The DING Family of Phosphate Binding Proteins in Inflammatory Diseases

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Abstract

Human paraoxonase 1 (hPON-1) is a protein that has been studied in relation to its antioxidant and anti-atherosclerotic properties. Despite extensive studies, the molecular mechanisms responsible for its functional properties remain unclear. During the last decade, a new partner of hPON-1 has been identified. Hidden for a long time because of a similar molecular weight with hPON-1, this protein, termed human phosphate-binding protein (HPBP), may contribute to the biological functions of hPON-1. Belonging to the DING protein, a sub-family of phosphate binding proteins (PBP or pstS), HPBP stabilizes hPON-1 and might prevent calcification of arteries in case of advance atherosclerosis. The role of other DING proteins in some calcification processes (*i.e.* nephrolithiasis) and the identification of HPBP in the atheroma plaque support this hypothesis. Nevertheless, the relevance of hPON-1/HPBP as well as the molecular determinants in atherosclerosis remains to be elucidated.

Keywords

DING proteins • HPBP • Inflammation • Phosphate-binding proteins

4.1 The Serendipitous Discovery of HPBP

Human paraoxonase-1 (hPON-1) is largely studied due to its relationship with human atherosclerosis. Nevertheless, the molecular determinants responsible of its anti-atherosclerotic properties remain unclear. While performing structural studies on hPON-1, Morales *et al*. serendipitously discovered a hPON-1 associated protein [1]. Astonishingly, from a supposedly "pure" sample of hPON-1,

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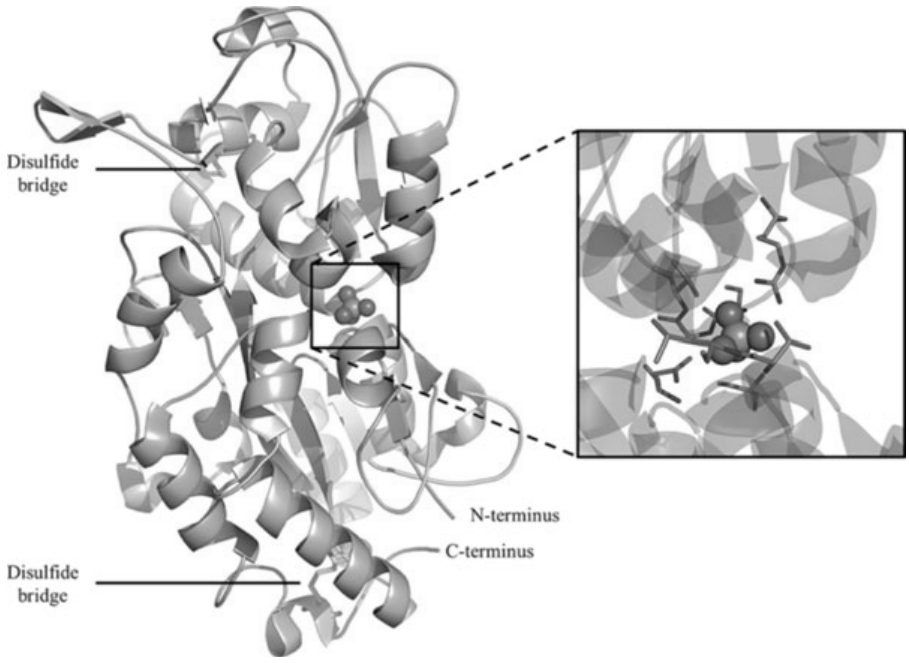

Fig. 4.1 Structure of HPBP (pdbid: 2V3Q). HPBP presents two globular domains colorized in *green* (*upper domain*) and *blue* (*bottom domain*). The disulfide bridges have been represented as sticks. The phosphate has been represented as spheres. At the interface of both domains, there is the phosphate binding cleft. Residues involved in phosphate binding have been represented as sticks and colorized using the same color-code as the domain

they crystallized a new protein, absent from genomic databases. This protein, termed human phosphate binding protein (HPBP), has been for a long time hidden behind hPON-1 glycosylated isoforms on SDS-PAGE because they have similar molecular weight (*i.e.* 38 kDa) [1]. Since HPBP was absent from genomic databases, the sequence of the protein has been determined using crystallographic data, coupled with mass spectrometry [2]. The obtained sequence and structure shows that HPBP belongs to the phosphate binding proteins (PBP) and more precisely to a sub-family called "DING proteins".

