A novel proteinase from human pancreas

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A cholesterol-binding protein was previously isolated from human pancreas [Sziegoleit (1982) *Biochem. J.* **207**, 573–582] and shown to consist of a single polypeptide chain with an apparent M_r of 28000 and an isoelectric point of pH 4.9. In further investigations, a proteolytic activity was observed to be present in preparations of this protein. The enzyme activity was not dissociable from the cholesterol-binding protein. It decreased in the presence of sodium dodecyl sulphate or urea parallel to degradation of the protein, indicating autodegradation in the presence of these denaturants. Glucagon digestion studies indicated the carbonyl bond of alanine to be a favoured site of the enzymic cleavage. The proteinase was inactive against chromogenic substrates relatively specific for elastase, trypsin and chymotrypsin, but was found to cleave benzyloxycarbonylalanine *p*-nitrophenyl ester efficiently. The enzyme was inactivated by phenylmethanesulphonyl fluoride and was thus classified as a serine proteinase. Autoradiographic studies demonstrated binding to serum α_1 -anti-trypsin and α_2 -macroglobulin in a similar manner to that observed with other pancreatic endo-proteinases. The collective results indicate that the isolated protein, provisionally named ‘cholesterol-binding pancreatic proteinase’, is a novel proteinase of the human pancreas. Quantitative measurements indicate that it comprises 4–6% of total protein in pancreatic secretions.

A previous paper has dealt with the isolation and characterization of a cholesterol-binding protein from human pancreas (Sziegoleit, 1982). On SDS/polyacrylamide-gel electrophoresis the protein yielded a single polypeptide chain with an apparent M_r of 28000 and was very stable towards the action of trypsin and chymotrypsin. Surprisingly, the polypeptide band disappeared almost completely on SDS/polyacrylamide gels when there was a delay of more than 2 min between the addition of SDS (1%, w/v) and boiling, indicating a proteolytic activity in the presence of SDS. After confirmation of this proteinase activity by hydrolysis of casein, experiments were undertaken to identify the proteolytic enzyme. Results of these studies are reported in the present paper and provide evidence that the cholesterol-binding protein itself is a hitherto undetected major proteinase

of the pancreas, which I provisionally refer to as ‘cholesterol-binding pancreatic proteinase’ (CBPP).

Experimental procedures

Chemicals

Unless otherwise stated, sources of chemicals were as given previously (Sziegoleit, 1982). Z-Lys-ONp, Z-Tyr-ONp and Z-Ala-ONp were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. Tos-Phe-CH₂Cl, Tos-Lys-CHCl, glucagon and the chromogenic substrates, Bz-Phe-ONap and Bz-Tyr-OEt (relatively specific for chymotrypsin), Bz-Arg-Nan and Tos-Arg-OMe (relatively specific for trypsin), and Ac-Ala-Ala-Ala-Nan (relatively specific for elastase), were purchased from Serva, Heidelberg, Germany. Casein, trypsin and α -chymotrypsin as well as soya-bean trypsin inhibitor and di-isopropyl phosphorofluoridate were from Merck, Darmstadt, Germany. Phenylmethanesulphonyl fluoride was from Boehringer, Mannheim, Germany. Fluorescamine (Fluoram) was from Hoffmann–La Roche, Basel, Switzerland. Precoated plastic sheets for t.l.c., Polygram CEL

Abbreviations used: CBPP, cholesterol-binding pancreatic proteinase; SDS, sodium dodecyl sulphate; Z-, benzyloxycarbonyl-; Bz-, benzoyl-; Ac-, acetyl-; Tos-, tosyl- (toluene-*p*-sulphonyl-); -ONp, *p*-nitrophenyl ester; -ONap, 2-naphthyl ester; -OEt, ethyl ester; -OMe, methyl ester; -Nan, *p*-nitroanilide.

300, were from Machery–Nagel, Düren, Germany. All other chemicals were reagent grade.

