

202. Coexistence but Independent Biosynthesis of Catechyl and Guaiacyl/Syringyl Lignin Polymers in Plant Seeds

Fang Chen^{1,2*} (fang.chen@unt.edu), Yuki Tobimatsu,³ Jin Nakashima,⁴ Luis L. Escamilla-Treviño,^{1,2} Lisa Jackson,⁴ Richard A. Dixon,^{1,2} John Ralph^{3,5} and Paul Gilna² (BESC PI)

¹University of North Texas, Denton; ²BioEnergy Science Center, Oak Ridge National Laboratory, Oak Ridge, Tennessee; ³University of Wisconsin-Madison, Madison; ⁴The Samuel Roberts Noble Foundation, Ardmore, Oklahoma, and ⁵Great Lakes Bioenergy Research Center, Wisconsin Energy Institute, Madison, Wisconsin

<http://bioenergycenter.org> <http://glbrc.org>

Project Goals: The BioEnergy Research Centers BESC and GLBRC are focused on the fundamental understanding and elimination of biomass recalcitrance. BESC's approach to improve accessibility to the sugars within biomass involves 1) designing plant cell walls for rapid deconstruction and 2) developing multi-talented microbes for converting plant biomass into biofuels via consolidated bioprocessing. Addressing the roadblock of biomass recalcitrance will require a greater understanding of plant cell walls from synthesis to deconstruction. This understanding will generate models, theories and finally processes in order to understand and overcome biomass recalcitrance. This grand challenge calls for an integrated research approach as illustrated by the project described below. BESC research is multi-disciplinary by design and multi-institutional in composition. GLBRC has similar aims and approaches that are both different and complementary, such as the joint BESC- GLBRC study described here.

Lignins are phenylpropanoid polymers, derived from monolignols, commonly found in terrestrial plant secondary cell walls. During lignin biosynthesis, the monolignol precursors are functionalized by aromatic hydroxylation and *O*-methylation to generate, successively, *p*-hydroxyphenyl (H), catechyl (C), guaiacyl (G), 5-hydroxyguaiacyl (5H), and syringyl (S) units. Natural lignins in dicotyledonous angiosperms consist of approximately equal amounts of G and S units, with less than 2% of H units. Lignins from gymnosperms are composed of G-units only with minor amounts of H units. C and 5H units are not considered as natural lignin subunits. 5H units are found in lignins in transgenic plants in which the second methylation step is blocked by mutation or gene silencing, but C units do not accumulate in the lignin of angiosperms in which the first or both methylation steps are similarly blocked. We recently reported evidence of an unanticipated catechyl lignin homopolymer (C-lignin) derived solely from caffeyl alcohol in the seed coats of several monocot and dicot plants. Here we report the identification of several dicot plants (*Euphorbiaceae* and *Cleomaceae*) that produce C-lignin together with traditional G/S lignins in their seed coats. Solution-state NMR analyses, along with an *in vitro* lignin polymerization study, determined that there is no copolymerization detectable, indicating that the synthesis and polymerization of caffeyl alcohol and conventional monolignols *in vivo* is spatially and/or temporally separated. C-lignin is structurally similar to the polymer synthesized *in vitro* by peroxidase-catalyzed polymerization of caffeyl alcohol. Circular dichroism spectroscopy did not detect any optical activity in the seed polymer. These data support the contention that the C-lignin polymer is produced *in vivo* via combinatorial oxidative radical coupling that is under simple chemical control, a mechanism analogous to that theorized for classical lignin biosynthesis. Of particular interest, the deposition of G and C lignins in *Cleome hassleriana* seed coats is developmentally regulated during seed maturation; C-lignin appears successively after G lignin within the same testa layers, concurrently with loss of the functionality of *O*-methyltransferases that are key enzymes for the conversion of C to G lignin

precursors. This study exemplifies the flexible assembly of different types of lignin polymers in plants, dictated by substantial, but still poorly understood, control of monomer supply by the cells in nature. A major challenge in current lignin bioengineering is achieving tight regulation of precursor synthesis to enable the flexible design of cell wall lignins with controlled structures. In this direction, future studies will focus on understanding the molecular genetic mechanisms that regulate the spatiotemporally specific production of C-lignin and the apparently abrupt cessation of *O*-methylation activity. The engineering of C-lignin as a novel, renewable, high-value polymer is also being explored.

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