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Activation of Human Pancreatic Proteolytic Enzymes: The Role of Enteropeptidase and Trypsin

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ABSTRACT

Objective: The role of enteropeptidase and trypsin in the process by which pancreatic proteolytic zymogens are converted into active enzymes has been investigated in the past, using purified enzymes and proenzymes of animal origin. In the present study, we wanted to study this process under conditions which come near to the physiological situation, which prevails in the human duodenum and upper small intestine.

Patients and Methods: Duodenal contents were collected from 2 patients with intestinal enteropeptidase deficiency. The samples expressed no tryptic activity and were used as the source of zymogens. Enteropeptidase or trypsin was added to these samples and the process of zymogen activation was followed by measuring trypsin and chymotrypsin activities.

Results: When exogenous trypsin was added to the duodenal contents of patients with enteropeptidase deficiency, having no tryptic activity, activation of intrinsic trypsinogen was not observed. When purified porcine or human enteropeptidase was added to the same samples of duodenal contents, this resulted in a rapid, dose-dependent activation of trypsinogen followed by the activation of chymotrypsinogen.

Conclusion: The study underlines the key role of enteropeptidase in the cascade process, which leads to the presence of active proteolytic enzymes in the human small intestine. The results also explain why patients with congenital deficiency of enteropeptidase are unable to activate trypsinogen by alternative pathways and therefore suffer from a severe disturbance of protein digestion with failure to thrive at young age, hypoproteinemia, and anemia.

Key Words: enterokinase, enteropeptidase, trypsin, chymotrypsin, zymogen activation, enteropeptidase deficiency

What Is Known

- Congenital enteropeptidase deficiency leads to severe hypoproteinemia, anemia, and failure to thrive shortly after birth. The molecular cause of enteropeptidase deficiency is mutations in the proenteropeptidase gene. In duodenal contents of these patients, all pancreatic proteolytic enzymes are present in their inactive form (zymogens). Studies performed with purified proenzymes of animal origin have shown that, In vitro, trypsinogens can be activated by trypsin via an autocatalytic process or by enteropeptidase.

What Is New

- When duodenal contents of patients with deficiency of intestinal enteropeptidase were used as the source of proteolytic zymogens, no activation of trypsinogens by the addition of trypsin was observed and proteolytic zymogens remained inactive. The addition of purified enteropeptidase of porcine or human origin resulted in a rapid and complete activation of trypsinogens followed by the activation of chymotrypsinogens. The yield of trypsin formed in this process was proportional to the amount of enteropeptidase used for activation.

How Might It Impact on Clinical Practice in the Foreseeable Future?

- The study underlines the key role of enteropeptidase in the cascade process leading to the presence of active proteolytic enzymes in the human intestinal lumen. In the therapy of patients with congenital enteropeptidase deficiency, substitution of the missing proteolytic enzymes by oral gifts of pancreatin in addition to easily absorbable protein hydrolysates containing amino acids and peptides has been successful. The addition of enteropeptidase to duodenal contents of patients and the assessment of trypsin activity before and after the addition of enteropeptidase to the sample are an indispensable tool for a precise diagnosis of enteropeptidase deficiency.

INTRODUCTION

Enteropeptidase (E.C.3.421.9, formerly enterokinase) was discovered at the beginning of the last century by Schepowalnikov (1). The enzyme is located in the brush border membrane of duodenal and upper small intestinal enterocytes (2–11). In the duodenal fluid, it is present in a soluble form, or attached to small fragments of brush border membrane released from intestinal epithelial cells (10,11). This localization of the enzyme is found in many animal species (2,6,8,10,11) and in man (4,5,7,9).

Enteropeptidase is a serine proteinase. It is produced as a single-chain precursor (proenteropeptidase), sorted to the apical membrane of cells (12–14) and activated by duodenase (15) or trypsin (14).