The structure of HPBP is composed of two globular domains linked together with a flexible hinge (Fig. 4.1). The phosphate anion binding site is located at the interface of both domains. Due to its ability to fix phosphate (with sub-micromolar affinity), HPBP constitutes the first phosphate transporter identified from human tissues, being homologous to the prokaryotic periplasmic PBP associated with the ABC transporter system (*i.e.* PstS) [3]. Noteworthy, some differences are noticed between HPBP's and PstS' structure, such as the presence of two disulfide bridges and four protuberant loops (Fig. 4.1). Nevertheless, the physiological role of these differences remains to be elucidated.

4.2 Properties of DING Proteins

DING proteins constitute a poorly characterized family of proteins, having usually 38–40 kDa, which were named for their eponymous N-terminus extremity (D-I-N-G-G-G) [1]. The first DING proteins were identified in animals and in some plants at the end of the 1990s. The genomic era has greatly increased the number of new DING protein sequences, especially in bacteria. Moreover, it allowed the first identifications of DING protein's genes in prokaryotes (and mainly from genus *Pseudomonas*). Today, more than 50 DING proteins (and genes) have been identified in the prokaryote kingdom [4, 5].

The situation is different in eukaryotes: although DING-coding sequences were amplified from eukaryotic genomic sequences; no open

reading frame nor locus has yet been identified on published eukaryotic genomes [5]. This critical absence of eukaryotic genetic information constitutes a major barrier which has drastically hampered their studies. As a consequence, eukaryotic DING proteins were serendipitously identified due to their implication in pathological processes and, in particular, in inflammation processes [6].

In humans, six different DING proteins have been identified; HPBP, synovial stimulatory Protein (SSP), X-DING-CD4, crystal adhesion inhibitor (CAI), genistein-binding protein and a DING protein from GSS database. In the next part, we will focus on DING proteins which have been clearly involved in inflammatory diseases [1, 7–11].

4.2.1 Implications of DING Proteins in Inflammatory Processes

4.2.1.1 Rheumatoid Arthritis

DING proteins were discovered because of their involvement with rheumatoid arthritis (RA), an inflammatory auto-immune disease causing a deformation or destruction of articulations. In the aim to unravel the poorly understood origin of RA, the composition of rheumatoid fluids were widely studied, leading to the identification of the synovial stimulatory protein (SSP) [7]. This protein has been partially sequenced, revealing the characteristically N-terminus "D-I-N-G-G-G" and leading to the first isolation of a DING protein in a human disease. SSP has been shown to interact with human sera containing rheumatoid factor (*i.e.* auto-antibody) and to possess stimulatory capacity of T cell proliferation. Both abilities suggest that SSP is involved in the RA inflammatory process [7].

4.2.1.2 Nephrolithiasis

The implication of DING proteins in inflammatory processes is not only limited to SSP, and numerous examples show that this family of proteins is tightly correlated with inflammation [9, 12]. Nephrolithiasis, also known as kidney stone disease is a common derangement with an increasing prevalence in the population (up to 5 %) [13]. This illness is caused by the aggregation of calcium oxalate or calcium phosphate with proteins and/or bacteria [14]. Growth and migration of renal calculi into the lower urinary tract cause severe pain and in some cases complications and inflammation leading to death [14]. However, the exact mechanism of renal calculi formation remains unclear even if many theories have been proposed (saturation of urine, nanobacteria) [13, 15–17].

The crystal adhesion inhibitor (CAI) is a DING protein studied in relation to renal calculi formation. CAI is a 39 kDa protein which has been isolated in monkey renal epithelial cells [10, 18]. It has been demonstrated that CAI inhibits renal calculi growth by significantly reducing the binding of calcium oxalate monohydrate (COM) onto crystal [10]. Indeed, CAI sequesters/covers crystal surface avoiding its growth. This fixation capability may be due to CAI affinity towards phosphate (like other DING) since renal calculi crystals are often composed of calcium phosphate [13, 14]. Even whether the implication CAI in inhibition of renal calculi growth is clear, other proteins seem to be more crucial (for instance the Tamm-Horsfall Protein) [18].