Materials

Pancreatic secretions were collected by endoscopic retrograde catheterization of the pancreas after intravenous injection of 1 unit of secretin/kg body wt. Samples were stored for several weeks at -20°C .

Staphylococcus aureus α -toxin was prepared as described previously (Füssle *et al.*, 1981).

General methods

Electroimmunoassays, SDS/polyacrylamide-gel electrophoresis, amino acid analysis and protein determination were performed as described previously (Sziegoleit, 1982).

The isolation of CBPP followed essentially the previous description adapted to a large-scale purification starting with 25–35 necrobiotic human pancreas organs. Dialysis of the solubilized and delipidated tissue extract (5 litres) against water was performed by use of a haemodialysis cell (Diasart 4k; Sartorius, Göttingen, Germany), thus shortening the time required to about 5 h. Thereafter 0.5 M-Tris/HCl buffer, pH 6.0, was added to give a final concentration of 10 mM, and the material was applied to a 1.7-litre column of DEAE-Sepharose CL-6B (Pharmacia, Uppsala, Sweden) equilibrated with the same buffer. After application of the material, protein was eluted with 4 litres of a linear 100–600 mM-NaCl gradient in Tris/HCl buffer, pH 6.0, at a flow rate of 72 ml/h, and 18 ml fractions were collected. CBPP-containing fractions were detected by fused rocket immunoelectrophoresis with specific antiserum, concentrated by pressure dialysis and subsequently applied to a Sepharose 6B (Pharmacia) column (2.6 cm $\times$ 100 cm), equilibrated with 10 mM-Tris/50 mM-NaCl medium, pH 8.2, containing 1 mM-EDTA and 15 mM- NaN_3 , and finally to a Sephacryl S-200 (Pharmacia) column (0.9 cm $\times$ 60 cm) equilibrated with the Tris/NaCl medium without EDTA/Tris/NaCl/ NaN_3 medium. The applied material for gel chromatography was less than 2% of the gel bed volume.

Autoradiographic analyses (Bjerrum & Bhakdi, 1977) were employed to investigate the specific binding of CBPP to serum proteins. CBPP was labelled with ^{125}I by the chloramine-T technique (Greenwood *et al.*, 1963). A 10 μl portion of normal human serum was incubated with 2 μl of ^{125}I -labelled CBPP, corresponding to 3×10^5 c.p.m., for 30 min at room temperature. Thereafter 2 μl was examined by crossed immunoelectrophoresis with a polyspecific antiserum to human serum proteins (code 100 SF; DAKO, Copenhagen, Denmark) (10 $\mu\text{l}/\text{cm}^2$). The plates were washed,

dried and covered with an X-ray film, which was developed after 2 days of exposure. Identification of the immunoprecipitates causing a blackening on the film was achieved with specific antisera incorporated into an intermediate gel. To ascertain that radioactivity indeed derived from CBPP, radio-labelled CBPP was analysed by SDS/polyacrylamide-gel electrophoresis. The gel was dried and the distribution of the radioactivity established by autoradiography.

Proteolytic digest

Enzymic digests of casein were performed in 10 mM-Tris/50 mM-NaCl medium, pH 8.2, at 37°C . Casein solution (50 μl ; 2 mg/ml) was incubated with 10 μl of CBPP preparation (0.1–0.5 mg/ml). After 10 min, 20 μl was added to 50 μl of aq. (2%, w/v) SDS preheated to 95°C , and the temperature was maintained at 95°C for a further 10 min before electrophoresis in SDS/polyacrylamide gels.