Enteropeptidase is responsible for the activation of trypsinogen to trypsin. The mechanism of this activation process and its kinetics have been studied by Kunitz and his coworkers (16–18),

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McDonald and Kunitz (19), Maroux et al (20), and Baratti et al (21,22) using purified zymogens of animal origin. More recently Varón et al (23) studied the kinetics of these activation cascades. Similar studies were carried out using samples of the normal human duodenal or pancreatic fluid as the source of enzymes or zymogens (24–32). The enteropeptidase catalyzed activation process is highly specific. In this process, an octapeptide with the structure N-Ala-Pro-Phe-Asp-Asp-Asp-Asp -Lys is released from trypsinogen, the trypsinogen activation peptide (33) and, by a conformational change trypsinogen is converted into the active enzyme trypsin. The tetra-aspartate motif in trypsinogen has been shown to be important for the control of the autocatalytic activation of trypsinogen (34) and for the recognition of the activation peptide in trypsinogen by enteropeptidase. Maroux et al (20) have shown that the polyaspartyl sequence of this domain in trypsinogen is an obstacle for the autocatalytic process, as trypsin does not attack model peptides containing this sequence. In recent studies, doubts have been raised as to whether the polyaspartate structure is the only domain necessary for recognition of the trypsinogen activation peptide by enteropeptidase. Other structures interacting with the heavy chain of enteropeptidase might be involved in this recognition process (34). The autocatalytic activation of trypsinogen by trypsin can occur under certain conditions in vitro (31,35). In the normal human duodenal contents, it is unlikely to occur in the absence of enteropeptidase, unless alternative forms of trypsinogen are present caused by gain of function mutations (36).

Duodenal fluid collected from patients with enteropeptidase deficiency that contains zymogens before activation represents an opportunity to study the activation process of proteolytic zymogens under conditions, which are close to the physiological situation in the lumen of the human duodenum.

PATIENTS AND METHODS

Uncontaminated duodenal contents of 2 patients with enteropeptidase deficiency were used as the source of zymogens. The samples were collected from a boy with congenital enteropeptidase deficiency at the age of 10 months (patient 1) and from his older sibling, a girl with enteropeptidase deficiency at her age of 3 years, 11 months (patient 2).

The clinical features of both patients have been described by Haworth et al (37), the underlying mutations in the proenteropeptidase gene by Holzinger et al (38).

Reagents and Enzymes

Bovine crystalline trypsin (lot Nr. 93610), chymotrypsin (lot Nr. 93610), trypsinogen (lot Nr 93630), and chymotrypsinogen (lot Nr. 93610) were purchased from Fluka (Fluka Chemie AG, 9470 Buchs, Switzerland). Purified porcine enteropeptidase was a gift from Opochimie (Monte Carlo, Monaco). Human enteropeptidase was partially purified from human duodenal juice by passing it over a Sephadex G-200 column and collecting the fractions containing enteropeptidase. These fractions were free of trypsin, and the specific activity of these samples was 50 Enteropeptidase Units (EpU) per gram of dry weight after lyophilization. One Enteropeptidase Unit is the amount of enteropeptidase that liberates 1 μg trypsin from trypsinogen per minute at pH 5.6 and 25°C (39). Trypsin and chymotrypsin activity were measured according to Hummel (40) using as the substrates N-alpha-tosyl-L-arginine methylester (TAME) and N-alpha-benzoyl-L-tyrosine ethylester (BTEE), respectively. In the gel filtration experiments, tryptic activity was measured with the chromogenic substrate Nα-Benzoyl-DL-arginine-4-nitroanilide (BAPNA) according to the method of Erlanger et al (41). Tryptic and chymotryptic activity were expressed in microgram of bovine crystalline enzyme after comparison of the activity with standard curves obtained with different concentrations of purified enzymes. TAME, BTEE, and BAPNA were purchased from Sigma-Aldrich (Merck & Cie, 8200 Schaffhausen, Switzerland). Enteropeptidase activity was measured by incubating in a plastic tube 0.1 mL Na-citrate buffer, 50 mM, pH 5.6, and 100 μL trypsinogen solution containing 2 mg bovine trypsinogen per ml of 0.001 N HCl. The volume was adjusted to 0.65 mL with distilled water. The incubation at 25°C was started by adding the trypsinogen solution. Aliquots for trypsin determination were removed after 10, 20, 30, and 40 minutes. In the aliquots further activation of trypsinogen was immediately stopped by adding 200 μL HCl 0.006 N. The 100-μL aliquots were adjusted to 300 μl with distilled water. To this mixture the trypsin substrate with appropriate buffers were added and tryptic activity was measured with the substrate TAME or BAPNA (40,41). For calculation of enteropeptidase activity, trypsin standard curves were obtained by incubating various amounts of purified trypsin with the substrate according to Erlanger et al (41).

