Identification of a Second Rat Pancreatitis-Associated Protein. Messenger RNA Cloning, Gene Structure, and Expression during Acute Pancreatitis[†,‡]

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ABSTRACT: The pancreatitis-associated protein (PAP) is a lectin-related secretory protein present in small amounts in the rat pancreas and overexpressed during the acute phase of pancreatitis. On the other hand, PAP is constitutively expressed in the intestinal tract but not in other tissues. We cloned from a pancreatic cDNA library two overlapping cDNAs encoding a protein structurally related to PAP. This second PAP, which was called PAP II, was the same size as the original PAP (PAP I) and showed 74.3% amino acid homology. Studies on gene expression demonstrated that PAP II mRNA concentration increased within 6 h following induction of pancreatitis, reached maximal levels (>200 times control values) at 24–48 h, and decreased thereafter, similar to PAP I. However, PAP II mRNA could not be detected in the intestinal tract or in other tissues. We also isolated a PAP II genomic DNA fragment which was characterized over 2.7 kb of gene sequence and 1.9 kb of 5′ flanking sequence. The 5′ end of the coding sequence was determined by primer extension of the PAP II mRNA. The PAP II coding sequence spanned six exons separated by five introns. Several potential regulatory elements were identified in the promoter region, including two glucocorticoid-response elements and one IL-6-response element. Antibodies raised to a synthetic peptide of PAP II detected a single band in Western blot analysis of the pancreatic secretory proteins from rats with pancreatitis, with a M_r compatible with the theoretical M_r of PAP II. PAP I and PAP II are members of a new family of pancreatic secretory proteins, structurally related to C-type lectins, that might be classified among the acute-phase proteins.

We have recently reported that a novel rat pancreatic secretory protein, the pancreatitis-associated protein (PAP), was specifically secreted by the pancreas during the acute phase of pancreatitis (Keim et al. 1991). PAP was not detectable in the pancreas of control animals. It could be evidenced in juice 6 h after induction of experimental acute pancreatitis, reached a maximum during the acute phase (24–48 h), and disappeared during recovery (Keim et al., 1991). Cloning of the rat PAP mRNA provided the primary structure of the encoded protein (Iovanna et al., 1991b), which comprised 175 amino acids including a 26 amino acid signal peptide. Gene expression of PAP and several other secretory proteins was monitored in rat pancreas during experimental acute pancreatitis (Iovanna et al., 1991a). A rapid and massive rearrangement of the pattern of pancreatic gene expression was observed (Iovanna et al., 1991a). Concentrations of most messenger RNAs encoding pancreatic enzymes decreased significantly, whereas PAP mRNA concentration increased dramatically. A moderate increase was also observed for lithostathine (formerly called PSP) (Sarles et al., 1990), which was the only described pancreatic protein with significant structural similarity to PAP (Rouquier et al., 1991). In addition, both proteins displayed marked homology with the carboxy-terminal region of C-type animal lectins (Drickamer, 1988), which probably explains their capacity for aggregating bacteria (Iovanna et al., 1991b, 1993a).

Analysis of pancreatic juice proteins by two-dimensional gel electrophoresis revealed that PAP, by far the most prominent new spot appearing after induction of pancreatitis, was surrounded by several minor spots also absent from normal juice (Keim et al., 1991). This observation suggested that several proteins were overexpressed during pancreatitis. We describe in this report one of those proteins, whose mRNA and gene were sequenced. It was called PAP II because of its structural homology with PAP and similar pattern of expression during pancreatitis.

EXPERIMENTAL PROCEDURES

Isolation and Sequencing of cDNAs. Total pancreatic RNA was prepared as recommended by Chirgwin et al. (1979) from rats sacrificed 24 h after induction of an experimental acute pancreatitis. Poly(A)$^+$ RNA was purified by affinity chromatography on oligo(dT)–cellulose (Aviv & Leder, 1972) and used to direct cDNA synthesis in the presence of reverse transcriptase. DNA polymerase was used in conjunction with RNase H to synthesize the complementary DNA strands. Double-stranded cDNA was ligated into the bacteriophage λ gt-11 DNA with *Eco*RI linkers and packaged using the Amersham packaging kit (Amersham, U.K.). The library contained a total of 3.5×10^5 independent recombinant clones. This library was screened by the plaque screening procedure (Huyng et al., 1985) in conditions adapted to heterologous hybridization. The R4 insert, corresponding to most of the rat PAP mRNA sequence (Iovanna et al., 1991b), was ^{32}P-labeled by random priming (Feinberg & Vogelstein, 1983) to a specific activity of 10^9 cpm/μg and used as probe to screen about 2.5×10^3 recombinant clones. Duplicate filters were

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‡ The nucleotide sequences reported in this paper have been submitted to the GenBank/EMBL Data Bank, accession numbers L10229 and L10230.

