Towards New Degradable Lignin Types

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Abstract
Lignin is an aromatic heteropolymer abundantly present in secondarily-thickened plant cell walls. It is a major limiting factor in the conversion of lignocellulosic biomass to liquid biofuels. In dicots, the lignin polymer is built up by the combinatorial radical coupling of mainly coniferyl and sinapyl alcohol, although a range of minor units are also present in the polymer. Radical coupling results in a variety of chemical bonds, the frequency of which depends on the relative abundance of the various monomers, on the chemical characteristics of the monomers, and the local environment in the cell wall. For applications, such as the conversion of lignocellulosic biomass to fermentable sugars in the process to bioethanol, cell walls would ideally contain less lignin, and lignin rich in bonds that are easily cleaved [1,2].

The goal of the project is to identify natural products (called “target molecules”) that can be biosynthesized in energy crops, translocated to the cell wall and cross-coupled with lignin units such that the final lignin polymer is more susceptible to chemical cleavage, or is more hydrophilic, or is less cross-linked with hemicelluloses. Ideally, the structures of the target molecules are very similar to traditional monolignols so that they can be exported to the wall using the same transport system. A variety of such monomer substitutes was evaluated, some with positive effects on lignin degradability [3,4].
The project is divided into four tasks. Task 1 aims at defining which molecules are good targets for engineering and targeting to plant cell walls. We have identified more than 100 of such target molecules from literature. Several of these molecules are being cross-coupled in vitro with normal lignin monomers to make small synthetic lignin polymers, by the GCEP funded group of John Ralph. We have developed and published a new mass-spectrometry based method to sequence small lignin polymers, a method that will also be used to determine how the new lignin monomer substitutes couple into the growing lignin polymer [5,6]. Furthermore, we have developed and published a mathematical model of monolignol coupling and lignin polymerization [7]. This method should help in designing new lignin structures. Task 2 aims at cloning biosynthetic genes for target molecules and overexpressing these in bioenergy crops. We have identified two Arabidopsis genes that, when knocked out, steer the flux through the phenylpropanoid pathway into the biosynthesis of ferulate-derivatives. This is interesting, because incorporation of ferulate into the wall gives rise to labile acetal bonds that should reduce the cost of biomass pretreatment [8]. NMR analysis, however, did not reveal any incorporation of ferulate in the lignin polymer, indicating that engineered rerouting of these ferulate derivatives to the cell wall will be needed. One of these mutants has an increased incorporation of p-coumaryl alcohol units (H-units) in the cell wall, and has improved saccharification potential. Task 3 aims at rerouting a selected number of target molecules of which the biosynthetic pathway and subcellular localization are already partially known, to the cell wall in transgenic plants. Because several of the proposed target molecules are thought to be located in the vacuole, we have identified putative transporters that are involved in translocation/retention of several target molecules into the vacuole. Arabidopsis knock-out mutants in these putative transporters genes are currently being analyzed by vacuolar metabolomics. This technique, developed for the first time, has also provided insight into the subcellular localization of particular classes of monolignol-derived compounds. Task 4 aims at identifying biosynthetic pathways for promising target molecules by a combination of genetics and metabolomics in Arabidopsis. To this end, we have metabolically profiled 250 natural Arabidopsis accessions and identified several of our target molecules in this model system. We have used association genetics to identify SNPs in genes that influence the abundance of these compounds, and that may be involved in their biosynthesis.

References


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