The Preparation and Properties of Bovine Enterokinase*

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Bovine enterokinase was purified from duodenal mucosa. The purification included an initial extraction with 2% deoxycholate, ammonium sulfate fractionations, DEAE-cellulose chromatography, and affinity chromatography on basic pancreatic trypsin inhibitor (Kunitz) (PTI)-Sepharose. The purified enzyme contained 35% carbohydrate; it had a molecular weight of 150,000, with a heavy (115,000) and light (35,000) chain connected by one or more disulfide bonds. Enterokinase hydrolyzed lysine and arginine substrates and slowly reacted with the trypsin active site titrant 4-methylumbelliferyl-p-guanidinobenzoate. The enzyme activated bovine trypsinogen with kinetic parameters similar to those of other preparations of enterokinase.

Bovine enterokinase was inhibited by Kunitz pancreatic trypsin inhibitor with a \( K_{\text{in}} \) of \( 2 \times 10^{-8} \) M\(^{-1}\) and only weakly by other proteinase inhibitors. The amino acid composition differed from bovine enterokinase isolated from duodenal contents (Anderson, L. E., Walsh, K. A., and Neurath, H. (1977) Biochemistry 16, 3354-3360). The mucosal enzyme and the duodenal contents enzymes also differed in the size of the heavy and light chains. The mucosal enterokinase more closely resembled the properties of porcine enterokinase (Baratti, J., Maroux, S., Louvard, D., and Desnuelle, P. (1973) Biochim. Biophys. Acta 315, 147-161). The amino acid composition and size of the light chain were also similar to bovine trypsin.

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Enterokinase (enteropeptidase, EC 3.4.21.9) is one of many hydrolases located in the brush border membrane of the intestinal duodenum (1, 2). The enzyme initiates the intestinal digestion of foodstuffs by activating pancreatic trypsinogen, which then activates pancreatic chymotrypsinogen, proelastase, procarboxypeptidase, and prolipase (3-5). In the activation process, enterokinase catalyzes the hydrolysis of the Lys 6-Ile 7 bond of bovine trypsinogen with the release of the NH\(_2\)-terminal hexapeptide (4, 6). The newly formed trypsin catalyzes the hydrolysis of a single bond in the otherzymogens, converting each to an active enzyme. The activation process has a large amplification since a small amount of enterokinase produces large amounts of active trypsin and other digestive enzymes (7).

The molecular and enzymatic properties of porcine enterokinase have been reported by the Desnuelle group in Marseilles (4, 8, 9). The enzyme was purified from intestinal mucosa in a multistep procedure. The purified porcine enzyme has a molecular weight of 195,000 and contains about 40% carbohydrate. The protein has a heavy and light chain linked by one or more disulfide bonds. The enzyme is a serine proteinase and components of the active site are only found in the light chain. Enterokinase and bovine trypsinogen have similar specificities since both enzymes are capable of activating trypsinogen (4, 8, 9). However, trypsinogen is a poor activator of trypsinogen, while enterokinase is very much better (\( k_{\text{cat}} \) is 2000 times larger, \( K_{\text{m}} \), about 6 times smaller).

Recently, the purification, specificity, and some molecular properties of enterokinase isolated from the intestinal contents of the cow have been reported by Anderson et al. (10). The purified bovine enzyme has a molecular weight of 145,000, which is much less than that of the porcine enzyme, and both the heavy and light chains also differ from the molecular weights of the porcine enzyme.

It is surprising that the molecular weights of bovine and porcine enterokinase differ to such an extent since these species are closely related on the evolutionary scale (11). We purified enterokinase from bovine duodenal mucosa and, in light of the above differences in size, we examined the properties of the purified enzyme and paid particular attention to these comparative aspects. We wish to describe our purification procedure, which utilizes affinity chromatography, and to report on the size of the enzyme, its amino acid composition, carbohydrate content, and several enzymatic properties toward large and small molecules. We will compare these properties with those reported by the Seattle and Marseilles laboratories.