4.2.1.3 Atherosclerosis

Atherosclerosis represents the leading cause of mortality/morbidity in western countries [19]. The molecular mechanisms involved in the formation of the atheroma plaque are complex, and a key phenomenon is the accumulation of oxidized low density lipoprotein particles (oxLDL) in the inner walls of arteries [20, 21]. The atherosclerotic plaque, once unhooked, may lead to ischemic stroke/heart attack [22, 23]. The accumulation of oxLDL in the atheroma plaque is counteracted by high density lipoproteins (HDL) that modify the balance oxLDL/LDL, slowing down the formation of atherosclerotic plaque [24]. Particularly, it has been found that hPON-1, a HDL-associated protein, plays a protective role by degrading peroxidized lipids and reducing the level of oxLDL in human plasma [25–28]. Nevertheless, the exact the molecular determinants remain unclear.

Recent studies showed that HPBP, a DING protein, is associated with hPON-1 [1, 29], and

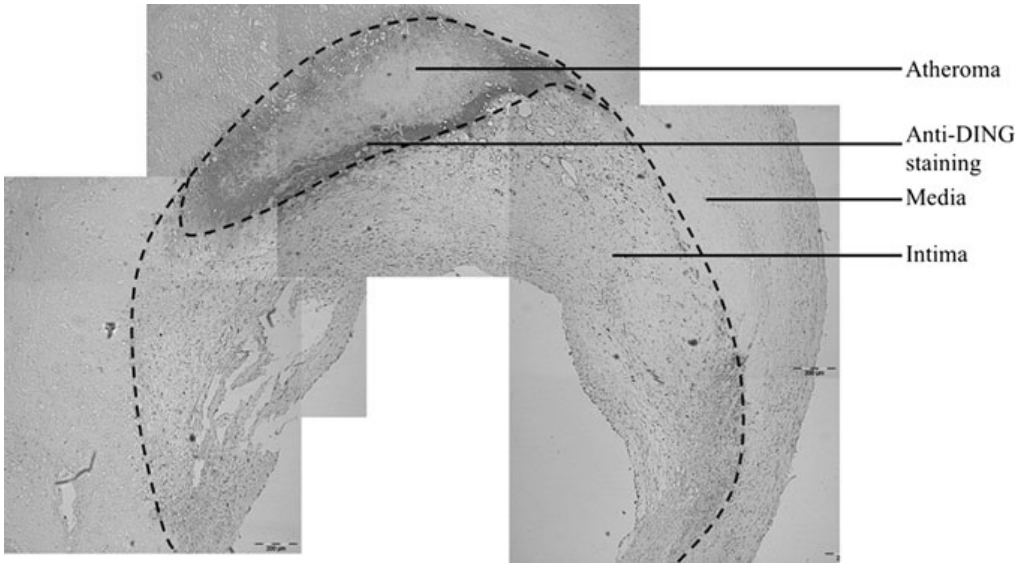

Fig. 4.2 Immunohistochemical staining of DING proteins in an atheroma. The endothelium is sub-divided in two parts, the intima and the media. The separation between the two layers is represented as a *dashed line*. The atheroma has been limited with *dashed line*

stabilizes the active conformation of this enzyme [29–31]. The association hPON-1/HPBP may thus influence the related anti-atherosclerotic properties of hPON1. Moreover, since DING proteins belong to the phosphate binding protein superfamily, it has been hypothesized that HPBP may have a role in preventing phosphate salt formation, specifically with calcium. HPBP may thus be implicated in calcification processes which are widely common in advance atherosclerosis. This hypothesis is consistent with the co-localization of DING proteins and hPON1 within the atheroma plaque.

Even if the biochemical mode of action of HPBP is yet unclear, the relationships between inflammation and other DING proteins closely related to HPBP have been recently investigated. DING proteins have been related to the phosphorylation of several proteins in the mitogen-activated protein (MAP) kinase pathway, including extracellular signal-regulated kinases (ERK) 1 and 2 and c-Jun N-terminal kinases (JNK) [32]. This impacts their downstream targets like the signal transducer and activator of transcription (STAT) 3, the cyclic AMP response element-binding (CREB), c-Jun and more importantly NF-κB [32]. Moreover, it has been shown that some DING proteins interact with the p50 sub-unit of NF-κB [33], a major pathway in inflammatory response. Indeed, the production of numerous pro-inflammatory cytokines has been shown to be NF-κB-dependent in atherosclerosis [34]. The modulation of the association of NF-κB subunits may thus lead to the modulation of inflammatory response in atherosclerosis process. Furthermore, DING proteins interact with the CCAAT/enhancer-binding protein (C/EBP) β binding site altering its nuclear localization. This was demonstrated by DNA band-shift assay, that showed that the presence of DING proteins reduces significantly the ability of C/EBP β to bind to DNA [35]. C/EBP β is important in the regulation of genes involved in immune and inflammatory responses and has been shown to bind to regulatory regions of several acute-phase and cytokine genes, and is critical for normal macrophage functioning, and in the modulation of inflammatory cytokine such as interleukins-1 and -6 [35, 36]. All these molecular mechanistic insights, and the presence of DING proteins *in situ* (*i.e.* in atheroma, Fig. 4.2) suggests that DING proteins may play a role in the pathophysiology of atherosclerosis.

