HIGH-RESOLUTION SOLUTION-STATE NMR OF UNFRACTIONATED PLANT CELL WALLS

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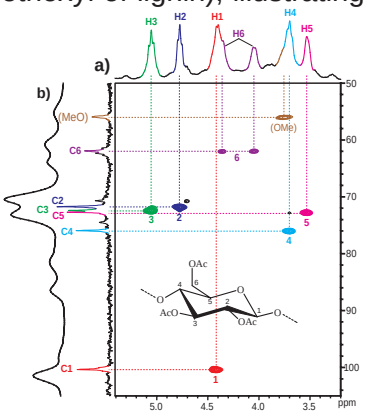
Abstract

Detailed structural studies on the plant cell wall have traditionally been difficult. NMR is one of the preeminent structural tools, but obtaining high-resolution solution-state spectra has typically required fractionation and isolation of components of interest. With recent methods for dissolution of, admittedly, finely divided plant cell wall material, the wall can now be studied by solution-state NMR. Exploiting the dispersion of 2D (and even 3D) NMR allows strikingly detailed structural analysis of the wall components without the need for isolation and fractionation. The initial method utilized acetylation of the wall to prepare optimal samples for NMR, but more recently we avoid the acetylation step; spectra from unacetylated walls have improved resolution of some components and allow natural acetylation (of polysaccharides and lignins) to be readily identified. The structural "fingerprint" of the cell wall produced by 2D ^{13}C–^{1}H correlative NMR experiments is potentially unmatched by any other spectroscopy. Dissolution/NMR methods therefore potentially lend themselves well to chemometrics methods once the issues of dealing with multivariate analyses on 2D NMR data have been addressed. Improved throughput (in principle, toward 50 samples per day) seems possible.

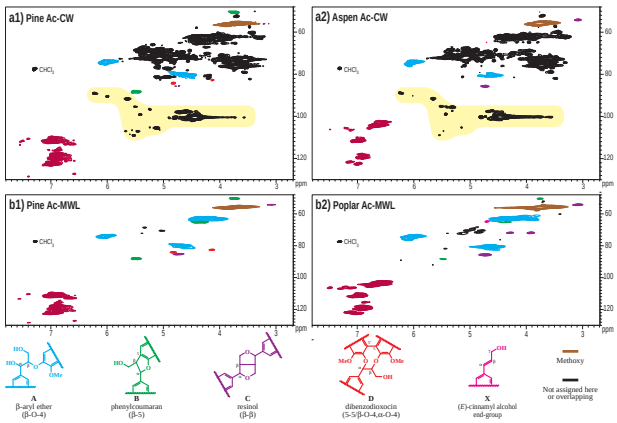
The Original Dissolution Method

Two H-bond-disrupting solvent systems to fully dissolve finely divided whole cell walls, DMSO-N-methylimidazole (DMSO-NMI) and DMSO-tetrabutylammonium fluoride (DMSO-TBAF) were introduced in a cover article in *The Plant Journal*.[1]

When walls in DMSO-NMI are acetylated, as is readily accomplished simply by adding acetic anhydride to the solvent after dissolution, the resultant acetylated cell walls (Ac-CWs) dissolved readily in common NMR solvents (e.g. $CDCl_3$, DMSO-d_6) allowing the application of high-resolution solution-state NMR methods. 2D ^{13}C–^{1}H correlative NMR at low level shows just the cellulose (and the methoxyl of lignin), illustrating how the entire 6 correlation-contour pattern represents a (unique) profile for cellulose. Also, in (b), the solid-state ^{13}C NMR spectrum is shown, illustrating how much sharper the solution-state



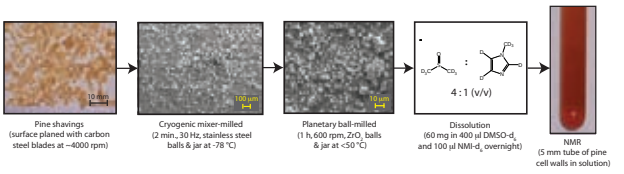
spectra in (a) are. But it is 2D spectroscopy that has the spectral dispersion to make profiling the wall so powerful.