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prehybridized for 4 h at 65 °C in a solution containing 6× SSC (SSC is 150 mM NaCl and 15 mM sodium citrate), 0.1% bovine serum albumin, 0.1% Ficoll, 0.1% poly(vinylpyrrolidone), 0.5% SDS, and 100 μg/mL denatured herring sperm DNA. Overnight hybridization was conducted in the same buffer in the presence of 5×10^6 cpm/mL cDNA probe, at 65 °C. Filters were washed 2 × 15 min with 6× SSC and 0.1% SDS at 50 °C (low-stringency conditions). Twenty-five positive clones were selected after two rounds of screening.

Identification of the Rat PAP II cDNA. Phage suspensions (1 μL) corresponding to the 25 PAP-positive clones were spotted onto a lawn of Y 1090 cells and incubated at 37 °C overnight. Phage DNAs were transferred to Hybond membranes (Amersham, U.K.), and the filters were prehybridized and hybridized at 65 °C as described above, again with labeled 4R DNA as probe. Then the filters were washed 2 × 60 min with 0.1× SSC and 0.2% SDS at 68 °C (high-strigency conditions). Two clones (VOIS-D and VOIS-E) were selected on the basis of positivity after washing under low-stringency conditions and negativity after washing under high-stringency conditions.

Subcloning and Sequencing of cDNAs Encoding Rat PAP II. Purified inserts from VOIS-D and VOIS-E were subcloned in the M13 mp18/mp19 system (Messing et al., 1981), and the single-stranded recombinant phages were sequenced in both directions (Sanger et al., 1977) with Sequenase version 2.0 (USB), following the recommendations of the manufacturer. Sequencing was initiated by the universal M13 primer or by appropriate synthetic oligonucleotides.

Genomic Library Screening. A genomic library constructed in the phage λ Charon 4A (Clontech) was screened with the PAP II cDNA (VOIS-E clone), following standard methods (Maniatis et al., 1982). Appropriate DNA fragments were subcloned into pBluescript KS^+/SK^+ phagemid (Stratagene) vectors for sequencing.

Primer Extension. A synthetic oligonucleotide primer complementary to nucleotides 159–182 of the PAP II mRNA sequence was 5′ end-labeled as already described (Maniatis et al., 1982). Hybridization of the oligonucleotide (40 000 cpm) to 10 μg of total pancreatic RNA was performed at 30 °C for 16 h in 40 mM PIPES, 1 mM EDTA, 400 mM NaCl, and 80% deionized formamide, pH 6.4 RNA was precipitated, and cDNA was synthesized from the primer by the addition of 50 units of avian myeloblastosis virus reverse transcriptase over 60 min at 42 °C. The samples were analyzed by electrophoresis on 6% acrylamide/urea (sequencing) gels.

Genomic Southern Hybridization Analysis. High molecular weight DNA from rat liver was isolated as described (Maniatis et al., 1982). DNA was digested with various restriction endonucleases, fractionated by electrophoresis on agarose gels, and transferred to Hybond membranes (Maniatis et al., 1982). Filters were prehybridized in 5× SSPE (SSPE is 180 mM NaCl, 1 mM EDTA, and 10 mM NaH_2PO_4, pH 7.5), 0.5% SDS, 0.1% Ficoll, 0.1% poly(vinylpyrrolidone), 0.1% bovine serum albumin, and 200 μg/mL denatured herring sperm DNA for 4 h at 68 °C. They were hybridized in a solution of the same composition except that the final concentration of SDS was 0.2%. Hybridization was allowed to proceed for 16 h at 68 °C. The hybridization probe was prepared by random-primed labeling of the cloned 885-bp PAP II cDNA (VOIS-E). The filters were washed twice in 2× SSPE and 0.1% SDS for 30 min each at room temperature and twice in 0.1× SSPE and 0.1% SDS for 30 min each at 65 °C, allowed to dry, and exposed to Hyperfilm TM (Amersham, U.K.) at −80 °C.

Animal Experiments. Acute pancreatitis was induced in male Sprague-Dawley rats by retrograde injection of 200 μl of 1% sodium taurocholate into the main pancreatic duct, as described by Lankisch et al. (1974). The animals were killed after 0, 1.5, 3, 6, 12, 24, 48, 96, 168, and 240 h. Pancreases were removed and quickly trimmed free of fat, and fragments of the glands were processed according to Chirgwin et al. (1979) for RNA purification. In a separate experiment, acute pancreatitis was induced by the same method. Two rats were sacrificed after 24 h, and the RNA from stomach, duodenum, jejunum, ileum, cecum, colon, brain, kidney, liver, submaxillary gland, heart, testis, spleen, skeletal muscle, lung, and prostate was extracted. Also, hepatic acute phase was induced in two rats by subcutaneous injection of turpentine at two sites in the dorsolumbar region, as described by Schreiber et al. (1986). The rats were sacrificed after 24 h, and RNA from the liver was purified. Finally, acute renal failure was induced in two rats by an intraperitoneal injection of 500 mg/kg folic acid in 150 mM $NaHCO_3$ (Cowley et al., 1989). RNA was extracted from the kidneys after 36 h.