Proteolysis of glucagon was used to gain insight into the specificity of the enzyme reaction. A 1.6 mg portion of glucagon was added to 60 μl of CBPP in water (0.32 mg/ml) and incubated at 37°C . After 6 h, 5 μl was withdrawn and the proteolytic products were separated by the fingerprint technique on cellulose thin-layer plates by the procedure of Boege *et al.* (1980). Peptides were located by using fluorescamine (Perham, 1978), and the spots were scraped into 0.5 ml portions of 6 M-HCl containing 0.02% mercaptoethanol in 1.5 ml plastic vials, which were shaken for 15 min. Cellulose was then removed by centrifugation, and supernatants were transferred into glass tubes, which were sealed under vacuum. Amino acid analyses of the 24 h hydrolysates at 110°C were performed in an Autogamma amino acid analyser.

Enzyme assay

Hydrolysis of Bz-Tyr-OEt and Tos-Arg-OMe was measured by using standard procedures (Walsh & Wilcox, 1970). Bz-Arg-Nan, Bz-Phe-ONap and Ac-Ala-Ala-Ala-Nan were dissolved to 20 mM in dimethyl sulphoxide, and 10 μl of substrate solution was added to 2 ml of 0.1 M-sodium phosphate buffer, pH 7.2. Then 20 μl of CBPP, trypsin or chymotrypsin (0.5 mg/ml) was added, and the enzyme reaction followed in a fluorescence spectrophotometer (Perkin-Elmer 204; λ_{exc} 335 nm, λ_{em} 415 nm) for 20 min at room temperature (Bieth & Wermuth, 1973; Rinderknecht & Fleming, 1975).

Hydrolysis of Z-Lys-ONp (dissolved in water), Z-Tyr-ONp and Z-Ala-ONp (dissolved in acetone) was tested by measuring the spectral changes in the range 360–405 nm associated with the formation of free *p*-nitrophenol. Substrate stock solutions (10 mM) were added to 2 ml of buffer to give final

substrate concentrations of 10–160 μM . The initial velocity after addition of the enzyme was corrected for spontaneous hydrolysis (Ascenzi *et al.*, 1980, 1982). The following buffers were used: 0.1 M-sodium acetate, pH 3.5–6, 0.1 M-sodium phosphate, pH 6–8, and 0.1 M-Tris/HCl, pH 7–9.

With regard to Z-Ala-ONp, which was found to be the most suitable substrate for CBPP, the following standard test was used. A 20 μl volume of Z-Ala-ONp (10 mM in acetone) was mixed with 2 ml of 0.1 M-Tris-HCl buffer, pH 7.9; 10 μl of CBPP (0.01–0.4 mg/ml) was added and substrate hydrolysis was monitored at 405 nm (Unicam SP.1800 spectrophotometer). The temperature was held constant at 23–25°C. Ca^{2+} was not present in the reaction mixture; control experiments with added CaCl_2 (up to 10 mM) showed no effect on the enzyme activity.

Inhibition assays

The effect of various proteinase inhibitors on the esterolytic activity of CBPP was studied by incubating the pancreas protein with the appropriate inhibitors at room temperature. Stock solutions (10 mg/ml) of Tos-Lys- CH_2Cl and Tos-Phe- CH_2Cl were prepared in 50 mM-Tris/HCl buffer, pH 7.0, and acetone respectively. Phenylmethanesulphonyl fluoride (100 mM) and di-isopropyl phosphorofluoridate (100 mM) were prepared in propan-2-ol, and soya-bean trypsin inhibitor (10 mg/ml) was in 1 mM-HCl. A 10 μl portion of inhibitor solution was incubated with 90 μl of CBPP (0.2 mg/ml) in 50 mM-Tris/HCl buffer, pH 7.0. The activity against Z-Ala-ONp was determined in the standard test in comparison with non-treated CBPP over a period of up to 4 h.

Determination of CBPP in pancreatic secretions

The concentration of CBPP in pancreatic secretions was determined by single radial diffusion in agarose containing 0.75 μl of sheep anti-CBPP serum/ cm^2 . Purified CBPP was used for calibration.

