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Abstract

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Title: PZP and PAI-2: Structurally-diverse, functionally similar pregnancy proteins?

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Abstract

Pregnancy zone protein (PZP) and plasminogen activator inhibitor type 2 (PAI-2) are two multifunctional proteins that are elevated in normal pregnancy and numerous other inflammatory states. Both proteins were originally identified as protease inhibitors, but current evidence supports the notion that they may also function as modulators of T-helper cells and/or extracellular chaperones. Exacerbated inflammation, fibrinolytic disturbances and misfolded proteins are all implicated in the pathology of preeclampsia, a leading cause of maternal and foetal mortality and morbidity. Notably, reduced levels of PZP or PAI-2 are associated with preeclampsia and clarification of their diverse functions in normal pregnancy could provide much needed insight regarding the pathogenesis of this disorder. Given that inflammation and protein misfolding underlie the pathology of a very large number of disorders, the contributions of PZP and PAI-2 to extracellular proteostasis and immunoregulation could be broad-reaching.

Keywords (4)

Pregnancy zone protein

Plasminogen activator inhibitor type 2/SERPINB2

Preeclampsia

protein misfolding

1. Introduction

Despite their original identification many decades ago, the precise biological importance of both pregnancy zone protein (PZP; also known as α_2 -pregnoglobulin and pregnancy-associated α_2 -glycoprotein) and plasminogen activator inhibitor type 2 (PAI-2; also known as SERPINB2) remains unresolved in the broader biological context. Analysis of the relevant literature shows a striking disparity between the number of studies of PZP and PAI-2 compared to the number of studies of other members of the α -macroglobulin (α M) and serpin protein families, respectively. It may have been the case that these “poor cousins” were considered functionally redundant with respect to other α M and serpin family members, but it is clear that PZP and PAI-2 blood levels are markedly enhanced in pregnancy, which supports the conclusion that they have fundamentally important roles during human gestation. Moreover, their upregulation in a broad range of other inflammatory states is consistent with PZP and PAI-2 having generalised roles as immunomodulators or stress responders. Although PZP and PAI-2 share no overt structural similarity (Fig. 1), they share some striking functional similarities and may work together in a synergistic or complementary manner in extracellular fluids.

2. PZP

a. Structure

PZP belongs to the highly conserved α M protein family which includes a number of protease inhibitors and complement components. Typically α M family members are formed by one or more subunit (~180 kDa) which contains a series of macroglobulin domains and a reactive thioester bond (Fig. 1A). In humans, the *PZP* gene (12p12-13, 36 exons) encodes a 1482 bp transcript which is translated and secreted as a highly glycosylated, disulfide-linked

homodimer (360 kDa) that shares extensive amino acid sequence identity (71%) with α_2 -macroglobulin (α_2 M) (Devriendt et al., 1991). The latter is a homotetramer (720 kDa) formed by the non-covalent association of disulfide-linked dimers. Each subunit of PZP and α_2 M comprises a receptor binding domain for the low density lipoprotein receptor-related protein (LRP) and a bait region that contains multiple protease cleavage sites (Fig. 1A). Cleavage of the intramolecular thioester bond of PZP or α_2 M by reaction with proteases or small amine molecules results in a major conformational change that causes the proteins to become more compact, referred to as the transformed conformation. It is well known that transformation of α_2 M reveals the cryptic binding site for LRP, on the other hand, while it is known that transformed PZP (i.e. in complex with proteases) is a ligand of LRP, the mechanism by which this is achieved is not fully characterised (Chiabrando et al., 2002). Additional, currently unidentified high affinity receptors for PZP have also been proposed (Jensen et al., 1988, Chiabrando et al., 2002). Current information on the multi-domain fold of PZP is limited; at best, its tertiary structure can be estimated from a low resolution (4.3-Å) structure of transformed α_2 M (Marrero et al., 2012) (Fig. 1A).

b. Expression, activation and turnover

PZP is known to be expressed by a number of different tissue types in humans including the liver, uterus and brain, and is present in cerebral spinal fluid (CSF), synovial fluid and blood plasma (Tayade et al., 2005). The majority of plasma PZP is believed to be synthesised by the liver (Tayade et al., 2005) with plasma levels in both men and women normally < 0.03 mg/ml, but these levels can reportedly increase to ~ 3 mg/mL in some women during pregnancy (Ekelund and Laurell, 1994). At these levels PZP is one of the most abundant proteins in blood plasma. This supports the notion that PZP plays an important role in pregnancy that is not fulfilled by α_2 M, which is constitutively present in blood plasma at high

levels (1.5-2.0 mg/ml). Nevertheless, the expression of PZP is extremely variable between individuals and a fraction of women have PZP levels < 0.5 mg/ml throughout otherwise normal pregnancy (Ekelund and Laurell, 1994). Studies of PZP levels in women administered contraceptives indicate that PZP expression is controlled by estrogen (Damber et al., 1976), but alternative promoters are predicted to exist. For example, it has been proposed that PZP is the acute phase α M in humans (a role performed by α_2 M in other mammalian species including rodents) (Sand et al., 1985), which would explain its enhanced levels in a number of inflammatory disorders (discussed below). Similar to transformed α_2 M, transformed PZP is very rapidly cleared from the bloodstream via LRP, which is highly expressed by hepatocytes in the liver (Gliemann et al., 1986).

