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Review

Plant cell walls: Protecting the barrier from degradation by microbial enzymes

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ABSTRACT

Plant cell walls are predominantly composed of polysaccharides, which are connected in a strong, yet resilient network. They determine the size and shape of plant cells and form the interface between the cell and its often hostile environment. To penetrate the cell wall and thus infect plants, most phytopathogens secrete numerous cell wall degrading enzymes. Conversely, as a first line of defense, plant cell walls contain an array of inhibitors of these enzymes. Scientific knowledge on these inhibitors significantly progressed in the past years and this review is meant to give a comprehensive overview of plant inhibitors against microbial cell wall degrading enzymes and their role in plant protection.

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1. Introduction

1.1. The plant cell wall: not only about size and shape

The most visible role of plant cell walls is to determine the size and shape of cells. By differentially resisting and yielding to turgor pressure, the plant cell wall prescribes the growth and shape of the cells [1]. It is also the first obstacle that a pathogen needs to overcome in order to penetrate the plant cell. As the plant cell wall is such an important interface between the plant and the pathogen, millions of years of coevolution have led to plants developing various strategies to protect their cell walls and to pathogens developing ways to circumvent these strategies.

Pathogens secrete numerous cell wall degrading enzymes to breach the plant cell wall and use it as a source of nutrients. As a first line of defense, plant cell walls contain several inhibitors to specifically block the activity of these microbial enzymes. The pathogen attack can be recognized by the plant through the detection of elicitors, such as hydrolyzed plant cell wall components, pathogen cell wall components and microbial enzymes, among which the plant cell wall degrading enzymes themselves [2]. Upon pathogen detection, plant cells react in several ways, ranging from the production of pathogenesis-related proteins, such as microbial cell wall hydrolases and proteases [3], to cell death [4]. Pathogens can counteract by the production of inhibitors of hydrolases and proteases and the use of proteins that interfere with the plant response,

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which in turn can be detected by the plant and result in even more defense responses. Above these attack and counterattack strategies, pathogens and plants adapt their 'arms' in a constant hide-and-seek battle.

This review specifically addresses the current knowledge on the first line of defense in the plant cell wall: how plants protect their cell walls against degradation by microbial enzymes. Excellent reviews have been written on other aspects of plant defense, such as the general outline of the plant immune system [5], plant defense proteins [6] and the influence of plant cell wall composition on pathogen vulnerability [7].

1.2. Composition of the barrier

The primary cell wall is composed of approximately 10% proteins and 90% polysaccharides, which can be divided into three groups: cellulose, hemicellulose and pectin [8]. While the composition of polysaccharides can vary among species, most notably in grasses [9], typical values are approximately 30% cellulose, 30% hemicellulose and 35% pectin [10].

Cellulose is a linear polymer of (1–4)-linked β-D-glucose and in the cell wall several cellulose chains are packed in ordered, crystalline aggregates (microfibrils) [11]. Microfibrils are deposited around each cell in a random framework and interlocked by hemicelluloses, leading to a strong yet resilient network [12,13]. The most abundant hemicellulose in dicots is xyloglucan, a linear backbone of (1–4)-linked β-D-glucose substituted at regular sites with D-xylosyl residues that can be further extended by galactosyl, fucosyl or arabinosyl residues [8,14]. The major hemicellulose in monocots is xylan, which is a polymer of (1–4)-linked β-D-xylose [15]. This backbone can be decorated with various substituents, including arabinose, glucuronic acid, 4-*O*-methylglucuronic acid and acetyl side groups. The arabinosyl residues can also be esterified with ferulic and *p*-coumaric acid [16,17].

The cellulose/hemicellulose network is embedded in a jelly-like matrix of pectin, the most complex class of wall polysaccharides. The most abundant and best studied pectic polysaccharides are homogalacturonan, rhamnogalacturonan-I and rhamnogalacturonan-II [18]. Homogalacturonan is a linear chain of (1–4)-linked α-D-galacturonic acid residues in which some of the carboxyl groups are methylesterified. The backbone of rhamnogalacturonan-I is composed of alternating (1–2)-linked α-L-rhamnosyl and (1–4)-linked α-D-galactosyluronic acid residues [19]. 20–80% of the rhamnosyl residues have attached side chains, predominantly linear and branched chains of arabinosyl and galactosyl residues [20,21]. Rhamnogalacturonan-II has a backbone of (1–4)-linked α-D-galactosyluronic acid residues that is substituted with four different side chains [22–24]. The side chains contain 12 different glycosyl residues, among which some very rare monosaccharides such as 2-*O*-methyl-L-fucose, 2-*O*-methyl-D-xylose, apiose, aceric acid, Kdo and Dha. Rhamnogalacturonan-II exists in the primary wall as a dimer that is covalently cross-linked by a borate ester [25]. Despite the complexity of this polysaccharide, its structure is highly conserved among plants [22,25].

