ABSTRACTS (MASTER THESIS)

Analysis of interaction of 12-mer peptides and carbohydrate-binding module of cellulase with lignin

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Lignin, a heterogeneous aromatic polymer provides plant cell walls structural rigidity and biological resistance. Lignin is the sole large-volume renewable feedstock composed of an aromatic skeleton. Highly selective degradation of lignin is pivotal for industrial production of paper, biofuels, chemicals, and materials. However, few studies have examined natural and synthetic molecular components recognizing the heterogeneous aromatic polymer. Recently lignin-binding 12-mer peptides were identified using a phage display technique[1]. The selected peptides were found to possess a characteristic sequence and exhibit structure-dependent high-affinity binding to the lignin isolated from softwood and hardwood. A consensus sequence was found in several lignin-binding peptides, but the outer amino acid sequence affected the binding affinity of the peptides. To characterize the key amino acid residues in the lignin-binding peptides, a peptide designated as C416 and its single-residue substituted peptides were subjected to the binding analysis with milled wood lignin (MWL) from softwood and hardwood by surface plasmon resonance. Substitution of the 7th residue of phenylalanine with isoleucine in the lignin-binding peptide (C416) decreased the affinity of the peptide for softwood lignin without changing its affinity for hardwood lignin, indicating that the peptide recognized structural differences between the lignin. Circular dichroism spectroscopy demonstrated that C416 adopted a highly flexible random coil structure, allowing key residues to be appropriately arranged in relation to the binding site in lignin. In this study binding of the lignin-binding peptides with β-*O*-4 lignin dimer and oligomer model compounds and also with milled wood lignin (MWL) was studied using NMR. Spectral changes were found on addition of these lignin model compounds and isolated lignin depending on concentration of the additives, and the results were interpreted based on the structures of the peptide sequence. In this study carbohydrate-binding module (MWL) of cellobiohydrolase were expressed in *Pichia pastoris*, and binding to the MWL was analyzed using NMR. Differences were found for the binding behavior between cellohexaose and MWL. These binding analysis provides a useful platform for designing synthetic and biological catalysts selectively bind to lignin. Analysis of the binding between peptides/CBM and lignin would also give important information to unveil the molecular interaction between polysaccharide hydrolases and lignin, which is a key factor to suppress unproductive binding of the enzymes with lignin during enzymatic saccharification of lignocellulosic biomass for production of biofuels and chemicals.

Reference

[1] Yamaguchi, A., K. Isozaki, M. Nakamura, H. Takaya, T. Watanabe, Discovery of 12-mer peptides that bind to wood lignin, *Scientific Reports*, 6, 21833, 2016.