A Family of 16-kDa Pancreatic Secretory Stress Proteins Form
Highly Organized Fibrillar Structures upon Tryptic Activation*

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A group of 16-kDa proteins, synthesized and secreted by rat pancreatic acinar cells and composed of pancreatic
stone protein (PSP/reg) and isoforms of pancreatitis-associated protein (PAP), show structural homologies,
including conserved amino acid sequences, cysteine residues, and highly sensitive N-terminal trypsin cleavage sites, as well as conserved functional responses in conditions of pancreatic stress. Trypsin activation of recombinant stress proteins or counterparts contained in rat pancreatic juice (PSP/reg, PAP I and PAP II) resulted in conversion of 16-kDa soluble proteins into 14-kDa soluble isoforms (pancreatic thread protein and pancreatitis-associated thread protein, respectively) that rapidly polymerize into insoluble sedimenting structures. Activated thread proteins show long lived resistance to a wide spectrum of proteases contained in pancreatic juice, including serine proteases and metallopeptinases. In contrast, PAP II, following activation with trypsin or pancreatic juice, does not form insoluble structures and is rapidly digested by pancreatic proteases. Scanning and transmission electron microscopy indicate that activated thread proteins polymerize into highly organized fibrillar structures with helical configurations. Through bundling, branching, and extension processes, these fibrillar structures form dense matrices that span large topological surfaces. These findings suggest that PSP/reg and PAP I and III isoforms consist of a family of highly regulated soluble secretory stress proteins, which, upon trypsin activation, convert into a family of insoluble helical thread proteins. Dense extracellular matrices, composed of helical thread proteins organized into higher ordered matrix structures, may serve physiological functions within luminal compartiments in the exocrine pancreas.

Pancreatic juice in vertebrates contains a group of 16-kDa proteins without known enzyme, proenzyme, or inhibitor function in the digestive process. This group, without defined function, is composed of the following protein species. Pancreatic stone protein (PSP/reg) is a 16-kDa acidic protein with an isoelectric point in the range of pH 5.5–6. A truncated form of this protein was originally isolated from calcium carbonate stones surgically removed from the main pancreatic duct of humans with chronic pancreatitis (1). For several years it was believed that PSP/reg served as an inhibitor of calcium carbonate precipitation in pancreatic juice, and it was proposed that its name should be changed to "liithostathine" (2). However, it was later shown that PSP/reg has no more crystal inhibitory activity than several of the pancreatic digestive enzymes (3, 4). Other studies have demonstrated that the expression of PSP/reg protein is increased during the regeneration of islets after nicotinamide treatment and partial pancreatectomy (5, 6). These observations led to the conclusion that PSP/reg may be a protein involved in regeneration (7) and furthermore may act as a growth mediator stimulating the proliferation of β-cells. Tissue culture studies implied a mitogenic activity of PSP/reg on the growth of various cell types (8, 9), and application of PSP/reg was observed to partially ameliorate diabetes in NOD mice (10). Recently, a receptor was cloned from regenerating islets that binds PSP/reg and causes an increase in proliferation of cells transfected with a vector containing the receptor cDNA (11). Reg II (PAP I) appears to be involved in regeneration of motor neurons by acting as a Schwann cell mitogen (12).

Still other investigations sought to implicate PSP/reg in the digestive process. However, recent studies did not show regulated PSP/reg synthesis and secretion in response to changes in nutritional substrates in the diet (13).

Pancreatitis-associated protein (PAP) is a 16-kDa basic protein with an isoelectric point in the range of pH 6.5–7.6. Although most species contain a single PAP form, rat contains three isoforms, PAP I, PAP II, and PAP III, transcribed from three separate genes (14–16). PAP levels increase in pancreatic juice during experimental (17) and clinical (18) pancreatitis. Although showing an acute phase response under conditions of pancreatic disease, the function of PAP remains unknown. PSP/reg and PAP forms, cloned in the rat, mouse, cow, and man, show similarities in amino acid sequence. At the C terminus there is a C-type lectin binding sequence, and it has been proposed that this site might confer bacterial resistance on PAP (14, 19). Recent studies have demonstrated that PSP/reg and PAP both act as acute phase reactants in pancreatic juice under a variety of conditions including acute pancreatitis (20), chronic pancreatitis in male WBN/Kob rats, and during the regenerating protein; CTL, C-type lectin domain (Ca²⁺-dependent carbohydrate-recognition domain); PAP, pancreatitis-associated protein; PATP, pancreatitis-associated thread protein; PTF, pancreatic thread protein; SEM, scanning electron microscope; STEM, scanning/transmission electron microscope; PCR, polymerase chain reaction; MES, 4-morpholinethanesulfonic acid; PAGE, polyacrylamide gel electrophoresis.

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1 The abbreviations used are: PSP/reg, pancreatic stone protein,
post-weaning period (21). Trypsin cleavage of PSP/reg and PAP has resulted in the appearance of precipitated proteins believed to represent insoluble thread structures in humans (22) and cows (23). However, it remains difficult to understand how precipitation properties could serve useful functions in pancreatic physiology, and it is not clearly known whether these precipitated protein forms demonstrate specific or nonspecific structures.

By taking a different approach in this study, we have attempted to define the functional as well as structural similarities shared between these molecules as a means to generate clues related to their function. To this end we have cloned, expressed, and purified recombinant forms of PSP/reg, PAP I, PAP II, and PAP III in the rat (24, 25). These purified reagents have allowed us to investigate the structural and functional properties of these proteins before and after trypsin cleavage to search for a unifying hypothesis that might explain the function of these proteins in pancreatic physiology and pathology.

In this paper we have studied the structural/functional consequences of trypsin activation on these proteins with respect to (i) resistance of the processed forms to trypsin as well as to sequences of trypsin activation on these proteins with respect to properties of these proteins before and after trypsin cleavage to have allowed us to investigate the structural and functional properties of these proteins before and after trypsin cleavage to search for a unifying hypothesis that might explain the function of these proteins in pancreatic physiology and pathology.

In the heterogeneous mixture of proteases in pancreatic juice, (ii) the kinetics of conversion from soluble to insoluble protein forms, (iii) the kinetics of assembly of protein subunits into polymerized thread structures, and (iv) the morphology of polymerized thread structures by scanning, transmission scanning electron microscopy.

**EXPERIMENTAL PROCEDURES**

**Recombinant PSP/reg—**Recombinant PSP/reg was produced in the baculovirus system and purified as described previously (3, 24). Monospecific antibodies directed against rat PSP/reg were generated in rabbits as described earlier (24).

**Construction of Recombinant PAP Isoforms in Ficha pastoris Vectors—**PAP I cDNA and PAP II cDNA were amplified using the polymerase chain reaction (PCR) from a rat pancreas cDNA library using PAP I- and II-specific primers and confirmed by DNA sequencing. PAP III cDNA was isolated by reverse transcriptase-PCR using rat ileum mRNA as a template. The cDNAs were subsequently digested with restriction enzymes to check for inserts, and some were subsequently sequenced to confirm the correct orientation of the coding sequence.

