FEBS Letters 504 (2001) 36–40 FEBS 25167

Pregnancy-associated plasma protein-A (PAPP-A) cleaves insulin-like growth factor binding protein (IGFBP)-5 independent of IGF: implications for the mechanism of IGFBP-4 proteolysis by PAPP-A

Lisbeth S. Laursen[a], Michael T. Overgaard[a], Rikke Søe[a], Henning B. Boldt[a], Lars Sottrup-Jensen[a], Linda C. Giudice[b], Cheryl A. Conover[c], Claus Oxvig[a,*]

[a] *Department of Molecular and Structural Biology, University of Aarhus, Science Park, Gustav Wieds Vej 10C, DK-8000 Aarhus C, Denmark*
[b] *Department of Gynecology and Obstetrics, Stanford University, Stanford, CA 94305, USA*
[c] *Endocrine Research Unit, Mayo Clinic and Foundation, Rochester, MN 55905, USA*

Received 20 June 2001; revised 30 July 2001; accepted 30 July 2001

First published online 9 August 2001

Edited by Jacques Hanoune

Abstract Pregnancy-associated plasma protein-A (PAPP-A) has recently been identified as the proteinase responsible for cleavage of insulin-like growth factor binding protein (IGFBP)-4, an inhibitor of IGF action, in several biological fluids. Cleavage of IGFBP-4 by PAPP-A is believed to occur only in the presence of IGF. We here report that in addition to IGFBP-4, PAPP-A also cleaves IGFBP-5. Cleavage occurs at one site, between Ser-143 and Lys-144 of IGFBP-5. In the presence of IGF, IGFBP-4 and -5 are cleaved with similar rates by PAPP-A. Interestingly, cleavage of IGFBP-5 by PAPP-A does not require the presence of IGF, but is slightly inhibited by IGF. These findings have implications for the mechanism of proteolysis of IGFBP-4 by PAPP-A, suggesting that IGFBP-4 binds IGF, which then becomes a PAPP-A substrate. Using highly purified, recombinant proteins, we establish that (1) PAPP-A cleavage of IGFBP-4 can occur in the absence of IGF, although the rate of hydrolysis is very slow, and (2) IGF is unable to bind to PAPP-A. We thus conclude that IGF enhances proteolysis by binding to IGFBP-4, not by interaction with PAPP-A, which could not previously be ruled out.

Key words: Pregnancy-associated plasma protein-A; Insulin-like growth factor; Insulin-like growth factor binding protein-5; Insulin-like growth factor binding protein-4; Proteolysis

1. Introduction

The biological activity of insulin-like growth factors (IGFs)-I and -II [1] is tightly regulated by six different, but homologous, IGF binding proteins (IGFBP-1–6) [2,3]. Cleavage in the central region of IGFBPs causes dissociation of bound IGF, and such limited proteolysis is a powerful means by which the bioavailable concentration of IGF is locally regulated [4,5]. Although a large number of biological fluids and conditioned media have been shown to contain specific proteolytic activity against one or more of the IGFBPs, the identities of the proteinases have remained unknown in most cases. One exception is the IGFBP-4 proteinase secreted by human fibroblasts, which has recently been identified as pregnancy-associated plasma protein-A (PAPP-A), a high-molecular-weight protein not previously connected with the IGF system [6]. Several other sources of IGFBP-4 proteolytic activity have now been investigated, and PAPP-A has been shown to be the responsible proteinase [6–10].

Based primarily on the presence of an elongated zinc-binding motif in the amino acid sequence, PAPP-A belongs to the metzincin superfamily of metalloproteinases [11–13]. The PAPP-A monomer of 1547 residues is secreted as a dimer of 400 kDa [6,8], but in pregnancy serum the majority of PAPP-A ($>99\%$) is found in a 2:2 disulfide bound complex with the proform of eosinophil major basic protein [8,14], which functions as a proteinase inhibitor [8]. Except for a recently discovered homolog, PAPP-A2, which cleaves IGFBP-5 [15], PAPP-A does not show global similarity to any known proteins. IGFBP-4 is the only known PAPP-A substrate.

Intriguingly, cleavage of IGFBP-4 by PAPP-A is believed to occur only in the presence of IGF. Since this observation was made [16–18] before the identity of the IGFBP-4 proteinase was known, the mechanistic basis for the requirement of IGF has not been defined. On one hand, IGF might be required as a co-factor for the activation of PAPP-A [18]. On the other hand, it might be required that IGFBP-4 binds IGF in order to become a substrate for PAPP-A [19].

