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Review Article

AN INSIGHT INTO THE MOLECULAR STRUCTURE AND FUNCTION OF POLYGALACTURONASE INHIBITING PROTEIN (PGIP)

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Abstract: Plants lack the system of circulating antibodies, thus defense mechanism in plants depends on the capability of recognition and interaction with the invading pathogenic microorganisms and neutralising their effect via specific interactions. The first barrier of defense in plants is the cell wall made up of pectin consisting of homogalacturonan, rhamnogalacturonan I and rhamnogalacturonan II. The major component of pectin is homogalacturonan. Pathogenic fungi secrete polygalacturonase (PG) to degrade the homopolygalacturonan of cell wall. Thus plants have pathogenesis-related protein in the cell wall to neutralise the effect of PG known as polygalacturonase inhibiting protein (PGIP). The interaction between PGIP and PG is very specific and effective and differs in different pathogenic fungi and plant species due to the components of plant system. The article presents a critical review on the molecular association of PGIP with PG in nature. An insight has been provided for the use of PGIP in the extracellular localization of mature proteins, in the inhibition of fungal infection, as an elicitor of immune response in plants with great economic and agricultural importance across the world.

Keywords: Polygalacturonase; Polygalacturonase inhibiting protein; *Phaseolus vulgaris*; Fungi

Introduction

Infections may occur in plants due to pathogens like bacteria, fungi, viruses, nematodes and insects. Plants successfully protect themselves from the attack of wide range of infectious microorganisms. Since they lack a system of circulating antibodies, plant defense depends on recognizing a pathogen eliciting appropriate defense response against it (Matteo *et al.*, 2003). Generally plants recognise pathogen associated molecular pattern (PAMP) using cell surface and intracellular receptors and elicit defense response against the invader (Federici *et al.*, 2006). Polygalacturonase inhibiting protein (PGIP) is present at the cell surface of many plants (Cervone *et al.*, 1997). PGIPs are members of the leucine-rich repeat (LRR) protein family that in plants play crucial roles in development, defense against pathogens and recognition of beneficial microbes. It is a pathogenesis related (PR) glycoprotein which can bind and inhibit polygalacturonase (PG) of the pathogens and thus prevents its entry into the plant cell (Federici *et al.*, 2006; Gomathi and Gnanamanickam, 2004).

Plant cell wall serves as the first barrier to invading microorganisms. It contains pectin made up of homogalacturonan (HGA), rhamnogalacturonan I and rhamnogalacturonan II. Polygalacturonase is a pectin lytic enzyme that can depolymerise HGA. Pathogens secrete polygalacturonase (PG) during the early stages of infection to depolymerise HGA and get entry into the host cell (De Vries and Visser, 2001). Endopolygalacturonase (EC 3.2.1.15) is an important cell wall degrading enzyme secreted by phytopathogenic fungi that cleaves the linkage between $_{D}$-galacturonic acid residues in

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homogalacturonan of cell wall (De Lorenzo and Ferrari, 2002). Degradation of plant cell wall by phytopathogenic fungi initially requires the action of polygalacturonase followed by other degrading enzymes. PGIP is present in plant cell wall and checks the entry of pathogenic fungi and other microorganism by interacting with polygalacturonase enzyme (De Lorenzo *et al.*, 2001).

Structure

PGIPs belong to a super-family of leucine-rich repeat (LRR) proteins (Kobe and Kajava, 2001). The LRR is a structural motif responsible for many protein-protein interactions because of its versatility in different recognition specificities. Proteins having this motif participate in many cellular functions like receptor dimerization, regulation of adhesion, domain repulsion and binding events (Buchanan and Gay, 1996). PGIP of molecular mass of 43 kDa has been purified from pear fruit and mass was decreased to 34 kDa by chemical deglycosylation (Stotz, 1993). Partial amino acid sequence of pear PGIP have been used to amplify a corresponding cDNA that encodes a 36.5 kDa polypeptide having a 24 amino acid signal sequence and 7 N-glycosylation sites (Stotz, 1993). The crystal structure of PGIP of *Phaseolus vulgaris* and the crystal structure of endopolygalacturonase from the phytopathogenic fungus *Fusarium moniliforme* has been solved and is shown in Figure 1 (Matteo *et al.*, 2003; Federici *et al.*, 2001). Table 1 represents the primary structure of the former.

The crystal structure of only PGIP 2 from *Phaseolus vulgaris* reveals a typical curved and elongated shape. Eight β strands (with one long β strand, B1, at the N-terminal end) comprise the inner concave face of the curved surface. On the opposite side of the β sheet, there are nine 3_{10} helices that are almost parallel to the β sheet. The concave surface is known to bear residues necessary for binding and recognition specificity in this class of protein (Matteo *et al.*, 2003).

