THREE TRYPSINOGENS FROM RAT PANCREAS

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1. Introduction

In [1] we described 3 trypsinogens in mouse, however, 2 trypsinogens have been described in rat [2], human [3], pig [4], cattle [5] and dog [6]. Although closely related, the multiple forms of trypsinogens between rat and mouse are not consistent. Therefore, we have examined the heterogeneity of rat trypsinogens and describe here the existence of 3 distinct trypsinogens.

2. Materials and methods

Wistar King A inbred rat strain was kindly donated by Dr R. Shoji (Department of Development of this Institute) and all rats used were 2–4 months old.

Benzoyl-L-arginine ethyl ester (BAEE), tosyl-L-arginine methyl ester (TAME) and benzoyl-L-tyrosine ethyl ester (BTEE) were obtained from Nakarai Chemical Ltd. DEAE-cellulose type 52 was the product of Whatman Ltd. Enterokinase from porcine intestine and trypsin from bovine pancreas were purchased from Sigma Chemical Co. Other reagents were commercial preparations of the highest purity available.

All preparatory procedures were performed at 0–4°C. Pancreas from a freshly killed rat was homogenized in 5 vol. buffer A (50 mM Tris–HCl buffer, at pH 8.0), and was centrifuged for 30 min at 25 000 × g. The clear supernatant was loaded on a column of DEAE-cellulose (1 × 10 cm) equilibrated with buffer A, and the column was eluted with a linear NaCl gradient from 0–0.6 M in buffer A. The reservoir and mixing chambers each contained 100 ml of the appropriate solution. The flow rate was 21 ml/h and 5 ml fractions were collected. Portions (0.5 ml) of each fraction were activated by the addition of 50 μg enterokinase and 25 mM $CaCl_2$ at room temperature for 30 min. Activation of chymotrypsinogen was performed by addition of 50 μg trypsin and 25 mM $CaCl_2$ under the same conditions. Tryptic and chymotryptic activities were determined by the spectrophotometric method in [1,7].

To estimate the M_r-values of each trypsinogen by gel filtration, fractions with high amounts of trypsinogen were pooled and precipitated by addition of ammonium sulfate to 70% saturation. The precipitates were dissolved in 1 ml 0.1 M Tris–HCl buffer (pH 8.0). The solutions were loaded on a column of Sephadex G-100 (2.5 × 65 cm) equilibrated with 0.1 M Tris–HCl buffer (pH 8.0) and eluted with the same buffer.

3. Results

The extract from rat pancreas was applied to the DEAE-cellulose column and a NaCl gradient was used for elution. The tryptic and chymotryptic activities were measured using BAEE and BTEE as substrates, respectively. As shown in fig.1, 3 peaks of trypsinogens and a single peak of chymotrypsinogen were obtained. The peak of chymotrypsinogen, Chy-I, was eluted at 0.13–0.17 M NaCl. The peaks of trypsinogens were designated Try-III, Try-I and Try-II in the order of elution from the column according to the designation of mouse trypsinogens. Try-III was present in the void volume. Try-I and Try-II were eluted at 0.15–0.21 M NaCl and 0.26–0.32 M NaCl, respectively.

When tryptic activities of the 3 trypsinogens were measured using TAME as substrate, their activities were not identical with the activities obtained in the presence of BAEE (table 1). Only Try-III showed the almost identical activities in both substrates. How-

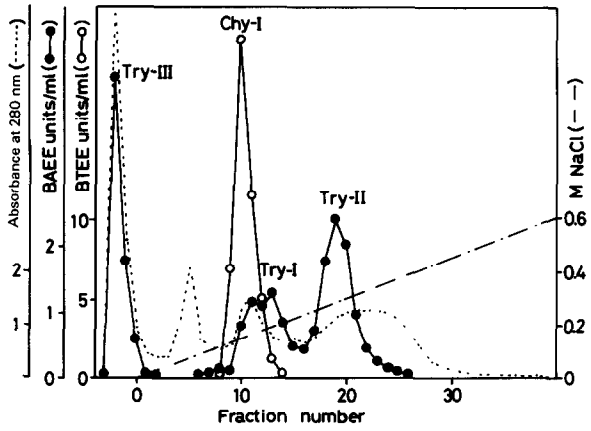

Fig.1. DEAE-cellulose column chromatography of trypsinogens (●——●) and chymotrypsinogen (○——○) from extract of rat pancreas. The supernatant fluid from 0.7 g pancreas of one rat was applied to the DEAE-cellulose column (1 × 10 cm). The column was washed with 20 ml buffer A. The NaCl gradient and assay were as in section 2.

ever, the activity of Try-I was much higher in BAEE than in TAME, and the activity of Try-II was much higher in TAME than in BAEE. Thus, the % activities of each trypsinogen against the total activity was very different between BAEE and TAME. When BAEE was used as substrate, the 3 trypsinogens showed the almost same % activity. Whereas, in the presence of TAME, Try-II showed much greater % activity and Try-I showed very low % activity.

