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Cathepsin B Activates Human Trypsinogen 1 but Not Proelastase 2 or Procarboxypeptidase B

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Key Words
Cathepsin B · Tryptase · Trypsinogen · Procarboxypeptidase B · Proelastase · Acute pancreatitis

Abstract
Background/Aims: Activation of trypsinogen to trypsin is a crucial step in the development of acute pancreatitis. The cause of this activation is not known although suggested explanations include autoactivation, cathepsin B-mediated activation and activation by mast cell tryptase. The aim of this study was to investigate cathepsin B and tryptase activation of pancreatic zymogens. ***Methods:*** Trypsinogen-1, proelastase, and procarboxypeptidase B were purified from human pancreatic juice. Human cathepsin B and βI-tryptase are commercial products. Activation and degradation of zymogens were measured by activity towards specific substrates for trypsin and pancreatic elastase, ELISAs for procarboxypeptidase B and its activation peptide, and a radioimmunoassay for the trypsinogen activation peptide. ***Results:*** Cathepsin B caused activation of trypsinogen-1 with a trypsin yield of about 30% of that produced by enterokinase. Proelastase and procarboxypeptidase B was not activated by cathepsin B. None of the zymogens were inactivated by cathepsin B. Neither monomeric nor tetrameric tryptase could activate any of the examined zymogens. ***Conclusion:*** Cathepsin B is a competent activator of trypsinogen-1, although not as efficient as enterokinase. If cathepsin B is to play a role in protease activation in acute pancreatitis, this most probably occurs by activation of trypsinogen.

Introduction

The presence of free active trypsin is considered crucial for the development of acute pancreatitis. Once activated, trypsin is thought to activate proinflammatory cascade systems and other pancreatic digestive enzymes, causing autodigestion. However, the mechanism behind the premature activation of trypsinogen to trypsin is still being debated.

Today there is important evidence supporting the hypothesis that cathepsin B is responsible for the activation of trypsinogen during acute pancreatitis [1–7]. A recent study by Halangk et al. [8] has questioned the role of trypsin in trypsinogen activation in acute pancreatitis. In isolated rat acini, specific inhibition of trypsin activity did not diminish the rate of trypsinogen activation after supramaximal cerulein stimulation. When the trypsin in-

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hibitor was washed out, trypsin activity was four times higher than after cerulein stimulation alone [8]. These findings have led to the controversial hypothesis that active trypsin can be a protective mechanism under certain circumstances through degradation and inactivation of active enzymes, including trypsin itself. As a consequence, investigation is currently underway into the possible activators of the pancreatic digestive enzymes other than trypsin. In this context, cathepsin B has been proposed. However, the ability of cathepsin B to activate other pancreatic zymogens is poorly investigated in contrast to its trypsinogen-activating properties. Although bovine cathepsin B has been shown to activate porcine proelastase [9], we know of no studies on the action of human cathepsin B on other human pancreatic zymogens than trypsinogen.

Another possible activator of trypsinogen and the other pancreatic proenzymes is mast cell tryptase. Previous studies have provided evidence for the importance of mast cells during the early phase of acute pancreatitis [10–12]. Tryptase is a tetrameric serine protease stored in the mast cell secretory granules. It is structurally related to trypsin and shows an overlapping substrate specificity [13]. Tryptase has been shown to form tetramers with the active site directed towards the central pore of the tetramer [14, 15]. This pore is not accessible to large substrates or inhibitors, e.g. trypsinogen. However, in a recent study it was shown that tetramers of human tryptase can, under physiological conditions, dissociate into active monomers. These monomers markedly exhibit enhanced activity towards macromolecular substrates [16]. Active monomers could therefore constitute possible activators of pancreatic zymogens.

The aim of this study was to investigate the ability of cathepsin B and monomeric tryptase to activate pancreatic zymogens. Trypsinogen, procarboxypeptidase B and proelastase were selected because they are known to be involved in the pathophysiology of acute pancreatitis where they are believed to have harmful effects. The capability of cathepsin B to degrade the pancreatic zymogens was also investigated.

