Structural Basis for the Specific Activation of Human Cationic Trypsinogen by Human Enteropeptidase*

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Human cationic trypsinogen is activated by human enteropeptidase much more readily than bovine trypsinogen, the ratios $k_{cat}/K_m$ being 330 and 11 mm$^{-1}$ s$^{-1}$, respectively. Conversely, porcine enteropeptidase activates bovine trypsinogen much more readily than bovine trypsinogen ($k_{cat}/K_m = 2.4$ mM$^{-1}$ s$^{-1}$). The primary structure of the activation region of human cationic trypsinogen has been investigated in an attempt to elucidate the basis for these findings. The sequence of the first 12 residues at the NH$_2$-terminus of human cationic trypsinogen has been shown to be Asp-Lys-Ile-Val-Gly-Gly-Val-Tyr-Asn-Cys-Glu-Glu. Furthermore, the activation peptide derived from human cationic trypsinogen has been isolated and shown to be the dipeptide Asp-Lys. This result is in contrast to the Val-(Asp)$_2$-Lys activation peptide from bovine trypsinogen and demonstrates that human cationic trypsinogen does not contain the (Asp)$_2$ sequence present in many other mammalian trypsinogens. It is proposed that the high degree of specificity for activation of human cationic trypsinogen by human enteropeptidase is due to the preferential recognition of the novel activation peptide sequence in the human zymogen. Thus, these two functionally related proteins, cationic trypsinogen and enteropeptidase, may have evolved in a parallel manner in the human lineage.

PANCREATIC TRYPSIN PLAYS A KEY ROLE IN THE PROCESS OF PROTEIN DIGESTION, SINCE IT ACTIVATES TRYPSINOGEN AS WELL AS SEVERAL OTHER ZYMOMGENS PRESENT IN PANCREATIC SECRETION, INCLUDING CHYMOTRYPSINOGEN, PROCARBOXYPEPTIDASES A AND B, AND PROLASTASE (1). ACTIVATION OF THE PANCREATIC ZYMOMGENS IS INITIATED BY THE ACTIVATION OF TRYPSINOGEN BY Tryptase ENZYMATICALLY (2). ACTIVATION OF BOVINE TRYPSINOGEN IS ACCOMPLISHED BY CLEAVAGE OF AN NH$_2$-TERMINAL HEXAPEPTIDE WITH THE UNUSUAL SEQUENCE Val-Lys-Tyr-Val-Gly-Glu. TRYPSIN CLEAVAGE IS CATALYZED BY TRYPSIN (4), THE RATE CONSTANT FOR ACTIVATION OF BOVINE TRYPSINOGEN BY PORCINE ENZYMATICALLY IS APPROXIMATELY 3 ORDERS OF MAGNITUDE GREATER THAN THAT FOR AUTOACTIVATION (5, 6). MAROUX ET AL. (6) HAVE SUGGESTED THAT THIS UNIQUE SPECIFICITY IS DUE TO THE INTERACTION OF PORCINE ENZYMATICALLY WITH THE (ASP)$_2$ SEQUENCE IN THE ACTIVATION PEPTIDE. BUT, ALTHOUGH ENZYMATICALLY IS A SERINE PROTEASE WITH APPARENT SPECIFICITY TOWARD ARGinine OR LYSINE RESIDUES WITH RESPECT TO HYDROLYSIS OF ESTER OR AMIDE SUBSTRATES (6, 7), TRYPSINOGEN APPEARS TO BE THE ONLY KNOWN PROTEIN SUBSTRATE FOR THIS ENZYME (6).

AMINO ACID SEQUENCES HAVE BEEN DETERMINED FOR THE ACTIVATION PEPTIDES DERIVED FROM SEVERAL OTHER TRYPSINOGENS (6-12). IN EACH CASE, THE (ASP)$_2$ SEQUENCE IS PRESENT. THE ONLY KNOWN EXCEPTIONS ARE THE ACTIVATION PEPTIDES FROM AFRICAN LUNGFISH TRYPSINOGENS A AND B (13). IT HAS BEEN SUGGESTED THAT THIS SERIES OF ACIDIC RESIDUES CONSTITUTES A SPECIFIC RECOGNITION SITE FOR ACTIVATION OF TRYPSINOGEN BY ENZYMATICALLY (6), AND THAT THE SEQUENCE HAS, THEREFORE, BEEN HIGHLY CONSERVED DURING EVOLUTION (13).


