Trypsin Inhibitor from Bovine Pancreatic Juice*

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SUMMARY

A trypsin inhibitor has been isolated in 54% yield from bovine pancreatic juice by gel filtration on Sephadex G-75 at pH 8.1 and by elution chromatography on DEAE-cellulose at pH 9.0. It appears to be homogeneous by equilibrium chromatography, equilibrium sedimentation ultracentrifugation, and amino acid analysis, and on the basis of the stoichiometry of its interaction with trypsin. The polypeptide inhibitor has a molecular weight of 6155 and has the following amino acid composition: Asp, Thr, Ser, Glu, Pro, Gly, Ala, Cys, Val, Met, Ile, Leu, Tyr, Lys, and Arg.

The inhibitor is secreted in the pancreatic juice in the free form (not in a complex with trypsin) and it prevents the trypsin-catalyzed activation of the proteolytic zymogens. The amount of inhibitor is equivalent to 1% of the total potential trypsin in pancreatic juice.

Although two trypsin inhibitors have been isolated from acid extracts of the gland, only one (Kazal type) is present in the secretion. This suggests that the inhibitors are segregated at the subcellular level in the pancreatic acinar cells.

EXPERIMENTAL PROCEDURE

Collection of Pancreatic Juice

Bovine pancreatic juice was collected by direct cannulation of the main pancreatic duct (24). Cannulation does not completely deprive the animal of the secretion because the cow has a functional accessory duct system (25). The daily secretion, 4 to 8 liters of pancreatic juice containing 0.5 to 2% protein, was collected into bottles at 4°. Four 5-ml aliquots were frozen immediately for the later determination of enzyme and zymogen concentrations, and the remainder was treated with DFP (0.1 ml of 10% isopropyl alcohol, 10 ml per liter of juice) for 1 hour with continuous stirring, and was then lyophilized. The animal was maintained on a normal ration and was permitted water ad libitum. Electrolyte loss was compensated by the addition of 30 g each of NaCl and NaHCO₃ to the feed.

Determination of Enzyme Activity

Trypsin—The rate of hydrolysis of p-toluensulfonyl-L-arginine methyl ester was determined at pH 7.8 and 26° in a pH-
stat (Radiometer T'11Te, SHR-2e, ABU-ia, 0.25-ml piston). Four ml of a solution containing 0.01 m TAME, 0.1 m KCl, 0.02 m CaCl2, 0.005 m Tris hydrochloride, and 1 ml of enzyme were used for each assay. The rate of hydrolysis was essentially linear with respect to time for at least the first 40% of the reaction; a linear relationship between initial rate and trypsin concentration was observed within the range of 2 to 20 μmoles per min. The standard deviation of replicate determinations was 1 and 2%. Activity was expressed as micromoles of TAME hydrolyzed per min.

In order to determine the amount of active enzyme in our trypsin preparations we have used the spectrophotometric benzoylarginine methyl ester assay and the specific activity reported by Kassell et al. (15) for chromatographically purified trypsin which combined stoichiometrically with homogeneous basic trypsin inhibitor. The specific activities of two trypsin preparations were determined and the percentage of active trypsin was calculated by dividing the observed specific activity by the value reported by Kassell et al. (51 units per mg). On this basis 100% active trypsin has a specific activity of 410 ± 34 units per mg in the titrmetric assay used in this study.

Trypsin Inhibitor—A stock trypsin solution (Worthington TRL 6257 or TRL 6261) containing approximately 1 mg per ml in 0.01 ml KCl-HCl at pH 2.9, stored at 4° for 2 to 3 weeks, was used to prepare fresh trypsin daily. The standard trypsin solution contained approximately 10 units per ml in 0.1 ml KCl, 0.015 ml Tris, 0.020 ml CaCl2 at pH 7.8. No decrease of enzyme activity was observed when the trypsin standard was stored for 2 hours at 25° or 8 hours at 4°. The trypsin standard was incubated with the test solution (usually 1 ml of each) at 25° for 3 min and the activity remaining was determined on 1 ml of the incubation mixture. The difference in activity between the test solution and the diluted control is taken as trypsin inhibition. Inhibitor concentration was adjusted so as to inhibit 25 to 75% of the trypsin. The enzyme inhibitor complex was not dissociated by the large excess of substrate for at least the first 5 min of the assay. One inhibition unit is the amount of inhibition that caused the reduction of TAME hydrolysis by 1 μmole per min. Inhibitor specific activity is defined as inhibitor units per A1em.