**EXPERIMENTAL PROCEDURES**

**Materials**

Trypsin, trypsinogen, chymotrypsinogen A, ovalbumin, pepsin, and soybean trypsin inhibitor were obtained from Worthington. Bovine serum albumin was obtained from Pentex. Kunitz pancreatic trypsin inhibitor (Trasylol) was a generous gift from Dr. E. Trueschleit of the Bayer Werk Co., while other trypsin inhibitors were a gift from Prof. M. Laskowski, Jr. of Purdue University.

DEAE-cellulose (DE52) was purchased from Whatman; Sepharose 4B and Con A-Sepharose from Pharmacia; and Ultrogel AcA 22 from LKB. Tos-Arg-OMe, Bz-Arg-OEt, sodium deoxycholate, sodium docyl sulfate, and Triton X-100 were products of Sigma. Tos-Lys-OMe, Oda-Bz-ONp, and TLCK were from Cytochrome 5a-xythamide was purchased from Mann. Dithioerythritol and guanidine hydrochloride (Heico) were obtained from Pierce and urea (ultrapure).

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*The abbreviations used are: Tos-Arg-OMe, N\(_2\)-tosyl-l-arginine methyl ester; Bz-Arg-OEt, N\(_2\)-benzoyl-l-arginine ethyl ester; Tos-Lys-OMe, N\(_2\)-tosyl-l-lysine methyl ester; TLCK, N\(_2\)-tosyl-l-lysine chloromethyl ketone; Gdn-Bz-ONp, p-nitrophenyl-p'-guanidinobenzoate; Gdn-Bz-OMMe, 4-methylumbelliferyl-p'-guanidinobenzoate; STl, soybean trypsin inhibitor (Kunitz); PTI, basic pancreatic trypsin inhibitor (Kunitz); SDS, sodium dodecyl sulfate; Con A, concanavalin A.*

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from Schwarz/Mann. Dimethylsuberimidate and cyagen bromide were obtained from Aldrich. Gdn-Bz-OMum was synthesized by the procedure of Chase and Shaw (12) for their synthesis of Gdn-Bz-ONp. 4-Methylumbelliferone was purchased from Eastman. Naphthyl diazo blue was obtained from K & K.

**Methods**

**Assays—**Trypsin activity was measured potentiometrically at pH 7.9 and 25°C with 0.01 M solutions of Tos-Arg-OMe, Bz-Arg-OMe, or Tos-Lys-OMe containing 0.05 M calcium chloride (13). A trypsin unit is the amount of enzyme hydrolyzing 1 μmol of Tos-Arg-OMe/min. ρm values were calculated from the rate of hydrolysis of the substrate and the molarity of the trypsin in the solution. The operational molarity of enterokinase was calculated from a titration with the enzyme at the active site-directed reagent Gdn-Bz-OMum (14). Typically, 10 to 15 μl of a solution of enterokinase in 3 ml of 0.1 M Veronal, pH 8.3, containing 0.02 M CaCl₂, were rapidly mixed with 0.0 ml of Gdn-Bz-OMum (0.4 mg/ml of dimethyl formamide). A Perkin-Elmer MPF-2A spectrophotometer was used to follow the reaction, with excitation at 360 nm and emission at 450 nm. The time for the “burst” lasted 2 min before a steady state turnover of substrate was reached. The fluorescence burst was standardized with a trypsin solution whose molarity of active sites had been determined by titration with Gdn-Bz-ONp (12).

Enterokinase activity was determined from the activation of trypsinogen (3). Activation mixtures contained enterokinase, 0.1 M sodium acetate, pH 5.0, 0.05 M calcium chloride, and 0.1 ml of trypsinogen solution (1.0 mg/ml of 1 M HCl) in a total volume of 1 ml. After 30 min at 35°C, the reaction was quenched with 0.1 ml of 1 M HCl, and the trypsin activity was measured with 0.01 M Tos-Arg-OMe. An enterokinase unit is the amount of enzyme producing 1 trypsin unit/ml of activation mixture. The assay was linear from 0.5 to 7 enterokinase units.

