RESEARCH PAPER

Tension required for pectate chemistry to control growth in Chara corallina

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Abstract

Recent work showed that polygalacturonate (pectate) chemistry controlled the growth rate of the large-celled alga Chara corallina when turgor pressure (P) was normal (about 0.5 MPa). The mechanism involved calcium withdrawal from the wall by newly supplied pectate acting as a chelator. But P itself can affect growth rate. Therefore, pectate chemistry was investigated at various P. A pressure probe varied P in isolated walls, varying the tension on the calcium pectate cross-links bearing the load of P. When soluble pectate was newly supplied, the wall grew irreversibly but the pectate was inactive below a P of 0.2 MPa, indicating that tension was required in the existing wall before new pectate acted. It was suggested that the tension distorted some of the wall pectate (the dominant pectin), weakening its calcium cross-links and causing the calcium to be preferentially lost to the new pectate, which was not distorted. The preferential loss provided a molecular mechanism for loosening the wall structure, allowing the polymers to be more rapidly displaced by tension, and accelerating growth. The newly supplied pectate then developed cross-links with the existing wall that together with free Ca2+ entering the wall re-tightened the structure. Growth decelerated. The balance of these loosening and tightening actions in the same pectate molecules controlled the rate of wall extension.

The balance is important because the cross-links bear the load of P (Proseus and Boyer, 2006c). If most of the calcium was removed from the cross-links, the wall burst (Proseus and Boyer, 2006c). The deposition of new wall typically prevents bursting. Plant cell walls grow about 10–100-fold and would become much thinner if new wall were not added (Roberts, 1994). Wall thickness is usually maintained to within a factor of about two during the growth process (Taiz, 1984; Kutschera, 1990, and references therein; Bret-Harte et al., 1991). Using labelled substrates in living cells, new wall was deposited at a rate linked to the rate of growth driven by P (Cleland, 1967; Proseus and Boyer, 2006a). Proseus and Boyer (2005, 2006a) found that the P acted to concentrate polysaccharides in the periplasm next to the inner wall face. The

Key words: Cell enlargement, cell wall, pectin, turgor pressure.

Introduction

This work was undertaken to determine whether turgor pressure (P) affects the chemistry of pectate in cell walls. Proseus and Boyer (2006c) reported that the chelation chemistry of calcium-linked pectate (polygalacturonate) controlled the growth rate of Chara corallina cells when turgid. Calcium and magnesium were the only inorganic elements detected in the walls, and the calcium served to cross-link neighbouring pectate to form some of the gel-like structure of the wall matrix. Magnesium appeared inactive in this structure. Newly supplied pectate removed calcium from the cross-links, loosening the structure, allowing the polymers to be more rapidly displaced by tension, and accelerating growth. The newly supplied pectate then developed cross-links with the existing wall that together with free Ca2+ entering the wall re-tightened the structure. Growth decelerated. The balance of these loosening and tightening actions in the same pectate molecules controlled the rate of wall extension.
pectate began to gel and the gelling was promoted by calcium, creating a cross-linked gel-like matrix at the inner wall face that also penetrated the existing wall and would surround cellulose microfibrils being produced at the same time (Proseus and Boyer, 2005, 2006a). Because the microfibrils are transverse to the long axis of the cell (Baskin, 2005), they prevent lateral expansion and force the pectin-containing wall matrix to extend longitudinally.

The finding that a wall constituent such as pectate also controlled growth rate suggested a molecular link between growth and wall deposition (Boyer and Proseus, 2006c). Further evidence for a link was seen with isolated walls, which do not deposit new wall. At first the wall grew rapidly, but after 2 h it lost most of its activity even though growth-promoting P was present (Proseus and Boyer, 2006b). The loss was overcome by supplying soluble pectate to the wall as though the cytoplasm had done so. A single exposure rejuvenated wall growth for several hours (Proseus and Boyer, 2006b). If sufficient Ca\(^{2+}\) was added for the pectate to gel and bind to the wall, the rejuvenating activity was immediately quenched (Proseus and Boyer, 2006c). This indicated that cytoplasmic release of new pectate would control growth initially but be followed by deposition of new wall.