Results

Evidence for a proteolytic activity in CBPP preparations

Incubation of CBPP preparations with various protein substrates consistently led to degradation of the proteins. Fig. 1 depicts SDS/polyacrylamide-gel electrophoresis patterns of CBPP (gel a), casein (gel b) and staphylococcal α -toxin (gel d). After the exposure of casein or α -toxin to CBPP these proteins were degraded to small- M_r products (gels c and e respectively). The digestion of α -toxin is particularly striking, since this protein is relatively

Fig. 1. Proteolytic activity of CBPP against casein and staphylococcal α -toxin

The Figure shows the SDS/polyacrylamide-slab-gel (12.5% polyacrylamide) electrophoretograms of (a) purified CBPP (10 μg), (b) casein (40 μg), yielding two protein bands, (c) casein (40 μg) incubated with CBPP (1 μg) in 10 mM-Tris/50 mM-NaCl medium, pH 8.2, for 10 min at 37°C, (d) staphylococcal α -toxin (15 μg), (e) staphylococcal α -toxin (20 μg) incubated with CBPP (2 μg) in 10 mM-Tris/50 mM-NaCl medium, pH 8.2, for 30 min at 37°C.

resistant towards the action of a number of proteinases (Watanabe & Kato, 1978).

Specificity of proteolytic cleavage by CBPP preparations

In order to gain insight into the specificity of peptide cleavage exhibited by CBPP preparations, I analysed products of the digestion of glucagon and studied the action of the proteinase on various chromogenic substrates.

Peptides in the digests of glucagon were separated by t.l.c. on cellulose acetate. As shown in Fig. 2, 26 peptides were discernible, of which nine predominating peptides were selected for amino acid analysis (Table 1). The amino acids of peptides 1–4 and 6–9 are compatible with distinct sequences of the glucagon molecule, as indicated in Fig. 3.

Fig. 2. Peptide map of CBPP digest of glucagon

The peptides obtained by digestion of 133 μ g of glucagon with 1.6 μ g of CBPP for 6 h at 37°C were separated on cellulose thin-layer plates. First-dimension electrophoresis (right to left) was in pyridine/acetone/acetic acid/water (2:15:4:79, by vol.), and second-dimension chromatography (bottom to top) in pyridine/butan-1-ol/acetic acid/water (1:5:33:4, by vol.). The dotted line indicates the solvent front. Predominating peptides 1–9 were eluted for amino acid analyses.

Table 1. Amino acid analysis of peptides 1–9 from glucagon digested by CBPP

Amino acid	Peptide	Amino acid composition (residues/100 residues)								
		1	2	3	4	5	6	7	8	9
Asp		21.8	38.3	29.1	1.7	9.5	1.5	12.4	14.0	10.6
Thr		–	39.1	26.3	3.7	11.9	20.3	2.3	1.3	1.3
Ser		2.1	5.9	7.0	4.7	16.5	18.0	16.7	12.7	10.3
Gln		18.4	1.6	4.7	19.3	6.8	21.6	4.2	3.7	2.9
Gly		1.9	4.0	7.2	4.1	7.2	21.7	7.0	6.2	5.1
Ala		1.8	3.7	3.0	3.5	5.6	1.7	15.9	12.6	11.0
Val		21.3	–	–	5.2	–	–	–	–	–
Met		–	–	9.6	8.7	–	–	–	–	–
Leu		–	–	–	32.8	5.6	–	–	9.1	11.0
Tyr		–	–	–	–	9.5	–	–	–	8.4
Phe		20.5	–	–	5.0	6.8	–	–	–	–
Lys		5.9	1.4	4.5	2.9	4.7	0.9	1.4	2.5	4.9
Arg		–	–	–	–	10.4	1.3	31.8	25.5	19.2

