Quantification of Proteolytically Active Pregnancy-Associated Plasma Protein-A with an Assay Based on Quenched Fluorescence

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Background: Maternal serum concentrations of pregnancy-associated plasma protein-A (PAPP-A, pappalysin-1, EC 3.4.24.79) are used to predict the occurrence of Down syndrome. In pregnancy, PAPP-A primarily circulates as a covalent 2:2 complex with the proform of eosinophil major basic protein (proMBP), which inhibits the proteolytic activity of PAPP-A. At term, however, ~1% of PAPP-A exists as an active, uncomplexed dimer with proteolytic activity directed specifically toward insulin-like growth factor binding protein (IGFBP)-4 and IGFBP-5. No assays have been developed that allow quantification of PAPP-A proteolytic activity.

Methods: We developed a sensitive and specific immunocapture assay for PAPP-A activity based on intramolecular quenched fluorescence. We used a 26-residue synthetic peptide derived from IGFBP-4 in which specific positions on each side of the PAPP-A cleavage site were substituted with 3-nitrotyrosine and \(\text{O-aminobenzoic acid}\).

Results: The assay detected the activity of recombinant PAPP-A as well as PAPP-A in serum samples from pregnant women. The limit of detection (mean signal of blank plus 3 SD) of the active PAPP-A subunit was 13 pmol/L, and the intra- and interassay CVs were <10% and <15%, respectively. Interestingly, the fraction of active PAPP-A decreased gradually from week 7 to week 19 of pregnancy.

Conclusions: This method allows the measurement of PAPP-A enzymatic activity and because of its specificity it is relevant to the study of the biological function of PAPP-A. The method may also be useful in the diagnosis of pregnancy disorders.

Many studies have documented the diagnostic value of detecting decreased amounts of pregnancy-associated plasma protein-A (PAPP-A) antigen in the 1st and 2nd trimester, particularly in combination with other markers (1, 2). The presence of PAPP-A antigen in the circulation of pregnant women was first reported in 1974 (3), but it was only recently shown that PAPP-A is a metalloproteinase of the metzincin superfamily (pappalysin-1, EC 3.4.24.79) (4, 5). Active PAPP-A, a dimer of 400 kDa, specifically cleaves insulin-like growth factor binding protein (IGFBP)-4 (4) and IGFBP-5 (6), which modulate the biological activities of insulin-like growth factor (IGF)-I and IGF-II (7). While bound to an IGFBP, the IGFs cannot stimulate the IGF receptor; however, proteolytic cleavage at a single site in the central domain of the IGFBPs results in the release of bioactive IGF (8). The IGFs have multiple roles in fetal development and postnatal growth in both health and disease (9, 10). Data supporting the role of PAPP-A as a regulator of IGF activity include the finding that targeted disruption of the PAPP-A gene in mice caused a 40% reduction in birth weight compared with wild-type littermates (11). In humans, low amounts of circulating PAPP-A antigen are associated with low birth weight (12).

In the circulation of pregnant women, PAPP-A predominantly exists as a 500-kDa heterotetrameric 2:2 complex with the proform of eosinophil major basic protein (proMBP), denoted PAPP-A/proMBP. The PAPP-A (200 kDa) and proMBP (50 kDa) subunits of this complex...
cannot dissociate, because they are covalently bound to each other by disulfide bonds (13, 14). ProMBP is a physiological inhibitor of the proteolytic activity of PAPP-A, and therefore the PAPP-A/proMBP complex is inactive (15). Only the uncomplexed, dimeric form of PAPP-A, estimated as ~1% of the total PAPP-A at term (15), shows proteolytic activity against IGFBP-4 and -5 (16).

PAPP-A is ubiquitously expressed in human tissues, although at much lower concentrations than in the placenta (17). PAPP-A antigen is present in ovarian follicular fluid (18), the epidermis of healing human skin (19), and atherosclerotic plaques (20). In the circulation or other body fluids and tissues, however, the balance between proteolytically active dimeric PAPP-A and the inactive PAPP-A/proMBP complex is unknown. To allow measurement of PAPP-A proteolytic activity, we developed a sensitive and specific assay using a modified peptide derived from IGFBP-4.

