PHYSICAL CHEMISTRY 2018

14th International Conference
on Fundamental and Applied Aspects of
Physical Chemistry

Proceedings
Volume I

September 24-28, 2018
Belgrade, Serbia

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ISBN 978-86-82475-36-1
Title: Physical Chemistry 2018 (Proceedings)
Editors: Željko Čupić and Slobodan Anić
Published by: Society of Physical Chemists of Serbia, Studentski Trg 12-16, 11158, Belgrade, Serbia
Publisher: Society of Physical Chemists of Serbia
For Publisher: S. Anić, President of Society of Physical Chemists of Serbia
Printed by: ″Jovan″, <Printing and Publishing Company, 200 Copies
Number og pages: 550+6, Format B5, printing finished in September 2018

Text and Layout: ″Jovan″

200 - *Copy printing*

ORIENTATION OF CELL WALL POLYMERS IN THE *Arabidopsis thaliana* STEM

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ABSTRACT
Mechanical and physical propreties of plant fibres are dependent on the orientation of constituent polymers (cellulose, hemicellulose, lignin). Fourier transform infrared (FTIR) microscopy was used to examine the orientation of the main plant polymers in transversal and longitudinal direction of the isolated cell wall of the *Arabidopsis thaliana* stem. The polarised FTIR measurements indicated that xylan and glucomannan have parallel orientation with regard to the orientation of cellulose, as well as lignin.

INTRODUCTION
Plant cell wall (CW) can be considered as a nano-composite in which cellulose, lignin and hemicelluloses are interconnected in a specific manner. It is well recognised that the cell wall development and expansion and deposition implies an anisotropic arrangement of the cell wall components [1]. Structural organisation of the cell wall and related polymers is important for both mechanical properties of plants and chemical reactions occurring in the wall space, especially in the response to stress. Understanding the arrangement and anisotropy of the polymers in cell wall is important for understanding the mechanical properties of plant, which has implications in plant response to stress. By using imaging FT-IR microscopy, run in transmission mode and at different polarisation modes (from 0° to 90°), it is possible to follow the chemical variability and the orientation of cell wall polymers [2]. The orientation of cellulose, hemicellulose (xylan and glucomannan) and lignin, as essential components of the plants, were analysed by FTIR with regard to the sample axis.

EXPERIMENTAL
The purified isolated cell wall material was obtained from *Arabidopsis thaliana* stem by methanol extraction and subsequent purification using a

series of solvents (phosphate buffer, 1% Triton X-100, 1M sodium chloride, distilled water, methanol, acetone) [3]. FTIR microscopy measurements were carried out using a Spectrum Spotlight 400 FTIR Imaging System (Perkin Elmer Inc, Shelton, CT, USA). Spectral resolution: 8 cm^{-1}; spectral range: from 1800 cm^{-1} to 720 cm^{-1}. Polarisation: the incident IR radiation was polarised by a gold wire grid polariser from 0° to 90° polarisation in relation to the fibre orientation with intervals of 5°. The sample was mounted on the sample stage as parallel as possible to the orientation of the 0° polarisation. The IR spectra were processed by the software Spotlight 1.5.1, HyperView 3.2 and Spectrum 6.2.0 (Perkin Elmer Inc., Shelton, CT, USA) [2].

RESULTS AND DISCUSSION

From the in-depth study of polymer orientation, three areas from the sample were selected. The transmission spectra recorded at 0° and 90° polarisation modes were processed using a ratio function to produce an orientation spectrum ($R = A_{0°} - A_{90°}$), where R is the anisotropy spectra, indicating the orientation of components, $A_0{}^o$ is the absorbance spectra recorded at 0° and $A_{90°}$ is the absorbance spectra recorded at 90°. Spectral signals related to absorptions from cellulose, xylan, glucomannan and lignin in the wavenumber range between 1800 cm^{-1} and 720 cm^{-1} can be identified. Figure 1 (left) shows average absorbance spectra at 0° and 90° polarization angle and Figure 1 (right) shows the average orientation spectra for the *Arabidopsis thaliana* stem cell wall. In Figure 1 (right), the positive signals indicate that their corresponding functional groups are arranged in a more parallel orientation to the fibre axis, and the negative signals indicate that their corresponding functional groups are arranged in a perpendicular orientation to the fibre axis.