Each of these features was identified in living cells and their isolated walls at normal, steady, growth-promoting P while the cells or walls remained in the culture medium in which they were grown. If P is reduced, however, it is well known that wall extension decreases (Cleland, 1971; Taiz et al., 1981; Taiz, 1984). Below a critical threshold, growth ceases. Any molecular mechanism for growth must account for this P dependency. In the following work, P-induced tensions are varied in walls isolated from Chara cells without altering any other condition, and the effects on pectate chemistry are monitored.

Materials and methods

Chara corallina (Klien ex. Wild., em. R.D.W.) was cultured in the laboratory as described previously (Zhu and Boyer, 1992; Proseus et al., 1999). Briefly, the rhizoids of Chara were anchored in sediment above which the thallus extended into water occasionally supplied with 1 mM chloride salts of Na, K, Mg, and Ca. The sediment and plants kept the cultures at pH 7–8 and the walls accumulated large amounts of Ca and Mg from the medium (Proseus and Boyer, 2006c). The plants grew at about 23 °C in continuous photosynthetically active radiation of 10–15 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) from sunlight and fluorescent bulbs. The medium was dilute, having an osmotic potential of about –0.01 MPa. In this situation, P essentially balanced the osmotic potential of the cytoplasm, and growth-induced water potentials were negligible (Boyer and Silk, 2004). The experiments were conducted at 23 °C. Young internode cells were obtained from the apex of the thallus to ensure the cells were growing and contained primary walls. The cell walls were isolated from the young cells by opening one end and removing the cytoplasm with gentle stroking in the culture medium until no green remained and the walls had been gently rinsed. Walls prepared in this way are free of organelles and detritus visible by confocal microscopy (Proseus and Boyer, 2005, 2006a). The walls remain permeable to small solutes, which readily moves uniformly from the lumen through the wall to the external medium (Proseus and Boyer, 2005, 2006a).

**P generation and control**

The walls were glued to the tip of a microcapillary while in the culture medium, as detailed in Proseus and Boyer (2005, 2006a). The microcapillary was mounted on a pressure probe (Steudle and Zimmermann, 1974) and the wall was held in a shallow plastic cup with 6 ml of culture medium. Mineral oil from the pressure probe was injected to fill the lumen previously occupied by the cytoplasm, as shown in Proseus and Boyer (2005). ‘Artificial’ P as high as 0.5 MPa was generated in the lumen by moving a rod into or out of the oil reservoir in the pressure probe. A P of 0.5 MPa is similar to that causing growth in the living cells before harvest. Stable P developed in the isolated walls because the oil was trapped inside the lumen by the surface tension between the oil and water at the inner wall face, and the oil did not enter the wall structure. The same forces prevent the plasma membrane from entering the wall structure in living cells.

The pressure probe included an automatic turgor control system consisting of a small electric motor attached to a programmable switch that could be set to maintain a steady P or to change P rapidly.

**Pectate chemistry from growth measurements**

When P develops in the lumen, the wall immediately deforms elastically in a reversible fashion (Proseus et al., 1999). Superimposed on this deformation is a slower, time-dependent deformation that is irreversible and considered to be ‘growth’. Because the cellulose microfibrils in the wall prevent most lateral deformation, the growth is mostly longitudinal. The growth responses immediately to calcium withdrawal from the wall by newly supplied pectate, demonstrated by Proseus and Boyer (2006b, c). Consequently, measuring the longitudinal growth of the isolated wall indicated the activity of the calcium pectate. In the present work, the pectate activity was measured with a radial position transducer attached to the free end of the isolated but oil filled and pressurized wall, as described by Proseus et al. (2006b). A datalogger (CR7X; Campbell Scientific, Logan, UT, USA) and strip chart recorder monitored the output every 5 s from the position transducer, pressure probe, and a thermocouple immersed in the medium around the isolated wall. A computer continually displayed the change in wall length \(\Delta L\) and elongation rate \(dL/d\tau\). The data in the datalogger were downloaded to a computer for processing.

