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

Amy R. Wyatt[a,b], Jordan H. Cater[a,b], Marie Ranson[a,b,*]

[a]Illawarra Health and Medical Research Institute, University of Wollongong, Wollongong, 2522, Australia
[b]School of Biological Sciences, University of Wollongong, Wollongong, 2522, Australia.

∗Corresponding author at: School of Biological Sciences, University of Wollongong, Wollongong, 2522 Australia. E-mail address: mranson@uow.edu.au (M. Ranson).

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 (αM) and serpin protein families, respectively. It may have been the case that these "poor cousins" were considered functionally redundant with respect to other αM 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 αM protein family which includes a number of protease inhibitors and complement components. Typically αM 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_2M) (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_2M 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_2M 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_2M 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_2M (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_2M, 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 promotors are predicted to exist. For example, it has been proposed that PZP is the acute phase αM in humans (a role performed by α_2M 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_2M, 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_2M, but compared to α_2M 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_2M are uniquely regulated by hypochlorite (an oxidant produced in high concentrations during inflammation), which induces the dissociation of the native α_2M

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_2M 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_2M dimers and PZP compared to the native α_2M 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 phylogeneticially 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_2M (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_2M 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_2M

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_2M dimers compared to the native α_2M tetramer (Wu et al., 1998) (Fig. 2). It is likely that levels of PZP exceed that of hypochlorite-induced α_2M 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 (Asn^{54}, Asn^{69}, Asn^{246}, Asn^{392}, Asn^{406}, Asn^{753}, Asn^{875}, Asn^{932}, Asn^{997}, Asn^{1430}) 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_2M based on the structure of α_2M 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 (Asn^{120}, Asn^{404}, Ser^{413}) and B (Asp^{120}, Lys^{404}, Cys^{413}). Hashtags denote N-glycosylation sites (Asn^{75}, Asn^{115}, Asn^{339}). 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_2M 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_2M

(generated at low centration 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.***

Acknowledgements

A.R.W is grateful to the National Health and Medical Research Council for financial support.

References

Al-Ejeh, F., Croucher, D.Ranson, M. 2004. Kinetic analysis of plasminogen activator inhibitor type-2: urokinase complex formation and subsequent internalisation by carcinoma cell lines. Exp. Cell Res. 297, 259-271.

Amaral, L. M., Cunningham, M. W., Cornelius, D. C.LaMarca, B. 2015. Preeclampsia: long-term consequences for vascular health. Vasc. Health Risk Manag. 11, 403-415.

Baker, M. S., Green, S. P., Goss, N., Katrantzis, M.Doe, W. F. 1990. Plasminogen activator inhibitor 2 (PAI-2) is not inactivated by exposure to oxidants which can be released from activated neutrophils. Biochem. Biophys. Res. Commun. 166, 993-1000.

Belin, D. 1993. Biology and facultative secretion of plasminogen activator inhibitor-2. Thromb. Haemost. 70, 144-147.

Boncela, J., Przygodzka, P., Wyroba, E., Papiewska-Pajak, I.Cierniewski, C. S. 2013. Secretion of SerpinB2 from endothelial cells activated with inflammatory stimuli. Exp. Cell Res. 319, 1213-1219.

Booth, N. A., Reith, A.Bennett, B. 1988. A plasminogen activator inhibitor (PAI-2) circulates in two molecular forms during pregnancy. Thromb. Haemost. 59, 77-79.

Buhimschi, I. A., Nayeri, U. A., Zhao, G., Shook, L. L., Pensalfini, A., Funai, E. F., Bernstein, I. M., Glabe, C. G.Buhimschi, C. S. 2014. Protein misfolding, congophilia, oligomerization, and defective amyloid processing in preeclampsia. Sci. Transl. Med. 6, 245ra292.

Chiabrando, G. A., Vides, M. A.Sanchez, M. C. 2002. Differential binding properties of human pregnancy zone protein- and alpha2-macroglobulin-proteinase complexes to low-density lipoprotein receptor-related protein. Arch. Biochem. Biophys. 398, 73-78.

Coolman, M., de Groot, C. J., Steegers, E. A., Geurts-Moespot, A., Thomas, C. M., Steegers-Theunissen, R. P.Sweep, F. C. 2006. Concentrations of plasminogen activators and their inhibitors in blood preconceptionally, during and after pregnancy. Eur. J. Obstet. Gynecol. Reprod. Biol. 128, 22-28.

Damber, M. G., von Schoultz, B., Solheim, F.Stigbrand, T. 1976. A quantitative study of the pregnancy zone protein in sera of woman taking oral contraceptives. Am. J. Obstet. Gynecol. 124, 289-292.