**Purification and Analysis of Recombinant Proteins—**High yield media were collected by centrifugation at 1500 × g for 5 min at 4 °C. The supernatants were centrifuged again at 5000 × g for 15 min. The supernatants were diluted 1:3 with chilled MilliQ filtered water (Millipore) and adjusted to pH 3.6 with HCl. Diluted protein solution (1200–1600 ml) was applied at a rate of 5 ml/min to a cation exchange column (SP-Sepharose, Amersham Pharmacia Biotech, 26 × 80 mm) with a bed volume of 40 ml. The column was washed with 2 volumes of starting buffer (50 mM MES, 10 mM LiCl, pH 5.3). The proteins were eluted with a linear LiCl gradient (0–35% elution buffer: 50 mM MES, 2 mM LiCl, pH 6.3), generated by an AKTA purifier system (Amersham Pharmacia Biotech).

**Analysis of Purified Recombinant Proteins by Mass Spectrometry and N-terminal Sequencing—**To verify the identity of each of the isoforms, both mass spectrometry (electrospray mass analysis, PerkinElmer Life Sciences) and N-terminal amino acid sequencing was performed. The secreted, purified forms of PAP I, II, and III were desalted by ultrafiltration. To identify the new N terminus of the trypsin-resistant 14-kDa protein, each isoform was digested with trypsin as described below. The solutions were centrifuged to pellet the fibrils and remove the cleared supernatant fraction. For PAP II, the solution was filtered after digestion with an ultrafiltration device that retained the C-terminal peptide (10-kDa molecular weight cut-off, Centricon, Millipore). For mass analysis, aliquots were adsorbed to C18 Ziptips (Millipore), eluted with 78% methanol, 1% formic acid, and injected into the analyzer.

**Production of Antiserum against PAP—**One hundred micrograms of recombinant PAP II were injected in Freund's complete adjuvant into several subcutaneous deposits in the back of a New Zealand White rabbit and a guinea pig. After 1 month the animals were boosted with 50 μg of PAP II in Freund's incomplete adjuvant followed by a similar boost a month later. Antibody titers were monitored in serum after venopuncture of the ear vein. Terminal bleeding was performed under anesthesia (Ketamine/Xylazine) by heart puncture. The antibody directed against recombinant PAP II reacts with PAP II (100%), PAP III (35%), PAP I (<10%), PATP II (80%), PATP III (<10%), and PATP I (<10%) but does not react with PSP/reg.
Tube, and the pellet fraction was washed with 50 μl of Tris-Calcium buffer and centrifuged as described above. The pellets were dissolved in Tris-Calcium buffer in the original volume of the sample. They were prepared for electrophoresis by adding a 0.5 v/v volume of 3-fold concentrated SDS-sample buffer (150 mM Tris-HCl, pH 6.8, 10% SDS, 0.1% bromphenol blue, 15% glycerol v/v) followed by heating for 5 min at 90°C. To maximize immunoreactivity of the activated protein forms, β-mercaptoethanol was omitted from the buffer solutions.

**Tryptic Activation of PAP, PSP/reg, and Pancreatic Juice for Morphological Analysis**—Activation of the recombinant proteins was performed in a volume of 100 or 200 μl in Tris-Calcium buffer. Proteins (10 or 20 μl) were activated with 0.5–1 μg of trypsin (Worthington) for 30 min at 37°C.

**SDS-PAGE and Western Blotting**—Standard 15% polyacrylamide gels were prepared in SDS. Protein samples were heat-denatured at 90°C in SDS-sample buffer (50 mM Tris-HCl, pH 6.8, 1% SDS, 0.005% bromphenol blue, and 5% glycerol) in the presence or absence of β-mercaptoethanol as indicated above. Proteins resolved in gels were stained with Coomassie Brilliant Blue (0.1% in 30% methanol, 10% acetic acid, Bio-Rad). For Western blot analysis, the proteins were transferred to polyvinylidene difluoride membranes (Bio-Rad) on a semidry blotting apparatus (Amersham Pharmacia Biotech). The membranes were blocked with 1% bovine serum albumin in Tris-buffered saline (20 mM Tris, pH 7.5, 150 mM NaCl). Guinea pig anti-PAP II, diluted 1:3000 (256), and phosphorilated anti-guinea pig IgG (Sigma), diluted 1:1-100,000 (Sigma), were employed to detect PAP and PAPT. For the detection of PSP/reg and PTP, chemiluminescence (ECLplus, Amersham Pharmacia Biotech) was employed. The primary rabbit anti-PSP/reg antibody was diluted 1:20,000 in Tris-buffered saline containing 1% bovine serum albumin. The membranes were washed in Tris-buffered saline containing 0.05% Tween 20. The secondary antibody, peroxidase-coupled goat anti-rabbit IgG (Sigma) was diluted 1:25,000 in the same buffer. Development and detection followed the manufacturer’s recommendations.

**Densitometry**—Relative quantities of individual protein bands were estimated by densitometry. Coomassie Blue-stained gels or Western blots were scanned with a Scanjet 6300C (Hewlett-Packard). The files were imported into Adobe Photoshop and quantified using ImageQuant (Molecular Dynamics) software. Intensities were expressed in percent of the maximal value as indicated in the figure legends.

**Differential Centrifugation of Thread Proteins**—Recombinant thread proteins were diluted to 10 μg/50 μl in Tris-Calcium buffer and centrifuged in Microfuge tubes (Beckman) using a table top ultracentrifuge (JSM-25S II) for 30 min at 4°C. Following centrifugation of the sample to 1000 × g (4°C, 30 min), the supernatant fractions were transferred to new tubes and centrifuged at 10,000 × g for 30 min at 4°C. The resulting supernatant fractions were centrifuged at 100,000 × g for 30 min at 4°C. The pellets were washed with Tris-Calcium buffer (described above) and redissolved in the original volume. Pellet and supernatant fractions were denatured in the presence of SDS-sample buffer, including 1% β-mercaptoethanol, for 5 min at 90°C and submitted to SDS-PAGE.

**Analysis of Protein Matrices by Scanning Electron Microscopy** (SEM) for electron microscopy—Microscopy of a layer of nylon mesh, 3 cm², were produced by folding a small piece of nylon mesh. The edges were heat-sealed, except for a small hole through which the samples could be introduced. The activation mixtures were transferred into the pouch, closed with a clamp, and submerged in 50 mM sodium cacodylate, pH 7.5, for 30 min at 4°C. The pouch was then transferred to 2% glutaraldehyde in 100 mM sodium cacodylate, pH 7.5. After fixation at 4°C overnight, the pouches were opened and processed for SEM analysis using standard procedures. The samples were viewed on a JEOL (JSM-25S II) scanning electron microscope. Photographs were taken via an attached computer using the software DISS (Digital Image Scanning System, Prophysics, Switzerland).