MATERIALS AND METHODS

HUMAN CATIONIC TRYPSINOGEN WAS PREPARED AS DESCRIBED IN THE ACCOMPANYING REPORT (14). CROFIBER ENZYMATICALLY WAS OBTAINED AS A LYPHOLYZED POWDER FROM MILES LABORATORIES. BOVINE TRYPSINOGEN WAS PURCHASED FROM SIGMA. CONAUM всей А-СЕФАХИЛ и DEAE-Sephadex A-50 were supplied by Pharmacia. Ultragel ACA-22 was obtained from LKB. DEAE-cellulose (Whatman, DE52 microgranular) was obtained from Reeson Angel. L-Aspartyl-L-lysine was purchased from Bachem.

Preparation of Porcine Enteropeptidase—Porcine enteropeptidase was purified by a procedure based on that of Liopinio and Light (16). Two grams of crude enteropeptidase powder were suspended in

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Activation of Human Cationic Trypsinogen

20 ml of 10 mM Mes\(^{-}\) buffer (pH 6.0) containing 20 mM NaCl, stirred for 2 h, clarified by centrifugation, dialyzed versus two changes of the same buffer, and applied to a column (4 x 20 cm) of DEAE-
 cellulose in 10 mM Tris.HCl (pH 7.6) equilibrated with 200 ml of 10 mM sodium acetate (pH 5.5). Column fractions containing enteropeptidase activity were pooled, concentrated by ultrafiltration, and reap-
equilibration buffer, the column was eluted with a linear gradient of
0.05 to 0.5 M NaCl in the same buffer. Enteropeptidase activity, which was eluted in the first quarter of the salt gradient, was pooled, concentrated by ultrafiltration, and applied to a column (2 x 90 cm) of Ultrogel CA-22 (an agarose-polyacrylamide gel filtration resin) equilibrated in 10 mM sodium acetate (pH 5.5). Column fractions containing enteropeptidase activity were pooled, concentrated by ultrafiltration, and reap-
equilibrated to the same solution. The resulting supernatants were combined and
stored at 4\(^\circ\)C. Activation of Human Cationic Trypsinogen

The final preparation contained no detectable aminopeptidase activity, as described by Barns and
Elmslie (17). The pass-through fractions containing enteropeptidase activity were pooled, concentrated by ultrafiltration, and stored at 4\(^\circ\)C. The preparation was not homogeneous by the criterion of discontin-
uous sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis (19) followed by autoradiography according to the method of Baratti et al. (18). The material was extracted by homogenization in 1% (w/v) sodium deoxycholate (pH 7.5) and centrifuged at 10,000 g for 30 min. Bovine trypsinogen was separated from enteropeptidase by chromatography on a column (2 x 20 cm) of DEAE-
Sephadex A-50 equilibrated with 10 mM Tris.HCl (pH 8.0) contain-
ing 50 mM NaCl and eluted with a linear gradient of 0.02 to 0.5 M NaCl in the same buffer. Enteropeptidase activity eluted as a single peak early in the salt gradient.

The material from DEAE-cellulose chromatography was pooled, concentrated by ultrafiltration, and applied to an Ultrogel CA-22 column (4 x 20 cm) equilibrated with 10 mM Tris.HCl containing 50 mM NaCl. Enteropeptidase activity was eluted from this column as described above for porcine enteropeptidase. Fractions containing enteropeptidase activity (partially separated from ami-
nopeptidase activity) were pooled and passed through a column of concanavalin A-Sepharose as described above. While essentially all of the enteropeptidase activity passed through the column, approxi-
ately 40% of the aminopeptidase activity was retained. The re-
maind aminopeptidase activity was separated from enteropeptidase activity by chromatography on a column (2 x 20 cm) of DEAE-
Sephadex A-50 equilibrated with 10 mM Tris.HCl (pH 8.0) contain-
ing 50 mM NaCl and eluted with a linear gradient of 0.02 to 0.5 M NaCl in the same buffer. Enteropeptidase activity eluted as a single peak early in the salt gradient.