We here show that PAPP-A is capable of cleaving IGFBP-5, in addition to IGFBP-4, and we identify the PAPP-A cleavage site in IGFBP-5. Interestingly, proteolysis of IGFBP-5 occurs in the absence of IGF and is slightly inhibited by IGF. This finding prompted further analysis of the proteolysis of IGFBP-4 by PAPP-A. The availability of highly purified reagents has allowed us to distinguish between the two models of IGF-dependent IGFBP-4 proteolysis.

2. Materials and methods

2.1. Tissue culture and protein expression

Human embryonic kidney 293T cells (293tsA1609neo) [20] were cultured in high glucose Dulbecco's modified Eagle's medium with 10% fetal bovine serum, 2 mM glutamine, non-essential amino acids, and gentamicin (Life Technologies). Cells (2.5×10^6 on 6 cm plates containing 4 ml medium) were transfected by calcium phosphate co-precipitation [21] with 10 μg of empty vector, or constructs containing cDNA encoding human PAPP-A [8], human IGFBP-4 [13], or human IGFBP-5 [15]. The PAPP-A construct was based on pcDNA3.1+ (Invitrogen), and the IGFBP-4 and -5 constructs were both based

*Corresponding author. Fax: (45)-86123178.
E-mail address: co@mbio.aau.dk (C. Oxvig).

0014-5793/01/$20.00
PII: S0014-5793(01)02760-0

on pcDNA3.1/*Myc*-His(-)A (Invitrogen), as detailed previously [8,13,15]. 48 h post-transfection the supernatants contained approximately 10 μg/ml of recombinant proteins. IGFBP-4 and -5 were both expressed as C-terminally *c-myc* and His-tagged proteins, increasing the molecular weights by 3.1 and 2.9 kDa, respectively. PAPP-A-containing supernatants were harvested after 48 h and cleared by centrifugation. To facilitate purification, cells transfected with IGFBP-4 and -5 cDNA were maintained for another 48 h in serum-free medium (293 SFM II, Life Technologies).

2.2. Protein purification

Recombinant IGFBP-4 and -5 were both purified from serum-free media by successive steps of (1) affinity chromatography on a metal chelate column (2 ml NTA-Sepharose, Pharmacia) charged with nickel ions, (2) gel filtration in 50% formic acid (pH < 1) on a Superdex 75 HR 10/30 column (Pharmacia), and (3) reversed-phase high pressure liquid chromatography (RP-HPLC) on a column (4×250 mm) of Nucleosil C4 500-7 (Macherey-Nagel), as described recently for IGFBP-5 [15]. The purified protein eluting from the RP-HPLC column was lyophilized and redissolved in 50 mM Tris, pH 7.5. The amounts of purified proteins (approximately 100 μg per run) were determined by amino acid analysis [22]. Recombinant PAPP-A in standard serum-containing medium was immobilized on protein G agarose beads (Life Technologies) using a non-inhibitory PAPP-A monoclonal antibody, 234-2 [23]. The beads were washed extensively with PBS containing 1000 mM sodium chloride, and then equilibrated with PBS. Immobilized PAPP-A was used directly (for experiments shown in Figs. 3 and 4), or bound PAPP-A was eluted with 200 mM glycine, 1 mM calcium chloride, pH 3.0, into 1 M Tris to a final pH of 7.5 (for experiments shown in Fig. 2, 5, and 6). As evaluated by reducing SDS–PAGE, a variable fraction (typically about 50%) of the eluted PAPP-A was autocleaved and thus inactive, as recently reported [13]. The concentration of PAPP-A (40 ng/μl) was determined by amino acid analysis [24].

