Amino Acid Sequence of Human Pregnancy-Associated Plasma Protein-A Derived from Cloned cDNA†,‡

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ABSTRACT: The amino acid sequence of human pregnancy-associated plasma protein-A (PAPP-A), a component of the circulating complex with the proform of eosinophil major basic protein (proMBP), has been determined from partial protein sequencing and from sequencing of cloned cDNA. The PAPP-A monomer contains 1547 amino acid residues, but is derived from a larger precursor of placental origin. PAPP-A contains 82 Cys residues, which are all bridged, 14 putative sites for N-glycosylation, and 7 putative sites for attachment of glycosaminoglycan groups. The C-terminal part of PAPP-A contains 5 approximately 60-residue motifs related to the short consensus repeats of complement proteins and selectins. The SCRs presently known can be grouped into three classes: complement-type, class I; selectin-type, class II; PAPP-A-type, class III. PAPP-A further contains three approximately 26-residue motifs, related to the lin–notch motifs of proteins regulating early tissue differentiation, and, in addition, a putative Zn^{2+} binding site similar to that found in many metalloproteinases has been identified. Apart from these features, the PAPP-A sequence is not related to other known protein sequences.

Using immunological procedures, four proteins termed pregnancy-associated plasma proteins A, B, C, and D (PAPP-A, -B, -C, and -D),[1] respectively, were identified in the early 1970's in human late pregnancy serum (Gall & Halbert, 1972; Lin et al., 1974). While PAPP-C and -D represented pregnancy-specific β_1-glycoprotein and placental lactogen, respectively (Lin et al., 1974; Bohn, 1971), PAPP-A and -B were novel proteins. PAPP-B has recently been described as a 1300-kDa octadecamer of presumed identical 74-kDa subunits (Bossi et al., 1993).

PAPP-A was previously thought to be a tetramer composed of approximately 200-kDa subunits, but recent work has shown that PAPP-A is probably a heterotetramer composed of two PAPP-A subunits disulfide-bonded to each of two mutually disulfide-bridged 50–90 kDa proMBP subunits (Oxvig et al., 1993). The term PAPP-A/proMBP is hereafter used for what has been known earlier as PAPP-A.

PAPP-A/proMBP has α_2 mobility, and contains approximately 19% carbohydrate including glycosaminoglycan; it binds to heparin and metal chelate columns and contains zinc (Lin et al., 1974; Bischof, 1979; Sutcliffe et al., 1980; Folkersen et al., 1981a; Davey et al., 1983; Sinosich et al., 1982, 1983, 1990). It has recently been found that both the PAPP-A and proMBP subunits are extensively glycosylated (unpublished results).

PAPP-A/proMPB can be detected 4–6 weeks after conception, and it is present at approximately 50 mg/L in pooled late pregnancy serum (Folkersen et al., 1981b; Westergaard et al., 1983a). Postpartum PAPP-A/proMBP disappears from the circulation with a half-life of 3–4 days (Lin et al., 1976). PAPP-A/proMPB, which is not present in the fetus, has been localized to the trophoblastic tissue of the placenta (Lin & Halbert, 1976; Wahlström et al., 1981; Tornehave et al., 1984), which is considered to be the main source of circulating PAPP-A/proMBP. In nonpregnant women, PAPP-A/proMBP has been reported to be present in low amounts in the granulosa cells and the follicular fluid of the ovary, fallopian tube mucosa, cervical mucosa, and endometrium (Sinosich et al., 1984; Sjöberg et al., 1984a,b, 1986). In men, low levels of PAPP-A/proMBP have been detected in testes and seminal fluid (Bischof et al., 1983; Sjöberg et al., 1984c; Sinosich et al., 1987). With the exception of the rare cases of Cornelia de Lange Syndrome (Westergaard et al., 1983b), monitoring circulating levels of PAPP-A/proMBP is not considered of value in predicting the outcome of threatened pregnancies (Westergaard et al., 1983a, 1985; Stabile et al., 1989). However, it was indicated recently that depressed PAPP-A/proMBP levels correlate with cases of Down's Syndrome (Wald et al., 1992). The PAPP-A gene has been assigned to the q33.1 region of human chromosome 9 (Silahtaroglu et al., 1993).

The biological role of PAPP-A/proMBP is not known. However, to provide the biochemical background for studying the properties of PAPP-A and its gene expression, we report here the amino acid sequence of the 1547-residue PAPP-A subunit deduced from protein and cDNA sequence analyses. Evidence is presented that the PAPP-A subunit present in circulating PAPP-A/proMBP is derived from a large precursor. It is shown that stretches of the PAPP-A sequence are homologous with the SCRs of several complement-regulating proteins and that sequence elements are present which are similar to the LNRs, found in the homeotic genes lin-12 and glp-1 of *Caenorhabditis elegans*, notch of *Drosophila melanogaster* and rat, and xotch of *Xenopus laevis* (Kidd

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‡ The cDNA and protein sequences reported here have been deposited with the GenEMBL Data Bank under Accession Number X68280.