**Pectate characteristics**

Polygalacturonic acid [poly-(1–4)-D-galacturonate, 80% potassium salt, denoted as PGA] was used for the experiments. Obtained from citrus albedo (Sigma, St Louis, MO, USA), its molecular weight was 170 kDa (about 945 saccharide residues) measured by size exclusion chromatography at the Complex Carbohydrate Research Center, Athens, GA, USA. The PGA was added to culture medium taken directly from the Chara cultures filtered to 0.2 \(\mu\)m and, if necessary, adjusted to pH 7 before use. In the calcium-containing medium, the PGA remained soluble unless additional Ca\(^{2+}\) was added, as shown by Proseus and Boyer (2006c).

**Results**

**Tension and pectate action**

Proseus and Boyer (2006b) found that young isolated walls grew lengthwise in a P-dependent manner like that
in live cells. In the present work, the isolated walls behaved similarly in the presence of PGA, showing an obvious dependence of growth on $P$. If $P$ was low at 0.26 MPa and subsequently increased to 0.47 MPa (Fig. 1A), the length increased dramatically and settled to an accelerated but steady lengthening (Fig. 1B). If $P$ was returned to its original low level of 0.26 MPa, the original rate returned, but the steady component at high $P$ was seen as an increased length, thus showing that the steady component at high $P$ was irreversible. The persistence of this increase in length with PGA was identical to the behaviour previously observed in live cells and isolated walls by Proseus and Boyer (2006b) and considered to be growth.

It was possible to measure the response of growth to $P$ in these walls, then add PGA and repeat the measurement in the same walls. The response was first determined during $P$ steps-down, then PGA was added and the response repeated during $P$ steps-up as shown in Fig. 2. For a single isolated wall, the growth rates decreased (Fig. 2B) as $P$ was stepped down (Fig. 2A) until growth became zero at 0.42 MPa. The growth resumed slowly if PGA was added (arrow, Fig. 2B). Further steps-down were required before the PGA-induced growth became zero at 0.34 MPa. If $P$ was stepped up, cell extension accelerated markedly. This flush of extension then settled to a steady, rapid rate. At the final $P$ identical to the original $P$, growth with PGA was faster than the original rate. If PGA was not added, the response during steps-down was reiterated during steps-up, and the growth at the final $P$ was equal to the original rate [data not shown, but see Proseus et al. (2000) for this behaviour in live cells]. The temperature was steady during these manipulations, indicating that the growth response was caused by PGA and not a thermal change (Fig. 2C). Mature cells did not respond to PGA (Proseus and Boyer, 2006c).

The same experiment in many walls showed that growth without PGA began at $P$ above 0.34 MPa, as shown by the open circles in Fig. 3A. This represented a threshold $P$ at about 75% of the normal $P$ in a live cell (open circles in Fig. 3B). If PGA was supplied as in Fig. 2, the same walls grew more rapidly, but the growth again required $P$ above a threshold of about 0.2 MPa (closed circles, Fig. 3A). The threshold was 45% of the original $P$ (closed circles, Fig. 3A).

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**Fig. 1.** Irreversible growth of isolated wall of *Chara* internode in culture medium containing PGA (170 kDa, 6 mg ml$^{-1}$, 35 μM, pH 7): (A) turgor pressure ($P$) applied to oil in wall lumen; (B) wall lengthening ($\Delta L$).

**Fig. 2.** Growth of isolated *Chara* cell wall before and after PGA was added to the culture medium: (A) turgor pressure ($P$) applied to oil in wall lumen; (B) wall extension ($\Delta L$); (C) temperature ($T$). The point at which PGA (170 kDa, 12 μM, pH 7.0) was added is indicated by an arrow.
Fig. 3B), i.e. it appeared lower than in the absence of PGA.