2.3. Measurement of proteolytic activity

Proteolysis of purified ^{125}I-labeled IGFBP-4 or -5 was carried out in 50 mM Tris, pH 7.5, as described for IGFBP-5 [15] and IGFBP-4 [13], using 0.3 ng PAPP-A (1 μl of a culture supernatant containing about 8 μg/ml PAPP-A, diluted approximately 25 times in PBS), and 50 000 cpm of the binding proteins per lane (approximately 5 ng) (Fig. 1). The total reaction volume was 20 μl. Incubation at 37°C was carried out with or without the addition of 40 nM IGF-II (Bachem), and with PAPP-A polyclonal antibodies (A0230, DAKO, Denmark, diluted 1:20 to 0.4 mg Ig per ml) or irrelevant antibodies (anti-α2-macroglobulin, A0033, DAKO) at the same concentration as specified. The reaction mixture was analyzed by non-reducing SDS–PAGE (16%) followed by autoradiography. For quantitative analysis (Figs. 2, 5, and 6), purified PAPP-A (10–30 ng) eluted from beads was used, and 1 μg unlabeled IGFBP-4 or -5 was added. The degree of cleavage was determined by measuring band intensities with a PhosphorImager (Molecular Dynamics) [13]. Medium from mock-transfected cells was processed similarly to serve as a negative control to be subtracted from all values. In other experiments (Figs. 3 and 4), using larger amounts of proteins, the proteolysis of purified IGFBP-4 and -5 was performed with immobilized PAPP-A in 50 mM Tris, pH 7.5, with or without added IGF-II. The amounts of protein used and the length of incubation are specified in the text. Cleavage was assessed by non-reducing SDS–PAGE (16%) followed by Coomassie staining.

2.4. Miscellaneous procedures

SDS–PAGE was performed in Tris–glycine gels (10–20% or 16%) [25]. For cleavage-site determination, affinity purified IGFBP-5 (20 μg) was digested (37°C, 2 h) with immobilized PAPP-A (approximately 1 μg). Edman degradation of protein (> 50 pmol) separated by SDS–PAGE and blotted onto a polyvinylidene fluoride (PVDF) membrane was performed as described earlier [26]. IGF-I and -II (Bachem) were ^{125}I-labeled [27] using lactoperoxidase [28] rather than chloramin-T. To evaluate the binding of IGF to IGFBP-4 and PAPP-A, radiolabeled IGF-I or -II (approximately 40 000 cpm contained in 10 μl) was incubated (1 h at room temperature) with proteins immobilized to protein G agarose (20 μl) via monoclonal anti-*c-myc* (clone 9E10, ATTC) or PAPP-A mAb 234-2, respectively. The amounts of PAPP-A and IGFBP-4 bound per 20 μl protein G beads were 0.9 μg and 0.1 μg, respectively, corresponding to the same molar amounts. Prior to incubation, PBS containing 0.1% bovine serum albumin (BSA) was added to obtain a final volume of 300 μl. The amount of bound IGF following four rounds of washing in Eppendorf tubes with PBS/0.1% BSA (500 μl) was determined using a γ counter. A variant of this experiment was carried out in which unlabeled IGFBP-4 (0.1 μg) was added, and inactive PAPP-A (E483Q) [13] was used rather than wild-type PAPP-A to prevent cleavage of IGFBP-4.

3. Results and discussion

3.1. PAPP-A cleaves IGFBP-5

We have found that in addition to IGFBP-4, PAPP-A is capable of cleaving IGFBP-5 (Fig. 1). Like IGFBP-4, IGFBP-5 is apparently cleaved into two fragments of similar size. Surprisingly, in contrast to the proteolysis of IGFBP-4, the cleavage of IGFBP-5 by PAPP-A occurs without the addition of IGF (Fig. 1, lanes 2 and 3 vs. 5 and 6). The radiolabeled binding proteins used for these experiments had been subjected to gel filtration at pH < 1 to ensure the absence of contaminating IGF [15,29]. However, PAPP-A contained in medium with 10% fetal bovine serum was diluted in buffer and added to the reaction mixture (5000 times final dilution compared to undiluted bovine serum), inarguably introducing traces of IGF into the system. But, because these traces were not sufficient to allow proteolysis of IGFBP-4 (Fig. 1, lane 2), we draw the provisional conclusion (further addressed below, see Section 3.3) that proteolysis of IGFBP-5 does not depend on IGF.

A time course experiment showed that IGFBP-5 and -4 are cleaved with similar rates by PAPP-A in the presence of IGF-II, although IGFBP-5 consistently disappeared faster initially (Fig. 2).