RESULTS

For the diagnosis of enteropeptidase deficiency, possible interference of trypsin and enteropeptidase in the activation process of trypsinogen must be avoided. Therefore, duodenal contents of patients and controls were subjected to gel filtration on Sephadex G-200, separating the different enzymes (Fig. 1). In the normal control, 2 peaks are visible, the first one (elution volume 5–6 mL) corresponding to enteropeptidase and the second peak (8–10 mL) corresponding to tryptic activity. In the patient, no activity of enteropeptidase or trypsin was found. After addition of purified porcine enteropeptidase to each sample of elution fluid from the patients' duodenal contents, a peak corresponding to tryptic activity appeared between 8 and 9 mL of elution volume. This result indicated that the sample did contain trypsinogen before activation. This elution profile is diagnostic for enteropeptidase deficiency (42) and was found in both patients participating in this study (37).

Activation of Trypsinogen by Exogenous Trypsin

Figure 2 shows the effect of the addition of bovine crystalline trypsin to duodenal contents of enteropeptidase deficient patient 1. To 4 samples of duodenal juice, 4 different amounts of bovine trypsin were added and trypsin activities were determined at different times after incubation at 25°C. No buffers were added to the samples. The pH was 7.5 and remained unchanged during the incubation. The concentrations of various ions present in the sample of duodenal juice are listed in the legend to Figure 2. In none of the 4 samples, was there an apparent activation of trypsinogen. A considerable part of the added active trypsin was immediately inactivated as is shown in Figure 2. The amount of trypsin which was inactivated was almost the same for the 4 samples. The loss of activity is due to intrinsic trypsin inhibitors. The residual activity of trypsin which escaped inhibition remained constant for 60 minutes except in one sample that received the highest amount of trypsin initially. In this sample a loss of tryptic activity was observed during the first 60 minutes of incubation.

The activation of trypsinogen by enteropeptidase in duodenal fluid of patient 1 was studied in the samples described above by adding after 60 minutes of incubation with trypsin alone, 1 mL of purified human enteropeptidase (1.5 EpU/mL). This resulted in rapid activation of trypsinogen. The final amount of trypsin formed was highest in the 2 samples which initially received only small amounts of trypsin. In the 2 samples with the higher amount of trypsin initially added the yield of trypsin formed from trypsinogen was smaller.

The kinetics of the activation of trypsinogen by enteropeptidase in duodenal fluid of patient 2 is shown in Figure 3. Different amounts of purified porcine enteropeptidase were added to samples of duodenal contents. Two phenomena were observed: first, the initial