**Discussion**

**Pectate requires tension for activity**

Pectate, when newly supplied as PGA, expressed growth activity only if the cell wall was under tension from $P$. The tension had to exceed a threshold, thus resembling the threshold turgor that must be exceeded before growth begins in live cells of *Chara* (Proseus *et al.*, 2000) and other plants (Cleland, 1971; Taiz, 1984). The growth exhibited the same irreversible behaviour reported by Proseus and Boyer (2006b) for live cells and untreated walls. This behaviour is diagnostic for the growth of young plant cells. Therefore, PGA appeared to operate on the same molecular properties of the wall that controlled growth in the live cells.

How could tension affect the activity of newly supplied pectate? In the isolated wall, calcium is a critical constituent bearing the longitudinal load for growth because removing >95% of wall calcium caused the isolated wall to burst (fig. 9 in Proseus and Boyer, 2006c). This finding is unequivocal evidence for load-bearing by wall calcium.

Newly supplied pectate removed only moderate amounts of wall calcium (fig. 4A in Proseus and Boyer, 2006c). With partial removal, the wall did not burst and instead grew faster. When the walls were returned to the same culture medium but without pectate (containing 0.61 mM Ca$^{2+}$), growth immediately reverted to the original rate (figs 1 and 3 in Proseus and Boyer, 2006c). Clearly, the removed calcium had vacated sites in the wall that could be re-filled by normal levels of Ca$^{2+}$ in the culture medium.

Plant cell walls typically contain large amounts of pectin (Morrison *et al.*, 1993; Ridley *et al.*, 2001; Popper and Fry, 2003). In charophyte walls, the pectin is mostly PGA with a small amount of polyglucuronic acid (Morrison *et al.*, 1993; Popper and Fry, 2003). Other forms such as rhamnogalacturonans appear to be rare or absent (O’Neill *et al.*, 2004). The galacturonic acid

![Fig. 3. Growth of isolated cell walls when $P$ was varied (open circles), then repeated in the same walls after PGA was added to the culture medium (closed circles): (A) growth rates ($dL/dt$) for each wall at various $P$; (B) same as (A) but normalized to the original growth rate and original $P$ for each wall. Details for one wall are shown in Fig. 2. Many walls were isolated from young *Chara* internodes and are represented by individual data in this figure. PGA was 170 kDa, 12 μM, pH 7.](image)

![Fig. 4. Pectate structure and suggested distortion in *Chara* cell walls under tension. (A) Ladder-like structure of polygalacturonate (PGA). Hydrogen and hydroxyl groups are not shown. (B) Two anti-parallel, cross-linked PGA chains before tension was applied. The bold portion of pectate in (A) binds two of the 10–20 calcium pectate cross-links for a junction zone in magnified view (with calcium as black ovals). (C) Same as (B) but chains are in moderate tension (small arrows). (D) Same as (B) but chains are in high tension (large arrows). It is proposed that high tension distorts the cross-links, causing calcium to be released more readily (dashed arrow) than from undistorted links.](image)
residues in PGA are mostly unmethylated (Anderson and King, 1961; Morikawa and Senda, 1974; Gillet et al., 1992, 1998; Morrison et al., 1993; Gillet and Liners, 1996; Popper and Fry, 2003). The weakly dissociating carboxyl groups form non-covalent associations with divalent cations, especially calcium (Grant et al., 1973; Morris et al., 1982; Powell et al., 1982; Jarvis, 1984; Jarvis and Apperley, 1995; Ralet et al., 2001; Willats et al., 2001). Removing wall PGA removes the ability of the charophyte wall to bind calcium (Gillet et al., 1992). Therefore in the charophyte wall, load-bearing by calcium is shared with pectate.