Previously, it was suggested that PAPP-A did not degrade IGFBP-5 based on the lack of inhibition of IGFBP-5 proteo-

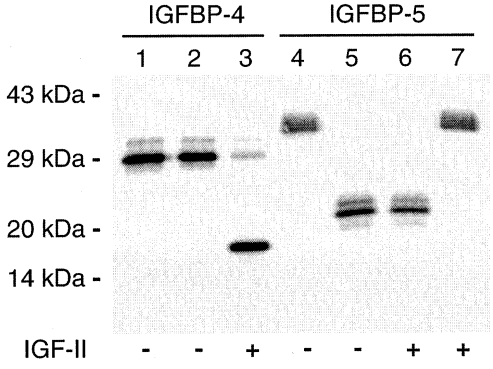

Fig. 1. PAPP-A proteolysis of IGFBP-4 and -5 visualized by non-reducing SDS–PAGE followed by autoradiography. Radiolabeled IGFBP-4 was incubated (16 h) with medium from cells transfected with empty vector (lane 1), or with medium from cells transfected with PAPP-A cDNA in the absence (lane 2) and presence (lane 3) of added IGF-II. Likewise, radiolabeled IGFBP-5 was incubated (16 h) with medium from cells transfected with empty vector (lane 4), or with medium from cells transfected with PAPP-A cDNA in the absence (lane 5) and presence (lane 6) of IGF-II, and with PAPP-A polyclonal antibodies in the presence of IGF-II (lane 7). Irrelevant antibodies at the same concentration did not inhibit proteolysis (not shown). The concentration of PAPP-A was 0.07 nM, the concentration of the binding proteins were 9 nM, and the concentration of IGF-II was 40 nM. The absence (−) or presence (+) of IGF-II is indicated below each lane. The C-terminal tag on both of the binding proteins causes their cleavage products to co-migrate, and thus appear as one band. Both binding proteins show faint, less abundant, glycosylation variants of higher molecular weight.

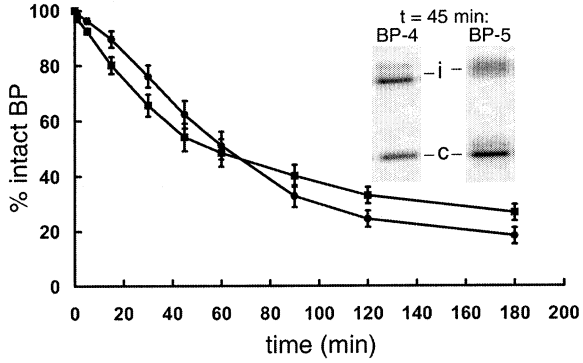

Fig. 2. Degradation of IGFBP-4 (filled circles) and IGFBP-5 (filled squares) by PAPP-A as a function of time. Purified PAPP-A (15 ng, 0.3 nM) was incubated with IGFBP-4 or -5 (1 μg, 144 nM and 130 nM, respectively), in the presence of excess IGF-II (0.3 μg, 167 nM). Trace amounts of radiolabeled IGFBP-4 or -5 were added to allow quantification of cleavage. The total reaction volumes were 240 μl. Samples were taken out at various time points, and the degree of cleavage was determined by densiometry after separation by SDS–PAGE. The insert shows one lane of IGFBP-4 and -5 after 45 min of incubation (intact binding proteins (i) and cleavage products (c) are indicated). Values are average of three independent experiments ± standard deviation. Similar curves were obtained with PAPP-A first incubated alone for 4 h at 37°C (not shown).

lytic activity in ovarian follicular fluid using polyclonal antibodies [7], but other results have pointed at PAPP-A as a possible IGFBP-5 proteinase in non-fractionated pregnancy serum [30]. We find here that PAPP-A proteolysis of IGFBP-5 can be inhibited by polyclonal PAPP-A antibodies (Fig. 1, lane 7). However, efficient inhibition required more antibody than inhibition of IGFBP-4 proteolysis [6], probably reflecting that antibody binding to overlapping, but non-identical sets of PAPP-A epitopes abrogate proteolysis of IGFBP-4 and -5, respectively, and that not all epitopes can be occupied by antibody at the same time.

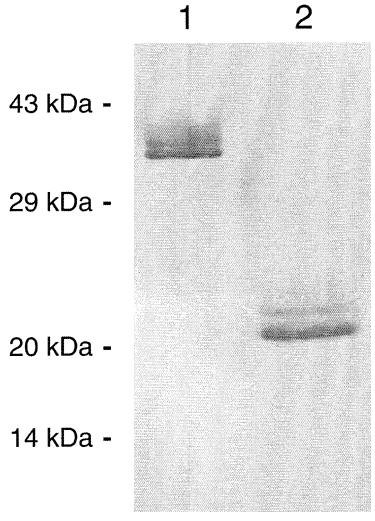

Fig. 3. Preparative digest of IGFBP-5 for determination of the PAPP-A cleavage site. Purified IGFBP-5 (10 μg) was digested with immobilized PAPP-A. A fraction (1 μg) of the reaction mixture (lane 2) and the same amount of undigested IGFBP-5 (lane 1) is shown. Edman degradation of the cleavage products blotted onto PVDF membrane showed that cleavage occurs between Ser-143 and Lys-144 of IGFBP-5. The two distinct cleavage bands (just above 20 kDa) are both C-terminal fragments that differ in size due to glycosylation. The N-terminal fragment does not appear as a distinct band, but rather co-migrates as a smear around the two C-terminal fragments.