Deby-Dupont, G., Croisier, J. L., Camus, G., Brumioul, D., Mathy-Hartert, M., Sondag, D., Deby, C.Lamy, M. 1994. Inactivation of alpha(2)-Macroglobulin by Activated Human Polymorphonuclear Leukocytes. Mediators Inflamm. 3, 117-123.

Devriendt, K., Van den Berghe, H., Cassiman, J.-J.Marynen, P. 1991. Primary structure of pregnancy zone protein. Molecular cloning of a full-length PZP cDNA clone by the polymerase chain reaction. Biochim. Biophys. Acta. 1088, 95-103.

Dougherty, K. M., Pearson, J. M., Yang, A. Y., Westrick, R. J., Baker, M. S.Ginsburg, D. 1999. The plasminogen activator inhibitor-2 gene is not required for normal murine development or survival. Proc. Natl. Acad. Sci. USA. 96, 686-691.

Ekelund, L.Laurell, C. B. 1994. The pregnancy zone protein response during gestation: a metabolic challenge. Scand. J. Clin. Lab. Invest. 54, 623-629.

Gandley, R. E., Rohland, J., Zhou, Y., Shibata, E., Harger, G. F., Rajakumar, A., Kagan, V. E., Markovic, N.Hubel, C. A. 2008. Increased myeloperoxidase in the placenta and circulation of women with preeclampsia. Hypertension. 52, 387-393.

Gliemann, J., Moestrup, S., Henning Jensen, P., Sottrup-Jensen, L., Busk Andersen, H., Munck Petersen, C.Sonne, O. 1986. Evidence for binding of human pregnancy zone protein-proteinase complex to alpha 2-macroglobulin receptors. Biochim. Biophys. Acta. 883, 400-406.

Guller, S., Tang, Z., Ma, Y. Y., Di Santo, S., Sager, R.Schneider, H. 2011. Protein composition of microparticles shed from human placenta during placental perfusion: Potential role in angiogenesis and fibrinolysis in preeclampsia. Placenta. 32, 63-69.

Horne, C. H., Briggs, J. D., Howie, P. W.Kennedy, A. C. 1972. Serum -macroglobulins in renal disease and preeclampsia. J. Clin. Pathol. 25, 590-593.

Hunt, B. J., Missfelder-Lobos, H., Parra-Cordero, M., Fletcher, O., Parmar, K., Lefkou, E.Lees, C. C. 2009. Pregnancy outcome and fibrinolytic, endothelial and coagulation markers in women undergoing uterine artery Doppler screening at 23 weeks. J. Thromb. Haemost. 7, 955-961.

Jankova, L., Harrop, S. J., Saunders, D. N., Andrews, J. L., Bertram, K. C., Gould, A. R., Baker, M. S.Curmi, P. M. 2001. Crystal structure of the complex of plasminogen activator inhibitor 2 with a peptide mimicking the reactive center loop. J. Biol. Chem. 276, 43374-43382.

Jensen, P. E., Hagglof, E. M., Arbelaez, L. F., Stigbrand, T.Shanbhag, V. P. 1993. Comparison of conformational changes of pregnancy zone protein and human alpha 2-macroglobulin, a study using hydrophobic affinity partitioning. Biochim. Biophys. Acta. 1164, 152-158.

Jensen, P. H., Moestrup, S. K., Sottrup-Jensen, L., Petersen, C. M.Gliemann, J. 1988. Receptors for alpha 2-macroglobulin- and pregnancy zone protein-proteinase complexes in the human placental syncytiotrophoblast. Placenta. 9, 463-477.

Lee, J. A., Cochran, B. J., Lobov, S.Ranson, M. 2011. Forty years later and the role of plasminogen activator inhibitor type 2/SERPINB2 is still an enigma. Semin. Thromb. Hemost. 37, 395-407.

Lee, J. A., Yerbury, J. J., Farrawell, N., Shearer, R. F., Constantinescu, P., Hatters, D. M., Schroder, W. A., Suhrbier, A., Wilson, M. R., Saunders, D. N., et al. 2015. SerpinB2 (PAI-2) Modulates Proteostasis via Binding Misfolded Proteins and Promotion of Cytoprotective Inclusion Formation. PLoS One. 10, e0130136.

Marrero, A., Duquerroy, S., Trapani, S., Goulas, T., Guevara, T., Andersen, G. R., Navaza, J., Sottrup-Jensen, L.Gomis-Rüth, F. X. 2012. The Crystal Structure of Human α2-Macroglobulin Reveals a Unique Molecular Cage. Angew. Chem. Int. Ed. 51, 3340-3344.