For scanning transmission electron microscopy (STEM, Philips CM 120) analysis, a small amount of fibrillar material was removed from the surface of the pouch and plated with gold. To exclude gold-derived artifacts, the following pilot study was performed. After glutaraldehyde fixation, the pouch (see above) was immersed in 2% OsO₄, 0.1 M sodium cacodylate buffer for 3 h. The pouch was rinsed and processed for SEM and STEM. In the absence of gold plating, the quality of resolution of the protein matrix was inferior compared with the gold-plated sample. However, it was concluded that the structure of the unprotected sample was comparable to the structure of the gold-plated sample.

**Analysis of Protein Matrices by Transmission Electron Microscopy**—To prepare thin sections of fibrils for examination in the electron microscope, the activation mixtures were initially embedded in Epon. For scanning electron microscopy, pouches of nylon mesh, 3 cm², were produced by folding a small piece of nylon mesh. The edges were heat-sealed, except for a small hole through which the samples could be introduced. The activation mixtures were transferred into the pouch, closed with a clamp, and submerged in 50 mM sodium cacodylate, pH 7.5, for 30 min at 4°C. The pouch was then transferred to 2% glutaraldehyde in 100 mM sodium cacodylate, pH 7.5. After fixation at 4°C overnight, the pouches were opened and processed for SEM analysis using standard procedures. The samples were viewed on a JEOL (JSM-25S II) scanning electron microscope. Photographs were taken via an attached computer using the software DISS (Digital Image Scanning System, Prophysics, Switzerland).

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**Analysis of Protein Matrices by Transmission Electron Microscopy**—To prepare thin sections of fibrils for examination in the electron microscope, the activation mixtures were initially embedded in Epon. However, this approach required extensive centrifugation after each medium change and a polymerization step at 60°C. The resulting preparations appeared amorphous due to the loss of the fibrillar architecture. To circumvent this problem, we omitted centrifugation and used an alternative procedure that did not require polymerization at 60°C. The dried sheets were stained with 2% glutaraldehyde and left at 4°C overnight. Then OsO₄ was added to a final concentration of 2 mM, and fixation was continued at room temperature for another hour. The fibrils demonstrated a black appearance and settled to the bottom of the vessel. Unircyl (British Biocell Int., Cardiff, UK), an embedding medium that polymerizes at low temperatures under UV light, was used according to the manufacturer’s recommendation. This section was cut with a diamond knife and examined in an electron microscope (Philips 400). As a negative control for this procedure, buffer was substituted for the activation mixture and processed as described above. Although some amorphous material was generated during OsO₄ fixation, the fibrillar structures were not observed in the control.

For negative contrast staining of fibrils, a small drop of activated sample was placed on the surface of a grid, dried, exposed to phosphotungstic acid (27), and examined in the electron microscope.

**Computer Programs for Sequence Analysis**—The multiple sequence alignment was created by “PileUp,” an algorithm for progressive, pairwise sequence alignments by Feng and Doolittle (28). The same program produces a dendrogram that depicts the clustering relationships. The determination of sequence similarity and identity was performed by pairwise analysis using the “gap” program of Genetics Computer Group (Madison, WI). GCG is run on a UNIX system (Silicon Graphics) maintained by the Rechenzentrum der Universität Zürich.

**RESULTS**

Conservation of the N-terminal and C-terminal Peptides in a Family of secretory stress proteins...- Fig. 1 shows the sequence alignment of the N-terminal and C-terminal regions of three isoforms of PAP with PSpreg in the rat. The mature secretory proteins are defined by two peptide domains separated by a highly conserved trypsin cleavage site. In three of the four secretory stress proteins (PSPreg, PAP I, and PAP III), the Arg¹¹-Ile¹² bond represents the most sensitive cleavage site for trypsin. Upon trypsin cleavage the N-terminal undecapeptide is separated from the C-terminal peptide, which varies from 138 residues in PAP to 133 residues in PSpreg.

In addition to similarities in size, these proteins show similarities in sequence and protein domains. PSpreg shows 43% identity and 54% similarity with PAP I, 45% identity and 52% similarity with PAP II, and 42% identity and 57% similarity with PAP III. PSpreg is 7 amino acids shorter than the three PAP isoforms. The conservation of six cysteine residues suggests that three disulfide bonds are conserved. At the C terminus there is a conserved sequence indicating a C-type lectin domain (Fig. 1). Although the function of this signature sequence has not been elucidated, CTLs have been found in a variety of proteins that demonstrate diverse functions in different cellular and extracellular compartments (29). However,
Trypsin activation of pancreatic juice generates 14-kDa products that are protease-resistant and sediment under conditions of low speed centrifugation. Pure pancreatic juice from a rat with high levels of PSP/reg and PAP was activated with enterokinase. Aliquots were withdrawn at the indicated time points (min), and reactions were stopped by the addition of FOY-305, a protease inhibitor. The samples were centrifuged, and the supernatant fractions were transferred to a fresh tube. The pellet (P) and supernatant (S) fractions were analyzed separately by SDS-polyacrylamide gel electrophoresis. The top panel of the figure shows the Coomassie Blue-stained gel. M, marker proteins with the sizes indicated in kDa. The middle panel (PSP) shows an immunoblot of the same gel analyzed for the proteolytic conversion of PSP/reg to PTP (arrowheads indicate their position). The bottom panel shows an immunoblot analyzed for the proteolytic conversion of PAP to PATP (arrowheads).

The secreted and proteolytic processed forms of recombinant PSP/reg and recombinant PAP shown in Fig. 1 were analyzed by mass spectrometry and N-terminal sequencing. The secreted forms of PAP I, II, and III all conformed with the expected amino acid sequence (first 10 amino acids determined), starting with a glutamine residue in each case. The processed forms of PAP also conformed with the expected sequence (first 5–7 amino acids determined) starting with an isoleucine (PATP I, III) or a threonine (PATP II) residue. Mass analysis yielded the following measurements: PAP I, 16623.6 (theoretical 16623.6); PATP I, 15414.0 (15414.2); PAP II, 16404.2 (16403.2); PATP II, 15203.9 (15203.9); PAP III, 16247.8 (16248.0); and PATP III, 15021.5 (15019.7). Mass determinations indicated that PSP/reg and each of the PAP isoforms were not modified by glycosylation or other posttranslational mechanisms.

Trypsin Activation of Rat Pancreatic Juice Generates Protease-resistant Products with Low Solubility—By utilizing purified recombinant PSP/reg and recombinant PAP shown in Fig. 1, we have recently demonstrated that trypsin activation leads to the formation of 14-kDa trypsin-resistant products (25). In order to determine if the 14-kDa products are resistant to the wide spectrum of proteases observed in activated pancreatic juice, we studied the identity and longevity of these products generated from rat pancreatic juice containing high levels of PSP/reg and PAP (116 μg/ml). Fig. 2 shows the Coomassie Blue and immunostained products that appear in soluble and insoluble fractions of pancreatic juice, diluted into 20 volumes of buffer and activated with enterokinase at 37 °C, over a period of 960 min (16 h).

