101. Stoichiometric and kinetic modeling of phenylpropanoid metabolism in Arabidopsis

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Project Goals: We propose to develop a kinetic model for the shikimate and phenylpropanoid pathways. Kinetic models provide insights into the distribution of flux control, thus permitting more intelligent, predictive and effective design of experiments to modulate fluxes towards pathway end products. For this work, we will compare flux measurements in wild-type Arabidopsis plants to plants that are mutant or down-regulated for genes of the lignin biosynthetic pathway, and, those that have been metabolically engineered to bypass the shikimate dependent branch or direct carbon away from lignin biosynthesis to the production of 2-phenylethanol. The outcomes of our proposed kinetic modeling are to identify what remains unknown about the regulation and control of metabolic fluxes to lignin, and to allow development of strategies and predictions of what targets are the most promising candidates for alteration of metabolic flux to lignin.

Lignin, a major component of the plant cell wall, is an inhibitory factor for lignocellulosic biofuel production because of its recalcitrance to degradation. Although progress has been made in altering plant lignin levels and composition, there still remains a lack of systematic rational design for manipulating lignin biosynthesis towards achieving optimum biofuel yield. We utilized stoichiometric modeling to analyze the energetic and carbon costs and yields of the major cell wall polymers lignin, cellulose and hemicellulose. Based upon the carbon and energetic cofactor requirements, the stoichiometric model provides quantitative constraints for computing optimal alterations to biomass composition through metabolic engineering.

In order to get a deeper mechanistic understanding of the control of lignin biosynthesis and its allosteric regulation, a preliminary kinetic model was generated starting with phenylalanine, and ending with synthesis of three lignin monomers, *p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol. The parameters for the model were initially derived from *in vitro* enzymatic values and adjusted based on analysis of Arabidopsis plants with knockdown or knockout of several enzymatic steps, namely phenylalanine ammonia lyase (PAL), 4-coumarate:CoA ligase (4CL), hydroxycinnamoyl CoA:shikimate hydroxycinnamoyl transferase (HCT), *p*-coumaroyl shikimate 3'-hydroxylase (C3'H), caffeoyl shikimate esterase (CSE) and ferulate 5-hydroxylase (F5H). We compared the relative change of the ratio of lignin monomers as well as some intermediates between mutant and wild-type plants as predicted by the model against experimental results from the literature. Generally, the model is consistent with knockout experiments of C3'H, CSE and F5H, and also fits well with 4CL. However, the model was not predictive in the other knockdown cases. We are currently further refining the structure of the model and adjusting parameters through ^{13}C ring labeled Phe feeding experiments. In addition to modeling native phenylpropanoid metabolism, we are simulating the production of 2- phenylethanol, a promising biofuel molecule by introducing the heterologous pathway that competes for the common substrate phenylalanine. The maximum attainable flux ratio towards 2-phenylethanol was computed from both the stoichiometric and kinetic models.

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