

Summarized version of doctoral degree

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Preparation and advanced characterization of lignocellulose using multidimensional NMR (多次元 NMR 法のためのリグノセルロースの調製及び解析技術の高度化)

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Background

Biorefinery, a process converting biomass to various chemicals, has a high potential to provide sustainable resources substituting mining resources. Considerable attention has been paid in various research fields to improve its efficiency and production. Efforts have been put to increase the utilization efficiency of biomass including lignocellulose which is its main compositions located in plant cell wall. Lignocellulose is composed of cellulose, hemicellulose, and lignin. Specifically, cellulose acts like reinforcing bars due to its tough physical property, whereas hemicellulose and lignin act like the concrete surrounding around the cellulose. Yet, the detail structure of lignocellulose is not clear. The key-to-success for understanding supramolecular structures of lignocellulose is to analyze them without breaking them down to monomer molecules.

For the purpose of contribution of lignocellulose study, I characterized ^{13}C -labelled lignocellulose components via the lignocellulose solubilization, using multidimensional nuclear magnetic resonance (NMR). Two kinds of solubilization system were chosen. The first was an ionic liquid enabling structural changes of cellulose, and the second was dimethylsulfoxide (DMSO)/pyridine for characterization of lignocellulose components of poplar. Then, NMR signals of lignocellulose components were assigned by the combination of ^{13}C -labeling and multidimensional NMR techniques.

Exploring the conformational space of amorphous cellulose using NMR chemical shifts

Cellulose is the most common chemical polymer on land and has been used in diverse materials. It is composed of crystalline and amorphous structures. Crystalline cellulose has been investigated intensively, and its atomic structures have been classified by Infra-red (IR), Raman, and NMR spectroscopy, X-ray, diffraction, and molecular dynamics simulation. Among these various techniques, the solid-state NMR spectroscopy enables us to observe not only crystalline but also amorphous structures of cellulose. Although the C4 chemical shift, which is a marker for classifying crystalline and amorphous structures, has been detected by NMR, complete NMR assignments for ^{13}C atoms in amorphous cellulose have not been achieved yet. The understanding of local atomic structures of amorphous cellulose is expected to obtain knowledge about the detailed structure of amorphous cellulose. However, very little knowledge on these structures is known about. Therefore, I performed the complete NMR assignments for ^{13}C -labelled amorphous cellulose using solid-state ^{13}C - ^{13}C incredible natural abundance double quantum transfer experiment (INADEQUATE) NMR. The ^{13}C -regenerated amorphous cellulose was obtained by ionic liquid treatment. Detection of a computer-simulated candidate of a local structure for amorphous cellulose that is consistent with the both experimental and quantum chemistry-assisted theoretical chemical shifts. The candidate local structure of amorphous cellulose possesses a surface that is significantly less hydrophobic than that of crystalline cellulose.

Multidimensional high-resolution magic angle spinning and solution-state (HR-MAS) NMR characterization of ^{13}C -labeled plant metabolites and lignocellulose

Next, poplar, one of the main sources of plant biomass which is easily transformed, was selected as multicomponent realistic biomass system. However, in many cases the NMR spectra of metabolites and lignocellulose components were ambiguously assigned because of the overlapping of chemical shift peaks. Using ^{13}C -labeled poplar samples, I demonstrated that overlapping peaks could be resolved by three-dimensional NMR to more accurately assign chemical shifts compared with two-dimensional NMR measurements. Metabolites of the ^{13}C -labelled poplar were measured by HR-MAS NMR, which provides easy sampling without solvent extraction. Lignocellulose components of ^{13}C -labelled poplar that were ball-milled and then dissolved in DMSO/pyridine solvent were measured by solution-state NMR. By these methods, I was able to appropriately assign chemical shifts of metabolites and lignocellulose components of ^{13}C -labeled plants using the HCCH correlation spectroscopy (COSY) and HCCH total correlation spectroscopy (TOCSY), respectively. Application of this method enabled us to compare the lignocellulose components between

two transgenic poplar samples (*VND6* and *VND7*), which produce clearly modified plant cell wall components. The poplar samples were grown for short durations, so that, the solubilized lignocellulose components of *VND6* were lesser amount than those in the wild-type due to expression of *VND6* gene in protoxylem. In contract, the *VND7* components were abundant than those in the wild-type due to expression of *VND7* gene in metaxylem.

Conclusions

In this study, the ^{13}C -labelled amorphous cellulose and ^{13}C -labelled lignocellulose components of plant were evaluated, using multidimensional NMR. Regarding lignocellulose study, the utilization of lignocellulose solubilization shows to be an effective approach for characterizing structural changes of lignocellulose. It can be expected that my approach will allow us to improve the utilization and productivity of biomass in the future.