学位論文内容の要旨

博士の専攻分野名称：博士（農学）　　氏　名： Qiang LI

学位論文題名

Elucidation of xylan function in lignin formation using artificial wood cell wall

（人工木材細胞壁を用いたリグニン形成におけるキシランの機能解明）

There are still unclear subjects about the formation of wood cell wall and the functions of its components. An aim of this study is to elucidate xylan functions in the wood cell wall formation *in vitro*, lignin formation in particular, using a model, artificial wood cell wall, which mimics the structure and formation process of wood cell wall. So far, two opposite hypotheses have been proposed on xylan function in lignin formation in the secondary cell wall: One is that xylan affects lignin formation, while the other is that xylan does not. In addition to the above objective, the second aim in this study is to investigate the effects of xylan and lignin deposition onto cellulosic framework of the artificial cell wall on the mechanical properties of cellulosic framework.

1. Fabrication of cellulosic films as a framework of artificial wood cell wall and adsorption of xylan on the films

Two types of cellulosic films as framework of artificial wood cell wall were firstly made of bacterial cellulose (BC), the structure of which was the same as native cellulose with cellulose I polymorphism. One was honeycomb-patterned BC film (HPBC), the morphology of which mimicked wood cell wall array. This was prepared by the culture of a cellulose-producing bacterium, *Gluconacetobacter xylinus* (ATCC53582), on an agarose gel with the surface of honeycomb-patterned grooves. The other was flat BC film (FBC) without big pores as a reference to the former one. The FBC as a bacterial pellicle was prepared by the culture of *G. xylinus* in the liquid media.

Both BC films were immersed in aqueous beech xylan solutions at different concentrations to yield xylan-adsorbed HPBC and FBC. The xylan concentrations before and after immersion were measured by HPLC, and adsorption isotherms were plotted against the initial concentrations. It showed that the adsorption of xylan on HPBC was much higher than that on FBC at any xylan concentrations, suggesting that HPBC had larger area accessible to xylan that FBC did. The distribution of xylan in HPBC and FBC was observed by a combined method of immunolabeling and

fluorescent spectroscopy. Xylan was immunolabeled with two primary anti-xylan antibodies, LM 10 and LM 11, and a secondary antibody with fluorophore. The images displayed that xylan was distributed not only on the surface but also inside the films. Thus, xylan-deposited cellulosic films were successfully prepared as a second process for creation of artificial wood cell wall.

2. Formation of dehydrogenation polymer (DHP) in the xylan-deposited cellulosic films

An endwise type of dehydrogenative polymerization, "Zutropfverfahren," of coniferyl alcohol as a monolignol was attempted in FBC and HPBC with and without xylan. DHP location in the films was observed under a SEM-EDXA after staining the lignin with bromine. It was found that DHP was generated inside and outside the xylan-deposited films, whereas DHP was deposited only outside the films without xylan. In both of the DHP and xylan-deposited cellulosic films, colocalization of xylan and DHP was confirmed by a combination of above SEM-EDXA and immunolabeling methods with xylan release during DHP formation. Therefore, it was concluded that xylan acts as a scaffold for DHP deposition in the polysaccharides matrix comprised of xylan and cellulose.

The amount of the generated DHP in the films was measured by acetyl bromide method, and the frequency of ary-ether interunitary linkage, representative *β-O-4*, was estimated from the yield of alkaline nitrobenzene oxidation. DHP formed in the xylan-deposited films was much larger than that in the films without xylan. This result indicates that xylan is a scaffold for DHP deposition. In addition, the frequency of ary-ether linkage in DHP was also increased by the xylan deposition, suggesting that xylan acts as a structure-regulator for the formation of *β-O-4* linkages.

Mechanical properties of the cellulosic films, such as tensile strength and modulus of elasticity (MOE), were found to be strengthened by the deposition of xylan and DHP. Especially, DHP deposition remarkably enhanced MOE. Therefore, both xylan and lignin can improve the mechanical strength of cellulosic framework, particularly rigidity upon lignin deposition.

In conclusion, I empirically proved xylan functions in the lignin formation by using biomimetic models to wood cell wall structure: xylan induced deposition of a monolignol, coniferyl alcohol, into the polysaccharides matrix, and regulated the structure of its polymer, DHP. In addition, deposition of xylan and lignin onto cellulosic framework strengthened the mechanical properties of cellulose.