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[1] Abbreviations: PAPP, pregnancy-associated plasma protein; proMBP, proform of eosinophil major basic protein; SCR, short consensus repeat; LNR, lin–notch repeat; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; HPLC, high-performance liquid chromatography.

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et al., 1986; Yochem et al., 1988; Yochem & Greenwald, 1989; Coffman et al., 1990; Weinmaster et al., 1991). In addition, a Zn^{2+} binding motif similar to that found in metalloproteinases (Woessner, 1991) is also present.

MATERIALS AND METHODS

Proteins. PAPP-A/proMBP and PAPP-A subunit material were prepared from pooled pregnancy serum as described by Oxvig et al. (1993). Tosylphenylalanine chloromethyl ketone-treated trypsin was from Cooper Biomedicals. Restriction enzymes, polynucleotide kinase, T4 DNA ligase, terminal transferase, and mung bean nuclease were from Boehringer Mannheim or Amersham. *Escherichia coli* ligase and DNA polymerase were from New England Biolabs, and RNase H was from Bethesda Research Laboratories.

Column Materials. DEAE-Sephacel and oligo(dT)–Sepharose were from Pharmacia. Nucleosil 100-5 C18 was from Machery-Nagel and was packed into a 8 × 250 mm column.

Chemicals and Kits. cDNA synthesis and cloning system kits and radionucleotides ([α-^{32}P]dATP and [α-^{35}S]dATP) were from Amersham. [γ-^{32}P]ATP was from ICN. Random-primed DNA labeling kits were from Boehringer Mannheim. The Sequenase version 2 kits were from U.S. Biochemical Corp. Nitrocellulose filters were purchased from Millipore Corp. and Schleicher & Schuell. Standard chemicals were from Sigma, Merck, Serva, and BDH.

Miscellaneous Procedures. SDS–PAGE, gel staining, and electroblotting were carried out as previously described (Kristensen et al., 1990). Peptides were purified by anion-exchange chromatography and reversed-phase HPLC as described earlier (Kristensen et al., 1986). Edman degradations were carried out on a Beckman 890C instrument (2–25 nmol samples) or on an Applied Biosystems 477A sequencer (10–200 pmol samples), and the PTH-amino acids were analyzed as described earlier (Sand et al., 1985; Kristensen et al., 1990). Analysis of amino acids and amino sugars was done by cation-exchange chromatography using postcolumn derivatization with sodium hypochlorite/*o*-phthaldialdehyde (Sottrup-Jensen, 1993).

Preparation of Tryptic Peptides for Sequence Analysis. Approximately 18 mg of reduced and carboxymethylated PAPP-A/proMBP was digested with 0.37 mg of trypsin for 2 h at room temperature. The peptides were initially separated on DEAE-Sephacel as described earlier (Kristensen et al., 1986). Of 52 pools made, 6 were further separated by reversed-phase HPLC on Nucleosil C18 (not shown), resulting in 15 pure peptides. These were sequenced partially or completely, and seven degenerate oligodeoxynucleotide probes were synthesized on the basis of the obtained sequences.

PAPP-A/proMBP (3 mg/mL in 50 mM Tris-HCl/50 mM NaCl, pH 8.0) was treated with 1:100 (w/w) trypsin for 30 min to obtain partial fragmentation. After inactivation of trypsin with 1 mM phenylmethanesulfonyl fluoride, the digest was separated on reducing 8% and 16% Tris/Tricine gels, blotted onto PVDF membranes, and stained, and the major bands were subjected to N-terminal sequencing.

Isolation of RNA. Normal-term placentas were obtained after Caesarean sections. Total RNA was purified from the placental tissues by the guanidinium thiocyanate method (Chirgwin et al., 1979). RNA to be used for cDNA library construction was 1- or 2-fold poly(A^+)-selected by chromatography on oligo(dT)–cellulose columns (Aviv & Leder, 1972).

For construction of a random-primed cDNA library enriched for copies of large (>28 S) RNA molecules, 1-fold poly(A^+)-selected RNA was size-selected by ultracentrifugation in step gradients of sucrose (Wetsel et al., 1990).

Construction of cDNA Libraries. Two cDNA libraries were constructed: The first library [oligo(dT)-primed] was constructed from 2-fold poly(A^+)-selected RNA in the pCDV1-PL/pSP62-K2 vector system (Noma et al., 1986; Boel et al., 1990). The second random-primed library constructed from poly(A^+) RNA greater than 28 S was cloned in the pUEX1 vector using *Bam*HI/blunt-end adaptors in the cloning process as previously described (Gubler & Hoffman, 1983; Haymerle et al., 1986).

