[Frontiers in Bioscience E1, 242-249, June 1, 2009]

Enteropeptidase, a type II transmembrane serine protease

X. Long Zheng[1], Yasunori Kitamoto[2], J. Evan Sadler[3]

[1]Department of Pathology and Laboratory Medicine, The Children's Hospital of Philadelphia and The University of Pennsylvania School of Medicine, Philadelphia, PA 19104, [2]Department of Internal Medicine, Sendai Shakai-Hoken Hospital, 3-16-1 tsutsumi-machi, Aoba-ku Sendai 9818501, Japan, [3]Department of Medicine, Washington University School of Medicine, St. Louis, MO 63110

TABLE OF CONTENTS

1. ABSTRACT

Enteropeptidase, a type II transmembrane serine protease, is localized to the brush border of the duodenal and jejunal mucosa. It is synthesized as a zymogen (proenteropeptidase) that requires activation by another protease, either trypsin or possibly duodenase. Active enteropeptidase then converts the pancreatic precursor, trypsinogen, to trypsin by cleavage of the specific trypsinogen activation peptide, Asp-Asp-Asp-Asp-Lys↓Ile that is highly conserved in vertebrates. Trypsin, in turn, activates other digestive zymogens such as chymotrypsinogen, proelastase, procarboxypeptidase and prolipase in the lumen of the gut. The important biological function of enteropeptidase is highlighted by the manifestation of severe diarrhea, failure to thrive, hypoproteinemia and edema as a result of congenital deficiency of enteropeptidase activity in the gut. Conversely, duodenopancreatic reflux of proteolytically active enteropeptidase may cause acute and chronic pancreatitis.

2. INTRODUCTION

Enteropeptidase [(E.C.3.4.21.9)], also named enterokinase, was first discovered by N.P. Schepovalnikow in the laboratory of I.P. Pavlov, who was awarded the 1904 Nobel Prize in Medicine or Physiology for his studies of gastrointestinal physiology. Enteropeptidase is a type II transmembrane serine protease that is localized to the brush border of the duodenal mucosa. It cleaves trypsinogen to generate active trypsin, which in turn activates other digestive enzyme precursors including chymotrypsinogen, procarboxypeptidase, proelastase, and prolipase in the lumen of the intestine (Figure 1). The activation peptide (Asp-Asp-Asp-Asp-Lys) is highly conserved in the trypsinogens found in all vertebrates (1). The intestinal activation of pancreatic pro-enzymes is a physiologic mechanism to prevent the proteolytic damage of the pancreas or pancreatic duct system. The importance of the proenteropeptidase (or enteropeptidase)-trypsinogen (or trypsin) activation pathway is highlighted by the development of severe intestinal malabsorption and

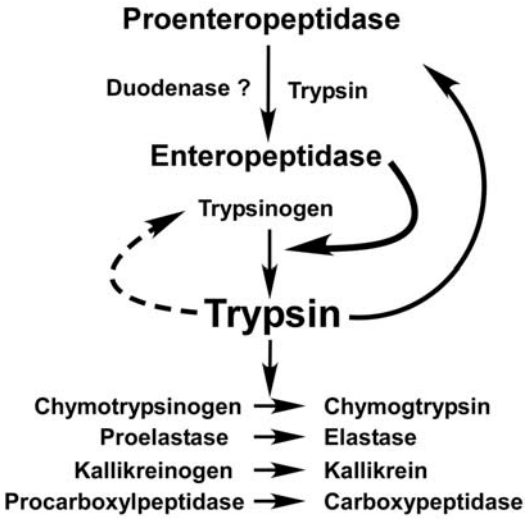

Figure 1. Pancreatic zymogen activation cascade. It remains unknown what activates proenteropeptidase. Trypsin can readily activate proenteropeptidase, but other proteases such as duodenase may make the first cut. Autoactivation of trypsinogen is relatively slow (dashed arrow) compared to the activation of trypsinogen by enteropeptidase.