Chymotrypsin—The rate of hydrolysis of 0.01 ml acetyl-L-tyrosine ethyl ester in 0.1 ml KCl, 0.020 ml CaCl2, and 0.005 ml Tris-hydrochloride containing 1% v/v acetonitrile was measured at pH 7.8 at 25° in a pH-stat as described previously for trypsin. A linear relationship existed between initial rate (first 20% of reaction) and chymotrypsin A concentration within the range of 2.0 to 15 mmoles per min. Activity was expressed as micromoles of ATE hydrolyzed per min (initial rate).

Ribonuclease—The hydrolysis of cytidine 2',3'-cyclic phosphate (Schwarz, Lot 6301) was measured at 286 mμ by means of a Beckman DB spectrophotometer fitted with a logarithmic conversion circuit and potentiometric recorder. The reaction was measured at pH 7.0 at 25° by the procedure of Murdock et al. (20). One unit of activity is the change in absorbance at 286 mμ per min (initial rate) with a 1-em light path.

Activation of Trypsinogen and Chymotrypsinogen (cf. Fig. 2)—As soon as possible after fractions were collected, 100 μl of 0.5 m CaCl2 were added to each even numbered tube. Ten microliters of a trypsin solution (1 mg per ml in 0.1 m KCl, pH 2.9) were added to 1.0-ml aliquots of each fraction and the tubes were stored at 4°. The solutions were assayed for ATE and TAME activity at 0, 24, and 48 hours after addition of trypsin.

Preparation of Material for Amino Acid Analyses—The column effluents containing inhibitor were lyophilized and the concentrated solutions were dialyzed in 23/32 NoJax Visking cellulose casings at 4° for 42 hours against two changes of 0.1 m KCl, and for an additional 52 hours against four changes of deionized water (27). The material retained was lyophilized and stored at -22°. Samples of 1 to 1.5 mg of protein were hydrolyzed in an evacuated, sealed tube with 1 ml of twice distilled, constant boiling HCl. The tubes were held at 110 ± 0.2° for 22 and 52 hours.

Amino acid analysis of the hydrolysates was performed according to the method of Spackman, Stein, and Moore (28). The long column was run for 30 min after the elution position of phenylalanine to permit observation of glucosamine. The cysteic acid content of a sample oxidized by the method of Moore was determined on a 22-hour hydrolysate (29). The ammonia content was determined by correcting the observed value for traces of ammonia in acid and buffers as well as for serine and threonine decomposition. The correction amounted to less than 15% of the observed value.

Ultraviolet Absorption Spectra—A Zeiss PMQ-II spectrophotometer was used for absorbance measurements. Absorbance indices, A1em (1% solution), were selected from the literature: bovine pancreatic juice, 18.0 (5); trypsin, 14.4 (30); ribonuclease A, 6.98 (31); basic trypsin inhibitor, 11.98 (15); Kazal's trypsin inhibitor, 8.5 (8); bovine pancreatic juice trypsin inhibitor, 5.89 (this study). Absorption spectra were determined with a Cary recording spectrophotometer, model 14, at 24°. Acetyltirosinamide from Cycle Chemical Company (Lot R 4898) (Dumas nitrogen; found, 12.41% (theory, 12.6%); εtyr.5 1400) was used without further purification. Reagent grade urea (Mallinckrodt) was recrystallized from ethanol before use (32). Protein concentrations for spectral studies were determined from the 22-hour acid hydrolysates by amino acid analysis in the usual manner.