Northern and Dot-Blot Analysis. For Northern blot hybridization, 10 μg of denatured RNA was loaded onto each gel lane and electrophoretically separated on 1% agarose gel (Maniatis et al., 1982). The RNA was transferred to Hybond filters, which were baked and prehybridized for 4 h at 42 °C in buffer containing 50% deionized formamide, 5× SSPE, 0.1% bovine serum albumin, 0.1% Ficoll, 0.1% poly(vinylpyrrolidone), 0.5% SDS, and 200 μg/mL denatured herring sperm DNA. Hybridization was conducted at 42 °C for 16–20 h in the same buffer in the presence of the ^{32}P-labeled VOIS-E probe. Duplicate membranes were hybridized with β-actin as a control. Then the filters were washed four times, 5 min each time, at room temperature in 2× SSC and 0.1% SDS, twice for 15 min at 50 °C in 0.1× SSC and 0.1% SDS, and once for 30 min in 0.1× SSC. Filters were dried and exposed to X-ray films at −80 °C for 16 h. Quantitative analysis of RNAs was performed according to White and Bancroft (1982), as previously described (Renaud et al., 1986). RNA samples (5, 2.5, 1.25, and 0.625 μg/sample) were denatured and dotted onto prewashed nitrocellulose filters using a Minifold apparatus (Schleicher and Schuell Inc.). The filters were washed as described for Northern blots. Filters were exposed to Hyperfilm TM for autoradiographic detection. The autoradiographs of the blots were scanned with a Multiscan apparatus (LKB Instruments), and the results were analyzed as previously described (Calvo et al., 1991), the slopes of the regression lines giving an estimate of the mass of the probed mRNA/μg of total RNA.

Western Blot Analysis. The predicted amino-terminal peptide (EDSQKAVPSTR) of the protein was chemically synthesized (Neosystem, Strasbourg, France) and used to immunize New Zealand white rabbits. Pancreatic secretory proteins from rats with pancreatitis (Lankisch et al., 1974) or from control rats were subjected to polyacrylamide gel electrophoresis, transferred to nitrocellulose, and probed with the rabbit antibodies as previously described (Orelle et al., 1992).

RESULTS

Isolation and Sequencing of PAP-Related Clones. PAP II cDNA clones (VOIS-D and VOIS-E) were detected in a pancreatic cDNA library from pancreatitis-induced rats by low-stringency screening with 4R cDNA (see Experimental Procedures). VOIS-D and VOIS-E cDNAs were subcloned into M13 vectors and sequenced (Figure 1). Inserts from both clones had overlapping nucleotide sequences, although

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atcccagatcactgcaaggcagaccttagcaaagcagagatggggctaaga      51

ctagtgtatccctgaagacctaggcaaagggagagagggccctcgtttgct     102

tgttctcgttgactacactctctgtgccctgtttgctgcttccttgaagac     153

aag ATG CTG CCT CGT CTG TCC TTC AAC AAT GTG TCC TGG      192
    Met Leu Pro Arg Leu Ser Phe Asn Asn Val Ser Trp       12

ACG CTG CTC TAC TAC CTG TTC ATA TTT CAG GTA CGA GGT      231
Thr Leu Leu Tyr Tyr Leu Phe Ile Phe Gln Val Arg Gly      25

GAA GAC TCC CAG AAG GCA GTG CCC TCT ACA CGA ACC AGC      270
Glu Asp Ser Gln Lys Ala Val Pro Ser Thr Arg Thr Ser      38

TGC CCC ATG GGC TCC AAG GCT TAT CGT TCT TAC TGC TAT      309
Cys Pro Met Gly Ser Lys Ala Tyr Arg Ser Tyr Cys Tyr      51

ACC TTG GTC ACG ACA CTC AAA TCC TGG TTT CAA GCA GAT      348
Thr Leu Val Thr Thr Leu Lys Ser Trp Phe Gln Ala Asp      64

CTG GCC TGC CAG AAG AGA CCT TCA GGA CAC CTT GTG TCT      387
Leu Ala Cys Gln Lys Arg Pro Ser Gly His Leu Val Ser      77

ATT CTT AGT GGA GGT GAG GCT TCC TTT GTG TCT TCC CTG      426
Ile Leu Ser Gly Gly Glu Ala Ser Phe Val Ser Ser Leu      90

GTG ACA GGC AGA GTG AAC AAC AAC CAA GAC ATC TGG ATT      465
Val Thr Gly Arg Val Asn Asn Asn Gln Asp Ile Trp Ile     103

TGG CTC CAT GAT CCA ACA ATG GGT CAA CAA CCC AAT GGA      504
Trp Leu His Asp Pro Thr Met Gly Gln Gln Pro Asn Gly     116