In this study, about 31% of the di-galactosyl carboxyl groups of pectate were occupied by calcium (Appendix table 2 in Proseus and Boyer, 2006c). Although removing nearly all of this calcium caused the wall to burst, 95% of it could be removed without losing wall integrity, i.e. only 1.6% of the possible calcium pectate bonds bore the entire load of tension in the wall (figs 1, 3, and 4A in Proseus and Boyer, 2006c). Thus, in normal conditions, load-bearing calcium pectate is surrounded by a large reservoir of calcium pectate that is not load-bearing. When new pectate removed moderate amounts of calcium from the wall and accelerated growth, its action necessarily included the small group of load-bearing bonds that controlled growth rates.

This behaviour was mimicked by the strong calcium chelator EGTA [ethylene glycol-bis(beta-aminoethyl ether) N,N,N’,N”-tetracetic acid]. When supplied at low concentration, EGTA removed 95% of the wall calcium (fig. 4A in Proseus and Boyer, 2006c). Growth accelerated (figs 1 and 3 in Proseus and Boyer, 2006c). Removing the EGTA by returning the walls to the culture medium quenched this activity (medium contained 0.61 mM Ca^{2+}). Also, when the medium contained the EGTA, adding 50 mM Ca^{2+} quenched the activity. Newly supplied PGA acted similarly and thus may be considered a chelator.

All of the results in the present work were obtained with isolated walls and were independent of the cytoplasm. The chelating activity of PGA thus acted only on the wall itself. After isolation, the walls could have contained active wall enzymes but the growth of the walls was unaffected by boiling, eliminating wall enzymes as contributors (figs 5C and 6C in Proseus and Boyer, 2006b). It appeared instead that physico-chemical properties of the wall structure governed the activity of newly supplied PGA.

**Proposed molecular mechanism**

Given the chelating activity of PGA for calcium, tension is likely to affect the binding affinity between PGA and calcium. PGA has a ladder-like chemical structure with rungs of galacturonic acid and a single side rail consisting of the α-(1–4)oxygenic bonds between rungs (Ridley et al., 2001). Figure 4A shows that the side rail alternates
from one side of the ladder to the other between adjacent rungs. The carboxyl groups also alternate. As a consequence, it has been proposed that two anti-parallel ladders create an ‘egg-box’ structure that sequesters Ca\(^{2+}\) with coordination bonds as in Fig. 4B, shown for two calcium cross-links (Grant et al., 1973; Morris et al., 1982; Powell et al., 1982: Jarvis, 1984; Jarvis and Apperley, 1995; Ralet et al., 2001; Willats et al., 2001). When 10–20 of these calcium cross-links are adjacent in the egg-box, a junction zone forms that holds the pectate ladders together, with sufficient strength to gel. The junction zones would be the sites of load-bearing in the wall.

With the alternating oxygenic side rail of the molecular ladder, PGA is likely to be highly flexible between junction zones (Fig. 4B). When placed under tension, the PGA would stretch and become constrained in its motion with a tendency to straighten (Fig. 4C). Further stretching would distort the ladder structure, increasing the distance between adjacent galacturonic residues as in Fig. 4D. The distortion would lengthen the coordination bonds with calcium, weakening them. Ca–PGA has a normal pK for dissociation of about 3.5 (Van Cutsem and Gillet, 1983; Jarvis, 1984), but, with weakening, the pK would be lower and calcium would dissociate more readily (Fig. 4D, dashed arrow).

Any stretching of calcium pectate bonds would tend to lower the activation energy for bond breakage and give this result. In thermodynamic terms, calcium in the wall would exist in a mixed population of unstretched (pK lower than 3.5) and stretched PGA (pK lower than 3.5), and would tend to migrate toward the bonds with higher pK. It is for this reason that more calcium moved from the wall to the strong chelator EGTA (pK 11) than to the weaker, externally supplied PGA (fig. 4A in Proseus and Boyer, 2006c).