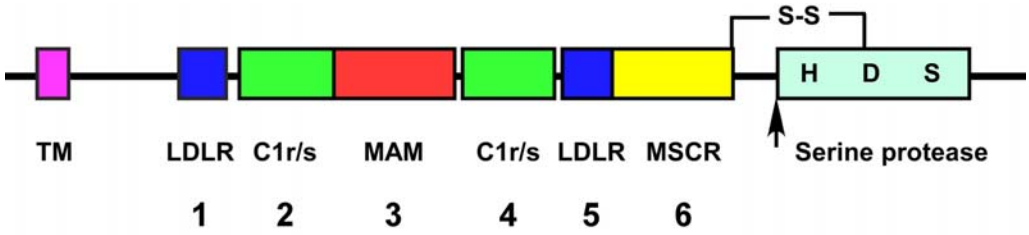

Figure 2. Diagram of human enteropeptidase domain structure. Full-length human enteropeptidase consists of a proposed transmembrane domain (TM), followed by a heavy chain and light chain. The enteropeptidase light chain is a serine protease domain with active site Histidine (H), Aspartate (D) and Serine (S) residues. The locations are shown for the cleavage site between the heavy and light chains (arrowhead) and of the predicted disulfide bond that connects them. The enteropeptidase heavy chain contains repeated motifs (numbered 1-6) that are homologous to domains of other proteins: LDLR, a low density lipoprotein receptor cysteine-rich repeat; C1r/s, a repeat type found in complement components C1r and C1s and also found in the *Drosophila* dorsal-ventral patterning gene *tolloid*; MAM, a domain homologous to members of a family defined by motifs in the mammalian metalloprotease meprin, the *X. laevis* neuronal protein A5, and the protein tyrosine phosphatase μ1; MSCR, macrophage scavenger receptor-like domain.

diarrhea as a result of congenital deficiency of enteropeptidase.

3. GENE AND PROTEIN STRUCTURE

3.1. Proenteropeptidase gene

The complete cDNA sequences of bovine (2), porcine (3), mouse (4), rat (5), Japanese rice fish (6) and human (7) enteropeptidase have been determined. Enteropeptidase cDNA sequences have been assembled from genomic data for several other vertebrates including opossum (XP_001373037), chicken (XP_425539), chimpanzee (XP_514836), dog (XP_544824) zebrafish (XP_001919639), and platypus (XP_001510441). The structural features of enteropeptidase are well conserved among these various species. In humans, the enteropeptidase gene is located at chromosome 21q21 (7). The gene spans ~90 kb and contains 25 exons (8). The cDNA sequence of human proenteropeptidase contains an open reading frame of 3,057 nucleotides, encoding a protein of 1,019 amino acid residues. The coding regions of the human and bovine nucleotide sequences are 85% identical, and encoded amino acid sequences are 82% identical (2, 7).

3.2. Proenteropeptidase domain structure

The human enteropeptidase protein consists of two polypeptide chains: a heavy chain (784 amino acids) and a light chain (235 amino acids) linked by a disulfide bond (Figure 2). The heavy chain contains a potential amino terminal myristoylation site at Gly2 (9). In addition, a transmembrane sequence is present at the amino terminus, followed by six structural motifs in the heavy chain. These motifs include two copies of low-density lipoprotein (LDL) receptor, two copies of complement component C1r/s, one copy of the metalloprotease meprin, and one copy of the macrophage scavenger receptor (MCSR). The light chain is a typical chymotrypsin-like serine protease (Figure 2).

4. BIOSYNTHESIS AND TRAFFICKING

4.1. Localization of enteropeptidase

In all animal species studied, enteropeptidase activity is highest in duodenum and rapidly decreases aborally, becoming undetectable by the distal jejumum. In humans (10), rats (11) and mice (4), this distribution is established late in fetal life. Enteropeptidase mRNA was detected in differentiated enterocytes and goblet cells of human duodenum (4), and also in some duodenal tumors (12), and the protein was associated with the brush border of enterocytes and some goblet cells (13-15). Substantial amounts of free enteropeptidase also occur in mucinous secretions of bovine (13), porcine (16) and human (17, 18) small intestine. The intraluminal enteropeptidase may be derived from the brush border enzyme due to the action of biliary or pancreatic proteases, and possibly to local effects of secretagogues such as gastrointestinal hormones. *In vivo*, cholescystokinin-pancreozymin (CCK-PZ) and/or secretin have been shown to increase the enteropeptidase activity in the duodenal fluid of rats (19-22) and humans (21, 23) even in the absence of bile salts or pancreatic secretions, suggesting that the enteropeptidase shedding from the enterocytes may be regulated by these physiological stimuli.

4.2. Polarized trafficking

The molecular basis of human enteropeptidase localization to the brush border membrane is not well characterized. Once it is synthesized in the endoplasmic reticulum and transferred to Golgi apparatus, full-length bovine enteropeptidase is directly targeted to the apical domain of polarized cells such as Madin-Darby canine kidney (MDCK) cells (24). Multiple signals within the protein appear to determine the polarity of enteropeptidase targeting (24). At least, one of the signals that direct the apical sorting of enteropeptidase resides in an amino terminal 27-amino acid residue region consisting of two short *O*-glycosylated mucin-like repeats (repeat A and repeat B) (25). Either mucin-like repeat A or repeat B is sufficient to direct apical targeting of non-polarized green fluorescent reporter protein (GFP) (25). However, *O*-linked glycosylation is not sufficient for apical targeting because a fusion to a different *O*-glycosylated motif does not alter the random targeting of GFP (25). In addition, several mutations in either repeat A or repeat B caused random targeting of GFP in MDCK cells, but did not prevent *O*-glycosylation. Furthermore, mucin-like repeat B appears to contain an apical sorting signal that functions in the absence of glycosylation (25).