GGT GGA TGG GAG TGG AGT AAC TCT GAC GTA CTG AAT TAT      543
Gly Gly Trp Glu Trp Ser Asn Ser Asp Val Leu Asn Tyr     129

CTC AAC TGG GAT GGG GAT CCT TCC TCT ACT GTC AAC CGT      582
Leu Asn Trp Asp Gly Asp Pro Ser Ser Thr Val Asn Arg     142

GGT AAC TGT GGC AGT CTA ACA GCT ACC TCG GAG TTT CTG      621
Gly Asn Cys Gly Ser Leu Thr Ala Thr Ser Glu Phe Leu     155

AAG TGG GGA GAC CAT CAC TGT GAT GTG GAA TTA CCT TTT      660
Lys Trp Gly Asp His His Cys Asp Val Glu Leu Pro Phe     168

GTC TGC AAG TTC AAG CAG tagacccgcagcatcctgagtttatca      705
Val Cys Lys Phe Lys Gln                                 174

tgaaggtcaccgtgacaagggatgtacatgacaaggctgtacttgcttcac     756

agtcctgcatcagacttgctgttatgattttccatcttccttcatccgttt     807

tcttccccccatttcaggctttttttggtatagttcctgctttgcaatctt     858

gaagataaataatacataaaatcaaaa                             885
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FIGURE 1: Complete nucleotide sequence of PAP II and amino acid sequence of its predicted protein product. The nucleotide sequence of the mRNA was deduced from that of the cDNA insert of clone VOIS-E. The 5′ and 3′ untranslated regions are given in lowercase letters. The open reading frame encoding rat PAP II is given in capital letters. The variant sequence GATAAA, a possible polyadenylation signal, is underlined.

the VOIS-D insert was shorter. A single open reading frame was found in the corresponding mRNA sequence. The predicted PAP II protein contained regions of extensive sequencing similarity with PAP I (Figure 2). The VOIS-E clone contained the initiating methionine codon preceded by the consensus translational start site GACAAG (Kozac, 1984), followed by a cluster of hydrophobic amino acids, which conforms to the general pattern previously observed for leader sequences (von Heijne, 1984). The 3′ region consisted of a TAG stop codon at position 679 followed by 200 nucleotides of untranslated message containing a putative polyadenylation recognition site (GATAAA). The VOIS-E clone contained 156 nucleotides of 5′ untranslated region. The encoded polypeptide comprised 174 amino acids and showed an overall similarity of 74.3% to rat PAP I. Positions of the six cysteine residues were conserved. Homologies with lithostathine/reg cDNA and the calcium-dependent animal lectins (C-type) observed in several regions of the rat PAP I sequence (Iovanna et al., 1991b) were also found in the PAP II sequence (data not shown). Computer analysis of the sequence indicated that mature PAP II protein had a molecular weight of 16 538 and an isoelectric point of 6.79.

Transcription Initiation Site. To map the site of initiation of PAP II transcription, a synthetic oligonucleotide complementary to the PAP II mRNA leader sequence was hybridized with total RNA from pancreas with acute pancreatitis, and the heteroduplex was extended with reverse transcriptase. The length of the extended product was 182 nucleotides, as determined by comparison with a sequence ladder in a denaturing gel. These results show that the VOIS-E clone contained the whole transcribed sequence. No extension was observed in a control experiment carried out under identical conditions with intestinal RNA, from which PAP II mRNA is absent (Figure 3).

Southern Analysis of Rat Genomic DNA. A rat genomic blot was prepared as described under Experimental Procedures, hybridized with the VOIS-E cDNA, and washed under high-stringency conditions. The probe hybridized to a small set of DNA fragments (Figure 4), suggesting that PAP II gene is present in low copy numbers, perhaps as a single copy. The hybridization pattern for PAP II was different from that of PAP I (unpublished results).

Structure of the PAP Gene. About 5×10^5 phage plaques were screened. Clone B31 was selected after three rounds of screening. The intron–exon boundaries and sizes were determined by DNA sequence analysis employing oligonucleotides as primer and subcloned restriction fragments of the genomic clones as template. The gene spanned about 3 kb and contained six exons ranging in size from 22 bp (exon I) to 268 bp (exon VI) and five introns ranging from 175 bp (intron 1) to 723 bp (intron 2). All splice junction sequences flanking the introns conformed to the consensus splice junction sequences and the GT/AG splice rule (Breathnach &

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PAP II  MLPRLSFNNVSWTLLYYLFIF-QVRGEDSQKAVPS    1 - 34
PAP I   MLHRLAFPVMSWMLLSCLMLLSQVQGEDSPKKIPS    1 - 35