Materials and Experimental Procedures

Cathepsin B, purified from human liver, 780 mg/l (specific activity >200 U/mg protein), was purchased from Biomol. Immediately before each experiment cathepsin B was activated with 1 m*M* *DL*-dithiothreitol on ice for 30 min. Proper activation was verified using the fluorogenic substrate Nα-CBZ-Arg-Arg 7-amid-4-methylcoumarin. The pH profile of cathepsin B activity was investigated and was not found to differ from what has been described previously in the literature [17].

Recombinant human βI-tryptase was purchased from Promega (Madison, Wisc., USA). Active monomers of human β-tryptase were obtained as previously described [16].

Enterokinase was obtained from Miles Laboratories. *DL*-Dithiothreitol, dimethyl sulfoxide, Nα-benzolyl-*DL*-Arg-*p*-nitroanilide (BAPNA) and Nα-CBZ-Arg-Arg 7-amid-4-methylcoumarin were purchased from Sigma. S-2288 (H-*D*-Ile-Pro-Arg-*p*-nitroanilide) was purchased from Chromogenix (Mölndal, Sweden). The EnzChek® Elastase Assay Kit was purchased from Molecular Probes, this kit contained both DQ-elastin and N-methoxysuccinyl-Ala-Ala-Pro-Val-chloromethyl ketone. Polybrene (hexadimethrine bromide, 1,5-dimethyl-1,5-diazaundecamethylene polymethobromide) was obtained from Janssen Chimica (Beerse, Belgium). Aprotinin (Trasylol®) was purchased from Bayer (Leverkusen, Germany). E64d was purchased from Bachem.

Rabbit anti-sera against trypsinogen-1, trypsinogen activation peptide (TAP), procarboxypeptidase B, procarboxypeptidase B activation peptide (CAPAP), and proelastase/elastase were available at our laboratory.

All reported results are based on experiments on pancreatic juice from one single patient who had her pancreatic duct drained in connection with pancreatic surgery. The findings have been confirmed in experiments on pancreatic juice from other patients. Harvested juice was stored at –20°C. Written informed consent was obtained from all patients. The study was approved by the local ethics committee (LU Dnr 616/2004).

Purification of Trypsinogen-1

Pancreatic juice was centrifuged and the supernatant was transferred to a 0.02-*M* Tris-HCl buffer (pH 7.5) using a PD-10 column. The elute was added to a Mono Q column (HR 5/5 Pharmacia Biotech), equilibrated with 0.02 *M* Tris-HCl (pH 7.5), connected to a high-performance liquid chromatography system (LKB Pharmacia). Elution was performed using a linear gradient with the NaCl concentration increasing from 0 to 0.5 *M*. Trypsin activity after addition of enterokinase to the samples was detected by spectrophotometry (405 nm) using BAPNA as a substrate. Trypsinogen-1 and 2 appeared as two separate peaks and were distinguished using polyclonal antibodies and immunoelectrophoresis. The fractions containing trypsinogen-1 were pooled.

Purification of Procarboxypeptidase B

Procarboxypeptidase B was purified from pancreatic juice as described previously by Appelros et al. [18].

Purification of Proelastase

Trypsin and trypsinogen were removed from the pancreatic juice as described for the purification of procarboxypeptidase B [18]. Filtered pancreatic juice, devoid of trypsin and trypsinogen, was applied to a Mono Q column using 0.02 *M* Tris-HCl (pH 7.5) as running buffer. The effluent was then applied to a Mono S column. Elution was performed with 0.02 *M* Tris-HCl (pH 7.5) used as a running buffer and a linear gradient of NaCl from 0 to 0.25 *M*. Elastase-like activity in the fractions was investigated after activation by trypsin, using the substrate N-succinyl-Ala-Ala-Pro-Leu P-nitroanilide. Two peaks of activity towards this substrate were found, elastase-2 was identified by a specific antibody [19]. The identification of elastase-2 was confirmed by the subsequent find-