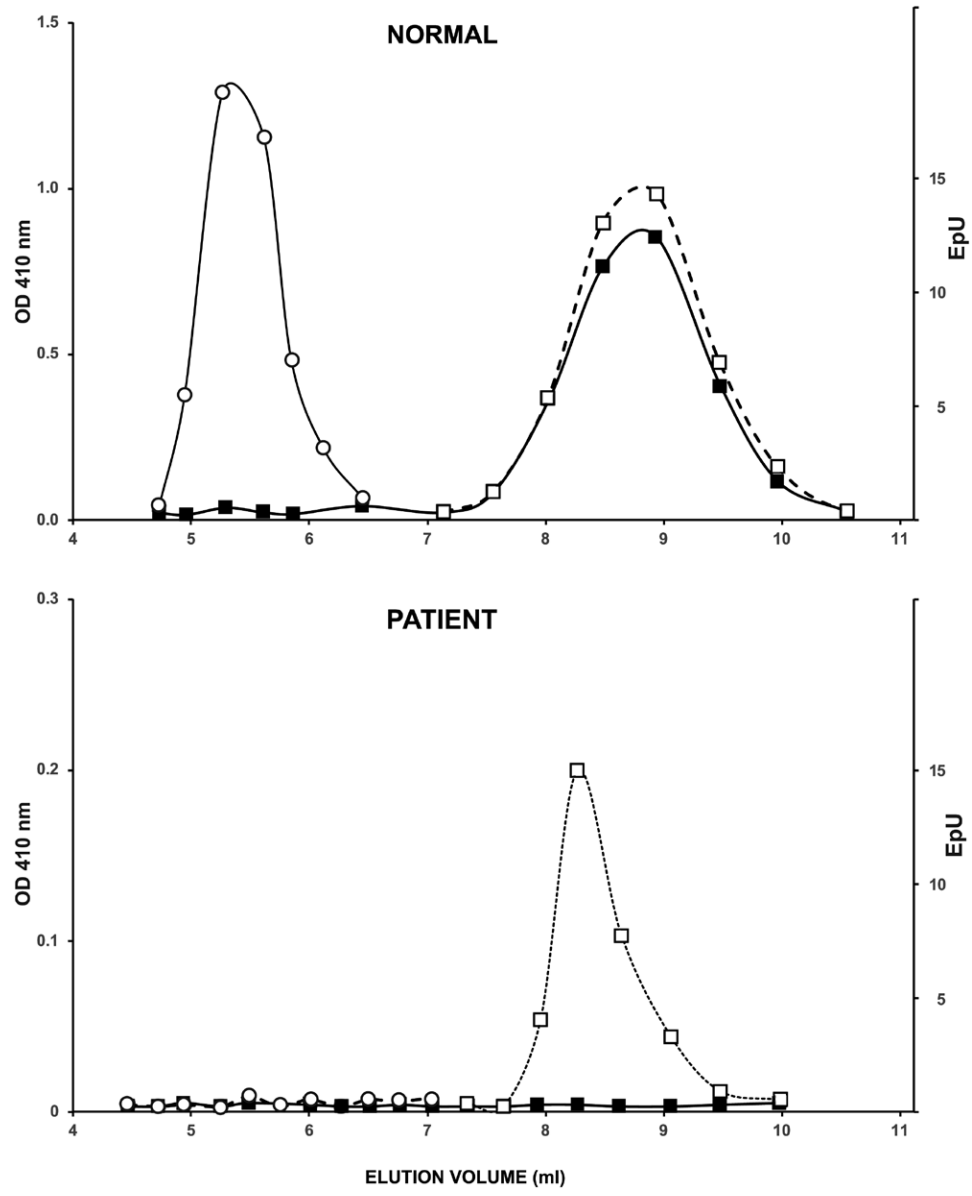

FIGURE 1. Diagnosis of enteropeptidase deficiency. Elution profile and enzymatic activities in samples eluted from a Sephadex G-200 column. Eight hundred µL of duodenal fluid of patient 2 was applied to a 61 × 1.6 cm column and eluted with 50 mM TRIS buffer at pH 7.5. Upper half: duodenal fluid of a patient with normal intestinal and pancreatic function. Lower half: duodenal fluid of patient 2 with enteropeptidase deficiency. ○ enteropeptidase activity, ■ tryptic activity, □ tryptic activity after addition of enteropeptidase. Ordinate: activity of trypsin: optical density at 410 nm (substrate BAPNA) and enteropeptidase activity in EpU/100 µL of elution fluid. Abscissa: elution volume, mL.

slope of the activation curve depended on the amount of enteropeptidase added to the incubation mixture. Second, the final amount of trypsin formed from a constant amount of trypsinogen was proportional to the amount of active enteropeptidase present.

The activation of chymotrypsinogen in duodenal fluid of patient 1 with enteropeptidase deficiency is shown in Figure 4. Chymotryptic activity was measured in the same samples of duodenal fluid that is represented in Figure 2. Activation of chymotrypsin was observed only in those samples where tryptic activity was present, either because it had escaped inhibition (immediate activation) or was formed as a result enteropeptidase addition. The final amount of chymotrypsin formed did not depend on the amount of enteropeptidase or trypsin present in the incubation mixture. In the 2 samples to whom 15 or 50 µg of trypsin were added, a small activity of trypsin persisted.

DISCUSSION

The experiments presented here demonstrate that no activation of trypsinogen occurred when trypsin was added to the duodenal fluid of patients with enteropeptidase deficiency. Active trypsin was formed only after the addition of enteropeptidase to the samples of duodenal fluid. Autoactivation of trypsinogen had not occurred because the samples collected from patients with enteropeptidase