An overall 300-fold increase in specific activity was obtained with respect to the initial extract.

Preparation of Human Enteropeptidase - Scrapings of duodenal mucosa were homogenized in 0.1 M sodium succinate (pH 6.0) containing 50 mM NaCl and 0.2 mg/ml of concanavalin A and then passed through a column (2 x 20 cm) of DEAE-
Sephadex A-50 equilibrated with 10 mM Tris.HCl containing 50 mM NaCl. Fractions containing only enteropeptidase activity were pooled, concentrated by Diflo ultrafiltration, and stored at 4\(^\circ\)C. The preparation was not homogeneous by the criterion of discontin-
uous sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis (19) followed by autoradiography according to the method of Bonner and Laskey (20). A single band of radioac-
tivity was observed for each enzyme, indicating that both prepara-
tions under the assay conditions employed (14).

Active Site Titration of Enteropeptidase Preparations - The active site titration of enteropeptidase was performed according to the method of Crestfield et al. (21) at pH 5.6 in 28 mM sodium succinate containing 1 mM CaCl, for 4 h at 150\(^\circ\)C in order to recover
TLCK-trypsin, the resulting sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis (19) followed by autoradiography according to the method of Bonner and Laskey (20). A single band of radioac-
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tions under the assay conditions employed (14).

An overall 300-fold increase in specific activity was obtained with respect to the initial extract.

Preparation of Human Enteropeptidase - Scrapings of duodenal mucosa were homogenized in 0.1 M sodium succinate (pH 6.0) containing 50 mM NaCl. The dialysate was clarified by centrifugation and loaded onto a column (4 x 18 cm) of DEAE-cellulose equilibrated with the same buffer. After washing nonadsorbed material through the column, proteins were eluted with a linear gradient of 0.02 to 0.5 M NaCl in the same buffer. Enteropeptidase activity eluted as a single peak early in the salt gradient.

The preparation was not homogeneous by the criterion of discontin-
uous sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis (19) followed by autoradiography according to the method of Bonner and Laskey (20). A single band of radioac-
tivity was obtained with respect to the specific activity of the initial extract.

Aliquots of \([\text{H}]\text{DIPF-human and porcine enteropeptidases were subject}

2 The abbreviations used are: Mes, 2-(N-morpholino)ethanesulfonic acid, TosArgOMe, diisopropylfluorophosphate; NPGB, \(p\)-nitrophenyl-\(p\)-guani-
dobenzoate HCl; TLCK, \(N\)-tosyl-L-lysine chloromethyl ketone; \(D\)-tocr, trypsin reacted with TLCK; PTH, phenylthio-
lycysteine; Dn or dansyl, 5-dimethylaminonaphthalene-1-sulfonyl. The final preparation contained no detectable aminopeptidase activity, as described by Barns and
Elmslie (17). The pass-through fractions containing enteropeptidase activity were pooled, concentrated by ultrafiltration, and stored at 4\(^\circ\)C. The preparation was not homogeneous by the criterion of discontin-
uous sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis (19) followed by autoradiography according to the method of Baratti et al. (18). The material was extracted by homogenization in 1% (w/v) sodium deoxycholate (pH 7.5) and centrifuged at 10,000 g for 30 min. Bovine trypsinogen was separated from enteropeptidase by chromatography on a column (2 x 20 cm) of DEAE-
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The material from DEAE-cellulose chromatography was pooled, concentrated by ultrafiltration, and applied to an Ultrogel CA-22 column (4 x 20 cm) equilibrated with 10 mM Tris.HCl containing 50 mM NaCl. Enteropeptidase activity was eluted from this column as described above for porcine enteropeptidase. Fractions containing enteropeptidase activity (partially separated from ami-
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tivity was observed for each enzyme, indicating that both prepara-
tions under the assay conditions employed (14).

