Ion induced changes in the structure of bordered pit membranes

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INTRODUCTION

Self-acting hybrid materials are used in a wide range of micro-devices ranging from flux control in microfluidic systems using gels (Beebe et al., 2000) to shape memory actuators (Vyawahare et al., 2008). The growing interest in the use of "smart materials" results from the fact that such materials do not require external triggers, rather they simply respond to changes in their surroundings. Such autonomously acting materials occur within the plant vascular network. Trees must move large volumes of water from soil to atmosphere to sustain photosynthetic activity. Because the supply of water in terrestrial ecosystems is often limiting, the ability to tune the distribution of water across plant's dendritic structure may play an important role in optimization of resource use (Holbrook and Zwieniecki, 2005; Zwieniecki and Holbrook, 2009; Shackel et al., 2010). The movement of water through the plant vascular system occurs via specialized micro-channels composed of dead cells arranged in series. The ability of these cells to respond to the ionic concentration in the transpiration stream allows the seemingly dead xylem micro-channels to modulate their hydraulic properties (Zwieniecki et al., 2001). Bordered pit membranes create a porous boundary between adjacent micro-channels, that prevents the spread of embolism that could halt water transport from soil to leaves and ultimately cause plant death. Pit membranes are a composite material that includes cellulose, lignin, hemicelluloses, and pectin hydrogels (Zwieniecki and Holbrook, 2006; Liu et al., 2004). It also provides a significant hydraulic resistance to xylem sap flow that was shown to be variable in response to sap ion concentration.

Research on the structure and function of bordered pit membranes most often consists of either the indirect study of their properties by inferring them from analysis of liquid fluxes across plant stem segments (Zwieniecki et al., 2001) or scanning electron microscope (SEM) observation of dry plant material (Choat et al., 2006). This comes from the fact that bordered pit membranes are very small (~5 μm diameter) and hidden among overarching heavy cellulose-lignin walls that prevent easy access to membrane surface. More importantly, bordered pit membranes in vivo are immersed in water and in an intact plant, cations in the surrounding solution "open" the bordered pit membrane, facilitating the movement of water. This observed hydraulic activity is the bases for the hydrogel hypothesis that the positive ion shrinks the hydrogel (pectin) covering the nanofibrils of membrane changing its porosity. However, hydraulic measurements do not provide direct evidence of physical bases for the altered flow resistance change. SEM makes it possible to image the nano-fibers but this is usually done using imprints or visualizing dried samples imaged in high vacuum (Jansen et al., 2009). Using environmental SEM it is possible to image bordered pit membranes in humid air at some level of hydration but never under water and never with the resolution needed (Kirk et al., 2009). Recently, new insights into bordered pit membrane structural properties have been obtained using atomic force microscopy (AFM) on air-dry material (Pesacreta et al., 2005;
Dute and Elder, 2011). The obvious next step is to use liquid phase AFM to investigate how the structure of bordered pit membranes changes in response to the ionic composition of surrounding solution and what properties of this composite material are responsible for the changes in its hydraulic properties.

**MATERIALS AND METHODS**

**SAMPLE PREPARATION**

Tobacco plants (*Nicotiana tabacum* L.) were grown in a greenhouse under natural light conditions until stem diameters exceeded 10 mm. A 5-cm-long stem segment was excised and the xylem sectioned longitudinally into 50 μm thick sections, while supplying DI water to prevent samples from drying out. Bordered pit membranes are covered by a secondary wall having 1 by 5 μm elliptical openings. To access and image the pit membrane in AFM, the secondary wall has to be removed without damaging the pit membrane underneath. Longitudinal sectioning sometimes separates from the primary walls leaving intact bordered pit membranes (Figure 1A). As localization of these regions using AFM is exceedingly difficult, the sections were first examined with a 50X optical microscope to locate areas with exposed and undamaged bordered pit membranes. These areas were marked so that they could be located in the AFM. Sections were stored in DI water upon transfer from the light to the AFM microscope. Sample preparation proved to be extremely difficult. Most often the entire process resulted in sections that were unsuitable for AFM scans due to tilt, unexposed or torn bordered pits, problems impossible to detect in light microscope. Thus only a small number of replicates gave useful results despite hundreds of attempts. For visualization purpose only we also used environmental SEM (99% relative humidity in 0.01 MPa pressure) to image exposed bordered pit membranes prepared in the same way as for AFM analysis.

**AFM OBSERVATIONS**

A MFP-3D-BIO™ Atomic Force Microscope (Asylum Research Inc.) was used in the tapping mode to image hydrated bordered pit membranes. Sectioned samples were mounted in the closed fluid cell of the AFM using double-sided tape (3M). Bordered pit samples were submerged and imaged first in DI water followed by imaging in 50 mM KCl (Sigma Aldrich, NE). The cantilever tip was kept in same position to allow for visualization of pit membrane structure resulting from alterations in the ionic concentration of the liquid medium. The sample and AFM cantilever were aligned using an Olympus 1 × 81 inverted microscope (Olympus Inc). A 60 μm long silicon nitride bio-lever (Olympus Inc) provided the necessary flexibility for working with submerged samples. Scan rate was 0.3 Hz, around four to five times slower than with dry samples, with scan speed corresponding to the size of the image. The second harmonic resonance frequency was 51.8 kHz and the image resolution was 512 by 512.

