

Metabolic Analysis of the Cinnamate/Monolignol and Lignan Pathways

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Fossil resource-based industrial society has provided us prosperity, especially in the developed countries. However, it also has brought serious negative impacts on the global environment due to the increase in the atmospheric concentration of carbon dioxide, accompanied by a number of pollution problems. Therefore, it is becoming very important to establish a sustainable and recycling-based society, which depends on renewable resources.

Wood biomass is the most abundant and potentially renewable resource. However, utilization of wood biomass has been based on the paradigm of a fossil resource-based society. Therefore, it is necessary to establish the sustainable production and utilization system for forest resources, so that a sustainable, recycling-based society can be established.

Wood biomass is composed mostly of secondary xylem which is formed through two metabolic events, cell-wall formation and heartwood formation. Hence, biochemical and molecular biological studies of secondary xylem formation provide the basic knowledge for establishing systems for sustainable production of forest resources or wood biomass.

Because both cell wall and heartwood formation comprise a wide variety of biochemical reactions, it is not an easy task to elucidate their integrated biochemical and molecular biological mechanisms. Fortunately, however, phenylpropanoid biosynthesis occurs commonly in both cell wall and heartwood formation as principal metabolic events. For example, lignin is one of the major cell wall components, while lignans and norlignans are deposited in significant amounts in heartwood regions of trees.

During the last decade, due to the recent development of molecular biological techniques, significant advances have been made in the biosynthetic studies of phenylpropanoid. This has presented many new questions to be elucidated in this field. For example, phenylpropanoid biosynthesis involves several pathways where many possible and parallel routes can be envisaged, and identification of a true, physiological route among the possibilities are still open to question.

In the present study, the author focused on the identification of the physiological route by the use of comprehensive metabolite quantitation (metabolic profiling). First, the author worked on the biosynthetic route for yatein from matairesinol in the lignan pathway [1], and then characterizing the cinnamate/monolignol pathway in relation to lignan biosynthesis [2].

A dibenzylbutyrolactone lignan, yatein, is of interest because it is not only a typical heartwood lignan but also a key biosynthetic intermediate for an antitumor lignan, podophyllotoxin. In 1980s, it was shown that matairesinol was a precursor of podophyllotoxin [3]. Later, the biosynthesis of matairesinol from coniferyl alcohol was established [3]. However, the route from matairesinol to yatein remains unknown. The conversion of matairesinol to yatein involves four steps, and many possible orders of their occurrence can be envisaged, suggesting that a metabolic grid might be present in the transformation.

First of all, the author synthesized deuterium-labeled and unlabeled standards for 10 lignans which are the possible precursors of yatein. Then, using these standards, the author established a comprehensive quantitation system for the precursors of yatein based on a stable-isotope-dilution method.

The system was applied to characterizing the yatein biosynthesis in *Anthriscus sylvestris*, which contains significant amounts of yatein and other lignans. In addition, deuterium-labeled lignan and [U-ring-¹³C₆]phenylalanine were administered to the plant, and stable isotope incorporation into downstream metabolites were examined comprehensively. Taken together, the results of these experiments established two independent branch pathways from matairesinol; one giving rise to yatein and the other to burshehennin [1].

Next, the author expanded the quantitation system to the cinnamate/monolignol pathway, because, in the post-genomic era, much attention has been focused on the overall understanding of the integrated control mechanisms for the pathway in relation to xylem formation.

Again, the author synthesized deuterium-labeled and unlabeled standards for all the compounds on the pathway, and established a comprehensive metabolite quantitation system for the pathway. Using the system and that for lignan biosynthesis established previously [1], the author measured comprehensively the amounts of the metabolites on the cinnamate/monolignol and lignan pathways in the *C. tinctorius* cv.

Round-leaved White seeds. In addition, the author carried out the simultaneous administration of two distinctly-labeled compounds to the seeds. Taken together, the results of these experiments suggested strongly the intermediacy of ferulic acid in lignan biosynthesis in *C. tinctorius* seeds. This is in sharp contrast to the current view of lignin biosynthesis that ferulic acid is not involved in lignin biosynthesis as a precursor [4].

In conclusion, the present study established for the first time the precise and comprehensive metabolite quantitation (metabolic profiling) systems for the cinnamate/monolignol and lignan pathways by the use of a stable-isotope-dilution method. The system was successfully applied to the metabolic analysis of lignan pathway in *Anthriscus sylvestris*, and the pathway from matairesinol to yatein was established. In addition, the intermediacy of ferulic acid in lignan biosynthesis in *Carthamus tinctorius* cv. Round-leaved White was strongly suggested. The quantitation systems would be useful tools for elucidating integrated mechanisms for xylem formation in trees.

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