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Immunochemical properties of PAPP-A isolated from human atherosclerotic coronary arteries

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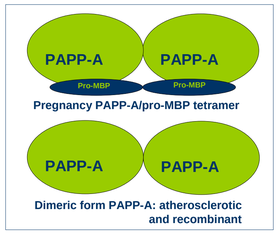
Introduction

Pregnancy Associated Plasma Protein-A (PAPP-A) is a high-molecular-weight glycoprotein originally isolated from human pregnancy serum. In the blood of pregnant women PAPP-A circulates as a heterotetrameric 2:2 complex with the proform of eosinophil major basic protein (PAPP-A/proMBP). Recently, it was shown that another form – homodimeric (dPAPP-A) exists in unstable atherosclerotic plaques.

Recent studies indicate that increased serum level of dPAPP-A is associated with development of acute coronary syndrome. Thus **dPAPP-A** was suggested **as a marker** of some cardiovascular diseases, such as unstable angina, myocardial infarction.

However, there are certain methodological difficulties for reliable measurements of this protein, associated with shortage of information about biochemical features of this protein and its low concentration in patients' serum (about 0.7 ng/ml).



<u>In this study we planned to:</u>

1. Develop method of **dPAPP-A** isolation from human coronary atherosclerotic vessels
2. Study its biochemical and immunological properties in comparison with human recombinant dPAPP-A
3. Develop method of dPAPP-A quantitative immunodetection

Materials and Methods

Tissue PAPP-A extraction: Samples of human atherosclerotic coronary vessels were stored at -70℃ until used. dPAPP-A wa s extracted from the atherosclerotic coronary arteries in 50 mM tris-HCl buffer, pH=7.5.

Human recombinant dPAPP-A : HEK 293 cells were transfected by the PAPP-A expression plasmid in the presence of cationic lipid transfection reagent.

Protein purification: Tissue and recombinant dPAPP-A were purified by means of affinity chromatography. Out of several tested monoclonal antibodies specific to PAPP-A (MAbs), **MAb 4G11** was selected for the affinity matrix preparation.

Protein analysis: dPAPP-A from atherosclerotic coronary arteries (tissue dPAPP-A), recombinant dPAPP, and PAPP-A from human pregnancy serum were analyzed by **SDS-PAGE** in reducing conditions.

All PAPP-A forms were also analyzed by **Western blotting** with several PAPP-A-subunit-specific MAbs: **18A10, 7A6 and 3C8**, and by liquid chromatography/tandem mass spectrometry analysis (**MS-MS**).

Monoclonal antibodies specific to PAPP-A and pro-MBP were obtained from **HyTest Ltd** (Finland).

Sandwich immunofluorescent assay (IFA): MAbs with different antigen (subunit) specificity were used for the development of in-house sandwich IFAs.

Specificity	Specific to PAPP-A subunit in heterotetrameric and homodimeric forms	Specific to pro-MBP subunit only	Specific to dPAPP-A only
MAbs	7A6, 10E1	5H9	PAPP2

Detection antibodies were labeled with Eu3+-chelate. The fluorescence was measured by Victor 1420 multilabel counter (Wallac-Percin Elmer, Finland).

Results and Discussion

We have elaborated a method of PAPP-A isolation from human atherosclerotic coronary arteries. This method enables to obtain 70-90 % of initial PAPP-A amount from the tissue extract. Content of dPAPP-A was determined as 8 µg per 100 g of vessel tissue, containing atherosclerotic plaques.

Using analysis by SDS-PAGE and Western blotting we have shown that the major band of protein with molecular mass about 200 kDa was PAPP-A subunit. Another band displaying PAPP-A immunoreactivity had molecular mass about 45 kDa, so we concluded that it was a proteolytic fragment of PAPP-A. The purity of PAPP-A preparation was about 80%.

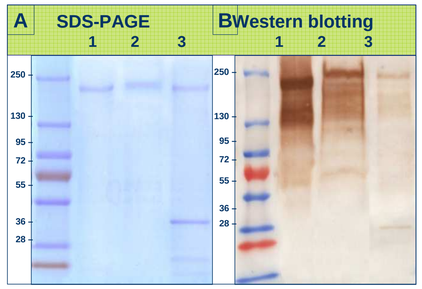


Figure.1 A – SDS-PAGE analysis in reducing conditions of PAPP-A purified from atherosclerotic arteries. Protein bands were visualized by Coomassie R250 staining.
Lane 1, heterotetrameric form of PAPP-A (PAPP-A/proMBP);
Lane 2, recombinant dPAPP-A;
Lane 3, tissue form of PAPP-A.
ProMBP released from heterotetrameric PAPP-A upon reduction is not stained by Coomassie R250 due to its extensive glycosilation.
B – Western blotting analysis of PAPP-A purified from atherosclerotic tissue. For immunostaining mixture of PAPP-A-specific MAbs (18A10, 7A6 and 3C8) was used. Lanes are noted as in Fig.1 A.

It was shown that under reducing conditions the apparent molecular mass of PAPP-A subunit purified from atherosclerotic tissue was equal to molecular masses of recombinant PAPP-A and PAPP-A from pregnancy serum - about **200 kDa** (Fig. 1A). Identity of proteins was also confirmed by Western blotting analysis with PAPP-A-specific MAbs (Fig. 1B).