When expressed by itself, the carboxyl terminal light chain of enteropeptidase also is targeted apically, and the apical targeting of the light chain requires *N*-linked glycosylation (24). Density gradient centrifugation indicated that, unlike several other apically targeted membrane and soluble proteins, apical sorting of bovine enteropeptidase or mucin-GFP chimeric proteins does not appear to utilize lipid rafts as platforms (24, 25).

5. ZYMOGEN ACTIVATION

Purified porcine (1), bovine (26), and human (27) enteropeptidases from duodenal juices consists of a heavy chain of 100~140 kDa and a light chain of 35~62 kDa, linked by the disulfide bond. Enteropeptidase is synthesized as a zymogen, containing a well-conserved activation site Val-Ser-Pro-Lys ↓ Ile (cattle and pig) or Ile-Thr-Pro-Lys↓Ile (human) or Val-Gly-Pro-Lys↓Ile (rat). These are very poor sites for autoactivation, but excellent sites for cleavage by trypsin and many related proteases. When expressed in baby hamster kidney (BHK) cells with a prothrombin signal peptide in place of its transmembrane domain, recombinant bovine enteropeptidase was secreted as a single-chain molecule of ~150 kDa with no detectable proteolytic activity toward peptide substrates (28). Incubation of this soluble proenteropeptidase with a trace amount of trypsin (~5 nM) rapidly converted it into an active enzyme composed of two fragments of ~133 kDa and ~43 kDa (28). However, enteropeptidase is believed to initiate a protease activation cascade for digestive enzymes: if enteropeptidase activates trypsinogen, then is trypsin really the physiological activator of proenteropeptidase? It is possible that enough trypsin is present in pancreatic fluid to activate a small amount of proenteropeptidase, after which the strong positive feedback between trypsinogen activation by enteropeptidase and proenteropeptidase activation by trypsin (Figure 1) is sufficient. The active trypsin then activates downstream zymogens including chymotrypsinogen and proelastase. Such a positive feedback mechanism is analogous to the serine protease-mediated cascades of blood coagulation and fibrinolysis.

Alternatively, another serine protease purified from duodenal mucosa such as duodenase has been proposed to make the "first cut" on proenteropeptidase. Duodenase is a chymase-like enzyme, composed of 226 amino acids (30 kDa) (29, 30). It is localized to the secretory epithelial cells of Brunner's glands of the proximal segment of duodenum (29, 30). Duodenase prefers peptide sequence containing Lys residue at P1 and Pro residue at P2, which matches the activation cleavage sequence of proenteropeptidase (30). However, the rate of activation of purified bovine recombinant proenteropeptidase by purified duodenase (*kcat/km* value of 2,700 $M^{-1}s^{-1}$) is approximately 100 fold slower than that by trypsin (30). Furthermore, duodenase is synthesized as a zymogen that appears to require activation by yet another protease. Therefore, the proposed role for duodenase as an activator of proenteropeptidase remains to be established.

6. SUBSTRATE SPECIFICITY

Enteropeptidase activates trypsinogen by cleaving the Val-Asp-Asp-Asp-Asp-Lys↓ILe (or VDDDDK↓I in single letter code) peptide bond. This cleavage sequence consists of a basic residue at the P1 position and acidic residues at the P2–P5 sites. The cleavage peptide (DDDD↓K) is highly conserved among vertebrate trypsinogens (31, 32). It was hypothesized that such remarkable sequence specificity may provide an important mechanism to ensure that trypsinogen is activated only by enteropeptidase after it has been secreted into the small intestine, but not by other proteases in pancreas, because an excessive activation of trypsinogen in

pancreas may lead to devastating conditions such as acute pancreatitis (33, 34).

Unexpectedly, recent studies demonstrated that a tetra-Ala19-22 trpysinogen mutant devoid of acidic residues in the activation peptide is still a highly specific substrate for human enteropeptidase, although not for bovine enteropeptidase (35). However, an intact Asp19-22 motif inhibits the autoactivation of trypsinogen. Single Ala substitutions of Asp19 or Asp21 increased the rate of autoactivation 2-3 fold, whereas Ala substitution for Asp22 increased the rate of autoactivation 66 fold. These effects were multiplicative in the tri-Ala19-21 and tetra-Ala19-22 mutants (35). These data suggest that in human cationic trypsinogen the Asp19-22 motif per se is not required for enteropeptidase recognition, whereas it is essential for maximal suppression of autoactivation.

The determinants of enteropeptidase substrate specificity may not be confined to serine protease domains. For instance, proteolytic removal or recombinant truncation of the transmembrane domain appears to have little effect on trypsinogen activation by bovine (28, 36) or porcine (37) enteropeptidase. Full-length and soluble bovine enteropeptidase had similar K_m and k_{cat} values. However, further deletion of the heavy chain of bovine enteropeptidase significantly impaired the proteolytic activation of trypsinogen, but not the cleavage of the small peptide substrate Gly-$(Asp)_4$-Lys-β-naphthylamide (28). A construct containing a light chain of enteropeptidase cleaved trypsinogen relatively slowly, with increased *Km* by 24 fold and reduced k_{cat} by 20~40 fold, compared to full-length soluble bovine enteropeptidase (28). These data suggest that the heavy chain of enteropeptidase has little influence on the recognition of small peptides, but is required for efficient macromolecular substrate recognition.