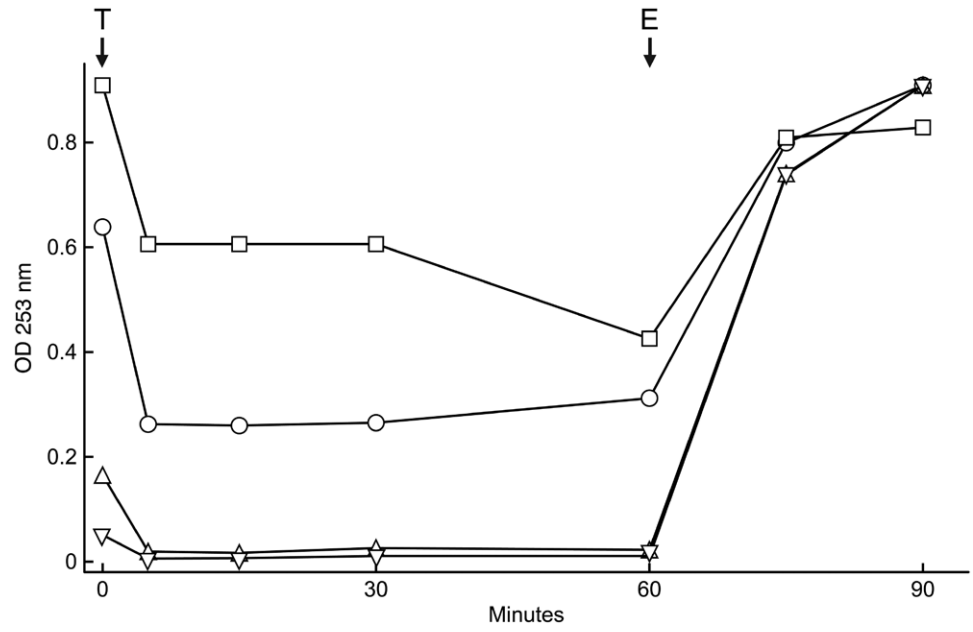

FIGURE 2. Effect of trypsin and enteropeptidase on activation of trypsinogen in duodenal contents of patient 1 with enteropeptidase deficiency. To 4 samples of duodenal fluid, different amounts of bovine trypsin were added and tryptic activities were determined by the method of Hummel (40) during incubation at 25°C. Amount of trypsin added (T): □ 225 µg, ○ 125 µg, △ 50 µg, ▽ 15 µg; no buffers were added; pH and ionic environment in the samples were pH 7.5 (unchanged during the experiment), Na^+ 112 mM, K^+ 6.7 mM, Cl^- 43 mM, Ca^{++} 2.0 mM (ionic strength I = 0.109). After 60 minutes, 1 mL of human purified enteropeptidase (1.5 EpU/mL) was added (E) and tryptic activity was measured in the 4 samples. Ordinate: trypsin activity measured as optical density at 253 nm with substrate: N-alpha-tosyl-L-arginine methylester (TAME). Abscissa: incubation time in minutes.