Kinetic Measurements - In order to determine the parameters \(K_m\) and \(V_{max}\) for trypsinogen activation by human and porcine enteropeptid-
ases, the assay conditions of Baratti et al. (21) were employed.

Varying concentrations of human cationic trypsinogen or bovine trypsinogen were incubated at 25\(^\circ\)C in a total volume of 0.5 ml in polypropylene tubes containing 28 mM sodium succinate (pH 5.60), 1.0 or 10 mM CaCl\(_2\), and enteropeptidase as indicated. Incubation was terminated after 15 min for bovine trypsinogen, or 10 min for human cationic trypsinogen, by addition of 10 \(\mu\)l of 2 N HCl. The amount of trypsin formed was then determined spectrophotometrically
with TosArgOMe (22).

In all kinetic measurements, duplicate determinations were made at treated with TLCK by the procedure of Shaw (32) in order to
minimize autodigestion. The resulting TLCK-trypsin was then re-
duced and alkylated with iodoacetic acid as described above.

The PTH derivatives derived from each seconquenator cycle were identified by at least two of the following methods: high pressure
liquid chromatography, thin layer chromatography, amino acid analysis, and thin layer chromatography. High pressure liquid chromatography was performed on a Waters Association liquid chromatography using a micro Bondapak C18 column (33). Gas-liquid chromatography was performed on a Varian 1500 A chromatograph (34). Prior to amino acid analysis, PTH derivatives were hydrolyzed in 6 N HCl containing 0.1% SnCl\(_2\) for 4 h at 150\(^\circ\)C in order to recover the free amino acids as described by Mendez and Lai (35). Thin layer chromatography was performed following the procedure described by Nummers et al. (36). Cysteine was identified as PTH-cysteine by high pressure liquid chromatography and as aspartic acid by amino acid analysis
Activation of Human Cationic Trypsinogen

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Isolation of Activation Peptide—A sample of human cationic trypsinogen (4.5 mg in 5 ml) was allowed to autoactivate in 50 mM N-ethylmorpholine acetate (pH 6.0), 1 mM CaCl₂, until apparent full trypsinogen obtained during the course of this work were mixture was then adjusted to pH 3.5 and applied to a column (2 cm) of Sephadex G-25. Aliquots of fractions obtained were monitored for peptide material with fluorescamine (37). A single, somewhat skewed peak of fluorescamine-positive material was eluted near the included volume of the column. Fractions containing peptide material were pooled and lyophilized.

RESULTS

NH₂-terminal Amino Acid Residue of Human Cationic Trypsinogen—Three separate preparations of human cationic trypsinogen obtained during the course of this work were subjected to NH₂-terminal amino acid determination as described under "Materials and Methods." In each case, a single major Dns-Asp residue was identified after acid hydrolysis, indicating that the NH₂-terminus of human cationic trypsinogen is aspartic acid or asparagine.

Activation Peptide of Human Cationic Trypsinogen — The peptide fraction isolated from activated human trypsinogen was found to contain a single major component, detected with fluorescamine (38), after paper electrophoresis in pyridine acetate buffers at pH 3.5 and pH 6.5. The electrophoretic mobility at pH 6.5 was consistent with that of a neutral peptide. Amino acid analysis of a sample of the peptide fraction after hydrolysis in 6 N HCl for 24 h at 110⁰ yielded aspartic acid and lysine in a molar ratio of 1:1.3, with minor contamination from several other amino acids. NH₂-terminal analysis indicated a single major spot of Dns-Asp after thin layer chromatography. Amino acid analysis prior to acid hydrolysis yielded a single component which was eluted in the second buffer, between the elution times of isoleucine and leucine. A sample of commercially obtained L-aspartyl-n-ly-sine was eluted at the same position. From these results, it was concluded that the single major peptide isolated from autoactivated human cationic trypsinogen has the sequence Asp-Lys.