The sequence of mature polygalacturonase from the phytopathogenic fungus *Fusarium moniliforme* (*Fm*PG) after the processing of the signal peptide (residues 1–24) includes 349 aa (residues 25–373). It consists of a right-handed parallel β helix (Yoder *et al.*, 1993), resulting from the tandem repetition of 10 coils, each formed by three or four β strands. The crystal structure of PGIP from *Phaseolus vulgaris* and endopolygalacturonase from the phytopathogenic fungus *Fusarium moniliforme* is represented in Figure 1.

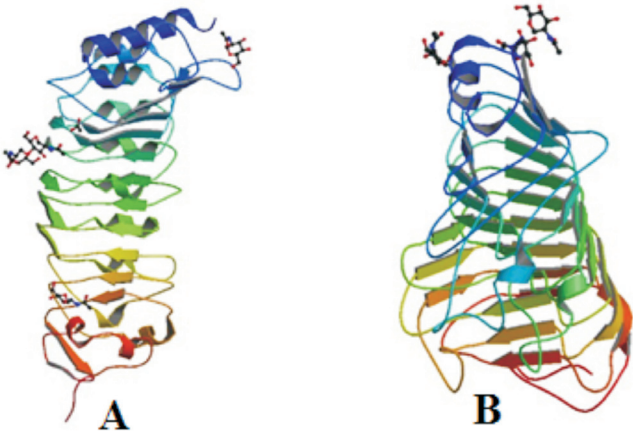

Figure 1: **The crystal structure of (A) PGIP of *Phaseolus vulgaris*, PDB ID: 1OGQ and (B) endopolygalacturonase from the phytopathogenic fungus *Fusarium moniliforme*, PDB ID: 1HG8. Jmol was used to depict the three-dimensional structure**

PGIP– PG Interaction

PGIP2 inhibits fungal PGs through the formation of bimolecular complexes, and the residues of PGIP2 critical for its affinity and recognition capability are located in sheet B1 (Leckie *et al.*, 1999). The interaction between the PG of *F. moniliforme* (*Fm*PG) and PGIP2 is mediated by at least two positively charged residues of the enzyme (Arg-267 and Lys-269), which are located at the edge of its active site and are involved in substrate binding. The involvement of these two residues in the interaction with PGIP2 provides an explanation for the competitive inhibition observed (Federici *et al.*, 2001). Examination of the electrostatic potential surface of PGIP2 reveals a negative pocket formed by the charged residues Asp-131, Asp-157, and Asp-203 and the polar residues Ser-133, Thr-155, and Thr-180, located approximately in the centre of sheet B1. Interestingly, the three aspartic residues are highly conserved in all PGIPs (De Lorenzo et al., 2001). The pocket is sufficiently large and deep to accommodate the positively charged residues Arg- 267 and Lys-269 on the surface of the enzyme

and may completely cover its active site, thus preventing access to the substrate. The residue Gln-224 of PGIP2, which is crucial for the specificity of the inhibitor toward *Fm*PG, is located in sheet B1 immediately above the negative pocket putatively involved in PG binding.

Table 1
Sequence information of PGIP of *Phaseolus vulgaris* (Toubart P *et al.*, 1992)

Gene	*pgip*
Amino acid	342
Signal peptide	1 to 29
Protein sequence ID (Uniprot ID)	P35334 (PGIP1_PHAVU)
Mature peptide	30 to 342
Protein sequence	1 mtqfnipvtm ssslsiilvi lvslrtalse lcnpqdkqal lqikkdlgnp ttlsswlptt61 dccnrtwlgv lcdtdtqtyr vnnldlsghn lpkpypipss lanlpylnfl yigginnlvg121 pippaiaklt qlhylyitht nvsgaipdfl sqiktlvtld fsynalsgtl ppsisslpnl181 ggitfdgnri sgaipdsygs fsklftamti srnrltgkip ptfanlnlaf vdlsrnmleg241 dasvlfgsdk ntkkihlakn slafdlgkvg lsknlngldl rnnriygtlp qgltqlkflq301 slnvsfnnlc geipqggnlk rfdvssyann kclcgsplps ct

The protein feature view of PGIP-2 of *Phaseolus vulgaris* has been shown in Figure 2.

