Pectin – lignin interactions in plant cell walls and model composites

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Project Goals: The development of renewable biofuels is a key mission of the DOE Genomic Science program. Lignocellulosic biomass has the potential to be an abundant, renewable source material for production of biofuels and other bioproducts. The use of organic solvents to optimize biomass pretreatment has shown considerable promise, but their disruption of microbial membranes is key to toxic effects limiting fermentation titers. The Oak Ridge National Laboratory (ORNL) Scientific Focus Area (SFA) Biofuels Program utilizes multi-length scale imaging with neutron scattering complemented by high performance computer simulations, NMR, biochemistry and targeted deuteration to provide fundamental knowledge about the molecular forces that drive solvent disruption of the critical assemblies of biomolecules that comprise plant cell walls and microbial biomembranes.

Lignin – carbohydrate complexes (LCCs) are hypothesized to form through interactions of lignin with polysaccharides such as pectin and hemicellulose in the plant cell wall. Here, we report on putative LCCs formed between lignin and the pectin homogalacturonan (HG). A recent study showed that HG is found in the middle lamella and cell wall corner region that is also reported to be the location where lignification is initiated.[1,2] In addition, a transgenic switchgrass with reduced pectin, formed by suppressing galacturonic acid synthesis (GAUT4-kd), was shown to have reduced recalcitrance and released higher sugar compared to the wild-type (WT) switchgrass.[3] These studies provided the motivation to investigate the potential for LCCs formed due to interactions between lignin and pectin.

We compared the structural properties of WT and GAUT4-kd mutant switchgrass using small-angle neutron scattering (SANS). No differences were found in either the cellulose microfibril structure or the arrangement of the cell wall matrix copolymers in the native plants indicating that this particular mutation has negligible effects on the structure of the cell wall. However, after hot water pretreatment the GAUT4-kd variant formed significantly more lignin aggregates that were greater in size compared to wild-type switchgrass. Our data shows that despite being subjected to the same pretreatment conditions, more lignin is re-distributed to form aggregates in GAUT-kd and suggests that interactions between lignin and HG could decrease lignin aggregation in switchgrass. To further investigate lignin – HG interactions, we synthesized a model composite by polymerizing coniferyl alcohol to form a lignin-like polymer (i.e., dehydrogenation polymer (DHP)) in the presence of HG. Small-angle X-ray scattering (SAXS) of the composite showed a network-like structure unlike that obtained from a physical mixture of the individual polymers. Fourier transform infrared spectroscopy showed a unique absorption band in the ester region (~1730 cm^{-1})[4] that was only present in the composites and not in the HG, DHP or physical mixture

of HG and DHP. Furthermore, solid state nuclear magnetic resonance (SSNMR) analysis provides dynamic evidence of interaction between the synthetic lignin polymer and homogalacturonan.

Overall, studies of intact mutant and wild-type switchgrass and model composite materials provide evidence for formation of a lignin-polysaccharide complex. Interactions of polysaccharides with lignin, and the role polysaccharides play in changing lignin morphology during pretreatment, are critical to understand to improve pretreatment regimes and for conversion of these plant cell wall polymers to bioproducts.

References

1. DeMartini, J. D et al, Application of monoclonal antibodies to investigate plant cell wall deconstruction for biofuels production. *Energy & Environmental Science* 2011, *4* (10), 4332-4339.
2. Li, Y. et al, Optically transparent wood from a nanoporous cellulosic template: combining functional and structural performance. Biomacromolecules 2016, 17 (4), 1358-1364.
3. Biswal, A. K et al, Sugar release and growth of biofuel crops are improved by downregulation of pectin biosynthesis. 2018, 36 (3), 249-257.
4. Haitao Yang et al, Elucidation of the bonds between cellulose and dehydrogenation polymer with carbon-13 isotopic tracer method. Cellulose Chemistry and Technology 2013, 47 (3-4), 143-151.

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