SEPARATION OF HUMAN BLOOD PLATELET ELASTASE AND PROELASTASE BY AFFINITY CHROMATOGRAPHY.

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Received January 9,1975

SUMMARY

Platelet proelastase and elastase were separated and purified by a two step procedure : 1) elastase was adsorbed to mixed bed cellulose elastin columns and eluted as a single slow migrating protein band on acrylamide disc gel electrophoresis. Proelastase was not adsorbed to this column and was further purified by preparative acrylamide disc gel electrophoresis.

The separation of proteolytic enzymes present in human blood platelets (cathepsins, trypsin, chymotrypsin and elastase) by precipitation with ammonium-sulphate followed by gel filtration was reported (1). Incubation with trypsin strongly enhanced the elastolytic activity of a platelet extract and it was possible to dissociate a proelastase activated by trypsin (associated with the proteins precipitated at 40 % ammonium sulphate) from an elastase not activated by trypsin (associated with the proteins precipitated at 70 % ammonium sulphate (1-2). It was shown that pancreatic elastase can be quantitatively adsorbed on fibrous elastin (3). On the other hand there is some analogy between platelet elastase and pancreatic elastase as far as both of them appear as inactive precursors (proelastase) activable by limited proteolysis with trypsin (4, 5, 6, 7, 8). We therefore tempted to separate platelet elastase and proelastase by affinity chromatography on a cellulose-elastin mixed bed column followed by polyacrylamide preparative disc gel electrophoresis.

Abbreviations : $AcAla_3OMe$: N-acetyl-L-alanyl-L-alanyl-L-alanine methyl ester. AcTyrOEt : N-acetyl-L-tyrosine ethyl ester. BAPNA : N-benzoyl D-L-arginine-p-nitro anilide.

TABLE I

)istribution of proteins, proelastase and elastase, trypsin and chymotrypsin-.ike esterases in a platelet extract (supernate of centrifugation of a)latelet lysate by Triton-X-100 0.5 % final concentration) in two fractions)btained by gel filtration on G-150 Sephadex column (100 x 5 cm) of proteins)recipitated from the extract by ammonium sulphate at 40 % and 70 %)aturation and in peaks A and B obtained by affinity chromatography on :ellulose elastin of"40 % fraction" and "70 % fraction" and of fraction 3)btained by preparative acrylamide gel electrophoresis from pooled peak A-)aterial. Substrates used are mentioned under method. Esterolytic activities :re expressed in equivalents (micrograms) of crystalline elastase, trypsin, hymotrypsin per mg of protein (see methods).

FRACTION	PROTEIN (mg) *	ELASTASE	PROELASTASE **	TRYPSIN-LIKE ESTERASES	CHYMOTRYPSIN-LIKE
PLATELET EXTRACT	6080	0.28	0.37	0.10	0.05
40 % PEAK I	410	2.40	15.60	0.30	0.14
40 % PEAK A	330	1.13	17.8	0.92	0.55
40 % PEAK B	2.95	0.15	0	0.13	0.06
70 % PEAK II	52.8	15.5	3.0	6.0	4.1
70 % PEAK A	42.9	1.57	1.3	9.3	1.8
70 % PEAK B	4.2	23.5	0	0.65	0.41
Fraction 3	27.6	9.52	30.0	0.26	0.20

* Total protein content in each fraction

** Trypsin added for activation : 10 µg/mg protein

METHODS

Enzymatic determinations

The elastase activity was measured after incubation (20 min. at 37°C) with 10 µg of trypsin (2x cryst., Sigma Chem. Co, St-Louis, Mo, USA) per mg of platelet proteins in 0.1 ml of buffer (pro-elastase activity), or with the equivalent amount of buffer (elastase activity). The esterolytic activity of elastase and chymotrypsin-like enzymes were determined using respectively

TABLE II

Elastolytic activity recovered in the protein bands eluted from gel 1 and 2 of Figure 1. Elution with 0.15 M KCl. Substrate = $AcAla_3OMe$. Results expressed in equivalents of elastase/ml of eluate. Trypsin added = 1 µg.

	ELASTASE ACTIVITY	PROELASTASE ACTIVITY
GEL 1		
Band P	9.5	180
Band E	15	15
GEL 2		
Band E	200	200

$AcAla_3OMe$ (8) and AcTyrOEt by a titrimetric method (1).