Disc Electrophoresis—Electrophoresis was carried out in 15% acrylamide by the method of Reisfeld, Lewis, and Williams (33) at pH 8.0 as described by Davis (34) at 4 mμ per tube for 120 min at 4°. After fixing and staining with Amido schwarz in 7% acetic acid excess stain was removed by diffusion. The gels were photographed by transmitted light and the negative was examined by densitometry. The data were corrected for the film response (Kodak Super XX) to calibrated variable light intensities.

Equilibrium Ultracentrifugation—The Spinco model E analytical ultracentrifuge was equipped with a scanning photoelectric absorption system and Rayleigh interference optics. The analytical runs were made in a three-channel Rexelite aluminum-epoxy cell of 3-mm column height at 30,000 rpm at 22°. Solutions containing 0.085, 0.0425, and 0.017% protein were examined at pH 4.28 (0.01 m CaCl2, 0.0158 m KCl, 0.005 m citric acid-NaOH) and at pH 7.81 (0.01 m CaCl2, 0.026 m KCl, and 0.005 m Tris-HCl). Weight average molecular weights were obtained from plots of the logarithm of observed absorbance as a function of the square of the radius. The interference optics were used to obtain z-average molecular weights by a modification of Method II of Van Holde and Baldwin (35).
Preparation of Inhibitor

Gel Filtration on G-75—Sephadex G-75, bead form, was swollen in 50% acetic acid and the fines were removed by decantation. Lyophilized pancreatic juice was dissolved in distilled water containing $10^{-4} \text{M}$ DFP to a final concentration of 1.5 to 2% protein. The solution, 600 to 800 ml, was applied to a Sephadex G-75 column, 7.6 x 175 cm, previously equilibrated with 0.5 M KCl, 0.01 M Tris-hydrochloride, and $10^{-4} \text{M}$ DFP at pH 8.1. A flow rate of 4.8 cm hr$^{-1}$ was reduced to 2.6 cm hr$^{-1}$ in the presence of the protein sample. Effluent was collected in 60-ml fractions. Gel filtration and all subsequent manipulations were carried out at 2-4°C.

Effluent fractions with an inhibitor specific activity in excess of 5 (Fig. 1, Region 1) were pooled and lyophilized. The effluent corresponding to Region 2 from 23 preparative columns (2.5 to 5 specific activity units) was lyophilized and submitted again to gel filtration (Fig. 1, bottom). Effluent tubes with more than 12 specific activity units were pooled, lyophilized, and combined with Fraction 1. This combined pool is denoted "low molecular weight fraction."

Chromatography on DEAE-cellulose—DEAE-cellulose was treated with 0.1 M KOH-0.25 M KCl until the filtrate was colorless. After washing with 0.025 M KCl and 0.02 M Tris-hydrochloride, the resin was equilibrated by suspending the basic form in 5 volumes of 0.025 M KCl and 0.020 M Tris-hydrochloride, pH 9.0, and correcting the pH to 9.0. A column, 7.6 x 40 cm, was poured (36) and was washed with 0.0025 M potassium chloride, 0.01 M KCl, 0.01 M Tris-hydrochloride, and 10$^{-4}$ M DFP (0.1% isopropyl alcohol) and dialyzed in 23/32 No-Jax Visking casings against distilled water. The material retained was lyophilized and resuspended in 550 ml of distilled water. The suspension was clarified by centrifugation at $13,200 \times g$ for 30 min. The pH and conductivity of the combined supernatants were adjusted to those of equilibrating buffer with concentrated buffers.

Equilibrated sample, 615 ml (8,550 $A_{650}$, 105,000 inhibitor units), was applied to the DEAE-cellulose column, 7.6 x 40 cm. Elution was carried out with equilibrating buffer; flow rates of 540 to 600 ml per hr were achieved at 10 p.s.i. Fractions of 60 ml were collected. At tube 231 at tube 278 the eluting solutions were changed to 0.025 M KCl, 0.020 M Tris-hydrochloride, pH 9.0, and 1.0 M KCl, respectively.