3.2. Cleavage site determination

For determination of the PAPP-A cleavage site, IGFBP-5 (Fig. 3, lane 1) was digested with PAPP-A and separated by SDS–PAGE (Fig. 3, lane 2). Edman degradation of digested, blotted material showed two sequences of IGFBP-5, K(144)FVGG and L(1)GXFV (N-terminus of mature IGFBP-5), demonstrating that PAPP-A cleaves IGFBP-5 between Ser-143 and Lys-144, (R137KKLTQS↓KFVGGAE). Sequence analysis directly on the reaction mixture, without prior separation by SDS–PAGE, showed no other sequences, verifying that cleavage occurs only at one site. Comparison with the reported IGFBP-4 cleavage site of PAPP-A (S129TSGGKM↓KVNGAPR) [31], shows that a Lys residue is present in both of the P1′ positions, hydrophobic residues occupy the P2′ positions, and a Gly residue is present in both P4′ positions. Interestingly, PAPP-A2 has recently been found to cleave IGFBP-5 at the same site [15].

3.3. IGF does not promote proteolysis of IGFBP-5 by PAPP-A

Although the cleavage of IGFBP-5 by PAPP-A appeared independent of IGF (Fig. 1), we further asked whether it would occur in the absence of possible traces of IGF (see Section 3.1), and whether the rate of proteolysis would be affected by IGF. Using both IGFBP-5 and PAPP-A in highly purified forms, we thus compared partial digests with and without IGF-II (Fig. 4A). This experiment demonstrates that the proteolysis of IGFBP-5 by PAPP-A does not depend on IGF and is not dramatically affected by its presence. Several previous studies [32–34] have reported moderate inhibition of IGFBP-5 proteolysis by IGF. To evaluate whether IGFBP-5 proteolysis by PAPP-A was also inhibited by IGF, we performed a time course experiment in the presence and

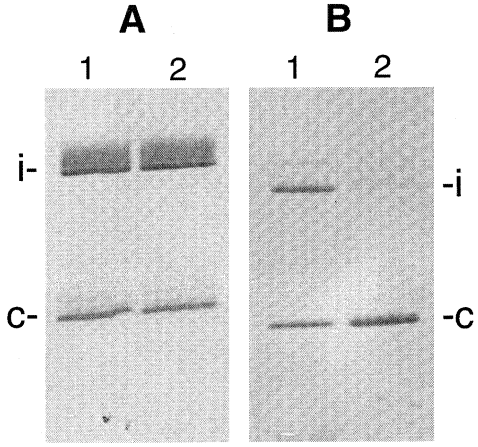

Fig. 4. Effect of added IGF on partial proteolysis of IGFBP-5 and -4. A: Purified IGFBP-5 (1 μg, 694 nM) was digested (2 h) partially with purified PAPP-A (0.1 μg, 11 nM) in the absence (lane 1) and presence (lane 2) of 500 nM IGF-II, showing that IGFBP-5 proteolysis by PAPP-A does not depend on IGF. The total reaction volume was 45 μl. B: Purified IGFBP-4 (1 μg, 766 nM) was digested (8 h) partially with purified PAPP-A (0.1 μg, 11 nM) in the absence (lane 1) and presence (lane 2) of 500 nM IGF-II. This shows that IGFBP-4 proteolysis by PAPP-A can occur in the absence of IGF, although it is promoted by IGF. The total reaction volume was 45 μl. Both gels are stained with Coomassie blue. The positions of intact (i) and cleaved (c) IGFBP-5 and -4 are indicated.

