Human Pancreatitis-associated Protein

Messenger RNA Cloning and Expression in Pancreatic Diseases

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Abstract

A human pancreatic cDNA library was screened with the cDNA encoding rat "pancreatitis-associated protein" (PAP). The selected clone encoded a secretory protein structurally related to rat PAP. The protein had the same size as rat PAP and showed 71% amino acid identity, the six half-cystines being in identical positions. Domains of the proteins showing homologies with calcium-dependent lectins were also conserved. In addition, expression in pancreas of the genes encoding the human protein and rat PAP showed similar characteristics: both were expressed at very low levels in control tissue and overexpressed during the acute phase of pancreatitis, contrary to most secretory products. The human protein was therefore named human pancreatitis-associated protein (PAP-H). Antibodies raised to a synthetic peptide of PAP-H detected a single band with an M_r compatible with PAP-H in Western blot analysis of proteins extracted from a pancreas presenting with acute pancreatitis. In that tissue, the protein could be immunolocalized to the apical regions of acinar cells. An immunoassay was also constructed to quantify the protein in serum. Elevated PAP-H levels were observed in patients with acute pancreatitis and in some patients with chronic pancreatitis. Values were close to background in healthy subjects and in patients with other abdominal diseases. These results confirm that PAP-H synthesis increases during inflammation and suggest a possible use of the protein as biological marker of acute pancreatitis. (*J. Clin. Invest.* 1992. 90:2284–2291.) Key words: lectin • pancreatic inflammation • acute pancreatitis • acute phase • biological marker

Introduction

Acute pancreatitis is characterized by edema, leukocyte infiltration, hemorrhage, and cellular necrosis (1). When that episode is not fatal, it is followed by a total recovery of the initial morphology and functional capacity of the gland (1). During the acute phase, damaged acinar cells show many ultrastructural modifications such as fragmentation and dilatation of the rough endoplasmic reticulum, reduction in the number of zymogen granules, and formation of cytoplasmic vacuoles. Morphological alterations are accompanied by important changes in secretory parameters. Studies in rats showed that content and secretion of pancreatic secretory proteins were generally reduced during the acute phase of pancreatitis (2), whereas a limited number of proteins were secreted in higher amounts (2–4). These modifications result from a change in the pattern of gene expression programmed to help the tissue survive the acute phase of inflammation (2). The most dramatic increase was observed for the "pancreatitis-associated protein" (PAP),[1] which was merely detectable in control pancreas and represented up to 5% of secreted protein at the climax of the acute phase. Return to control levels paralleled regression of tissue inflammation (5). The rapid and strong induction of the PAP gene is reminiscent of the response to stress of acute phase proteins (3). Yet, contrary to such proteins, PAP is an exocrine protein synthesized on the rough endoplasmic reticulum of acinar cells and stored in zymogen granules before being secreted, as demonstrated by subcellular fractionation and immunoelectron microscopy (5). Several domains of homology with calcium-dependent animal lectins were found in the primary structure of rat PAP and the protein was shown to induce extensive bacterial aggregation in vitro (3), suggesting that it is involved in the control of bacterial proliferation. PAP appears therefore as an important component of the mechanism of defense against pancreatic aggression which deserves being characterized in human pancreas. It might also prove a very useful marker of pancreatic inflammation. Purification of such a protein by classical procedures is hindered by the hazards associated with pancreatic juice collection from patients presenting with acute pancreatitis. We developed an alternative strategy based on the assumption that mRNAs encoding the rat and human PAPs were similar enough to allow screening a human cDNA library with the rat probe.

We report here the cloning and sequencing of human PAP (PAP-H) cDNA, from which was deduced the primary structure of the protein. The human clone was used to monitor PAP gene expression in diseased pancreas. In addition, antibodies to the synthetic amino-terminal peptide of PAP-H were used to determine PAP-H levels during acute pancreatitis.

Methods

Library screening. A human pancreatic cDNA library in the expression vector λgt-11 containing 1.4×10^6 different recombinant clones was obtained from Clontech Laboratories, Inc. (Palo Alto, CA). That library was screened by the plaque screening procedure (6), in conditions adapted to heterologous hybridization. The R4 insert (3), ^{32}P-labeled by random priming (7) to a sp act of 10^9 cpm/μg, was used as probe to screen ~ 3×10^5 recombinant clones. That fragment corresponds to nucleotides 1–765 of the rat PAP cDNA (3). Duplicate filters

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1. *Abbreviations used in this paper:* PAP, pancreatitis-associated protein; PAP-H, human PAP; PCR, polymerase chain reaction.

were prehybridized 4 h at 65°C in a solution containing 6× standard saline citrate (SSC) (SSC is 150 mM NaCl, 15 mM sodium citrate), 0.1% BSA, 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 0.5% SDS, and 100 μg/ml of denatured herring sperm DNA. Overnight hybridization was conducted in the same buffer in the presence of 5×10^6 cpm/ml of cDNA probe, at 65°C. Filters were washed 2 × 15 min with 6× SSC, 0.1% SDS at 65°C. A single clone (QH1) was selected through three rounds of screening.

Biohazards associated with the experiments described in this publication have been previously examined by the French National Control Committee.

Enzymatic amplification for sequencing QH1 DNA. About 0.5 μg of QH1 DNA was incubated in 50 μl of 50 mM KCl, 10 mM Tris HCl pH 8.3, 1.25 mM $MgCl_2$, 200 μM of each deoxynucleotide triphosphate, 0.1% (wt/vol) gelatin and 2.5 U of *Taq* DNA Polymerase (Appligene, Illkirch, France) containing 20 pM of each of the synthetic oligonucleotides (*A*) 5′GGTGGCGACGACTCCTGGAGCCCG 3′ and (*B*) 5′TTGACACCAGACCAACTGGTAATG 3′, corresponding to regions of the λgt 11 DNA flanking the EcoRI cloning site. Polymerase chain reaction (PCR) was performed as follows for 25–30 cycles in a thermocycler (Crocodile; Appligene): denaturation at 94°C for 10 s, annealing at 55°C for 2 min, and DNA synthesis at 74°C for 3 min. In the last cycle DNA synthesis lasted 10 min. Amplified DNA was purified by electroelution after separation on polyacrylamide gel followed by phenol/chloroform/isoamyl alcohol (50:48:2) extraction and ethanol precipitation.

