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Uronic polysaccharide degrading enzymes

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In the past several years progress has been made in the field of structure and function of polysaccharide lyases (PLs). The number of classified polysaccharide lyase families has increased to 23 and more detailed analysis has allowed the identification of more closely related subfamilies, leading to stronger correlation between each subfamily and a unique substrate. The number of as yet unclassified polysaccharide lyases has also increased and we expect that sequencing projects will allow many of these unclassified sequences to emerge as new families. The progress in structural analysis of PLs has led to having at least one representative structure for each of the families and for two unclassified enzymes. The newly determined structures have folds observed previously in other PL families and their catalytic mechanisms follow either metal-assisted or Tyr/His mechanisms characteristic for other PL enzymes. Comparison of PLs with glycoside hydrolases (GHs) shows several folds common to both classes but only for the β-helix fold is there strong indication of divergent evolution from a common ancestor. Analysis of bacterial genomes identified gene clusters containing multiple polysaccharide cleaving enzymes, the Polysaccharides Utilization Loci (PULs), and their gene complement suggests that they are organized to process completely a specific polysaccharide.

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Introduction

Alongside nucleic acids, lipids and proteins, carbohydrates are the essential building blocks of living organisms. They are present in all three kingdoms of life and the chemical variety of their monosaccharides by far exceeds that of the standard amino acids, notwithstanding the multitude of ways they are joined into oligo-saccharide or polysaccharide chains [1]. Glycans are involved in a wide range of biological activities, such as cell and tissue architecture, immunity, metabolism and pathogenesis. The structural variety of the oligosaccharides derives from the diversity of enzymes dedicated by various organisms to their synthesis and degradation [1,2]. Significant efforts led to the identification of *C*arbohydrate *A*ctive en*Zy*mes (CAZymes) in all sequenced genomes and their classification into families based on sequence similarities (www.cazy.org) [3,4,5••].

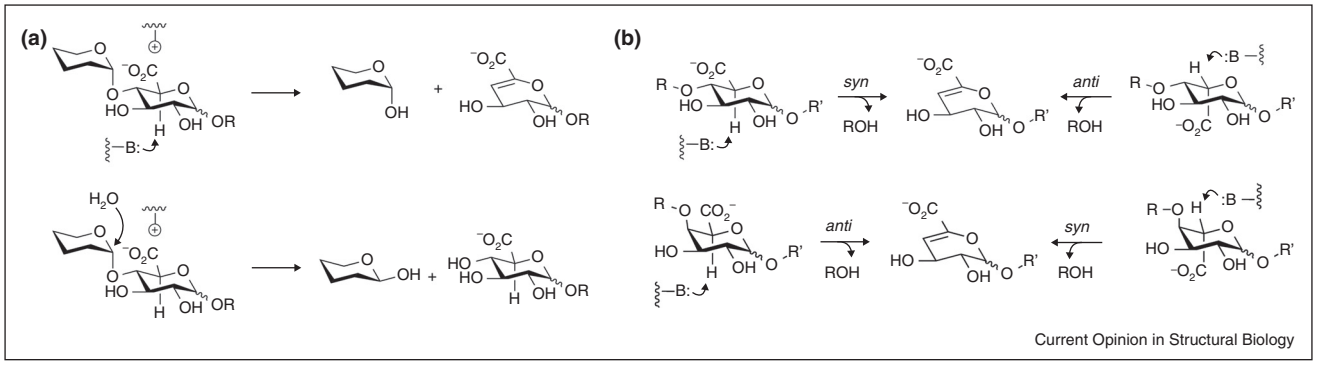
Two kinds of enzymes are involved in polysaccharide degradation that break the *O*-linkage (C1-O-Cn) between two sugars, namely glycoside hydrolases (GHs) and polysaccharide lyases (PLs). GHs utilize a water molecule to break the anomeric C1–O bond (Figure 1) and are presently classified into 133 families and form the most abundant group of CAZymes. PLs are specific to the polysaccharides containing uronic acid and break the O–C4 bond to the uronic acid employing a β-elimination mechanism, which yields a 4,5-unsaturated sugar at the new non-reducing end of the product (Figure 1a). There are presently 23 PL families within the CAZy database, significantly fewer than the GH families, likely reflecting the smaller range of substrates. Two general enzymatic mechanisms were identified in PLs: the metal-assisted mechanism predominant in pectin degradation, and the histidine/tyrosine mechanism used by enzymes families involved in *syn* and/or *anti* β-elimination (Figure 1b) [6••].