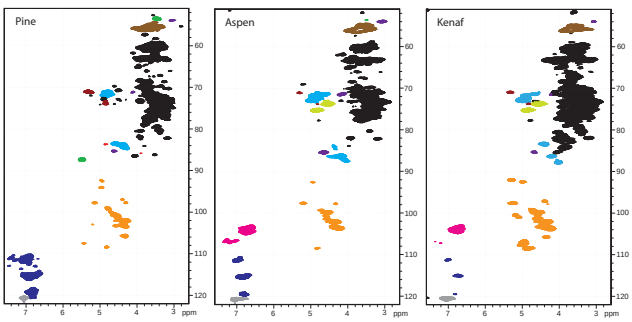
At lower levels, NMR shows the entire wall profile and that, even in the presence of overwhelming polysaccharides, allows the identification of all the common lignin structures (colored). The syringyl/guaiacyl composition can be deduced from the aromatic region, and that the polysaccharides can potentially be distinguished, particularly in the anomeric region (yellow highlighting).

Direct in-NMR-tube Dissolution

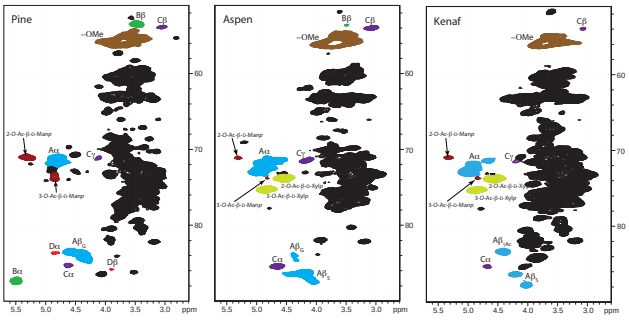
A logical extension to the method came once NMI-d_6 was synthesized;[2] — the finely-ground sample could be dissolved directly, in the NMR tube.



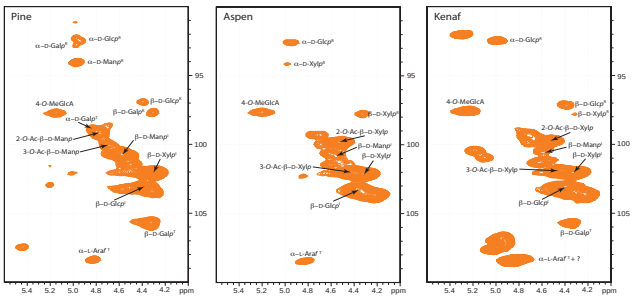
Pre-grinding steps are not normally required, but ball milling to about 5 micron is crucial.
The resulting spectra were superior in some ways to those from the Ac-CWs. Some peaks we more dispersed (although others were less dispersed).



In particular, naturally acetylated polysaccharide units could be identified. These are important as they can limit saccharification. Here we see the natural acetylation of mannans and xylans (and the 2- and 3-positions of each).

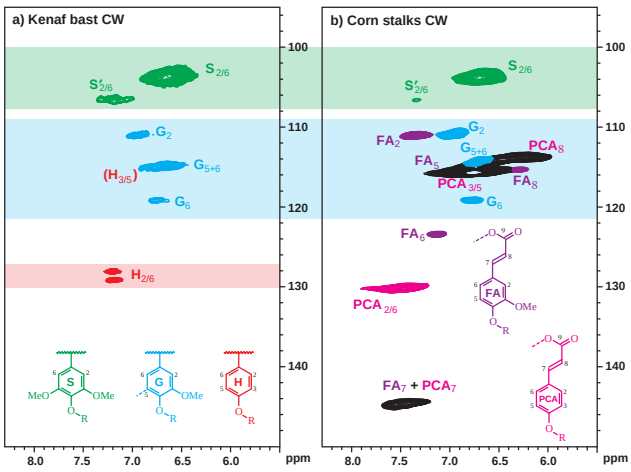


The anomeric region was also particularly well dispersed, allowing the polysaccharides, including uronics, to be profiled; assignments remain incomplete, however.