Generation of single-stranded DNA by PCR. Asymmetric DNA amplification was performed as described by Gyllenstein and Erlich (8). About 10 ng of amplified DNA was incubated in the PCR reaction mixture described above, with the exception that the concentrations of synthetic oligonucleotides were 50 pmol for *A* and 0.5 pmol for *B*. A single strand in the opposite direction was obtained with the reverse ratio of oligonucleotide concentrations. The reaction mixture was subjected to 20 cycles of 10 s at 94°C, 2 min at 55°C, and 3 min at 74°C.

Sequencing single-stranded DNA. The amplification mixture was adjusted to 300 μl with H_2O, applied onto a 5-ml column (Bio-Gel P-30; Bio-Rad Laboratories, Richmond, CA) and spun at 2,000 rpm in a rotor (HB-4; Sorvall Instruments Div., Du Pont Co., Newton, CT) to remove excess dNTP and buffer components and the DNA ethanol precipitated. After resuspension in H_2O, the ssDNA was sequenced in both directions with a DNA sequencing kit (Sequenase version 2.0; U.S. Biochemical Corp., Cleveland, OH) following recommendations of the manufacturer and using appropriate synthetic oligonucleotides as primers (see Results).

Sequence comparisons. The complete sequence of human PAP mRNA was compared with the sequences listed in Genbank (9). The search was conducted with the BISANCE system (CITI2; Centre Interuniversitaire d'informatique, Paris, France) using the program of Goad and Kanehisa (10). The complete amino acid sequence was compared with the sequences listed in the National Biochemical Research Foundation data bank, using the FASTP program (CITI2).

Genomic analysis. Human genomic DNA was purified from lymphocytes (11) and digested in separate reactions with the restriction endonucleases BamHI, EcoRI, PstI, HindIII, and BglII. The samples were fractionated by electrophoresis through a 1% agarose gel, and the DNA was transferred to a membrane (Hybond N; Amersham Int'l., Amersham, UK). The membrane was baked in a vacuum oven at 80°C for 2 h and prehybridized for 4 h at 65°C in 6× SSC, 0.1% BSA, 0.1% Ficoll, 0.1% polyvinylpirrolidone, 0.5% SDS, and 100 μg/ml denatured herring sperm DNA. The membrane was hybridized in the same solution containing as probe the QH1 insert amplified by PCR (12) and ^{32}P-labeled (7) to a sp act of 1×10^9 cpm/μg. The blot was washed twice for 15 min at room temperature in 2× SSC, 0.1% SDS, and then twice for 30 min at 65°C in 0.1× SSC, 0.1% SDS before autoradiography.

RNA analysis. RNA was extracted from tissue fragments immediately frozen in liquid nitrogen after surgery and stored at −80°C (13). Fragments of pancreas, liver, spleen, and kidney were obtained from cadaver kidney transplant donors. Other fragments of pancreas were obtained from the Service de Gastroentérologie, Hôpital Sainte Marguerite, Marseille, France (Dr. J. Sahel). One of them was obtained from a patient with a severe necrohemorrhagic pancreatitis who died 5 d after the onset of abdominal pain. Two samples were taken at surgery from patients presenting with obstructive pancreatitis due to an adenocarcinoma of the biliary tract and an adenocarcinoma of the head of the pancreas, respectively. Two samples were obtained from patients presenting with chronic pancreatitis and one from a patient presenting with a pancreatic endocrine tumor. Samples of pancreatic adenocarcinomas were obtained from two patients. Samples were submitted to routine histological examination. Frozen pellets of Capan I and Mia-Paca cells were a generous gift of Dr. A. Estival (U.151 INSERM, Toulouse, France).

RNA samples were size fractionated on a formaldehyde agarose gel as described (11). Northern blot analysis was performed by blotting RNAs onto nylon membranes (Biodyne; Pall BioSupport Corp., Glen Cove, NY). The filters were prehybridized then hybridized in 50% formamide, 5× sodium chloride sodium phosphate EDTA buffer (180 mM NaCl, 1 mM EDTA, 10 mM NaH_2PO_4, pH 7.5), 0.1% BSA, 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 0.5% SDS, and 200 μg/ml of denatured herring sperm DNA at 42°C in the presence of the ^{32}P-labeled QH1 insert. Then the filters were washed four times for 5 min at room temperature in 2× SSC, 0.1% SDS, twice for 15 min at 50°C in 0.1× SSC, 0.1% SDS, and for 30 min in 0.1× SSC.

Production of antibodies to QH1. The predicted amino-terminal peptide (EEPQAY) of the protein was chemically synthesized (Neosystem, Strasbourg, France), coupled to BSA, hemoglobin, or lactoferrin as carriers using bis-diazotized benzidine (14), and used as immunogen. Rabbits were injected subcutaneously with a suspension of 100 μg of the immunogen coupled to albumin (100 μg in 1 ml saline) mixed with 1 ml CFA. The same injection, except that incomplete adjuvant was used, was repeated three times at 2-wk intervals with the antigen coupled with hemoglobin, lactoferrin, and albumin again. 10 d after the last injection, antisera were collected by puncture of the ear vein. Immunoglobulins were purified from the sera as already described (5).

Immunodetection of QH1 in pancreatic homogenates. Fragments of pancreas (~ 300 mg) were homogenized in 1 ml of 50 mM Tris buffer, pH 8.0. The homogenate was centrifuged at 100,000 *g* for 2 h. 25 μl of supernatant was loaded onto a 15% polyacrylamide-SDS gel and processed for Western blotting with the antibodies to the NH_2-terminal end of QH1 as already described (5). Immunolocalization was performed in thin sections of pancreas using the peroxidase-antiperoxidase method of Sternberger et al. (15). The antiserum to the NH_2-terminal end of QH1 was used at a 1:100 dilution.