A separate sample of human cationic trypsinogen was activated with 0.1% by weight of human enteropeptidase for 10 min at 25⁰ in 20 mM sodium succinate (pH 5.2) containing 20 mM CaCl₂. Autoactivation by trypsin produced during the incubation was minimized, but not prevented, under these conditions. A single major peptide was isolated from Sephadex G-25 gel filtration of this incubation mixture. This activation peptide had properties identical with those described above.

NH₂-terminal Sequences of Human Cationic Trypsinogen and Trypsin—The results obtained from sequenator analyses of samples of human cationic trypsinogen, and of cationic trypsin prepared by activation of trypsinogen by enteropeptidase, are presented in Fig. 1. Unambiguous identification of PTH-derivatives released during sequential Edman degradation was obtained up to residue 19 in cationic trypsinogen and residue 10 in cationic trypsin. The results clearly show that the NH₂-terminal Asp-Lys constitutes the activation peptide of human cationic trypsinogen, confirming the results obtained by isolation of peptide material from autoactivated cationic trypsinogen. The first 16 residues in the sequence of bovine trypsinogen (39) are presented for comparison. Three differences in sequence were observed, in addition to the change in the activation peptide, at positions 6, 8, and 9 in the numbering system shown in Fig. 1.

Kinetic Parameters for Activation of Human and Bovine Trypsinogens by Human and Porcine Enteropeptidases—Initial attempts at demonstrating activation of human cationic trypsinogen by a partially purified preparation of porcine enteropeptidase suggested that bovine trypsinogen was activated much more readily than human cationic trypsinogen under the same conditions. Other experiments indicated that a preparation of human enteropeptidase, obtained by extraction of duodenal scrapings, activated human cationic trypsinogen much more rapidly than bovine trypsinogen.

The initial observations were confirmed with preparations of human and porcine enteropeptidase purified free of interfering enzyme activities (trypsin, aminopeptidase) following published procedures, as described under "Materials and Methods." Although both human and porcine enteropeptidases were highly purified when compared with the initial extracts, neither preparation was considered to be homogeneous. Thus, active site concentrations determined by titration with [111]DTPF were employed in order to estimate kcat values. Kinetic parameters were determined using the conditions described by Baratti et al. (21) except that the CaCl₂ concentration was increased to 10 mM in order to limit autoactivation of human trypsinogen, which was shown to occur readily at pH 5.6 in 1 mM CaCl₂ (14). In all instances, the rate of activation was linear for the times of incubation indicated (see "Materials and Methods"). The results obtained in two of the kinetic measurements, plotted according to Lineweaver and Burk (25), are shown in Fig. 2.

Table I shows the kinetic parameters obtained for activation of bovine and human cationic trypsinogens by porcine or human enteropeptidases. Anderson et al. (40) have recently demonstrated that the kinetic parameters for hydrolysis of bovine trypsinogen by bovine enteropeptidase are similar to those reported by Maroux et al. (6) and by Baratti et al. (21) for activation of the same zymogen by porcine enteropeptidase. Thus, the term homologous will be applied to the combination of bovine trypsinogen and porcine enteropeptidase as well as to the corresponding human proteins. The values of Kₘ and kcat shown for the homologous combinations were derived from statistical analysis of kinetic data obtained at two levels of enteropeptidase as described under "Materials and Methods." For the heterologous combinations, neither Kₘ nor kcat could be determined.
bovine trypsinogen by porcine and human enteropeptidases. The line for human enteropeptidase was drawn graphically from the data points shown (A—A). The line for porcine enteropeptidase (×—×) was derived from the slope and intercept estimated by computer analysis as described under "Materials and Methods."

**Figure 2.** Lineweaver-Burk double reciprocal plots for activation of bovine trypsinogen by porcine and human enteropeptidases. The line for human enteropeptidase was drawn graphically from the data points shown (A—A). The line for porcine enteropeptidase (×—×) was derived from the slope and intercept estimated by computer analysis as described under "Materials and Methods."

