Novel methods to investigate metal interactions with plant cell walls

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Abstract
Low concentrations of some metals markedly reduce root elongation rate and cause ruptures to root rhizodermal and outer cortical cells in the elongation zone. The interactions between metals and plant components responsible for these effects are not well understood but may be linked to changes in water uptake, cell turgor, and cell wall extensibility. Bacterial cellulose (BC)-pectin composites, used as plant cell wall analogs were used as a model system to investigate metal interactions with plant cell walls. Experiments were conducted to examine changes in hydraulic conductivity of BC-pectin composites with metal treatment and the effect of aluminium (Al) on BC-pectin composites uniaxial tensile properties. Hydraulic conductivity of the composites was reduced to ≈ 30 % of the initial flow rate by 39 μM Al and 0.6 Cu μM, ≈ 40 % by 4.6 μM La, 3 μM Sc and 4.4 μM Ru, and ≈ 55 % by 3.4 μM Gd. Aluminium had no measurable effect on the uniaxial tensile properties of the BC-pectin composites.

Key Words
Cell wall, bacterial cellulose, aluminium, toxicity, tensile testing, hydraulic conductivity

Introduction

The toxicity of cationic trace elements to plant roots increases with decreasing soil pH, which is particularly known to lead to aluminium (Al) toxicity in acid soils since Al constitutes ca. 7 % of mineral soils. Toxicities of other trace metals are less common due to low concentrations in parent materials, except in some instances. A distinct symptom of metal toxicity is inhibition of root elongation, and the consequent development of a stunted root system. It has been extensively hypothesised that metals interact with the cell wall through interactions with pectin (Blamey, 2003; Kopittke *et al.,* 2008; Wehr *et al.,* 2004) which makes up 30 % by weight of primary plant cell walls. The apoplast, which is composed of cell walls and inter-cellular spaces, constitutes 5 % or less of root tissue volume but it is important for uptake and transport of water, nutrients, and growth regulating components. The direct consequences of the interactions of metals with the cell wall are not well understood and hotly debated. Physical properties of primary cell walls of plants are difficult to investigate due to their small cell size and heterogenous composition. Yet, BC-pectin composites in their natural hydrated state (typically more than 90 % water) mimic the hydration state of primary plant cell walls, and provide a useful model system for plant cell walls.

Methods

Bacterial composite preparation and characterisation

Gluconacetobacter xylinus strain ATCC 53524 from the American Type Culture Collection (Manassas, VA, USA) was used to form a pectin composite or pellicle (Chanliaud and Gidley 1999). The bacterial strain was cultured in modified Hestrin and Schramm (HS) medium containing 5.5 g l^{-1} peptone, 5.5 g l^{-1} yeast extract, 11.4 g l^{-1} potassium hydrogen phthalate, 0.16 g l^{-1} NaOH and 2 % (w/v) glucose (Hestrin and Schramm 1954). Citrus pectin (Sigma-Aldrich, Australia) (0.5%), de-esterified via alkali treatment to degree of esterification of 33%, and 12.5 mM $CaCl_2$ was added to this medium (initially at pH 4.0). Static incubations were performed at 30 °C for 96 h in 70 mL sterile specimen jars with a diameter of 42 mm, with the resulting pellicle floating on the surface of the medium. The BC-pectin composites were then harvested, gently shaken to dislodge some of the embedded cells, and rinsed in ice-cold 12.5 mM $CaCl_2$ under gentle agitation. Pellicles were stored at 4 °C in 0.02 % NaN_3 until required (i.e. within 2 weeks).

Pectin incorporation into the pellicle (dry weight basis) was determined using the modified colorimetric assay for galacturonic acid (Filisetti-Cozzi and Carpita 1991) after prehydrolysis for 1 h in concentrated H_2SO_4-borate at 0 ºC. The chromophore 3-phenylphenol was used, and sulfamic acid was added as the colour depressant for neutral sugars. The BC-pectin composites all consisted of approximately 30 % pectin by weight, (data not shown) which is consistent with optimum composite formation (Chanliaud and Gidley, 1999) and also reflects the pectin content of primary plant cell walls.

Hydraulic conductivity measurements

The concentrations of metals selected were those found to reduce elongation of cowpea (*Vigna unguiculata* L.) roots by approximately 50 %; 30 μM Al, 0.3 μM Cu, 2 μM La, 1.2 μM Gd, 1.3 μM Ru and 1.8 μM Sc (Kopittke *et al.,* 2008; Kopittke *et al.,* 2009). To measure saturated hydraulic conductivity, pellicles were placed in a flow cell (Figure 1). The glass frit funnel, flow cell and burette was filled with 1 mM $CaCl_2$ solution (ca. pH 5.4), and the burette inserted through the top rubber seal of the flow cell. Thereafter, the burette stopcock was opened and the flow rate through the pellicle was measured by recording the level of $CaCl_2$ solution in the burette every 5 min for 45 min to determine initial flow rate. Then, 5 ml of either 1 mM $CaCl_2$ (controls, pH ≈ 5.4) or metal chloride solution adjusted to pH 4.0 was injected through the rubber septum at the side of the flow cell using a hypodermic syringe. The flow rate was then recorded every 5 min for another 45 min. The treatments were replicated four times. Hydraulic conductivity, *K,* was calculated using the falling head method (Klute and Dirksen, 1982) (1);

