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A zymogen of phospholipase A in human pancreatic juice

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The presence of phospholipase A activity (EC 3.1.1.4) in pancreatic tissue and in pancreatic secretion of different species is now well established[1-7].

An inactive precursor of the enzyme was first demonstrated in porcine pancreatic homogenate by De Haas *et al.*[8] who isolated and purified the enzyme and its precursor. The complete amino acid sequence of the zymogen has recently been determined[9].

The occurrence of an enzymically inactive precursor of phospholipase A in the external pancreatic secretion of the rat has been demonstrated by Arnesjo *et al.*[10], and confirmed by Belleville and Clement[11]. In addition, the latter found phospholipase A activity in human pancreatic juice, and they demonstrated that this activity increased after activation by trypsin. But this particular sample of juice contained more than 50% of total phospholipase A activity before activation, so that it was not possible to conclude that all of the phospholipase was present as zymogen in human external pancreatic secretion. We have succeeded in obtaining human pancreatic juice devoid of tryptic activity. Pure pancreatic juice was obtained from patients during or after operations which involved draining the main pancreatic duct. In this paper, we report the identification and some properties of the zymogen of phospholipase A present in human pancreatic juice.

The phospholipase A activity was determined by potentiometric titration using a TTT 1 Radiometer autotitrator at 40° with 0.1 M NaOH on egg yolk emulsion as described by De Haas[12] (who determined the good specificity of this substrate) but with modifications in the concentrations of $CaCl_2$ and sodium deoxycholate.

The activity was defined as the amount of NaOH μequiv liberated per min. The highest specific activity (activity per mg of protein) was found when the assay was performed with $6 \cdot 10^{-3}$ M sodium deoxycholate and $1 \cdot 10^{-3}$ M $CaCl_2$.

When phospholipase A was assayed directly in human pancreatic juice, no detectable activity could be measured. This activity appeared only after incubation of pancreatic juice with trypsin. We can conclude therefore that all the phospholipase is present as zymogen in human pancreatic juice.

The activation of zymogen was studied as a function of different quantities of

trypsin and the kinetics are shown on Fig.1. In all cases, phospholipase A activity progressively increased to the same plateau of maximal activity corresponding to the complete conversion of zymogen into active enzyme. But we observed a decreasing latent period corresponding to increasing quantities of trypsin. This latent period is presumably due to the large amount of trypsin inhibitor present in human pancreatic juice[13,14].

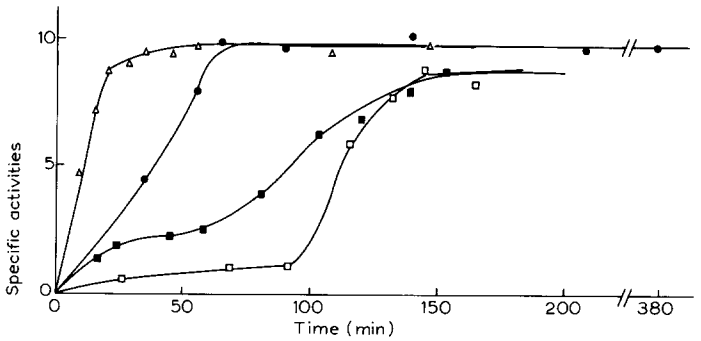

Fig.1. Kinetics of activation of prophospholipase A in human pancreatic juice at 0°.

The human zymogen was heat stable at acidic pH as was the case with prophospholipase A of porcine pancreas[8] and other phospholipases[3]. When the human pancreatic juice was treated at 70° for 3 min at pH 4.0, the potential phospholipase A activity did not change; lipase activity, however, was completely destroyed.

As we have already reported[14], chromatography of desalted human pancreatic juice on a DEAE-cellulose column in 5 mM Tris–HCl buffer (pH 8.0), separates "cationic" (*i.e.* nonadsorbed proteins) and "anionic" fractions. The cationic fraction contains prophospholipase A with lipase, ribonuclease, chymotrypsinogen 1, proelastase and trypsin inhibitor. The chromatography of these proteins on a Sephadex G-100 column (1.2 cm x 90 cm) equilibrated with 5 mM Tris–HCl buffer (pH 6.5) is presented in Fig.2. Lipase activity eluted with the first peak of proteins is clearly separated from potential phospholipase A activity eluted with the second peak of proteins. This experiment demonstrates the existence of two distinct lipolytic enzymes with different molecular weights in human pancreatic juice.