Quantification of PAP-H by competitive ELISA. The solid phase was prepared by adding 100 μl of the peptide coupled to albumin (10 μg/ml) (14) in solution A ($NaHCO_3$ 100 mM, pH 8.5) into each well of ELISA microplates (Nunc, Roskilde, Denmark). The plates were incubated 2 h at 25°C in a humid atmosphere. The wells were then washed with buffer B (0.5% Tween 20 (vol/vol) in PBS). The antibody capture assays were performed by incubating serum samples (50 μl, 25 μl, 12.5 μl, and 6.25 μl) with purified IgG in 100 μl of solution C (Tris 100 mM, pH 7.4, 1% Tween 20, and 1.5% BSA), 2 h at 25°C in a humid atmosphere. Incubation continued for 2 h in the antigen-coated plates. The wells were washed with buffer B and fixed antibodies were detected by goat anti-rabbit peroxidase-labeled IgG. Finally, the plates were washed and the peroxidase reaction conducted as already described (5). Quantification was made by comparison with a calibration curve obtained with serial dilutions of the peptide. That ELISA allowed the detection of 12.5 fmol of QH1 equivalent. Specificity of the QH1 assay in serum was controlled by fractionating serum from healthy subjects and patients with acute pancreatitis on an HPLC column (TSK 3000, System Gold apparatus; Beckman Instruments, Inc., Fullerton, CA). No immunoreactivity was observed in fractions from control serum. In patients with acute pancreatitis, immunoreactivity appeared under a single peak and was completely inhibited by competition with the synthetic QH1 NH_2-terminal peptide (not shown).

PAP-H was assayed in the serum of patients admitted to the Service

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cgggagagtgactcctgattgcctcctcaagtcgcagacact ATG CTG    48
                                            Met Leu     2

CCT CCC ATG GCC CTG CCC AGT GTA TCT TGG ATG CTG CTT    87
Pro Pro Met Ala Leu Pro Ser Val Ser Trp Met Leu Leu    15

TCC TGC CTC ATG CTG CTG TCT CAG GTT CAA GGT GAA GAA   126
Ser Cys Leu Met Leu Leu Ser Gln Val Gln Gly Glu Glu    28

CCC CAG AGG GAA CTG CCC TCT GCA CGG ATC CGC TGT CCC   165
Pro Gln Arg Glu Leu Pro Ser Ala Arg Ile Arg Cys Pro    41

AAA GGC TCC AAG GCC TAT GGC TCC CAC TGC TAT GCC TTG   204
Lys Gly Ser Lys Ala Tyr Gly Ser His Cys Tyr Ala Leu    54

TTT TTG TCA CCA AAA TCC TGG ACA GAT GCA GAT CTG GCC   243
Phe Leu Ser Pro Lys Ser Trp Thr Asp Ala Asp Leu Ala    67

TGC CAG AAG CGG CCC TCT GGA AAC CTG GTG TCT GTG CTC   282
Cys Gln Lys Arg Pro Ser Gly Asn Leu Val Ser Val Leu    80

AGT GGG GCT GAG GGA TCC TTC GTG TCC TCC CTG GTG AAG   321
Ser Gly Ala Glu Gly Ser Phe Val Ser Ser Leu Val Lys    93

AGC ATT GGT AAC AGC TAC TCA TAC GTC TGG ATT GGG CTC   360
Ser Ile Gly Asn Ser Tyr Ser Tyr Val Trp Ile Gly Leu   106

CAT GAC CCC ACA CAG GGC ACC GAG CCC AAT GGA GAA GGT   399
His Asp Pro Thr Gln Gly Thr Glu Pro Asn Gly Glu Gly   119

TGG GAG TGG AGT AGC AGT GAT GTG ATG AAT TAC TTT GCA   438
Trp Glu Trp Ser Ser Ser Asp Val Met Asn Tyr Phe Ala   132

TGG GAG AGA AAT CCC TCC ACC ATC TCA AGC CCC GGC CAC   477
Trp Glu Arg Asn Pro Ser Thr Ile Ser Ser Pro Gly His   145

TGT GCG AGC CTG TCG AGA AGC ACA GCA TTT CTG AGG TGG   516
Cys Ala Ser Leu Ser Arg Ser Thr Ala Phe Leu Arg Trp   158

AAA GAT TAT AAC TGT AAT GTG AGG TTA CCC TAT GTC TGC   555
Lys Asp Tyr Asn Cys Asn Val Arg Leu Pro Tyr Val Cys   171

AAG TTC ACT GAC  tagtgcaggagggaagtcagcagcctgtgtttggt  602
Lys Phe Thr Asp                                      175

gtgcaactcatcatgggcatgagaccagtgtgaggactcaccctggaagaga  654

atattcgcttaattcccccaacctgaccacctcattcttatctttcttctgt  706

ttcttcctccccgctagtcatttcagtctcttcattttgtcatacggcctaa  758

ggctttaaagagcaataaaattttagtctgcaaaaaaa                797
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Figure 1. Nucleotide sequence of QH1 (PAP-H) mRNA and deduced amino acid sequence of the encoded preprotein. The initiation codon (nucleotides 43–45) was selected by analogy with rat PAP mRNA sequence (3). Noncoding sequences are in lowercase letters and the polyadenylation site AATAAA is underlined. These sequence data are available from EMBL/GenBank® under accession number M8433.

de Gastroentérologie, Hôpital Sainte Marguerite (Dr. J. Sahel) or the Departement de Chirurgie, Hôpital d'Adultes d'Avignon, France (Dr. G. Angelvin). Diagnosis of acute pancreatitis was established by typical abdominal pain, accompanied by increased serum amylase and lipase levels and ultrasonography or computed tomography. Patients classified in Fig. 10 in the group of abdominal diseases other than pancreatitis included seven main bile duct lithiasis, two cancers of the main bile duct, four pancreatic cancers, two pancreatic cysts, three biliary stenosis, five ulcerative colitis, one duodenal tumor, one liver cirrhosis, one hyperamylasemia of unknown origin, one renal insufficiency with increased amylasemia, and three duodenitis.

