ISOLATION, PURIFICATION AND PROPERTIES OF AORTIC ELASTASE

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1. Introduction

The slow fragmentation of elastic fibers has been considered by many authors as an important factor in the ageing process of elastic tissues such as blood vessels, lungs or cartilage [1–3]. This lysis can be induced experimentally by immunising rabbits with κ-elastin [4] or by intravenous injection of pancreatic elastase [5]. Elastase can be considered as an enzyme capable of hydrolysing more or less specifically elastic fibers. Pancreatic elastase was first isolated by Balo and Banga [6] and recently other elastases have been extracted and characterised from blood platelets [7] and leucocytes [8]. Arguments in favour of the existence of an elastase-like protease in arterial tissues were given by Loeven [9]. We have recently described the isolation of an elastolytic enzyme from human and pig aorta [10, 11]. We wish to report here on the purification and partial characterisation of an elastolytic protease isolated from pig aorta and on its comparison with its analogous pancreatic enzyme.

2. Materials

Elastase (EC 3447), trypsin (2X crystallized) and α *N*-benzoyl-DL-arginine-paranitroanilide HCl (BAPNA) were purchased from Sigma Chemical; achromobacter collagenase was a gift of Professor Keil (Institut Pasteur); human α_1-antitrypsin and α_2-macroglobulin were from Behringwerke (Marburg/Lahn) and *N*-succynoyl tri-alanine paranitroanilide was a gift of Dr Bieth.

3. Methods

3.1. *Determination of enzyme activities*

The elastinolytic activity of the enzyme solutions was determined using a 2% solution of κ-elastin as substrate in a gelified medium or ^{131}I-labelled polymeric elastin as previously described [10,14].

The esterolytic activity was measured using *N*-succynoyl trialanine paranitroanilide as synthetic substrate as recommended by Bieth [13].

4. Results

4.1. *Purification procedure of the aortic elastase*

All manipulations were carried out at cold room temperature. 900 g of pig aorta were used; the adventitia was carefully stripped off, cut into small pieces with a razor blade, homogenised and washed with NaCl 0.9% until the supernatant was colourless.

4.1.1. Extraction with sodium acetate 0.5 M pH 4.0, 0.2 M EDTA 0.02% w/v sodium azide

After centrifugation, the residue was extracted with 2 liters of the above buffer during 24 hours at 4°C under mechanical stirring. This extraction was repeated once more, and the supernatants were dialysed and lyophilised.

4.1.2. Purification by precipitation with ammonium sulphate

The lyophilised acetate extracts were solubilised in 1 liter of distilled water and an adequate amount of crystallised ammonium sulphate was added for a final