PAP II  TRTSCPMGSKAYRSYCITLVTTLKSWFQADLACQK    35 - 69
PAP I   ARISCPKGSQAIGSYCYALFQIPQTWFDAELACQK    36 - 70

PAP II  RPSGHLVSILSGGEASFVSSLVTGRVMNNQDIWIW    70 - 104
PAP I   RPEGHLVSVLNVAEASFLASMVKNTGMSYQYTWIG    71 - 105

PAP II  LHDPTMGQQPNGGGWEWSNSDVLNYLNWDGDPSST    105 - 139
PAP I   LHDPTLGGEPNGGGWKWSNNDIMNYVNWERNPSTA    106 - 140

PAP II  VNRGNCGSLTATSEFLKWGDHHCDVELPFVCKFKQ    140 - 174
PAP I   LDRGFCGSLSRSSGFLRWRDTTCEVKLPYVCKFTG    141 - 175
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FIGURE 2: Amino acid sequence comparison between pre-PAP II and pre-PAP I. Shaded areas correspond to amino acid identities.

Table I: Sequences of the Intron–Exon Boundaries in the Rat PAP II Gene[a]

exon	3′ acceptor	exon size (bp)	5′ donor	intron size (bp)
I		22	...CACTGCAAGGCAG**gt**aagtggga	175
II	ttccttcc**ag**ACCTTAGCAAAGC...	207	...CAG GTA CGA G**gt**aagtcttc	723
			...Gln Val Arg G	
III	ctccatcc**ag**GT GAA GAC TC...	119	...T CAA GCA GAT**gt**gtgtgatg	195
	ly Glu Asp se...		...e Gln Ala Asp	
IV	ccttaaat**ag**CTG GCC TGC C...	138	...T CCA ACA ATG**gt**gagattct	507
	Leu Ala Cys G...		...p Pro Thr Met	
V	t6actgtgc**ag**GGT CAA CAA C...	127	...GCT ACC TCG G**gt**aagaaatg	239
	Gly Gln Gln P...		...Ala Thr Ser G	
VI	ctctctcc**ag**AG TTT CTG AA...	268	...ACATAAAATCttttattttt	
	lu Phe Leu Ly...			

[a] The exon sequences are in capital letters; the intron sequences are in lowercase letters. Amino acids at the exon–intron junctions are indicated.

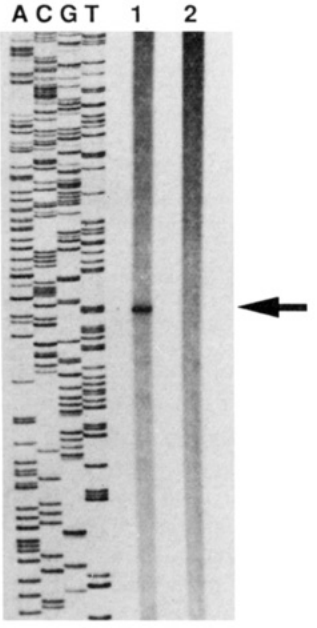

FIGURE 3: Determination of the 5′ end of the PAP II transcript by primer extension analysis. An end-labeled oligonucleotide (5′-TTGTTGAAGGACAGACGAGGCAGC-3′) complementary to the PAP II mRNA was annealed to 10 μg of RNA from rat pancreas in the acute phase of pancreatitis (1) or intestine (2) and extended with reverse transcriptase. The position of the labeled product is indicated by an arrow. An M13 mp18 ladder was used as a size marker.

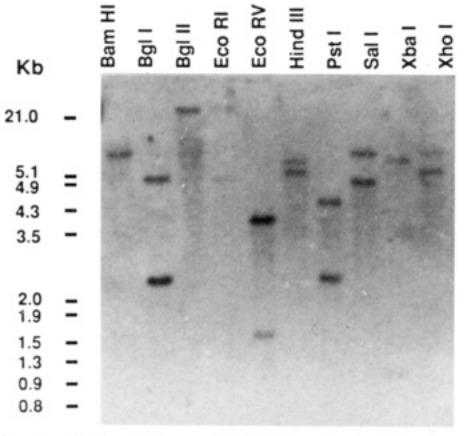

FIGURE 4: Analysis of the rat PAP II gene by Southern blotting. Fragments of rat genomic DNA generated by endonuclease restriction were probed with VOIS-E (PAP II) cDNA. Migration of size markers is indicated on the left.