**RESULTS**

Isolation of Trypsin Inhibitors

Low Molecular Weight Fraction Obtained by Gel Filtration on Sephadex G-75—A typical elution profile of lyophilized bovine pancreatic juice on Sephadex G-75 is presented at the top of Fig. 1. The inhibitor activity (•) is retarded relative to most of the secretory protein (○). The recovery of inhibitor activity was 100 ± 5%.

When DFP was omitted from the eluting buffers, the recovery of inhibitor activity remained high but there was evidence for the activation of thezymogens of carboxypeptidase A, carboxypeptidase B, chymotrypsin, and trypsin after the fractions had been collected.

The fast side of the inhibitor activity peak, Region 2, was submitted to gel filtration for a second time on the same column (Fig. 1, bottom). It had the same retention volume as the inhibitor activity in Region 1.

The designation low molecular weight fraction has been arbitrarily applied to a pool of material derived from several preparative columns corresponding to Region 1 (Fig. 1, top) and tubes 97 to 112 (Fig. 1, bottom). The protein composition of the low molecular weight fraction was studied by means of quantitative enzyme activation and assay after analytical scale gel filtration on G-75 (cf. Fig. 2). The retention volumes of the enzyme activities were the same as on the preparative columns but the resolution was improved. Trypsinogen was eluted in the same position as chymotrypsin.

Activation by trypsin was required for ATE and TAMe activity even when CaCl$_2$ was added immediately after collection of the fractions. There was no increase in enzyme activity after acidification. This procedure would dissociate enzyme inhibitor complexes of the Kunitz or Kazal type if present (11).

The recovery of protein, ribonuclease, and inhibitor activity was quantitative. Of the total protein in the fraction, 15.7% could be accounted for in the following manner: 14% ribonuclease, 1% trypsin inhibitor, 0.5% chymotryptsinogen A equivalents, and 0.16% trypsinogen. The weight assignments are based on activity determinations and the following specific activities: trypsin, 410 units per mg; chymotrypsin A, 600 units per mg;
ribonuclease A, 3 units per mg; trypsin inhibitor, 1600 units per mg.

**Elution Chromatography on DEAE-cellulose**—The elution diagram presented in Fig. 3B is typical of those obtained on both a preparative and analytical scale. After the sample was applied to the column, the chromatogram was developed by “elution analysis” (37), i.e. eluent composition was held fixed before, during, and after the development of the first activity peak. The inhibitor activity was quantitatively recovered in two regions. The major fraction, tubes 20 to 30, accounting for 86% of the activity (○), was eluted after 5 column volumes of buffer had passed through the column. The elution position varied between

![Fig. 2. Gel filtration of low molecular weight fraction of bovine pancreatic juice on Sephadex G-75.](image)

Fig. 2. Gel filtration of low molecular weight fraction of bovine pancreatic juice on Sephadex G-75. The column was 1.8 X 145 cm in 0.5 M potassium chloride, 0.01 M Tris-HCl at pH 8.1, 4°. Sample, 10 A°\textsubscript{280} X 11 ml. •—•, absorbance at 280 nm; X—X, hydrolysis of N-acetyl-l-tyrosine ethyl ester (ATE); △—△, hydrolysis of cytidine 2',3'-phosphate; ○—○, trypsin inhibition (TAME hydrolysis).

![Fig. 3. Chromatography on DEAE-cellulose.](image)

Fig. 3. Chromatography on DEAE-cellulose. The column was 0.9 X 40 cm in 0.002 M Tris-HCl, 0.0025 M KCl at pH 9.0, 4°. The columns were eluted with equilibrating buffer until tube 72 (Arrow I) when 0.025 M Tris-HCl, 0.025 M KCl, pH 9.0, was applied. At tube 126 (Arrow 2) 1 M KCl was applied to the top of the column. A, effluent conductivity; △, Column B; ▽, Column C. B, low molecular weight fraction of bovine pancreatic juice. Sample, 10.1 A°\textsubscript{280} X 10 ml. C, “Kazal” inhibitor (No. BD-1037-561-187B). Sample, 0.396 A°\textsubscript{280} X 5 ml.

