Evidence for the presence of large amounts of cathepsin E in rat spleen

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Although cathepsin E is present in trace amounts in spleen from several species, it was found in large amounts in rat spleen This observation can be correlated with the fact that spleen in the rat is an important organ in haemopoeisis

Cathepsin E *Rat spleen*

1. INTRODUCTION

The main intracellular aspartic proteinases are cathepsins D (EC 3 4.23.5) and E (EC 3.4.23.-) Whereas cathepsin D is widely distributed in all animal cells, cathepsin E is found to be present chiefly in polymorphonuclear cells and in small amounts in macrophages [1]. Cathepsin E was first isolated from rabbit bone marrow and characterized [2].

Cathepsins D and E share many properties. They are active at acid pH, they are strongly inhibited by pepstatin and by diazoacetylnorleucine methyl ester. However, cathepsin E has a much higher molecular mass (90 kDa) than cathepsin D (50 kDa). It possesses a higher negative charge and a lower pH optimum. Inhibitor from *Ascaris lumbricoides* inhibits cathepsin E but not cathepsin D (review [3]).

Although it was known that rabbit spleen contains cathepsin E only in traces [1], it was recently shown that rat spleen contains both cathepsin D and cathepsin E-like enzyme in almost equal amounts [4,5].

This work was undertaken to confirm and explain the presence of a significant amount of cathepsin E in rat spleen.

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2 MATERIALS AND METHODS

2.1. *Material*

Rat and rabbit spleens were obtained from exsanguinated animals. They were kept frozen at −15°C until use. Human serum albumin (HSA) was Cohn fraction V from Squibb USA, kindly given by the American Red Cross Before use, the albumin was dialyzed against water and lyophilized DEAE-Sephacel and cyanogen bromide were purchased from Pharmacia All other chemicals used were of reagent grade.

2 2. *Proteolytic activity*

Two methods were used to measure the proteolytic activity. In the first, human serum albumin was used as substrate as described in [2]; cathepsin D was measured at pH 3.5 and cathepsin E at pH 2.5. In the second method, hemoglobin was used as substrate at pH 3.3 as described in [5].

2.3. *Separation of cathepsins D and E*

Crude acetone-ether dried powder (3 71 g) of rat or rabbit spleen was suspended in 0.15 M NaCl, centrifuged and acidified at pH 5.0 with 6 N HCl as described in [6]. After centrifugation at 8000 × *g* for 1 h, supernatant was dialysed against 0.02 M phosphate buffer, pH 7.5, containing 0.15 M NaCl. It was then applied to a column (2 × 23 cm)

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of DEAE-Sephacel, which had been equilibrated with the same phosphate buffer.

A first fraction was eluted by washing the column with the same buffer until the absorbance at 280 nm returned to the baseline. A second fraction was eluted with 0 5 M NaCl in the same buffer.

2.4. *Isolation of cathepsins D and E*

Cathepsins D and E were isolated from rat spleen by the method of Yamamoto et al [4,5]

2.5. *Immunological procedures*

A rabbit received intramuscular injections two times at 3-week intervals with 1 5 mg cathepsin E emulsified with an equal volume of Freund's complete adjuvant. The animal was bled 20 days after the second injection. Antibodies were purified by affinity chromatography on Sepharose 4B-cathepsin E conjugate. The antibodies were eluted at pH 2.2.

Isolated cathepsin D or E was mixed with different concentration of antibodies, in a final volume of 0 5 ml in 0.02 M sodium phosphate buffer, pH 7.5, containing 0.15 M NaCl. The mixtures were incubated at 37°C for 5 min and at 4°C for 14 h After centrifugation at 10000 × *g* for 10 min the enzyme activity was determined in the supernatants by assay with bovine hemoglobin.

3. RESULTS

Fig 1 shows the elution profile on DEAE-Sephacel of rabbit spleen extract (A) and rat spleen extract (B) Cathepsin D was measured at pH 3 5 and cathepsin E at pH 2.5. Under the conditions used, cathepsin D was not adsorbed on the column and cathepsin E, which was adsorbed, was eluted by addition of 0.5 M NaCl in the buffer.

The active fractions were collected and table 1 gives the total amounts of enzyme isolated. One sees that whereas spleens from rabbit and rat contain large amounts of cathepsin D, only rat spleen contains a significant amount of cathepsin E.

Fig.2 shows that rabbit antibody anti-rat cathepsin E, isolated by immunoadsorption, precipitated with rat cathepsin E but not at all with rat cathepsin D.