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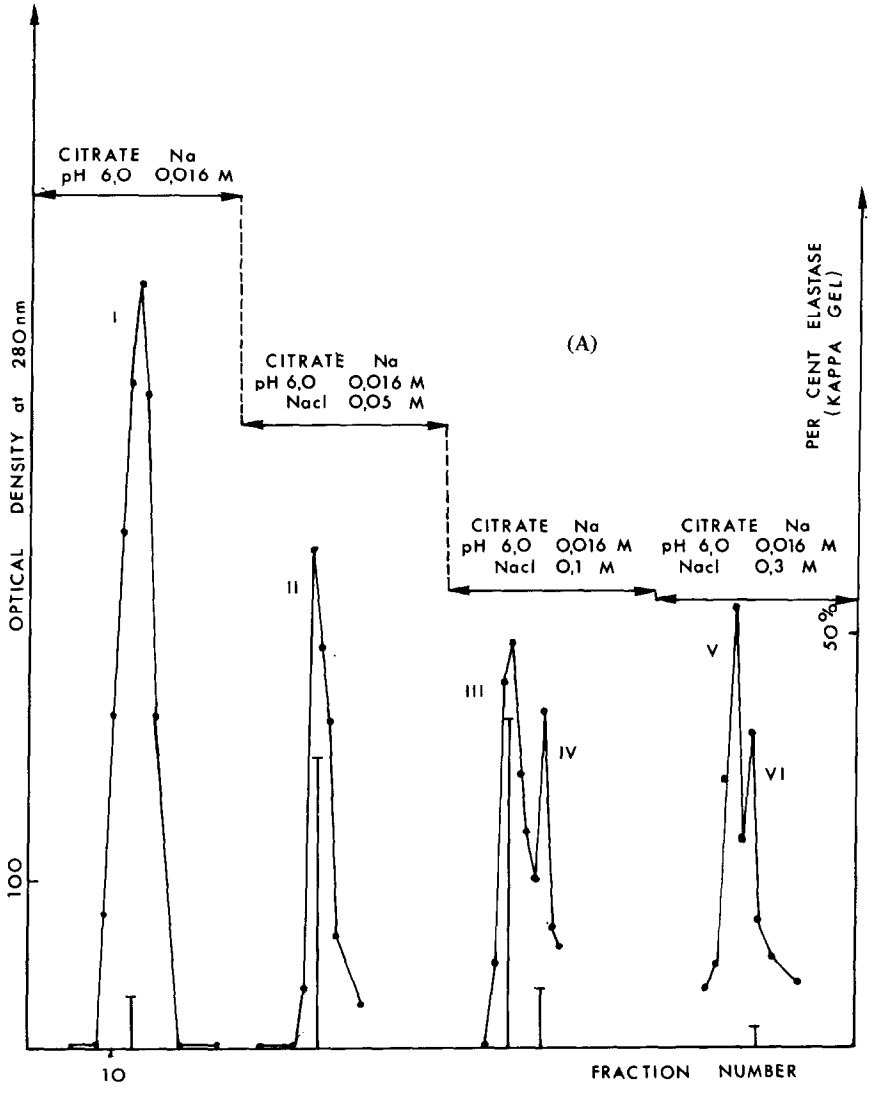
concentration of 60% of saturation. The reaction mixture was stirred 1 hr at room temperature and left overnight at 4°C. The pellet obtained by centrifugation at 40 000 *g* represented 186 mg protein, containing approx. 10 mg of elastase (expressed as equivalents of the pancreatic enzyme) determined by the method using κ-elastin as substrate in a gelified medium as previously described [10,11].

4.1.3. DEAE cellulose chromatography

The euglobulin precipitate was partially solubilised in 50 ml of tris buffer 0.01 M pH 7.3 and the supernatant applied to a DEAE cellulose column (50 X 2 cm) eluted first with the same buffer. When the absorbance reached the base line a stepwise gradient of NaCl was applied. Almost all the proteolytic activity was recovered in the breakthrough peak of the column. The ratio (R) of mg equivalents pancreatic enzyme/mg of protein of this fraction was 0.13.

4.1.4. CMC cellulose chromatography

This purification step is fully described in the legend of fig.1. Peaks II and III gave the major elastase activity and their respective R ratios as defined above are 0.35 and 0.39 respectively. The two eluted peaks gave two bands in the area of 23–30 000 daltons on