c. Biological Functions

PZP was originally described as a protease inhibitor analogous to α_2 M, but compared to α_2 M which can covalently trap a very broad spectrum of proteases, PZP inhibits far fewer proteases and the mechanism involved is much less efficient (Sand et al., 1985). It has been suggested that PZP is upregulated under conditions of increased cellular turnover to control the activities of intracellular proteases such as elastases and chymotrypsin-like enzymes, but there is currently little evidence to support this claim and it is feasible that the biological importance of PZP is related to an alternative role. One suggestion is that during pregnancy PZP works synergistically with placental protein-14 (PP14) to inhibit T-helper 1 (Th1) cell activation, thereby, protecting the allogenic foetus from maternal immune system attack (Skornicka et al., 2004) (Fig. 2).

The activities of α_2 M are uniquely regulated by hypochlorite (an oxidant produced in high concentrations during inflammation), which induces the dissociation of the native α_2 M

tetramer into dimers that bind to: (i) misfolded proteins (Wyatt et al., 2014); (ii) growth factors and pro-inflammatory cytokines (Wu et al., 1998), and (iii) the receptor LRP (Wu et al., 1997). It is tempting to speculate that PZP (predominately a dimer in biological fluids) will perform many of the same functions as α_2 M dimers (Fig. 2). This may be particularly valid in the case of functions that are largely mediated by hydrophobic interactions such as holdase-type chaperone activity, since this property is markedly enhanced on the surface of α_2 M dimers and PZP compared to the native α_2 M tetramer (Jensen et al., 1993, Wyatt et al., 2014).

3. PAI-2

a. Structure

PAI-2 (SERPINB2), a Clade B member of the serine protease inhibitor (serpin) superfamily, folds into the well-conserved serpin tertiary structure, including an exposed, flexible peptide reactive center loop (RCL), which contains the protease recognition site (Fig. 1B). The *SERPINB2* gene (18q21.3, 9 exons) encodes a 1884 bp mRNA which is translated into a 415 amino acid residue monomer (47 kDa) (Ye et al., 1989). PAI-2 has an inefficient internal signal sequence and thus tends to accumulate intracellularly (Belin, 1993). However, PAI-2 is found as a secreted high molecular weight (HMW) 60 – 70 kDa glycoprotein in biological fluids including pregnancy plasma (Coolman et al., 2006). Unlike other serpins, no pathological outcomes have been linked to PAI-2 polymerization nor does PAI-2 convert to a latent inactive state in the absence of certain cofactors (Lee et al., 2011).

b. Expression and turnover

Under normal physiological conditions PAI-2 is undetectable in extracellular fluids. In humans PAI-2 is acutely upregulated, from negligible levels up to 250 ng/mL in late

pregnancy (Coolman et al., 2006, Hunt et al., 2009, Guller et al., 2011). PAI-2 is expressed by numerous cell types including placental trophoblasts, endothelial cells, monocytes and macrophages, with large scale upregulation evident *in vitro* by a wide range of stimulants associated with inflammatory and/or fibrinolytic conditions (Lee et al., 2011). Unglycosylated PAI-2 can be secreted via exosomes by endothelial cells after inflammatory stimulation *in vitro* (Boncela et al., 2013) and via microparticles in placental perfusions (Guller et al., 2011), and this may account for the presence of LMW PAI-2 in biological fluids including pregnancy plasma (Booth et al., 1988). Both HMW and LMW PAI-2 specifically and efficiently inhibit urokinase plasminogen activator (uPA) and tissue plasminogen activator (tPA), which are responsible for the proteolytic activation of plasmin from plasminogen, via an irreversible suicide-substrate mechanism common to the serpins (Silverman et al., 2004). Upon inhibition of cellular receptor-bound uPA, the serpin-uPA complex is rapidly endocytosed via receptors of the LDL receptor family, including LRP (the only known receptor for PZP) and degraded (Al-Ejeh et al., 2004, Lee et al., 2011).