Isolation and Sequencing of PAPP-A cDNA Clones. The cDNA library screening, isolation, and sequencing of PAPP-A cDNA clones were as previously described (Kristensen et al., 1986). It was found crucial to grow clones in the pUEX1 vector below 30 °C because most inserts were partially or wholly deleted even at 34 °C. Sequences were compiled and examined using the Staden programs (Staden 1980, 1982a,b) and the GCG program package (Devereux et al., 1984). The final nucleotide and derived amino acid sequences were compared to the Genbank (release 77.0), the MIPSX (release 35), and the Swissprot (release 24) databases.

RESULTS AND DISCUSSION

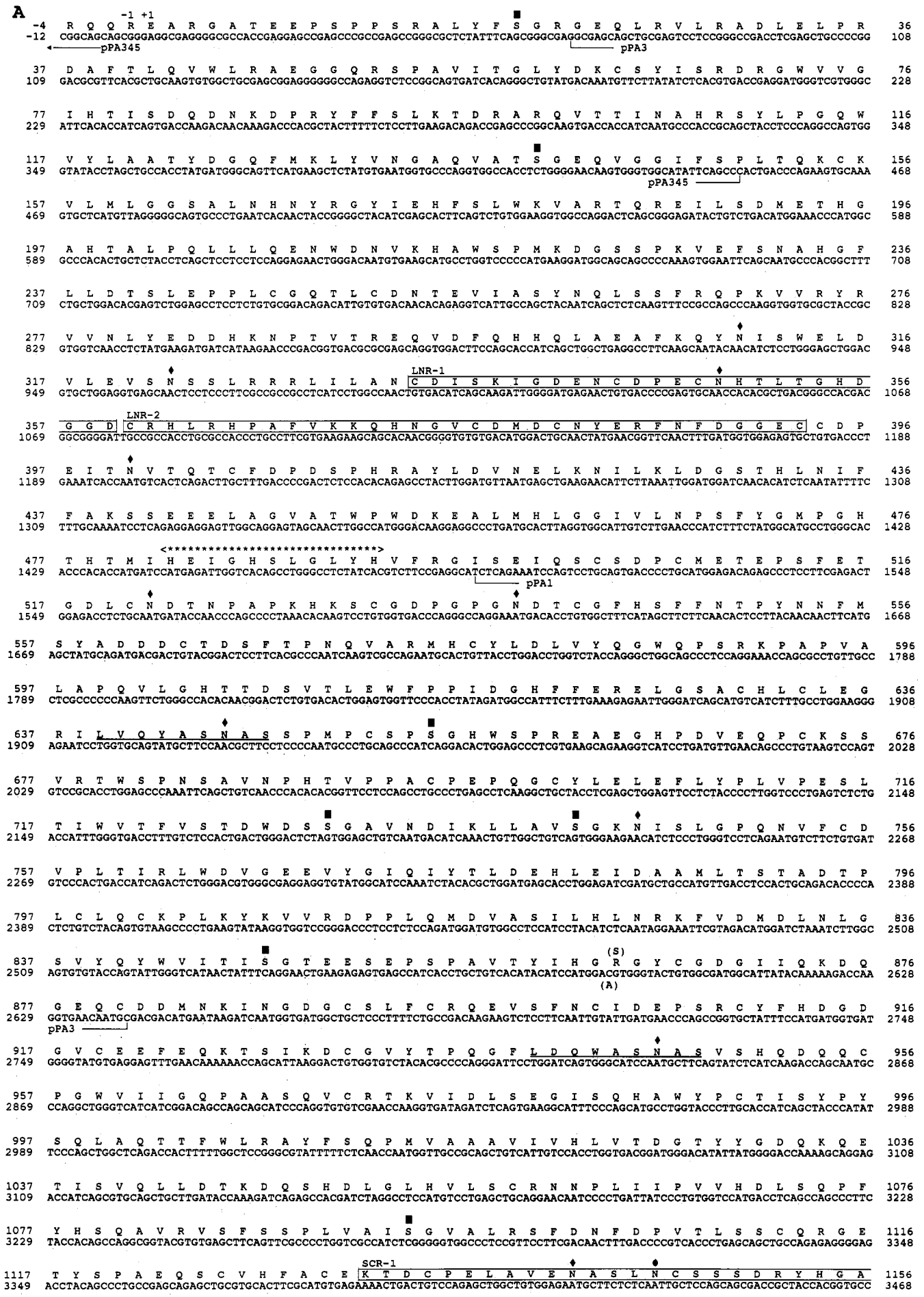
Partial Protein Sequence Analysis of the PAPP-A Subunit. Reduced and carboxymethylated PAPP-A subunit was purified as described by Oxvig et al. (1993). After SDS–PAGE and electroblotting, a single N-terminal sequence, Glu-Ala-Arg-Gly-Ala-Thr-Glu-Glu-Pro-Ser-Pro-, was found.

