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THE BIOCHEMISTRY AND STRUCTURAL BIOLOGY OF PLANT CELL WALL DECONSTRUCTIO

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ABSTRACT:

The cell walls of plants are the most abundant source of organic carbon on the planet. This photosynthetically fixed carbon is recycled by microbial enzymes that convert cell wall polysaccharides to monosaccharides and oligosaccharides, a process that is of biological and industrial importance. Plant cell walls are recalcitrant to biological depolymerization, as the extensive interactions between polysaccharides, and between polysaccharides and lignin, restrict access to the battery of microbial glycoside hydrolases, pectate lyases, and esterases that break down these composite structures. Since the early 1990s, there has been an explosion of structural information on both the catalytic and noncatalytic components of these enzymes. This review will provide an overview/update of the structure-function relationships of the enzymes that catalyze plant cell wall deconstruction. This review paper can also serve as a ready source of literature review for reaserchers and students involved in life science and Biochemistry. The aim of this paper is to review the Plant Cell Wall Deconstruction and the importance of the cell wall in the biochemistry and structural biology.

KEY WORD: *Plants, Cell Wall, Biochemistry.*

INTRODUCTION:

The plant cell wall

Plant cell walls are composed predominantly of the polysaccharides cellulose, hemicellulose, and pectin, although secondary walls are often rigidified by the impregnation of lignin, a heterogenous aromatic polymer. The structure of the plant cell has been

extensively reviewed previously and will be described briefly here (for an overview of plant cell wall structure.

Cellulose is a β-1,4-linked Glc molecule that is substantially crystalline. All hemicellulosic polysaccharides contain a β-linked sugar backbone. In xylans, mannans, and xyloglucans, the backbone sugars are β-1,4-d-Xyl, β-1,4-d-Man, and β-1,4-d-Glc, respectively, while in glucomannan, the backbone consists of randomly dispersed β-1,4-Glc and β-1,4-Man sugars. The backbones of hemicellulosic polysaccharides are decorated with a variety of sugars and acetyl groups, explaining why these polymers are not crystalline. There are three major forms of pectin: homogalacturonan, rhamnogalacturonan I, and rhamnogalacturonan II . Homogalacturonan consists of a polygalacturonic acid backbone . Rhamnogalacturonan I displays a backbone composed of an alternating disaccharide, $[(\alpha\text{-}1,4)\text{-d-GalA}\rightarrow(\alpha\text{-}1,2)\text{-l-Rha}]_n$, that contains extensive decorations at the *O4* of the Rha residues. Rhamnogalacturonan II is the most structurally complex of the three pectic polysaccharides, consisting of 13 different sugars and over 20 different linkages.

Cazy:

Enzymes that modify complex carbohydrates, together with their accessory noncatalytic carbohydrate-binding modules (CBMs), have been grouped into sequence-based families on the continuously updated Carbohydrate-Active EnZymes (CAZy) database Members of the same enzyme family display a common fold, while the catalytic apparatus and mechanism are similarly conserved. Currently, 44 of the 115 glycoside hydrolase families (GHs) contain enzymes that contribute to plant cell wall deconstruction. Crystal structures of relevant enzymes in 41 of these 44 GHs have been reported. With respect to polysaccharide lyase families (PLs) and carbohydrate esterase families (CEs), six out of 21 PLs and 11 out of 16 CEs contain enzymes that play a role in plant cell wall metabolism. Of the 59 CBM families, around half of these modules bind to components of the plant cell wall, and structural information is available for all but three of these families.

Mechanisam of plant cell wall deconstruction:

The vast majority of glycoside hydrolases cleave glycosidic bonds by either a single or double displacement mechanism, which leads to inversion or retention of anomeric configuration, respectively. Polysaccharide lyases cleave their scissile bond through a β-elimination mechanism. While carbohydrate esterases generally hydrolyze ester linkages through a double displacement mechanism in which or Asp in CE8 functions as the catalytic nucleophile, exceptions to this mode of action are apparent in CE4, where catalysis is metal dependent.