**Materials and Methods**

**Buffers and reagents**

We prepared and quantified recombinant human PAPP-A as described (15). A PAPP-A concentration of 12 mg/L corresponds to a molar concentration of 30 nmol/L PAPP-A dimer, i.e., 60 nmol/L PAPP-A subunit, each containing 1 active site. We purified PAPP-A/proMBP complex from pooled term pregnancy serum as described (21). The amino acid sequence of all modified peptides analyzed as candidate substrates was LQKHFAKIR-DRSTSGMKVNGAPRE, corresponding to residues 118 to 143 of human IGFBP-4 (22). In all peptides, the glycine residue at position 139 was substituted with a 3-nitrotyrosine residue [Tyr(NO2)]. The position of a-aminobenzoic acid–modified lysine [Lys(Abz)] was varied between positions 130 (peptide QF-I), 131 (peptide QF-II), 132 (peptide QF-III), and 133 (peptide QF-IV) (Fig. 1). All peptides were synthesized by Jerini Peptide Technologies (Germany). Enzymatic reactions were carried out in a buffer containing 50 mmol/L Tris, 1 mmol/L CaCl2, pH 8.0 (QF buffer).

**Quantification of PAPP-A antigen**

We measured the serum concentration of PAPP-A by use of a commercially available semiautomated PAPP-A TriFMA assay on the AutoDelfia® (Perkin-Elmer, Life Sciences) platform. This assay is based on the PAPP-A monoclonal antibodies 234–2 and 234–4 (23) and measures both PAPP-A/proMBP complex and the uncomplexed PAPP-A (15). The standard was WHO international reference preparation 78/610 for pregnancy-associated proteins, by definition containing a PAPP-A concentration of 100 IU/L. Intraassay and interassay variations were <5% and <10%, respectively. Using this assay, we found that a recombinant PAPP-A concentration of 12 mg/L corresponds to 21 400 mIU/L. Hence, based on a molecular weight of 400 kDa for the PAPP-A dimer, 1 mIU/L corresponds to 2.8 pmol/L of recombinant active PAPP-A. This is the

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**Fig. 1. PAPP-A activity assay.**

(A), quenched fluorescent assay with peptide QF-II, derived from PAPP-A substrate IGFBP-4 (residues 118–143). Residues known to be important for substrate binding (22) are indicated (dotted), and the cleavage site at Met-135/Lys-136 (22) in intact human IGFBP-4 is marked with an arrow. Peptide QF-II carries an a-aminobenzoic acid-modified lysine residue [Lys(Abz)] at the position of Ser-131 (circle), and a 3-nitrotyrosine residue [Tyr(NO2)] at the position of Gly-139 (rounded square), Light at 310 nm was used for excitation. In the uncleaved peptide, the emission of light at 420 nm from the Abz moiety was quenched by the Tyr(NO2) residue. In the cleaved peptide lack of quenching by the Tyr(NO2) residue caused emission of light at 420 nm. (B), recombinant wild-type PAPP-A (PAPP-A), recombinant inactive PAPP-A (E483Q), and PAPP-A/proMBP complex purified from pregnancy serum (PAPP-A/proMBP), all at 1 mg/L, were incubated with peptide QF-II (1 μmol/L). Readings of the fluorescence were taken at intervals of 20 s. Arbitrary fluorescence units (FU) are plotted.
basis for converting pmol/L to IU/L and for calculating percentage active in serum.

PREPARATION AND QUANTIFICATION OF PEPTIDE SUBSTRATE

We purified crude preparations of derivated peptides by reversed-phase HPLC using a 4 × 250 mm column packed with Nucleosil C18 300–5 (Macherey-Nagel). Gradients were formed from 1.0 mL/L trifluoroacetic acid (solvent A) and 0.75 mL/L trifluoroacetic acid in 900 mL/L acetonitrile (solvent B), increasing the amount of solvent B to 50% over 30 min at a flow rate of 1 mL/min. The column was operated at 50 °C, and the eluate was monitored at 220 nm. Fractions were collected manually. We carried out quantitative amino acid analysis by cation exchange chromatography after peptide hydrolysis at 110 °C for 18 h with 6 mol/L HCl containing, per liter, 1 g phenol and 50 mL thioglycolic acid (24). For confirmation of peptide identities, we acquired matrix-assisted laser desorption ionization mass spectra on a Voyager DE-PRO (Applied Biosystems).

