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学位規則第9条第2項により要約公開
Lignification Mechanism Involved in Coniferin Transport in Differentiating Xylem of Poplar and Japanese Cypress

(Summary)

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2015
Chapter 1
General Introduction

Lignin is one of the essential components of the cell wall of vascular plants, and necessary for the survival of plants in terrestrial environments. Lignin is a phenolic complex polymer, and the second most abundant biomaterial on land. It is a characteristic compound of wood, which comprises 15–35% lignin (Sarkanen and Ludwig, 1971).

Lignification proceeds in three steps: (1) the biosynthesis of lignin precursors in the cytosol, (2) transport of the precursors to the cell wall, and (3) dehydrogenative polymerization of the precursors in the cell wall (Boudet et al., 1995; Whetten and Sederoff, 1995).

Although the transport of lignin precursors is a crucial step in lignification, very little research on transport mechanisms has been conducted. For example, Miao and Liu (2010) reported that the transport of monolignols and their glucosides occurred in microsomal fractions from Arabidopsis leaves. However, in Arabidopsis leaves, lignification occurs in only a limited number of cells in the leaf vein, and the majority of microsomes are derived from mesophyll cells. On the other hand, the mechanisms of lignin precursor transport in the secondary xylem of wood, where the highest amount of lignin exists, are yet to be elucidated.

Similarly, another study on Arabidopsis described an ABC transporter as a $p$-coumaryl alcohol transporter (Alejandro et al., 2012). However, $H$ lignin derived from $p$-coumaryl alcohol is a minor lignin component, and the transport mechanisms of the...
major lignin components, G and S lignin precursors, need to be examined in angiosperms.

Here, the differentiating xylem tissues (in which most cells are actively lignifying) of several tree species were examined in monolignol transport experiments. Biochemical techniques were used to investigate the transport activities of microsomal fractions from differentiating xylems. Transport mechanisms were elucidated by observations of the inhibitors of some transporters, and the fractionation of membranes. These studies are described in Chapter 2.

Another aspect of this study addresses coniferin BGL, a key enzyme in the dehydrogenative polymerization of coniferin by laccases and/or peroxidases in the cell wall. Despite the importance of the activity and distribution of coniferin BGL in the lignification of cell walls, only a few studies have examined coniferin BGL in the differentiating xylem of angiosperms. Therefore, the activity and localization of coniferin BGL in the differentiating xylem of poplar were examined, as described in Chapter 3.

The transport mechanisms, and balance of supply and consumption of lignin precursors reflect the distributions of lignin and lignin precursors in differentiating xylem. In Chapter 4, the distributions of lignin and lignin precursors (coniferin, syringin, coniferyl alcohol, and sinapyl alcohol) in the differentiating xylem of Japanese cypress and poplar are described.
Chapter 2
Transport Activities of Lignin Precursors in Differentiating Xylem of Woody Plants

Lignin biosynthesis is an essential physiological activity of vascular plants if they are to survive under various environmental stresses on land. The biosynthesis of lignin proceeds in the cell wall by polymerization of precursors; the initial step of lignin polymerization is the transportation of lignin monomers from the cytosol to the cell wall, which is critical for lignin formation. There has been much debate on the transported form of the lignin precursor, either as free monolignols or their glucosides. In this chapter, biochemical analyses were performed to characterize the membrane transport mechanism of lignin precursors using angiosperms, *Populus sieboldii × Populus grandidentata* and *Populus sieboldii*, as well gymnosperms, Japanese cypress (*Chamaecyparis obtusa*) and Japanese red pine (*Pinus densiflora*). Membrane vesicles prepared from differentiating xylem tissues showed clear ATP-dependent transport activity of coniferin. By contrast, less than 4% of the coniferin transport activity was seen for coniferyl alcohol. Bafilomycin A1 and proton gradient erasers markedly inhibited coniferin transport in hybrid poplar membrane vesicles; in contrast, vanadate had no effect. Cis-inhibition experiments suggested that this transport activity was specific for coniferin. Membrane fractionation of hybrid poplar microsomes demonstrated that transport activity was localized to the tonoplast and endomembrane-rich fraction. Differentiating xylem of Japanese cypress exhibited almost identical transport properties, suggesting the involvement of a common
endomembrane-associated $\text{H}^+$/coniferin antiport mechanism in the lignifying tissues of woody plants, both angiosperms and gymnosperms.
Chapter 3
Coniferin β-glucosidase Localizes in Cell Wall in Differentiating Xylem of Poplar