c. Biological function

In pregnancy extracellular PAI-2 is believed to protect against premature placental separation and maintain haemostasis by controlling localized proteolysis and tissue destruction by counteracting the activity of uPA and tPA (Lee et al., 2011). These target protease specificities are shared by plasminogen activator inhibitor type 1 (PAI-1/SERPINE1) (Lee et al., 2011), even though PAI-1 and PAI-2 are phylogenetically distinct as denoted by their classification into different serpin clades (Silverman et al., 2004). The overall increased PAI levels in normal human pregnancy result in decreased overall fibrinolytic activity thus contributing to the hypercoagulable state associated with pregnancy (Coolman et al., 2006, Hunt et al., 2009). PAI-2 is not required for normal murine development, survival or fertility

(Dougherty et al., 1999). Considering that *Pai2* mRNA is only detected at significant levels in the murine placenta late in gestation (Dougherty et al., 1999), this limits the usefulness of murine models of pregnancy-related PAI-2 deficiency in humans.

PAI-2 has also been reported to have a number of other functions that appear to be independent of protease inhibitory interactions, similar to PZP. This includes modulation of T-cell responses (Schroder et al., 2010) and stabilisation of misfolded proteins similar to holdase-type chaperones (Lee et al., 2015) (Fig. 2).

4. Disease implications

PZP and PAI-2 are upregulated in large number of diverse inflammatory conditions where they are believed to exert cellular protective effects (Thomson and Horne, 1980, Lee et al., 2011). Here we focus on the reported association of low PZP and PAI-2 levels with preeclampsia (Horne et al., 1972, Lee et al., 2011) (Fig. 2). Preeclampsia is a leading cause of maternal and neonatal morbidity and mortality with currently unknown etiology. The disorder is characterised by high maternal blood pressure, proteinuria, aberrant placental development and haemostatic and fibrinolytic disturbances (Hunt et al., 2009, Amaral et al., 2015). It is known that PZP levels vary markedly between individuals (Ekelund and Laurell, 1994). The reasons for the significantly reduced levels of PAI-2 in preeclampsia compared to normal pregnancy are not known (Hunt et al., 2009, Wikstrom et al., 2009), but it possible that damage to endothelial cells is an important contributing factor.

Systemic inflammation in preeclampsia is much greater than that which occurs in normal pregnancy (Amaral et al., 2015), and it has been proposed that increased myeloperoxidase

(the enzyme that generates hypochlorite) in blood plasma and the placenta is an important source of oxidative stress in preeclampsia (Gandley et al., 2008). An increased PAI-1/PAI-2 ratio and placental oxidative stress have been reported as good predictors of preeclampsia (Wikstrom et al., 2009). Given the known susceptibility of protease inhibitors including PAI-1 (Baker et al., 1990) and α_2 M (Deby-Dupont et al., 1994) to inactivation by reactive oxygen species, it is plausible that PAI-2, which is resistant to inactivation by oxidants (Baker et al., 1990), is an important regulator of fibrinolytic activity under these conditions (Fig. 2). Currently the effect of oxidation on the protease inhibitory activities of PZP is unknown.

Substantial evidence supports the conclusion that enhanced Th1 cell activity is important in the pathology of preeclampsia (Amaral et al., 2015). Both α_2 M and PZP are reported to potentiate the ability of PP14 to inhibit Th1 cells, but comparatively, PZP appears to be more efficient at performing this role (Skornicka et al., 2004) (Fig. 2). The enhanced non-covalent binding of PZP to PP14 (a lipocalin) is likely to be the result of hydrophobic interactions. Experiments in PAI-2 knockout mice indicate that PAI-2 also plays a role in Th1 cell suppression (Schroder et al., 2010). The mechanism by which this is achieved is not known, but appears to be unrelated to PAI-2 protease inhibitory activity and could involve intracellular functions (Fig. 2).

It has recently been shown that misfolded proteins including the Alzheimer's disease-associated amyloid beta peptide accumulate in the placenta and biological fluids in preeclampsia (Buhimschi et al., 2014). The toxicity of misfolded proteins is incompletely characterised, but it is well-accepted that their accumulation (known to occur in over 40 human disorders) is deleterious. Hypochlorite-induced dissociation of the native α_2 M

tetramer into dimers dramatically enhances its ability to bind to misfolded proteins and deliver them to LRP for disposal (Wyatt et al., 2014). It is possible that upregulation of PZP is a strategy to enhance the chaperone capability of biological fluids, but this has yet to be proven. Furthermore, it is possible that PZP contributes to immunoregulation by non-covalently sequestering of a range of other ligands including TNF-alpha, IL-2 and IL-6, which all preferentially bind to α_2 M dimers compared to the native α_2 M tetramer (Wu et al., 1998) (Fig. 2). It is likely that levels of PZP exceed that of hypochlorite-induced α_2 M dimers formed *in vivo*. While the modest chaperone activity of PAI-2 could also contribute to the stabilisation and disposal of misfolded proteins at the placenta (Fig. 2), this is possibly overshadowed by the chaperone activity of the highly abundant PZP.