Results

Cloning human PAP messenger RNA. A radiolabeled rat PAP cDNA (R4) (3) was used to screen a human pancreatic cDNA library in λgt-11 at relatively low stringency as requested by the heterologous nature of the probe. The only positive clone after three successive screenings (QH1) was selected and directly sequenced after single-strand generation by asymmetric PCR amplification (Fig. 1). Sequence was completed with the help of five synthetic oligonucleotides, homologous, respectively, to regions 12 nucleotides upstream of the cloning site of the vector and positions 168–186, 327–345, and 528–546 of QH1 and, in the opposite direction, positions 798–765, 546–528, and 155–138 of QH1. The complete sequence comprised 790 nucleotides, exclusive of the poly-A tail. A putative polyadenylation signal (AATAAA) was present 13 nucleotides upstream from the poly-A extension. The approximate length of the mature transcript was estimated to 950 nucleotides by Northern blot analysis. The sequence reported here extends to 42 nucleotides in the nontranslated 5′ end of the transcript.

Sequence comparisons of QH1 with PAP mRNA and of the encoded protein with rat PAP and other lectins. Nucleotide sequence comparison of QH1 and the coding region of rat PAP mRNA revealed 74% identity. Regions outside the coding region only showed 55% identity (not shown).

A single open reading frame was found in QH1 mRNA, encoding a polypeptide of 175 amino acids which showed an overall similarity of 71% with rat PAP (Fig. 2). Positions of the six cysteine residues were conserved. In addition, the homology with calcium-dependent animal lectins (C-type lectins) observed in several regions of the rat PAP sequence was also found in the QH1 sequence. Fig. 3 shows the comparison of the carboxy-terminal portion of QH1 (residues 10–148) with several lectins including human lithostathine (16), human thrombomodulin (17), chicken hepatic lectin (18), rat hepatic lectin (19), rat cartilage proteoglycan (20), human tetranectin (21), human IgE receptor (22), human chondroitin sulphate core protein (23), rat Kupffer cell receptor (24), *Sarcophaga peregrina* lectin (25), dog pulmonary surfactant apoprotein (26), acorn barnacle lectin (27), and the A chain of the coagulation Factor IX/Factor X binding protein (28). Regions showing homology corresponded to domains potentially involved in lectin activity (29). Comparison with human pancreatic lithostatine (16) (Fig. 3) revealed a high degree of similarity, as already reported for the rat proteins (3). Again, similarity did

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PrePAPH  MLPPMALPSVSWMLLSCLMLLSQVQGEEPQRELPS    1 - 35
PrePAPR  MLHRLAFPVMSWMLLSCLMLLSQVQGEDSPKKIPS    1 - 35

PrePAPH  ARIRCPKGSKAIGSHCYALFLSPKSWTDADLACQK   36 - 70
PrePAPR  ARISCPKGSQAYGSYCYALFQIPQTWFDAELACQK   36 - 70

PrePAPH  RPSGNLVSVLSGAEGSFVSSLVKSIGNSYSYVWIG   76 - 105
PrePAPR  RPEGHLVSVLNVAEASFLASMVKNTGNSYQYTWIG   76 - 105

PrePAPH  LHDPTQGTEPNGEGWEWSSSDVMNYFAWERNPSTI  106 - 140
PrePAPR  LHDPTLGGEPNGGGWEWSNNDIMNYVNWERNPSTA  106 - 140

PrePAPH  SSPGHCASLSRSTAFLRWKDYNCNVRLPYVCKFTD  141 - 175
PrePAPR  LDRGFCGSLSRSSGFLRWRDTTCEVKLPYVCKFTG  141 - 175
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Figure 2. Sequence comparison of human (H) and rat (R) prePAPs. Sequence alignment was obtained without introducing deletions. Mature PAP-R starts with a Gln in position 27. Shaded areas correspond to amino acid identities.