The protease domain of enteropeptidase is also required for substrate specificity. The catalytic triad and substrate specificity pocket, the conserved sequence of four basic residues, R/KRRK, at positions between amino acid residues 96 and 99 in the light chain may all participate in substrate recognition. Molecular modeling based on three-dimensional structures from other serine proteases suggested that these basic residues may interact with the acidic P2–P5 residues within the trypsinogen activation sequence (2, 37). This was confirmed by the crystal structure of the light chain of enteropeptidase complexed with an analog of the trypsinogen activation peptide, the inhibitor Val-$(Asp)_4$-Lys-chromethane (38). As one of the major determinants of substrate recognition, the Lys99 residue in the light chain of bovine enteropeptidase makes extensive contacts with P2 and P4 Asp residues in the trypsinogen activation peptide. Substitution of Lys99 with Ala by site-directed mutagenesis, or acetylation with acetic anhydride, specifically prevented the proteolytic cleavage of trypsinogen or Gly-$(Asp)_4$-Lys-β-naphthylamide (38). Mutations at Lys96, Arg97, and Arg98 in bovine enteropeptidase had much less effect on proteolytic activity (38).

7. REGULATION OF ENZYMATIC ACTIVITY

Enteropeptidase is active at pH values between 6 and 9, with a broad optimum at about pH 8 (1, 28). Activity toward trypsinogen is inhibited by increasing ionic strength, due mainly to an increase in K_m (39). Calcium ions at concentrations between 4 and 10 mM stabilize enteropeptidase to heat denaturation (26, 40) and also modestly increase its activity (41-44). The calcium-dependent increase in trypsinogen activation may be due in part to the binding of calcium ions by the acidic trypsinogen activation peptide (45). Bovine enteropeptidase is not inhibited by chicken ovomucoid, soybean trypsin inhibitor or limabean trypsin inhibitor (1, 46, 47). It is, however, inhibited by Tos-Lys-CH_2Cl, diisopropyl phosphorofluoridate (DFP), p-aminobenzamidine, benzamidine (39), or bovine pancreatic trypsin inhibitor (47). The light chain of human enteropeptidase is inhibited by soybean trypsin inhibitor, bovine pancreatic trypsin inhibitior, leupeptin, antipain, benzamidine, and weakly by E-64; it is not inhibited by chymostatin, pepstatin A, or bestatin (48), porcine enteropeptidase appeared to be resistant to bovine pancreatic tyrpsin inhibitor (1, 49). A 60-kDa protein isolated from kidney bean can also inhibit enteropeptidase activity (50).

8. ASSAY METHODS

Several methods are used to assay trypsinogen activation by enteropeptidase; the trypsin product may be quantitated by cleavage of chromogenic substrate Bz-Arg-Oet (41), Z-lys-S-Bzl (51) or N-CBZ-Gly-Pro-Arg-*p*-nitroanilide at pH 5.6 and 8.0 (32), or trapped as a complex with chicken ovomucoid (52). A relatively specific enteropeptidase substrate, Gly-$(Asp)_4$-Lys-β-naphthylamide (GD_4K-NA) (28, 38), is commercially available and its cleavage can be assayed fluorometrically (53) or spectrophotometrically (28, 38). This substrate can also be used for the histochemical identification of enteropeptidase in tissue sections (15).

8.1. Trypsinogen activation assay

This assay contains trypsinogen (0-40 μM), 25 mM Tris-HCl, pH 8.4 or 50 mM sodium citrate, pH 6.0 in the presence of 10 mM $CaCl_2$ and 40 μM ovomucoid at 37 °C. The reaction is initiated by adding enteropeptidase (0.3 nM). The reaction is stopped by addition of 20 μl of 0.2 M HCl to achieve pH value of 2.0. This condition inactivates enteropeptidase and dissociates the trypsin and ovomucoid complex. The free trypsin generated is quantitated by addition of an equal volume of 500 μM S-2765 in 20 mM Tris-HCl, pH 8.4, and 150 mM NaCl, and absorbance at 405 nm is recorded as a function of time (28).

8.2. Cleavage of synthetic peptides

The proteolytic activity of enteropeptidase can also be determined by cleavage of synthetic peptide substrate GD4K-NA as previously described (28). Reaction (60 μl) contains 0.1-1 mM GD_4K-NA, 25 mM Tris-HCl, pH 8.4, and 10 mM $CaCl_2$ at 37 °C. The reaction is initiated by adding enteropeptidase (15 nM). At various time points, 10 μl samples are removed and added to 3.5 μl of 2 M HCl.