Gel-State Modifications

It is not necessary to fully dissolve the wall. Simply swelling the finely divided cell wall sample in DMSO produces gels that give surprisingly good spectra.[3]
In grasses, *p*-coumarates (pCA), and the important ferulate (FA) cross-linking agents are readily identified. Full 2D spectra from the viscous gels can be acquired in <30 min! If sample

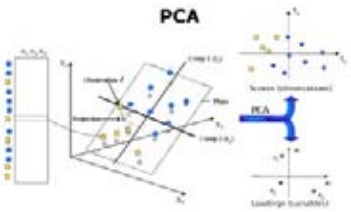


preparation can be streamlined, the potential for using this method for screening may become a reality.

Applications

The methods are relatively new, and work best with relatively modern NMR instrumentation with sensitive cryogenic probes, but have already been used in various studies on natural and transgenic plants,[4-6] and to delineate the mechanisms of brown rot fungi.[7] Entire-cell-wall 2D difference NMR spectroscopy has also become useful in, for example, establishing the incorporation of free ferulic acid into lignins in poplar deficient in cinnamoyl-CoA reductase (CCR),[4,6] and to improve assignments in Gingko lignins in difference spectra from natural and specifically ^{13}C-enriched Gingko.[8]

Chemometrics

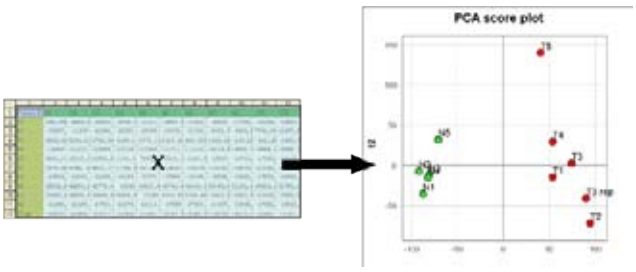


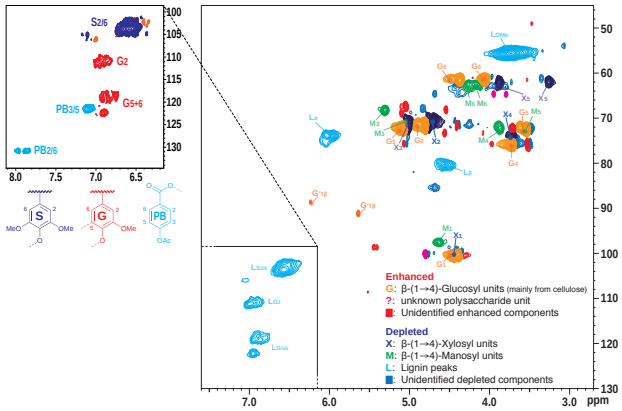
2D NMR spectroscopy has not previously been amenable to multivariate analysis (MVA). The Umeå group developed methods to allow the '2D NMR Profile' to be used like any 1D spectrum in chemometrics.[9]

Proof of Concept

Poplar tension wood cells are loaded with cellulose. Can MVA reveal this difference?

Spectra were acquired from 5 replicated samples of normal wood, and 5 tension wood samples. Principal component analysis (PCA) readily separated the normal (N) population from the tension wood (T) population, with the first principal component (PC1) accounting for 81% of the variation.[10]





In the PC1 2D NMR 'reconstructed spectrum' we can see that the cellulose is enhanced, as expected, with concomitant relative decreases in the levels of the hemicelluloses (mannan and xylan) and the lignin. An unknown polysaccharide is elevated — we are still tracking this down.

If just the aromatic region is subjected to PCA, we readily see that the syringyl units (and their attached *p*-hydroxybenzoates) are depleted in favor of guaiacyl units.

Further validation of the chemometrics methodology was by discovering, and subsequently authenticating by other methods, 5% relative level changes in lignin units (syringyl:guaiacyl) caused by downregulation of a pectin methyl esterase — an enzyme whose effect on the cell wall was not known.[10]

Conclusions

Detailing cell wall polymer composition and structure without the need for lengthy fractionation and component isolation is becoming a reality for some studies. Although there remain technical difficulties, and questions about whether some components are fully soluble (or fully swelled) and whether they are therefore fully represented in the spectra, the ability to now quickly profile the wall in a meaningful way is a significant advance. The NMR structural fingerprint coupled with mulitvariate analysis should allow correlations to be made between the profile and parameters in *any* process that depends on plant cell wall composition and structure!

Acknowledgements

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