![Fig. 4. Pancreatic juice trypsin inhibitor.](image)

Fig. 4. Pancreatic juice trypsin inhibitor. Rechromatography of inhibitor activity (cf. Fig. 3B, tubes 20 to 30) on DEAE-cellulose at pH 9.0. The column was 1.8 X 41 cm. Experimental conditions were the same as indicated for Fig. 3. Sample, 2 A°\textsubscript{280} X 27 ml. •—•, absorbance at 280 nm; ○—○, trypsin inhibition (TAME hydrolysis); △—△, specific activity.

![Fig. 5. Chromatography at pH 6.5.](image)

When the effluent corresponding to tubes 20 to 30 in Fig. 3B (specific activity, 2000) was rechromatographed under similar conditions, 60% of the inhibitor activity was eluted in the same position (tubes 50 to 55), but 40% was eluted in the position corresponding to the major peak (tubes 20 to 30).

When the effluent corresponding to tubes 20 to 30 in Fig. 3B (specific activity, 2000) was rechromatographed, inhibitor activity was recovered in a single peak of constant high specific activity, 2780. When it was chromatographed at pH 6.5 in 0.001 M potassium phosphate on DEAE-cellulose (Fig. 5), the activity was quantitatively recovered in a single peak of constant high specific activity, 2780.3

**Amino Acid Composition**

The amino acid composition of the pancreatic juice trypsin inhibitor is presented in Table I. Phenylalanine, histidine, tryptophan, and glucosamine were not detected in any acid hydrolysate. The low sensitivity of the analytical data for tryptophan is due to the small sample size and the acid lability of this amino acid. The absence of tryptophan in the sample

3 Inhibitor with a specific activity of 2750 has been prepared in the pH 9.0 system. The amino acid compositions of inhibitor prepared at pH 6.5 and pH 9.0 were identical.
has been confirmed by spectrophotometric procedures (cf. “Ultraviolet Spectra” below). The integral molar ratios of the constitutive amino acids and the inability to detect phenylalanine, histidine, and tryptophan may be taken as strong evidence for the homogeneity of the polypeptide.

The amino acid composition of the basic trypsin inhibitor is also presented in Table I. Although both bovine pancreatic trypsin inhibitors have high cystine content and are devoid of histidine and tryptophan, there is little other similarity in their amino acid composition.

**Ultraviolet Spectra**

A time-dependent change in the ultraviolet absorption spectrum was observed when pancreatic juice trypsin inhibitor was dissolved in 0.1 N NaOH (Fig. 6). Vestigial “acid” peaks of tyrosine at 278.0 and 284.7 nm were apparent even after 3 min. The 294.5 nm phenolate ion peak increased with time and reached a maximum value between 46 and 70 hours. There was no evidence for the 281 or 288 nm peaks present in the alkaline spectrum of tryptophan (cf. Fig. 7). A 6-min incubation with 8 M urea in 0.1 N NaOH removed most of the time dependence from the tyrosine ionization.

The shoulder between 320 and 330 nm formed during alkaline treatment was present in the 70-hour sample after neutralization to pH 6.9. The chromophore responsible for this shoulder is unknown but it may be related to the peak in this region observed after the tyrosinase-catalyzed oxidation of peptides and proteins (38–40).

Tyrosine and tryptophan were estimated by the spectrophotometric method of Goodwin and Morton (41) on a sample after 70-hour exposure to 0.1 N NaOH. The absorbances at 280.0 and

**Fig. 5. Chromatography of pancreatic juice trypsin inhibitor on DEAE cellulose at pH 6.5.** The column was 1.8 × 71 cm in 0.001 M potassium phosphate at 4°. Sample, 3.2 A₁α₂ × 6.1 ml.

**Fig. 6. The change in absorption spectra of 0.09 × 10⁻⁴ M pancreatic juice trypsin inhibitor with time in 0.1 N NaOH.** The 6-min urea treatment was in 8 M urea. All other curves are in the absence of urea.