Catalytic activity of CBPP against small- M_r synthetic substrates

The action of CBPP on several chromogenic substrates was tested in comparison with trypsin and chymotrypsin. The enzyme failed to hydrolyse the substrates Bz-Phe-ONap, Bz-Tyr-OEt, Bz-Arg-Nan, Tos-Arg-OMe and Ac-Ala-Ala-Ala-Nan, the classical substrates for chymotrypsin, trypsin and elastase (Walsh & Wilcox, 1970; Bieth & Wermuth, 1973). It was also inactive against Z-Tyr-ONp or Z-Lys-ONp, the latter of which is a substrate for a wide variety of proteinases (Ascenzi *et al.*, 1982). Of the substrates tested, only Z-Ala-ONp was rapidly hydrolysed. This finding agrees well with the previous results on glucagon cleav-

age, and indicates the carbonyl group of alanine as a favoured site of proteolytic attack.

These results discriminate the proteolytic action of CBPP preparations from trypsin, chymotrypsin and elastase activities. The availability of a suitable chromogenic substrate rendered possible a detailed study of the effect of SDS and urea on CBPP.

Effect of SDS and urea on CBPP

Enzyme activity was destroyed by 0.1% SDS in 20 min. The process of enzyme inactivation was paralleled by the disappearance of the 28 000- M_r protein band in SDS/polyacrylamide-gel electrophoresis. An identical behaviour, though following

Fig. 3. Action pattern of CBPP on glucagon, derived from the amino acid analysis of peptides 1-4 and 6-9

a different time course, was observed with urea. In 6M-urea a 50% decrease in enzyme activity occurred after 24h at room temperature. In 8M-urea autodigestion and loss of enzyme activity was complete within 60min. The time course of disappearance of the 28000- M_r protein and loss of esterolytic activity in 0.1% SDS and in 8M-urea are summarized in Fig. 4.

These results indicated that cleavage of CBPP in the presence of SDS or urea resulted from autodigestion of the protein in these denaturants. Since proteolytic activity must be an innate function of the cholesterol-binding protein, it is now designated as 'cholesterol-binding pancreatic proteinase' (CBPP).

Catalytic properties of CBPP

pH- and temperature-dependence. The pH-dependence of the esterolytic cleavage of Z-Ala-ONp (Ascenzi *et al.*, 1980) by CBPP showed a strong increase in activity at pH values above 7 with a maximum at pH 8.4. Elevating the temperature resulted in an acceleration of substrate cleavage, and maximal velocity was found at 41°C.

Determination of K_m and V_{max} .

The initial velocity of Z-Ala-ONp cleavage was determined at increasing substrate concentrations in 0.1 M-Tris/HCl buffer, pH 7.0, at 25°C. Kinetic parameters describing the course of the function were evaluated by applying a BMDP 77 program (program P3R; Biomedical Computer Programs, Los Angeles, CA, U.S.A.). This program estimates the parameters of a non-linear function by least squares with a Gauss-Newton algorithm and calculated a maximal molecular activity of $13.8s^{-1}$ and a K_m of 47.06 μM .

Effect of proteinase inhibitors

Tos-Phe-CH₂Cl and Tos-Lys-CH₂Cl did not interfere with the esterolytic activity of CBPP at an inhibitor/enzyme molar ratio of 300:1 (4h incubation). Soya-bean trypsin inhibitor was inactive in a 5-fold (w/w) excess. However, di-isopropyl phosphorofluoridate and phenylmethanesulphonyl fluoride, in a molar excess of 500:1, each inactivated CBPP within 4min.

Temperature- and pH-sensitivity of CBPP

At 4°C CBPP was stable over months without any loss of enzymic activity. CBPP stored at room temperature in 50mM-Tris/100mM-NaCl medium, pH 7.9, containing 15mM-NaN₃, with or without 10mM-CaCl₂ lost approx. 8% of its esterolytic activity per week. At 56°C there was a rapid inactivation and the esterolytic activity disappeared within 5min.