$$K = \frac{aL}{At}\log_e\left(\frac{h_1}{h_2}\right) \quad (1)$$

with *A*, cross sectional area of the sample; *L*, thickness of the specimen; *t*, time and *a*, the cross sectional area of the burette. These remained constant for each test, allowing the percentage difference in *K* to be calculated from the log of the ratio of initial and final head (h_1 and h_2). Solution within the flow cell was sampled at the end of the experiment (90 min) and analysed via ICP OES or ICP MS (no solution analysis was performed for Al).

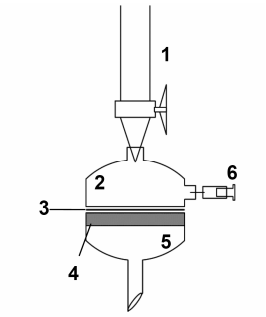

Figure 1. Schematic diagram of flow cell. A standard burette (1) attached to the inlet side of the flow cell (2). The BC-pectin pellicle (3) sits on top of the glass frit (4) which is part of the outlet side of the flow cell (5). A clamp is used to secure the pellicle between (2) and (5). A syringe is inserted through a sealed opening (6) to inject the metal solution at the start of the second 45 min period and to sample solution at the end. The diameter of the flow cell was 10 mm.

Tensile testing

After overnight equilibration in 500 mL of 40 μM, 80 μM or 160 μM $AlCl_3$ in a background of 12.5 mM $CaCl_2$ at pH 4.0, BC-pectin composites were removed from the beakers and tensile tested. Mechanical properties of hydrated bacterial cellulose-pectin pellicles were assessed by uniaxial tensile testing using an Instron 5543 (Instron, Melbourne, Australia) as in McKenna *et al.* (2009). Briefly, dumbbell shaped strips (of known dimensions) were cut using a dumbbell press (ISO 37-4). Three dumbbells were cut per pellicle, five pellicles were tested for each treatment. The two ends were placed directly between vice grips, and moved apart at a constant speed of 10 mm/min. A 5 N load cell was used to record the force required for extension as a function of time. From the geometrical measurements, force-deformation data were converted to stress-strain profiles. Engineering stress (σ) was calculated by F/A where A is the area measured and F is the force in N. Strain (ε) was calculated by ΔL/Lo where ΔL is exerted extension from the starting point Lo, and converted to a percentage. Data was plotted as stress/strain profiles (Figure 2(a)) for each treatment.

Results and discussion

Hydraulic conductivity

All metal treatments caused a significant reduction in hydraulic conductivity compared to the control ($P < 0.001$), with hydraulic conductivity in the control (Ca) treatment reduced to only 80 % (Table 1). However, the injection of metals into the flow cell reduced hydraulic conductivity to approximately 40 % for Sc and La, 45 % for Ru, 55 % for Gd, and to approximately 30 % for both Al and Cu (Table 1). We hypothesise that the observed reduction in hydraulic conductivity following metal treatment was a result of conformational change of the pectic fraction of the composite. Scanning electron micrographs (results not shown) indicated differences in the structure of the composites at the end of the experiment (McKenna *et al.,* 2009a).

Metal interaction with the pectin component of the composite is expected to take place either by the exchange of the metal ion tested with Ca^{2+} (as the reticulating cation of the gel), or by the adsorption to any protonated negative charges within the system. The high background of Ca within the system (see Table 2), meant it was not possible to distinguish between exchange or adsorption reactions. It would be expected that carboxyl groups within the pectin would be the predominant source of available binding sites. The magnitude of the reduction was similar for all metals. However, the concentrations of metals causing this reduction were very different. The metals, and their concentrations were selected based on the concentrations causing a 50 % reduction in root elongation in Kopittke *et al.* (2008; 2009). Similar levels of reduction in hydraulic conductivity seen across the trivalent metals suggest a relationship between these metals, which may contribute to the 50 % reduction in root elongation.

Table 1. Metal effects on relative saturated hydraulic conductivity and metal concentration in solution and in the composite at the completion of the experiment.

Metal and concentration (µM)	Relative flow rate (flow rate during second 45 min period relative to the first 45 min period) (%)*	Average metal concentration in flow cell after 90 min (µM)	Average metal concentration in digested pellicle (sampled at end of 90 min) (µg/g)
Al 39	28.3 a	Not measured	Not measured
Cu 0.6	29.3 a	Not detected (< 0.16 µM)	Not detected (< 10 µg/g)
La 4.6	41.9 ab	4.00	55
Sc 3.0	42.5 ab	1.10	8.0
Ru 4.0	45.3 ab	1.39	94
Gd 3.4	54.5 b	1.48	7.7
Ca (control) 1000	80.8 c	1000	52000

*For the mean percentage change in flow rate, means with same subscript are not significantly different at P <0.001 (LSD = 19.5).