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<th>Amino acid composition of bovine pancreatic trypsin inhibitors</th>
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**Table I**

**Total**                                | 565  | 58 |

Molecular weight                      | 6155 | 6513 |

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* Average of two determinations; deviation from average was 1% or less. The values for aspartic acid, glutamic acid, glycine, alanine, valine, and leucine were used as the basis for the calculation of molar ratios of constituent amino acids.

† Kassell et al. (15).

‡ Average of 22- and 52-hour hydrolysates unless otherwise indicated.

§ Fifty-two-hour hydrolysate.

¶ Value obtained by extrapolation to intercept at zero time.

‖ Cysteic acid determination on the oxidized protein yielded 6.06 moles.

|| Estimated lower limit of detection.

* Determined on 22-hour acid hydrolysate corrected for destruction of β-hydroxyamino acids and ammonia content of solvents.

* The calculated molecular weight is based on 8 amide groups per molecule.
who studied a system composed of ATE and 1.5% Schardinger's dextran. On the basis of this analysis and the time-dependent absorbance change in 0.1 N NaOH, it appears that both tyrosines are buried.

**Acrylamide Gel Electrophoresis**

Fig. 9 shows the result obtained when 140 µg of the pancreatic juice trypsin inhibitor were subjected to gel electrophoresis in 15% acrylamide gel. More than 95% of the material migrated as a single component.

**Molecular Weight Determination**

Weight average (M_w) and z-average (M_z) molecular weights have been calculated from absorption and interference measurements, respectively. Determinations were made over a 5-fold range of protein concentration. As shown in Fig. 10, all of the log concentration versus r^2 plots conform to a straight line as expected for a homogeneous material. Molecular weights were obtained from the slope of the line with the calculated partial specific volume 0.719. The values of M_w and M_z (each is the result of averaging results for three protein concentrations) were 6020 and 6220 at pH 4.28 and 5920 and 6270 at pH 7.81. The grand average of all the M_w and M_z values is 6100 and the standard deviation, ±260, is well within the ±8% error estimated for these experiments. The polypeptide could not be distinguished from a homogeneous monomer under the experimental conditions employed.

**Trypsin Inhibitor Activity**

Pancreatic Juice—The direct determination of trypsin inhibitor activity in bovine pancreatic juice is complicated by the presence of trypsinogen. The trypsin activity of the mixture first decreases and then rises sharply (Fig. 11, 0). The increase in activity is due presumably to the autocatalytic conversion of trypsinogen to trypsin. Inhibitor activity was stable for 1 to 2 hours when the partially purified low molecular weight fraction (Fig. 11, A) or pancreatic juice trypsin inhibitor was assayed. The time-dependent liberation of TAME activity did not interfere with the quantitative determination of inhibitor in pancreatic juice activity provided that the 1- and 2-min time points were taken (Fig. 12). No inhibition of chymotrypsin activity could

4 Under these conditions, 15 to 30 hours would be required to demonstrate "temporary" inhibition.
Pancreatic Juice Trypsin Inhibitor

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FIG. 10. Molecular weight determination by equilibrium centrifugation. Pancreatic trypsin inhibitor at an initial concentration of 0.085%. The equilibrium distribution of protein was determined by the scanning absorption system after 18 hours at 30,000 rpm and 22°C. The ordinate gives the logarithm of concentration (absorbance). On the abscissa are the values of the square of the distance (in centimeters) from the axis of rotation. The lines marked a and b refer to the top and bottom of the cell, respectively. $M_w = M_r = 6,230$.

be detected when 10 μg of α-chymotrypsin were incubated with 20 mg of bovine pancreatic juice protein.

Pancreatic Juice Trypsin Inhibitor—There is a linear relationship between the extent of trypsin inhibition and concentration of inhibitor until approximately 95% of the trypsin activity has been abolished (Fig. 13).

Extrapolation of the linear portion of the dose response curve yields $7.0 \times 10^{-7}$ m for inhibitor required to inactivate $9.9 \times 10^{-4}$ m "active" trypsin. Similarly, the highest specific activity observed for column effluents, 2,750, corresponds to an inhibitor to trypsin molar ratio of 0.07 when 23,800 and 6,155 are taken as the molecular weights of trypsin and inhibitor, respectively. These results are in good agreement with the 1:1 molar ratio usually observed for polypeptide trypsin inhibitors (13, 15, 46).

