

39. Metabolite Profiling of the Monolignol Biosynthesis Pathway Using Reversed Phase Liquid Chromatography Coupled with Tandem Mass Spectrometry

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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.

Abstract

Monolignols constitute the fundamental units of lignin that impart strength, vascular integrity and pathogen resistance to plants. It has been observed that altering lignin synthesis improves conversion efficiency of biomass into energy, food or other industrial chemicals. As a result, a multitude of research efforts have been invested in understanding the mechanism of monolignol biosynthesis via the phenylpropanoid pathway, making the quantification of the network intermediates invaluable. We present a novel and comprehensive method for profiling the metabolites of the monolignol biosynthesis pathway based on liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) in the negative ion mode using multiple reaction monitoring (MRM).

Method improvement by altering chromatographic conditions indicated low pH and low buffer concentration were conducive for obtaining better analyte responses using standards. *Arabidopsis thaliana* stem tissue was used as a model system for metabolite profiling. Refining and modifying extraction protocols revealed that vortexing combined with incubation at high temperatures resulted in an enhanced extraction of hydrophobic compounds. Process efficiency, constituting matrix effects (ion suppression) and extraction recovery, was evaluated by testing the recovery of metabolites from exogenously fed stems. Twelve of the seventeen pathway intermediates considered were detected and quantified in wild-type *A. thaliana* stem tissue. Ferulate-5-hydroxylase knockout and overexpression lines were used to validate the analytical method by analyzing the sinapoyl derivatives in the respective strains. The analytical method would be further extended to detect and quantify CoA ester intermediates of the pathway. The CoA esters such as p-coumaroyl-CoA and feruloyl-CoA constitute crucial junctions of the phenylpropanoid pathway, the abundances of which may be vital for understanding flux regulation to the monolignols.