Free β-naphthylamine concentration is determined spectrophotometrically (28, 38). Alternatively, enzyme activity is determined from the increase over time of free β-naphthylamine fluorescence (excitation at 337 nm, emission at 420 nm) (53).

9. CONGENITAL DEFICIENCY

The first case of congenital enteropeptidase deficiency was described in 1969 in a 3-week old female infant who suffered from diarrhea and failed to gain body weight (54). Since then, at least 10 additional cases of congenital enteropeptidase deficiency have been reported (54-59). Congenital enteropeptidase deficiency is an autosomal recessive disorder. In these patients, diarrhea, vomiting, edema, anemia, hypoproteinemia, and failure to gain weight are common symptoms in early infancy. Very low or no enteropeptidase activity can be detected in intestinal biopsies and duodenal fluid samples. Celiac disease was reported in a 40-year old patient with congenital deficiency of enteropeptidase (60). There is no evidence for a causal relationship between these disorders.

Mutations in the enteropeptidase gene were identified in patients with congenital enteropeptidase deficiency from two unrelated families (57). Two affected siblings from one family had compound heterozygous nonsense mutations, one in exon 18 (2135 C>G) and another in exon 22 (2569 C>T), resulting in premature stop codons at residues Ser712 within the MSCR domain and Arg857 within the serine protease domain, respectively (57). A patient from another family had a heterozygous nonsense mutation in exon 8 (781 C>T) and a heterozygous deletion mutation (2707-2708 delGT) in exon 23, resulting in a premature stop codon at residue Gln261 within the C1r/s motif and a frameshift at residue Gln902 within the serine protease domain, respectively (57). Thus, the mutations in proenteropeptidase gene result in either truncation or abolishment of the enzymatic function of the serine protease domain.

Because of the difficulty of obtaining and assaying duodenal contents for enteropeptidase activity, patients with congenital enteropeptidase deficiency may remain undiagnosed. Treatment with oral pancreatic extracts usually is effective. Interestingly, pancreatic replacement therapy usually can be discontinued in later life and patients live with essentially normal body weights and free of gastrointestinal symptoms (12), suggesting that sufficient digestive protease activity can be sustained by a trypsin-mediated feedback or autoactivation mechanism to meet the dietary needs of adults.

10. ACUTE PANCREATITIS

Acute pancreatitis is a complex inflammatory disease of the pancreas with uncertain pathogenesis and its severe form is devastating. Since the disease is rarely identified at its earliest stage and access to the pancreas at that time is generally not possible, studies using clinical materials have been limited. Instead, animal models have been used to elucidate the mechanism of the disease. Simultaneous injection of cerulenin, a pancreatic secretagogue, and enteropeptidase induces necrotizing acute pancreatitis (61). The inappropriate intraluminal activation of pancreatic trypsinogen has been thought to cause acute pancreatitis (62-65). On the other hand, a recent study (66) using cerulenin induced only a mild form of pancreatitis, suggesting that a lysosomal hydrolase may activate trypsinogen to trypsin, leading to activation of other lysosomal zymogens and pancreatic autodigestion. However, enteropeptidase may be required to induce severe necrotizing pancreatitis. For instance, reflux of enteropeptidase into the pancreatic duct was shown to cause the acute necrotizing pancreatitis (62). Therefore, the underlying mechanism that causes mild and severe acute pancreatitis may be quite different.

11. ACKNOWLEDGEMENT

This work is supported by grants from the National Institutes of Health to XLZ (HL079027) and JES (HL72917, HL89746).

12. REFERENCES

1. Maroux S, J. Baratti, and P. Desnuelle: Purification and specificity of porcine enterokinase. *J Biol Chem* 246, 5031-5039 (1971)

2. Kitamoto Y, X. Yuan, Q. Wu, D. W. McCourt, and J. E. Sadler: Enterokinase, the initiator of intestinal digestion, is a mosaic protease composed of a distinctive assortment of domains. *Proc Natl Acad Sci U S A* 91, 7588-7592 (1994)

3. Matsushima M, M. Ichinose, N. Yahagi, S. Tsukada-Kato, K. Miki, M. Omata, Y. T. Kim, H. Ito, T. Takahashi, Y. Sakurai, Y. Tsuchiya, S. B. Athauda, H. Inoue, and K. Takahashi: Purification and further characterization of enteropeptidase from porcine duodenum. *J Biochem (Tokyo)* 125, 947-951 (1999)

4. Yuan X, X. Zheng, D. Lu, D. C. Rubin, C. Y. Pung, and J. E. Sadler: Structure of murine enterokinase (enteropeptidase) and expression in small intestine during development. *Am J Physiol* 274, G342-G349 (1998)

5. Yahagi N, M. Ichinose, M. Matsushima, Y. Matsubara, K. Miki, K. Kurokawa, H. Fukamachi, K. Tashiro, K. Shiokawa, T. Kageyama, T. Takahashi, H. Inoue, and K. Takahashi: Complementary DNA cloning and sequencing of rat enteropeptidase and tissue distribution of its mRNA. *Biochem Biophys Res Commun* 219, 806-812 (1996)