ASSAY FOR PAPP-A PROTEOLYTIC ACTIVITY IN SOLUTION

We used a solution-based assay for kinetic analysis of PAPP-A substrate cleavage. We diluted conditioned medium (Dulbecco’s modified Eagle’s medium with 100 mL/L fetal bovine serum) containing recombinant PAPP-A in QF buffer to PAPP-A subunit concentrations of 0.75 nmol/mL or 5 nmol/mL and added 40 μL to the wells of a 384-well microplate (Optitrap 384 F, PerkinElmer). For measurement of enzyme activity at 37 °C, we added 10 μL of substrate (QF-I, QF-II, QF-III, or QF-IV) diluted in QF buffer from a stock solution of 100 μmol/L. We monitored peptide cleavage using a fluorescence plate reader (Synergy HTTR-I, Bio-TEK) by following the increase in fluorescence at 420 nm over a 1-h period with excitation at 310 nm.

ENZYME KINETICS AND SALT AND PH SENSITIVITY

We performed Michaelis-Menten analysis by making a dilution series of the substrate from 7.5 μmol/L to 10 nmol/L. We added PAPP-A (0.6 nmol/L) to the wells and monitored the fluorescence response for 1 h, carrying out 3 independent experiments. We converted the degree of cleavage to concentrations from a calibration curve obtained after total hydrolysis of the substrate. We plotted velocities against substrate concentrations and obtained kinetic parameters by fitting the Michaelis-Menten equation to the data using the Enzyme Kinetics Module of Sigma Plot 8.02. To analyze the effect of ionic strength on the reaction rate, we prepared QF buffer with 0.5 mol/L NaCl and diluted it in salt-free QF buffer to obtain a range of buffers with different concentrations of NaCl. In this experiment, a substrate (QF-II) concentration of 0.4 μmol/L was used. For analysis of pH dependency, we obtained a series of reaction buffers by mixing volumes of QF buffer adjusted from pH 6.0 to 10.0 using 6 mol/L NaOH or 6 mol/L HCl. In this experiment, a substrate (QF-II) concentration of 0.4 μmol/L was used.

IMMUNOCAPTURE ASSAY FOR PAPP-A PROTEOLYTIC ACTIVITY

We coated black microplates (Optiplate 384 F HB, PerkinElmer) with the PAPP-A specific monoclonal antibody 234–2 (23). We diluted the antibody in 100 nmol/L Na2HCO3, pH 9.8, and used 1 μg antibody in 40 μL buffer per well. Coating was performed by incubation for 2 h at 22 °C followed by 7 wash steps in PBS [150 mmol/L NaCl, 10 mmol/L Na2HPO4, pH 7.4, containing 50 mg/L Tween-20 (PBS-T)]. We diluted recombinant PAPP-A or samples of human serum (1:3 or 1:5) in PBS-T and added 40 μL of the dilutions per well. After incubation for 2 h at 22 °C, we washed the wells 5 times in PBS-T and 5 more times with QF buffer. We assessed activity by the addition of substrate (QF-II) at a concentration of 10 μmol/L in QF buffer and sealed the plates with Crystal Clear sealing tape (Hampton Research). We measured the fluorescence response at 420 nm with excitation at 310 nm after incubation at 37 °C for up to 48 h. We used a dilution series of recombinant PAPP-A made with 30% serum from nonpregnant persons in PBS-T to establish a calibration curve and expressed measured PAPP-A activity in concentration units (nmol/L) of PAPP-A subunit, each containing 1 active site. We constructed calibration curves by fitting a sigmoidal dose–response curve \( y = \frac{y_{ \text{max}} - y_{ \text{min}}}{[1 + 10^{(\log EC_{50} - x)}]} \) to the results obtained from serial dilutions of recombinant PAPP-A spanning the concentration interval from 5 nmol/L to 2.4 pmol/L PAPP-A subunit using Sigma Plot 8.02.

We calculated the limit of detection as the average signal from 10 measurements of samples from normal nonpregnant individuals diluted 1:3 (blank samples) plus 3 SD. We analyzed analyte recovery and possible interference with unknown sample components by adding recombinant PAPP-A into PBS or a 30% solution of serum from nonpregnant persons in PBS-T. In addition to mAb 234–2, this analysis was carried out with PAPP-A specific mAb 234–5 and a mAb specific for proMBP, 234–10 (23). We calculated intraassay imprecision from 6 separate measurements at 1 nmol/L and 0.37 nmol/L PAPP-A diluted in PBS-T and interassay imprecision at 1 nmol/L PAPP-A diluted in PBS-T using 10 different plates analyzed over a period of 6 weeks.