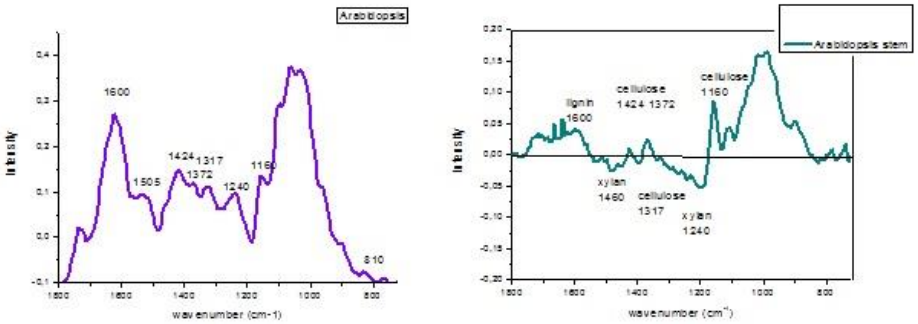

Figure 1. Average absorbance spectra of *Arabidopsis thaliana* stem cell wall (left); The average orientation spectra of *Arabidopsis thaliana* stem cell wall (right).

The relative absorbance spectra are presented (Figure 2) as specific absorption peaks ($RA = (I_p - I_{min}) / (I_{max} - I_{min})$) where RA is relative absorbance, I_p is intensity of the absorbed IR radiation at a given angle of the polarisation, P, I_{max} is maximal intensity observed for a given vibration and I_{min} is minimal intensity observed for a given vibration. These relative absorbance values were presented in relation to the angle of the incident IR polarisation (from 0° to 90°).

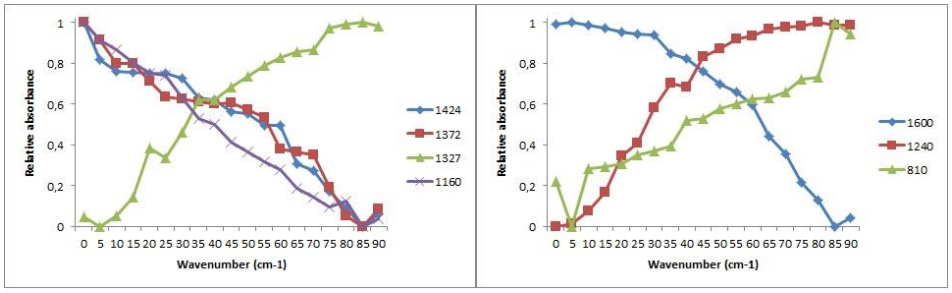

Figure 2. The relative absorbance of IR specific absorption wavenumbers plotted against the polarisation angle for the cellulose (left) and xylan, glucomannan and lignin (right) for *Arabidopsis thaliana* stem.

It is evident (Fig. 2) that the three cellulose peaks (1160 cm^{-1},1370 cm^{-1} and 1424 cm^{-1}) [3-5] had high absorption levels at low polarisation angles, which is a consequence of a more parallel orientation of the corresponding groups to the CW longitudinal axis. The fourth cellulose peak (the perpendicular signal at 1317 cm^{-1}) had the greatest intensity at a high polarisation angle, due to the perpendicular orientation of the corresponding group (Fig. 2). For the xylan, the characteristic band signal (1240 cm^{-1}) [4-6] increased with an increase in the polarisation angle. Due to the parallel orientation of these side groups in xylan, an orientation parallel to the longitudinal CW axis is indicated (Fig. 3). A glucomannan absorbance vibration, indicating the orientation of glucomannan, was observed at 810 cm^{-1} (equatorially aligned hydrogen on the C_2 atom in the mannose residue) [4, 6]. The peak was negative (Fig 3.). This group is oriented orthogonally to the glucomannan backbone, which is the reason why it may be concluded that glucomannan is also oriented in parallel to the CW longitudinal axis. For the lignin, the characteristic band signal (1600 cm^{-1}) [4-6] decreased with an increase in the polarisation angle (Fig. 3), indicating that lignin is organised in parallel with the longitudinal CW axis.

CONCLUSIONS

It has been demonstrated here that xylan and glucomannan are oriented in parallel to the cellulose and more or less parallel to the axis of a cell wall, in isolated CW fragments from *Arabidopsis thaliana* stem. There was also a clear indication of lignin being oriented in parallel to the longitudinal CW axis. This indicates that both lignin and hemicelluloses enhance the main role of cellulose in elastic/stiffness property of CW. It is believed that this structuring of the lignin in the S_2 layer of the cell wall might be the result of the spatial constrains within the cell wall, as occuring due to the previous deposited cellulose/hemicellulose organization of a strongly oriented assembly.

Acknowledgement

This work was supported by Ministry of Education and Science of Republic Serbia (Project #173017, 45012) and COST Action FP0802.

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