At the beginning of the experiment (0-, 0.5-, and 1.0-min time points), the major digestive enzymes and zymogens present in pancreatic juice may be easily identified (30, 31). These include amylase (major band at 55 kDa), procarboxypeptidase A and B isoforms at 40–45 kDa, and serine protease zymogens between 21 and 27 kDa. At later time points these bands disappear in response to the degradative effects of activated proteases. Despite the progressive degradation of the digestive enzymes and zymogens, a prominent Coomassie Blue-stained band appears at 14 kDa in the pellet fraction at 10 min and remains largely intact throughout the duration of the experiment.

In order to determine the composition of this 14-kDa protease-resistant band, we used polyclonal antibodies individually directed against PSP/reg and PAP to determine the representation and longevity of PTP and PATP isoforms in this region of the gel. The antibody directed against PSP recognizes both PSP and PTP. The antibody directed against PAP II reacts with PAP II (100%), PAP III (35%), PAP I (<10%), PATP II (80%), PATP III (<10%), and PATP I (<10%) but does not react with PSP/reg.

Between 1 and 10 min PSP/reg disappears from the supernatant fraction, and PTP appears in the pellet fraction. However, PTP appears in the supernatant fraction before it appears in the pellet fraction, suggesting that soluble PTP requires time to polymerize into insoluble PTP complexes. Similar findings are observed in the conversion of PAP to PATP, with two exceptions. First, whereas conversion of PSP/reg occurs between 1 and 10 min, the conversion of PAP occurs more slowly, largely between 10 and 60 min. Second, the PAP/PATP signal is less intense, due to the lower concentration of PAP isoforms and possibly compounded by a lower binding of the antibody to its target protein(s).

These findings indicate that trypsin activation leads to the rapid conversion of PSP/reg and PAP forms to trypsin- and protease-resistant forms of PTP and PATP, respectively. The longevity of PTP and PATP forms in the presence of fully activated pancreatic juice containing numerous protease forms is impressive and suggests that the two groups of thread proteins have evolved with similar properties of protease resistance that may be related to the ultimate function of these proteins in pancreatic physiology and pathology.

To gain more insight into the temporal aspects of activation and polymerization of thread proteins, the gels and blots were analyzed by densitometry. Fig. 3A shows the relative abundance of Coomassie Blue-stained products in the pellet fraction.
The sum of soluble and insoluble PTP at each time point. Maximum density was arbitrarily set at 100%.

Fig. 4) was not quantified. Similar activation kinetics were observed for assessed and processed forms with equal sensitivity. The 15-kDa band (see

The gel and immunoblots shown in Fig. 2 were analyzed by densitometry and plotted as a function of time. A, relative abundance of Coomassie Blue-stained 14-kDa bands (CBB) in the pellet fraction (E) at each time point. Maximum density was arbitrarily set at 100%. B, relative abundance of PSP/reg (O) and PTP in the soluble (ISO) and PPT in the insoluble (E) fractions. The sum of soluble and insoluble PTP at 10 min was set as 100%. C, PAP (O) and PATP (E) in the soluble and PATP in the insoluble (E) fractions were determined from the PAP immunoblot as described for B. The PAP value at 1 min was set at 100%; for PATP, the combined soluble and insoluble fraction at 10 min was set as 100%. Note that the antibodies do not recognize the unprocessed and processed forms with equal sensitivity. The 15-kDa band (see Fig. 4) was not quantified. Similar activation kinetics were observed for trypsin-activated pancreatic juice.

as a function of time. Fig. 3B shows the kinetic data for conversion of PSP/reg to PTP as well as the conversion of PTP from a soluble form to an insoluble form. The data demonstrate that proteolysis precedes the polymerization process by several minutes and identifies an intermediate state between soluble precursor and insoluble product. Furthermore, the data suggest that PTP occurs in a soluble form before its polymerization into insoluble thread structures. Fig. 3C shows kinetic data for the conversion of PAP to PATP and similarly identifies an intermediate state represented by soluble PATP prior to its polymerization into insoluble thread structures.

In the presence of fully activated pancreatic juice at 37 °C the half-life is 100–150 min for the PATP band, 800 min for the PFP band, and 400 min for the Coomassie Blue band. Thus, the activation of secretory stress proteins into insoluble thread proteins leads to strong resistance to the degradative effects of pancreatic proteases.

Kinetics of Tryptic Conversion of Secretory Stress Proteins to Soluble and Insoluble Thread Proteins in Pancreatic Juice. The results of trypsin activation on PAP I, PAP II, and PAP III and monitors proteolytic processing to smaller thread proteins in soluble and insoluble fractions. These kinetic studies demonstrate that PAP I and PAP III isoforms are rapidly, i.e. within 1 min, cleaved from 16-kDa precursors to 14-kDa products. 14-kDa products initially appear as soluble intermediate products but rapidly polymerize into insoluble sedimenting structures. The 50% threshold for formation of insoluble products was 3 and 0.8 min for PATP I and PATP III (Fig. 4, A and C), respectively. In contrast, although PATP II was rapidly processed to 15- and 14-kDa forms by trypsin (PAP II contains an additional trypsin cleavage site at Lys²–Ala⁶), these products remained soluble throughout the duration of the 16-h incubation period.

During proteolytic processing intermediate sized peptides (15 kDa) appeared transiently in the incubation mixtures containing PAP isoforms. The intermediates are consistent with proteolytic processing at lysine residues contained within the N-terminal undecapeptide (cf. Fig. 1). These intermediate forms are most prominent in the case of PAP II, which demonstrates an Arg-Thr trypsin cleavage site. This finding is consistent with Arg-Iso being a more active trypsin cleavage site than Arg-Thr, which is observed in PAP isoforms I and III.

Fig. 4, D–F, shows the survival of PATP isoforms after 16 h of incubation in the presence of (i) trypsin, (ii) activated pancreatic, and (iii) trypsin-activated PAP followed by addition of activated pancreatic juice (with low endogenous PSP/reg and PAP levels). These studies demonstrate that PATP I and PATP III isoforms show complete resistance to trypsin and partial resistance to the mixture of proteases contained in pancreatic juice. These proteases include serine proteases (trypsin, chymotrypsin, and elastase) and metalloproteinases (carboxypeptidases A and B). Thread proteins exposed to pancreatic proteases, after fibril formation had been completed, appeared to show greater resistance to pancreatic proteases (45 and 54% survival after 16 h of incubation for PATP I and PATP III, respectively) than those formed in the presence of pancreatic proteases (4 and 27% survival after 16 h of incubation for PATP I and PATP III, respectively). PATP III showed greater resistance to pancreatic proteases than PATP I. In contrast, although PATP II showed trypsin resistance, it was completely digested within 60 min of addition of activated pancreatic juice.