We analyzed the effect of increasing concentrations of PAPP-A/proMBP by the addition of purified PAPP-A/proMBP (0 to 180 mg/L) to a reaction containing 1 nmol/L active PAPP-A. We measured analyte stability by incubating serum samples from pregnant women or recombinant PAPP-A diluted 1:3 in PBS-T to concentrations of ~1 nmol/L. Before measurement of activity, samples were incubated at −20, 4, 23, 37, or 56 °C for 0, 5, 12, 24, 72, or 120 h.
CLINICAL SPECIMENS
We used samples from 104 different pregnant women in weeks 7 to 19 of gestation with a singleton pregnancy and normal outcome. The samples were collected as part of the 1st- and 2nd-trimester prenatal screening program for chromosomal abnormalities at Statens Serum Institut, Copenhagen. They were stored at −20 °C until analysis and retrieved from the Prenatal Screening Registry at Statens Serum Institut in accordance with institutional guidelines for the use of the registry. Samples of serum obtained from healthy nonpregnant women (n = 2) or men (n = 2) were pooled and used as the blank sample. We could detect no enzymatic activity in individual blank samples. Institutional review board approval was obtained for the study.

STATISTICAL ANALYSIS
Descriptive statistics and nonparametric data comparison tests were performed using Prism 4.0 (Graphpad Software).

DEVELOPMENT OF A DERIVATIZED PEPTIDE SUBSTRATE FOR PAPP-A
We synthesized 4 variants (QF-I to -IV) of a 26-residue peptide derived from human IGFBP-4, in which residues 130 to 133 were substituted individually with an o-aminobenzoic acid–modified lysine residue, Lys(Abz), and the glycine residue at position 139 was substituted with a 3-nitrotyrosine residue, Tyr(NO2). Analysis of purified peptides showed that QF-II (Fig. 1A), with Lys(Abz) at position 131, was superior to the other 3 peptides in cleavage efficiency (not shown). QF-II was cleaved efficiently by recombinant PAPP-A, but not by an inactive mutated PAPP-A variant (E483Q) (25) or PAPP-A/proMBP complex purified from pregnancy serum (21) (Fig. 1B).

CHARACTERIZATION OF PEPTIDE CLEAVAGE
Using peptide QF-II, we further characterized its cleavage by PAPP-A. Typical enzyme kinetic analysis (Fig. 2A) demonstrated that QF-II functioned efficiently as a PAPP-A substrate with a mean (SD) \( K_m \) value of 5.38 (0.32) \( \mu \text{mol/L} \), and a \( k_{cat}/K_m \) value of \( 8 \times 10^4 (0.5 \times 10^4) \) \( \text{mol L}^{-1} \text{s}^{-1} \). No inner filter effect (resulting from reabsorption of emitted radiation) was present, as linearity was observed at all substrate concentrations analyzed.

Measurement of reaction rates under different conditions showed that PAPP-A cleavage of QF-II is sensitive to ionic strength and pH (Fig. 2B). At pH 6, PAPP-A did not show any measurable activity against QF-II and increased approximately linearly from pH 6 to pH 9 and decreased at pH 10 (Fig. 2B).

CHARACTERIZATION OF AN IMMUNOCAPTURE ASSAY FOR PAPP-A PROTEOLYTIC ACTIVITY
Using the solution enzymatic assay, measurable activity against QF-II was observed in serum samples that did not contain detectable amounts of PAPP-A antigen (not shown). To exclude interference by other proteinases in pregnancy serum that might be capable of QF-II cleavage, we developed a plate assay based on immunocapture using a PAPP-A–specific monoclonal antibody and QF-II. A calibration curve was established (Fig. 3), and the limit of detection, defined as the signal of the blank plus 3 SDs, was found to be 13 pmol/L of PAPP-A subunit, corresponding to 4.6 mIU/L as measured by ELISA. The intra- and interassay CVs at 1 nmol/L (357 mIU/L) were 7.5% and 14.8%, respectively. At a PAPP-A concentration of 0.37 nmol/L (132 mIU/L), intraassay variation was 9.8%. Complete recovery and lack of interference from unknown serum components was confirmed by comparison of dilution curves in a buffer solution and a solution of 30% control sera from nonpregnant individuals. The 2 resulting curves of fluorescence response were found to coincide over the entire interval of PAPP-A concentrations analyzed (Fig. 3). Similar results were obtained using another PAPP-A–specific monoclonal antibody.
and measurement with an antibody against proMBP showed no activity (not shown).