The trypsin-like protease activity of platelet extracts was measured by the esterolysis of BAPNA during 6 minutes at 37°C in a spectrophotometer at 420 nanometers. All the results given in Table I and II are expressed as equivalents (in micrograms) of the crystalline porcine pancreatic enzyme as read on a standard curve obtained with crystalline pancreatic elastase (EC. 3.4.4.7), trypsin and chymotrypsin as described (1).

Gel filtration of platelet protein fractions

The preparation of a human platelet extract and the gel-filtration of the proteins precipitated by ammonium sulphate at 40 % and 70 % saturation from this extract were carried out as previously described (1).

Affinity chromatography on a cellulose elastin column

2 g of finely powdered elastin (bovine ligamentum nuchae 200 mesh Sigma) was added to a suspension of 98 g of cellulose (Schwarz-Mann) in one liter of 0.05 M pH 4.5 acetate buffer and stirred for 30 minutes. After complete homogeneisation, the mixture was packed in a Pharmacia column (75 x 5 cm) cooled to +4°C. The protein fractions were dialyzed against the same buffer, charged on the column and washed with the same buffer. A single peak was recorded at 280 nanometers (peak A). The molarity of the buffer was then increased to 1M, and a second protein peak was slowly eluted (peak B). Both fractions were dialyzed against

0.15 M KCl and tested for their protein content by the method of Lowry (10), proelastase and elastase activities, trypsin-like and chymotrypsin-like activities. Homogeneity was assessed by analytical polyacrylamide disc gel electrophoresis (1) in the absence or in the presence of 1 per mille SDS.

Preparative polyacrylamide disc gel electrophoresis

The Canalco equipment with the P.D. 320 (320 square mm of gel surface) upper column was used for the preparative polyacrylamide gel electrophoresis. A 10 cm high, 10 % acrylamide separating gel was polymerized and overtopped by a 2 cm stacking gel as described by the manufacturer. The current was run for at least one hour to eliminate the catalysts used for the polymerisation of the gels (ammonium persulphate for the separating gel and riboflavine for the stacking gel) and the sample (dialyzed against Tris glycine buffer) was then charged in the presence of sucrose. A constant current (450 volts) was applied to the gel. The gel was cooled to + 4°C. The elution was performed with a 0.1 M pH 8.4 Tris HCl buffer.

RESULTS

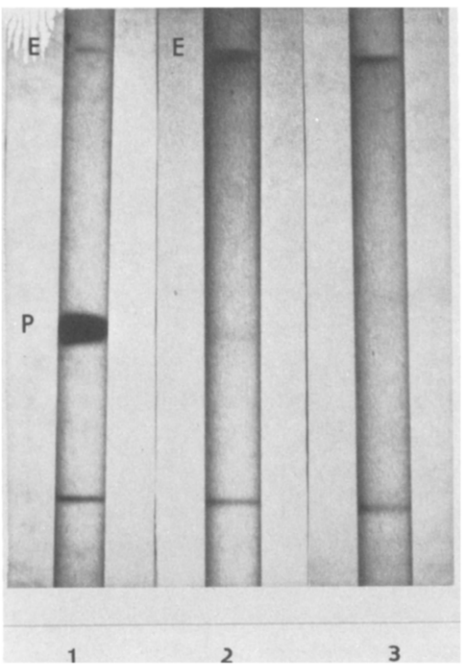
Gel filtration of the ammonium sulphate precipitates

Precipitation at 40 % ammonium sulphate of the platelet extract gave after gel filtration on G-150 Sephadex a single peak which contained most of the proelastase activity (40 % peak I in Table I)(1). The precipitation at 70 % ammonium sulphate of the 40 % ammonium sulphate supernatant gave 2 peaks ; the second one (70 % peak II, in Table I) was rich in elastase activity associated with non negligible amounts of trypsin-like and chymotrypsin-like esterases. These two fractions were used for further purification on cellulose-elastin columns.

Purification of platelet elastase by affinity chromatography

Each of the fractions charged on the column gave 2 peaks : one eluted with 0.05 M pH 4.5 acetate solution, "peak A", the other being eluted with 1 M pH 4.5 acetate solution "peak B". The results of the affinity chromatography are given on Table I. The greatest part of proelastase was found in the 40 % peak A. Elastase activity was recovered in the 70 % peak B. Thus elastase activity was retained on the cellulose-elastin column. Most of trypsin and chymotrypsin-like esterases are found in the 70 %

FIGURE 1



Acrylamide disc gel electrophoresis of fraction 3 incubated in the absence of trypsin (gel 1) ; incubated in the presence of trypsin - 10 ug per mg protein - (gel 2) ; and of a pool of lyophilized affinity peaks (gel 3). The thin lower band of each gel is bromophenol blue used as tracker dye. 7 % acrylamide gels stained with amidoblack were used. 1.5 Amp was applied to each gel.