4. DISCUSSION

Whereas cathepsin E was found only in trace amounts in the spleen of several species such as

Table 1

Total amount of cathepsin D and E obtained from rabbit and rat spleen by chromatography on DEAE-Sephacel

Spleen from	Units of		E/D
	Cathepsin D	Cathepsin E	
Rabbit	2695	208	0 08
Rat	680	1100	1 6

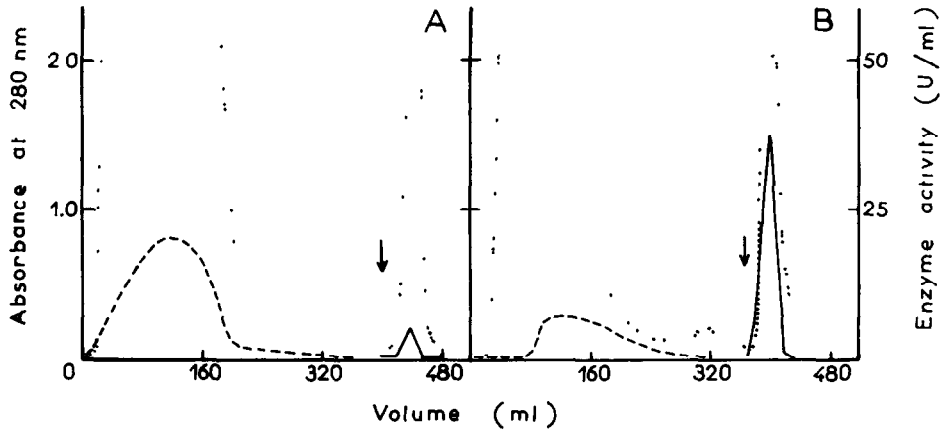

Fig 1 Chromatography on DEAE-Sephacel of extract of rabbit spleen (A) and rat spleen (B) The arrow indicates the change of concentration of NaCl (···) Protein concentration, (– – –) proteolytic activity on HSA at pH 3 5, (——) proteolytic activity on HSA at pH 2.5

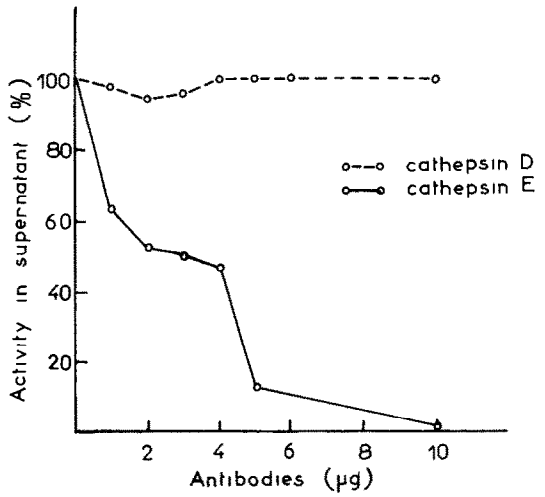

Fig 2 Purified rat cathepsins D and E were reacted with increasing amount of anti-rat cathepsin E antibodies Shown is the proteolytic activity of the supernatant after centrifugation

rabbit [2], guinea pig [6], beef [7], porcine and human spleens (unpublished), recent studies show its presence in a significant amount in rat spleen [4,7].

Cathepsin E isolated from rat spleen is different from rat cathepsin D, as was clearly demonstrated by immunoprecipitation. Antibodies against cathepsin E did not react with cathepsin D. This is in agreement with the studies of Yamamoto et al [8] showing that antibodies against rat cathepsin D do not react with rat cathepsin E These results do not confirm the hypothesis that cathepsin D could derive from cathepsin E [9].

Using isolated cells from rabbit blood, cathepsin E was found in large amounts in the polymorphonuclear cells, in small amounts in macrophages and only in traces in lymphocytes [1] For this reason bone marrow was chosen for the isolation of cathepsin E [2]. The absence of cathepsin E from spleen of different species is in keeping with the idea that cathepsin E arises chiefly from polymorphonuclear cells. The presence of large amounts of this enzyme in rat spleen, hitherto unexplained, is most probably due to the fact that the spleen in the rat is an important organ in haemopoiesis as well as in lymphogenesis [10].

This distribution of cathepsin E is in agreement with two other observations: (a) the presence of cathepsin E was found in human enlarged spleen which could have undergone myeloid transformation, thus becoming rich in polymorphonuclear cells [6,11], (b) the content of cathepsin E in rat spleen was very much increased by phenylhydrazine hydrochloride administration which provokes a large increase in the number of granulocyte cells [12].

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