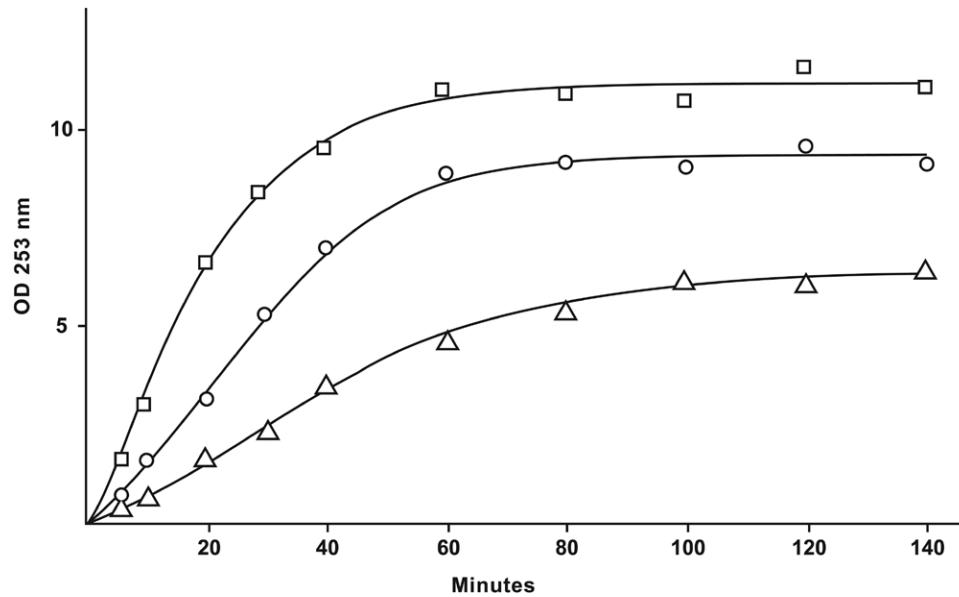

FIGURE 3. Velocity and yield of trypsinogen activation by various amounts of enteropeptidase. To 200 µL of duodenal fluid collected from patient 2 with enteropeptidase deficiency, various amounts of purified porcine enteropeptidase were added. Aliquots of the incubation mixture were harvested at different time intervals and tryptic activity was determined with the method of Hummel (40) using TAME as the substrate. Tryptic activity is expressed as µg/ml purified bovine trypsin. o 1.05 EpU, ○ 0.5 EpU, Δ 0.25 EpU. Ordinate: trypsin concentration measured as optical density at 253nm with substrate N-alpha-tosyl-L-arginine methylester (TAME). Abscissa: incubation time in minutes.

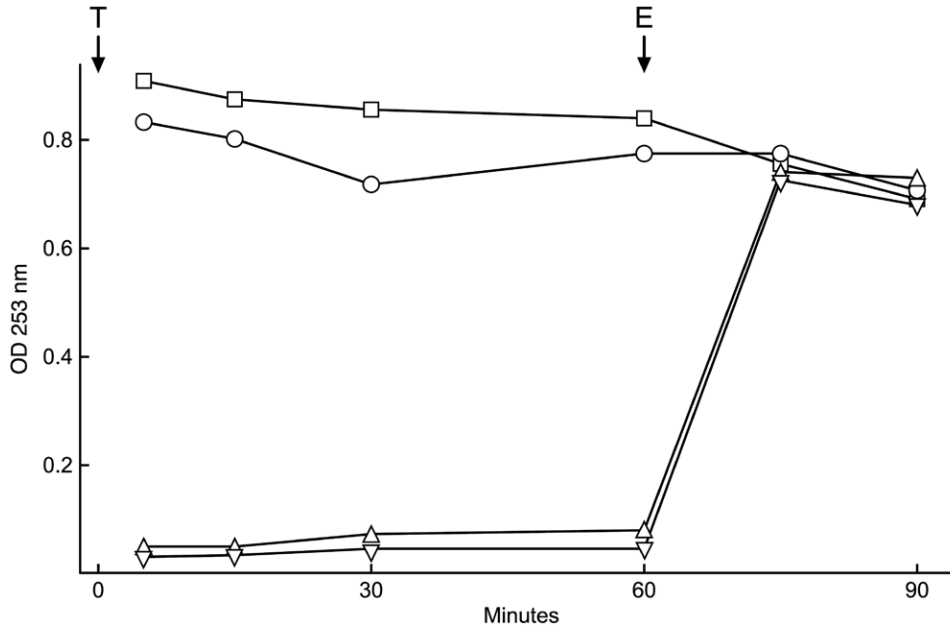

FIGURE 4. Activation of chymotrypsinogen by trypsin and by enteropeptidase. Duodenal juice of patient 1 with enteropeptidase deficiency. The activity of chymotrypsin [method of Hummel (40)] was determined in the same samples of duodenal contents as in Figure 2. Amount of trypsin added (T): □ 225 µg, ○ 125 µg, △ 50 µg, ▽ 15 µg. Addition (E) of 1 mL purified human enteropeptidase (1.5 EpU/mL). Ordinate: chymotrypsin concentration measured as optical density at 253 nm with substrate N-alpha-benzoyl-L-tyrosine ethylester (BTEE). Abscissa: incubation time in minutes.

deficiency lacked tryptic activity before the activation by exogenous enteropeptidase. These observations are in agreement with the in vitro findings of Maroux et al (20) obtained with pure bovine trypsinogen. They are also in accordance with earlier studies by Kunitz who used crystalline trypsinogen from beef pancreas and enterokinase isolated and concentrated from porcine duodenal contents (16–19). These classical investigations of Kunitz have shown that the catalytic action of enteropeptidase on trypsinogen is complicated by the partial transformation of trypsinogen into inert protein by trypsin itself. This process which reduces the yield of trypsin formed from trypsinogen is minimized in the presence of large amounts of enteropeptidase. Therefore, enteropeptidase is indispensable for a complete activation of trypsinogen. Inert protein formation may have occurred in our experiment shown in Figure 2 in the samples to which a higher dose of trypsin was added initially. In the activation of chymotrypsinogen (Fig. 4), the yield of chymotrypsin formed did not depend on the amount of trypsin present in the incubation mixture, when the yield of chymotrypsin formed in the samples receiving 225 and 125 µg trypsin were compared. This is in agreement with the findings of Kerr et al (43) who studied the mechanism of chymotrypsinogen activation by trypsin and found that inert protein formation was not complicating this process. In the samples to which 50 and 15 µg trypsin were added, a small amount of trypsin activity was detectable during the first 60 minutes, but no activation of chymotrypsin was observed.

