Developmental regulation of pectic polysaccharides in the root meristem of Arabidopsis

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Abstract

JIM 5, an antibody that recognizes a relatively unesterified pectic epitope, distinguishes between dividing (meristematic) and non-dividing (central cells of the quiescent centre) cells in the Arabidopsis root tip, indicating that non-dividing cell walls contain higher levels of relatively unesterified pectin than dividing cells. JIM 7, an antibody that recognizes a relatively methyl esterified epitope, labels all cell walls uniformly throughout the root, suggesting that there is little variation in the relatively methyl esterified pectic component in the two cell types. These observations suggest that the characteristics of cell walls in the root tip result in part from modulations in the amount of unesterified and non-methyl esterified pectin.

Key words: Pectin, quiescent centre, roots, Arabidopsis.

Introduction

Plant cells are surrounded by a wall that is composed of a matrix of complex polysaccharides. There are four main components of this matrix: (1) an array of cellulose microfibrils; (2) hemicellulosic polysaccharides; (3) pectic polysaccharides rich in polygalacturonic acid which are linked to a variety of other sugar moieties; and (4) glycoproteins (Roberts, 1989; Carpita and Gibeaut, 1993). Cellulose microfibrils are synthesized at the plasma membrane by a poorly characterized cellulose synthase complex. The other components of the matrix are secreted into the cell wall by Golgi-derived vesicles beginning at cell plate formation and continuing throughout the growth of the wall. Different components may be incorporated into the wall at different times during its subsequent growth and differentiation. For example, xyloglucan is incorporated into the developing cell plate of tobacco while rhamnogalacturonan-I appears in the wall much later (Moore and Staehelin, 1988). In addition to this temporal variation, there is well-documented variation in the localization of the different components between cells and between different regions of a single cell wall (McCann and Roberts, 1991).

There are three main classes of pectic polysaccharides: polygalacturonic acid (PGA), rhamnogalacturonan-I and II (RG-I and RG-II) (Levy and Staehelin, 1992). PGA is composed of long chains of 1,4-α-linked d-galacturonic acid residues that may be esterified at carbon 6 of each galactosylocturonosyl residue and the extent to which different pectic fractions are esterified can vary greatly. It is generally accepted that pectin is secreted into the wall in its esterified form and is subsequently de-esterified by the action of pectin methylesterases (Kauss and Hassid, 1967; Jarvis, 1984; Zhang and Staehelin, 1992).

It is clear that pectins can play an important mechanical role in the cell wall since cells adapted to growth in the presence of an inhibitor of cellulose synthesis, dichlorobenzonitrile (DCB), maintain their structural integrity with walls composed of predominantly pectic polysaccharides (Schedletzky et al., 1990, 1992). These cells lack almost their entire cellulose and hemicellulose network. The ability of pectin to maintain the integrity of the cell walls in such conditions is most probably a result of its ability to form large cross-bridged complexes (Schedletzky et al., 1992; McCann et al., 1994). The cell walls of the DCB-adapted cells are of normal thickness and porosity suggesting that it may be the pectic fraction that regulates these wall properties (Schedletzky et al., 1992).

The Arabidopsis root is remarkably simple in that the primary root contains an almost invariant number of cells in a precise spatial organization (Dolan et al., 1993). Since cells of all developmental stages are to be found along an individual root it is an ideal system in which to study the dynamic processes of cell wall formation because...
old cell walls can be easily distinguished from young cell walls. For example, many transverse walls in the meristematic zone are developmentally 'young' (laid down recently) while those in the elongation and differentiation zones will be developmentally 'older'. Detailed anatomical, and clonal analysis in combination with 3H-thymidine incorporation experiments have elucidated the cell fates and shown clearly the existence of a quiescent centre composed of four central cells in the primary root (Dolan et al., 1993). It is shown here that the pectic components in cell walls of the central cells of the quiescent centre display characteristics distinct from those of dividing cells.

Materials and methods

Plants and growth conditions

Arabidopsis Columbia seeds were sterilized in 5% Domestos and planted on to Murashige and Skoog medium containing 1% agar and 3% sucrose. Seeds were incubated at 4°C for 48 h and then grown in continuous illumination for 5 d as previously described (Dolan et al., 1993).