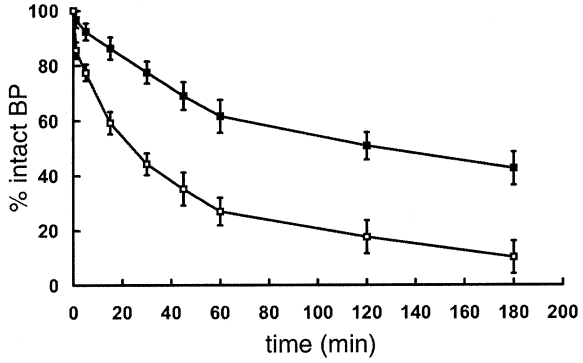

Fig. 5. Measurement of the effect of IGF on IGFBP-5 proteolysis by PAPP-A. Purified PAPP-A (10 ng, 0.2 nM) was incubated with IGFBP-5 (1 μg, 130 nM) in the absence (open squares) or presence (filled squares) of excess IGF-II (0.3 μg, 167 nM). Trace amounts of radiolabeled IGFBP-5 were added to allow quantification of cleavage. The total reaction volumes were 240 μl. Samples were taken out at various time points, and the degree of cleavage was determined by densiometry after separation by SDS–PAGE. Values are average of two independent experiments ± standard deviation.

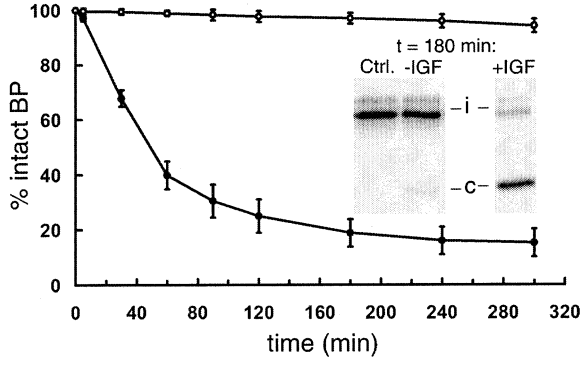

Fig. 6. Measurement of the effect of IGF on IGFBP-4 proteolysis by PAPP-A. Purified PAPP-A (30 ng, 0.6 nM) was incubated with IGFBP-4 (1 μg, 144 nM) in the absence (open circles) or presence (filled circles) of excess IGF-II (0.3 μg, 167 nM). Trace amounts of radiolabeled IGFBP-4 were added to allow quantification of cleavage. The total reaction volumes were 240 μl. Samples were taken out at various time points, and the degree of cleavage was determined by densiometry after separation by SDS–PAGE. The insert shows lanes of IGFBP-4 without PAPP-A, and with PAPP-A, in the absence or presence of IGF-II after 3 h of incubation (intact binding proteins (i) and cleavage products (c) are indicated). Values are average of three independent experiments ± standard deviation.

absence of IGF (Fig. 5). This clearly shows an inhibitory effect of added IGF, not evident from the previous experiment (Fig. 4A).

3.4. The role of IGF in proteolysis of IGFBP-4 by PAPP-A

The above findings have implications for the mechanism of IGF-dependent IGFBP-4 proteolysis [16–18], suggesting that PAPP-A is not activated by binding of IGF: because PAPP-A cleaves IGFBP-5 in the absence of IGF, and because proteolysis is not promoted by IGF, it is not likely that IGF activates PAPP-A or modulates its enzymatic activity towards IGFBP-4 – another substrate of the same enzyme. This could not previously be ruled out [18]. Therefore, the alternative model [19] that IGF binding to IGFBP-4 induces a conformational change that causes it to become a substrate for PAPP-A is more probable. In this model it is still possible that both components of the complex of IGF and IGFBP-4 interacts directly with PAPP-A.

Previous difficulties in discriminating between the two models of IGF-dependent IGFBP-4 proteolysis were caused firstly by not knowing the identity of the proteinase, secondly by its availability in only limited quantities that were not purified [6]. We thus asked whether cleavage of IGFBP-4 in the absence of IGF would occur using higher concentrations of purified enzyme and substrate. Cleavage of IGFBP-4 did occur (Fig. 4B, lane 1), and, as expected, added IGF-II increased the degree of proteolysis (Fig. 4B, lane 2). Compared to the standard experiment in which PAPP-A does not degrade IGFBP-4 without IGF (Fig. 1), the concentrations of both PAPP-A and IGFBP-4 were increased about 100-fold in this experiment (see figure legends for concentrations).

To more quantitatively determine the effect of IGF on proteolysis, a time course experiment using highly purified proteins in the absence and presence of IGF-II was performed (Fig. 6). This quantitatively illustrates the dramatic increase in proteolysis of IGFBP-4 caused by IGF: within the first 2 h of digestion it promotes the proteolysis 20-fold or more. It is thus questionable if IGF-independent IGFBP-4 proteolysis plays a role in vivo.