Catalytic modules of glycoside hydrolases

Currently, the crystal structure of the catalytic modules of representatives of nearly all the relevant GHs, PLs, CEs, and CBM families, which contribute to plant cell wall deconstruction, have been reported Some structural folds have given rise to a myriad of enzymes that display significant differences in specificity, exemplified by the GHs located in clan GH-A. Members of this clan display a $(\beta/\alpha)_8$-fold in which the catalytic residues are presented at the C terminus of β-strands 4 and 7 While the enzymes all hydrolyze an equatorial glycosidic bond, their mode of action (exo and endo), specificity for the sugar at the catalytic −1 subsite and more distal regions of the substrate-binding region (Xyl, Man, Glc, Ara*f*, Gal), and the linkage cleaved (e.g. β-1,4, β-1,3) vary between enzymes The same enzyme activity can often be found in multiple GHs, located in distinct clans, as a consequence of convergent evolution For example, cellulases are located in 11 GHs, with seven of these families distributed across four different clans, while four of these GHs currently are not linked to a clan. There have been several reviews on the three-dimensional structure of the catalytic modules of glycoside hydrolases, including plant cell wall-degrading enzymes. Therefore, this *Update* will provide a brief overview of the structures of these enzymes and a more detailed description of recent structural information.

Cellulesses:

Cellulose utilization is believed to be mediated by endo-β-1,4-glucanases, cellobiohydrolases (also called exo-β-1,4-glucanases), and β-glucosidases. Classically, cellulose hydrolysis, of which the *Hypocrea jecorina* (formerly *Trichoderma reesei*) system is the archetype, is viewed as a synergistic process; endo-acting cellulases create new ends from which the exo-acting cellobiohydrolases can release cellobiose from either the reducing (GH7 and GH48) or nonreducing (GH6) end of the cellulose chains . This model, however, is inconsistent with several features of cellulose degradative systems. Thus, biochemical and structural data indicate that GH6 cellobiohydrolases are not, exclusively, exo acting. Furthermore, some highly active cellulase systems lack a classic pair of cellobiohydrolases that act from the reducing and nonreducing ends of cellulose chains, respectively. Indeed, one of the most distinctive features of the cellulose-degrading bacterium, *Cytophaga hutchinsonii*, is the absence of GH6, GH48, or GH7 cellobiohydrolases , although it is possible that the bacterium contains novel cellobiohydrolases. An intriguing report by showed that a single endo-processive GH9 cellulase was essential for cellulose degradation in *Clostridium phytofermentans*. Given the redundancy in cellulase systems, demonstration that a single enzyme is essential for a functional degradative system is rare and questions the classical

synergy model. While there now does not appear to be a single unifying model for cellulose hydrolysis, recent studies, deploying atomic force microscopy to visualize the movement of cellulase molecules on its crystalline substrate, will likely provide novel insights into the mechanism by which these enzymes function

Xyloglucan:

The *β*-1,4-glucan backbone of xyloglucan is hydrolyzed by specific endoglucanases (i.e. endo-xyloglucanase or xyloglucan endo-hydrolases) from GH5, GH7, GH12, GH16, GH44, and GH74. GH12 enzymes can tolerate the side chains in xyloglucan. Indeed, GH5 and GH74 endoxyloglucanases can make productive interactions with the *α*-1,6-Xyl decorations and, in the case of the GH5 enzymes, Gal pendants of the Xyl residues Maybe the most interesting aspect of xyloglucan modification is found in GH16, where enzymes may display endoxyloglucanase activity or, in the case of XETs, remodel the structure of the polysaccharide through transglycosylation reactions. This article will not discuss these GH16 enzymes, which are covered in detail in the review by Eklöf .

Xylan degradation:

The xylan backbone is hydrolyzed primarily by GH10 and GH11 xylanases, while the Ara*f* side chains are removed by arabinofuranosidases from GH43, GH51, GH54, and GH62 (for review of xylan degradation. The uronic side chains are released from the nonreducing end of xylooligosaccharides by GH67 *α*-glucuronidases although recent data showed that GH115 *α*-glucuronidases remove the uronic acid decorations from the internal regions of xylan. Each of these families contains at least one structural representative, with the exception of GH62 and GH115 GH43 enzymes may display the highest level of substrate diversity, exemplified by the activity of two arabinofuranosidases from this family that remove the *O3* side chain from Xyl residues that are decorated at both *O2* and *O3* with Ara*f*. The crystal structure of this enzyme (H.J. Gilbert, unpublished data) reveals an extended substrate-binding pocket that interacts with both *O2*- and *O3*-linked Ara*f*. By contrast, an arabinoxylan-specific GH43 arabinofuranosidase, which removes *O2*- or *O3*-linked Ara*f* side chains from singularly substituted Xyl residues, contains a small substrate-binding pocket embedded in a shallow cleft that is optimized to bind the 3-fold helical structure of the xylan back. Recent protein crystallographic studies have shown that xylan side chains can be accommodated and can actually be exploited as specificity determinants while a GH5 xylanase displays an absolute requirement for 4-*O*-methyl-d-GlcUA appended to the Xyl positioned at the −2 subsite There are two structures of this enzyme however, the mechanism by which the enzyme recognizes the uronic acid side chain remains unclear.

