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# Viscoelastic properties of pectin/cellulose composites studied by QCM-D and oscillatory shear rheology

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ABSTRACT

The interactions between cellulose and pectin polysaccharides in primary plant cell walls are not fully understood, although several recent studies indicate that they might play an important role in wall properties. Studying polysaccharide interactions *in planta* is challenging, due to the complexity and heterogeneity of plant materials. Therefore, to investigate these interactions and the implications for the rheological properties of cell walls, we have taken a bottom-up approach in which cellulose/pectin composites are created either by adsorption of pectin polysaccharides (arabinan, galactan, homogalacturonan DE 69, homogalacturonan DE 33 and pectin DE 33) on cellulose-coated sensors in a quartz crystal microbalance with dissipation monitoring (QCM-D) or by incorporation of pectin during *in vivo* cellulose synthesis by *Komagataeibacter* bacteria. The viscoelastic behavior of the adsorbed layers was analyzed by applying the Voigt model to the QCM-D data, whilst the bulk viscoelastic properties of bacterial cellulose/pectin composites were studied by small amplitude oscillatory shear rheology. Our results show that all of the pectin polysaccharides studied have the ability to adsorb on the cellulose surfaces. The viscoelastic properties of the adsorbed layer varied depending on the substitution and degree of esterification of the pectin polysaccharides. Additionally, oscillatory rheology results showed that all bacterial cellulose-pectin composites had a gel nature ($G' > G''$) with moduli varying in line with QCM-D determined viscoelasticity. Our interpretation of the results provides a better understanding of pectin-cellulose interactions and has implications for primary plant cell wall material properties.



## 1. Introduction

Plant cell walls are renewable resources with many biotechnological applications. Whilst the chemical composition of primary plant cell walls is well established, the interactions of wall components and their role in cell properties requires more systematic studies. Such knowledge would improve the use of cell wall materials in food and the design of more sustainable processes to deconstruct cell walls to use its components as food ingredients or biomaterials. Cellulose, hemicelluloses (such as xyloglucans (XG) and arabinoxylan (AX)), and pectins are the main polysaccharides found in primary plant cell walls. Cellulose, the most conserved polysaccharide in primary plant cell walls, is composed of unbranched (1, 4)-linked β-D-glucan chains tightly connected by hydrogen bonds to form semi-crystalline microfibrils (Cosgrove, 2005; Waldron, Parker, & Smith, 2003). Cellulose has been proposed to have the main structural and load-carrying roles (Keegstra, 2010). Hemicelluloses have been shown to be able to bind directly to cellulose microfibrils, through hydrogen bonds (Lerouxel, Cavalier, Liepman, & Keegstra, 2006; Li, Jones, & McQueen-Mason, 2003). However, despite being one of the main components of the primary plant cell walls of dicots/gymnosperms and non-commelinid monocots (but not the commelinid monocots), little is known about the interactions of pectins with other cell wall components (Cosgrove, 2005). Pectin, the most complex and heterogeneous group of polysaccharides in the primary cell wall, is characterized by a high content of galacturonic acid (GalA). Pectins consist mainly of homogalacturonan (HG), rhamnogalacturonan I (RG I) and rhamnogalacturonan II (RG II), probably as a connected macromolecule containing all three components

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(Mohnen, 2008). Homogalacturonan (HG) is a negatively charged macromolecule which consists of α-1,4-linked galacturonic acid (GalA) that is often methyl-esterified at the C-O-6 carboxyl(Cosgrove, 2005). According to their degree of methylesterification (DE), pectins are classified as low DE (DE< 50%) and high DE (DE > 50%).

RG I consists of (1, 2)-α-L-Rha-(1, 4)-α- D-GalA residues which can be further substituted at O-4 by neutral side chains including arabinan and galactan (Harholt, Suttangkakul, & Scheller, 2010; Naran, Chen, & Carpita, 2008). Arabinan contains a backbone of (1, 5)-linked α-L-Ara (arabinose), while galactan consists of (1, 4)-linked β-D-Gal (galactose), occasionally containing 1-6-links (Mohnen, 2008). Recent studies indicate that pectins are in close proximity to cellulose in Arabidopsis cell walls (Wang, Zabotina, & Hong, 2012) suggesting that the interactions found between cellulose and pectins *in vitro* (Chanliaud & Gidley, 1999; Lin, Lopez-Sanchez, & Gidley, 2016) are also possible in the plant cell wall.

