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Contribution of hemicellulose network to plant cell wall properties

Videcoq P. (a), Assor C. (b), Arnould O. (b), Barbacci A. (a), Lahaye M. (a)

(a) UR 1268, PVPP, INRA, rue de la Géraudière, BP 71627, 44316 Nantes, France ; (b) LMGC, Université Montpellier 2, CNRS UMR5508, CC048 place Eugène Bataillon, 34090 Montpellier, France.

Plant cell walls are made of interacting networks of polysaccharides (cellulose, hemicelluloses, pectins) and some structural proteins. Cellulose-xyloglucan networks are considered to be the main source of structural strength in primary cell wall. Little is known about the contribution of each polysaccharide network to mechanical properties. The aim of this work is to evaluate the role of hemicellulose, especially xyloglucans, by monitoring plant cell wall mechanical properties during targeted enzymatic degradation and establishing links with xyloglucan chemical composition and structure.
Spatially homogeneous parenchyma from contrasted texture Golden Delicious (Go) and Granny Smith (Gr) apples were sampled and vacuum infused with enzymes in buffer solution aimed at maintaining and homogenising turgor pressure and limiting oxidation. Glucanases specific from xyloglucan and/or cellulose backbone and glycoside hydrolases (α-fucosidase, and β-galactosidase) were used to study the impact of xyloglucan and its side chains on parenchyma mechanical properties by dynamic mechanical analysis.
The results emphasize the key roles of side chains in the regulation of mechanical properties and are interpreted as resulting from direct modifications of polysaccharides conformations and interactions or indirect regulation of endogenous enzymes involved in the remodelling of networks assemblies. This study explores how xyloglucan can be related to the mechanical function emerging at the tissue scale and provides insight into structure-function relationships during plant organ development.

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Membrane Assembly and Interactions of Individual CesA Transmebrane Helices by Site-directed Spin-labelling EPR

Li L., Voynov M.A., Smirnov A.I.

Department of Chemistry, North Carolina State University, 2620 Yarbrough Drive, Raleigh, NC 27695-8204, USA.

Over the last decade site-directed spin-labelling (SDSL) EPR emerged as a powerful biophysical method for studying structure and local dynamics of proteins and other biomolecules without their crystallization. The method is based on a covalent modification of a protein side chain with small (molecular volume similar to phenylalanine) nitroxide molecular tags and analyzing EPR signals for effects of rotational dynamics, polarity, or dipolar interactions that are determined by distances to other nitroxides. Here we employ SDSL EPR to study membrane insertion and helix-to-helix interactions of selected transmembrane helices (TMHs) of CesA in order to verify CesA computational and homology models. A series TMH 4 and 5 with single point Cys mutations and no connecting loop were prepared using solid-state peptide synthesis, covalently modified with an EPR-active side chains at selected positions of the peptide sequence, and inserted into bilayers prepared from long-chain (DOPC), short chain (DLPC), or mixed DOPC/DLPC lipids. Formation of α-helices by these two TMHs was verified by CD. Local polarity experienced by the nitroxide-labeled side chains was measured based on the exquisite sensitivity of EPR parameters (i.e., g-factor and Aiso) to dielectric and hydrogen-bonding effects. Further, we investigated effects of a membrane-spanning α-helical WALP23 peptide on TMHs' membrane insertion and helix-to-helix interactions detected directly from EPR spectra. Polarity and depth parameter Φ profiles for bilayers of the same lipid compositions were separately calibrated using WALP23. One of the conclusions of this work is that the specific lipid composition of the bilayers and the presence of other membrane-spanning helices appear to be an important requirement for proper insertion of individual CesA TMHs in lipid bilayers.

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