The inhibition of enterokinase in the presence of proteinase inhibitors was determined by incubating 6.4 nm enzyme and 1.3 μm inhibitor in 0.1 M Veronal, 0.02 M CaCl₂, pH 8.3, for 30 min at room temperature. The determination of inhibition remaining was determined from a titration with Gdn-Bz-OMum. The ρm for the binding of enterokinase and bovine Kunitz PTI was determined by incubating 6.5 nm enzyme with varying amounts of PTI (from 0 to 19 nM) in the pH 8.3 buffer for 30 min at room temperature. The residual enzyme activity was determined with Gdn-Bz-OMum.

Amino peptidease was detected with a spot test procedure (15). A 10-μl sample was mixed with 0.5 ml of 0.3 M leucyl β-naphthylamide in 0.05 M Tris, pH 8.5, containing 0.005 M CaCl₂, and incubated for 30 min at room temperature. After 0.2 ml of naphthyl diazo blue (5 mg/ml of water) was added, the immediate appearance of a pink color was taken as a positive test.

**Preparation of PTI-Sepharose—**Seapharose-bound Kunitz pancreatic trypsin inhibitor was prepared by the method of March & al. (16). Approximately 25 ml of washed Sepharose 4B were activated with 1 g of cyanogen bromide at 0°C for 2 min. The activated gel was mixed with 60 mg of PTI in 30 ml of 0.2 M NaHCO₃, pH 5.5, for 18 h at 0°C. Ethanolamine was added to 0.1 M and the reaction mixture was kept at room temperature for 4 h for the reaction with the remaining activated sites.

**Analysis—**Amino acid analyses were performed on a Durrum model D-500 amino acid analyzer. Samples of enterokinase or the S-carboxymethylated heavy and light chains were hydrolyzed in duplicate in 6 N HCl at 108°C for 22, 48, 72, and 120 h in evacuated, sealed tubes. Norleucine was added to the hydrolysates as an internal standard (17). Half-cystine was determined as cysteic acid after performic acid oxidation (18) or as S-carboxymethyl cysteine. Tryptophan was determined spectrophotometrically (19) and after alkaline hydrolysis (20). Glucosamine and N-acetylgalactosamine were estimated from amino acid analyses after extrapolation to zero time to correct for partial destruction. Neutral sugars were determined by the orcinol/ H₂SO₄ procedure (21). Galactosamine was used as a reference standard. Reduced and carboxymethylated proteins were prepared by the method of Hirs (22).

**SDS Gel Electrophoresis—**Polyacrylamide gel electrophoresis, with gels varying in per cent acrylamide, was carried out essentially as described by Weber and Osborn (23). Electrophoresis was performed at 6 mA/tube and continued until the tracking dye was approximately 1 cm from the bottom. Gels were stained with Coomassie brilliant blue G solution and destained in 10% glacial acetic acid. The method of Segrest and Jackson (24) was used for the molecular weight determination of the light chain of enterokinase. The reference proteins and their molecular weights are: the monomer and dimer of bovine serum albumin (67,000 and 134,000), ovalbumin (43,000), pepsin (35,000), chymotrypsinogen A (25,000), cross-linked bovine serum albumin (67,000, 134,000, and 201,000), and cross-linked ovalbumin (43,000, 86,000, 201,000, and 172,000). Cross-linked serum albumin and ovalbumin were prepared as described by Carpenter and Harrington (25). The chains of enterokinase were cross-linked by the method of Davies and Stark (26).

**Preparation of Enterokinase—**Fresh bovine duodenum were obtained from a local slaughterhouse and stored at −20°C. Frozen bovine intestinal duodena were thawed overnight at room temperature, excess fat was removed, and the duodena were sliced down the middle. A spatula was used to remove the mucosa lining the intestinal wall by gentle scraping. The mucosal cells were stored at −20°C until needed.