**RESULTS**

**BORDERED PIT MEMBRANE IN DI WATER**

Atomic force microscopy revealed that the hydrated pit membrane is covered with a relatively disorganized material. Surprisingly, distinct microfibrils, which are usually quite obvious in SEM images (Figure 1A), were not observed. Instead, elevation images reveal a bumpy, but relatively featureless surface across the entire pit membrane (Figure 1B). The roughness of the surface was determined using: 

$$\bar{y} = \frac{1}{N} \sum_{i=1}^{N} y_i^2,$$

where $n$ is the number of analyzed pixels, $y$ is measured height and $\bar{y}$ is the height average. Average roughness was 12.6 nm with SD = 2.18 and $N = 10$ ($N$ is the number of independent roughness determinations). The surface of the border pits lacked any visible holes or voids, which are often reported from analysis of dry samples.

The relatively featureless structure of the material covering border pit membranes and lack of the visible cellulosic fibers found on dry imprints of the membranes in SEM studies raises the question of the physical properties of this surface covering. We believe that the microfibrils are hidden inside this material and that under dry conditions the material collapses, behaving as hydrogel. To determine if this hypothesis was true we adjusted the AFM tip position such that the AFM tip penetrated deeper into the covering material. During the tapping mode, AFM is designed to keep the position from the sample by feedback control. The position from the surface is determined by the set point, which is typically 80% of free amplitude (a delicate tap that results in very low tip displacement). To probe deeper into the pit membrane we reduced the set point from analysis of dry samples.

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**BORDERED PIT MEMBRANE IN KCl**

Replacing DI water with a 50 mM KCl solution resulted in an immediate and marked change in membrane structure. We chose...
Mechanism of Hydraulic Actuation in Xylem

Figure 2 | AFM height images with set point of AFM tip position at (A) 80% of free amplitude (B) 55% of free amplitude. In (B) the AFM tip penetrates the soft material of the bordered pit membrane, exposing the nanofiber structure (seen as straight lines in the red circle). The greater number of scratches in the deeper image (B) is the result of adhesion between AFM tip and the soft matrix.

Figure 3 | Typical AFM height images with (A) DI water and (B) 50 mM KCl taken at the same position. Application of KCl both reduces the height of the border pit membrane and reveals much more structured surface.

50 mM KCl based on previous experience that this concentration results in the maximum effect on membrane hydraulic properties (Zwieniecki et al., 2001). Upon exposure to 50 mM KCl, the cantilever tip had to be moved downward around several hundred nanometers to have the same set point. This suggests that the top surface of the bordered pit membrane also moved downward. This may indicate the collapse of the soft material. In support of this, the surface was much harder and the microfibrils more exposed (Figure 3). We did not observe the formation of any holes or cracks in the membrane that could have contributed to a lower hydraulic resistance. Instead, the major changes observed included a drop in height which can be interpreted as a decrease in overall membrane thickness (Figure 4) and a significant increase in surface roughness. The average roughness of bordered pit surface increased from 12.6 (in DI water) to 16.6 nm (SD = 1.36 and N = 10; in 50 mM KCl). This change was significant with t-value = 4.94, df = 18, and p < 0.001. The increase in mean roughness is consistent with a reduction in membrane thickness. We also observed a change in the signal to noise ratio from 4.2 to 5.5 determined as mean to SD. This change in signal to noise ration was consistent with the presence of harder structures, such as microfibrils, covered by a thinner layer of hydrogel.

Figure 4 | Schematic representation of a bordered pit membrane in cross section (A) before adding KCl and (B) after adding KCl. The reduction in hydraulic resistance results in part from shortening the water path across the membrane (gray microfibril (blue) pectin hydrogel). Also depicted is the change in surface roughness in 50 mM KCl.

Discussion
Comparative analysis of pit membrane surface properties in DI water and 50 mM KCl revealed several important and unexpected properties that enhance our understanding of how water moves through this hybrid material. The current paradigm portrays pit membranes as a microfiber network with discrete holes (Kiss et al., 2009). This view is supported by SEM observations of dry material. In contrast our observation of fully hydrated pit membranes did
not reveal any holes, but instead a continuous surface of soft materi-

covering fibers with well pronounced elevation features. Upon

transfer from DI water to 50 mM KCl, this soft surface underwent a

conformational change in its physical properties from soft to hard

and from thick to thin, a behavior that is consistent with the prop-

erties of hydrogel (Tanaka, 1981). We believe that this observation

provides strong support for the notion that bordered pit mem-

branes are composite materials formed from fibrous structure

submerged in amorphous gel.