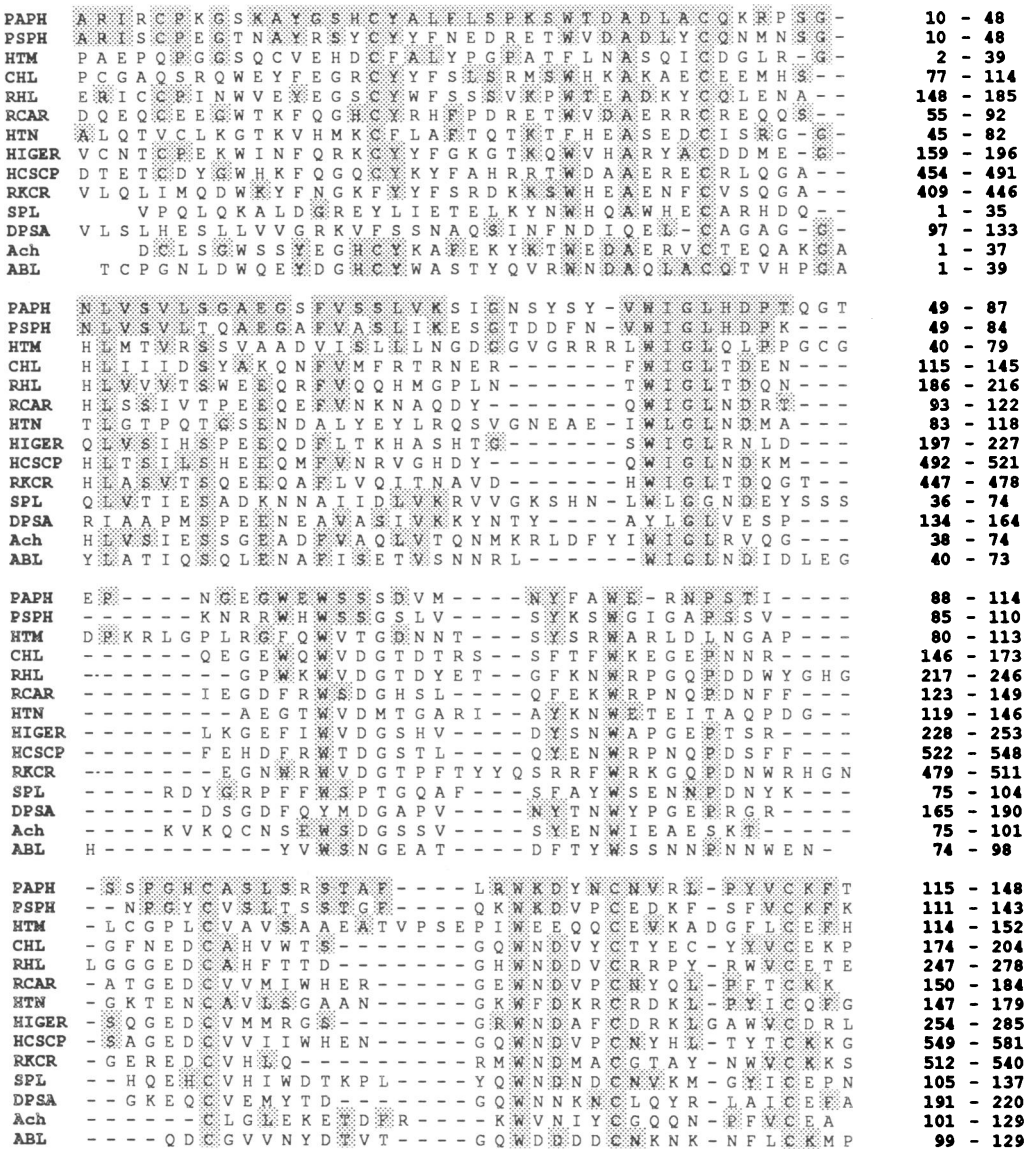

Figure 3. Sequence comparison of PAP-H and several lectins. The PAP-H sequence was aligned with sequences of human pancreatic lithostathine (PSPH) (16) and the following lectins: HTM, human thrombomodulin (17); CHL, chicken hepatic lectin (18); RHL, rat hepatic lectin (19); RCAR, rat cartilage proteoglycan (20); HTN, human tetranectin (21); HIGER, human IgE receptor (22); HCSCP, human chondroitin sulphate core protein (23); RKCR, rat Kupffer cell receptor (24); SPL, *Sarcophaga peregrina* lectin (25); DPSA, dog pulmonary surfactant apoprotein (26); Ach, A chain of coagulation Factor IX/Factor X binding protein (28); ABL, acorn barnacle lectin (27). Shaded areas correspond to amino acid identities. Amino acid numbering of PAP-H is based on the numbering of PAP-R (Fig. 2).

not extend through the NH_2-terminal peptide of lithostathine which bears the inhibitory activity of the protein toward $CaCO_3$ crystal growth.

Comparison of the amino-terminal end of rat PAP with the QH1 sequence (Fig. 2) suggested that the two proteins were provided with prepeptides of the same size (26 amino acids), since the methionine in position 1 of QH1 was the only one to confer the structural requirements of prepeptides, i.e., a charged amino-terminal end and a highly hydrophobic core (30) and since amino acid identity extended through the prepeptide cleavage site of prePAP-R. Computer analysis of the sequence indicated that mature QH1 protein had a molecular weight of 16,566 and an isoelectric point of 7.94.

Expression of the QH1 mRNA in pancreas and other tissues. Pancreatic RNAs from undiseased tissue and acute necrohemorrhagic pancreatitis were probed in Northern blot analysis with QH1 and trypsinogen I cDNA as control (Fig. 4). QH1 mRNA was not detectable in control pancreas, whereas trypsinogen I mRNA was abundant. Conversely, trypsinogen I mRNA expression was low in the diseased pancreas, whereas QH1 transcripts were very abundant.

Fig. 5 shows that QH1 transcripts could be detected in two cases of chronic pancreatitis and two cases of obstructive pancreatitis but not in control pancreas, pancreatic adenocarcinomas, or pancreatic endocrine tumor. Trypsinogen I mRNA levels were comparable in all RNA samples studied. The QH1 transcript could not be evidenced in mRNA extracted from human liver, kidney, spleen, or mammary gland, nor in mRNA from the pancreatic cancer cell lines Mia-Paca and Capan I (Fig. 6).

Southern blot analysis of the QH1 gene. Fig. 7 shows a Southern blot analysis of human genomic DNA probed with QH1 after restriction with five endonucleases. The blot was prepared as described in Methods and washed under conditions of high stringency. The limited number of restriction fragments revealed by the probe suggest that the QH1 gene is present in low copy numbers, perhaps as a single copy.

Localization of QH1 to the human pancreas. The antibodies raised to the synthetic NH_2-terminal peptide of QH1 were used for detecting the protein in the diseased pancreas. Western blot analysis of pancreatic homogenate supernatant (Fig. 8) revealed a single polypeptide, with an apparent mol wt of

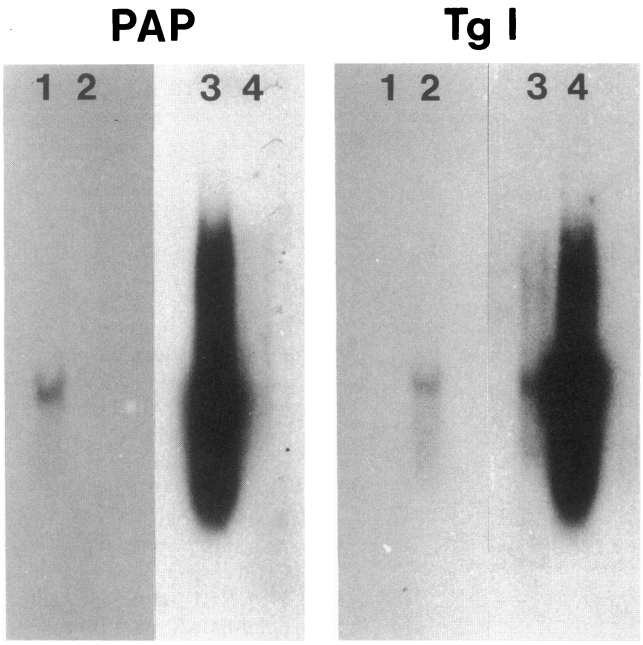

Figure 4. Detection of the PAP-H transcript in pancreatic RNA from control tissue and acute pancreatitis by Northern blot analysis. 25 μg of pancreatic RNA from acute pancreatitis (lanes *1* and *3*) or control tissue (lanes *2* and *4*) were submitted to electrophoresis, transferred onto nylon membranes, and probed with QH1 (PAP-H) cDNA (*left panel*) or human trypsynogen I cDNA (*right panel*). In each panel, lanes *1* and *2* corresponded to a 4-h exposure and lanes *3* and *4* to a 3-d exposure.