**Table I**

<table>
<thead>
<tr>
<th>Enteropeptidase</th>
<th>Trypsinogen substrate</th>
<th>$K_m$ (mM)</th>
<th>$k_{cat}$ (s$^{-1}$)</th>
<th>$k_{cat}/K_m$ (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>Human</td>
<td>7.2 x 10$^{-3}$</td>
<td>2.3</td>
<td>330</td>
</tr>
<tr>
<td>Human</td>
<td>Bovine</td>
<td>4.5 x 10$^{-3}$</td>
<td>2.8</td>
<td>630</td>
</tr>
<tr>
<td>Porcine</td>
<td>Bovine</td>
<td>2.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Porcine</td>
<td>Human</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

be estimated with any degree of confidence. In each case, the ratio $k_{cat}/K_m$ was estimated graphically from the slope of a plot of $v$ versus $[S]$ at the lowest values of substrate determined.

The results presented in Table I clearly show that much more favorable kinetic parameters were obtained with each homologous combination of enteropeptidase and trypsinogen. In particular, the ratio $k_{cat}/K_m$ obtained with human enteropeptidase and human trypsinogen was 30-fold greater than that observed for activation of bovine trypsinogen by the same enzyme. Porcine enteropeptidase preferred bovine trypsinogen to an even greater degree; thus, the ratio $k_{cat}/K_m$ was reduced by a factor of 980 when human cationic trypsinogen was employed as substrate. However, from the data available we could not determine whether both $K_m$ and $k_{cat}$ were reduced. More accurate kinetic studies with heterologous combinations were precluded by limitations on the maximum trypsinogen concentration that could be employed without interference from autoactivation.

**Discussion**

The activation peptide of human cationic trypsinogen has been shown to be the dipeptide Asp-Lys by two separate approaches. First, the activation peptide was isolated following autoactivation or enteropeptidase-catalyzed activation of the zymogen and was shown to be Asp-Lys. No other peptides were observed after Sephadex G-25 gel filtration of the activation mixtures followed by high voltage paper electrophoresis of the peptide material. In addition, the NH$_2$-terminal sequence of human cationic trypsinogen was shown to be identical with that of human cationic trypsin except for the addition of the Asp-Lys dipeptide.

The Asp-Lys sequence of the activation peptide of human cationic trypsinogen is quite different from those reported for several other mammalian trypsinogens, all of which contain the sequence (Asp)$_n$-Lys. While no evidence of heterogeneity in the NH$_2$-terminus of cationic trypsinogen has been observed, it is possible that chemical hydrolysis or partial cleavage by acid proteases of a longer activation peptide occurred during isolation of the zymogen. Bovine trypsinogen was employed in order to assess the stability of the activation peptide sequence to the purification steps carried out at low pH (14) as follows. A sample of bovine trypsinogen was incubated at pH 2.6 for 24 h at 4°C with 5% by weight of a dialyzed extract of acetone powder of human pancreas (concentrated by (NH$_4$)$_2$SO$_4$ precipitation). A second sample of bovine trypsinogen was incubated in pH 2.6 buffer alone for 24 h at 4°C. In both cases, a single NH$_2$-terminal valine was found, as was the case with an unincubated control sample of bovine trypsinogen. This result demonstrated that the (Asp)$_n$ sequence of the bovine zymogen is not hydrolyzed under these conditions. Although this does not exclude the possibility that a putative human cationic trypsinogen with an extended NH$_2$-terminus would be degraded under the same conditions, it is consistent with the contention that the human zymogen isolated in this laboratory has not been altered during purification. Finally, the possibility of autolytic partial degradation of trypsinogen by neutral proteases prior to preparation of acetone powders cannot be excluded.