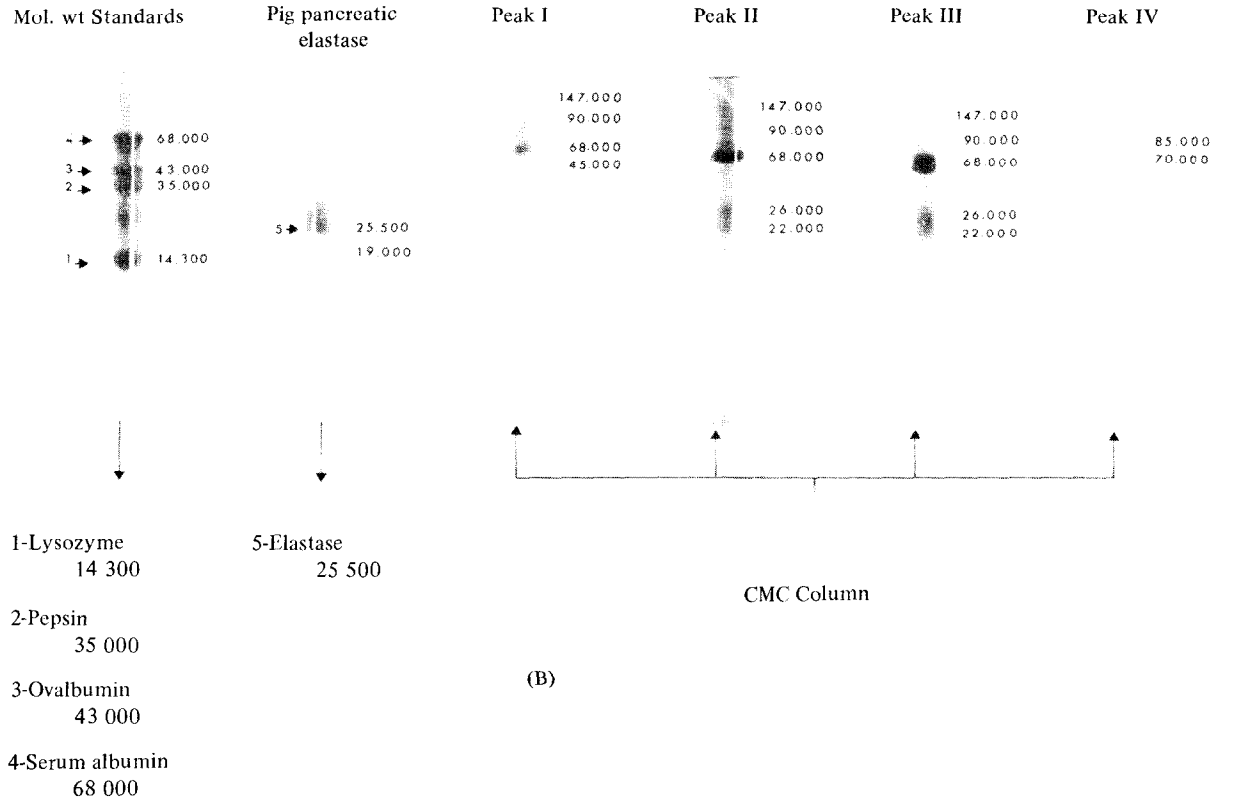


Fig.1.(A). Elution diagram from a carboxymethyl-cellulose column (70 × 2 cm) of pig aorta extract purified by DEAE cellulose chromatography as described in the text. Elution: sodium citrate pH 6.0, 0.016 M and stepwise gradient of sodium chloride in the same buffer as indicated in the figure. Temperature 4°C. Abscissa: Fraction number. Ordinates: Optical density at 280 nm (.—.) (—) Elastase activity expressed as percent of equivalents in mg of pancreatic elastase per mg of protein. (B) SDS polyacrylamide gel electrophoresis of pig aorta extract purified as described in the text by CMC cellulose column chromatography. The peak numbers indicated above the gels correspond to the peaks eluted as shown in Fig.1(A).

SDS acrylamide gel electrophoresis 12 as well as a strong band at about 68 000 daltons and several weaker higher molecular weight bands (see fig. 1/b).

4.1.5. Sephadex G-100 chromatography

Peaks II and III from the CMC cellulose column were further purified on Sephadex G-100 (50 × 1.2 cm) eluted with tris buffer 0.01 M pH 8.9. Both peaks gave a similar chromatographic pattern consisting in two major peaks. One was excluded from the column and was inactive on κ-elastin gel; the other eluted at the same position as trypsin, possessed all the activity. The R ratio for active peak obtained from peaks II and III of the CMC-column (see above), was equal to 0.95. The apparant molecular weight of this peak calculated from its position of elution from the G-100 column is about 23 000.

4.2. *Properties of the aortic elastase*

Fig.2 shows that by the Ouchterlony method a precipitation line was obtained between a rabbit antiserum against pancreatic pig elastase and the partially purified pig aorta elastase at the G-100 step (see above) as well as the 60% ammonium sulphate precipitate. No precipitation lines were observed with crystalline trypsin, alpha-chymotrypsin and collagenase.