3.5. IGF does not bind to PAPP-A

Finally, by incubating radiolabeled IGF-II with immobilized PAPP-A or IGFBP-4, we demonstrated that PAPP-A does not bind directly to IGF-II (Table 1). Labeled IGF-II efficiently binds to IGFBP-4, but no binding to PAPP-A was observed. Further, no binding of radiolabeled IGF-II to an inactive mutant of PAPP-A (Glu-483 substituted with Gln) [13] was observed in the presence of IGFBP-4 (not shown). Similar results were obtained with IGF-I (not shown).

Our finding that proteolysis of IGFBP-4 by PAPP-A can

Table 1
Binding of radiolabeled IGF-II to immobilized PAPP-A and IGFBP-4

Beads[a]	Immobilized protein[b]	Radioactivity (cpm)[c]	% Bound[d]
Protein G-anti-PAPP-A	control	58	0.68
Protein G-anti-PAPP-A	PAPP-A	47	0.55
Protein G-anti-*c-myc*	control	29	0.34
Protein G-anti-*c-myc*	IGFBP-4-*c-myc*	8531	100

[a]Agarose beads with monoclonal antibody against PAPP-A (234-2) or the *c-myc* epitope (9E10) were prepared.
[b]Beads with bound antibody were saturated with medium from cells transfected with empty vector (control), or with PAPP-A or IGFBP-4-*c-myc* cDNA to contain equimolar amounts of PAPP-A and IGFBP-4. The beads were washed with PBS prior to the addition of radiolabeled IGF-II.
[c]Radioactivity after washing with PBS. Background (25 cpm) is subtracted.
[d]Comparison of radioactivity bound to immobilized IGFBP-4. The radiolabeled IGF bound to IGFBP-4 represents the functional fraction of the labeled IGF.

occur in the absence of IGF (Fig. 4B), although it is relatively slow (Fig. 6), does not fully discriminate between the two models of IGF-dependent IGFBP-4 proteolysis. The fact that PAPP-A cleaves IGFBP-5 in the absence of IGF, and that this reaction is not enhanced by IGF (Fig. 4A), strongly suggests that PAPP-A does not require activation by IGF. However, by also demonstrating that PAPP-A does not bind IGF, we can definitively conclude that IGF binds IGFBP-4, which then becomes a (better) PAPP-A substrate.

3.6. Concluding remarks

We have demonstrated a second substrate for PAPP-A, IGFBP-5. Proteolysis of IGFBP-5 does not require the presence of IGF, and is not promoted by the presence of IGF. PAPP-A cleaves IGFBP-5 at the same site as PAPP-A2, between Ser-143 and Lys-144. We further found that, in contrast to common belief, PAPP-A is capable of cleaving IGFBP-4 in the absence of IGF. The rate of hydrolysis, however, is very slow and increases dramatically in the presence of IGF. Because PAPP-A is unable to bind IGF, we conclude that IGF enhances proteolysis by binding to IGFBP-4, not by interaction with PAPP-A. PAPP-A, IGFBP-4, and IGFBP-5 are present at the same time in several tissues. Further studies are required to delineate the dynamics of proteolysis of IGFBP-4 and -5 by PAPP-A. In addition to the concentration of IGFs, at least concentrations of individual components and relative affinities for the IGFs must be considered. In several biological fluids PAPP-A appears to be the only IGFBP-4 proteinase. Likewise, PAPP-A may be the only IGFBP-5 proteinase in some systems.

Acknowledgements: This work was supported by grants (to C.O.) from the Danish Medical Research Council, the Novo Nordic Foundation, and from the Alfred Benzon Foundation, and by a Grant (HD31579-07 to L.C.G., C.O., C.A.C.) from the National Institute of Health.