Fig. 4. Autodigestion of CBPP in the presence of SDS or urea

CBPP (1 mg/ml) was incubated with 0.1% SDS or 8M-urea in 10mM-Tris/50mM-NaCl medium, pH 8.2, at room temperature. At the time intervals given, 20 μ l portions were withdrawn, added to preheated SDS (2%, w/v) for SDS/polyacrylamide-gel electrophoresis and tested for hydrolytic activity towards Z-Ala-ONp. Apart from the time course of autodigestion, similar results were obtained with SDS and urea.

pH-sensitivity was tested by incubation of CBPP (0.1 mg/ml) in 50mM-NaOH, 50mM-HCl or 50mM-acetic acid for 4 h at room temperature. Thereafter the esterolytic activity was determined in the standard test and compared with that of non-treated CBPP. CBPP proved to be relatively resistant towards acid insofar as the activity decreased by 20% by incubation in acetic acid and by 60% in HCl. All activity was destroyed by NaOH treatment.

Binding of CBPP to serum proteins

The proteinase nature of CBPP was further indicated through its interaction with serum proteinase inhibitors. Autoradiographs revealed binding of the pancreas protein to two serum components, which were identified as α_2 -macroglobulin and α_1 -antitrypsin by the use of specific antisera (Figs. 5a–5c). When antiserum to CBPP was incorporated into the intermediate gel, antitrypsin-associated CBPP became precipitated in the lower gel, disso-

ciated from the serum protein. In contrast, α_2 -macroglobulin-bound CBPP did not react with the CBPP-specific antibodies and no dissociation was found in this case (Fig. 5d).

To exclude the possibility that a proteinase impurity could be responsible for the radiolabelling of the proteinase inhibitors, 125 I-labelled CBPP was electrophoresed in a SDS/polyacrylamide gel. When 125 I-labelled CBPP was added to preheated SDS, radioactivity coincided with the Coomassie Blue-stained band of CBPP (Figs. 6a and 6b). As with native CBPP, a delay of 5 min after addition of CBPP and heating induced a complete disappearance of the 28000- M_r band and the appearance of traces of radioactivity in smaller- M_r regions of the gel (Fig. 6c).

Concentration of CBPP in pancreatic secretions

The total protein content in ten pancreatic secretions from patients lacking pathological pancreas parameters varied from 3.2 to 6.5 mg/ml. The con-

Fig. 5. Interaction of CBPP with serum proteins

(a) Two-dimensional immunoelectrophoresis of human serum, incubated with ^{125}I -labelled CBPP. First-dimension electrophoresis was performed for 45 min at 10 V/cm. Second-dimension immunoelectrophoresis was performed at 2 V/cm for 16 h in gels containing antibodies to human serum proteins (code 100 SF; DAKO) ($10\ \mu\text{l}/\text{cm}^2$). The autoradiograms of the dried plates are shown below the corresponding Coomassie Blue-stained immunoplates, where arrows point at the radioactive immunoprecipitates. (b) and (c) Identification of the radioactive immunoprecipitates by specific antibodies in intermediate gels. (b) contained antiserum against α_1 -antitrypsin (a-AT) (DAKO) ($2\ \mu\text{l}/\text{cm}^2$). (c) contained antiserum against α_2 -macroglobulin (a-MG) (DAKO) ($2\ \mu\text{l}/\text{cm}^2$). With CBPP-specific antibodies (a-CBPP) ($2\ \mu\text{l}/\text{cm}^2$) only CBPP associated with α_1 -antitrypsin was precipitated in the lower gel and was dissociable from the serum protein (d).

Fig. 6. SDS/polyacrylamide-gel electrophoresis of CBPP and autoradiography of radiolabelled CBPP

(a) Coomassie Blue-stained SDS/polyacrylamide-gel electrophoretogram of CBPP. (b) and (c) Autoradiography of ^{125}I -labelled CBPP (b) immediately boiled in SDS and (c) after incubation in 1% SDS for 5 min at room temperature. The left track shows a marker protein mixture containing carbonic anhydrase (M_r 30000), soya-bean trypsin inhibitor (M_r 20100) and lactalbumin (M_r 14400).

centration of CBPP was determined to be 0.17–0.36 mg/ml. On an average CBPP was estimated to comprise 4–6% of the total protein in pancreas secretions.