Chambon, 1981) (Table I). Nucleotide sequences of all exons were in complete agreement with the cDNA sequence. A region comprising 1893 nucleotides upstream from the

FIGURE 5: Nucleotide sequence of the 5′ flanking region of the rat PAP II gene (sense strand). The transcription initiation site determined by primer extension (Figure 3) is marked with a bent arrow, and the proposed cap site is numbered +1. A TATA-like box is underlined (solid line), two contiguous CACCTG boxes (transcription activators of the pancreas-specific genes) are boxed, a puttive acute-phase reaction signal (IL-6-response element) is underlined (straight arrow), and two glucocorticoid-response element are shaded. A C-rich region is underlined (dashed line).

initiation site for transcription was sequenced (Figure 5). Neither TATA nor CAAT boxes were found, but a TATA-like sequence (ATAAAA) was present at position –30, within the region expected for TATA boxes (–20 to –30 bp). Two contiguous CACCTG motifs, involved together with a pyrimidine-rich stretch in the binding of the Pan-1 transcription activators in pancreas-specific genes (Meister et al., 1989), were located at positions –204 and –210. However, such a pyrimidine-rich stretch was not found in the vicinity. In addition, the sequence CTGGGAA, identical to the putative acute phase reaction signal (IL-6-response element), was located at position –127 (Hattori et al., 1987). Moreover, two classes of glucocorticoid-response elements appeared at positions –608 (TGTTCT) (Sheidereit et al., 1986) and –1415 (AGAACAT) (Payvar et al., 1983). Finally, a C-rich region was present at position –645.

Regulation of PAP II mRNA Expression during the Course of Acute Pancreatitis. Northern blot analysis of PAP II mRNA was performed by probing identical amounts of pancreatic RNA from controls and rats sacrificed 48 h after induction of acute pancreatitis. The hybridization pattern revealed a very low signal with RNA from healthy control

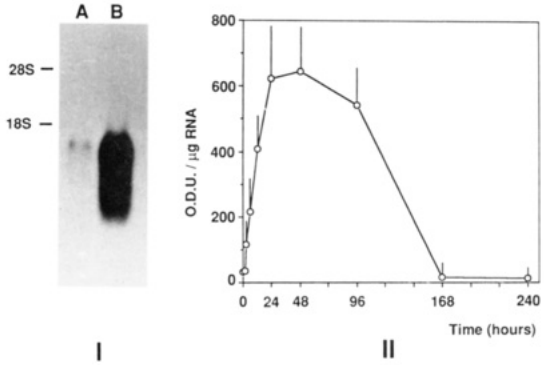

FIGURE 6: Panel I: Northern blot analysis of mRNA encoding PAP II in total pancreatic RNA obtained from controls (A) and after induction of acute pancreatitis (B). Panel II: Change in PAP II mRNA concentration as a function of time following induction of acute pancreatitis. Values were obtained by dot-blot hybridization of equal amounts of pancreatic RNA to ^{32}P-labeled cDNA (VOIS-E) probe. They were estimated by scanning the autoradiograms of the blots and expressed as optical density units, (ODU)/μg RNA. Values were expressed as means ± SEs (II).

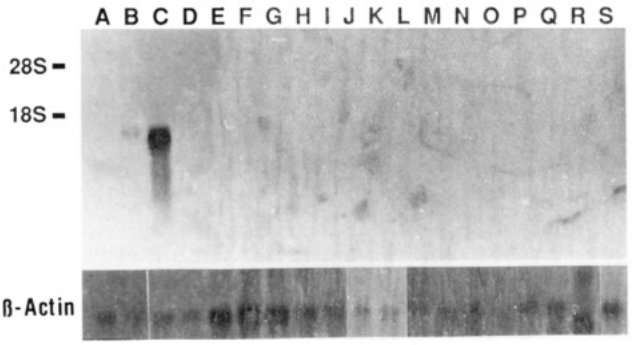

FIGURE 7: Tissue specificity of rat PAP II mRNA expression. RNA samples subjected to analysis were prepared from the following tissues: (A) AR 42J cell line, (B) normal pancreas, (C) pancreas in the acute phase of pancreatitis, (D) stomach, (E) duodenum, (F) jejunum, (G) ileum, (H) cecum, (I) colon, (J) brain, (K) kidney, (L) liver, (M) submaxillary gland, (N) heart, (O) testis, (P) spleen, (Q) skeletal muscle, (R) lung, and (S) prostate. Ten micrograms of RNA was separated on an agarose gel and blotted to a nylon membrane. The blot was probed with the random primer labeled cDNA (VOIS-E) or β-actin cDNA as described under Experimental Procedures.

animals but a strong signal in acute pancreatitis-induced rats (Figure 6). Quantification of the increase in PAP II mRNA concentration during the acute phase of pancreatitis was performed by dot-blot hybridization (Figure 6). PAP II mRNA concentration increased within 6 h following induction of pancreatitis, reached maximal levels (>200 times control values) at 24–48 h, and decreased thereafter. Levels at 7–10 days were similar to control values.

Tissue Expression of mRNA Encoding PAP II. To determine the tissue distribution of PAP II gene expression, total RNA was extracted from several rat tissues (stomach, duodenum, jejunum, ileum, cecum, colon, brain, kidney, liver, submaxillary gland, heart, testis, spleen, skeletal muscle, lung, prostate, and AR 42J cell line) and analyzed by RNA blot hybridization using VOIS-E cDNA as probe (Figure 7). PAP II mRNA could not be detected in any of them. Similar results were observed when we analyzed these rats tissues from animals with acute pancreatitis. In addition, no signal was obtained after probing with PAP II RNAs extracted from liver after induction of an acute phase or from kidney with acute experimental failure.