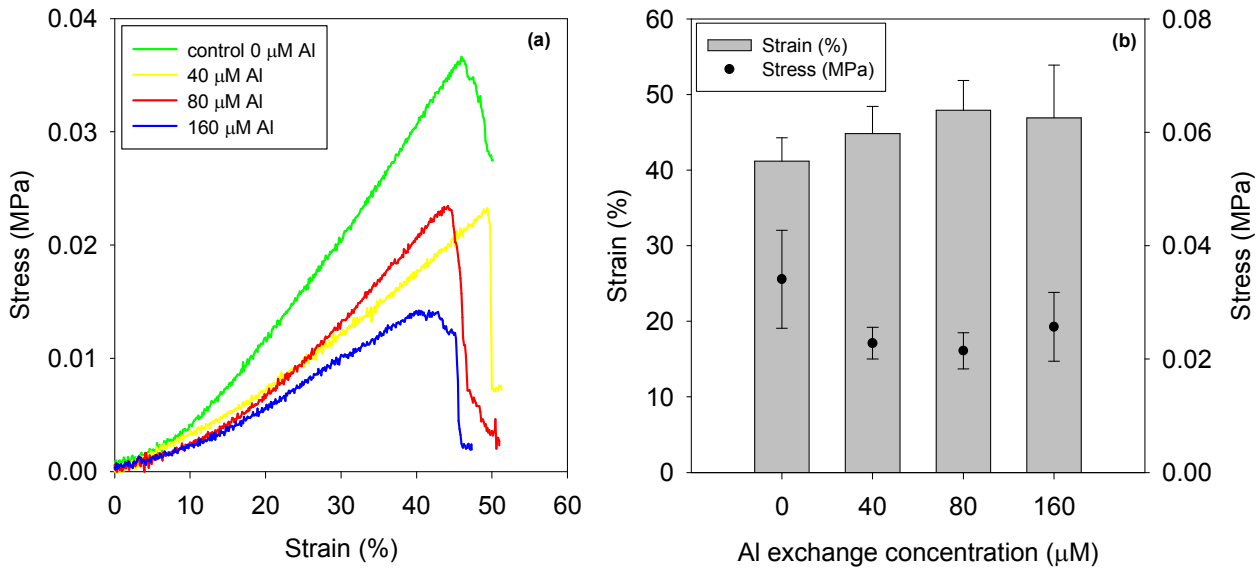

Figure 2. (a) Example stress versus strain plots for control BC-pectate pellicles and three Al treatments as indicated on the graph. (b) Average stress (scatter plots) and strain (bars) values at the three Al concentrations.

Tensile testing

Overall, the presence of Al, at the concentrations tested, had no significant effect on the tensile properties of BC-pectate composites (Figure 2(b)). The current widely-accepted cell wall model (for Type I cell walls) depicts the plant cell wall containing three structurally independent but interpenetrating networks (Carpita and Gibeaut, 1993). In this model pectin is not considered a 'load-bearing' component, instead this function is fulfilled by the cellulose-xyloglucan 'scaffolding' network. Cellulose is able to reinforce cell walls under stress by orientating in the direction of stress and it is the mean orientation of cellulose that has been shown to determine wall mechanical properties (Kerstens *et al.,* 2001). Chanliaud *et al.* (2002), using BC-pectate composites showed, that upon removal of the pectin component of the composite, tensile deformation profiles remained largely the same, indicating that cellulose was the main contributor to the tensile strength of the composite. However, the presence of pectin modified the arrangement of the cellulose microfibrils as they were being deposited which is analogues to the deposition of the plant cell wall (Chanliaud *et al.,* 2002).

The results of the current study further support these past findings, because despite changes in the pectin component of the composite, by the addition of Al, no effect was evident on the stress and strain profiles. However, it is still likely that changes to the pectin component of the composite occurred, through the adsorption of Al. Mimmo *et al*. (2005), showed, with FT-IR, the adsorption of Al onto preformed Ca-pectate gels weakened the overall structure of the gel. Modifications of pectin within plant cell walls has shown to have an impacts on the strength of the cell wall, by modulating access of wall-modifying enzymes to load bearing hemicellulose and cellulose fraction (Ben-Shalom, 1986).

Conclusions

Bacterial cellulose-pectin composites have been used, for the first time, as a model system to study metal interactions with plant cell walls. Metal interactions with the pectic component of the composite showed a marked decrease in hydraulic conductivity but the magnitude of reduction was similar for all metals suggesting a relationship between the metals which may contribute to the 50 % reduction in root elongation they cause in plant roots. There was no change in the tensile properties of the composites, after treatment with Al. This finding is consistent with the current cell wall model in which cellulose is the major load bearing cell wall component, and hence changes in the pectic component (through binding of Al) is unlikely to have an impact on uniaxial tensile properties. BC-pectin composites offer a novel system to further investigate hypotheses about metal interactions with plant cell walls.

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