Sand, O., Folkersen, J., Westergaard, J. G.Sottrup-Jensen, L. 1985. Characterization of human pregnancy zone protein. Comparison with human alpha 2-macroglobulin. J. Biol. Chem. 260, 15723-15735.

Schroder, W. A., Le, T. T., Major, L., Street, S., Gardner, J., Lambley, E., Markey, K., Macdonald, K. P., Fish, R. J., Thomas, R., et al. 2010. A Physiological Function of Inflammation-Associated SerpinB2 Is Regulation of Adaptive Immunity. J. Immunol. 184, 2663-2670.

Silverman, G. A., Whisstock, J. C., Askew, D. J., Pak, S. C., Luke, C. J., Cataltepe, S., Irving, J. A.Bird, P. I. 2004. Human clade B serpins (ov-serpins) belong to a cohort of evolutionarily dispersed intracellular proteinase inhibitor clades that protect cells from promiscuous proteolysis. Cell. Mol. Life Sci. 61, 301-325.

Skornicka, E. L., Kiyatkina, N., Weber, M. C., Tykocinski, M. L.Koo, P. H. 2004. Pregnancy zone protein is a carrier and modulator of placental protein-14 in T-cell growth and cytokine production. Cell. Immunol. 232, 144-156.

Tayade, C., Esadeg, S., Fang, Y.Croy, B. A. 2005. Functions of alpha 2 macroglobulins in pregnancy. Mol. Cell. Endocrinol. 245, 60-66.

Thomson, A. W.Horne, C. H. 1980. Biological and clinical significance of pregnancy-associated alpha2-glycoprotein (alpha/-PAG)-a review. Invest. Cell. Pathol. 3, 295-309.

Wikstrom, A. K., Nash, P., Eriksson, U. J.Olovsson, M. H. 2009. Evidence of increased oxidative stress and a change in the plasminogen activator inhibitor (PAI)-1 to PAI-2 ratio in early-onset but not late-onset preeclampsia. Am. J. Obstet. Gynecol. 201, 597 e591-598.

Wu, S. M., Boyer, C. M.Pizzo, S. V. 1997. The binding of receptor-recognized alpha2-macroglobulin to the low density lipoprotein receptor-related protein and the alpha2M signaling receptor is decoupled by oxidation. J. Biol. Chem. 272, 20627-20635.

Wu, S. M., Patel, D. D.Pizzo, S. V. 1998. Oxidized alpha2-macroglobulin (alpha2M) differentially regulates receptor binding by cytokines/growth factors: implications for tissue injury and repair mechanisms in inflammation. J. Immunol. 161, 4356-4365.

Wyatt, A. R., Kumita, J. R., Mifsud, R. W., Gooden, C. A., Wilson, M. R.Dobson, C. M. 2014. Hypochlorite-induced structural modifications enhance the chaperone activity of human alpha2-macroglobulin. Proc. Natl. Acad. Sci. USA. 111, E2081-2090.

Ye, R. D., Ahern, S. M., Le Beau, M. M., Lebo, R. V.Sadler, J. E. 1989. Structure of the gene for human plasminogen activator inhibitor-2. The nearest mammalian homologue of chicken ovalbumin. J. Biol. Chem. 264, 5495-5502.

Figure 1

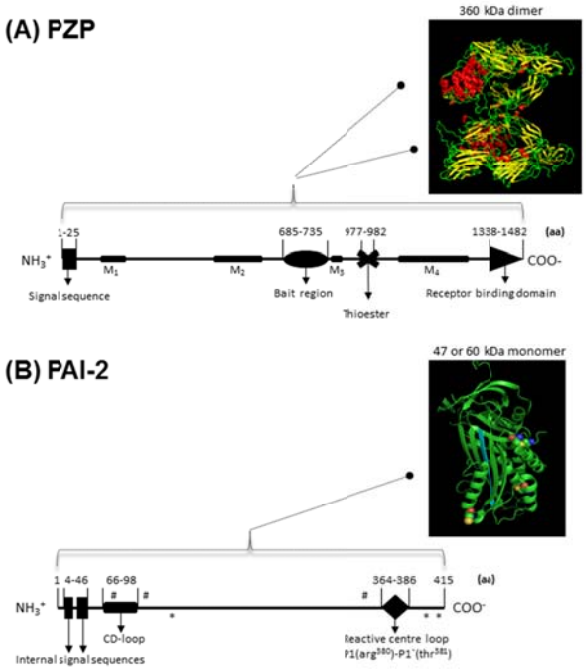

Figure 2