The difference in sequence between the activation peptides of human and bovine trypsinogens is reflected in the kinetics of activation of these zymogens by human and porcine enteropeptidases. Thus, the kinetic data presented in Table I provide strong evidence that human cationic trypsinogen, with an Asp-Lys activation peptide, is a much better substrate for human enteropeptidase than is bovine trypsinogen. Indeed, the kinetic parameters obtained with the homologous combinations of zymogen and enteropeptidase, as shown in Table I, are quite similar, suggesting that human enteropeptidase recognizes the Asp-Lys sequence in human cationic trypsinogen as effectively as porcine enteropeptidase recognizes the (Asp)$_n$ sequence in bovine trypsinogen. This kinetic evidence provides strong support for the contention that the human cationic trypsinogen isolated in this laboratory is identical with that secreted by the human pancreas. On the other hand, human trypsinogen appears to be a very poor substrate for porcine enteropeptidase. This finding is consistent with the data presented by Maroux et al. (6) indicating that the peptide Asp-Lys-Ile-Val-Gly, which corresponds to the NH$_2$-terminus of human trypsinogen, does not significantly inhibit the activation of bovine trypsinogen by porcine enteropeptidase.

The (Asp)$_n$ sequence of bovine trypsinogen has been proposed by Maroux et al. (6) to be a highly specific recognition site for activation by porcine enteropeptidase. The finding of an (Asp)$_n$ sequence in activation peptides from trypsinogens of several other species (8–12) supports this view. However, Feolde et al. (41) recently presented evidence that prococoncin, which contains the sequence -Asp-Asp-Gly-Gly-Lys in its activation peptide, is activated by porcine enteropeptidase. In addition, Maroux et al. (6) showed that the peptide (Asp)$_n$-Lys-Ile-Val-Gly is hydrolyzed readily by porcine enteropeptidase. These results suggest that the (Asp)$_n$ sequence is not an absolute requirement for peptide hydrolysis by porcine enteropeptidase.

The NH$_2$ terminal amino acid sequence Ile-Val-Gly-Gly-Tyr is present in all trypsins analyzed to date, including human cationic trypsin. This NH$_2$-terminal sequence has, therefore,
been more highly conserved than the (Asp) sequence in the activation peptide and may be involved in the binding of the zymogen to enteropeptidase. Furthermore, the kinetic data presented in this report strongly suggest that the binding site of human enteropeptidase differs from that of the porcine enzyme. This difference between the two enteropeptidases is currently under investigation in this laboratory.

The three internal sequence differences shown in Fig. 1 can be accounted for by point mutations resulting from single base changes. When compared to bovine trypsinogen, the loss of several amino acids in the human activation peptide could be due to a deletion mutation. An alternative mechanism is suggested by the work of Devillers-Thiery et al. (42) who have shown that in a cell-free protein synthesis system canine trypsinogen is synthesized via a precursor containing approximately 14 additional residues. If a similar mechanism is operative in vivo, then a mutation affecting the processing of a pretrypsinogen polypeptide might result in the formation of human trypsinogen with the Asp-Lys activation peptide. Thus, a change in the sequence of the human trypsinogen activation peptide of human cationic trypsinogen has presumably been accompanied by a mutation affecting the kinetic properties of human enteropeptidase. One other case of parallel evolution has recently been described in a brief report by Ferguson-Miller et al. (43). These workers reported that parallel evolution has recently been described in a brief report by Abita et al. (46) have briefly reported on the sequence of human trypsinogen with the Asp-Lys activation peptide. Conversely, these two proteins are related. Thus, the degree to which the anionic trypsinogen instability of anionic human trypsin (44), suggests that cationic trypsinogen depends also on the effectiveness of human enteropeptidase. Furthermore, the kinetic data presented in this report strongly suggest that the binding site of the activation peptide and may be involved in the binding of the zymogen to enteropeptidase. Additionally, the primate proteins. It is of some interest to speculate on the activation peptide of primate oxidase, bovine cytochrome c is less active than bovine protein with bovine cytochrome c oxidase. Conversely, the activation of other human pancreatic zymogens. The favorable functional relationship between human enteropeptidase and cationic trypsinogen described in this work is consistent with this view.

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