

“Ideal lignin” facilitates full biomass utilization

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Project Goals: The mission of the GLBRC is to perform the basic research that generates technology to convert cellulosic biomass to advanced biofuels. In combining aims for the “design” of superior plants with lignins that allow efficient depolymerization, we have the following goals: 1) Delineate the features of an “ideal lignin” for streamlined processing of lignocellulosics in a biorefinery; 2) Demonstrate high-yield lignin conversion to a small array of phenolic monomers after the usual processing to derive monomeric sugars (and/or liquid fuels) from the polysaccharides.

Lignin, one of the major components of lignocellulosic biomass, is crucial to plant growth and development, but poses a major impediment to biomass utilization in various processes. It is, however, increasingly recognized that valorizing lignin is essential for sustainably and profitably converting biomass to biofuels and coproducts.¹ Hydrogenolysis, i.e., the hydrogenation of unprocessed solid biomass by a solid noble metal catalyst, remains one of the most promising methods for producing aromatic monomers. By cleaving the major linkage (β -ether) in native lignin, this process can produce simple monomeric products in high yields. However, hydrogenolysis still suffers from some issues. In most wild-type biomass, the lignin polymer is composed of three phenylpropanoid subunits, *p*-hydroxyphenyl (**H**), guaiacyl (**G**), and syringyl (**S**), derived by combinatorial radical coupling from the three main monolignols, *p*-coumaryl, coniferyl, and sinapyl alcohols. Although **H**-units are typically low-level, this results in at least three different types of monomers (**H**, **G**, and **S**) each with ethyl, propyl, and propanol sidechains as the primary hydrogenolysis products, which makes monomer separation and utilization difficult. Lignin's principal alkyl-aryl-ether units with their β -O-4 inter-unit bonds (45-85%) can be selectively cleaved, but other linkages including β -5 (1-12%), β - β (5-12%), 5-5 (1-9%), 4-O-5 (~2%) and β -1 (1-2%) that are also present in lignins remain largely intact;¹ carbon-carbon (C-C) and diaryl ether (4-O-5) units typically result in dimeric or higher oligomeric products. The use of extracted lignins rather than whole biomass has the advantage that the material can be fully

dissolved in organic solvents and may facilitate catalyst recovery and continuous processing. However, industrial lignin fractionation, particularly by acidolytic methods, is known to cause some β -ether cleavage and result in the condensation between units via the electrophilic substitution of acid-generated carbocation intermediates on the electron-rich aromatic rings,² limiting hydrogenolytic depolymerization yields.

With the revelations regarding lignins' structural malleability from studies on lignin pathway mutants and transgenics as well as on various 'natural' plants discovered to possess unusual lignins, researchers have been able to contemplate actually designing lignins for improved utilization.³ It is now a realistic juncture to posit the characteristics for an "ideal lignin" archetype for biomass processing. For the depolymerization of the lignin polymer to monomers, lignin should have the following three necessary and sufficient characteristics. First, it should contain only ether (C–O) inter-unit linkages in its backbone. Second, it should be stable under acidic conditions to prevent condensation and the generation of undesired new C–C bonds, as well as the sidechain truncation that often accompanies hydrolysis, during pretreatment. Last, it should be generated *in planta* from a single phenylpropanoid monomer to allow the production of the simplest array of compounds.

The recently discovered catechyl lignin (C-lignin),⁴ which is essentially a homopolymer biosynthesized purely by β -O-4-coupling of caffeyl alcohol with the growing polymer chain to produce benzodioxanes as the overwhelmingly dominant unit in the polymer, might represent such an ideal lignin that overcomes biomass fractionation issues and expands the opportunities for lignin valorization. Here we discovered that C-lignin is highly acid resistant and is able to maintain its native structure even after harsh acidic treatments. Hydrogenolysis of C-lignin resulted in the cleavage of essentially all benzodioxane structures to cleanly produce catechylpropane and catechylpropanol, and a small amount of a chromane, in high yield. The unique properties make the valorization of C-lignin compatible with traditional inexpensive acid pretreatment methods and allow continuous upgrading of the extracted lignin. Such valuable properties suggest that there is unrecognized value in certain biomass residues, and highlight the merit in pursuing genetic means to modify biomass to produce C-lignin.

References

1. Rinaldi, R.; Jastrzebshi, R.; Clough, M. T.; Ralph, J.; Kennema, M.; Bruijninx, P. C. A.; Weckhuysen, B. M., Paving the way for lignin valorisation: Recent advances in bioengineering, biorefining and catalysis. *Angewandte Chemie (International Edition)* **2016**, *55* (29), 8164-8215.
2. Shuai, L.; Amiri, M. T.; Questell-Santiago, Y. M.; Héroguel, F.; Li, Y.; Kim, H.; Meilan, R.; Chapple, C.; Ralph, J.; Luterbacher, J. S., Formaldehyde stabilization facilitates lignin monomer production during biomass depolymerization. *Science* **2016**, *354* (6310), 329-333.
3. Mottiar, Y.; Vanholme, R.; Boerjan, W.; Ralph, J.; Mansfield, S. D., Designer lignins: harnessing the plasticity of lignification. *Current Opinion in Biotechnology* **2016**, *37* (1), 190-200.
4. Chen, F.; Tobimatsu, Y.; Havkin-Frenkel, D.; Dixon, R. A.; Ralph, J., A polymer of caffeyl alcohol in plant seeds. *Proceedings of the National Academy of Science USA* **2012**, *109* (5), 1772-1777.

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