The effect of increasing the concentration of inactive PAPP-A/proMBP at a fixed concentration of active PAPP-A was analyzed. At 1 nmol/L active PAPP-A, no decrease in activity was observed after the addition of up to 50 mg/L (100 IU/L) PAPP-A/proMBP. The addition of PAPP-A/proMBP to a concentration of 50 to 180 mg/L resulted in a gradual reduction in measured activity (10% reduction at 180 mg/L) (not shown). Hence, the PAPP-A activity in serum samples containing high concentrations of PAPP-A/proMBP will not be underestimated using this assay.

The analyte was found to be highly stable in the temperature interval from 20 to 37 °C for at least 5 days, with no decline in measurable proteolytic activity (Fig. 4). Importantly, as the measurable activity was constant for samples incubated at or ≤37 °C, we conclude that incubation does not cause the formation of PAPP-A/proMBP complex from the active PAPP-A fraction, which would have caused a reduction in activity. At 56 °C, the activity dropped rapidly, probably as a result of protein denaturation.

**Determination of PAPP-A Proteolytic Activity in Serum Samples of the 1st and 2nd Trimester of Pregnancy**

We assessed the proteolytic activity in a set of 104 serum samples drawn from women in week 7 to 19 of pregnancy using the immunocapture assay and plotted as a function of gestational age (Fig. 5A). Measured PAPP-A activity was found to increase with gestational age (Table 1).

For each sample, we measured the total amount of PAPP-A antigen (the sum of active PAPP-A and inactive PAPP-A/proMBP) by ELISA, which measures both species, and the percentage of active PAPP-A was calculated for each sample and plotted against the antigen concentration (Fig. 5B). Surprisingly, this showed that the proportion of active PAPP-A is not constant during pregnancy. Samples containing <5000 mIU/L of PAPP-A antigen, corresponding to 90% of the samples from gestational weeks 7 to 13, showed a specific activity that markedly increased (average of 3-fold) compared with samples containing a PAPP-A antigen concentration >5000 mIU/L.

**Discussion**

The clinical usefulness of PAPP-A for prenatal screening is increasingly investigated. All studies carried out so far have assessed the maternal serum concentration of total PAPP-A by immunoassays. Because PAPP-A has recently been shown to be a metalloproteinase (4, 5), the potential value of knowing PAPP-A activity in serum deserves study. Many proteolytic enzymes closely related to PAPP-A, such as the family of matrix metalloproteinases, are capable of hydrolyzing low–molecular-weight chromogenic substrates, but no such PAPP-A substrate has been found (5). For PAPP-A, only gel-based qualitative or semiquantitative assays of activity have been devised (6), and no current method allows the measurement of specific PAPP-A activity in biological samples. We therefore developed an activity assay based on quenched fluorescence using a synthetic peptide spanning the cleavage site of the PAPP-A substrate IGFBP-4 (Fig. 1A). Fluid-phase kinetics analyses demonstrated that the peptide QF-II was cleaved efficiently by PAPP-A (Fig. 2) and showed that no activity was measured in a control experiment using a PAPP-A variant inactivated by a mutation in the active site (Fig. 1B).
PAPP-A cleavage of QF-II was found to decrease with increasing ionic strength (Fig. 2B). This indicates that substrate binding depends on interactions between charged residues and emphasizes the importance of assay conditions in which ionic strength is maintained at a constant level. This finding is in accordance with previous studies of PAPP-A substrate specificity using full-length IGFBP-4, which demonstrated that several basic residues are important for substrate cleavage (22). No cleavage of QF-II occurred below pH 6, and an apparent linear increase in activity was observed from pH 6 to pH 9. This is reasonable, as PAPP-A belongs to the superfamily of metzincin metalloproteinases in which the active site zinc ion is coordinated by 3 histidine residues that become protonated upon lowering of the pH.

To avoid possible interference by other proteins, including cleavage of the QF-II peptide by unknown proteinases, we developed an immunocapture assay. This assay allowed the measurement of PAPP-A activity in human serum samples with a detection limit of 13 pmol/L of PAPP-A subunit, corresponding to a PAPP-A antigen concentration of ~4.6 mIU/L. We observed no interference from unknown sample components present in serum from nonpregnant persons (Fig. 3).