Fifteen random tryptic peptides obtained after anion-exchange chromatography followed by reversed-phase HPLC were completely or partially sequenced. Twelve of the peptides were located in the PAPP-A sequence: TP1, DAFTLQVWLR (37–46); TP2, LYVNGAQVATSGEQV (130–144); TP3, FNFDGGECCDPEITNVTQTC (386–405); TP4, LDGSRDLNIFFAK (427–439); TP5, GISEIQSCSDPCMETEPS (496–513); TP6, ILVQYASNASSPMPCSPSG (638–656); TP7, EAEGHPDVEQPCK (662–674); TP8, SQTGPSVTVTCTEGK (1179–1193); TP9, VGSFCK (1289–1294); TP10, LQCPDGYAIGSECATSCLDH (1410–1429); TP11, DIPHWLNPTR (1444–1453); TP12, VVCTAGLK (1457–1464). The underlined sequences show the stretches on which degenerate oligodeoxynucleotide probes were based (two contiguous 17-mers were synthesized from the sequence of TP3). Three peptides were located in the preproMBP sequence: TproMBP1, FQWVDGSR (172–179);[2] TproMBP2, AHCLR (210–214); TproMBP3, RLPFICSY (215–222).

Limited digestion of PAPP-A/proMBP with 1/100 (w/w) trypsin for 30 minutes yielded well defined cleavage products seen on SDS–PAGE (not shown). The SDS–PAGE bands were sequenced and gave the following sequences: 1) GATEEPSPPSRALYF (4–528) with the underlined sequence being used as basis for a degenerate oligodeoxynucleotide probe; 2) HKSXGDPGPGNDT (529–925); 3) SQTGPSVTVTXT (1179–1308); KSKKRAFKTQ (1309–1547); and 5) a mixture of two sequences, one starting at position 1309, the other being TSIKDXGVYTPQ (926–1178).

PAPP-A Nucleotide and Amino Acid Sequences. Screening the two cDNA libraries with the oligodeoxynucleotide probes yielded several clones which were isolated and characterized. The overlapping nucleotide and derived amino acid sequences of pPA1 (3.5 kb), pPA3 (2587 bp), and the approximately 500 bp 3' end of pPA345 (3.5 kb) are shown in Figure 1A.

[2] Numbering of preproMBP.