Fig.2 shows plots of initial velocity of the 3 trypsinogens against BAEE and TAME concentrations. Among these enzymes, the velocity curves were slightly different in both substrates. The K_m-values against BAEE were 8 μM in Try-III, 18 μM in Try-I and 2 μM in Try-II. Against TAME, the values of 20, 8 and 5 μM were obtained for Try-III, Try-I and Try-II, respectively. Thus, Try-II showed the lowest K_m against both substrates, while the K_m-values of

Table 1
Substrate specificity of the 3 trypsinogens and % of each activity of the 3 trypsinogens against total activity

	TAME/BAEE ratio	% against total activity	
		BAEE	TAME
Try-III	1.10	35	25
Try-I	0.51	26	8
Try-II	2.78	39	67

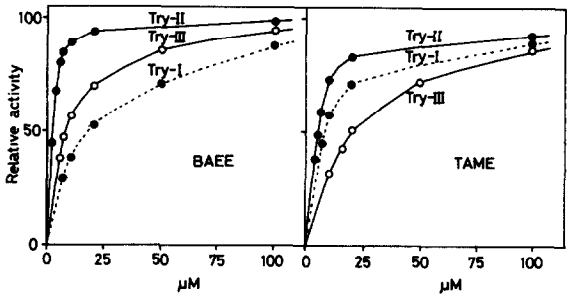

Fig.2. Effect of BAEE and TAME concentrations on initial velocity of the 3 trypsinogens, Try-III (○——○), Try-I (●– – –●) and Try-II (●——●). The incubation mixtures consisted of the various concentrations of BAEE or TAME, 50 mM Tris–HCl buffer (pH 8.0) and 25 mM $CaCl_2$.

Try-III and Try-I were higher and converse each other between the substrates.

The activities of the 3 trypsinogens were compared over pH 5.9–10.2. The pH activity curves of the 3 enzymes were almost identical (fig.3). The optimum activities were observed at pH 8–10, and fell off gradually to 20% of the maximum at pH 6.0.

Molecular sizes of the 3 trypsinogens were determined by gel filtration on Sephadex G-100 using blue

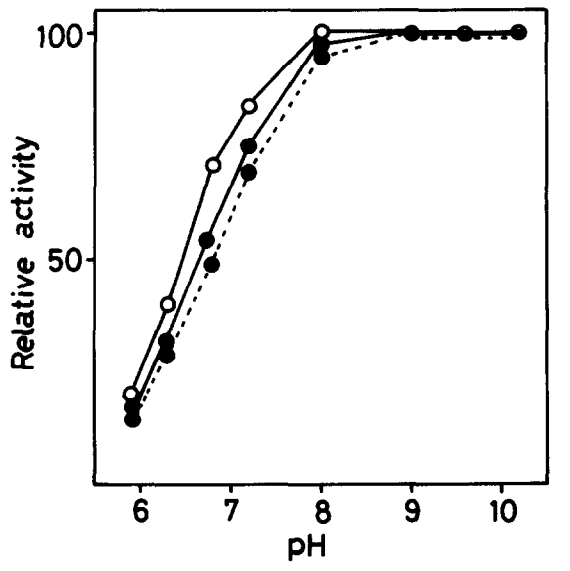

Fig.3. Effect of pH on the activity of the three trypsinogens, Try-III (○——○), Try-I (●– – –●) and Try-II (●——●). The following buffers were used at 0.1 M, citrate at pH 5.8–6.8, Tris–HCl at pH 7.2–9.0 and glycine–NaOH at pH 9.5–10.0, containing 25 mM $CaCl_2$ and 0.5 mM BAEE.

dextran, bovine serum albumin, ovalbumin, bovine chymotrypsin and cytochrome *c* as standards. Small amount of each enzyme was layered on a calibrated Sephadex G-100 column equilibrated with 0.1 M Tris–HCl buffer (pH 8.0). Here, the enzyme activity was eluted as 1 distinct peak in all 3 trypsinogens. The M_r-values are estimated as 21 000 in Try-III, 25 000 in Try-I and 24 000 in Try-II.

4. Discussion

Two trypsinogens have been described in a large number of mammalian species. In rat, after 2 trypsinogens were reported, purification and enzymatic studies had been done [8.9]. However, 3 trypsinogens have been demonstrated here, using a different method of fractionation. We have separated the 3 trypsinogens by using cation exchange DEAE-cellulose chromatography. However anion exchange chromatography was used in [1.8] where Try-I and Try-II could not be separated.

Here, 3 trypsinogens were detected in rat pancreas of a genetically pure inbred strain. Therefore, the 3 enzymes are not derived from genetic heterogeneity, but exist as isoenzymes. Furthermore, since the substrate specificities against BAEE and TAME are clearly different among these enzymes, the existence of 3 trypsinogens is well tested. The physiological function of the 3 trypsinogens in vivo would be of interest.

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