The data presented here demonstrate that enteropeptidase plays the key role for the cascade of activation processes which lead to active proteolytic enzymes in the human intestine. The data also show that patients with enteropeptidase deficiency are unable to activate trypsinogen by an alternative pathway. In the absence of enteropeptidase activity proteolytic pancreatic enzymes remain inactive, and the consequence is a severe disturbance of protein digestion leading to hypoproteinemia, anemia and failure to thrive at young age.

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REFERENCES

1. Schepowalnikov NP. P of the intestinal juice (summary of thesis, translated from Russian). In: Maly R, von Bergmann VJF, eds. *Jahresbericht über die Fortschritte der Thier-Chemie*. Vol. 29. 1900:378–380.
2. Lobley RW, Holmes R. The localization of enterokinase to the brush border membrane of the guinea pig small intestine. *Gut*. 1970;11:1059.
3. Nordström C, Dahlqvist A. The cellular localization of enterokinase. *Biochim Biophys Acta*. 1970;198:621–622.
4. Eggermont E, Molla AM, Rutgeerts L, et al. The source of human enterokinase. *Lancet*. 1971;2:369.
5. Eggermont E, Molla AM, Tytgat G, et al. Distribution of enterokinase activity in the human intestine. *Acta Gastroenterol Belg*. 1971;34:655–662.
6. Nordström C, Dahlqvist A. Rat enterokinase: the effect of ions and the localization in the intestine. *Biochim Biophys Acta*. 1971;242:209–225.
7. Hermon-Taylor J, Perrin J, Grant DA, et al. Immunofluorescent localisation of enterokinase in human small intestine. *Gut*. 1977;18:259–265.
8. Louvard D, Maroux S, Baratti J, et al. On the distribution of enterokinase in porcine intestine and on its subcellular localization. *Biochim Biophys Acta*. 1973;309:127–137.
9. Schmitz J, Preiser H, Maestracci D, et al. Subcellular localization of enterokinase in human small intestine. *Biochim Biophys Acta*. 1974;343:435–439.
10. Götze H, Adelson JW, Hadorn HB, et al. Hormone-elicited enzyme release by the small intestinal wall. *Gut*. 1972;13:471–476.
11. Fonseca P, Light A. The purification and characterization of bovine enterokinase from membrane fragments in the duodenal mucosal fluid. *J Biol Chem*. 1983;258:14516–14520.
12. Kitamoto Y, Yuan X, Wu Q, et al. Enterokinase, the initiator of intestinal digestion, is a mosaic protease composed of a distinctive assortment of domains. *Proc Natl Acad Sci U S A*. 1994;91:7588–7592.