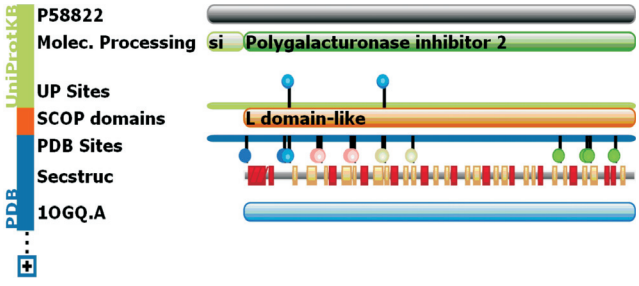

Figure 2: **Molecular description of PGIP-2 of *Phaseolus vulgaris* (From Protein Database)**

Mode of Action

PGIP is an innate defense protein present in cell wall of plants and secreted in the apoplast to block the cleavage of pectin of middle lamella by polygalacturonase of pathogenic bacteria and fungi (Roper *et al.*, 2007). PGs are produced by bacteria, fungi, nematodes and insects (De Lorenzo and Ferrari, 2002; Jaubert *et al.*, 2002; Girard and Jouanin, 1999) and their involvement in pathogenesis has been demonstrated for several fungi such as *Botrytis cinerea* (ten Have *et al.*, 1998; Kars *et al.*, 2005), *Aspergillus flavus* (Shieh *et al.*, 1997), *Alternaria citri* (Isshiki *et al.*, 2001), *Claviceps purpurea* (Oeser *et al.*, 2002) and *Sclerotinia sclerotiorum* (Li *et al.*, 2004) and bacteria such as *Ralstonia solanacearum* (Tans-Kersten *et al.*, 2001) and *Agrobacterium tumefaciens* (Rodriguez-Palenzuela *et al.*, 1991). PGs from salivary glands of phytophagous insects are considered a main cause of plant damage (Girard and Jouanin, 1999; Boyd *et al.*, 2002; Frati *et al.*, 2006). Many plants produce extracellular polygalacturonase-inhibiting proteins (PGIPs) that specifically recognize and inhibit fungal and insect PGs (De Lorenzo and Ferrari, 2002; D'Ovidio *et al.*, 2004). The PG–PGIP interaction limits the destructive potential of polygalacturonases and leads to the accumulation of elicitor active oligogalacturonides as demonstrated *in vitro*. These oligosaccharides may activate plant defence responses such as synthesis of phytoalexins, lignin and ethylene, expression of proteinase inhibitor I and b-1,3-glucanase and production of reactive oxygen species (Ridley *et al.*, 2001). Studies have shown that during infection, the gene encoding PGIP is significantly up-regulated and PG encoding gene is down regulated (Matteo *et al.*, 2006).

Specificity

The activities of PGIP from different plant are specific for different PGs of invading pathogenic fungi. Studies have shown that four *pgip* genes in *Phaseolus vulgaris* are responsible for diverse and recognition specific functions (Matteo *et al.*, 2003). Individual members of PGIP family are differentially regulated by separate transduction pathways (Ferrari *et al.*, 2003). PGIPs from different or same plant differ in their inhibitory activity towards the same PG (Maulik *et al.*, 2009).

PGIP of several plants form specific complex with PG of pathogenic fungus and help in the accumulation of oligogalacturonides to elicit plant defense response (Cervone *et al.*, 1997). The four PGIPs of *Phaseolis vulgaris,* designated PGIP1-4,

have varied but overlapping specificities for different polygalacturonases (PGs) (D'Ovidio *et al.*, 2004). PGIP-1 inhibits polygalacturonases from *A. niger*, whereas PGIP-2 inhibits polygalacturonases from both *A. niger* and *F. moniliforme* (Leckie *et al.*, 1999). Thus PGIP-1 is monospecipic while PGIP-2 is dual specific in action (Table 2). Studies have shown that when the glutamine at position 253 of the PGIP-2 sequence is mutated to a lysine, the dual specificity of PGIP-2 is lost. Conversely, the monospecific PGIP-1 can be converted to dual specificity by replacement of the lysine at position 253 by glutamine (Matton *et al.*, 1999).

To accommodate pathogenesis to different environmental conditions and on various hosts, fungi produce PG isoenzymes variable in terms of sequence, specific activity, pH optimum and substrate preference (De Lorenzo *et al.*, 2001; Poinssot *et al.*, 2003). Conversely, plants have evolved PGIPs with different recognition specificities (Table 2) encoded by differentially regulated pgip genes (De Lorenzo and Ferrari, 2002; Ferrari *et al.*, 2003). Also plants produce PGs that play a role in the cell wall development (Torki *et al.*, 2000) but these PGs do not interact with PGIPs, suggesting that the inhibitors are specialized for plant defence (Federici *et al.*, 2001).