Secretory Thread Proteins Show Differential Sedimentation Properties—In order to compare the physical properties of insoluble thread proteins, we activated purified recombinant forms of PSP/reg, PAP I, PAP II, and PAP III. Trypsin cleavage converted these recombinant forms into PTP, PATP I, II, and III, respectively. In previous studies we had observed that PTP, PATP I, and PATP III sediment under conditions of centrifugation at 10,000 × g for 10 min at 4 °C. In contrast, PATP II could not be sedimented under these conditions.

To explore in greater detail the sedimentation properties of these four activated proteins, we studied their sedimentation at varying conditions of centrifugation. Fig. 5A shows the results of sedimentation of trypsin-activated proteins (10 µg of protein/50 µl) at 1,000, 10,000, and 100,000 × g for 30 min at 4 °C. Pellet fractions from the three sedimentation conditions and the final supernatant fractions were submitted to SDS-PAGE for each of the recombinant forms.

The data indicate that 67% of PATP I is sedimented at 10,000 × g, an additional 14% is sedimented at 100,000 × g, and 11% remains in the final supernatant fraction. Under these conditions PATP III shows greater insolubility with 87% sedimented at 10,000 × g and minimal amounts observed in the 100,000 × g fraction and the final supernatant fraction.
PTP appears to sediment over a wider range of g forces, demonstrating 25% in the 1,000 × g fraction, 52% in the 10,000 × g fraction, 23% in the 100,000 × g fraction, and negligible amounts in the final supernatant fraction. In contrast, PATP II appears to be largely soluble under these conditions: 14% sedimented up to 100,000 × g and 86% was observed in the final supernatant fraction.

In order to investigate whether PATP II enters into insoluble aggregates at acidic or alkaline pH, we suspended trypsin-cleaved PATP II at different pH values, and we monitored aggregation with SDS-PAGE after centrifugation at 10,000 × g for 10 min. Fig. 5 demonstrates the absence of sedimenting complexes at all pH values tested under these conditions.

These data confirm the insoluble nature of PTP, PATP I, and PATP III and the soluble nature of PATP II. Questions now arise whether these insoluble complexes are specific or nonspecific and whether they serve functions important in pancreatic physiology.

Trypsin Activation of Purified Recombinant Secretory Stress Proteins Generate Matrices of Highly Organized Fibrils—To characterize the morphological structures of the insoluble thread proteins (PTP, PATP I, and PATP III), the recombinant precursors were activated with trypsin and analyzed by a variety of electron microscopy methods. Previous studies that attempted to elucidate the structures of PTP-derived fibrils were hampered by fibril fragmentation and the collapse of fibrils when they were placed onto a surface and dried down. Collapse of fibrils resulted in a nonspecific amorphous appearance of the matrix complex without distinguishable markings. To circumvent the collapse of the three-dimensional architecture, we constructed small pouches of nylon mesh, which were then filled with fibril-containing solutions. The pouch could then be transferred into the various washing and fixation solutions without the need for attachment to a glass support. Fig. 6 shows fibrils generated in this manner and examined in the scanning electron microscope. With the exception of PATP II, which did not form visible fibrils, all other forms produced a matrix of highly organized fibrils.
Fig. 5. Members of the family of secretory thread proteins show differential sedimentation. A, pancreatic thread proteins were centrifuged at increasing g force as follows: initially at 1,000 x g for 30 min at 4°C, then the supernatant fractions were transferred and centrifuged at 10,000 x g for 30 min at 4°C, and finally at 100,000 x g for 30 min at 4°C. The supernatant and the pellet fractions were dissolved in sample buffer, and proteins were resolved by SDS-PAGE. The bands were analyzed by densitometry and expressed as percent of the total (pellet fractions plus the supernatant fraction). The top panel shows the section of the gel used for densitometric analysis. B, pH-independent solubility of PATP II. PAP II was digested with trypsin, acidified to pH 3, and neutralized by buffer exchange (AN). Aliquots were then mixed with buffers of various pH and centrifuged. Proteins in the pellet and supernatant were resolved by SDS-PAGE. M, markers at 14 and 18 kDa.

Fig. 6. Trypsin activation of purified recombinant secretory stress proteins generates a matrix of highly organized fibrils. Scanning electron microscopy of PTP and PATP generated in vitro. The micrographs demonstrate fibrous networks following activation of PAP I, PAP III and PSP/reg with trypsin. A and D show the matrix obtained with PATP I (A, bar 70 μm; D, 3 μm). B and E show the matrix obtained with PATP III (B, 20 μm; E, 7 μm). C and F show the matrix obtained with PTP (C, 40 μm; F, 4 μm). Inset in B gives a higher magnification micrograph showing individual fibrils that emerge from the dense matrix. A and C, part of the plastic mesh of the pouch can be seen.

Fig. 6 shows scanning electron micrographs of matrices formed from PATP I (A and D), PATP III (B and E), and PTP (C and F). Low power micrographs (A–C) indicate the appearance of tight matrices covering the nylon mesh in the pouch. Matrices appear to attach to the nylon mesh. At higher power differences were observed in matrices formed from activation of the three recombinant proteins. PATP I (D) shows tight bundling of filaments into larger diameter fibers that contribute to the meshwork. PATP III (E) shows loose bundling of filaments in a nodular distribution particularly near the edges. PTP (F) shows more delicate filaments comprising the meshwork and less bundling. The inset in Fig. 5B shows that individual fibrils appear to “grow out” of the densely polymerized matrix. Extensive branching appears in each of the observed matrices.

These studies demonstrate that insoluble thread proteins form dense, highly organized three-dimensional matrices.
Through bundling, branching, and extension processes, these fibrillar threads achieve higher ordered matrix structures that span large topological surfaces.

**High Resolution Analysis of Fibrillar Structures Using Transmission Electron Microscopy and Scanning Transmission Electron Microscopy**—In order to determine the fine structure of individual filaments, we examined the morphological appearance of matrices using a variety of techniques that employ transmission EM and scanning transmission EM (Fig. 7, A–E).

In Fig. 7A, a PTP matrix analyzed by scanning transmission electron microscopy is shown to examine the surface and branching properties. To exclude artifacts caused by gold plating, we explored whether the size and surface features of filaments were altered by gold spraying methods. Osmium-fixed samples were examined (i) without prior gold treatment, (ii) with lightly sprayed gold treatment (shown in A), and (iii) with the standard gold treatment. The morphology of the filaments showed little or no change as a function of gold treatment. However, the visualization of the filaments was improved with gold spraying.

Fig. 7, B (PTP) and C (PATP III), shows filaments that were negatively stained with phosphotungstic acid. This procedure, which causes extensive fragmentation of filaments, compares favorably with negatively stained micrographs that appear in the literature (22, 23).