In a typical preparation, 4 kg of thawed duodenal mucosa were extracted with 12 liters of 2% deoxycholate containing 0.05 M Tris, pH 8.0, for 2 h with continuous stirring. All operations were performed at 4°C. The mixture was centrifuged at 11,000 × g for 1 h in a Beckman J-21 centrifuge with a JA-10 rotor. The supernatant was acidiﬁed to pH 6.0 with 1 N acetic acid which precipitated the bulk of the deoxycholic acid and some impurities. The precipitate was removed by centrifugation at 11,000 × g for 30 min. Fat particles were removed by filtration through glass wool. The supernatant was adjusted to pH 8 and brought to 40% saturation with solid ammonium sulfate, stirred 1 h, and centrifuged at 14,000 × g for 1 h. The supernatant was then brought to 70% saturation with ammonium sulfate and centrifuged at 15,000 × g for 30 min. The precipitate was dissolved in 3 liters of water, brought to 30% saturation with ammonium sulfate, and centrifuged. The supernatant was made 60% saturated with ammonium sulfate and centrifuged. The precipitate was dissolved in 1 liter of water and dialyzed against 0.005 M Tris, pH 6.0, containing 0.02 M NaCl.

The dialyzed sample was mixed with 700 ml of settled DEAE-cellulose (DE53) equilibrated with 0.01 M Tris, pH 6.0, containing 0.04 M NaCl, and stirred for 2 h. The cellulose was recovered by centrifugation at 1500 × g. The cellulose was suspended in the equilibration buffer, poured into a column, and packed by gravity flow. After washing with 4 to 6 column volumes of the pH 6.0 buffer, the enterokinase was eluted with 0.01 M Tris, pH 6.0, containing 0.12 M NaCl (Fig. 1).

The active fraction was adjusted to pH 8.0 and applied to a PTI-Sepharose column (1.6 × 23 cm) equilibrated with 0.05 M KH₂PO₄, pH 8.0, containing 0.5 M NaCl. The column was washed with at least 20 column volumes of the equilibration buffer and then with 0.1 M sodium formate, pH 3.0, containing 0.1 M NaCl (Fig. 2). Fifteenmilliliter fractions of the pH 3 eluent were collected in vials containing 4-Methylumbelliferone was purchased from Eastman.

**FIG. 1.** Purification of enterokinase. Chromatography of the dialyzed ammonium sulfate precipitate on DEAE-cellulose (5 × 48 cm). The buffer was 10 mM Tris/acetate, pH 5.0, containing 40 mM NaCl, and was changed at 3 to 0.12 M and at 2 to 0.25 M NaCl. The flow rate was 250 ml/h and 20-ml fractions were collected. See text for details on the initial batch adsorption of the sample on DEAE-cellulose. The solid line is absorbance at 280 nm and the dashed line is enterokinase activity (units/ml X 10⁻⁴).

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Purification of bovine enterokinase from 4 kg of duodenal mucosa

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Protein*</th>
<th>Total activity*</th>
<th>Specific activity</th>
<th>Yield</th>
<th>Purification fold</th>
</tr>
</thead>
<tbody>
<tr>
<td>2% Deoxycholate extract</td>
<td>1.3 × 10^6</td>
<td>1.2 × 10^7</td>
<td>9 (100)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>pH 6 supernatant</td>
<td>4.0 × 10^5</td>
<td>1.0 × 10^6</td>
<td>25</td>
<td>85</td>
<td>2.5</td>
</tr>
<tr>
<td>60% saturation (NH₄)₂SO₄ precipitate</td>
<td>5.9 × 10^4</td>
<td>9.6 × 10^4</td>
<td>162</td>
<td>82</td>
<td>16</td>
</tr>
<tr>
<td>DEAE-cellulose chromatography</td>
<td>4.7 × 10^3</td>
<td>5.3 × 10^4</td>
<td>1,130</td>
<td>45</td>
<td>113</td>
</tr>
<tr>
<td>PTI-Sepharose</td>
<td>11.5</td>
<td>3.22 × 10^6</td>
<td>280,000</td>
<td>28</td>
<td>28,000</td>
</tr>
</tbody>
</table>

* Protein was determined spectrophotometrically, assuming an E₂₈₀ of 10.