Table 2
Specificity of PGIPs from plants for varying PG of pathogens

Plant PGIP	*Similarity*	*Action*	*Fungal PG*	*Reference*
PGIP1 from *Phaseolus vulgaris*	99% with PGIP2	Cannot inhibit	PG from *Fusarium moniliforme*	Maulik *et al.*, 2009
PGIP-1 from *Phaseolus vulgaris*	Differs by 8 amino acids from PGIP-2 of bean	Inhibits	PG from *Aspergillus niger*	Leckie *et al.*, 1999
PGIP2 from *Phaseolus vulgaris*	-	Inhibits	PG from *Fusarium moniliforme*	Maulik *et al.*, 2009
PGIP-2 from *Phaseolus vulgaris*	-	Inhibits	PG from both *Aspergillus niger* and *Fusarium moniliforme*	Leckie *et al.*, 1999
PGIP3 from Glycinemax	Less than 88% similar with PGIP2	Inhibits	PG from *Fusarium moniliforme*	Maulik *et al.*, 2009
Transgenic *Arabidopsis* plants over-expressing PGIPs	-	Inhibits	PG from *Botrytis cinerea*	Matteo *et al.*, 2006

Interaction of PGIP and PG in Nature

1. PGIP gene can be utilized for the secretion and accumulation of mature proteins outside the cell. In a study, the leader sequence of PGIP gene (from *Phaseolus vulgaris*) was joined upstream of the DNA fragment coding for C-terminal peptide LL-37 of the human Cathelicidin antimicrobial protein for the extracellular localization of the mature protein to enhance the disease resistance in Chinese cabbage. For this the 87 base pair signal sequence for PGIP peptide has been amplified with primers containing restriction sites *BamHI* and *SacI* using PCR for *Agrobacterium* transformation (Jung *et al.*, 2012).
2. PGIPs from tomato have shown to inhibit polygalacturonides from *Ralstonia solanacearum* (Schacht *et al.*, 2011).
3. A *Phaseolus vulgaris* gene encoding a polygalacturonase-inhibiting protein (PGIP) antagonistic to pathogen polygalacturonase can protect transgenic tobacco against oomycetes (*P. parasitica* and *Peronospora hyoscyami*) (Borras-Hidalgo *et al.*, 2012).
4. The *in vitro* inhibition of the hydrolysis of polygalacturonic acid by a plant cell wall-

associated, proteinaceous inhibitor PGIP results in prolonged existence of oligogalacturonides large enough to act as elicitors. This suggests that the inhibitor plays a role in the resistance of plants to fungal pathogens. It has been hypothesized that high-level, constitutive production of the inhibitor in transgenic plants may render these plants fungus resistant (Toubart *et al.*, 1992).

5. Soybean (*Glycine max* (L.) Merr.) is a legume crop of great economic and agricultural importance across the world (Chai *et al.*, 2013; Chaudhary and Dantu, 2011; Kumar and Kayastha, 2010). Soybean yields are significantly reduced due to *Phytophthora* root and stem rot caused by *Phytophthora sojae*. The disease leads to 1-2 billon dollars in damage globally every year (Tyler *et al.*, 2007). PGIP antagonistic to the pathogen may protect the Soybean plant against infection. Constitutive expression of *Vitis vinifera* polygalacturonase-inhibiting protein 1 (Vvpgip1) has been shown to protect tobacco plants against *Botrytis cinerea*. Evidence points to additional roles for VvPGIP1, beyond the classical endopolygalacturonase (ePG) inhibition mechanism, in providing protection against fungal infection (Ona *et al.*, 2013).
6. Although PGIPs are not classified as PRs, their expression can also be induced by both biotic (phytopathogenic fungi and insects) and abiotic (wounding, phytohormones) elicitors, and PGIPs play an active role in plant defense (Li *et al.*, 2003).

Conclusion

Polygalacturonase inhibitor protein present in the cell wall of plants serve as a defense protein to neutralize the damaging effect of degrading enzyme polygalacturonase secreted by phytopathogenic fungi and bacteria. Structure-function analysis has shown that PGIP is very specific in its action against the fungal PG. The structure of PGIP from *Phaseolus vulgaris* is fully characterized and even a single amino acid change in PGIP structure may alter its function. Also the action of PGIP differs from plant of different or same species towards the PG of different phytopathogenic microorganisms.

Abbreviation

PGIP: Polygacturonase inhibitor protein; PG: Polygalacturonse; HGA: homogalacturonan; LRR: leucine-rich repeat.

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