Immunodetection of PAP II. Antibodies were raised against a synthetic peptide which corresponded to the NH_2-terminal

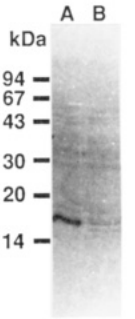

FIGURE 8: Immunodetection of PAP II in pancreatic juice from rat with acute pancreatitis (A) and control (B). The antibody raised to the NH_2-terminal peptide of PAP II was used to detect the protein in pancreatic juice (Western blot analysis). The positions of molecular weight markers are indicated to the left.

sequence of rat PAP II. Two out of three immunized rabbits (xb and xc) produced sera that reacted in Western blot experiments with a ~15-kDa pancreatic secretory protein that is absent from the pancreatic juice of control animals (Figure 8).

DISCUSSION

PAP was originally identified as a lectin-related protein overexpressed during the acute phase of pancreatitis (Iovanna et al., 1991b; Orelle et al., 1992) and constitutively expressed by the intestinal tract (Iovanna et al., 1993b). This report describes the isolation of a new protein, related to PAP, which was characterized by determining the structures of its mRNA and gene and by analyzing its expression during pancreatitis. The protein is very similar to PAP (57.1% identity and 74.3% similarity) without being an allelic form of it since the two cDNAs were cloned from the same library and hybridized to different gene fragments in Southern blot analysis. The protein was called PAP II because, like PAP, it is strongly overexpressed during acute pancreatitis. As a consequence, PAP will henceforth be called PAP I.

Analysis of PAP II gene expression revealed several interesting features. Pancreatic expression was very low in control animals, whereas substantial amounts of PAP II mRNA were present in pancreas during the acute phase of pancreatitis (Figure 6). Expression was not detected in tissues other than pancreas under normal conditions (Figure 7) or after induction of acute pancreatitis (not shown). Neither was it detected in liver or kidney after an acute response of these tissues was generated (not shown). PAP II expression therefore seems specific to the pancreas in the acute phase of pancreatitis. Structural analysis of the promoter region of the PAP II gene provided some support for this observation. All genes expressed in the exocrine pancreas described so far contain in their promoter region a conserved sequence of 20 bp, called the "pancreas-specific sequence" (Boulet et al., 1986). That sequence was not detected in the PAP II gene, in agreement with the very weak expression in normal conditions. Another regulatory element described for pancreas-specific genes, to which the transcriptional activator PTF 1 binds (Cockell et al., 1989), consists of two noncontiguous consensus sequences (Kruse et al., 1988; Meister et al., 1989). Only one of them, the CACCTG box, was found in the promoter region, as a tandem repeat at position –210. That sequence alone is obviously not sufficient for constitutive expression in pancreas. Two glucocorticoid-response elements and one IL-6-response element (Figure 5) are other specific DNA sequences present in the promoter region which might bind activation factors synthesized during the course of acute

pancreatitis and trigger PAP II gene activation in pancreas after the onset of pancreatitis. A situation comparable to acute pancreatitis has been described in liver, where synthesis of four major acute-phase proteins, serum amyloid A protein, fibrinogen, C-reactive protein, and α-2 macroglobulin, increases up to 1,000-fold in response to tissue injury. Both IL-6 and glucocorticoids are required for the full induction of the α-2 macroglobulin gene (Hattori et al., 1987) and other acute-phase proteins (Kageyama et al., 1987; Kunz et al., 1989; Oliviero & Cortese, 1989; Arcone et al., 1988; Poli & Cortese, 1989). These presence of potential cis-acting sequences in the 5′ flanking region of the PAP II gene might therefore be associated with the fact that PAP II is expressed predominantly in pancreas and is transcriptionally upregulated during the acute phase of pancreatitis. However, actual assessment of their role must await functional studies involving deletions and mutant reporter gene fusion constructs.

PAP I and PAP II are members of a new family of pancreatic secretory proteins structurally related to C-type lectins, and on the basis of their gene expression, both proteins might, on the other hand, be classified among the acute-phase proteins. PAP II is probably not directly involved in normal pancreatic function but participates in the early response to acute pancreatitis. Although the function of PAP I is not known, its high concentration in pancreatic juice during pancreatitis and its ability to induce aggregation of several bacterial strains suggests that it has a protective role against infection (Iovanna et al., 1991b), a frequent complication of pancreatitis. The amino acid sequences established for PAP I and PAP II suggest that the two pancreatitis-associated proteins might be functionally related.

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REFERENCES

Arcone, R., Gualandi, G., & Ciliberto, G. (1988) *Nucleic Acids Res. 16*, 3195–3207.

Aviv, H., & Leder, P. (1972) *Proc. Natl. Acad. Sci. U.S.A. 69*, 1408–1412.