"Trypsinogen (0.1 mg/ml) was activated at pH 5, 35°C, for 30 min. Trypsin activity was determined with Tos-Arg-OMe."
A fraction of enterokinase (15 to 40%) did not bind to PTI (Fig. 2) and remained unbound on repeated chromatography on P11-Sepharose under the above conditions. The fraction was further purified on DEAE-cellulose (5 × 31.5 cm) with a shallow salt gradient at pH 8.0, on Ultrogel A-A 29 (0.9 × 105 cm), and on Con A-Sepharose (1.5 × 8.5 cm). The active fraction was recovered from Ultrogel just before the major protein fraction, and it was unretarded on Con A while a large fraction of the protein remained bound. The partly purified sample now had properties typical of the purified enterokinase. The sample could be bound to PTT-Sepharose and displaced with the pH 3 buffer. On a molar basis it had a trypsinogen-activating activity of 46,000 enterokinase units/nmol, identical with enterokinase. The ratio of the molar specific activity toward lysine and arginine substrates was the same as the homogeneous enzyme. On SDS electrophoresis, with and without mercaptoethanol present, bands were detected with the same mobility shown for the homogeneous enzyme (see below). The bound and unbound fractions appear to be identical; only a single species of enterokinase is present in mucosal cells. Difficulties due to heterogeneity of the carbohydrate structure of enterokinase or from other causes were not observed in the purification of the enzyme.

**Molecular Weights**—Purified enterokinase showed a single band on SDS-gel electrophoresis and two bands when mercaptoethanol was present (Fig. 3). These observations suggest that enterokinase is a two-chain structure with one or more intermolecular disulfide bonds. Table II gives a summary of the molecular weight determinations of the intact protein and the two chains. Although a reliable molecular weight is difficult to obtain for a glycoprotein containing 35 to 40% carbohydrate (see below), we selected procedures that minimized abnormal behavior. The Segrest and Jackson methodology (24) was satisfactory with the light chain; a constant molecular weight at 10% acrylamide or greater was found (Fig. 4). The heavy chain hardly moved and gave continually variable values at gels higher than 6% acrylamide.

The molecular weights of the S-carboxymethylated chains of enterokinase were estimated from their elution volumes on Sepharose 4B in 6 M guanidine hydrochloride (Fig. 5). The molecular weight of the intact protein could not be estimated on gel electrophoresis or on molecular sieves because of abnormal behavior; the protein hardly moved on electrophoresis and it eluted with the void volume on Sephadex G-200. Instead, satisfactory results were obtained with cross-linked enterokinase after the protein was reduced and carboxymethylated. The cross-linked chains were chromatographed on a calibrated column of Sepharose 4B in 6 M guanidine hydrochloride (Fig. 5). Electrophoresis on SDS gels was used to identify the separated components as disulfide intact, heavy chain, and light chain. The cross-linked protein and heavy chain were poorly resolved and a DuPont 310 curve resolver was used to give better estimates of their elution volumes. The molecular weight determined as the sum of the two chains (150,000) is in excellent agreement with the value of the intact protein (147,000).

The molecular weight of the two bovine preparations is the same and 25% smaller than the porcine enzyme (Table II). The individual chains showed greater differences. Both chains of the porcine enzyme are larger than the corresponding bovine chains. In comparing the bovine enterokinase preparations, our heavy chain is larger than the one described by the Seattle group while the reverse is true for the light chain.

**Table II**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Intact</th>
<th>Heavy</th>
<th>Light</th>
<th>Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine enterokinase</td>
<td>147,000†</td>
<td>115,000†</td>
<td>35,000†</td>
<td>150,000</td>
</tr>
<tr>
<td>Bovine enterokinase</td>
<td>145,000</td>
<td>82,000</td>
<td>57,000</td>
<td>139,000</td>
</tr>
<tr>
<td>Porcine enterokinase</td>
<td>195,000</td>
<td>134,000</td>
<td>62,000</td>
<td>196,000</td>
</tr>
</tbody>
</table>

† From chromatography on Sepharose 4B in 6 M guanidine hydrochloride of cross-linked enterokinase.  
‡ From chromatography on Sepharose 4B in 6 M guanidine hydrochloride of S-carboxymethylated chains.

