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Research article

Cathepsin B cleavage of the trypsinogen activation peptide

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Abstract

Background: Cathepsin B is thought to play a central role in intrapancreatic trypsinogen activation and the onset of pancreatitis. A recent investigation of the cathepsin B mediated activability of wildtype trypsinogen and their mutations N29I, N29T and R122H, which are associated to hereditary pancreatitis, revealed no differences. This action seems to be restricted to the K23-I24 peptide bond, which is the trypsinogen activation bond. Here we investigated the influence of the mutations D22G and K23R of the trypsinogen activation peptide on the cleavability by cathepsin B.

Methods: To investigate the functional impact of the TAP mutations on cathepsin B mediated cleavage of the trypsinogen activating K23-I24 bond, the corresponding peptides pWT, APFDDDDKIVGG; pD22G, APFDDDGKIVGG; and pK23R, APFDDDDRIVGG were digested with cathepsin B for 30 min at pH 3.8 and 5.0, and the fragments were analysed by high-performance liquid chromatography.

Results: Without cathepsin B, less than 1 % of the peptides were hydrolysed. After a 30-minute digestion with cathepsin B at pH 5, 96% of pWT, 48% of pK23R, but only 2.4% of pD22G were hydrolysed. At pH 3.8, the cathepsin B cleavage of pWT and pK23R was less than at pH 5, whereas the cleavage of pD22G was completely inhibited

Conclusions: Cathepsin B mediated trypsinogen activation seems not to be a crucial pathogenic step in hereditary pancreatitis patients with the trypsinogen mutations D22G and K23R.

Background

In families with hereditary pancreatitis, a cationic trypsinogen mutation is a predominant risk factor for the manifestation of chronic pancreatitis [1]. Almost all disease-associated mutations are located within the active enzyme ([http://www.uni-leipzig.de/pancreasmutation]); three other gained interest, as they change the amino acid sequence of the trypsinogen activation peptide (TAP), but not the active trypsin molecule. In detail, the A16V mutation alters the cleavage bond A15-A16 of the signal peptide [2]. The D22G variant destroys the highly conserved tetraaspartic group of TAP [3], whereas K23R directly affects the trypsinogen activation bond K23-I24 [4] (table 1). As reported earlier, D22G and K23R facilitate the hydrolysis of the K23-I24 bond by active trypsin, what could lead to pancreatitis by enhanced trypsinogen autoactivation [3].

Table 1: Sequences of synthetic peptides in the 1-letter-code; underlined letters represent the mutated amino acids. The tryptic cutting site K23-I24 of the peptides are marked with dashes. pWT, wild type peptide; pD22G, peptide D22G; pK23R, peptide K23R.

peptide	amino acid sequence
pWT	APFDDDDK-IVGG
pD22G	APFDDDGK-IVGG
pK23R	APFDDDDK-IVGG

The lysosomal cysteine protease cathepsin B is thought to play a central role in intrapancreatic trypsinogen activation and the onset of pancreatitis. It activates human trypsinogen in vitro [5] and was found to be redistributed to a zymogen-granule enriched subcellular compartment during the early course of experimental pancreatitis [6]. Studies with cathepsin B deficient mice presented evidence, that the premature and intracellular activation of trypsinogen largely depends on the presence of cathepsin B [7]. A recent study showed that cathepsin B is abundantly present in the secretory compartment of the healthy human pancreas and is secreted together with trypsinogen and active trypsin into the pancreatic juice of patients with chronic pancreatitis [8]. The authors conclude, that trypsinogen and cathepsin B could potentially interact. This interaction may be modified by pancreatitis associated trypsinogen mutations. The investigation of the wild type trypsinogen molecule and three distinct mutants (i.e., N29I, N29T and R122H) found that cathepsin B mediated trypsinogen activation was not influenced by the respective mutants [8].

The studies by Kukor *et al.* imply, that the proteolytic cleavage of trypsinogen by cathepsin B is restricted to the K23-I24 trypsinogen activation peptide bond in the TAP [8]. In contrast to the mutations N29I, N29T and R122H, which are located away from this cleavage site, trypsinogen mutations affecting the K23-I24 bond could potentially affect the cathepsin B action on trypsinogen activation. The aim of this study was to evaluate the cathepsin B mediated cleavage of the K23-I24 bond in the presence of the pancreatitis associated TAP mutations D22G and K23R.

Materials and Methods

Unless otherwise indicated, all reagents were obtained from Sigma-Aldrich (Deisenhofen/Germany). Human liver cathepsin B (Sigma-Aldrich, catalog number C8571, enzymatic activity 3,930 units/mg protein) was used in a final concentration of 0.14 ng/µl. The oligopeptides shown in table 1 were commercially synthesized (MWG Biotech, Ebersberg, Germany) and purified by high-performance liquid chromatography (HPLC), and their molecular masses were verified by mass spectrometry. To investigate the functional impact of the TAP mutations on cathepsin B mediated cleavage of the trypsinogen activating K23-I24 bond, the corresponding peptides pWT, pD22G and pK23R were digested with cathepsin B. These dodecapeptides are homologous to the N-terminal part of wild type human cationic trypsinogen and the pancreatitis associated mutations D22G and K23R and include the activation peptide cleavage site (table 1).

pH 3.8 has been previously used in the activation of human trypsinogen [8]. The concentration of cathepsin B in lysosomes is high (about 1 mM) [9] and pH 5 mimics the approximate intralysosomal pH, the putative cellular compartment of trypsinogen activation in acute pancreatitis [10–14]. Therefore, all experiments have been carried out at both pH 3.8 and 5.0.