A
-1 +1
pPA345
pPA3
pPA345
LNR-1
LNR-2
pPA1
pPA3
(S)
(A)
SCR-1

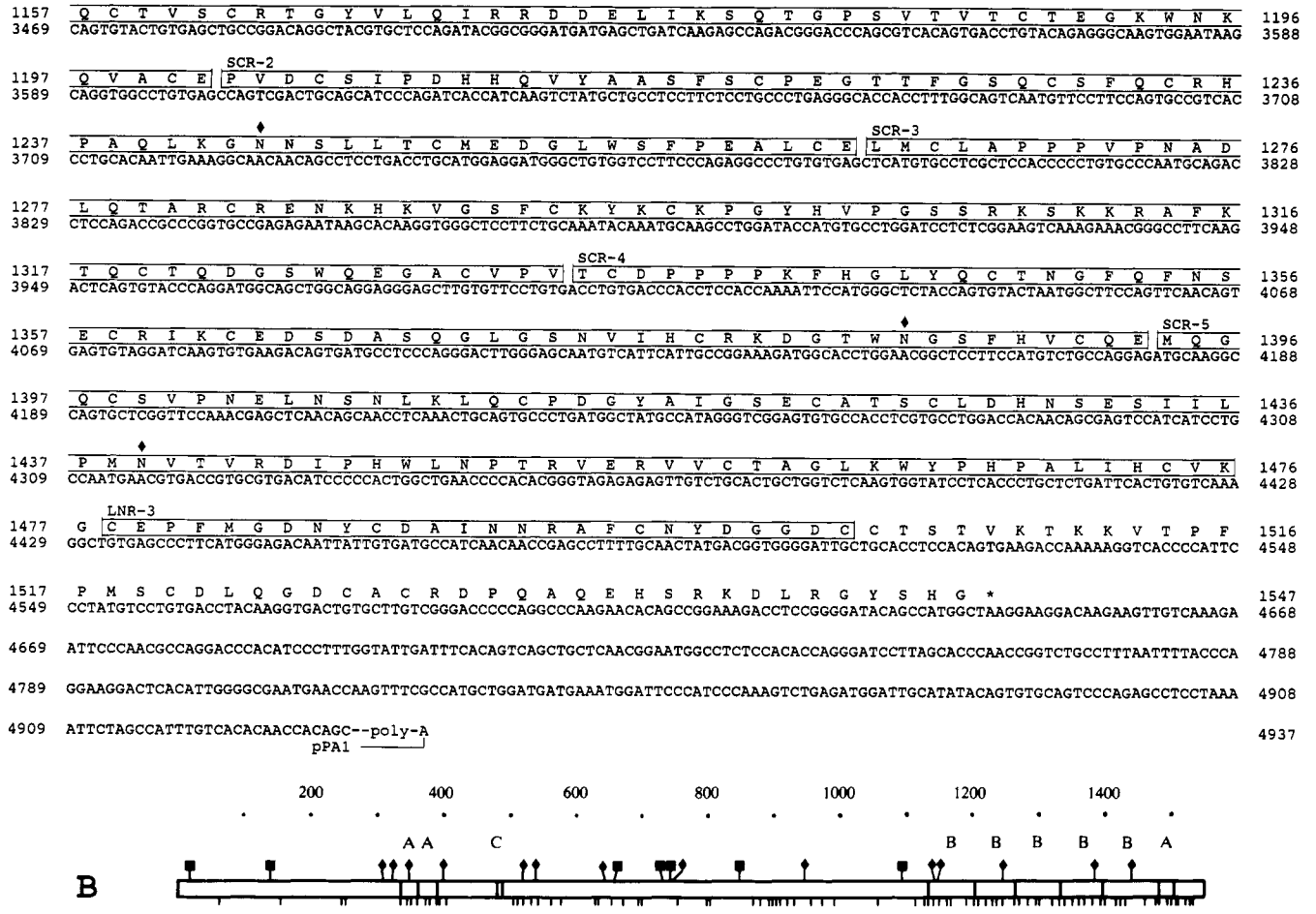


FIGURE 1: (A) Nucleotide sequence and deduced amino acid sequence of the PAPP-A subunit. Regions homologous with other proteins are boxed (SCR-1 to SCR-5, LNR-1 to LNR-3), and a putative Zn^{2+} binding site is shown (<*******>). Two nearly complete nine-residue repeats are underlined. Filled diamonds indicate putative N-glycosylation sites, and filled squares indicate putative sites of glycosaminoglycan attachment. (B) Schematic representation of the PAPP-A subunit showing the locations of the 82 half-cystine residues (vertical bars below line), LNR-related motifs (A), SCR sequence elements (B), a putative Zn^{2+} coordination site (C), 14 putative N-glycosylation sites, and 7 putative sites for attachment of glycosaminoglycans.

The clones were identified as PAPP-A clones as all sequenced PAPP-A peptide stretches were located within the deduced amino acid sequence. Two additional PAPP-A cDNA clones, pPA331 and pPA18, not shown here and also covering the overlap between pPA3 and pPA345, were partially sequenced, and it was found that they contained intron sequences.

The 4949 bp nucleotide sequence shown covers 4653 bp encoding an open reading frame of 1551 amino acids, followed by a stop codon (UAA) and 293 bp of 3'-untranslated sequence. The poly(A) signal may be CCTAAAA (4903–4909) positioned 35 bp upstream from the poly(A) tail. Only one sequence difference was found at nucleotide position 2590, the first position of a codon, where a C was determined in pPA3 and an A in pPA1, leading to arginine and serine, respectively, in the corresponding amino acid sequences.

The N-terminus of PAPP-A starts at residue 5 in the sequence shown in Figure 1A. At present, 80 residues of amino acid sequence derived from the pPA345 nucleotide sequence upstream from this site are known.[3] However, a putative signal peptide sequence was not revealed. The presence of Arg residues at positions −1 and −4 indicated that serum PAPP-A is the result of cleavage of a proform of PAPP-A, by a proteinase recognizing the Arg-X-X-Arg motif (Molloy et al., 1992).

[3] Only the portion of pPA345 encoding the sequence of mature PAPP-A plus four additional residues is shown. The full sequence of the propiece of PAPP-A will be reported later.

Further support of this came from Northern blots of placental poly(A^+) RNA and total RNA hybridized with pPA1 cDNA or synthetic oligodeoxynucleotides, since one major RNA band greater than 12 000 nucleotides and a minor band of about 8500 nucleotides were seen (Figure 2). To ensure that both bands represented PAPP-A or PAPP-A-like mRNA, we hybridized the Northern blots with several unique oligodeoxynucleotides from different parts of the sequence shown in Figure 1A and obtained identical results. Furthermore, the washes of the Northern blots were performed at different stringencies, and it was found that the two bands were present in all cases, showing that there was no cross-hybridization with other mRNAs.