To examine the higher ordered structure of polymerized thread proteins, fibrils were fixed in glutaraldehyde and osmium, embedded in low temperature Unicryl, sectioned, and examined with transmission EM. Under these conditions filaments (Fig. 7, D, PTP, and E, PATP III) appeared to be composed of subunits assembled into helical fibrillar structures. Favorable sections in this micrograph also suggested the presence of cavities within PATP III filamentous structures.

The data indicate that, regardless of the method employed or the protein species examined (PTP, PATP I, and PATP III), filaments exhibit a consistent diameter of ~15–20 nm.

**Morphological Structure of Filaments and Matrices Generated from Activated Pancreatic Juice**—In order to determine if matrices and filaments formed from pancreatic juice mimic those observed from activated recombinant stress proteins, we activated rat pancreatic juice and prepared it for examination by scanning EM using the nylon pouch method described above.

We chose samples of pancreatic juice obtained from male WBN/Kob rats, which contain high levels of PSP/reg (150 μg/ml) and PAP (50 μg/ml) and spontaneously develop chronic pancreatitis. Fig. 8, A and C, shows low power and high power micrographs, respectively, of matrices generated by trypsin activation of pancreatic juice from normal Wistar rats (PSP/reg, 14 μg/ml; PAP, 1.4 μg/ml). The morphological appearance of the filaments and three-dimensional architecture of the matrix appear to be intermediate between those observed for pure PTP and pure PATP I in Fig. 6.

Fig. 8, B and D, shows low power and high power micrographs, respectively, of matrices generated by trypsin activation of pancreatic juice from normal Wistar rats (PSP/reg, 14 μg/ml; PAP, 1.4 μg/ml). The morphological appearance of the filaments and three-dimensional architecture of the matrix appear to be intermediate between those observed for pure PTP and pure PATP III in Fig. 6. We also noted that the amount of matrix covering the nylon mesh pouch was considerably less in the sample shown in B than that of A and correlated with the levels of secretory stress proteins in the two pancreatic juice samples.

These data indicate conclusively that trypsin activation of pure pancreatic juice can generate insoluble matrices of filamentous thread proteins that have the capacity to attach to and cover large surface areas in a manner similar to that observed for matrices generated from purified recombinant secretory stress proteins.

**DISCUSSION**

This study investigates a group of 16-kDa proteins present in rat pancreatic juice without known enzymatic or inhibitory function in the digestive process. In addition to the similarity in size between PSP/reg and PAP isoforms, a comparison of amino acid sequences reveals significant structural homologies, including conservation in the position of six cysteine residues, which implies the conservation of three disulfide bonds. The conserved structural features of PSP/reg and PAP, including the locations of tryptophan residues, suggest that the members of this group of secretory proteins demonstrate highly conserved three-dimensional structures.2 In the rat all three of the PAP genes and the PSP/reg gene colocalize to the same segment on chromosome 4 (32), observations that suggest a common ancestral gene. Taken together, the conserved structural

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2 The published sequence of rat PAP II contains an error: residue 79 of the mature secreted protein is glycine rather than tryptophan and should read WIGLH. This was verified by DNA sequencing and confirmed by mass spectroscopy analysis.
features in this group of genes suggest that they form a gene family generated through gene duplication processes.

The structural similarities in these genes are augmented by functional homologies. First, each of these proteins is synthesized and secreted by pancreatic acinar cells. Second, their synthesis and secretion is increased in response to conditions of pancreatic stress as follows. During experimental acute pancreatitis, PAP (33, 34) is increased in pancreatic juice and PSP/\textit{reg} mRNA is increased in pancreatic tissue (35). During chronic pancreatitis, both in man and in an animal model, the male WBN/Kob rat, PAP and PSP/\textit{reg} levels are increased in pancreatic tissue (21, 36–38) and juice (21). During maximal caerulein stimulation and supramaximal caerulein-induced pancreatitis, PAP and PSP/\textit{reg} levels are increased in pancreatic tissue (34) and juice. In the post-weaning period of pancreatic organ growth, PSP/\textit{reg} levels are increased in pancreatic juice, and PAP levels show transient increases in tissue (39). During pancreatic regeneration following partial pancreatectomy, PSP/\textit{reg} levels increase in pancreatic tissue (5, 40) (PAP levels have not been measured in this experimental protocol). The similarity in tissue response and secretion between PSP/\textit{reg} and PAP isoforms under conditions of stress due to (i) post-weaning glandular growth including experimental pancreatic regeneration, (ii) hormone stimulation, and (iii) experimental and clinical disease suggests homologies of function for this family of proteins.

The wide divergence of proposed functions for this group of secretory proteins, summarized in the Introduction, suggests that the primary function of this gene family remains to be elucidated. In this study we have focused on an important structural feature, the conserved trypsin cleavage site (Arg^{11–Ile^{12}}) in PSP/\textit{reg}, PAP I, and PAP III isoforms and Arg^{11–Thr^{12}} in PAP II. This cleavage site is conserved in all proteins related to this gene family, both within a single species as well as across species lines. These proteins include human, rat, and mouse PSP/\textit{reg} and PAP, bovine pancreatic thread protein, the trypsin cleavage product isolated from bovine pancreatic homogenates treated with trypsin, and PSP/\textit{reg} protein isolated from pancreatic tissue in the hamster. This list may be extended to include islet neogenesis-associated protein-related protein (42). These proteins all share the C-type lectin binding domain, conserved amino acid sequences, including six cysteine residues, and a conserved trypsin cleavage site at the N terminus. In their activated state PSP/\textit{reg} and PAP isozymes remain soluble during their secretory passage through the pancreatic duct and their delivery to the intestinal milieu. Cleavage at the conserved Arg^{11–Ile^{12}} site leads to the removal of an N-terminal undecapeptide from the C-terminal polypeptide. This proteolytic processing feature has not been observed in any other proteins bearing a C-type lectin domain.

By using rat PSP/\textit{reg} and PAP activated by trypsin, we have demonstrated in this study that the corresponding C-terminal peptides or thread proteins (pancreatic thread protein and pancreatitis-associated thread protein, respectively) remain largely resistant to proteases contained in pancreatic juice, including serine proteases (trypsin, chymotrypsin, and elastase) and metalloproteases (carboxypeptidases A and B). Despite the presence of numerous potential cleavage sites within their amino acid sequence, thread proteins show impressive protease resistance and survive the proteolytic degradation of the proteases themselves. Longstanding protease resistance implies that thread proteins undergo a significant change in conformational structure following activation and enter into tightly folded structures relatively impervious to the effects of exogenous proteases.

When pancreatic juice containing high levels of PSP/\textit{reg} and PAP was activated, PTP and PATP survived through the 16-h time period (960 min). By utilizing antibodies to track the conversion of stress proteins to thread proteins, the half-lives of PATP forms and PTP were 100–150 and 800 min, respectively. Our recombinant studies indicated that PATP I and PATP III were entirely resistant to trypsin and substantially resistant to the mixture of pancreatic proteases after fibril formation. PATP III was resistant to pancreatic proteases both during and after fibril formation. In contrast, although PATP II was resistant to trypsin, it was completely degraded by pancreatic proteases within 60 min of adding pancreatic juice.