This seemingly simple observation has important implications

for our understanding of the hydraulic properties of bordered

pit membranes. In particular, it suggests that water does not flow

through discrete holes or pores, but instead has to pass through

the soft gel (pectin) matrix. Thus, the physical description of

water transport across pit membranes in most angiosperms may

be similar to what occurs in bio-inspired materials that can sus-

tain tension and operate in a similar manner to xylem or leaves

(Noblin et al., 2008; Wheeler and Stroock, 2008). In particular,

we may need to change our models for how tension is gener-

ated in leaves, as well as how the embolism spreads in the xylem,

from ones dominated by surface tension and meniscal failure to

ones that emphasize the osmotic forces that can occur within

hydrogels.

This study also shifts our understanding of how pit membrane

hydraulic permeability is altered in response to ionic strength of

xylem sap. Increase in hydraulic conductance may result from

changes in membrane thickness (shortening the water path across

the membrane) and changes in permeability of the charged

hydrogel (Mattern et al., 2008), while previously proposed changes

in the dimension of channels might be less influential (Zwieniecki

et al., 2001). This finding underscores the role of pit membranes in

protection from embolism as ionic strength does not necessarily
decrease the pressure threshold for the air to pass from one vessel
to another (Cochard et al., 2010). In addition, the observed com-

posite nature of the pit membrane explains their ability to recover

from mechanical damage (Sperry and Hacke, 2004), as changes in

gel hydration allow cellulose microfibrils to shift within the

network and thus prevent the formation of permanent holes or

cracks. Such healing properties are especially important for plants

that sustain multiple cycles of embolism and embolism removal
during drought stress (Sperry and Hacke, 2004). The conforma-

tion response of pit membranes to variation in ions reported here

suggests that we need to replace the current emphasis on the struc-
tural framework provided by the cellulose microfibrils in flow

models dominated by the properties of the gel matrix (Wheeler

and Stroock, 2008).

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REFERENCES

Beebe, D. J., Moore, J. S., Bauer, J. M., Yu, Q., Liu, R. H., Devadoss, C., and

Jo, B. H. (2000). Functional hydro-
gel structures for autonomous flow

control inside microfluidic channels.

Nature 404, 588–590.

Chao, B., Brodie, T. W., Cobb, A. R., Zwieniecki, M. A., and Holbrook, N. M.

(2006). Direct measurements of inter-

vessel pit membrane hydraulic resist-

ance in two angiosperm tree species.

Am. J. Bot. 93, 993–1000.

Cochard, H., Herbette, S., Hernandez,

E., Holotta, T., and Mencuccini, M.

(2010). The effect of sap ionic com-

position on xylem vulnerability to


Force microscopy of torus-bearing

pit membranes. IAWA J. 32, 415–430.

Holbrook, N. M., and Zwieniecki, M. A.


Physiol. 141, S302–S302.

Jansen, S., Chao, B., and Pfeeters,

A. (2009). Morphological vari-

ation of intervessel pit membranes and

implications to xylem func-

tion in angiosperms. Am. J. Bot. 96,

409–419.

Kirk, S., Skepper, J., and Don-


of environmental scanning electron

microscopy to determine biological

surface structure. J. Microsc. 233,

205–224.

Kiss, J. Z., Chao, B., Pittermann, J.,

Strullu-Derrien, C., Rioult, J. P., and


into bordered pit structure and cavi-

tation resistance in angiosperms and


Liu, L. S., Cooke, P. H., Coffin, D. R.,

Fishman, M. L., and Hicks, K. B.

(2004). Pectin and poylacrylamide

composite hydrogels: effect of pectin

on structural and dynamic mechan-

ical properties. J. Appl. Polym. Sci. 92,

1893–1901.

Mattern, K., Nakornchai, C., and


of agarose-glycosaminoglycan gels

analyzed using fiber-mixture and

Donnan models. Biophys. J. 95,

648–656.

Noblin, X., Mahadevan, L.,

Coomaraswamy, I. A., Wex, D. A.,

Holbrook, N. M., and Zwienie-


density in artificial and real leaves.


9140–9144.

Pesacreta, T. C., Groom, L. H., and


microscopy of the intervessel pit

membrane in the stem of Sapinum

sebiferum (Euphorbiaceae). IAWA J.

26, 397–426.

Shackel, K. A., Brodersen, C. R., McEl-

rone, A. J., Chao, B., and Matthews,

M. A. (2010). The dynamics of

embolism repair in xylem: in vivo

visualizations using high-resolution

computed tomography. Plant Phys-

iol. 154, 1088–1095.


Analysis of circular bordered pit

function – I. Angiosperm vessels

with homogenous pit membranes.


Vyasahare, S., Sitaula, S., Martin, S.,


Electronic control of elastomeric

microfluidic circuits with shape

memory actuators. Lab. Chip 8,

1530–1535.

Wheeler, T. D., and Stroock, A. D.

(2008). The transpiration of water at

negative pressures in a synthetic tree.

Nature 455, 208–212.

Zwieniecki, M. A., and Holbrook, N. M.

(2000). Bordered pit structure and

resistance in two angiosperm tree


Zwieniecki, M. A., and Holbrook, N. M.

(2001). Hydrogel control of xylem hydraulic resistance in


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