Northern blots of poly(A^+) RNA from heart, lung, skeletal muscle, brain, liver, kidney, and pancreas showed no detectable PAPP-A mRNA by hybridization. Negative results were also seen using PCR amplification of first-strand-synthesized cDNA from liver, granulocytes, and lung fibroblasts. The presence of PAPP-A mRNA in testes and follicular cells is currently being investigated.

The mature PAPP-A subunit is 1547 residues long and contains 14 putative N-glycosylation sites, which are probably all occupied. Further, seven Ser residues for putative attachment of glycosaminoglycans (Oldberg et al., 1987) are present, some of which are occupied, but no galactosamine-based carbohydrate groups are present (not shown).

PAPP-A contains 82 Cys residues. Radioalkylation experiments with PAPP-A/proMBP did not reveal incorporation

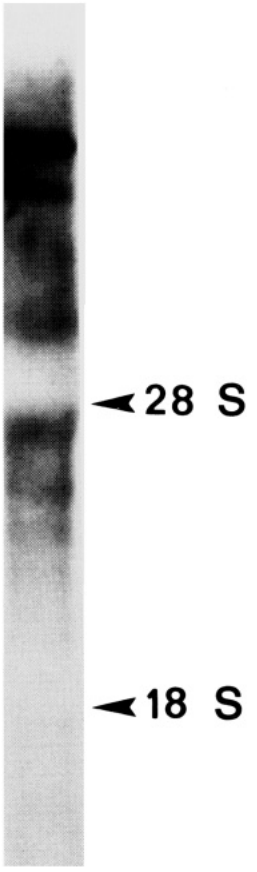


FIGURE 2: Northern blot of total placental RNA (20 μg loaded) hybridized with ^{32}P-labeled pPA1 insert.

of radioactivity, indicating that no free cysteinyl SH groups are present in PAPP-A/proMBP (unpublished results).

The observed structural motifs in PAPP-A (see below) indicated in Figure 1A as well as the positions of Cys residues and putative glycosylation sites are outlined schematically in Figure 1B. The Cys residues are grouped in several relatively large clusters, indicating possible structural domains. However, they could not be confirmed as limited digestion of PAPP-A by trypsin resulted in cleavage at residues 3, 528, 925, 1178, and 1308, all sites, except residue 3, located within extensively disulfide-bridged regions.

Identification of Sequence Motifs Present in PAPP-A and Found in Other Proteins. A comparison of the PAPP-A nucleotide and derived amino acid sequences with sequences contained in the Genbank, MIPSX, and Swissprot databases did not reveal any global similarities with known nucleotide or amino acid sequences. However, local similarities with SCR- and LNR-containing proteins were seen. Searching for internal repeating motifs using the Staden DIAGON program (Staden, 1982b), two nine amino acid stretches, five SCRs, and three LNR-like regions were seen.

The SCR-containing proteins comprise a large group of plasma and membrane proteins, which includes several complement-regulating proteins and the selectins (Kristensen et al., 1987; Reid & Day, 1989), but only a small group of presumed membrane proteins contains LNRs. That group is represented by five gene products: lin-12 and glp-1 (both from *C. elegans*); notch (*D. melanogaster* and rat); xotch (*X. laevis*) (Kidd et al., 1986; Yochem et al., 1988; Yochem & Greenwald, 1989; Coffman et al., 1990; Weinmaster et al., 1991).

PAPP-A SCRs. Aligning the sequences of the five SCRs in PAPP-A (Figure 3A) revealed the pattern of conserved residues known from other SCRs (Kristensen et al., 1986; Reid & Day, 1989). In particular, six Cys, one Pro, one Gly, and one Trp residue(s) are conserved in the same relative positions within all five SCRs. Compared with the complement SCRs which have four Cys residues disulfide-bridged in a 1 to 3 and 2 to 4 pattern (local numbering of complement SCRs) (Bendixen et al., 1992; Janatova et al., 1989; Hess et al., 1991), the PAPP-A SCRs contain two additional Cys residues placed between Cys-1 and Cys-2 (Cys-1a and Cys-1b). That pattern is also found in the selectin SCRs. Preliminary results indicate that the PAPP-A SCRs have the same 1 to 3 and 2 to 4 disulfide bridge pattern as the complement SCRs and that the additional two Cys residues form a bridge (not shown).

The degree of similarity among the PAPP-A SCRs was much lower than between the well-conserved selectin SCRs. However, except for the addtitional two Cys residues, the pattern of conserved residues in the PAPP-A SCRs and their diversity of distances are very similar to those of the complement SCRs. From these observations, we define three classes of SCRs (Figure 3B).

