146. Development of Crucial Tools for Lignin Research

Claudia Cardenas,[1] Utku Avci,[1] Yiki Tobimatsu,[2] Ruili Gao,[2] Alden Voelker,[2] Matt Regner,[2] Fachuang Lu,[2] **Fang Chen**,[3] **Steven Decker**,[4] **John Ralph**,[2] and **Michael G. Hahn**[1]* (hahn@ccrc.uga.edu)

[1]Complex Carbohydrate Research Center, University of Georgia, Athens, GA; [2]Department of Biochemistry and the Wisconsin Energy Institute, University of Wisconsin, Madison, WI; [3]Department of Biological Sciences, University of North Texas, Denton, TX; [4]National Renewable Energy Laboratory, Golden, CO

Project Goals: Research into aspects of lignification is currently stymied by lack of access to powerful modern methods to answer fundamental and practical questions. The three primary objectives are to: a) Develop a set of monoclonal antibodies to specific structures in lignins – for structural and localization studies; b) Develop a robust and flexible system for producing polymer-supported lignin monomers and oligolignols – for antibody screening, reactivity determination, elucidation of cross-coupling propensities, and beyond; and c) Develop fluorescent-tagged monolignols – to aid in lignin localization studies and to help elucidate monolignol transport mechanisms. These developments will provide researchers with an improved arsenal of tools to delve into today's most pressing and recalcitrant problems in lignin research, in projects ranging from very fundamental to those applied to improving pulp and papermaking or efficient biomass conversion to biofuels.

Abstract: We report here on progress in four goals for the project. First, in order to produce protein-bond phenolics for use in both raising antibodies and for screening, we have developed robust methodology for attaching phenolic compounds (including lignin models of interest here) to proteins (1). Along the way, we developed an improved method for derivatizing proteins such as the standard BSA as its cBSA (amines converted to acids with a small linker) derivative – cBSA is expensive commercially and only about 90% converted – we seem to be able to do it basically 100%, cleanly, and cheaply from BSA.

Second, a range of 'normal' and transgenic lignins with characteristics of interest have been isolated and used for both raising antibodies and for screening. A few of these will be noted below.

Third, we have synthesized and tested improved ('second-round') fluorescence-tagged monolignols, *p*-hydroxycinnamyl alcohols γ-linked to fluorogenic dyes such as aminocoumarin or nitrobenzofuran derivatives, as photoprobes for *in vitro* and *in vivo* studies of cell wall lignification. To illustrate the use of the probes for analysis of monolignol-protein interactions, we successfully monitored the complexation of aminocoumarin-tagged monolignols with horseradish apoperoxidase by Förster resonance energy transfer (FRET); the hindered binding affinity of this peroxidase- related protein towards syringyl substrates was first demonstrated experimentally by this method. In addition, to test imaging approaches, fluorescence-tagged monolignols were fed into various plant systems such as *Arabidopsis*, pine, and maize, and the localization of incorporated probes was readily visualized by fluorescence microscopy. The methods appear to be excellent for 'lighting up' actively lignifying zones, and appear to be useful in monolignol transport studies, e.g., using protoplasts. The paper was accepted as the featured cover article in *The Plant Journal* (2). [As it is now published, this aspect will not be highlighted on the poster here]

The main topic of this poster is to report on new lignin-directed monoclonal antibodies. Mice were immunized with solubilized purified lignin polymers from aspen/poplar, including from a high-syringyl transgenic line; the lignins are therefore referred to a S-L (a syringyl-only lignin) and GS-L (a guaiacyl/syringyl lignin as typically found in all monocots and dicots (including hardwoods such as

poplar, and *Arabidopsis*). Spenocytes harvested from the immunized mice were used to generate hybridoma lines that secrete S-lignin-directed monoclonal antibodies. Here we report the initial characterization of two of these antibodies, termed GLIMs for **G**eorgia **LI**gnin **M**onoclonals, namely GLIM6 and GLIM10. These antibodies were tested against several plant lignin preparations. Interestingly, GLIM6 recognizes a lignin substructure present in aspen and poplar, but not in corn, pine, or vanilla seed (a catechyl lignin) lignins. GLIM10 binds to aspen, poplar and corn lignin, suggesting a more ubiquitous S-lignin epitope is being recognized.

GLIM6 and GLIM10 were also used for immunolocalizations in w.t. and mutant *Arabidopsis* lines carrying mutations in the lignin biosynthetic pathway. GLIM6 immunoflorescence labeling was observed in *C4H::F5H* (S-enriched lignin phenotype), whereas no labeling was displayed on *fah1-2* (G-enriched lignin phenotype); on the other hand, GLIM10 recognizes a lignin substructure present in both G- and S-enriched lignin phenotypes. GLIM6 and GLIM10 also exhibit different labeling patterns on inter-fascicular fibers and vascular bundles as a function of plant development. Lastly, the effects of enzyme and alkaline pretreatment of the sections on GLIM6 and GLIM10 labeling patterns were studied. GLIM6 and GLIM10 labeling intensities increase after xylanase M1, pectin methylesterase or alkaline treatment, but no effect was noticed after polygalacturonase I and II treatments. The immunolocalization patterns observed with GLIM6 and GLIM10 show some striking (and never before noted) features that will require explanation and additional research.

References

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