4.3 Conclusion

Results from recent research show that DING proteins are probably involved in inflammatory process. In the case of human atherosclerosis, the association between HPBP and hPON1 may be relevant since it contributes to stabilize this antioxidant and anti-inflammatory enzyme. However, numerous questions remain and future structural/mechanistic studies on HPBP/hPON-1 complexes may unravel new potential functions of DING proteins in atherosclerosis.

References

1. Morales R, Berna A, Carpentier P, Contreras-Martel C, Renault F, Nicodeme M, et al. Serendipitous discovery and X-ray structure of a human phosphate binding apolipoprotein. Structure. 2006;14:601–9.
2. Diemer H, Elias M, Renault F, Rochu D, Contreras-Martel C, Schaeffer C, et al. Tandem use of X-ray crystallography and mass spectrometry to obtain ab initio the complete and exact amino acids sequence of HPBP, a human 38-kDa apolipoprotein. Proteins. 2008;71:1708–20.
3. Luecke H, Quiocho FA. High specificity of a phosphate transport protein determined by hydrogen bonds. Nature. 1990;347:402–6.
4. Berna A, Scott K, Chabriere E, Bernier F. The DING family of proteins: ubiquitous in eukaryotes, but where are the genes? Bioessays. 2009;31:570–80.
5. Bernier F. DING proteins: numerous functions, elusive genes, a potential for health. Cell Mol Life Sci. 2013;70:3045–56.
6. Berna A, Bernier F, Chabriere E, Elias M, Scott K, Suh A. For whom the bell tolls? DING proteins in health and disease. Cell Mol Life Sci. 2009;66:2205–18.
7. Adams L, Davey S, Scott K. The DING protein: an autocrine growth-stimulatory protein related to the human synovial stimulatory protein. Biochim Biophys Acta. 2002;1586:254–64.
8. Belenky M, Prasain J, Kim H, Barnes S. DING, a genistein target in human breast cancer: a protein without a gene. J Nutr. 2003;133:2497S–501.
9. Ivanova A, Shilpi RY, Sachdeva R, Li G, Simm M. Native X-DING-CD4 protein secreted by HIV-1 resistant CD4+ T cells blocks activity of IL-8 promoter in human endothelial cells infected with enteric bacteria. Innate Immun. 2012;18:571–9.
10. Kumar V, Yu S, Farell G, Toback F, Lieske J. Renal epithelial cells constitutively produce a protein that blocks adhesion of crystals to their surface. Am J Physiol Renal Physiol. 2004;287:F373–83.
11. Lesner A, Shilpi R, Ivanova A, Gawinowicz MA, Lesniak J, Nikolov D, et al. Identification of X-DING-CD4, a new member of human DING protein family that is secreted by HIV-1 resistant CD4(+) T cells and has anti-viral activity. Biochem Biophys Res Commun. 2009;389:284–9.
12. Mukerjee R, Deshmane SL, Darbinian N, Czernik M, Khalili K, Amini S, et al. St. John's Wort protein, p27SJ, regulates the MCP-1 promoter. Mol Immunol. 2008;45:4028–35.
13. Hall PM. Nephrolithiasis: treatment, causes, and prevention. Cleve Clin J Med. 2009;76:583–91.
14. Worcester EM, Coe FL. Nephrolithiasis. Prim Care. 2008;35:369–91.
15. Drancourt M, Jacomo V, Lepidi H, Lechevallier E, Grisoni V, Coulange C, et al. Attempted isolation of Nanobacterium sp. microorganisms from upper urinary tract stones. J Clin Microbiol. 2003;41:368–72.
16. Kumar V, Farell G, Yu S, Harrington S, Fitzpatrick L, Rzewuska E, et al. Cell biology of pathologic renal calcification: contribution of crystal transcytosis, cell-mediated calcification, and nanoparticles. J Investig Med. 2006;54:412–24.
17. Khullar M, Sharma SK, Singh SK, Bajwa P, Sheikh FA, Relan V, et al. Morphological and immunological characteristics of nanobacteria from human renal stones of a north Indian population. Urol Res. 2004;32:190–5.
18. Kumar V, Pena De La Vega L, Farell G, Lieske JC. Urinary macromolecular inhibition of crystal adhesion to renal epithelial cells is impaired in male stone formers. Kidney Int. 2005;68:1784–92.
19. Go AS, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, Borden WB, et al. Heart disease and stroke statistics-2013 update: a report from the American heart association. Circulation. 2013;127:e6–245.
20. Steinbrecher UP, Parthasarathy S, Leake DS, Witztum JL, Steinberg D. Modification of low density lipoprotein by endothelial cells involves lipid peroxidation and degradation of low density lipoprotein phospholipids. Proc Natl Acad Sci U S A. 1984;81:3883–7.
21. Yoshida H, Kisugi R. Mechanisms of LDL oxidation. Clin Chim Acta. 2010;411:1875–82.
22. Bui QT, Prempeh M, Wilensky RL. Atherosclerotic plaque development. Int J Biochem Cell Biol. 2009;41:2109–13.
23. Lusis AJ. Atherosclerosis. Nature. 2000;407:233–41.
24. Assmann G, Gotto Jr AM. HDL cholesterol and protective factors in atherosclerosis. Circulation. 2004;109:III8–14.
25. Shih DM, Gu L, Xia YR, Navab M, Li WF, Hama S, et al. Mice lacking serum paraoxonase are susceptible to organophosphate toxicity and atherosclerosis. Nature. 1998;394:284–7.
26. Tward A, Xia YR, Wang XP, Shi YS, Park C, Castellani L, et al. Decreased atherosclerotic lesion formation in human serum paraoxonase transgenic mice. Circulation. 2002;106:484–90.
27. Mackness B, Mackness MI, Arrol S, Turkie W, Durrington PN. Effect of the human serum paraoxonase