Class I SCRs comprise the complement SCRs (Kristensen et al., 1986; Reid & Day, 1989; Kotwal & Moss, 1988). The selectin SCRs (Johnston et al., 1989; Stamenkovic et al., 1989; Bevilacqua et al., 1989) are defined as class II SCRs, while the PAPP-A SCRs differing from both of these classes are defined as class III SCRs. Whether PAPP-A SCRs are merely structural units or whether they play a role in recognition or binding of proMBP or as yet unknown ligands is unknown.

PAPP-A LNRs. The PAPP-A sequence also contains three shorter mutually similar stretches (residues 334–360, 361–393, and 1478–1503) as shown in Figure 1. A consensus sequence was deduced (Figure 3C). In the 26–34-residue motifs, 10 positions are occupied by identical or chemically similar amino acid residues. These motifs are similar to the LNRs found until now only in five homeotic gene products. LNRs, of which there are three tandemly arranged in each of these proteins, are located in their presumed extracellular parts (Yochem & Greenwald, 1989). Although comparison of the five consensus sequences (Figure 3D) shows a marked difference between the PAPP-A LNR motifs on one side and the other known LNRs on the other side, their sequence similarity is evident. Determination of intron–exon boundaries as well as of the disulfide bridges in the LNRs should provide a better understanding of these structural units. The function of LNRs is unknown.

A Putative Zn^{2+} Coordination Site in PAPP-A. The sequence I(481)HEIGHSLGLYH (Figure 1A) is nearly identical with that containing the active-site Zn^{2+} of the matrix metalloproteinases (Woessner, 1991) and quite similar to those of other metalloproteinases (Vallee & Auld, 1992). However, upon comparison of the PAPP-A sequence with the available metalloproteinase sequences, only scattered sets of identically placed residues were revealed.

To explore the possibility that PAPP-A/proMBP might have proteolytic activity, reduced and carboxymethylated albumin, ovalbumin, fibrinogen, casein, and ribonuclease A were separately incubated with PAPP-A/proMBP at a 1:50 weight ratio for 4 h at 37 °C in the presence of 20 μM zinc acetate at pH 7.5. As judged from SDS–PAGE, PAPP-A/proMBP did not have general proteolytic activity. Preincubating PAPP-A/proMBP with trypsin for 1 h at a 1:100 weight ratio, followed by inactivation of trypsin by a 30-fold molar excess of Kunitz pancreatic trypsin inhibitor, did not cause the appearance of proteolytic activity in PAPP-A/proMBP as tested against the above substrates (not shown).

Furthermore, by incubating PAPP-A/proMBP for 4 h at 37 °C with 0.5 mM aminophenylmercuric acetate or $HgCl_2$,

A

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SCR-1    KT-DC--PELAVENASLN---CSSSDRYHGAQCTVSCRTGYVLQIRRDDELIKSQTGPSVTVT------CT-EGK-WNKQVA---CE
SCR-2    PV-DCSIPDHHQVYAAS-FS-CPEGTT-FGSQCSFQCRHPAQLKGNNSLLT----------------CMEDGL-WSFPEAL--CE
SCR-3    LM--CLAPPPVPNADL-QTARCRENKHKVGSFCKYKCKPGYHVPGSSRKSKKRAFKTQ----------CTQDGS-W-QEGA---CVPV
SCR-4    T---CDPPPPKFHG-LYQ---CTNGFQFN-SECRIKCEDSDASQGLGSNVIH----------------CRKDGT-WNGSFHV--CQE
SCR-5    MQGQCSVPNELNSNLKLQ---CPDGYAI-GSECATSCLDHNSESIILPMNVTVRDIPHWLNPTRVERVVCTA-GLKWYPHPALIHCVK
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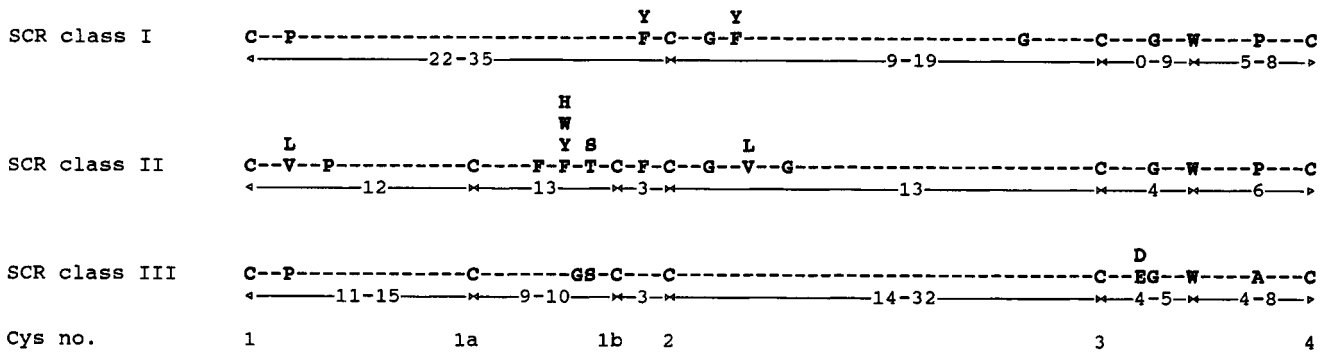
B