This tension-based model suggests several predictions. First, two kinds of bonds should be detectable when P stretches the wall: weak ones that control coiling and orientation of wall polymers, and strong ones that maintain the configuration of the calcium cross-links. Proseus et al. (1999) and Proseus and Boyer (2006b) detected both of these kinds of bonds, with weak ones seen when the wall underwent reversible elastic stretching by tension (Proseus et al., 1999). This stretching had an undetectable temperature response (Proseus et al., 1999), indicating that the coiling/orientation bonds were weak with a low activation energy. The bonds were thus readily reversed when tension was relieved (Proseus and Boyer, 2006b). The strong ones were observed when tension increased. A threshold tension (threshold P) was required that began to break the strong bonds irreversibly, with a high temperature response and high activation energy in the isolated walls demonstrated in fig. 9 of Proseus and Boyer (2006b). Because this indicates that only a few bonds were under load, these few bonds were the ones accelerating the growth observed here.

A second prediction is that a loss of calcium from the load-bearing bonds would diminish their density per unit of wall, accelerating growth and decreasing the threshold P for growth, which was observed in the present work.

Thirdly, a fraction of the pectate should become oriented when charophyte walls are placed under tension. Using optical methods, Morikawa and Senda (1974) and Morikawa et al. (1974) found that some of the wall pectate aligned parallel to the long axis of the cell if tension was applied to isolated walls of the charophyte Nitella. Richmond et al. (1980) similarly observed increasing order in the walls when placed under tension.

A fourth consequence of PGA distortion is its progressive nature, with greater distortion occurring as tension incrementally increases above the threshold. The increased tension would further weaken load-bearing cross-links, and the wall would irreversibly extend more rapidly. Growth would thus display an upwardly curvilinear response to P. Such a response was observed both in the isolated walls of the present work and in live cells (Fig. 5).

Fifth, the population of PGA molecules in the wall matrix would contain a mix of distorted cross-links (load-bearing under high tension) and normal cross-links (low or no tension) as in Fig. 6A. If this population encounters new PGA (N in Fig. 6A), the new PGA would bind calcium from the distorted chain 2 preferentially (dashed arrow in Fig. 6A). The calcium removed would be tightly bound to chain N because the chain is not distorted (N in Fig. 6B). The loss of calcium from chain 2 would remove its load-bearing function, allowing the chain to relax. The population of wall PGA would extend until chain 4, already stretched elastically, began to distort as it took up further load. The newly distorted link would be susceptible to preferential calcium loss to chain 2, whose relaxed configuration returns its tight binding affinity for calcium (dashed arrow in Fig. 6B).

It follows that further extension of the wall would occur while wall calcium migrates in this fashion throughout the pectate matrix. The action of chain N would thus be catalytic and generate accelerated wall extension for a long time, as observed in fig. 8 of Proseus and Boyer (2006b) where growth was accelerated for several hours in isolated walls of Chara during a single exposure to new PGA. The catalytic activity would be quenched by additional Ca\(^{2+}\) entering the existing wall and/or by incorporating the newly supplied pectate (chain N is now Ca–PGA; Fig. 6B) into the existing wall by cross-linking, as reported by Proseus and Boyer (2006a, c). The resulting new gel structure would be capable of bearing some of the load. The new gel structure would constitute new wall.

In a similar fashion, Ca\(^{2+}\) supplied to growing oat coleoptiles was able to quench part of the accelerating action of auxin, which had increased the supply of pectin (and other matrix precursors) to the walls (Baker and Ray,
1965; Ray and Baker, 1965). Also, calcium chelators enhanced deformation of tomato epidermis when under tension, and Ca\(^{2+}\) abolished the effect (Thompson, 2005). Ca\(^{2+}\) appeared to alter biochemical wall-loosening in oat coleoptiles (Cleland and Rayle, 1977). Passioura and Fry (1992) and Passioura (1994) suggested a tension-based model for plant enlargement that resembled the present concepts, but based on hydrogen bonding by xyloglucans rather than coordination bonding by calcium pectate. In view of the load-bearing ability of calcium pectate in *Chara* and prevalence of pectins in plants, these observations suggest that calcium pectate chemistry might play a wide role in plant enlargement.

