Structure and Packing of Complex Carbohydrates in Native Plant and Fungal Cell Walls from Solid-State NMR and MAS-DNP

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Introduction

The carbohydrate-rich cell wall in plant and fungi is a unique and versatile material that concurrently provides the cell with sufficient mechanical strength to keep its integrity and morphology under stress, and retains remarkable plasticity to extend during cell growth. At the same time, such a property also poses a challenge for post-harvest processing and utilization (plant) and for antifungal studies. A better understanding of the cell wall architecture will guide us for improving the efficiency of converting biomass into biofuels and facilitate the design of better anti-fungal agents to fight against fatal diseases caused by pathogenic fungi. Wall polymers are typically insoluble and disordered, thus requiring multidimensional solid-state NMR and MAS-DNP spectroscopy for high-resolution structural characterization.

Experimental

The 600 MHz/395 GHz MAS-DNP, the 800 MHz #1 and 600 MHz #2 NMR spectrometers at the NMR facility were used. We have obtained ^{13}C, ^{15}N-labeled fungi and ^{13}C-labeled plants and measured a series of 2D ^{13}C-^{13}C and ^{13}C-^{15}N correlation spectra to systematically determine the composition, conformational structure, packing, hydration and mobility of polysaccharides in intact cells. DNP has been used to overcome sensitivity limitations for detecting long-range intermolecular correlations between different polymers, as well as measuring natural abundance 2D correlation spectra on unlabeled biomaterials.

Results and Discussion

Three publications and two manuscripts under review are produced over the last year. The first collaborative paper revealed the high-resolution supramolecular architecture of the cell wall in Aspergillus fumigatus [1], a pathogenic fungus causing invasive infections to more than 200,000 patients annually. The structure that emerged is stunningly beautiful, unexpected, and full of possibilities for novel biochemistry and microbiology. Chitin and α-1,3-glucans tightly pack to form a highly rigid and hydrophobic scaffold that is surrounded by a soft matrix of diversely linked β-glucans and capped by a shell formed by glycoproteins and the bifunctional molecule α-1,3-glucan (**Fig. 1**). This study establishes the structural frame for further investigations of drug response and fungal virulence, from the molecular perspective and in intact cells. The second paper systematically presents the protocol for preparing ^{13}C, ^{15}N-labeled fungi and plants and how to process them for NMR and DNP studies. The third paper integrates natural-abundance 2D ^{13}C-^{13}C correlation spectroscopy, as enabled by MAS-DNP, with statistical analysis of chemical shifts to investigate the cellulose structure in unlabeled cotton[2]. This method opens many possibilities for elucidating the carbohydrate structure in samples without isotope-labeling.

Conclusions

These studies advance our understanding of the functional structure of complex carbohydrates and the relevant biological systems. The protocol and method developed here will substantially facilitate future studies of biomaterials and benefit the research community. These studies are enabled by the high-field NMR and DNP magnets at NHMFL.

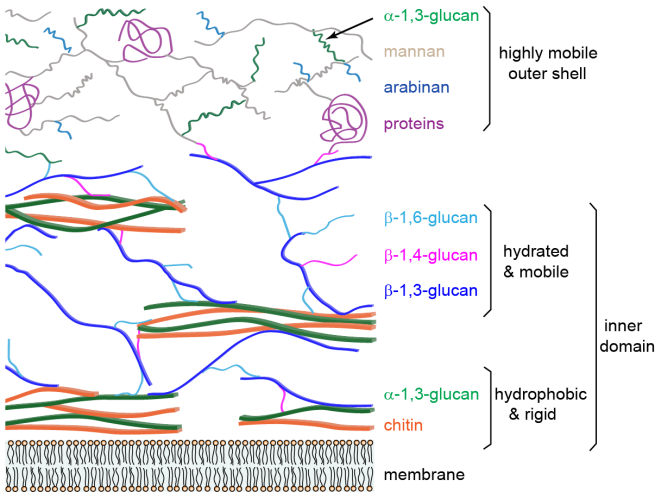

Fig.1 NMR-derived structural model of fungal cell walls. The figure is adapted from Ref. [1].

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References

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