C

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LNR-1    C D I S K I G D E N - - - - - - - C D P E C N H T L T G H D G G D -
LNR-2    C R H L R H P A F V K K Q H N G V C D M D C N Y E R F N F D G G E C
LNR-3    C E P F M G D N Y - - - - - - - - C D     C N Y - - - - - D G G D C
                                           AINNRAF
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D

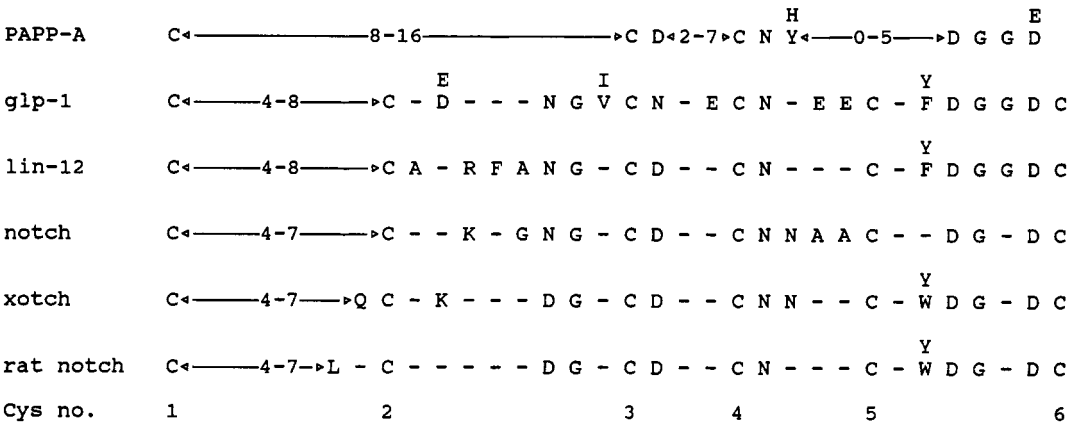

FIGURE 3: Comparison of the PAPP-A SCR and LNR motifs with SCRs and LNRs from other proteins. Panel A: Alignment of the five PAPP-A SCRs. Residues conserved in at least four SCRs are in bold-face types. Panel B: SCR class I, complement SCRs; SCR class II, selectin SCRs; SCR class III, PAPP-A SCRs. Amino acid residues conserved in more than 85% of the SCRs are in boldface type. The distances measured in amino acid residues between the conserved Cys and Trp residues of the three classes of SCR are shown. The class I SCRs contain four Cys residues (1–4), and the two additional Cys residues in SCR class II and class III are labeled 1a and 1b. Panel C: Alignment of the three PAPP-A LNRs. Conserved residues (including chemically similar residues) are in boldface type. Panel D: Comparison of the PAPP-A LNR consensus pattern with five different patterns having six conserved Cys residues (numbered). The variability in length of certain segments of the LNRs is shown. Space bars indicate that no residues are present (all species except PAPP-A).

2 mM dithiothreitol, 0.5 M urea, and 0.2 M KSCN, reagents that all can cause activation of latent collagenases (Springman et al., 1990), we observed no fragmentation of PAPP-A indicative of autolysis as seen in metalloproteinases.

The results indicate that the PAPP-A/proMBP complex is devoid of proteolytic activity. Whether PAPP-A in itself is proteolytically active, and proMBP functions as a proteinase inhibitor, is an intriguing possibility. From a comparison of the different classes of metalloproteinases, their sequences appear to be related only through their conserved Zn^{2+} binding sites.

In conclusion, in this report the sequence of the 1547-residue PAPP-A subunit, part of the PAPP-A/proMBP complex found in serum during pregnancy, has been described. The results show that PAPP-A is not related to the α-macroglobulins as suspected earlier (Sutcliffe et al., 1980). The major part of the PAPP-A sequence is not related to known protein sequences, but two types of sequence motifs indicative of a modular design of PAPP-A, in addition to a putative Zn^{2+} binding site, have been revealed.

Our results indicate that PAPP-A is synthesized as a precursor with the protein present in circulating PAPP-A/

proMBP constituting the C-terminal part of the precursor. The biological role of PAPP-A/proMBP in pregnancy is not known, but the presence in PAPP-A of SCR and LNR motifs, and in proMBP of motifs related to C-type lectins (Patthy, 1989), would suggest a role in molecular recognition phenomena associated with placental growth and function.

The isolation of the propart of PAPP-A from placental tissue and the determination of its sequence are in progress.

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