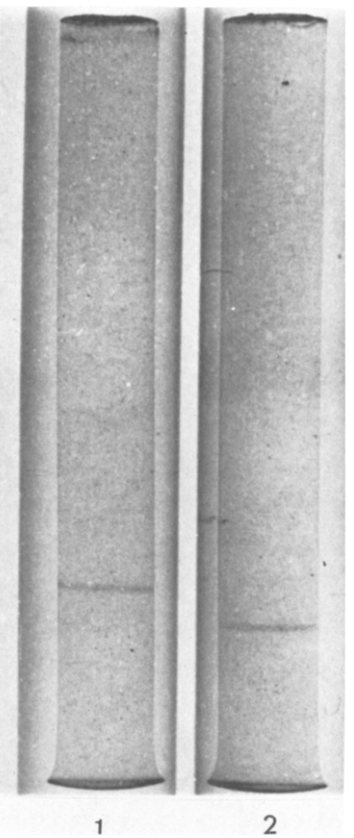
"peak A". The 40% and the 70 % peaks A materials were further purified by preparative acrylamide gel electrophoresis.

The 70 % peak B revealed on acrylamide disc gel electrophoresis one single slow migrating band (Fig. 1) presenting the same mobility as the 40 % peak B-material. These two fractions were then pooled and lyophilized and were shown to represent highly purified platelet elastase.

Purification of proelastase by preparative acrylamide disc gel electrophoresis

The preparative acrylamide disc gel electrophoresis of the elution peak A-material pooled from several experiments (40 mg

FIGURE 2



SDS acrylamide disc gel electrophoresis of fraction 3 incubated in the absence (gel 1) and in the presence of trypsin - 10 ug per mg protein - (gel 2). 7 % acrylamide gels containing 1 per mille SDS, stained with Coomassie blue were used.

protein in 8 ml buffer) gave three major protein fractions (fraction 1, 0.94 mg protein ; fraction 2 : 3.55 mg protein and fraction 3 : 27.6 mg protein). Most of the proelastase activity, associated with some elastase activity was recovered in the third fraction (fraction 3, Table I).

The electrophoretic mobility of fraction 3 has been studied before and after incubation with trypsin (Fig. 1) ; incubation with trypsin results in a change in migration (compare gel 1 and gel 2) ; the trypsin generated elastase (gel 2) has the same mobility as trypsin independent elastase (gel 3), in regard with the migration of bromophenol blue used as a tracker dye.

The trypsin generated elastase has been identified after elution with 0.15 M KCl from 2 mm slices of unstained gels run in parallel with gels 1 and 2. The values presented in Table II (total elastase activity found in 1 ml of eluate) show that fraction 3 contains the purified platelet proelastase (band P) together with approximately 9 % of elastase activity. This fraction could be stored after lyophilisation without loss of potential activity.

Mobility of fraction 3 on SDS acrylamide disc gel electrophoresis

Fig. 2 shows that the anodic mobility on SDS acrylamide gels of the proelastase associated with fraction 3 is greater when incubated with trypsin (gel 2) than with buffer (gel 1). This change in the mobility may be the result of a limited proteolysis during the activation of the proelastase, similar to the activation of pancreatic elastase (3, 4, 5).

CONCLUSION

Affinity chromatography enables the separation of platelet proelastase from platelet elastase. Only the elastase activity was adsorbed on the elastin-cellulose mixed bed column and recovered in the "affinity peak" (peak B), as a single protein band on acrylamide disc gel electrophoresis (gel 3, Fig. 1). Thus, in our conditions, proelastase was not adsorbed on elastin. These results suggest that the active site of this enzyme is involved in its adsorption on elastin. Proelastase behaves as the zymogen form of platelet elastase. The in vivo mechanism of activation of platelet proelastase into elastase is not yet known and the identification of the endogenous activating system (2) is now in progress.

ACKNOWLEDGEMENT

This work was supported by DGRST (71-7-2809), INSERM (ATP 72-44-20-7), CNRS (ERA 335, ER 53) and CNAMTS.

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