3150	165.0	19.1	100
Effluent and washings (E1)	170	1380	104.5	13.2	43.8
Eluate with 0.5 M NaCl (E2)	100	1250	11.0	113.6	39.7
Pancreatic tissue					
Extract applied	12	1944	164.4	11.8	100
Effluent and washings (E1)	120	473	108.0	4.4	24.3
Eluate with 0.5 M NaCl (E2)	90	1000	13.0	76.9	51.4

TABLE II

ACTION ON VARIOUS SUBSTRATES OF HUMAN PANCREATIC JUICE AND PORCINE PANCREATIC ELASTASES

Substrates	Elastase (units/mg protein)		
	E2	E1	Porcine
Human aortic elastin	253	44	1 015
Porcine aortic elastin	82	20	680
Bovine neck ligament elastin	145	34	1 086
Suc(Ala)$_3$pNA	105	840	16 760

Properties and relative activities of human pancreatic juice elastase

Elastase fraction E2 showed an optimum pH at 8.3. The activity decreased by 50% at pH values of 7.3 and 9.2. The enzyme preparation was shown to be free from trypsin and chymotrypsin, because it could not attack Tos-Arg-OMe and the elastolytic activity was not affected by Tos-Phe-OClMe, a chymotrypsin inhibitor. The enzyme was unstable and the activity decreased on dialysis over several days at +4° C. Repeated freezing and thawing of the enzyme solution also inactivated the enzyme.

The relative activities of the enzyme on several substrates are presented in Table II. For comparison, the sawdust non-adsorptive elastase (fraction E1) was further purified on columns of DEAE-Sephadex A-50 and Sephadex G-75, according to Largman et al. [2], and the porcine pancreatic elastase was purified according to Hartley and Shotton [9]. The relative activities on several substrates were also examined. Comparison of specific activities of human pancreatic elastases and porcine pancreatic elastase was made on reported $E^{1\%}_{280nm}$ values of 24.5 for elastase fraction E1 [10] 20.2 for elastase fraction E2 [2] and 23.6 for porcine pancreatic elastase [10] as $E^{1\%}_{280nm}$. Table II shows that fraction E2 was much more active than fraction E1 in hydrolysing several of

TABLE III

EFFECT OF VARIOUS DIVALENT METALS ON ACTIVITIES OF ELASTASES ISOLATED FROM HUMAN PANCREATIC JUICE

Enzyme solutions were dialyzed against $2 \cdot 10^{-2}$ M Tris-HCl $2 \cdot 10^{-3}$ M EDTA-NH_4 buffer pH 7.6, at 4° C for 24 h. Metals added, $4 \cdot 10^{-3}$ M. Activities of E1 and E2 before dialysis were both 5 units/ml. Substrate used was Suc(Ala)$_3$pNA. Activities are expressed as percent changes of activity before dialysis.

Metal salt (acetate)	Relative activities	
	E1	E2
None	5	51
Mg	77	85
Ca	80	88
Sr	59	48
Ba	36	52
Mn	35	92
Zn	28	32
Cu	42	50

the elastins. However, the activity of the former enzyme toward Suc(Ala)$_3$pNA was much less.

Effect of various metals and inhibitor

Fractions E1 and E2 showed decreased activity when solutions were dialyzed against solutions containing EDTA. However, the dialyzed enzymes were reactivated to considerable extents by addition of Ca^{2+} and Mg^{2+} (Table III).

20 μg elastase fraction E2 was added to $1 \cdot 10^{-3}$ M phenylmethanesulfonyl fluoride in $5 \cdot 10^{-2}$ M Tris-HCl buffer pH 8.3 and incubated for 30 min. Activity of elastase fraction E2 toward Suc(Ala)$_3$pNA was completely inhibited.

Discussion

One of the components of elastase of human pancreatic juice was obtained in a highly purified state by repeated chromatography on a sawdust column. This effective phenomenon may be due to specific adsorption of the enzyme onto the lignin moiety of sawdust. In solutions of lignin of concentration from 0.1—10 mg/ml, both porcine and human pancreatic juice elastase were inactivated. The degree of inactivation by lignin proceeded along a parabolic curve with respect to the concentration of lignin. At concentrations of lignin of 5—10 mg/ml, the enzyme was completely inactivated and, in fact, was precipitated. However, the resulting precipitate was readily redissolved by addition of 0.5 M NaCl and solution showed almost the original activity. Human pancreatic juice elastase fraction E2 could be further purified by gel filtration. However, no increase in the specific activity was shown, suggesting that the non-elastase protein was a result of autodigestion of the elastase protein.

Elastase fractions E1 and E2 were considered to be identical with elastases 1 and 2 reported by Largman et al. [2], because our fraction E1 and E2 elastases were similar to elastases 1 and 2 in their behavior on ion exchangers, and in their molecular weights and isoelectric points.

In the present study, the value of absorbance of a 1% solution of elastase fraction E2 was not exactly determined due to its poor recovery. Porcine pancreatic elastase contained both elastolytic and esterase activities. In comparison to human pancreatic elastases, fraction E1 seems worthy of the name, esterase, while fraction E2 is more like an elastase. Further study will be necessary to confirm these suggestions.

The present study demonstrated characteristic properties of human pancreatic elastases. The effects of chelating agents and metal ions on the esterase activities of both enzymes showed that human pancreatic elastases are dependent on the presence of divalent metal ions.

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