A residual activity of 3 to 4% persists even when the molar ratio of inhibitor to trypsin is 2.0. When this ratio is equal to 7, the residual activity is 2%. Experiments are currently under way to distinguish between two possible explanations for residual activity when the molar ratio of inhibitor to trypsin is greater than 1.0. (a) The inhibitor-trypsin complex has activity equiva-

Fig. 11. Time course of inhibition of a standard trypsin solution by: • O •, bovine pancreatic juice, 0.95 $A_{280}^m$; and ▲ ▲ ▲ , low molecular weight fraction, 0.244 $A_{280}^m$. The incubation solution was 0.02 M CaCl₂, 0.1 M KCl, 0.015 M Tris-HCl, pH 7.8, at 25°C.

Fig. 12. Inhibition of trypsin by bovine pancreatic juice. Conditions were the same as indicated for Fig. 11. The incubation period was 1 min.
respect to trypsinogen was achieved and the fraction contained no detectable activity toward TAMe or ATE. The initial fractionation presented by Finkenstadt and Laskowski (49), gel filtration on G-75 in the presence of DFP at the pH of pancreatic juice commercial preparations and the recent evidence for peptide bond cleavage during trypsin-soybean trypsin inhibitor complex formation were employed to separate free inhibitor from an 80-fold molar excess of inhibitor under the same conditions no inhibition of chymotryptic activity was observed.

DISCUSSION

Although trypsin inhibitor activity has been demonstrated in dog (47), rat (9), and human (10) pancreatic juice, this study is the first chemical characterization of homogeneous polypeptide trypsin inhibitor from pancreatic juice. The original procedures for the isolation of bovine trypsin inhibitors involved acid or acid-alcohol extracts of whole pancreas obtained as by-products of commercial insulin preparations. In view of the reports of Plummer and Hirs (48) and Eaker, King, and Craig (31) of structural homologues of pancreatic enzymes isolated from commercial insulin preparations, the evidence for peptide bond cleavage during trypsin-soybean trypsin inhibitor complex formation presented by Finkenstadt and Lackowski (40), gel filtration on G-75 in the presence of DFP at the pH of pancreatic juice was employed to separate free inhibitor from an 80-fold molar excess of trypsinogen. A 2000-fold purification of inhibitor with respect to trypsinogen was achieved and the fraction contained no detectable activity toward TAMe or ATE. The initial fractionation by molecular size also permitted the isolation of a new component of pancreatic juice that contains 10 mg per ml of protein. It is present as a free monomer and does not interact with other proteins in the mixture under the conditions of gel filtration examined. The role of the inhibitor in preventing the trypsin-mediated activation of zymogens was demonstrated by the activation of the proteases after the inhibitor was removed during gel filtration in the absence of DFP.

The secretion of inhibitor was correlated with the protein content of the juice and not with the secretion of water. This confirms the observations of Kaiser and Grossman (47) on canine pancreatic juice and supports their conclusion that the inhibitor is a constituent of the protein secretion that is synthesized and secreted by the acinar cells. It is reasonable to assume that the Kazal-type inhibitor is present in zymogen granules, i.e., the intracellular storage sites for the pancreatic secretion (4, 6), and one may anticipate that there is segregation of the two types of trypsin inhibitors at the subcellular level.

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REFERENCES


Inhibitor activity in tubes 131 and 132 is probably an artifact due to eluting the column with 1 M KCl.

Note—Kazal-type trypsin inhibitors have recently been isolated from human, dog, pig, and cow pancreatic juice (55, 56). An acidic trypsin inhibitor with an amino acid composition similar to the one reported in this paper has been isolated from bovine pancreas by Burk, Cerwinsky, and Grinnan (Abstracts of the American Chemical Society Meeting, New York, September 1966, No. 272).
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