Previously, we discussed the relationship between the structural fold, substrate specificity and catalytic mechanism of the 21 PL families identified at that time [6••]. Known PL structures adopted only six folds, with two families having no structural representatives. There is now at least one structural model for each of the 23 PL families. These new structures provided insight into the protein fold for family PL12 (heparinase III), PL17 (alginate lyases), PL22 (oligo-galacturonate lyase) and PL23 (viral chondroitin lyases). Moreover, the enzymatic mechanisms of PL4 and PL22 are now partially elucidated. Here we review the recent structural and functional data on PL enzymes and compare them with uronic polysaccharide hydrolases to find possible evolutionary relationships between GHs and PLs. Finally, we explore the new lyase activities identified among the 'non classified' lyases in CAZy database.

The subfamily classification of PLs

A more comprehensive sequence analysis, which incorporated maximum likelihood phylogenetic analysis was

Figure 1



Action of polysaccharide lyases and glycoside hydrolyses. **(a)** The hydrolase and lyase catalyzed breakdown of hexose-uronate disaccharide. Cleavage resulting via elimination severs the C4–O bond of the glucuronyl residue and introduces a double bond into the +1 ring. Cleavage resulting from glycoside hydrolysis severs the C1–O bond of the adjacent (−1) sugar residue; an inverting glycosidase reaction is shown; R = H or another sugar residue. **(b)** Elimination from D-glucoronide and L-iduronide residues proceeds via *syn* and *anti*-pathways, respectively, R = another sugar residue, R′ = H or another sugar residue.

applied to the sequences of PLs and led to the subdivision of the families into 61 subfamilies (Table S1). This new hierarchical classification demonstrated better correlation between the subfamily and the substrate specificity; 90% of the subfamilies for which functional data are now available appear to be mono-specific [5••,7••]. In this extended classification many PL subfamilies have no functionally or structurally characterized members and it is expected that novel specificities will be discovered and correlated with their structures.

Modularity and cooperativity of PLs

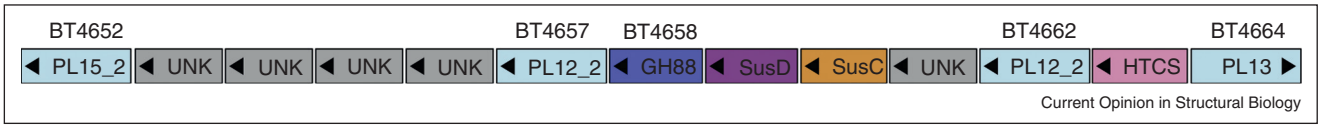
As with other CAZymes, PLs are modular enzymes frequently associated with Carbohydrate Binding Modules (CBMs). CBMs are small domains involved in glycan recognition and interaction [8]. Several of the 69 classified CBM families have been found associated with PLs but no CBM family is associated exclusively with PLs. CBMs perform diverse functions related to substrate recognition [9,10•], yet the role of CBMs in PL function is poorly understood. The prediction of the CBMs specificity is crucial for determining the role of the CBM and function of its associated enzyme [11•]. Structures of several PLs with additional domains have been determined but none with CBMs. Poly-functional PLs, are also known, where the lyase domain is associated with another enzymatic domain, often with complementary activities. For example, in *Saccharophagus degradans* 2–40, three putative alginate lyase domains (PL6_3, PL7_5 and PL6_3) are associated together within one polypeptide chain (GenBank accession number ABD82130.1).

Another level of organization was found in *Bacteroidetes* where genes required for full degradation of specific substrates form clusters named Polysaccharides Utilization Loci (PULs) (Figure 2) [12]. Approximately 90 PULs were identified in *Bacteroides thetaiotaomicron* [13] and 118 *Bacteroides cellulosilyticus* WH2 [14]. Gene clustering of sugar-cleaving enzymes have also been observed in *Firmicutes* [15••]. Although some PULs contain CAZymes with known activities, others contain unknown CAZymes. Participation of an unknown CAZyme in a specific PUL might help to identify its substrate.

Galacturonan lyases

Pectin forms a complex network made of galacturonan and other sugars, which together with cellulose and hemi-cellulose are the main components of plant cell walls [16]. Six PL families are involved in the degradation of the poly-galacturonan regions of pectin and are classified as pectate lyases (PL1, PL2, PL3, PL9, PL10) or oligo-galacturonan lyases (PL22). The other families

Figure 2



An example of a PUL. Heparin PUL from *Bacteroides thetaiotaomicron.* UNK are hypothetical proteins or non-CAZymes. Arrows indicate gene orientation. HTCS is Hybrid Two-Component System regulator. BTNNNN are locus tag identification.