~ 16,000, compatible with the predicted size of mature QH1. No signal was visible with normal pancreas. Immunolocalization on pancreatic sections from patients with acute pancreatitis (Fig. 9) revealed that the apical regions of acinar cells were strongly labeled whereas duct cells and fibroblasts were not stained. Again, no signal could be seen on sections of undiseased pancreas.

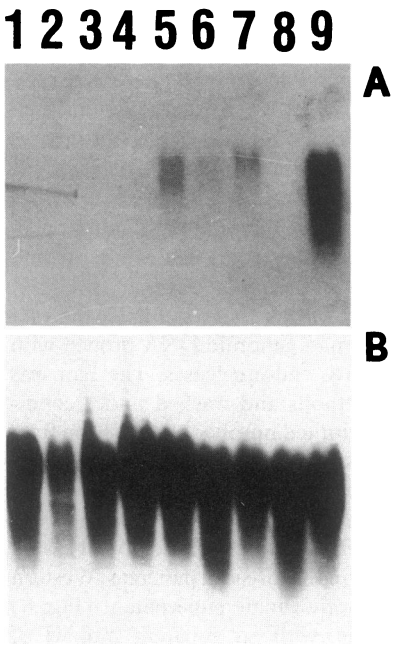

Figure 5. Detection of the PAP-H transcript in pancreatic RNA from patients with various diseases by Northern blot analysis. 25 μg RNA were loaded in each lane. Lanes *1* and *2*, control tissue; lanes *3* and *4*, pancreatic adenocarcinoma; lanes *5* and *6*, chronic pancreatitis; lanes *7* and *9*, obstructive pancreatitis; lane *8*, endocrine tumor. The filter was probed with QH1 (PAP-H) cDNA (*A*). The same filter was then dehybridized and probed with human trypsinogen I cDNA (*B*).

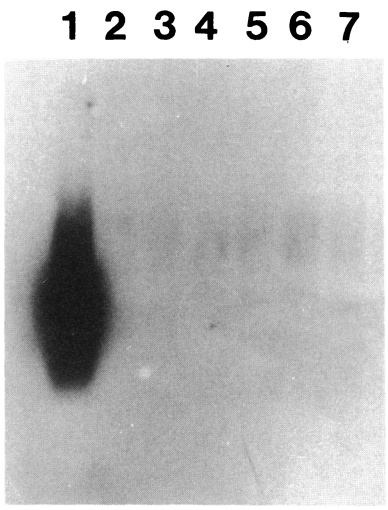

Figure 6. Detection of the PAP-H transcript in RNA from various human tissues and cell lines, by Northern blot analysis. 10 μg of RNA extracted from pancreas with acute pancreatitis (lane *1*), kidney (lane *2*), spleen (lane *3*), liver (lane *4*), mammary gland (lane *5*), and the cell lines Mia-Paca (lane *6*) and Capan I (lane *7*) were processed as described in Methods and probed with QH1 (PAP-H) cDNA.

Serum concentration of the QH1 protein. Serum concentration of QH1 was measured in healthy subjects and patients presenting with various pancreatic diseases (Fig. 10). QH1 concentration was below 0.6 pmol/ml in all samples from healthy subjects and from 26 patients with other abdominal diseases whose diagnoses are listed in Methods. In that group, two out of seven patients with stones in the common bile duct and two patients with cancer of the main bile duct showed moderate increase. On the contrary, all samples from patients with acute pancreatitis (19 cases) showed higher concentrations, values ranging from 15 to 82 pmol/ml. 7 of 13 patients with chronic pancreatitis showed elevated values of serum QH1 protein. In four of them hospitalized for recurrent pain, values were comparable to those of patients with acute pancreatitis.

Discussion

Management of acute pancreatitis has seen little improvement in the last decade. Future prospects seem better, however, because of the wealth of information recently obtained in experimental models, concerning regulation of gene expression (2), modifications in hormonal response (31), and alterations in the secretory pathway taking place during pancreatic inflammation (32). A novel secretory protein (PAP), whose expression increases sharply during acute pancreatitis contrary to other secretory products (5), was also described in the rat. Such a protein might be of clinical relevance as potential marker of the disease. Because many rat secretory proteins have their equivalent in human pancreas (33) we investigated whether a protein similar to PAP was expressed in human tissue during pancreatic inflammation. A classical strategy would have been to look for a PAP-H in the pancreatic juice of patients with acute pancreatitis, but juice collection in those patients is impossible for ethical reasons. We developed another strategy based on the assumption that if PAP-H does exist, human and rat PAP mRNA sequences should be similar enough to allow detection by heterologous screening.

A single full-length cDNA (QH1) was selected in the human cDNA library upon screening with the rat PAP cDNA and sequencing (Fig. 1). Analysis of its nucleotide sequence revealed that the region homologous to rat PAP cDNA, which