A solution of 2 mg dodecapeptide /mL of buffer (40 mM sodium acetate + 40 mM cysteine) with pH 3.8 and pH 5.0, was used [5]. Cathepsin B was added, and the mixture was incubated for 30 min at 37°C. As control, the peptides were incubated without addition of cathepsin B. The samples were then centrifuged using the Microcon centrifugal filter device YM-10 (catalogue no. 42406, Millipore, Bedford, MA) to remove cathepsin B from peptides or hydrolytic fragments. Next, 100 µl of the eluates was then separated by HPLC. After HPLC separation, the undigested peptides were eluted at a retention time of approximately 14 minutes. The N-terminal octapeptide was eluted after 11.6 minutes, and the residual C-terminal tetrapeptide was detected after 8.6 minutes. The method has been described in detail in reference [3].

Rates of hydrolysis were determined by integration of the respective area under each peak, and the digestion rates were indicated as percentages of initial dodecapeptide concentration.

Results and Discussion

Without cathepsin B, less than 1 % of the peptides was hydrolysed. After a 30-minute digestion with cathepsin B at pH 5, 96% of pWT, 48% of pK23R, but only 2.4% of pD22G were hydrolysed. At pH 3.8, the cathepsin B cleavage of pWT and pK23R was less than at pH 5, whereas the cleavage of pD22G was completely inhibited (figure 1).

In contrast to pancreatitis associated *trypsin* mutations, pancreatitis associated *TAP* mutations potentially prevent the hydrolytic activity of cathepsin B. The respective cleavage of the K23-I24 bond was reduced by 50% by the K23R mutation and largely inhibited by the D22G mutation.

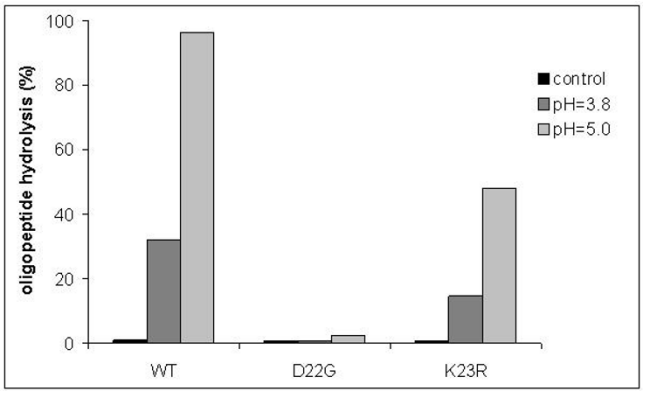

Figure 1
pH dependent hydrolysis of synthetic peptides by cathepsin B. Wild type and mutant (pD22G, pK23R) peptides were incubated with or without cathepsin B for 30 minutes. After chromatographic separation of the hydrolytic products, the percentage of peptide digestion was measured by integration of the areas under the respective peaks.

This indicates a high control of cathepsin B action on trypsinogen activation by the amino acid structure of TAP and highlights the particular functional importance of the D22 residue within the tetraaspartic group D19-D20-D21-D22.

An experimental setup using mutant dodecapeptides but not trypsinogen molecules might be questioned, as the structural organization of the N-terminal part of native trypsinogen is different from that of the synthetic oligopeptide. However, the recombinant expression of human trypsinogen is difficult and has been carried out by only two groups worldwide, with partially conflicting results due to different analytical conditions [8,15]. Crystallographic studies of the trypsinogen-to-trypsin conversion revealed that TAP can move freely in space and lacks a distinct secondary and tertiary structure [16]. In the past, the activation process of trypsinogen was analysed in numerous studies using oligopeptides [17] and the method used here has been proven to be highly reproducible [3].

The investigated TAP mutations have been discovered, as their carriers have chronic pancreatitis. With respect to that clinical picture it is highly unlikely, that a reduced activation of trypsinogen by cathepsin B provokes pancreatitis, but favors the role of trypsinogen autoactivation by active trypsin. Our data support the generally accepted hypothesis, that pancreatitis is caused by increased intrapancreatic trypsin activity. In contrast to this attractive hypothesis of gain-of-function trypsins in hereditary pancreatitis, recent *in vitro* studies have challenged these assumptions and suggested that a loss of trypsin function could impair the inactivation of other (more pancreatotoxic?) digestive enzymes [18,19]. These new insights suggest, that structural alterations, which impair the function of trypsin, could eliminate a *protective* mechanism rather than triggering an *aggressive* mechanism in initiating pancreatitis. However, our investigations on the cleavability of the trypsinogen activation bond bond by active trypsin [3] and cathepsin B provide further evidence, that autoactivation rather than cathepsin B mediated trypsinogen activation is the key pathogenic event in the development of hereditary pancreatitis in D22G and K23R-carriers.

In summary, two effects have been shown for the trypsinogen mutants D22G and K23R: (I) The trypsin mediated TAP-cleavage is facilitated. This could overwhelm the pancreatic protease-antiprotease-equilibrium and may lead to pancreatitis [3]. (II) The cathepsin B mediated TAP-cleavage is reduced. The resulting impaired cathepsin B mediated trypsinogen activation seems not be a pancreatitis promoting pathogenic step.

Competing interests
None declared.

Authors' contributions
NT carried out the peptide studies and drafted the manuscript. HB carried out the HPLCs. VK conceived of the study, and participated in its design and coordination. All authors wrote the manuscript.

All authors read and approved the final manuscript.

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