**Pectate cycle**

The catalytic, auto-propagating behaviour of calcium pectate under tension suggests an inherently cyclical action of its chemistry. The cycle would begin when tension decreases the strength of the coordination bonds with calcium. Removal of the weakly bound calcium would release the tension, relaxing the pectate and allowing the wall to extend. The relaxed pectate would then be free to re-bind calcium from other parts of the wall and complete the cycle. The auto-propagation inherent in this action would cause the wall to extend for long periods.

The cycle diagrammed in Fig. 7A is shown for a single junction zone considered to be two pectate molecules in anti-parallel orientation cross-linked by, say, 10 calcium bridges to give Pectate\(_2\)Ca\(_{10}\) in an isolated cell wall. Tension above the threshold would distort the junction zone in a few cross-links (Pectate\(_2\)Ca\(_{10}\) in Fig. 7A). Calcium in these cross-links would be preferentially lost to new undistorted PGA (XS Pectate in Fig. 7A). With this loss, wall growth would accelerate (1. Loosen in Fig. 7A), but the loss would relax the wall pectate, allowing it to accept calcium from other distorted cross-links and close the cycle (Fig. 7A). Accelerated growth would continue for as long as the calcium migrates.

In Fig. 7A and the present experiments, PGA was supplied to simulate the activity of the cytoplasm. Because of its large molecular weight, the PGA probably did not enter the wall, as shown by Proseus and Boyer (2005, 2006a). However, calcium had a small diameter and would readily move out of the wall. Load-bearing bonds throughout the wall would respond to PGA in the external solution. Richmond et al. (1980) reported that the inner wall bore most of the load of P, but the inner wall would lose calcium as readily as the other wall parts and thus respond to external PGA.

By supplying the PGA in excess, calcium removed from the walls did not gel the new PGA (Proseus and Boyer, 2006c). Instead, the removed calcium became distributed throughout the new PGA. Junction zones requiring 10–20 adjacent calcium cross-links could not form, and the XS Pectate remained in solution.

Live cells would follow this cycle but with new pectate supplied by the cytoplasm. The supply would not be in excess and instead would be deposited next to the inner wall face, where it would be concentrated by P as shown by Proseus and Boyer (2005, 2006a). As the pectate removed calcium from the wall, wall extension would accelerate as shown above (Proseus and Boyer, 2006c) (1. Loosen in Fig. 7B). Auto-propagation would begin as in Fig. 7A, but the concentrated pectate in the periplasm would begin moving into the existing wall [not shown in Fig. 7B but reported by Proseus and Boyer (2005, 2006a) for similar polysaccharides]. The new pectate (now calcium pectate) would bind to the calcium pectate in the existing wall (dashed arrow in Fig. 7B). Also, Ca\(^{2+}\) could enter from the medium. These actions would inhibit auto-propagation. The gel structure would be tightened [2. Tighten in Fig. 7B; demonstrated as gel-tightening and calcium-tightening by Proseus and Boyer (2006c)]. The binding and tightening by the new pectate would inevitably create new wall (3. New wall in Fig. 7B).
Accordingly, new wall would generate more rapidly when growth rates were rapid than when they were slow, as shown in figs 3 and 4 of Proseus and Boyer (2006a).

**Precautions**

The newly formed wall in Fig. 7B would strengthen the wall and counter the auto-propagation of Fig. 7A if the molecular weight of the new PGA was high enough to form junction zones. PGA of low molecular weight might not be large enough to form junction zones, although it can remove calcium from the wall and accelerate growth (see figs 6–8 in Proseus and Boyer, 2006c). But without the ability to form junction zones, the small PGA would probably allow auto-propagation to continue.