Table 1 summarised the properties of the purified aortic elastase compared with those of its homologous pancreatic enzyme: Aortic elastase possesses an elastolytic activity (on κ-elastin gel) similar to that of

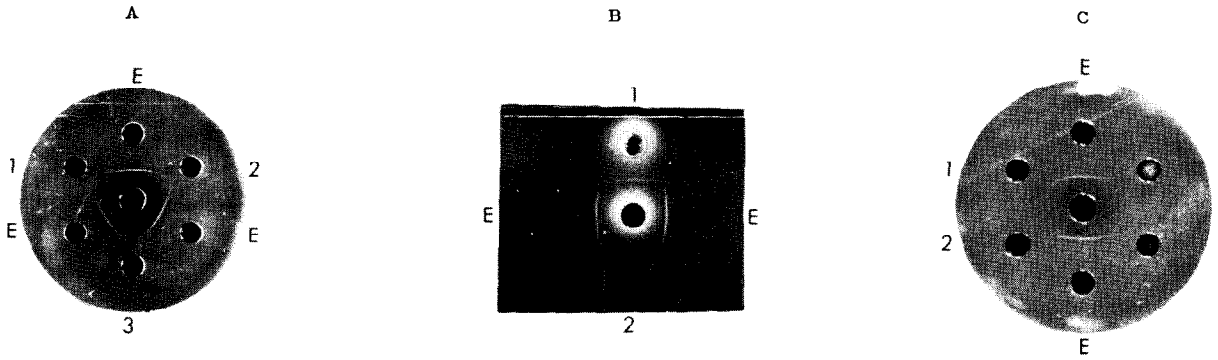


Fig.2. Immunodiffusion by Ouchterlony technique of pig aorta extracts purified as described in the text. Center well: rabbit antiserum to pig pancreatic elastase. Peripheral wells: A: E Pig pancreatic elastase. 1 Trypsin. 2 α_1-Chymotrypsin. 3 Collagenase. B: E Pig pancreatic elastase. 1 60% ammonium sulphate precipitate. 2 . 60% ammonium sulphate supernatant. C: E Pig pancreatic elastase. 1 Peak eluted from Sephadex G-100 column which possesses all the elastolytic activity. 2 Peak excluded from Sephadex G-100 column without elastolytic activity.

pancreatic elastase but a lower esterolytic activity (on *N*-succinoyl-trialanine paranitroanilide substrate [13]). This enzyme also exhibits a weak proteolytic activity on ^{131}I-labelled polymeric elastin [14] when compared to that of pig pancreatic elastase. Similar differences between the action of elastolytic proteases on synthetic (ester) substrates and fibrous elastin were reported for human platelet elastase [7] and for 'protease E' isolated from the pancreas [15].

The partially purified pig aorta elastase is inhibited by human α_2-macroglobulin and α_1-antitrypsin (Behringwerke, Marburg/Lahn) but to a different extent than the pancreatic enzyme [16]. Its mol. wt is about 23 000 as revealed by SDS polyacrylamide gel

Table 1
Comparison of some properties of pig aorta elastase as compared to pig pancreatic elastase

	Pig aorta elastase	Pig pancreatic elastase
Proteolytic activity on κ-gels[a] [10] (arbitrary units)	95	100
Proteolytic activity on polymeric elastin [14] (arbitrary units)	12	100
Esterolytic activity on synthetic substrate[b] (arbitrary units) [13]	57	100
Trypsin activity on BAPNA (arbitrary units)	1.4	0.0
Inhibition by α_1-antitrypsin (per cent) [16] I/E=0.6[c]	72	33
Inhibition by α_2-macroglobulin (per cent) [16] I/E=0.1[c]	61.4	15
Estimated molecular weight (SDS. gel electrophoresis and gel chromatography)	23 000	25 000
pH optimum on *N*-succinoyl-trialanine paranitroanilide	9-10	9.2

[a] κ-Elastin agarose gel [10,11].
[b] *N*-succinoyl-trialanine paranitroanilide [13].
[c] Molar ratio inhibitor to substrate.

and Sephadex gel chromatography and its esterolytic pH optimum is situated between pH 9 and 10 [16].

5. Conclusions

An elastase from pig aorta has been partially purified and characterised; it exhibits immunological cross reaction with pig pancreatic elastase. Its proteolytic (κ-elastin gel and polymeric elastin substrates) and esterolytic (*N*-succinoyl-trialanine paranitroanilide) activities as well as its degree of inhibition by serum protease inhibitors (α_1-antitrypsin and α_2-macroglobulin) differ sensibly from those of pancreatic elastase [14,16].

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