ON ZYMOGENS OF HUMAN PANCREATIC JUICE *

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1. Introduction

Pancreatic protein composition is now known in such diverse species as cattle [1,2], swine, dog [3], rat [4], chicken [5] and even in spiny dogfish [6], the most phylogenetically distant from man yet investigated. Comparison among these species shows that each has a full complement of enzymes and differs from the others only in enzymatic levels, chemical characteristics of proteins and number of homologous enzymes.

Little information has been published on human pancreatic enzymes. Studies of extracts of acetonic powder by immunodiffusion [7] and, very recently, an identification of pancreatic enzymes in human duodenal contents [8] contributed to our knowledge of human pancreatic proteins. Since the enzymes were present in their active forms, however, it was impossible to determine the number and the chemical characteristics of the zymogens secreted by the gland. Preliminary studies of the zymogens in human pancreatic homogenates [9] have so far been limited by paucity of material. Recently, Keller et al. [10] published a chromatographic study of proteins of human pancreatic juice, but some activation occurred during the experiment and raised some ambiguity about the number and nature of proteolytic zymogens.

We succeeded in obtaining human pancreatic juice devoid of free tryptic activity. Large amounts of naturally occurring inhibitor in human juice (nearly 1%) allowed the zymogens to remain in their native state during chromatography, on the condition that it be performed very quickly.

2. Material and methods

Fresh human pancreatic juice was collected in crushed ice, immediately lyophilised and stored at −20°C. All further experiments were performed at 4°C.

2.1. *Chromatographic material and techniques*

Pancreatic juice proteins (100 mg) were desalted by filtration on a Sephadex G 25 coarse column (2 × 60 cm) equilibrated with Tris 5 mM buffer of pH 8. Eluted proteins were concentrated by lyophilisation and chromatographed on a DEAE-cellulose column (0.9 × 100 cm) equilibrated with a Tris 5 mM – NaCl 5 mM buffer of pH 8. After the break-through peak containing cationic proteins, elution of anionic proteins was performed at pH 8 by a linear concentration gradient of NaCl from 5 mM to 300 mM.

2.2. *Activations of zymogens*

Fractions containing zymogens were activated with an amount of crystalline trypsin (Worthington) equal to 10% by weight, Ca^{++} 2.6×10^{-2} M was added to the trypsinogen activation mixture. Full

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Abbreviations used: ATEE, acetyl-L-tyrosine ethylester; BAEE, benzoyl-L-arginine ethylester; CGP, carbobenzoxy-glycyl-L-phenylalanine; HA, hippuryl-L-arginine; RNA, ribonucleic acid.

activation occurred in 1 hr at 0° for chymotrypsinogen and prophospholipase, 3 hr at 0° for trypsinogen, 30 min at 25° for proelastase and 30 min at 35° for procarboxypeptidases A and B.

2.3. *Assays of enzymes*

Amylase, lipase, chymotrypsins, trypsins and pancreatic inhibitor were assayed according to the classical methods described elsewhere [9]. Ribonuclease was assayed towards yeast RNA by the method of Anfinsen [11]. Carboxypeptidase A and B activities were measured spectrophotometrically using the substrates CGP [12] and HA [13]. Elastase was assayed against elastin following the method of Lamy [14] and phospholipase A against an emulsion of egg yolk by the potentiometric method of G.de Haas [15].

2.4. *Polyacrylamide gel electrophoresis*

Polyacrylamide gel electrophoresis was carried out in Tris-glycine buffer pH 8.6 for anionic proteins and in acetate-beta alanine buffer pH 5.0 for cationic proteins. All the gels were 7% with respect to the acrylamide.

3. Results and discussion

Cationic proteins account for 15% of total pancreatic proteins. They include lipase, ribonuclease and pancreatic inhibitor, which can be directly assayed, and three inactive precursors, chymotrypsinogen, proelastase and prophospholipase A, which need a previous activation by trypsin to be determined.