References

[1] Daughaday, W.H. and Rotwein, P. (1989) Endocr. Rev. 10, 68–91.
[2] Jones, J.I. and Clemmons, D.R. (1995) Endocr. Rev. 16, 3–34.
[3] Hwa, V., Oh, Y. and Rosenfeld, R.G. (1999) Endocr. Rev. 20, 761–787.
[4] Fowlkes, J.L. (1997) Trends Endocrinol. Metab. 8, 299–306.
[5] Poretsky, L., Cataldo, N.A., Rosenwaks, Z. and Giudice, L.C. (1999) Endocr. Rev. 20, 535–582.
[6] Lawrence, J.B., Oxvig, C., Overgaard, M.T., Sottrup-Jensen, L., Gleich, G.J., Hays, L.G., Yates III, J.R. and Conover, C.A. (1999) Proc. Natl. Acad. Sci. USA 96, 3149–3153.
[7] Conover, C.A., Oxvig, C., Overgaard, M.T., Christiansen, M. and Giudice, L.C. (1999) J. Clin. Endocrinol. Metab. 84, 4742–4745.
[8] Overgaard, M.T. et al. (2000) J. Biol. Chem. 275, 31128–31133.
[9] Qin, X., Byun, D., Lau, K.W., Baylink, D.J. and Mohan, S. (2000) Arch. Biochem. Biophys. 379, 209–216.
[10] Bayes-Genis, A. et al. (2001) Arterioscler. Thromb. Vasc. Biol. 21, 335–341.
[11] Kristensen, T., Oxvig, C., Sand, O., Moller, N.P. and Sottrup-Jensen, L. (1994) Biochemistry 33, 1592–1598.
[12] Stocker, W., Grams, F., Baumann, U., Reinemer, P., Gomis-Ruth, F.X., McKay, D.B. and Bode, W. (1995) Protein Sci. 4, 823–840.
[13] Boldt, H.B., Overgaard, M.T., Laursen, L.S., Weyer, K., Sottrup-Jensen, L. and Oxvig, C. (2001) Biochem. J., in press.
[14] Oxvig, C., Sand, O., Kristensen, T., Gleich, G.J. and Sottrup-Jensen, L. (1993) J. Biol. Chem. 268, 12243–12246.
[15] Overgaard, M.T., Boldt, H.B., Laursen, L.S., Sottrup-Jensen, L., Conover, C.A. and Oxvig, C. (2001) J. Biol. Chem. 276, 21849–21853.
[16] Conover, C.A. (1991) J. Clin. Invest. 88, 1354–1361.
[17] Fowlkes, J. and Freemark, M. (1992) Endocrinology 131, 2071–2076.
[18] Conover, C.A., Kiefer, M.C. and Zapf, J. (1993) J. Clin. Invest. 91, 1129–1137.
[19] Irwin, J.C., Dsupin, B.A. and Giudice, L.C. (1995) J. Clin. Endocrinol. Metab. 80, 619–626.
[20] DuBridge, R.B., Tang, P., Hsia, H.C., Leong, P.M., Miller, J.H. and Calos, M.P. (1987) Mol. Cell. Biol. 7, 379–387.
[21] Pear, W.S., Nolan, G.P., Scott, M.L. and Baltimore, D. (1993) Proc. Natl. Acad. Sci. USA 90, 8392–8396.
[22] Oxvig, C., Gleich, G.J. and Sottrup-Jensen, L. (1994) FEBS Lett. 341, 213–217.
[23] Qin, Q.P., Christiansen, M., Oxvig, C., Pettersson, K., Sottrup-Jensen, L., Koch, C. and Norgaard-Pedersen, B. (1997) Clin. Chem. 43, 2323–2332.
[24] Sottrup-Jensen, L. (1993) Biochem. Mol. Biol. Int. 30, 789–794.
[25] Laemmli, U.K. (1970) Nature 227, 680–685.
[26] Sottrup-Jensen, L. (1995) Anal. Biochem. 225, 187–188.
[27] Greenwood, F.C., Hunter, W.M. and Glover, J.S. (1963) Biochem. J. 89, 114–123.
[28] Oxvig, C., Thomsen, A.R., Overgaard, M.T., Sorensen, E.S., Hojrup, P., Bjerrum, M.J., Gleich, G.J. and Sottrup-Jensen, L. (1999) J. Biol. Chem. 274, 16953–16958.
[29] Hintz, R.L. and Liu, F. (1977) J. Clin. Endocrinol. Metab. 45, 988–995.
[30] Byun, D., Mohan, S., Yoo, M., Sexton, C., Baylink, D.J. and Qin, X. (2001) J. Clin. Endocrinol. Metab. 86, 847–854.
[31] Conover, C.A., Durham, S.K., Zapf, J., Masiarz, F.R. and Kiefer, M.C. (1995) J. Biol. Chem. 270, 4395–4400.
[32] Fielder, P.J., Pham, H., Adashi, E.Y. and Rosenfeld, R.G. (1993) Endocrinology 133, 415–418.
[33] Nam, T.J., Busby Jr., W.H. and Clemmons, D.R. (1994) Endocrinology 135, 1385–1391.
[34] Imai, Y., Moralez, A., Andag, U., Clarke, J.B., Busby Jr., W.H. and Clemmons, D.R. (2000) J. Biol. Chem. 275, 18188–18194.