Discussion

Investigations on the previously isolated cholesterol-binding protein disclosed an intrinsic proteolytic activity. The properties of this novel pancreatic proteinase, which I provisionally term 'cholesterol-binding pancreatic proteinase' (CBPP), differ markedly from those of the established pancreas proteinases trypsin, chymotrypsin and elastase. Thus CBPP did not cleave Bz-Arg-Nan and Tos-Arg-OMe or Bz-Tyr-OEt, the classical synthetic substrates for trypsin and chymotrypsin. The apparent preferential selectivity of CBPP for the carbonyl bond of alanine might be a property common with elastase (Narayanan & Anwar, 1969) or the elastase-resembling proteinase E described by Mallory & Travis (1975). Like CBPP, these enzymes cleave Z-Ala-ONp. However, CBPP did not hydrolyse Ac-Ala-Ala-Ala-Nan, a substrate relatively specific for elastase. The M_r values of elastase (30 500) and the protein-

ase E (31000) are higher than that of CBPP (28000). Moreover, the isoelectric point of elastase is between 7 and 8 (Scheele *et al.*, 1981), whereas that of CBPP is 4.9.

Thus CBPP is clearly not identical with elastase or proteinase E. Finally, autolytic digestion of CBPP in SDS, presumably due to partial unfolding of the molecule (Maroux & Desnuelle, 1969), is a unique property not known to be shared by other pancreas proteinases.

Among the proteinase inhibitors tested, only diisopropyl phosphorofluoridate and phenylmethanesulphonyl fluoride in a molar excess of 500:1 effected complete inhibition within a few minutes, classifying CBPP as a serine proteinase (Gold, 1965). Phenylmethanesulphonyl fluoride-treated CBPP was also found to be stable in SDS.

The amino acid analyses of the products of glucagon cleavage by CBPP reported in the present paper are as yet preliminary, because only a limited number of peptides were analysed. Nevertheless, the amino acid compositions of eight peptides were compatible with distinct sequences of the glucagon molecule and indicated a cleavage pattern different from those obtained with trypsin (Keil-Dlouha *et al.*, 1971) and chymotrypsin (Enenkel & Smillie, 1963). The apparent preferential selectivity for the carbonyl bond of alanine deduced from the present study is supported by the catalytic rate of esterolysis of the chromogenic substrate Z-Ala-ONp.

The somewhat surprising fact that CBPP has eluded detection to date probably derives from its unusual properties. Thus the proteinase would not be detected with the classical chromogenic proteinase substrates Bz-Arg-Nan, Tos-Arg-OMe and Bz-Tyr-OEt. Owing to its rapid autodigestion in SDS, the protein would not be detected in SDS/polyacrylamide gels unless special precautions were taken. Thus, using the two-dimensional SDS/polyacrylamide-gel electrophoresis separation system of Scheele *et al.* (1981), I failed to recover CBPP in the gel slabs (A. Sziegoleit, unpublished work). It is noteworthy that a discrepancy has indeed been observed between the proteolytic capacity of unfractionated pancreatic juices as opposed to the combined proteolytic activities of the established proteinases (Guy & Figarella, 1981; Kroghdahl & Holm, 1983). With the addition of CBPP to the list of pancreas proteinases, this discrepancy may become partly resolved. My present estimates indicate that CBPP indeed represents a major proteinase of the pancreas, constituting up to 6% of total protein in pancreatic secretions. Further investigations are required to elucidate the physiological role of CBPP and should disclose whether its capacity to bind cholesterol is simply an accidental property, or whether this property is of biological

relevance in gastroenteric protein-lipid metabolism.

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