5. Conclusion

Given the upregulation of PZP and PAI-2 in inflammatory states and their strong association with normal pregnancy and preeclampsia, it will be important to elucidate their function in the presence of relevant stressors. We propose that, far from being functionally redundant α -macroglobulin and serpin family members, the complementary activities of PZP and PAI-2 in fibrinolysis, the immune response and extracellular proteostasis are protective in pregnancy and may have global relevance in inflammation.

Figure legends

Fig. 1. Structure and known functional domains of PZP and PAI-2. (A) Schematic diagram of human monomeric PZP showing amino acid (aa) residue positions of the signal sequence, the bait region, the thiol-ester group and the receptor binding domain. Macroglobulin domains M1 – M4 are predicted using InterPro (EMBL-EBI). Multiple N-glycosylation sites (Asn⁵⁴, Asn⁶⁹, Asn²⁴⁶, Asn³⁹², Asn⁴⁰⁶, Asn⁷⁵³, Asn⁸⁷⁵, Asn⁹³², Asn⁹⁹⁷, Asn¹⁴³⁰) are present in PZP (not shown). PZP monomers (180 kDa) dimerize by disulfide bonds to form the native and functionally active 360 kDa homodimer. Image shows 3D structure of dimeric α_2 M based on the structure of α_2 M in the transformed conformation PDB ID: 4ACQ (Marrero et al., 2012). This is currently the best estimate of PZP tertiary structure. **(B)** Schematic diagram of human PAI-2 showing aa residue positions of the internal secretion signal, a unique to PAI-2 interhelical domain between helices C and D (CD-loop), the reactive center loop (RCL) which contains the protease recognition site. Asterisks denote polymorphism sites between two major variants of PAI-2; variant A (Asn¹²⁰, Asn⁴⁰⁴, Ser⁴¹³) and B (Asp¹²⁰, Lys⁴⁰⁴, Cys⁴¹³). Hashtags denote N-glycosylation sites (Asn⁷⁵, Asn¹¹⁵, Asn³³⁹). Image shows the 3D structure of PAI-2 based on PDB ID: 1JRR (Jankova et al., 2001). PAI-2 conforms to the general tertiary structure conserved across all known serpins (Silverman et al., 2004). The CD-loop and RCL are highly mobile or disordered structures and have not been resolved.

Fig. 2. Potential protective functions of PZP and PAI-2 in pregnancy. Normal pregnancy is associated with inflammation and oxidative stress, and elevated levels of PZP and PAI-2. (1) Reduced α_2 M and PAI-1 protease inhibitory activity occurs in response to hypochlorite generation, PAI-2 protease inhibitory activity is resistant to oxidative inactivation and contributes to the regulation of fibrinolysis. (2) Protein misfolding is enhanced by oxidative stress, PAI-2 acts locally as a chaperone for misfolded proteins at the placenta. Dimeric α_2 M

(generated at low concentration in response to hypochlorite) and PZP facilitate the clearance of misfolded proteins and pro-inflammatory cytokines. (3) PZP (in concert with PP14) and PAI-2 have inhibitory effects on Th1 cells, although the mechanisms involved remain enigmatic. Comparatively, the effect of PZP on Th1 cells is greater than that of α_2M . ***Low levels of PZP and PAI-2 could allow inflammation to become exacerbated which drives placental dysfunction and preeclampsia.***

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Figure 1

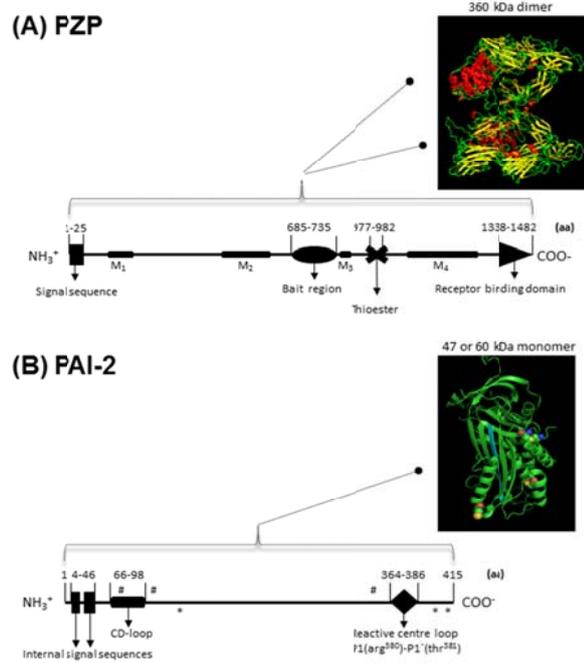


Figure 2