The complex and highly dynamic nature of plant cell walls makes it difficult to unravel the interplay between wall components and their individual functions. Therefore, to better understand cell wall architecture, simplified models are required as biomimetic tools. Among these simplified models to investigate physicochemical interactions are *in vitro* studies, which investigate adsorption of polysaccharides in solution (Chanliaud & Gidley, 1999; Gu & Catchmark, 2014; Hayashi, Marsden, & Delmer, 1987; Lin, Lopez-Sanchez, & Gidley, 2015, 2016; Whitney, Gothard, Mitchell, & Gidley, 1999; Zykwinska, Ralet, Garnier, & Thibault, 2005). These studies have shown that pectins, independent of their degree of substitution, are able to bind to cellulose (Zykwinska et al., 2005). Cellulose/polysaccharide composites based on bacterial cellulose (BC) have been widely accepted as model systems of relevance to understanding plant cell walls, since they are synthesised in an assembly manner that has some similarity to that proposed for plants. In these systems, cellulose is deposited into a (fermentation) medium containing polysaccharides, in the same way that cellulose synthesised at the plasma membrane in plants is deposited into an (extracellular) medium, which contains polysaccharides previously synthesised (Chanliaud & Gidley, 1999; Whitney et al., 1999). Although these models can generate knowledge about polysaccharide interactions and macromolecular properties, it is difficult to measure surface properties on a short time scale. Therefore, complementary techniques which make use of sensor technology are being used to investigate polysaccharide binding interactions (Cho, Frank, Kasemo, & Hook, 2010; Marx, 2003). Acoustic- and optical-based sensor techniques, such as surface plasmon resonance (SPR), ellipsometry, reflectometry and quartz crystal microbalance with dissipation monitoring (QCM-D), can quantitatively monitor changes in film thickness and mass without the addition of external labels (Huang, Chang, Chao, Wu, & Huang, 2014; Ogimoto, Selyanchyn, Takahara, Wakamatsu, & Lee, 2015; Takayama, Nasuno, Iimura, Morohoshi, & Kato, 2015; Wu, Ma, Gu, & He, 2015). Among these techniques, QCM-D uniquely captures the mass and energy-dissipating properties at the nanoscale, which can be related to the viscoelastic properties of the resulting films (Antosiewicz, Senkara, & Ciesla, 2015; Bruschi & Mistura, 2015). QCM-D allows quantification of low deposition levels and can help in understanding interaction mechanisms, and kinetics, at the solid-liquid interface (Ali et al., 2015; Mechler et al., 2007; Ogimoto et al., 2015).

In the present study, we investigated the adsorption behaviour of five different pectin polysaccharides (arabinan, galactan, homogalacturonan DE 69, homogalacturonan DE 33 and pectin DE 33) on cellulose membranes using QCM-D. The viscoelasticity of the resulting surfaces was analysed using the Voigt model. In addition, the viscoelastic properties of previously produced bacterial cellulose composites, containing the same type of pectins, were measured using a stress-controlled rheometer. The objective of this study is to enhance our understanding of pectin-cellulose interactions and their potential impact on primary plant cell wall material properties. Furthermore, the findings may aid the design of processes to enhance deconstruction of cell walls for diverse biotechnological applications.

## 2. Materials and methods

### 2.1. Polysaccharide solutions

In this study, five types of pectin polysaccharides were used: sugar beet arabinan (Megazyme, Ireland), potato debranched galactan (Megazyme, Ireland), commercial citrus pectin CU (Herbstreith and Fox HG, Neuenbürg/Württ, Germany), high DE homogalacturonan and low DE homogalacturonan (CP Kelco, Denmark). The composition of the polysaccharides was analysed using the alditol acetate method for neutral sugars and the total uronics assay for acidic sugars (Filisetti-Cozzi & Carpita, 1991; Lin et al., 2015). The detailed chemical composition of each pectin is described in Table 1. Polysaccharide solutions for QCM-D experiments were prepared by dissolving the polysaccharides in ultrapure water at a concentration of 1% (w:v). To remove air bubbles, prior to QCM-D experiments, all the solutions were sonicated using an ultrasonic dismembrator (Model 150 T, Thermo Fisher Scientific, Waltham, MA) in pulsed mode for 30 min at room temperature.

### 2.2. Viscosity measurements

The viscosities of 1% (w:v) polysaccharide solutions were measured in a stress-controlled rheometer (AR G2, TA Instruments, USA). Samples were measured at 25 °C using a cone-and-plate geometry with a cone diameter of 5 cm and an angle of 2.2°. The measurements were performed in steady flow mode with a shear rate between 1 and 100 $s^{-1}$. Samples were measured in triplicate.

### 2.3. Quartz crystal microbalance with dissipation monitoring (QCM-D) measurements

The properties of the adsorbed layers were investigated in a Q-Sense E4 system (Biolin Scientific AB, Sweden). The cellulose-coated sensors (QSX 334), with a fundamental resonance frequency of 4.95 MHz, were cleaned with two cycles of the following procedure before each usage: soaking in ultrapure water on a shaker at 50 rpm overnight and blow-drying with nitrogen gas. QCM-D experiments were carried out at 25 °C in exchange mode at a flow rate of 100 μL/min. The flow schedule was as follows: (i) background solution (ultrapure water) to establish a baseline; (ii) polysaccharide solution for testing; and (iii) background solution (ultrapure water) for rinsing. Once the frequency (F) and dissipation (D) signals were constant they were offset to zero, which were then monitored for an additional 10–15 min to obtain the

**Table 1**
Monosaccharide composition and viscosity (1%, w:v) of pectic polysaccharides. Viscosity values are the average and standard deviation of three replicates. Different letters in the same column denote significant differences.

| Samples | Ara | Gal | Rha | Xyl | Glu | GalA | Viscosity (mPa.s) |
|---|---|---|---|---|---|---|---|
| Arabinan | 71.0 | 26.0 | 3.0 | 0.0 | 0.0 | 0.0 | $1.48 \pm 0.05^{a}$ |
| Galactan | 3.0 | 88.0 | 3.0 | 0.0 | 0.0 | 6.0 | $1.62 \pm 0.02^{b}$ |
| Pectin DE33 | 10.9 | 26.8 | 3.1 | 1.8 | 0.0 | 56.4 | $72 \pm 0.1^{c}$ |
| HG DE69 | 2.8 | 3.6 | 1.0 | n.d. | 0.3 | 92.3 | $19 \pm 0.3^{d}$ |
| HG DE33 | 2.8 | 3.7 | 1.0 | n.d. | 0.2 | 92.3 | $56 \pm 0.5^{e}$ |