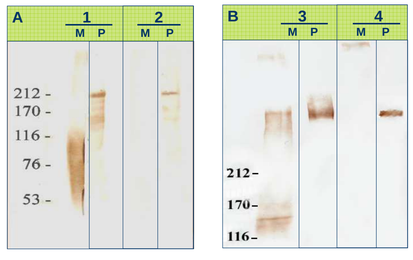


Figure 2. – proMBP analysis by Western blotting under reducing **(A)** and nonreducing **(B)** conditions.
Lanes 1, 3 – Heterotetrameric form of PAPP-A
Lanes 2, 4– Tissue form of PAPP-A
Proteins were stained with MAbs specific to PAPP-A subunit (marked as **P**) and with MAbs specific to proMBP (5H9, 11E4) (marked as **M**).
The absence of proMBP-specific staining in **Lanes 2M** and **4M** indicates the absence of PAPP-A/proMBP complex in PAPP-A preparation from atherosclerotic tissue.

ProMBP content analysis by Western blotting showed the **absence of pro-MBP** in tissue dPAPP-A preparation (Fig. 2A, 2B, *Lane 3*). We used mixture of three PAPP-A subunit-specific MAbs (18A10, 3C8, 7A6) and two MAbs specific to pro-MBP (11E4, 5H9) for PAPP-A and proMBP subunits immunodetection respectively.

Identity of tissue and recombinant dPAPP-A to PAPP-A from human pregnancy serum was also corroborated by liquid chromatography/tandem mass spectrometry analysis (MS-MS).

Using dPAPP-A as immunogen we generated three MAbs (PAPP2, PAPP7, PAPP8) specific to dPAPP-A only. Using these antibodies we have designed several ***dPAPP-A***-specific sandwich immunoassays**.**

These assays displayed good sensitivity (better than 0.3 ng/ml – preliminary data) with atherosclerotic and recombinant (dimeric) forms of the antigen and very low crossreactivity (less than 0.5%) with PAPP-A/proMBP complex from pregnancy blood, possibly because of the contamination of tetramer preparation with homodimeric form (Fig. 5). New assay could be used for dPAPP-A quantification in blood of ACS patients.

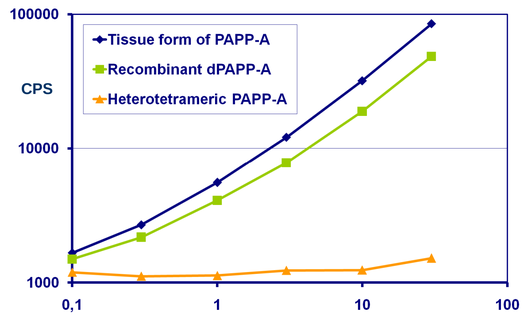


Figure 5. Calibration curves for dPAPP-A immunoassay (PAPP2 – 7A6-Eu). MAb ***PAPP2*** was used as a capture antibody. MAb ***7A6*** was used for detection. Detection limit of the assay was better than 0.3 ng/ml for dPAPP-A. This assay revealed low crossreactivity with heterotetrameric form of PAPP-A.

Characterization of atherosclerotic tissue PAPP-A as well as the measurement of its tissue content was carried out using several in-house sandwich immunoassays:

1. specific to **heterotetrameric** *(PAPP-A/proMBP)* **complex *5H9 - 7A6-Eu*** (Fig. 3A);
2. specific of **total PAPP-A** *(PAPP-A/proMBP complex and dPAPP-A)* ***10E1 -7A6-Eu*** (Fig. 3B);
3. specific to **dPAPP-A** only ***PAPP2 – 7A6-Eu*** (Fig. 3C).

Using ***5H9 - 7A6-Eu*** IFA we have demonstrated the absence of considerable amounts of heterotetrameric (PAPP-A/proMBP) complex in the PAPP preparation from atherosclerotic tissue as well as in recombinant dPAPP-A (Fig. 4).

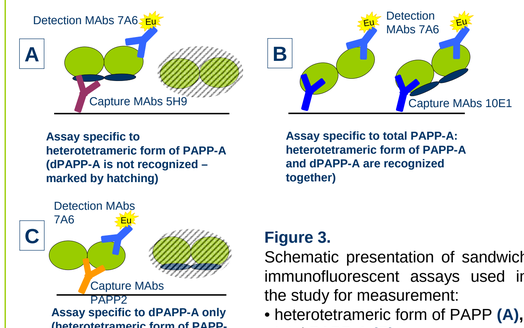


Figure 3.
Schematic presentation of sandwich immunofluorescent assays used in the study for measurement:
- heterotetrameric form of PAPP **(A),**
- total PAPP-A **(B),**
- dPAPP-A only **(C).**

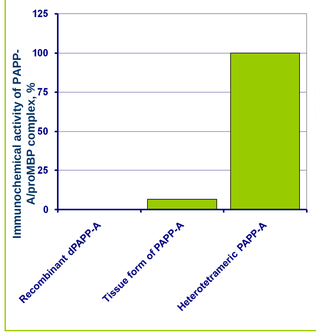


Figure 4. PAPP-A/proMBP measurements (assay ***5H9 – 7A6)*** in recombinant dPAPP-A preparation, atherosclerotic tissue dPAPP-A preparation and PAPP-A from pregnancy serum. Concentration of total PAPP-A measured by reference assay ***10E1 – 7A6*** was accepted as 100%.

Conclusions

1. **New method of dPAPP-A purification was developed.**
2. **dPAPP-A from human atherosclerotic tissue was characterized. Purified dPAPP-A could be used as calibrator in dPAPP-A assays.**
3. **New assay for selective dPAPP-A measurements was developed and described for the first time. This assay does not recognize PAPP-A/ proMBP tetrameric form and can be used for direct dPAPP-A quantification in human blood.**

For further information

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