Hemicelluloses: Molecular structure, interactions in woody tissues and properties

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WWSC is a joint research center at KTH and Chalmers
The Plant Cell Wall

Primary Plant Cell Wall

Secondary Plant Cell Wall

Biological and Technical Importance of Wood Heteropolysaccharides

Cell wall integrity, connectivity with cellulose/lignin, regulatory functions

Increase the material efficiency of lignocellulose biorefineries

Cellulose
Lignin
Extractives
Hemicelluloses

Wood Hemicelluloses:
Structural Definition and Heterogeneity

Teleman et. al., Carb. Res. (2000) 329 807; Shatalov et. al. Carb. Res. (1999), 320, 93 ...
Function of the Intramolecular Pattern in Hemicelluloses

Functions of hemicellulose intramolecular pattern

- Modulates macromolecular flexibility and **conformation**
- Modulates local **hydration, ionic strength** and **pH**
- Tailors **interactions** with wood components (cellulose and lignin)
- Prevents **enzymatic degradation** of cell walls
- Influences **macroscopic properties** (solubility, mechanical, rheology)

Picture courtesy of Prof. G. Henriksson (KTH)
Increased molecular understanding of structure-function-properties

**Plant molecular biology**

**Advanced molecular characterization (NMR/MS-based glycomics)**

**In silico molecular simulations**

*Grantham et al. Nature Plants (2017), 3, 859–865*

*Berglund et al. The Plant Journal (2016) 88 (1), 56-70*
### Platform for plant polysaccharide analysis

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<th>Intramolecular Substitution Pattern</th>
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**Techniques**

- **Mass spectrometry** (GC-MS, ESI, MALDI)
- **Liquid Chromatography** (HPAEC, HPLC, SEC)
- **Spectroscopy and Scattering** (NMR, IR, MALLS, DLS, X-ray)
- **Thermo-Mechanical and Imaging** (EM, AFM, tensile testing, DMA, rheometry)

Deciphering the ’Glycome’ of Plant Cell Walls

Scientific Questions

**Heterogeneity of hemicellulose molecular structure:** presence of regular motifs (not random)

**Conformation of plant heteropolysaccharides** in diluted and in arrested states (e.g. in the wood fibre)

**Water – polysaccharide interactions:** solubility, aggregation

**Interactions with cellulose and lignin:** cell wall architecture and recalcitrance

**Molecular heterogeneity limits the use of hemicelluloses in advanced material applications:**
- Need for improved extraction and purification processes
- Controlled modification/polymerization
Hemicellulloses in the WWSC
Impact of glycosidic linkage type on hemicellulose flexibility.

Molecular Dynamics (MD) simulations

Flexibility increases

Regular Decoration Motifs in Spruce Xylan Modulate Adsorption onto Cellulose Surfaces

**XynC GH30 glucuronoxylanase**

**Presence of regular intramolecular motifs in spruce xylan:**

Major domain with **even spacing** of substitutions (Araf and mGlcA)

Minor domain with **consecutive glucuronation**

Regular Decoration Motifs in Spruce Xylan Modulate Adsorption onto Cellulose Surfaces

3₁-fold flexible helical conformation in solution

X₆

X₆AmU

2₁-fold planar screw adsorbed onto cellulose surfaces

X₆

‘Hydrophilic’

(1-10)

‘Hydrophobic’

(200)

Ara and mGlcA decorations in xylan favour interactions with cellulose surfaces.

Adsorption of xylan is enhanced on the ‘hydrophobic’ surfaces: importance of ‘non-polar’ interactions

Figures prepared by Jennie Berglund and Jakob Wohlert.

Regular Decoration Motifs in Spruce Xylan Modulate Adsorption onto Cellulose Surfaces

- Effect of the **geometry of the cellulose microfibril** section and surfaces
- **Steric hindrance** of consecutive substitutions on the adsorption of additional xylan chains
- **Energetic balance**: anchoring effect of substitutions vs. stabilizing effect of multiple chains
- Impact on biomass recalcitrance: accessibility of cellulose for enzymatic saccharification
- Significance of *in vitro* adsorption studies


Fernandes et al. (2012) PNAS, 108: E1195-E1203

M. Busse-Wicher et al. (2016) Plant Physiology, 171 (4) 2418-2431
Sequential Extraction of Hemicelluloses from Birch Wood with Subcritical Water

Birch Chips → Defatting → SWE (170°C, pH 5) → Sequential Subcritical Water Extraction

Interconnected hemicellulose and lignin recalcitrance in birch hardwoods

Interconnected acetylation and glucuronation patterns in birch xylan.

Recalcitrance is correlated with lower acetylation and tighter glucuronation: connectivity with lignins through LCCs.

Enzymatic toolbox for hemicellulose modification and deconstruction


Effect of acetylation on hemicellulose properties

Acetylation inhibits microbial degradability of mannans

Chemical acetylation hinders mannannase activity

Reducing sugars (mM)

Time (h)

Interactions with wood polymers

Natural and chemically acetylated mannans

Biodegradability

Mannanase activity

Acetyl esterase activity

Regioselectivity of acetyl esterases

Figures prepared by Jennie, Berglund, Jenny Arling Bååth, Lauren McKee and Sylvia Klaubauf


S Klaubauf, LS McKee, F Ibatullin, L Olsson, F Vilaplana. In preparation
Galactoglucomannan Structure Impacts Degradation under Alkaline Conditions

Mechanisms for alkaline hydrolysis

Monitoring structural changes (molar mass/monosaccharide content)

Combined hydrolysis and peeling mechanisms in alkaline degradation. Stabilizing effect of Gal substitutions: ‘sandwich’ structure

Figures prepared by Jennie Berglund

Investigation of hemicellulose molecular (in)solubility using advanced scattering and microscopic techniques.

**Transient molecular solubility** is observed at low concentrations, whereas increased concentration results in phase separation and association into fractal aggregates.

Water is a poor solvent for hemicelluloses.
Outlook: Future Challenges

**Molecular structure and conformation *in planta***:
- Use of model biological systems (plant cell cultures, designer hemicelluloses through plant molecular biology ...)
- Integration of advanced molecular characterization, *in silico* modelling and *in situ* visualization

**Hemicellulose hydration and aggregation in diluted/semi-diluted systems**

**Overcoming the heterogeneity challenge for advanced material applications**:
- Hemicelluloses with high purity and controlled molecular structures
- Chemo-enzymatic toolbox for targeted modification/polymerization
- Hemicelluloses as molecular anchors for cellulose surface and wood fibre modifications.
Acknowledgements