6. Ogiwara K, T. Takahashi: Specificity of the medaka enteropeptidase serine protease and its usefulness as a biotechnological tool for fusion-protein cleavage. *Proc Natl Acad Sci U S A* 104, 7021-7026 (2007)

7. Kitamoto Y, R. A. Veile, H. Donis-Keller, and J. E. Sadler: cDNA sequence and chromosomal localization of human enterokinase, the proteolytic activator of trypsinogen. *Biochemistry* 34, 4562-4568 (1995)

8. Holzinger A, E. M. Maier, C. Buck, P. U. Mayerhofer, M. Kappler, J. C. Haworth, S. P. Moroz, H. B. Hadorn, J. E. Sadler, and A. A. Roscher: Mutations in the proenteropeptidase gene are the molecular cause of congenital enteropeptidase deficiency. *Am J Hum Genet* 70, 20-25 (2002)

9. Gordon JI, R. J. Duronio, D. A. Rudnick, S. P. Adams, and G. W. Gokel: Protein N-myristoylation. *J Biol Chem* 266, 8647-8650 (1991)

10. Antonowicz I, E. Lebenthal: Developmental pattern of small intestinal enterokinase and disaccharidase activities in the human fetus. *Gastroenterology* 72, 1299-1303 (1977)

11. Lebenthal E: Induction of fetal rat enterokinase (enteropeptidase EC. 3.4.21.9) in utero by hydrocortisone and actinomycin D. *Pediatr Res* 11, 282-285 (1977)

12. Imamura T, Y. Kitamoto: Expression of enteropeptidase in differentiated enterocytes, goblet cells, and the tumor cells in human duodenum. *Am J Physiol Gastrointest Liver Physiol* 285, G1235-G1241 (2003)

13. Fonseca P, A. Light: Incorporation of bovine enterokinase in reconstituted soybean phospholipid vesicles. *J Biol Chem* 258, 3069-3074 (1983)

14. Hermon-Taylor J, J. Perrin, D. A. Grant, A. Appleyard, M. Bubel, and A. I. Magee: Immunofluorescent localisation of enterokinase in human small intestine. *Gut* 18, 259-265 (1977)

15. Lojda Z, R. Gossrau: Histochemical demonstration of enteropeptidase activity. New method with a synthetic substrate and its comparison with the trypsinogen procedure. *Histochemistry* 78, 251-270 (1983)

16. Louvard D, S. Maroux, J. Baratti, and P. Desnuelle: On the distribution of enterokinase in porcine intestine and on its subcellular localization. *Biochim Biophys Acta* 309, 127-137 (1973)

17. Eggermont E, A. M. Molla, L. Rutgeerts, and G. Tytgat: The source of human enterokinase. *Lancet* 2, 369 (1971)

18. Hadorn B, M. J. Tarlow, J. K. Lloyd, and O. H. Wolff: Intestinal enterokinase deficiency. *Lancet* 1, 812-813 (1969)

19. Goldman RB, Y. S. Kim, R. S. Jones, and M. H. Sleisenger: The effect of glucagon on enterokinase secretion from Brunner's gland pouches in dogs. *Proc Soc Exp Biol Med* 138, 562-565 (1971)

20. Gotze H, J. Gotze, and J. W. Adelson: Studies on intestinal enzyme secretion; the action of cholecystokinin-pancreozymin, pentagastrin and bile. *Res Exp Med (Berl)* 173, 17-25 (1978)

21. Moss S, G. R. Birch, R. W. Lobley, and R. Holmes: The release of enterokinase following secretin and cholecystokinin-pancreozymin in man. *Scand J Gastroenterol* 14, 1001-1007 (1979)

22. Nordstrom C: Enzymic release of enteropeptidase from isolated rat duodenal brush borders. *Biochim Biophys Acta* 268, 711-718 (1972)

23. Lebenthal E, P. C. Lee: Effect of pancreozymin and secretin on intraluminal enterokinase, trypsin, and chymotrypsin activities of cystic fibrosis and control children. *Digestion* 23, 39-47 (1982)

24. Zheng X, D. Lu, and J. E. Sadler: Apical sorting of bovine enteropeptidase does not involve detergent-resistant association with sphingolipid-cholesterol rafts. *J Biol Chem* 274, 1596-1605 (1999)

25. Zheng X, J. E. Sadler: Mucin-like domain of enteropeptidase directs apical targeting in Madin-Darby canine kidney cells. *J Biol Chem* 277, 6858-6863 (2002)

26. Anderson LE, K. A. Walsh, and H. Neurath: Bovine enterokinase. Purification, specificity, and some molecular properties. *Biochemistry* 16, 3354-3360 (1977)