55 and 192 genetic polymorphisms on the protection by high density lipoprotein against low density lipoprotein oxidative modification. FEBS Lett. 1998;423:57–60.
28. Gupta N, Gill K, Singh S. Paraoxonases: structure, gene polymorphism & role in coronary artery disease. Indian J Med Res. 2009;130:361–8.
29. Renault F, Chabriere E, Andrieu JP, Dublet B, Masson P, Rochu D. Tandem purification of two HDL-associated partner proteins in human plasma, paraoxonase (PON1) and phosphate binding protein (HPBP) using hydroxyapatite chromatography. J Chromatogr B Analyt Technol Biomed Life Sci. 2006;836:15–21.
30. Rochu D, Chabriere E, Renault F, Elias M, Clery-Barraud C, Masson P. Stabilization of the active form(s) of human paraoxonase by human phosphate-binding protein. Biochem Soc Trans. 2007;35:1616–20.
31. Rochu D, Renault F, Clery-Barraud C, Chabriere E, Masson P. Stability of highly purified human paraoxonase (PON1): association with human phosphate binding protein (HPBP) is essential for preserving its active conformation(s). Biochim Biophys Acta. 2007;1774:874–83.
32. Bookland MJ, Darbinian N, Weaver M, Amini S, Khalili K. Growth inhibition of malignant glioblastoma by DING protein. J Neurooncol. 2012;107:247–56.
33. Lesner A, Li Y, Nitkiewicz J, Li G, Kartvelishvili A, Kartvelishvili M, et al. A soluble factor secreted by an HIV-1-resistant cell line blocks transcription through inactivating the DNA-binding capacity of the NF-kappa B p65/p50 dimer. J Immunol. 2005;175:2548–54.
34. Monaco C, Andreakos E, Kiriakidis S, Mauri C, Bicknell C, Foxwell B, et al. Canonical pathway of nuclear factor kappa B activation selectively regulates proinflammatory and prothrombotic responses in human atherosclerosis. Proc Natl Acad Sci U S A. 2004;101:5634–9.
35. Darbinian-Sarkissian N, Darbinyan A, Otte J, Radhakrishnan S, Sawaya BE, Arzumanyan A, et al. p27(SJ), a novel protein in St John's Wort, that suppresses expression of HIV-1 genome. Gene Ther. 2006;13:288–95.
36. Akira S, Isshiki H, Sugita T, Tanabe O, Kinoshita S, Nishio Y, et al. A nuclear factor for IL-6 expression (NF-IL6) is a member of a C/EBP family. EMBO J. 1990;9:1897–906.