2. Pectin degradation and defense

Pectin degrading enzymes weaken the plant cell wall and expose other polymers to degradation by hemicellulases and cellulases. They are the first cell wall degrading enzymes that are secreted by pathogens [26–28] and are important virulence factors [29–34]. Among the best known microbial pectic enzymes are polygalacturonases, pectate lyases, pectin lyases and pectin methylesterases. Polygalacturonases (E.C. 3.2.1.15) hydrolyze the linkage between galacturonic acids in unmethylated homogalacturonan. Pectate (E.C. 4.2.2.2) and pectin lyases (E.C. 4.2.2.10) cleave the same linkage, but cleavage occurs via a β-elimination reaction resulting in the formation of an unsaturated C4–C5 bond at the non-reducing end of the cleaved polysaccharide. Pectate lyases are specific for unmethylated substrates, while pectin lyases degrade the methylated forms. Pectin methylesterases catalyze the demethylesterification of homogalacturonan, thereby making the pectin available for degradation by polygalacturonases and pectate lyases. Remarkably, all pectic enzymes share a similar structure, i.e. a parallel β-helix motif [35,36].

2.1. Polygalacturonase-inhibiting proteins

The first report on a polygalacturonases-inhibiting protein (PGIP) was published almost 40 years ago [37] and since then PGIPs have been found in species throughout the plant kingdom, both in monocots and dicots [38]. They are effective against fungal polygalacturonases (PGs), but do not inhibit bacterial or plant PGs [39]. Some PGIPs also show weak inhibition against insect PGs [40,41]. PGs are secreted by almost all phytopathogens and a wide variety of isoenzymes exist. In turn, many plants have evolved multiple PGIP isoforms with different recognition specificities. For example, *Phaseolus vulgaris* cv. Pinto contains 4 PGIPs (PvPGIP1 to 4) and while *Botrytis cinerea* PG (BcPG) is inhibited by all 4 PGIPs, *Aspergillus niger* PG (AnPG) is inhibited only by PvPGIP1, 2 and 4 and only PvPGIP2 is effective against *Fusarium moniliforme* PG (FmPG) [40,42].

PGIPs belong to the superfamily of leucine-rich repeat (LRR) proteins, the structure of which is specialized for protein–protein interactions [43,44]. The consensus Lt/sGxIP in the repeating sequence puts PGIPs in the family of plant-specific LRRs, which mediate plant resistance to pathogen attack [45,46]. The crystal structure of PvPGIP2 shows a curved and elongated shape [47]. A long parallel β-sheet occupies the concave inner side, a regular array of short 3_{10} helices forms the convex face and an additional β-sheet is located between the two faces. The concave inner face has the highest probability to be involved in protein–protein interactions [48] and contains a negatively charged pocket [49]. In the interaction with FmPG, this pocket is thought to accommodate two positively charged enzyme surface residues (Arg267 and Lys269) [49]. As these residues are located at the edge of the active site, PvPGIP2 binding prevents access to the substrate, explaining the observed competitive inhibition of FmPG [50]. In contrast to FmPG, AnPG and *Colletotrichum lupini* PG are inhibited non-competitively by PvPGIP2 [51,52]. This is explained by a docking model that shows a different orientation for the AnPG-PvPGIP2-complex, leaving the active site accessible to the substrate [48]. Inhibition of BcPG occurs in a mixed mode, thus presenting a third mode of inhibition by PvPGIP2 [53]. While a docking model again shows enzyme binding at the convex face of the inhibitor, the active site of the enzyme is only partially covered, thereby still allowing substrate binding, but at decreased affinities [53].

Between the two β-sheets of PvPGIP2 is a positively charged patch. D'Ovidio et al. [49] noted that this patch consists of a cluster of regularly spaced Arg and Lys residues protruding in the solvent and creating a regular distribution of charges that resembles the one predicted for the pectate binding site in the apoplastic peroxidase APRX [54]. The interaction of these clustered residues with the negatively charged motif of homogalacturonan was confirmed later by site-directed mutagenesis [55]. Binding of PGIP to pectin is displaced *in vitro* by PGs, probably due to the close proximity of the two binding sites [49,55].

A stable interaction between PG and PGIP likely requires only one or very few strong contacts that "lock" the complex besides a network of multiple and relatively weak contacts [56]. In PvPGIP2,