The pectate cycle suggests that new wall would form in stoichiometric proportion to the rate of wall extension. While fully turgid cells do this (e.g. as reported in figs 3 and 4 by Proseus and Boyer, 2006a), P below the threshold relieves the distortion and would block the entire cycle. With blockage, the only remaining activity would be a continued release of new pectate by the cytoplasm. Proseus and Boyer (2006a) found that exocytosis to the wall continued for at least 30 min after low P completely blocked growth in Chara. Similar results were found with pea stems (Robinson and Cummins, 1976), tobacco pollen tubes (Kröh and Knuiman, 1985), and Micrasterias cells (Ueda and Yoshioka, 1976), which synthesized new matrix material, packaged it into vesicles, and secreted it via exocytosis during periods of low P, but without inserting it into the wall. This behaviour indicates that a strict stoichiometry between new wall formation and growth is unlikely to hold in all conditions. Other ways to interrupt the stoichiometry might be by starving the wall for precursors at normal P while the wall continues to extend, or by starving the wall for Ca$^{2+}$. Other cations might interrupt the chelation chemistry of pectate. These kinds of exceptions perhaps account for some of the differences previously noted between wall deposition and growth rates (Taiz, 1984; Kutschera, 1990).

In the present experiments, special care was taken to isolate, briefly wash, and subsequently mount the walls on the microcapillary without removing the walls from the culture medium in which the plants were grown. This precaution ensured that all wall structures and wall enzyme functions were preserved. By filling the wall lumen with oil, an ‘artificial’ P was generated that deformed the wall as it would in the live cell in the culture medium. Ca$^{2+}$ was present at normal low concentrations in the medium at all times (0.61 mM; Proseus and Boyer, 2006c). PGA was supplied to the culture medium.

Boiling the walls before gluing to the microcapillary demonstrated that wall enzymes were not involved in irreversible wall extension (Proseus and Boyer, 2006b), but a source of H$^+$ was absent that is normally present at the inner wall face because of the activity of plasma membrane H$^+$-ATPases (Hope and Walker, 1975; Metraux et al., 1980; Rayle and Cleland, 1992). In live cells, the plasma membrane normally maintains the pH between 4.5 and 5.0 at the inner wall face (Hope and Walker, 1975; Metraux et al., 1980), and an acid growth theory was proposed that loosened the walls at this pH (Rayle and Cleland, 1992). Exposing the isolated walls to this pH, Proseus and Boyer (2006c, their fig. 2A and B) found little if any effect on growth, probably because Ca$^{2+}$ from the medium replaced calcium displaced from the wall by H$^+$. Proseus and Boyer (2006c) also reported that the PGA remained active at pH between 3.5 and 11 in live cells. Therefore, in the culture medium, the primary growth mechanism did not appear to involve acidity and was stable over a range of pH.

**Conclusions**

This work extends the concept that a prominent constituent of the primary cell wall controls the rate of wall growth. The constituent was shown in earlier work to be calcium pectate, and the present results show that tension is required for its action. It is suggested that calcium pectate bonds can be distorted by tension, weakening the calcium bonding which holds the complex together. When tension is above a threshold, the distortion would cause calcium to be lost from the wall and bind to newly supplied pectate, which is not distorted. This molecular action would account for the turgor pressure requirement of plant growth.

With this activity, the wall would enlarge irreversibly because the density of load-bearing calcium pectate in the wall would be decreased by the calcium loss. In living cells, it is suggested that this accelerated growth would be controlled when the new pectate (now calcium pectate) forms new cross-links with the existing wall together with Ca$^{2+}$ entering from outside, thus strengthening the wall. This sequence indicates that a P-dependent pectate cycle may exist in walls of living cells, with deposition of new wall as a consequence.

An underlying feature of this chemistry is the relaxation of tension on the previously load-bearing pectate chains when they lose calcium. The resulting wall extension would cause other chains to come under tension and distort. The relaxed chains would then be able to accept calcium from the newly distorted ones, setting up a catalysis-like reaction of wall loosening auto-propagating throughout the wall. The initial calcium chelation by new pectate would thus act as a catalyst for long-term auto-propagation seen in isolated walls. In the live cell, auto-propagation must be controlled, and wall tightening by depositing new pectate in the wall or incorporating new Ca$^{2+}$ from the medium appears to be part of the control.
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References