Pectin degradation:

The structures of pectinases (polygalacturonases), pectate lyases, and pectin methylesterases have been extensively described and reviewed previously In general, these enzymes display a right-handed parallel β-helix topology. Exceptions include PL10 pectate lyases, which adopt an $(\alpha/\alpha)_6$ toroid conforand PL2 lyases, which display a $(\alpha/\alpha)_7$ barrel and utilize manganese rather than calcium in the active site. The catalytic apparatus in PL10, and those displaying a β-helix fold, is conserved, providing an example of convergent evolution. An Arg is the most likely candidate catalytic base in these PLs. The basic residue abstracts the C5 proton, which, in several PL families (PL2, PL9, and PL10), results in the formation of an enolate-enolate intermediate in which the two negatively charged oxygens are stabilized by calcium and hydrogen bonds. The collapse of the intermediate results in the cleavage of the scissile bond, although the mechanism by which the leaving group (glycosidic oxygen) is protonated remains unclear. An interesting variation of this catalytic mechanism has been proposed for PL1 lyases. It was suggested that the PL1 lyase generates an enol-enolate through donation of a proton by a nearby Lys to one of the oxygen atoms of the carboxylate. The authors suggest that through this intermediate, PL1 lyases are more active than PL10 and PL9 enzymes that can only generate the enolate-enolate intermediate.

Recent advances have also been made in understanding the processive mechanism displayed by pectin methyl esterases, which yield blocks of nonmethylated GalUA (GalA). Structural and biochemical data show that the enzyme demethylates the sugar at the +1 subsite and uses the negative charge of the carboxylate as a specificity determinant at the −1 subsite and to some extent at −2, while +3 makes hydrophobic contact with the methyl group of the esterified uronic acid. Thus, after removing the methyl group, the GalA generated then slides along the substrate-binding cleft to occupy the −1 site; thus, a new methylated GalA is presented in the crucial +1 subsite. This progressive sliding of pectin along the substrate-binding cleft is encouraged further by the specific content.

DISCUSSION:

Future perspectives:

In the last 15 years, there have been significant advances in the three-dimensional structural analysis of plant cell wall-degrading enzymes. The data have informed our understanding of the mechanism of both catalysis and substrate recognition, which has led to the identification of numerous “specificity motifs,” some of which are described in this article.

It is evident, however, that the explosion of genomic and metagenomic information is resulting in an exponential increase in the identification of CAZy enzymes. This has resulted in a significant imbalance between the number of enzymes in CAZy families and the biochemical/structural analysis of these proteins. Indeed, only around 3% of the proteins in CAZy have a characterized biochemical activity, while three-dimensional structural information is only available for 0.3% of these enzymes. It is estimated that we can safely predict the activities of no more than 20% of the proteins within CAZy. The situation is compounded further by the difficulties in determining the biochemical properties of plant cell wall-degrading enzymes, where the chemical complexity and requirement for a hierarchical degradative process create significant functional barriers. Notwithstanding these problems, continued biochemical and structural information is urgently required if we are to fully integrate information obtained from the "omics" technologies to understand the biology of plant cell wall deconstruction. Indeed, integrating structure, function, and phylogenetics to develop predictive models for ligand/substrate specificity is an important goal for structural biologists working on plant cell wall-modifying enzymes. An example of such an analysis was developed recently by Deploying CBM6 as a model system, they were able to identify two regions that appear to be "hot spots" of primary and tertiary structure variation, which confer functional specificity in these modules, a view supported by the recent characterization of a Xyl-specific CBM6. A more general phylogenetic analysis of endoglucanases belonging to several GHs was also insightful in providing a predictive platform for glycoside hydrolase activities. As discussed above, the characterization of glycosyltransferases that catalyze the synthesis of plant structural polysaccharides represents the biggest challenge in the cell wall field. It is evident that a significant investment is required to develop our understanding of the structure-function relationships of these enzymes, which is essential if we are to fully understand the mechanism for the biogenesis of the plant cell wall.

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