Lignin is crucial for the growth and persistence of vascular plants in diverse environments. Although biosynthesis and polymerization of lignin precursors have been subjected to in-depth studies, only limited information exists on the ways in which these precursors are transported. Recent research has demonstrated that V-ATPase dependent transport of coniferyl alcohol glucoside (coniferin) is a major metabolic function in differentiating xylem of both angiosperms and gymnosperms. Localization of β-glucosidase (BGL) involved in the cleavage of coniferin to coniferyl alcohol and glucose is important to clarify the role of coniferin in lignification; nevertheless, only little has been reported in angiosperms. Here, the presence of coniferin β-glucosidase activity was demonstrated in a cell wall ionically bound protein fraction extracted from differentiating xylem in poplar (Populus sieboldii × Populus grandidentata). Coniferin β-glucosidase localization is very likely in cell wall similar to previous reports in conifers. A putative poplar coniferin β-glucosidase was identified through phylogenetic analysis and named this protein “PtrBGL6.” Immunoprecipitation assays showed that the anti-PtrBGL6 antibody recognizes a coniferin β-glucosidase in a cell wall ionically bound protein fraction. Conserved coniferin β-glucosidases localized in cell wall in both angiosperm and gymnosperm implies their important roles in the formation of lignified cell wall.
Chapter 4

Distribution of Lignin and Lignin Precursors in Differentiating Xylem of Japanese Cypress and Poplar

Lignin is an integral component of the cell wall of vascular plants. The mechanism of lignin precursor supply from the cytosol into the cell wall of differentiating xylem has not yet been elucidated. The present chapter showed that a certain amount of coniferyl alcohol glucoside (coniferin) was occurred in the differentiating xylem of Japanese cypress (*Chamaecyparis obtusa*), as previously reported in gymnosperms. Coniferin content peaked in the early stages of secondary wall formation and decreased during lignification. In contrast to gymnosperms, coniferin content was limited in the differentiating xylem of poplar (*Populus sieboldii × Populus grandidentata*). Moreover, coniferyl alcohol was not detected in all specimens. In the differentiating xylem of poplar, a higher amount of sinapyl alcohol was occurred than glucoside (syringin). However, the phloem contained syringin and not sinapyl alcohol. The sinapyl alcohol content in the xylem peaked in the cells with ceasing cell wall formation, and decreased gradually towards the boundary of the annual ring, where the lignin content kept increasing. Sinapyl alcohol in the differentiating xylem of poplar may be used for the lignification of the xylem.
Chapter 5

Conclusions

This thesis aimed to elucidate the transport mechanisms of lignin precursors in differentiating xylem containing large amounts of lignin.

Chapter 2 describes biochemical analyses that were performed to characterize the membrane transport mechanism of lignin precursors using angiosperms and gymnosperms. Clear ATP-dependent transport activity of coniferin was shown in membrane vesicles prepared from the differentiating xylem tissues of both angiosperms and gymnosperms; in contrast, less than 4% of the coniferin transport activity was seen for coniferyl alcohol. Transport assays using inhibitors indicated that coniferin transport is mediated by a secondary transporter using the proton gradient created by V-ATPase rather than by an ABC transporter. The membrane fractionation of hybrid poplar microsomes showed that transport activity was localized to the tonoplast and endomembrane-rich fraction. The differentiating xylem of Japanese cypress exhibited almost identical transport properties, suggesting the involvement of a common endomembrane-associated H⁺/coniferin antiport mechanism in the lignifying tissues of woody plants, both angiosperms and gymnosperms.

Coniferin transported should be hydrolyzed by β-glucosidase (BGL) for polymerization of monolignols. In Chapter 3, it was demonstrated that the presence of coniferin BGL activity in a cell wall ionically bound protein fraction extracted from
differentiating xylem in poplar. Coniferin BGL localization is very likely in cell wall in differentiating xylem of poplar, as reported previously for conifers. Conserved coniferin BGLs localized in the cell wall of both angiosperm and gymnosperm implies their importance of their role in the formation of lignified cell wall.

Chemical analyses revealed the distribution of lignin and lignin precursors in differentiating xylem, which is described in Chapter 4. These analyses showed that a certain amount of coniferin is found in the differentiating xylem of Japanese cypress. Coniferin content peaked in the early stages of secondary wall formation and decreased with the proceeding of lignification, as previously reported in other conifers. In contrast to gymnosperms, coniferin content was limited in the differentiating xylem of poplar. Moreover, coniferyl alcohol was not detected in all specimens. A higher amount of sinapyl alcohol was contained in the differentiating xylem of poplar than its glucoside (syringin). Sinapyl alcohol in the differentiating xylem of poplar may be involved in the lignification of the xylem.

In conclusion, differentiating xylems of both angiosperms and gymnosperms have almost identical mechanism of coniferin transport, and identical localization of coniferin BGL. However, angiosperm and gymnosperm xylems differ in their amounts of stored coniferin, indicating that their coniferin supply and consumption balance differ. Coniferin might be transported to the cell wall and used for lignification of the cell wall where coniferin β-glucosidase is localized. This lignification mechanism involved in transport of coniferin may be common in woody plant.
References