27. Grant DA, A. I. Magee, and J. Hermon-Taylor: Optimisation of conditions for the affinity chromatography of human enterokinase on immobilised p-aminobenzamidine. Improvement of the preparative procedure by inclusion of negative affinity chromatography with glycylglycyl-aniline. *Eur J Biochem* 88, 183-189 (1978)

28. Lu D, X. Yuan, X. Zheng, and J. E. Sadler: Bovine proenteropeptidase is activated by trypsin, and the specificity of enteropeptidase depends on the heavy chain. *J Biol Chem* 272, 31293-31300 (1997)

29. Zamolodchikova TS, E. A. Sokolova, S. L. Alexandrov, I. I. Mikhaleva, I. A. Prudchenko, I. A. Morozov, N. V. Kononenko, O. A. Mirgorodskaya, U. Da, N. I. Larionova, V. F. Pozdnev, D. Ghosh, W. L. Duax, and T. I. Vorotyntseva: Subcellular localization, substrate specificity and crystallization of duodenase, a potential activator of enteropeptidase. *Eur J Biochem* 249, 612-621 (1997)

30. Zamolodchikova TS, E. A. Sokolova, D. Lu, and J. E. Sadler: Activation of recombinant proenteropeptidase by duodenase. *FEBS Lett* 466, 295-299 (2000)

31. Bricteux-Gregoire S, R. Schyns, and M. Florkin: Phylogeny of trypsinogen activation peptides. *Comp Biochem Physiol* B 42, 23-39 (1972)

32. Chen JM, Z. Kukor, M. C. Le, M. Toth, L. Tsakiris, O. Raguenes, C. Ferec, and M. Sahin-Toth: Evolution of trypsinogen activation peptides. *Mol Biol Evol* 20, 1767-1777 (2003)

33. Fernandez-del CC, J. Schmidt, A. L. Warshaw, and D. W. Rattner: Interstitial protease activation is the central event in progression to necrotizing pancreatitis. *Surgery* 116, 497-504 (1994)

34. Hartwig W, J. Werner, R. E. Jimenez, K. Z'graggen, J. Weimann, K. B. Lewandrowski, A. L. Warshaw, and C. C. Fernandez-del: Trypsin and activation of circulating trypsinogen contribute to pancreatitis-associated lung injury. *Am J Physiol* 277, G1008-G1016 (1999)

35. Nemoda Z, M. Sahin-Toth: Chymotrypsin C (caldecrin) stimulates autoactivation of human cationic trypsinogen. *J Biol Chem* 281, 11879-11886 (2006)

36. Light A, P. Fonseca: The preparation and properties of the catalytic subunit of bovine enterokinase. *J Biol Chem* 259, 13195-13198 (1984)

37. Matsushima M, M. Ichinose, N. Yahagi, N. Kakei, S. Tsukada, K. Miki, K. Kurokawa, K. Tashiro, K. Shiokawa, K. Shinomiya, and .: Structural characterization of porcine enteropeptidase. *J Biol Chem* 269, 19976-19982 (1994)

38. Lu D, K. Futterer, S. Korolev, X. Zheng, K. Tan, G. Waksman, and J. E. Sadler: Crystal structure of enteropeptidase light chain complexed with an analog of the trypsinogen activation peptide. *J Mol Biol* 292, 361-373 (1999)

39. Baratti J, S. Maroux: On the catalytic and binding sites of porcine enteropeptidase. *Biochim Biophys Acta* 452, 488-496 (1976)

40. Barns RJ, R. G. Elmslie: The active site of porcine enteropeptidase. Selective inactivation of the peptidase activity. *Biochim Biophys Acta* 350, 495-498 (1974)

41. Baratti J, S. Maroux, and D. Louvard: Effect of ionic strength and calcium ions on the activation of trypsinogen by enterokinase. A modified test for the quantitative evaluation of this enzyme. *Biochim Biophys Acta* 321, 632-638 (1973)

42. Grant DA, J. Hermon-Taylor: Hydrolysis of artificial substrates by enterokinase and trypsin and the development of a sensitive specific assay for enterokinase in serum. *Biochim Biophys Acta* 567, 207-215 (1979)

43. Magee AI, D. A. Grant, and J. Hermon-Taylor: The apparent molecular weights of human intestinal aminopeptidase, enterokinase and maltase in native duodenal fluid. *Biochem J* 165, 583-585 (1977)

44. Magee AI, D. A. Grant, and J. H. Taylor: Further studies on the subunit structure and oligosaccharide moiety of human enterokinase. *Clin Chim Acta* 115, 241-254 (1981)

45. Abita JP, M. Delaage, and M. Lazdunski: The mechanism of activation of trypsinogen. The role of the four N-terminal aspartyl residues. *Eur J Biochem* 8, 314-324 (1969)

46. Green GM: Use of trypsin inhibitors to prevent autoactivation in the assay of enterokinase. *Clin Chim Acta* 134, 363-367 (1983)