Boulet, A. M., Erwin, C. R., & Rutter, W. J. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 3599–3603.

Breathnach, R., & Chambon, P. (1981) *Annu. Rev. Biochem. 50*, 349–383.

Calvo, E., Dusetti, N. J., Cadenas, B., Dagorn, J.-C., & Iovanna, J. L. (1991) *Pancreas 6*, 150–156.

Chirgwin, J. M., Przybyla, A., MacDonald, R. J., & Rutter, W. J. (1979) *Biochemistry 18*, 5294–5299.

Cockell, M., Stevenson, B. J., Strubin, M., Hagenbuchle, O., & Wellauer, P. K. (1989) *Mol. Cell. Biol. 9*, 2464–2476.

Cowley, B. D., Chadwick, L. J., Grantham, J. J., & Calvet, J. P. (1989) *J. Biol. Chem. 264*, 8389–8393.

Drickamer, K. (1988) *J. Biol. Chem. 263*, 9557–9560.

Feinberg, A., & Vogelstein, B. (1983) *Anal. Biochem. 132*, 6–13.

Hattori, M., Abraham, L. J., Northemann, W., & Fey, G. H. (1987) *Proc. Natl. Acad. Sci. U.S.A. 87*, 2364–2368.

Huyng, T. V., Young, R. A., & Davies, R. W. (1985) in *DNA cloning: a practical approach* (Glover, D. M., Ed.) pp 49–78, IRL Press, Oxford.

Iovanna, J. L., Keim, V., Michel, R., & Dagorn, J.-C. (1991a) *Am. J. Physiol. 261*, G485–G489.

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

Iovanna, J. L., Frigerio, J.-M., Dusetti, N. J., Ramare, F., Raibaud, P., & Dagorn, J.-C. (1993a) *Pancreas* (in press).

Iovanna, J. L., Keim, V., Bosshard, A., Frigerio, J.-M., Dusetti, N. J., & Dagorn, J.-C. (1993b) *Am. J. Physiol.* (in press).

Kageyama, R., Kitamura, N., Ohkubo, H., & Nakanishi, S. (1987) *J. Biol. Chem. 262*, 2345–2351.

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

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

Kozak, M. (1984) *Nucleic Acids Res. 12*, 857–872.

Kruse, F., Komro, C. T., Michuoff, C. H., & MacDonald, R. J. (1988) *Mol. Cell. Biol. 8*, 893–902.

Kunz, D., Zimmermann, R., Heisig, M., & Heinrich, P. C. (1989) *Nucleic Acids Res. 17*, 1121–1138.

Lankisch, P. G., Winckler, K., Bokermann, M., Schmidt, H., & Creutzfeldt, W. (1974) *Scand. J. Gastroenterol. 9*, 725–729.

Maniatis, T., Fritsch, E. F., & Sambrook, J. (1982) in *Molecular cloning: A laboratory manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Meister, A., Weinrich, S. L., Nelson, C., & Rutter, W. J. (1989) *J. Biol. Chem. 264*, 20744–20751.

Messing, J., Crea, R., & Seeberg, P. H. (1981) *Nucleic Acids Res. 9*, 309–332.

Oliviero, S., & Cortese, R. (1989) *EMBO J. 8*, 1145–1151.

Orelle, B., Keim, V., Masciotra, L., Dagorn, J.-C., & Iovanna, J. L. (1992) *J. Clin. Invest. 90*, 2284–2291.

Payvar, F., De Franco, D., Firestone, G. L., Edgar, B., Wrange, O., Okret, S., Gustaffson, J. A., & Yamamoto, K. R. (1983) *Cell 35*, 381–392.

Poli, V., & Cortese, R. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 8202–8206.

Renaud, W., Giorgi, D., Iovanna, J. L., & Dagorn, J.-C. (1986) *Biochem. J. 235*, 305–308.

Rouquier, S., Verdier, J.-M., Iovanna, J. L., Dagorn, J.-C., & Giorgi, D. (1991) *J. Biol. Chem. 263*, 9557–9560.

Sanger, F., Nicklen, S., & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 5463–5467.

Sarles, H., Dagorn, J.-C., Giorgi, D., & Bernard, J.-P. (1990) *Gastroenterology 99*, 900–901.

Schreiber, G., Alred, A. R., Thomas, T., Birch, H. E., Dickson, P. W., Tu, G. F., Henrich, P. C., Northemann, W., Howlett, G. J., De Jong, F. A., & Mitchel, A. (1986) *Inflammation 10*, 59–66.

Sheidereit, C., Westphal, H. M., Carlson, C., Bosshard, H., & Beato, M. (1986) *DNA 5*, 383–391.

von Heijne, G. (1984) *EMBO J. 3*, 2315–2318.

White, B. A., & Bancroft, F. C. (1982) *J. Biol. Chem. 257*, 8569–8572.