Enzyme Engineering & Structural Biology - Research outputs - DTU Orbit (11/10/2019)

A carbohydrate-binding family 48 module enables feruloyl esterase action on polymeric arabinoxylan

Feruloyl esterases (EC 3.1.1.73), belonging to carbohydrate esterase family 1 (CE1), hydrolyze ester bonds between ferulic acid (FA) and arabinose moieties in arabinoxylans. Recently, some CE1 enzymes identified in metagenomics studies have been predicted to contain a family 48 carbohydrate-binding module (CBM48), a CBM family associated with starch binding. Two of these CE1s, wtsFae1A and wtsFae1B isolated from wastewater treatment surplus sludge, have a cognate CBM48 domain and are feruloyl esterases, and wtsFae1A binds arabinoxylan. Here, we show that wtsFae1B also binds to arabinoxylan and that neither binds starch. Surface plasmon resonance analysis revealed that wtsFae1B's Kd for xylohexaose is 14.8 μM and that it does not bind to starch mimics, β-cyclodextrin, or maltotriose. Interestingly, in the absence of CBM48 domains, the CE1 regions from wtsFae1A and wtsFae1B did not bind arabinoxylan and were also unable to catalyze FA release from arabinoxylan. Pretreatment with a β-D-1,4-xylanase did enable CE1 domain-mediated FA release from arabinoxylan in the absence of CBM48, indicating that CBM48 is essential for the CE1 activity on the polysaccharide. Crystal structures of wtsFae1A (at 1.63 Å resolution) and wtsFae1B (1.98 Å) revealed that both are folded proteins comprising structurally conserved hydrogen bonds that lock the CBM48 position relative to that of the CE1 domain. wtsFae1A docking indicated that both enzymes accommodate the arabinoxylan backbone in a cleft at the CE1-CBM48 domain interface. Binding at this cleft appears to enable CE1 activities on polymeric arabinoxylan, illustrating an unexpected and crucial role of CBM48 domains for accommodating arabinoxylan.

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Structural and functional aspects of mannanuronic acid-specific PL6 alginate lyase from the human gut microbe Bacteroides cellulosilyticus

Alginate is a linear polysaccharide from brown algae consisting of 1,4 linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) arranged in M, G, and mixed MG blocks. Alginate was assumed to be indigestible in humans, but bacteria isolated from fecal samples can utilize alginate. Moreover, genomes of some human gut microbiome-associated bacteria encode putative alginate-degrading enzymes. Here, we genome-mined a polysaccharide lyase family 6 alginate lyase from the gut bacterium Bacteroides cellulosilyticus (BcelPL6). The structure of recombinant BcelPL6 was solved by X-ray crystallography to 1.3 Å resolution, revealing a single-domain, monomeric parallel β-helix containing a 10-step asparagine ladder characteristic of alginate-converting parallel β-helix enzymes. Substitutions of the conserved catalytic site residues Lys-249, Arg-270, and His-271 resulted in activity loss. However, imidazole restored the activity of BcelPL6-H271N to 2.5% of that of the native enzyme. Molecular docking oriented tetra-mannuronic acid for syn attack correlated with M specificity. Using biochemical analyses, we found that BcelPL6 initially releases unsaturated oligosaccharides of a degree of polymerization of 2-7 from alginate and polyM, which were further degraded to di- and tri-saccharides. Unlike other PL6 members, BcelPL6 had low activity on polyMG and none on polyG. Surprisingly, polyG increased BcelPL6 activity on alginate 7-fold. LC-electrospray ionization (ESI)-MS quantification of products and lack of activity on NaBH4-reduced octa-mannuronic acid indicated that BcelPL6 is an endolyase that further degrades the oligosaccharide products with an intact reducing end. We anticipate that our results advance predictions of the specificity and mode of action of PL6 enzymes.
Plant cell wall glycosyltransferases: High-throughput recombinant expression screening and general requirements for these challenging enzymes

Molecular characterization of plant cell wall glycosyltransferases is a critical step towards understanding the biosynthesis of the complex plant cell wall, and ultimately for efficient engineering of biofuel and agricultural crops. The majority of these enzymes have proven very difficult to obtain in the needed amount and purity for such molecular studies, and recombinant cell wall glycosyltransferase production efforts have largely failed. A daunting number of strategies can be employed to overcome this challenge, including optimization of DNA and protein sequences, choice of expression organism, expression conditions, co-expression partners, purification methods, and optimization of protein solubility and stability. Hence researchers are presented with thousands of potential conditions to test. Ultimately, the subset of conditions that will be sampled depends on practical considerations and prior knowledge of the enzyme(s) being studied. We have developed a rational approach to this process. We devise a pipeline comprising in silico selection of targets and construct design, and high-throughput expression screening, target enrichment, and hit identification. We have applied this pipeline to a test set of Arabidopsis thaliana cell wall glycosyltransferases known to be challenging to obtain in soluble form, as well as to a library of cell wall glycosyltransferases from other plants including agricultural and biofuel crops. The screening results suggest that recombinant cell wall glycosyltransferases in general have a very low soluble: insoluble ratio in lysates from heterologous expression cultures, and that co-expression of chaperones as well as lysis buffer optimization can increase this ratio. We have applied the identified preferred conditions to Reversibly Glycosylated Polypeptide 1 from Arabidopsis thaliana, and processed this enzyme to near-purity in unprecedented milligram amounts. The obtained preparation of Reversibly Glycosylated Polypeptide 1 has the expected arabino-β-pyranose mutase and autoglycosylation activities.