In these activation studies we also measured the kinetics of conversion of soluble intermediate proteins into insoluble sedimenting forms. These studies demonstrate that PTP, PATP I, and PATP III rapidly appear in the pellet fraction as insoluble thread proteins. The 50% threshold for conversion of soluble
thread proteins to insoluble thread forms were 0.8, 3, and 5 min for PAP III, PTP, and PAP I, respectively. PATP II did not appear in an insoluble fraction. These findings suggest that, with the exception of PAP II, polymerization of soluble thread proteins (monomers) into insoluble forms (polymerized fibrils) is a specific assembly process that is dependent on time and protein concentration.

We have also studied the solubility characteristics of each of the rat pancreatic secretory thread proteins in detail and further confirmed that they fall into two groups. PATP I, PATP III, and PTP sediment at low speed centrifugation, whereas PATP II remains largely in the soluble fraction at either low speed or high speed centrifugation. Changes in incubation temperature had little effect on the physical characteristics of sedimentation for any of these isoforms. We conclude that PAP II is a non-sedimenting secretory stress protein.

Among pancreatic secretory stress proteins, PAP II shows a number of characteristics that distinguish it from the other forms, including (i) rapid digestion in the presence of pancreatic proteases, (ii) inability to polymerize into fibrils that may be isolated by low speed or high speed centrifugation, and (iii) undetectable fibrils analyzed by scanning and transmission electron microscopy. A dendrogram depicting the sequence relationship between PSP/reg and PAP isoforms of human, bovine, and rodent origin clearly demonstrates that PAP II is separated in a different evolutionary cluster distinct from either rodent PAP I and III isoforms or human PAP and bovine PAP. PAP II may serve as a facilitator or inhibitor in the coordinated assembly of insoluble thread structures during pancreatic stress reactions. Alternatively, PAP II may represent a non-functional secretory stress protein that has lost the critical sequences required for polymerization into fibrillar thread structures.

In humans and cows with chronic calcific pancreatitis, PTP has been identified as a major organic component of calcified stones. In both species thread proteins were isolated and shown to be fibrillar by negative staining in the electron microscope (22, 23). Purified PSP/ithostathine has been shown to form similar fibrils after trypsin cleavage in vitro (43), and prolonged incubation of PSP, purified by column chromatography, suggested that activation may occur by autoproteolysis. The studies reported here further extend the existence of fibrillar thread structures among pancreatic secretory proteins to include not only PTP but also PATP I and PATP III isoforms. This group of insoluble thread proteins appears to be generated from a family of pancreatic secretory stress proteins following trypsin activation.

The assembly of soluble thread proteins into insoluble fibrillar matrices by all of the 16-kDa proteins (PTP, PATP I, and PATP III), save one (PATP II), further substantiates the functional similarities in this family of secretory stress proteins. Analysis of the morphology of these fibrillar matrices by scanning and transmission electron microscopy, under a variety of staining and embedding methods, reveals highly organized structural features associated with extensively polymerized structures. Although features differ somewhat among individual thread matrices, there is a strong tendency for polymerized protein threads to bundle together. Bundling properties appear to be most developed (strong curvilinear bundles) in the case of PATP I filaments, less developed, and nodular, in the case of PATP III, and least developed (feathery matrix) in the case of PTP. Extensive branching of filaments in all three species leads to dense fibrillar matrices covering large topological surfaces.

High-resolution analysis of fibrillar matrices by transmission EM suggests the presence of subunit structures apparently assembled into a helical fibrillar structure. Certain favorable sections point to the presence of a cavity within these filamentous structures. However, further structural studies will be required to determine the precise subunit structures of these filaments and the nature of cavities, should they exist. Our investigations to date with purified recombinant proteins have been confined to the assembly of homologous filaments. Polymerization of heterologous mixtures of thread proteins forms the basis for a separate study.

We have demonstrated that samples of pancreatic juice, upon trypsin activation, also form dense fibrillar matrices similar to those observed with purified recombinant proteins. Both normal pancreatic juice (Wistar rat), containing small concentrations of stress proteins (14 μg/ml PSP/reg and 1.4 μg/ml PAP), and pathological juice (male WBN/Kob rats), containing elevated concentrations of stress proteins (150 μg/ml PSP/reg and 50 μg/ml PAP), form dense fibrillar matrices. However, the extent of matrix formation appears to correlate with the amount of stress proteins observed in samples of juice.

Based on the findings presented here we propose that PSP/reg and PAP form a family of secretory stress proteins which, upon trypsin or “trypsin-like” activation, convert to a family of insoluble thread proteins. We further propose that the activated thread proteins represent the active form of these molecules whereas the secretory proforms represent inactive precursors. In order to begin to unify the terminology in this complex area, we suggest that PSP/reg may refer to “pancreatic stress protein” as well as “pancreatic stone protein.”

Concerning their function, this family of secretory stress proteins may serve to form dense extracellular fibrillar complexes that attach to and span large topological surfaces during conditions of luminal stress. For example, these dense fibrillar structures may provide a luminal matrix from which the repair or regeneration of ductal structures may be orchestrated under conditions of stress. Since islet, acinar, and ductal cells are all derived from proliferating duct cells (44), it is possible that neogenesis of exocrine and endocrine tissue occurs as part of the more general process of ductal proliferation during pancreatic development or regeneration after partial surgical ablation of the gland. The mitogenic activity reported for PSP/reg (8) would be consistent with this notion. Further work is clearly needed to determine the role of luminal matrices during pancreatic development, growth, and repair and to determine the location of the activating enzyme, whether due to a soluble activity in pancreatic juice or a surface activator associated with ductal cells.