13. Zheng X, Sadler JE. Mucin-like domain of enteropeptidase directs apical targeting in Madin-Darby canine kidney cells. *J Biol Chem*. 2002;277:6858–6863.
14. Lu D, Yuan X, Zheng X, et al. Bovine proenteropeptidase is activated by trypsin, and the specificity of enteropeptidase depends on the heavy chain. *J Biol Chem*. 1997;272:31293–31300.
15. Zamolodchikova TS, Sokolova EA, Lu D, et al. Activation of recombinant proenteropeptidase by duodenase. *FEBS Lett.* 2000;466:295–299.
16. Kunitz M, Northrop JH. Isolation from beef pancreas of crystalline trypsinogen, trypsin, a trypsin inhibitor, and an inhibitor-trypsin compound. *J Gen Physiol*. 1936;19:991–1007.
17. Kunitz M. Purification and concentration of enterokinase. *J Gen Physiol*. 1939;22:447–450.
18. Kunitz M. Formation of trypsin from crystalline trypsinogen by means of enterokinase. *J Gen Physiol*. 1939;22:429–446.
19. McDonald MR, Kunitz M. The effect of calcium and other ions on the autocatalytic formation of trypsin from trypsinogen. *J Gen Physiol*. 1941;25:53–73.
20. Maroux S, Baratti J, Desnuelle P. Purification and specificity of porcine enterokinase. *J Biol Chem*. 1971;246:5031–5039.
21. Baratti J, Maroux S, Louvard D. 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*. 1973;321:632–638.
22. Baratti J, Maroux S. On the catalytic and binding sites of porcine enteropeptidase. *Biochim Biophys Acta*. 1976;452:488–496.
23. Varón R, Havsteen BH, Vázquez A, et al. Kinetics of the trypsinogen activation by enterokinase and trypsin. *J Theor Biol*. 1990;145:123–131.
24. Hadorn B. *Studies on human enterokinase* [PhD Thesis]. Australia: University of Melbourne; 1970.
25. Hadorn B, Sumida C, Tarlow MJ, et al. The activation of human pancreatic proteinases. Studies on human pancreatic juice and duodenal juice of four children with intestinal enterokinase deficiency. *Acta Paediatr Scand*. 1971;60:369.
26. Moss S, Lobley RW, Holmes R. Enterokinase in human duodenal juice following secretin and pancreozymin and its relationship to bile salts and trypsin. *Gut*. 1972;13:851.
27. Robinson LA, Kim WJ, White TT, et al. Trypsin in human pancreatic juice–their distributions as found in 34 specimens. Two human pancreatic trypsinogens. *Scand J Gastroenterol*. 1972;7:43–45.
28. Hadorn B. Pancreatic proteinases; their activation and the disturbances of this mechanism in man. *Med Clin North Am*. 1974;58:1319–1331.
29. Colomb E, Figarella C. Comparative studies on the mechanism of activation of the two human trypsinogens. *Biochim Biophys Acta*. 1979;571:343–351.
30. Roduner J. *Activation of human pancreatic proteolytic enzymes, the role of enterokinase and trypsin* [MD Thesis]. Medical Faculty, University of Berne; 1979.
31. Sarkany RP, Moreland BH. Enhancement of the autocatalytic activation of trypsinogen to trypsin by bile and bile acids. *Biochim Biophys Acta*. 1985;839:262–267.
32. Kukor Z, Toth M, Pal G, et al. Human cationic trypsinogen. Arg(117) is the reactive site of an inhibitory surface loop that controls spontaneous zymogen activation. *J Biol Chem*. 2002;277:6111–6117.
33. Guy O, Bartelt DC, Amic J, et al. Activation peptide of human trypsinogen 2. *FEBS Lett*. 1976;62:150–153.
34. Nemoda Z, Sahin-Tóth M. The tetra-aspartate motif in the activation peptide of human cationic trypsinogen is essential for autoactivation control but not for enteropeptidase recognition. *J Biol Chem*. 2005;280:29645–29652.
35. Kay J, Kassell B. The autoactivation of trypsinogen. *J Biol Chem*. 1971;246:6661–6665.
36. Sahin-Tóth M, Tóth M. Gain-of-function mutations associated with hereditary pancreatitis enhance autoactivation of human cationic trypsinogen. *Biochem Biophys Res Commun*. 2000;278:286–289.
37. Haworth JC, Hadorn B, Gourley B, et al. Intestinal enterokinase deficiency. Occurrence in two sibs and age dependency of clinical expression. *Arch Dis Child*. 1975;50:277–282.
38. Holzinger A, Maier EM, Bück C, et al. Mutations in the proenteropeptidase gene are the molecular cause of congenital enteropeptidase deficiency. *Am J Hum Genet*. 2002;70:20–25.
39. Green JR, Bender SW, Posselt HG, et al. Primary intestinal enteropeptidase deficiency. *J Pediatr Gastroenterol Nutr*. 1984;3:630–633.
40. Hummel BC. A modified spectrophotometric determination of chymotrypsin, trypsin, and thrombin. *Can J Biochem Physiol*. 1959;37:1393–1399.
41. Erlanger BF, Kokowsky N, Cohen W. The preparation and properties of two new chromogenic substrates of trypsin. *Arch Biochem Biophys*. 1961;95:271–278.
42. Hadorn B, Tarlow MJ, Lloyd JK, et al. Intestinal enterokinase deficiency. *Lancet*. 1969;1:812–813.
43. Kerr MA, Walsh KA, Neurath H. A proposal for the mechanism of chymotrypsinogen activation. *Biochemistry*. 1976;15:5566–5570.