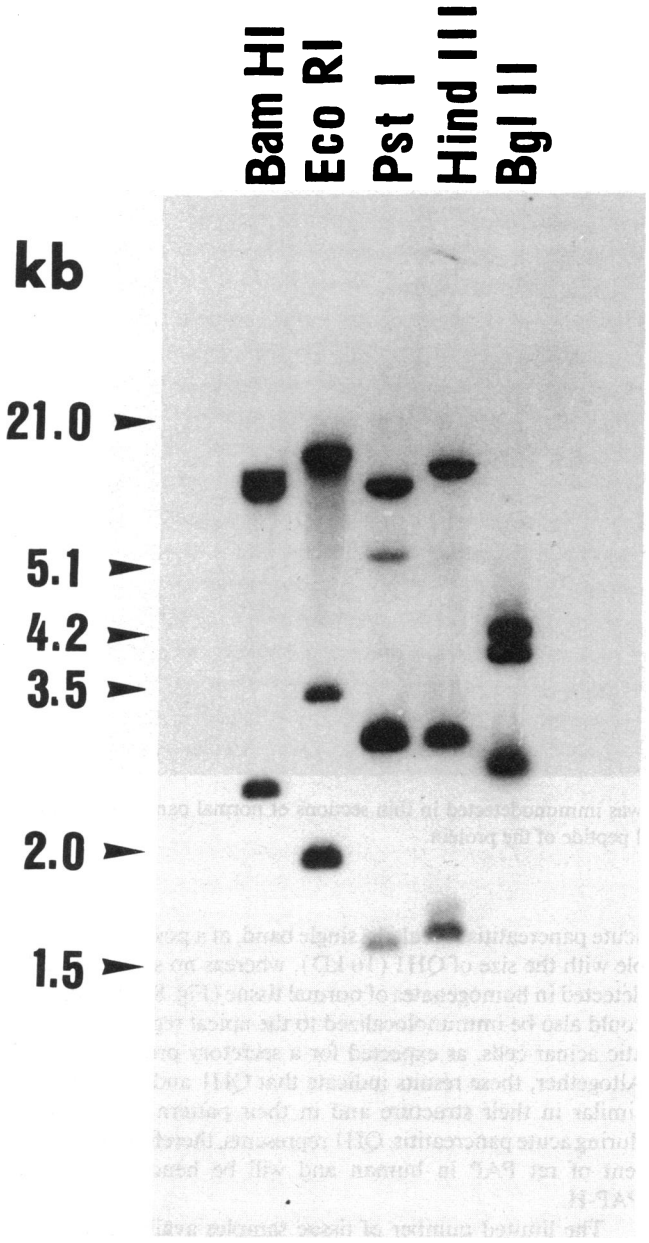

Figure 7. Analysis of the PAP-H gene(s) by Southern blotting. Fragments of human genomic DNA generated by endonuclease restriction were probed with QH1 (PAP-H) cDNA. Migration of size markers is indicated on the left.

allowed selective hybridization, extended from around nucleotide 45 to nucleotide 565, which corresponded in fact to the coding region of the transcript. The protein encoded by QH1 contained the same number of amino acids as rat PAP (Fig. 2) and the six cysteine residues were located in identical positions suggesting a similar organization of the disulphide bridges. The two sequences had an overall amino acid identity of 71%, which is in the range of values obtained when comparing related pancreatic proteins in rats and in humans (34). Relative conservation of the coding regions of QH1 and rat PAP mRNA and lack of homology between their noncoding regions could reflect differences in selective pressure. Genomic analysis (Fig. 7) revealed that the QH1 gene was present in low copy numbers, probably as a single copy, as shown for rat PAP (unpublished observations).

Whether the QH1 protein shares with rat PAP the ability to induce bacterial aggregation is impossible to verify at this time, because the purified protein is not available. It is noteworthy, however, that the QH1 protein has retained the structural domains specific of calcium-dependent lectins (Fig. 3), already characterized in rat PAP, which suggests a similar function.

The most important characteristic of the rat PAP gene, because of its potential implications, is a very low expression in normal pancreas and a dramatic increase during the acute phase of the disease. Presence of a single clone encoding QH1 in the cDNA library constructed with mRNA from control tissue was a first indication that the QH1 transcript was also expressed at a low level in the absence of inflammation. In fact, that transcript was not detectable by Northern blot analysis in control RNA, in conditions where trypsinogen gave a strong signal (Figs. 4 and 5). In a similar analysis of RNA extracted from a tissue fragment resected during necrohemorrhagic pancreatitis, the opposite situation was observed with a faint signal for trypsinogen and a strong hybridization with QH1 (Fig. 4). Decreased expression of trypsinogen and other pancreatic enzymes was reported in the rat during acute pancreatitis and considered as part of a defense mechanism including PAP overexpression (2). A similar mechanism might thus exist in humans, QH1 overexpression during acute pancreatitis being comparable to that of rat PAP during the acute phase of experimental pancreatitis. On the other hand, QH1 expression was not detectable in human pancreatic adenocarcinoma or in a pancreatic endocrine tumor, nor could it be detected in human cancer cell lines of pancreatic origin (Fig. 6) but was induced at low level in obstructive pancreatitis and some cases of chronic pancreatitis (Fig. 5). Such inductions are therefore probably caused by the focal inflammatory lesions associated with both diseases rather that to the primary diseases themselves. No ex-

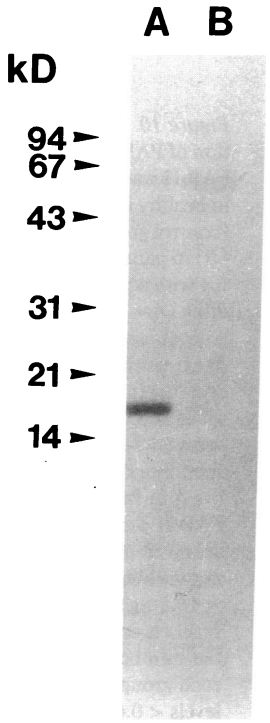

Figure 8. Immunodetection of PAP in pancreatic homogenate from acute pancreatitis (*A*) and control tissue (*B*). The antibody raised to the NH_2-terminal peptide of PAP-H was used to detect the protein in pancreatic homogenate supernatant (Western blot analysis). Position of molecular weight markers is indicated to the left.