Anionic proteins are eluted in seven peaks as shown in fig. 1. The first one has a potential activity towards ATEE (35% of total ATEE units). It seems to represent a second chymotrypsinogen probably distinct from cationic chymotrypsinogen (15% of total ATEE units) since it needs an increase of ionic strength of 0.1 to emerge from DEAE-cellulose. A third potential activity towards ATEE can be seen in the middle of the diagram and accounts for about 10% of total ATEE units. This last protein should be the homologue of porcine chymotrypsinogen C [16,17] and fraction II of bovine procarboxypeptidase A [18]. In the human as in the porcine pancreas, however, this ATEE-protein is free and not associated with carboxypeptidase A activity as in the bovine pancreas. Poor yield of total ATEE units (about 60%) is explained by a slow destruction of

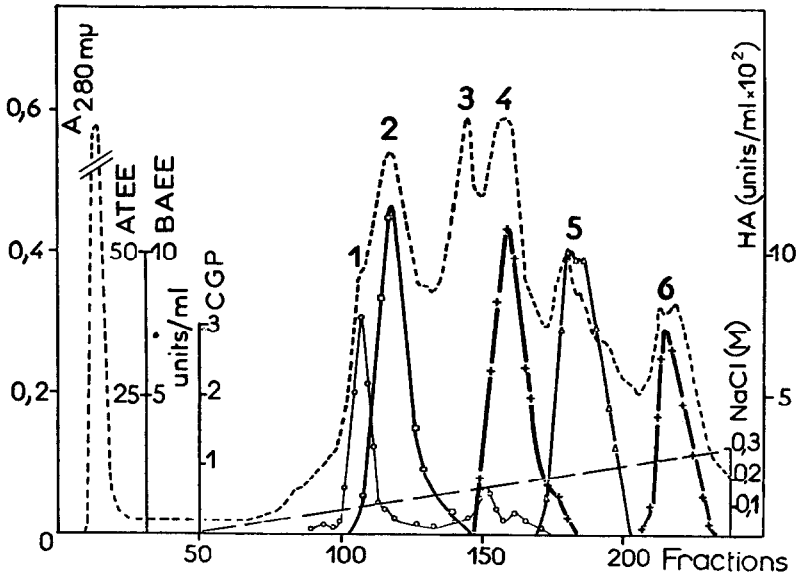

Fig. 1. Chromatography of human pancreatic juice on DEAE-cellulose at pH 8.0. – – – proteins. —— potential activities against ATEE (○), BAEE (+), HA (□) and CGP (△). Fraction volumes: 3.3 ml for fractions 0–250 and 2.8 ml for fractions 50–250. Volume of one chamber of gradient: 270 ml.

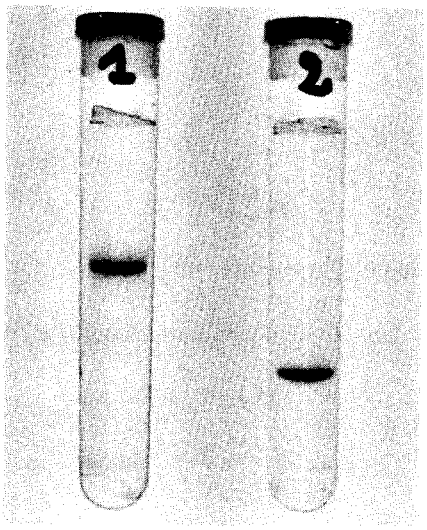

Fig. 2. Disc electrophoresis at pH 8.6 of trypsinogen 1 [1] and trypsinogen 2 [2].

chymotrypsinogen at pH 8, all the other enzymes being eluted with a higher yield (70–75%).

In this experiment, amylase emerges with the two first anionic proteins, chymotrypsinogen and procarboxypeptidase B, showing an anionic behaviour in the Tris buffer at pH 8, although it behaves like a cationic protein when 5 mM phosphate buffer is substituted for the Tris buffer at the same pH [19]. It is interesting to note that chymotrypsinogen (peak 1) does not change its behaviour under the same change of buffers. A fraction of pancreatic trypsin inhibitor emerges in peak 1 and we cannot yet say whether it is different from cationic inhibitor.

Four other proteins are displayed in the diagram: a procarboxypeptidase B (peak 2), a procarboxypeptidase A (peak 5) and two trypsinogens (peaks 4 and 6). The latter two zymogens are probably the precursors of the two trypsins characterized by Keller et al. and by Hadorn and coworkers. These two trypsinogens, cleanly separated by DEAE-cellulose, likewise behave differently on disc electrophoresis at pH 8.6 as fig. 2 shows. After activation by trypsin at 0° in presence of Ca^{++}, the two human trypsins have very similar specific activities (37 for trypsin 1 and 40 for trypsin 2), nearly the same as that of bovine trypsin (47 according to ref. 20). The ratio of potential units trypsinogen 1 to trypsinogen 2 is nearly 2.

No enzymatic activity has yet been demonstrated under peak 3. Contrary to its chromatographic behaviour this protein was found cationic on disc electrophoresis at pH 5.0. Further characterizations are in progress.

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