**Amino Acid Analysis**—The amino acid composition of enterokinase is summarized in Table III. The heavy chain has 763 and the light chain 222 amino acids. Both chains contain all types of amino acids and galactosamine and glucosamine. The agreement between the sum of the residue of the chains and of intact enterokinase is satisfactory except for glycine, half-cystine, and serine. Enterokinase contained equal...
Amino acid composition of enterokinase from the bovine duodenal mucosa, bovine duodenal contents, and porcine duodenal mucosa

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Bovine enterokinase</th>
<th>Bovine contents</th>
<th>Porcine enterokinase</th>
<th>Bovine enterokinase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>31.4</td>
<td>30.2</td>
<td>32.6</td>
<td>42.9</td>
</tr>
<tr>
<td>Arginine</td>
<td>16.6</td>
<td>10.5</td>
<td>15.1</td>
<td>21.7</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>31.4</td>
<td>52.0</td>
<td>66.8</td>
<td>66.3</td>
</tr>
<tr>
<td>Half-cystine</td>
<td>20.5</td>
<td>14.0</td>
<td>23.0</td>
<td>23.1</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>69.6</td>
<td>59.2</td>
<td>65.8</td>
<td>82.1</td>
</tr>
<tr>
<td>Glycine</td>
<td>53.6</td>
<td>43.4</td>
<td>44.2</td>
<td>75.7</td>
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<tr>
<td>Histidine</td>
<td>10.6</td>
<td>19.7</td>
<td>9.5</td>
<td>13.4</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>35.6</td>
<td>22.2</td>
<td>28.8</td>
<td>38.0</td>
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<tr>
<td>Leucine</td>
<td>48.4</td>
<td>26.1</td>
<td>46.8</td>
<td>42.3</td>
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<tr>
<td>Lysine</td>
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<td>17.5</td>
<td>20.4</td>
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<td>Methionine</td>
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<td>33.0</td>
<td>42.6</td>
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<tr>
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<td>33.0</td>
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<tr>
<td>Proline</td>
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<td>52.2</td>
<td>9.7</td>
<td>61.9</td>
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<tr>
<td>Serine</td>
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<tr>
<td>Threonine</td>
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<tr>
<td>Tryptophan</td>
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<tr>
<td>Tyrosine</td>
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<td>30.6</td>
<td>13.0</td>
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<tr>
<td>Valine</td>
<td>30.3</td>
<td>18.4</td>
<td>6.9</td>
<td>25.3</td>
</tr>
</tbody>
</table>

- Moles of amino acid in 100,000 g of protein.
- Enzyme preparation described under "Results."
TLCK and this is part of the evidence that suggests that enterokinase is a member of the serine proteinase class (4).

**DISCUSSION**

Enterokinase is a membrane-bound protein located in the brush border of the intestinal mucosa. The enzymatic activity is highest in the tops of the villi and decreases in cells closer to the crypts (2). The tips of the microvilli are continuously desquamated which gives rise to the enterokinase activity found in the duodenal contents (2, 35). In the duodenal contents, the activated forms of the pancreatic zymogens and intestinal hydrolases are also present and these enzymes working in concert are responsible for the terminal steps of protein, carbohydrate, and lipid digestion.

The porcine enterokinase and our bovine mucosal enzyme have many properties in common. The proteins have a heavy and light chain held together by one or more disulfide bonds (Fig. 3). Although the size differs, the amino acid composition of the heavy and light chains is very similar (Table IV). Noteworthy is the close resemblance of the light chains where all amino acids except the half-cystine, glutamic acid, glycine, and serine show good agreement. The similar composition of the light chains is significant since the light chain contains the residues of the active site. The Seattle group labeled the enzyme with $^{[35]S}$-lysopropyl phosphorofluoridate and found the modified serine in the light chain (8). Both enzymes are also inhibited with the active site reagent TLCK. The enzymatic properties are essentially the same toward trypsinogen ($K_{cat}$, $K_m$), Bz-Arg-OEt, and Tos-Arg-OMe (specific activities) (Table V). However, the bovine enzyme was inhibited by PTI (Table VI), while the porcine enzyme was not inhibited by STI or PTI.

