HOKKAIDO UNIVERSITY

Title	Functions of wood cell wall polysaccharides on lignification in vitro [an abstract of dissertation and a summary of dissertation review]
Author(s)	LYU, Yan
Citation	北海道大学. 博士(農学) 甲第15155号
Issue Date	2022-09-26
Doc URL	http://hdl.handle.net/2115/87244
Rights(URL)	https://creativecommons.org/licenses/by/4.0/
Type	theses (doctoral - abstract and summary of review)
Additional Information	There are other files related to this item in HUSCAP. Check the above URL.
File Information	Lyu_Yan_abstract.pdf (論文内容の要旨)

Instructions for use

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

学位論文内容の要旨

博士の専攻分野名称：博士（農学）　　氏　名：Yan LYU

学位論文題名

Functions of wood cell wall polysaccharides on lignification *in vitro*

（*in vitro*の木化における木材細胞壁多糖類の機能）

Wood cell walls are mainly composed of cellulose, hemicellulose, and lignin. During tree growth, lignin is biosynthesized by radical coupling of monolignols catalyzed by peroxidase/H_2O_2 and/or oxidase/O_2 in swollen polysaccharide matrices assembled with cellulose and hemicelluloses. However, it is still under discussion how cell wall polysaccharides affect the lignin formation, so-called lignification, such as, the morphology, substructure, and generated amount of resultant lignin.

An objective of this study is to elucidate the effects of wood cell wall polysaccharides on lignification through the fabrication of artificial cell walls mimicking the process of tree cell wall formation. The artificial cell walls were fabricated by dehydrogenative polymerizations of coniferyl alcohol (CA) and sinapyl alcohol (SA) with two enzymes, horseradish peroxidase (HRP) and recombinant cationic cell wall-bound peroxidase (rCWPO-C), in polysaccharide matrices comprised of cellulose and several kinds of hemicelluloses, water-soluble fraction of beech xylan (WXY), partially acetylated WXY with a degree of substitute (DS) of 0.50 (AcXY), galactoglucomannan (GGM) isolated from *Picea jezoensis*, and xyloglucan (XG) from tamarind seeds. XG is a well-known polysaccharide in the primary wall of wood, and WXY and GGM are representative hemicelluloses in the secondary wall. In hardwood, xylan is partly acetylated with the DS of 0.40–0.75. Thus, AcXY was chemically synthesized as an analog of native xylan in hardwood.

1. Elucidation of polysaccharides effects on HRP-catalyzed dehydrogenative polymerization of CA in bacterial cellulose (BC)-based matrices

Interactions between BC and hemicelluloses, WXY, GGM, and XG, were firstly investigated with equilibrium adsorptions. It was found that the affinity of hemicellulose with BC films was in the order of XG > GGM > WXY. Dehydrogenative polymerization of CA as a monolignol of softwood and hardwood was then attempted in the hemicellulose-adsorbed BC films, which was catalyzed by HRP as an often used enzyme for the polymerization. The largest amount of dehydrogenation polymer (DHP)

was generated in/on the XG-adsorbed BC matrix, indicating that XG facilitated DHP formation. From the nitrobenzene oxidation of DHP generated in different hemicellulose-adsorbed matrices, the DHPs formed in WXY- and GGM-adsorbed matrices showed higher frequency of aryl ether linkages (mainly *β-O-4'* linkage) than DHP formed in a BC film without hemicelluloses. By contrast, the DHP generated in the XG-adsorbed matrix was found to be enriched in *5–5'* interunitary linkages as one of condensed substructures. Since lignin in the primary wall has been reported to be rich in condensed substructures, XG must be a key polysaccharide to control the substructure of the lignin located in the primary wall.

2. Elucidation of polysaccharides effects on rCWPO-C-catalyzed dehydrogenative polymerization of CA and SA

HRP is not a tree enzyme, and thus should not involve lignification in tree. By contrast, a cationic cell wall-bound peroxidase discovered in poplar can oxidize monolignols, both CA and SA, and also polymeric lignin. Recently, the procedure of its recombinant preparation has been established, and thereby, the effects of wood cell wall polysaccharides on lignification was investigated through rCWPO-C-catalyzed dehydrogenative polymerization of CA and SA in polysaccharide matrices on a sensor of quartz crystal microbalance with dissipation (QCM-D). Firstly, cellulose nanofibers (CNFs) were anchored on the QCM-D sensor surface. Afterwards, hemicelluloses (WXY, AcXY, GGM and XG) and enzymes were successively deposited on the sensor. Finally, CA and SA buffered solutions with H_2O_2 were separately flowed into the sensors to generate DHP. These deposition processes mimicked the cell wall formation process in tree. The adsorption amounts of hemicelluloses, enzymes, and DHPs on the sensor during each process were estimated by QCM-D. The adsorption amount of hemicelluloses on CNFs was in the order of XG > GGM > WXY > AcXY. In addition, the largest amount of rCWPO-C adsorbed on AcXY among all polysaccharides-coated sensors, whereas HRP adsorbed on CNFs in the largest amount. The rCWPO-C-adsorbed sensors afforded spherical DHP of CA and SA with the larger quantity and size than HRP-adsorbed sensor. Particularly, the sensor containing AcXY yielded the largest amount and size of DHP from SA. This study clearly suggests a significant function of acetyl group in xylan for the lignification of hardwood.

This study clearly demonstrated the influence of cell wall polysaccharides, especially, XG in the primary cell wall and AcXY in the secondary wall of hardwood, on lignification *in vitro*. These findings on the hemicellulose functions must contribute to the clarification of wood cell walls, which are representative architecture in plant.