47. Liepnieks JJ, A. Light: The preparation and properties of bovine enterokinase. *J Biol Chem* 254, 1677-1683 (1979)

48. Gasparian ME, V. G. Ostapchenko, D. A. Dolgikh, and M. P. Kirpichnikov: Biochemical characterization of human enteropeptidase light chain. *Biochemistry (Mosc)* 71, 113-119 (2006)

49. Light A, H. Janska: Enterokinase (enteropeptidase): comparative aspects. *Trends Biochem Sci* 14, 110-112 (1989)

50. Jacob RT, P. G. Bhat, and T. N. Pattabiraman: Isolation and characterization of a specific enterokinase inhibitor from kidney bean (Phaseolus vulgaris). *Biochem J* 209, 91-97 (1983)

51. Mikhailova AG, V. V. Likhareva, I. A. Prudchenko, and L. D. Rumsh: Effect of calcium ions on enteropeptidase catalysis. *Biochemistry (Mosc)* 70, 1129-1135 (2005)

52. Janska H, A. Light: A direct high-performance liquid chromatography assay of the enzymatic activity of enterokinase (enteropeptidase). *Anal Biochem* 176, 132-136 (1989)

53. LaVallie ER, A. Rehemtulla, L. A. Racie, E. A. DiBlasio, C. Ferenz, K. L. Grant, A. Light, and J. M. McCoy: Cloning and functional expression of a cDNA encoding the catalytic subunit of bovine enterokinase. *J Biol Chem* 268, 23311-23317 (1993)

54. Hadorn B, M. J. Tarlow, J. K. Lloyd, and O. H. Wolff: Intestinal enterokinase deficiency. *Lancet* 1, 812-813 (1969)

55. Ghishan FK, P. C. Lee, E. Lebenthal, P. Johnson, C. A. Bradley, and H. L. Greene: Isolated congenital enterokinase deficiency. Recent findings and review of the literature. *Gastroenterology* 85, 727-731 (1983)

56. Green JR, S. W. Bender, H. G. Posselt, and M. J. Lentze: Primary intestinal enteropeptidase deficiency. *J Pediatr Gastroenterol Nutr* 3, 630-633 (1984)

57. Holzinger A, E. M. Maier, C. Buck, P. U. Mayerhofer, M. Kappler, J. C. Haworth, S. P. Moroz, H. B. Hadorn, J. E. Sadler, and A. A. Roscher: Mutations in the proenteropeptidase gene are the molecular cause of congenital enteropeptidase deficiency. *Am J Hum Genet* 70, 20-25 (2002)

58. Mann NS, P. K. Nair, and H. B. Russman: Enterokinase deficiency and vitamin B12 absorption. *Gastroenterology* 86, 384-385 (1984)

59. Tarlow MJ, B. Hadorn, M. W. Arthurton, and J. K. Lloyd: Intestinal enterokinase deficiency. A newly-recognized disorder of protein digestion. *Arch Dis Child* 45, 651-655 (1970)

60. Moroz SP, B. Hadorn, T. M. Rossi, and J. C. Haworth: Celiac disease in a patient with a congenital deficiency of intestinal enteropeptidase. *Am J Gastroenterol* 96, 2251-2254 (2001)

61. Hartwig W, M. Kolvenbach, T. Hackert, F. Fortunato, L. Schneider, M. W. Buchler, and J. Werner: Enterokinase induces severe necrosis and rapid mortality in cerulein pancreatitis: characterization of a novel noninvasive rat model of necro-hemorrhagic pancreatitis. *Surgery* 142, 327-336 (2007)

62. Grant D: Acute necrotising pancreatitis--a role for enterokinase. *Int J Pancreatol* 1, 167-183 (1986)

63. Hammond JB, N. S. Mann: Pancreatitis following the intraductal injection of partially purified enterokinase in dogs. *Am J Dig Dis* 22, 182-188 (1977)

64. Mann NS, R. S. Kadian, and K. Narenderan: Enterokinase induced pancreatitis: its prevention with Trasylol and soybean trypsin inhibitor. *Am J Proctol Gastroenterol Colon Rectal Surg* 29, 13-17 (1978)

65. Terry TR, D. A. Grant, and J. Hermon-Taylor: Intraduct enterokinase is lethal in rats with experimental bile-salt pancreatitis. *Br J Surg* 74, 40-43 (1987)

66. van Acker GJ, E. Weiss, M. L. Steer, and G. Perides: Cause-effect relationships between zymogen activation and other early events in secretagogue-induced acute pancreatitis. *Am J Physiol Gastrointest Liver Physiol* 292, G1738-G1746 (2007)

Key Words: Transmembrane Protein, Enteropeptidase, Enterokinase, Review

Send correspondence to: X. Long Zheng, The Children's Hospital of Philadelphia and The University of Pennsylvania Medical Center, 34th Street and Civic Center Blvd, 816G ARC, Philadelphia, PA 19104, Tel: 215-590-3565, Fax. 267-426-5165, E-mail: zheng@email.chop.edu

http://www.bioscience.org/current/vol1E.htm