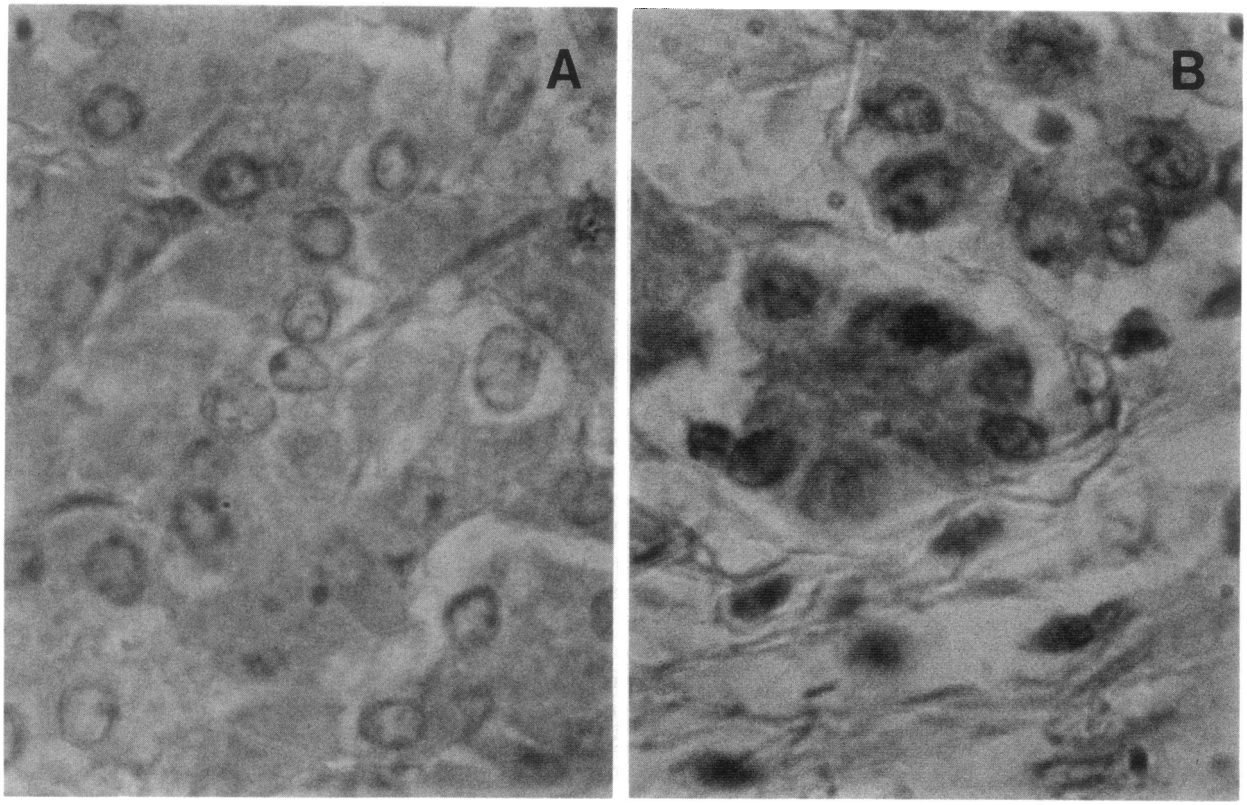

Figure 9. Immunolocalization of PAP-H in pancreatic tissue. The protein was immunodetected in thin sections of normal pancreas (*A*) and pancreas with acute pancreatitis (*B*), with the antibody to the NH_2-terminal peptide of the protein.

pression was observed in liver, spleen, kidney, or mammary gland (Fig. 6).

Further characterization of the QH1 protein was undertaken with antibodies raised to a synthetic peptide in the amino-terminal region of the QH1 sequence. Western blot analysis of proteins from pancreatic homogenates of patients with acute pancreatitis revealed a single band, at a position compatible with the size of QH1 (16 kD), whereas no signal could be detected in homogenates of normal tissue (Fig. 8). The protein could also be immunolocalized to the apical region of pancreatic acinar cells, as expected for a secretory protein (Fig. 9). Altogether, these results indicate that QH1 and PAP are very similar in their structure and in their pattern of expression during acute pancreatitis. QH1 represents, therefore, an equivalent of rat PAP in human and will be henceforth named PAP-H.

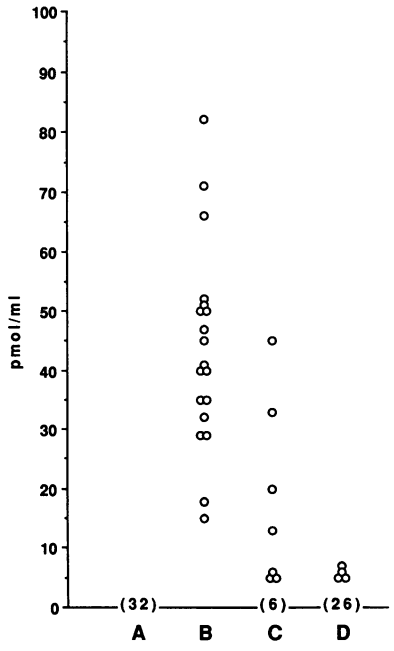

Figure 10. Quantification of PAP-H in serum. PAP-H was assayed (*A*) in healthy individuals (control group, $n = 34$); (*B*) in patients presenting with acute pancreatitis ($n = 19$); (*C*) in patients with chronic pancreatitis ($n = 13$); and (*D*) in patients with other abdominal diseases ($n = 30$, corresponding diagnoses are listed in Methods). PAP-H concentrations are given in picomoles per milliliter (1 pmol = 16.5 ng). Figures in brackets correspond to the number of cases in each group with PAP-H levels < 0.6 pmol/ml.

The limited number of tissue samples available for acute pancreatitis made it difficult to estimate the range of PAP-H expression during pancreatic inflammation, information obtained in the rat by measuring the rate of PAP synthesis (3, 5). We used an indirect approach, based on the fact that all pancreatic secretory proteins reach the bloodstream during the acute phase of pancreatitis. Measuring the increase in serum PAP-H concentration would provide therefore a conservative estimate of PAP-H overexpression in pancreas. An immunoassay was constructed using the antibody to the NH_2-terminal end of the protein and serum PAP-H was quantified in healthy subjects and a series of patients with pancreatitis and other abdominal diseases (Fig. 10). PAP-H serum levels were at near background values in healthy subjects and in patients with abdominal diseases other than pancreatitis but increased dramatically in all cases of acute pancreatitis and during acute exacerbations of chronic pancreatitis. Increases were 25–140 times over background, in good agreement with the 100–300 times increases observed in rat (3) and data on PAP-H gene expression (Figs. 4 and 5). These results confirm the important overexpression of

the protein during pancreatic inflammation. They also suggest that PAP-H, which is not detectable in serum of healthy individuals and appears in large amounts in patients with pancreatic inflammation, might be a very useful marker of acute pancreatitis.

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