Some preliminary attempts at purification of prophospholipase A have been made. Lyophilized cationic proteins (15 mg) were dissolved in distilled water (5 ml) adjusted to pH 4.0 with HCl and incubated at 70° for 3 min. After rapid cooling at 0° and centrifugation at 20000 x *g* in a IEC multispeed attachment at 0° for 45 min, the precipitate was discarded. The supernatant was adjusted to pH 8 with normal NaOH and assayed using egg yolk emulsion as substrate. No activity could be detected before trypsin activation, but after activation, the specific activity of prophospholipase A (150) indicated a 15-fold purification of the zymogen from the original pancreatic juice. In the next step, proteins in solution in the supernatant were chromatographed on a CM-Sephadex column (0.9 cm x 20 cm) equilibrated with a 50 mM phosphate buffer (pH 6.0) in the presence of 5 mM butylamine to prevent activation. After elution of a small peak of proteins containing trypsin inhibitor activity, a linear concentration gradient of

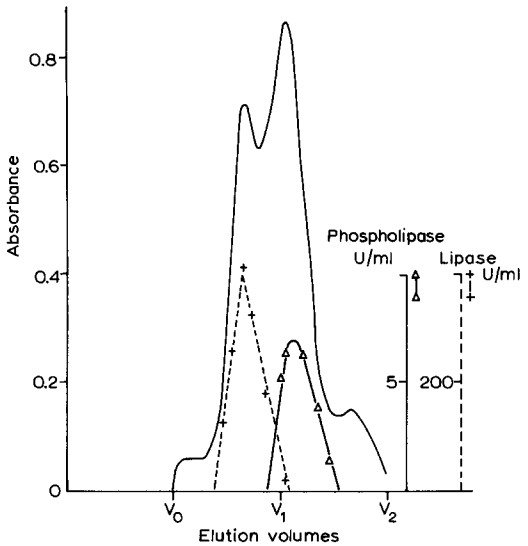

Fig.2. Filtration of cationic proteins on a Sephadex G-100 column (1.2 cm x 90 cm) in 5 mM Tris–HCl buffer (pH 6.5).

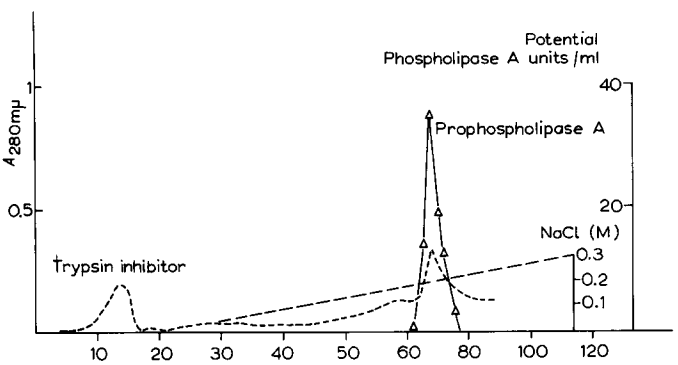

Fig.3. Chromatography of cationic proteins (after heat treatment) on a CM-Sephadex column (0.9 cm x 20 cm). Initial buffer: 50 mM phosphate, 5 mM butylamine, pH 6.0. Volume of one gradient chamber : 60 ml. Volume of fractions : 1.5 ml. Dotted line : proteins.

NaCl (from 0 to 300 mM) was applied to the column. As shown in Fig.3, prophospholipase A was eluted in one peak with 0.2 M NaCl concentration, with a satisfactory yield of 70% (maximal specific activity = 230). It gave one band by polyacrylamide gel electrophoresis at pH 4.3 (Fig.4).

While the isoelectric point of porcine prophospholipase and phospholipase are 6.5 and 7.4, respectively[8,12], human prophospholipase behaves as a cationic protein with ion-exchange chromatography and polyacrylamide gel electrophoresis.

These experiments demonstrate that no phospholipase A activity can be detected

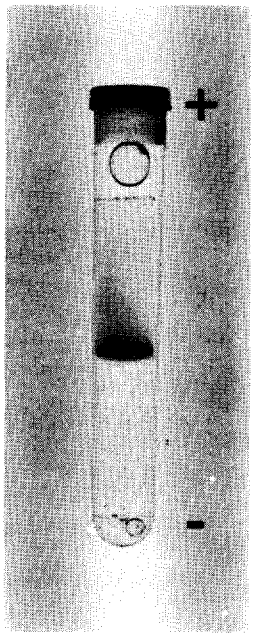

Fig.4. Polyacrylamide gel electrophoresis of prophospholipase A at pH 4.3.

in human pancreatic juice before tryptic activation. This was the case in subjects with and without chronic pancreatic disease. Moreover, human prophospholipase A seems to be more stable than its homologous precursors of other species. In porcine pancreas, De Haas *et al.*[12] noticed that traces of trypsin rapidly converted prophospholipase A into active enzyme at 0°. In lyophilized rat pancreatic juice, Arnesjo *et al.*[10] observed that phospholipase A activity was increasing with time spontaneously. This stability of human prophospholipase A is not due only to the presence of trypsin inhibitor. Indeed, during some chromatographic fractionations, we observed free chymotryptic activity without any free phospholipase activity. The activation bond is probably less exposed in prophospholipase than in chymotrypsinogen. More experiments are in progress to study the mechanism of activation of human prophospholipase A.

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