Activity of the isolated PAPP-A/proMBP complex against QF-II could not be measured (Fig. 1B). Although the PAPP-A/proMBP complex has been shown to be inactive toward full-length IGFBP-4 (26), the inability of PAPP-A/proMBP to cleave the much smaller QF-II peptide could not have been predicted. Thus, in a sample of pregnancy serum, which contains a mixture of active PAPP-A dimer and the PAPP-A/proMBP complex (15), cleavage of the QF-II peptide reflects the amount of PAPP-A dimer, previously estimated by chromatographic analysis to be ~1% at term (15).

We analyzed 104 serum samples drawn in weeks 7 to 19 from different pregnant women, all with normal outcome of pregnancy. The level of PAPP-A activity was found to increase with gestational age (Fig. 5A and Table 1). Surprisingly, however, when expressed as the fraction of active PAPP-A compared with total PAPP-A (the sum of PAPP-A and PAPP-A/proMBP complex), correlation coefficient: \( r = 0.828 \) (\( P < 0.0005 \)). The fraction of active PAPP-A was calculated based on the experimental finding that recombinant PAPP-A at 12 mg/L corresponds to 21 400 mIU/L (hence 1 mIU/L equals a subunit concentration of 2.8 pmol/L). Similar results were obtained in 3 independent experiments. The inset shows the same set of data in which the fraction of active PAPP-A is plotted against gestational age. Correlation coefficient: \( r = -0.594 \) (\( P < 0.0005 \)).

\[\text{Table 1. Reference ranges for PAPP-A activity in early human pregnancy.}\]

<table>
<thead>
<tr>
<th>Gestational age, weeks</th>
<th>n</th>
<th>Median, nmol/L</th>
<th>Interquartile range, nmol/L</th>
<th>Normal rangea, nmol/L</th>
<th>( P^b )</th>
</tr>
</thead>
<tbody>
<tr>
<td>7–8</td>
<td>27</td>
<td>0.07</td>
<td>0.033–0.152</td>
<td>0.064–0.126</td>
<td>NA</td>
</tr>
<tr>
<td>9–10</td>
<td>28</td>
<td>0.27</td>
<td>0.213–0.389</td>
<td>0.255–0.378</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>11–13</td>
<td>39</td>
<td>0.91</td>
<td>0.519–1.246</td>
<td>0.779–1.098</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>15–19</td>
<td>10</td>
<td>0.92</td>
<td>0.762–1.213</td>
<td>0.763–1.142</td>
<td>0.621</td>
</tr>
</tbody>
</table>

A PAPP-A activity level of 0.1 nmol/L corresponds to an antigen concentration of 35.7 mIU/L. NA, not applicable.

a 95% confidence interval.

b Mann–Whitney rank-sum test, comparison with previous group.
What determines the magnitude of the fraction of PAPP-A that is not complexed to proMBP and therefore active? First, the molar ratio between PAPP-A and proMBP may determine how much complex is formed. Although preliminary data indicate that proMBP is present in excess of PAPP-A throughout pregnancy, the molar concentration of proMBP rises more than PAPP-A (27). Second, proMBP is able to form a covalent complex with angiotensigen (27, 28), and angiotensigen thus potentially competes for proMBP, which may be relevant in conditions of pregnancy-induced hypertension and preeclampsia (27). Third, in vitro experiments have revealed that formation of the PAPP-A/proMBP complex is sensitive to the redox potential (26). This may be relevant under pathological conditions with locally altered redox potential, e.g., under conditions of hypoxia. Importantly, both PAPP-A and proMBP are expressed in the placenta during pregnancy (29), and formation of the PAPP-A/proMBP complex likely occurs in the placental microenvironment rather than in the circulation.

The measurement of PAPP-A activity is therefore of potential interest in several pathological conditions of pregnancy. In addition, PAPP-A is emerging as a potential marker of acute coronary syndromes and ischemic heart disease (20, 30–32), and because it has been reported that the increase in PAPP-A antigen in acute coronary syndromes is caused by uncomplexed PAPP-A (33), measurement of PAPP-A activity may be of considerable interest. Furthermore, the ability to quantify PAPP-A activity is likely to be useful in other tissues in which PAPP-A is believed to control the biological activity of IGF.

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