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REFERENCES
1. De Caro, A., Lohse, J., and Sarles, H. (1979) Biochem. Biophys. Res. Commun. 87, 1176–1182
2. Sarles, H., Dagorn, J. C., Giorgi, D., and Bernard, J. P. (1990) Gastroenterology 99, 900–901
3. Bimmerl, D., Graf, R., Scheele, G. A., and Frick, T. W. (1997) J. Biol. Chem. 272, 3073–3082
4. De Reggi, M., Gharib, B., Patard, L., and Stoven, V. (1998) J. Biol. Chem. 273, 4967–4971
5. Terazono, K., Uchiyama, Y., Ide, M., Watanabe, T., Yonekura, H., Yamamoto, H., and Okamoto, H. (1990) Diabetesologia 33, 250–252
6. Unno, M., Itoh, T., Watanabe, T., Miyashita, H., Morizumi, S., Teraoka, H., Yonekura, H., and Okamoto, H. (1992) Adv. Exp. Med. Biol. 321, 61–69
7. Watanabe, T., Yonekura, H., Suzuki, Y., Miyashita, H., Sugiyama, K., Morizumi, S., Unno, M., Tanaka, O., Kondo, H., Bone, A. J., Takasawa, S., and Okamoto, H. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 3599–3602
8. Zenilman, M. E., Magnuson, T. H., Swinson, K., Egan, J., Perfetti, R., and Shuldiner, A. R. (1996) Gastroenterology 110, 1208–1214
9. Fukui, H., Kinoshita, Y., Maekawa, T., Okada, A., Waki, S., Hassam, S., Okamoto, H., and Chiba, T. (1998) Gastroenterology 115, 1483–1493
10. Gross, D. J., Weiss, L., Reibstein, I., van den Brand, J., Okamoto, H., Clark, A., and Slavin, S. (1998) Endocrinology 139, 2369–2374
11. Kobayashi, S., Akiyama, T., Nata, K., Abe, M., Tajima, M., Shevani, N., J.,
Unno, M., Matsuno, S., Sasaki, H., Takasawa, S., and Okamoto, H. (2000) *J. Biol. Chem.* 275, 10723–10736

12. Livesey, F. J., O’Brien, J. A., Li, M., Smith, A. G., Murphy, L. J., and Hunt, S. P. (1997) *Nature* 390, 614–618

13. Bimmler, D., Angst, E., Valeri, V., Bain, M., Scheele, G. A., Frick, T. W., and Graf, R. (1999) *Pancreas* 19, 255–267

14. Iovanna, J., Orelle, B., Keim, V., and Dagorn, J.-C. (1991) *J. Biol. Chem.* 266, 24664–24669

15. Frigerio, J. M., Dusetti, N. J., Keim, V., Dagorn, J. C., and Iovanna, J. L. (1993) *Biochemistry* 32, 9236–9241

16. Frigerio, J. M., Dusetti, N. J., Garrido, P., Dagorn, J. C., and Iovanna, J. L. (1993) *Biochim. Biophys. Acta* 1216, 329–331

17. Keim, V., Rohr, G., Stockert, H. G., and Haberich, F. J. (1984) *Digestion* 29, 242–249

18. Keim, V., Iovanna, J. L., Orelle, B., Verdier, J. M., Busing, M., Hopt, U., and Dagorn, J. C. (1992) *Gastroenterology* 103, 248–254

19. Iovanna, J., Frigerio, J. M., Dusetti, N. J., Ramare, F., Raibaud, P., and Dagorn, J. C. (1993) *Pancreas* 8, 597–601

20. Keim, V., Iovanna, J. L., and Dagorn, J. C. (1994) *Digestion* 55, 65–72

21. Bimmler, D., Schiesser, M., Angst, E., Bain, M., Frick, T. W., and Graf, R. (1999) *Pancreas* 19, 416

22. Gross, J., Carlson, R. I., Brauer, A. W., Margolies, M. N., Warshaw, A. L., and Wands, J. R. (1985) *J. Clin. Invest.* 76, 2115–2126

23. Gross, J., Brauer, A. W., Bringhurst, R. F., Corbett, C., and Margolies, M. N. (1985) *Proc. Natl. Acad. Sci. U. S. A.* 82, 5627–5631

24. Bimmler, D., Frick, T. W., and Scheele, G. A. (1985) *Pancreas* 11, 63–76

25. Schiesser, M., Bimmler, D., Frick, T. W., and Graf, R. (2001) *Pancreas*, 186–192

26. Heery, D. M., Gannon, F., and Powell, R. (1990) *Trends Genet.* 6, 173–175

27. Livesey, F. J., and O’Brien, J. A. (1995) *Biochim. Biophys. Acta* 124, 103–110

28. Feng, D. F., and Doolittle, R. F. (1987) *J. Mol. Biol.* 202, 351–360

29. Drickamer, K. (1993) *Curr. Opin. Struct. Biol.* 3, 393–400

30. Scheele, G. A. (1975) *J. Biol. Chem.* 250, 5375–5385

31. Scheele, G., Bartelt, D., and Bieger, W. (1981) *Gastroenterology* 80, 461–473

32. Stephanova, E., Tissir, F., Dusetti, N., Iovanna, J., Spizirer, J., and Spizirer, C. (1996) *Cytogenet. Cell Genet.* 72, 83–85

33. Keim, V., and Loffler, H. G. (1984) *Clin. Physiol. Biochem.* 4, 136–142

34. Keim, V., Iovanna, J.-L., Rohr, G., Usadel, K. H., and Dagorn, J.-C. (1991) *Gastroenterology* 100, 775–782

35. Iovanna, J. L., Keim, V., Michel, R., and Dagorn, J. C. (1991) *Am. J. Physiol.* 261, G345–G348

36. Satomura, Y., Sawabu, N., Ohta, H., Watanabe, H., Yamakawa, O., Motoo, Y., Okai, T., Toya, D., Makine, H., and Okamoto, H. (1993) *Int. J. Pancreatol.* 13, 59–67

37. Su, S. B., Motoo, Y., Xie, M. J., Sakai, J., Taga, H., and Sawabu, N. (1999) *Pancreas* 19, 239–247

38. Keim, V., Hoffmeister, A., Teich, N., Halm, U., Scheurlem, M., Tannapfel, A., and Messner, J. (1999) *Pancreas* 19, 248–254

39. Bimmler, D., Schiesser, M., Scheele, G. A., Frick, T. W., and Graf, R. (1999) *Digestion* 60, 369

40. Smith, F. E., Bonner, W. S., Leahy, J. L., Laufergraben, M. J., Ogawa, Y., Rosen, K. M., and Villa, K. L. (1994) *Diabetologie* 37, 994–999

41. Rafaeloff, R., Pittenger, G. L., Barlow, S. W., Qin, X. F., Yan, B., Rosenberg, L., Duguid, W. P., and Vinik, A. I. (1997) *J. Clin. Invest.* 99, 2106–2109

42. Sasahara, K., Yamaoka, T., Moritani, M., Yoshimoto, K., Kuroda, Y., and Itakura, M. (2000) *Biochim. Biophys. Acta* 1500, 142–146

43. Cerini, C., Peyrot, V., Garnier, C., Duplan, L., Veesler, S., Le-Caer, J. P., Bernard, J. P., Boutelle, H., Michel, R., Vazi, A., Dupuy, P., Michel, B., Berland, Y., and Verdier, J. M. (1999) *J. Biol. Chem.* 274, 22266–22274

44. Githens, S. (1993) in *The Pancreas, Biology, Pathobiology, and Disease* (Go, V. L. W., DiMagno, E. P., Gardner, J. D., Lebenthal, E., Reber, H. A., and Scheele, G. A., eds) pp. 21–55, Raven Press, Ltd., New York
A Family of 16-kDa Pancreatic Secretory Stress Proteins Form Highly Organized Fibrillar Structures upon Tryptic Activation
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