In contrast, the Seattle bovine preparation differs in several characteristics from our duodenal mucosa enzyme and also from the porcine preparation. The most significant differences are the size of the heavy and light chains (Table II) and the amino acid compositions of the enzymes (Table IV). Unfortunately, the composition of the individual chains was not reported by the Seattle group. The molecular weight differences suggest that the heavy chain of the Seattle enzyme may represent a smaller degraded molecule than the one we found. However, the situation is reversed in comparing the molecular weights of the light chain. Since the light chain is the catalytic chain, and since the porcine enzyme and ours are similar in amino acid composition, it is difficult to imagine that the heavy chain of our enzyme is a degraded form of the Seattle chain. Furthermore, the size of the light chain of our enzyme is almost the same as bovine trypsin (23,600). It is more reasonable to consider the possibility that the Seattle enzyme and our enzyme are different forms of enterokinase.

We could only detect a single species displaying enterokinase activity in extracts of mucosal cells. Our purification scheme gave high recoveries of enzyme units at each step. Only a single active fraction was found following chromatography on DEAE-cellulose. However, affinity chromatography on PTI-Sepharose separated a bound fraction (major active component) from an unbound active fraction. After purification of the unbound fraction, it had properties identical with the homogeneous preparations of enterokinase.

In Marseilles, porcine enterokinase was isolated initially from duodenal contents (4) and later from duodenal mucosa (8). Unfortunately, the two preparations were not compared but the isolation procedure and enzymatic properties suggest that the two preparations are the same. Therefore, if two forms of bovine enterokinase exist, they must differ to a much greater extent than the porcine preparations. Either bovine enterokinase is elaborated as isoenzymes or differences in structure exist because of modifications caused by enzymes of the duodenal contents. Unfortunately, the lack of structural information on the two bovine preparations makes it impossible at this time to know if either of these explanations is true.

Since the light chain of enterokinase is the catalytic chain, it was of interest to see if the light chain bears any resemblance to bovine trypsin. The molecular weight of the light chain, after a correction is made for the carbohydrate content, is approximately 23,000. The amino acid composition of the light chain will be presented with the corresponding values of bovine trypsin given in parentheses. The light chain contains 15 alanine (14), 52 aspartic and glutamic acids (36), 27 glycine (25), 8 half-cystine (12), 5 histidine (3), 25 isoleucine and leucine (29), 14 lysine and arginine (16), 4 methionine (2), 5 phenylalanine (3), 13 proline (8), 34 serine and threonine (44), 12 tryptophan (4), and 7 tyrosine (10). The overall similarity of the composition of the two polypeptide chains is striking although proline and tryptophan differ to a large extent.

The light chain contains 8 half-cystine residues while trypsin has 12. Of the 8, 1 or 2 would be the minimum number linked in disulfide bridges to the heavy chain. We favor a minimum of two interchain disulfides since one disulfide would require 1 half-cystine remaining as a free sulfhydryl group. We have no evidence for a cysteine residue in the light chain. The remaining 6 half-cystine residues would pair to produce three intramolecular disulfides; trypsin has six disulfide bonds.4 The fewer disulfides of enterokinase are clearly within the range found with other serine proteinases. Human trypsin has five disulfides (36, 37), porcine pancreatic elastase-four (38), and bacterial proteinases three and two (39, 40).

In viewing enterokinase as a two-chain structure, it is clear that the light chain is the catalytic chain and contains the serine and histidine of the active site. The heavy chain, on the other hand, may anchor enterokinase in the brush border membrane. The size of the chain is sufficiently large that a part of the chain could be embedded in the membrane. It is also possible that the heavy chain may be necessary for the light chain to function catalytically; the heavy chain may provide a proper binding region for the biologically active conformation of the light chain. Further structural studies should clarify the mechanism of action of the enzyme.

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**REFERENCES**


2The half-cystine content is difficult to determine with high precision. If the recoveries were low and the number of residues is greater than 8, then the number of intramolecular disulfides must be increased. Since the light chain appears to be similar to trypsin, which has six disulfides (12 half-cystines), a larger half-cystine content would increase the resemblance still further.
The preparation and properties of bovine enterokinase.
J J Liepnieks and A Light


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