Fixation and immunofluorescence and immunoelectron microscopy

Whole seedlings were fixed in 2.5% glutaraldehyde in 50 mM cacodylate buffer pH 7 for 1 h at room temperature. Specimens were dehydrated in an ethanol series and after two changes of 100% ethanol the specimens were infiltrated and embedded in LR White resin. Thin sections were cut on an Ultracut-E microtome and picked up on gold grids for electron microscopy or on glass slides for immunofluorescence.

Grids were blocked in 3% BSA (bovine serum albumin) in TBS (TRIS-buffered saline pH 7) for 20 min before incubating in JIM 5 and JIM 7 monoclonal antibody as neat hybridoma supernatant for 1 h at room temperature (Knox et al., 1990). Grids were washed in three changes of BSA/TBS before incubation in gold-conjugated secondary anti-rat serum (Sigma) for 1 h at room temperature (Knox et al., 1990).

Indirect immunofluorescence with the JIM 5 antibody, which recognizes a relatively unesterified pectin epitope, reveals a generally patchy distribution of such pectin in meristematic cells just above the quiescent centre (Fig. 1B). What label there is, is largely restricted to three-way junctions (Fig. 2) although some labelling may be apparent elsewhere. These cells label uniformly with the JIM 7 antibody (Fig. 1A). As cells cease dividing (cells in the elongation and differentiation zone of the root), the JIM 5 labelling pattern becomes more uniform, note the continuous labelling of cells in the elongation zone of Fig. 1B.

There is a delay in the appearance of the JIM 5 epitope in new cell plates. This is illustrated in median longitudinal sections incubated with JIM 5 in which the cross walls that make T-junctions (arrows in Fig. 3) with adjoining longitudinal walls are not labelled, while the corresponding wall, one cell cycle later, that now forms Y-shaped junctions with the mother wall (arrowhead in Fig. 3), shows strong labelling. The low levels of labelling observed in the T-transverse walls is also to be seen in immunofluorescence images in which such walls label uniformly strong with JIM 7 but only weakly label with the JIM 5 antibody (compare T- and Y- walls in Fig. 1A and B).

Central cells of the quiescent centre are rich in relatively unesterified pectin

The central cells of the quiescent centre are the only cells in the internal region of the root tip that exhibit a uniform distribution of JIM 5 label on all facets (arrow in Fig. 1B and Fig. 4). Figure 4A shows a transverse section through the quiescent centre that has been immunogold labelled with the JIM 5 antibody. Central cells are located at the centre of the image and are flanked by cortical/endo-dermal initials which are, in turn, flanked by epidermal cells. Both these cell types divide more rapidly than quiescent cells. Figure 4B is a higher magnification of the walls between central cells and shows them to be heavily and uniformly labelled with the JIM 5 antibody indicating that these walls possess a uniform distribution of relatively unesterified pectin. Figure 4C shows walls between two endodermal/cortical initials and flanking epidermal cells which exhibit the characteristic decreased levels of JIM 5.
Fig. 1. Immunofluorescent labelling of medial longitudinal sections with JIM 7 and JIM 5. (A) Uniform labelling of a medial longitudinal section with the JIM 7 antibody that recognizes a relatively esterified pectin epitope in the cell wall. (B) Uneven labelling of the next section in the series with the JIM 5 antibody that recognizes a relatively unesterified pectin epitope in the cell wall. Large arrows indicate the central cells of the quiescent centre that exhibit uniform labelling on all walls with JIM 5. The small arrow indicates a transverse wall making a Y-junction with adjacent transverse walls. The arrowhead indicates a transverse wall that makes a T-junction with adjacent longitudinal walls. Bar = 10 μm.

labelling. Quantification of the density of this labelling in two independent samples is shown in Fig. 5A and B. Walls of the quiescent centre cells exhibit almost ten times more JIM 5 labelling than walls of the cortical/endodermal initials and epidermal cells. JIM 7 labelling in the central cells, endodermal/cortical initials and epidermal cells does not vary (Fig. 1A). Figure 6 shows the uniform heavy JIM 7 labelling of the central cells of the quiescent centre.

These data indicate that while all cell walls in the vicinity of the quiescent centre are labelled uniformly with JIM 7, only the central cells of the quiescent centre exhibit such uniform labelling with JIM 5.

Discussion

Antibodies against relatively unesterified pectic polysaccharides (JIM 5) and methyl esterified pectic polysaccharides (JIM 7) were used to examine the spatial distribution of two forms of pectin at the Arabidopsis root tip. The results indicate that the central cells of the quiescent centre express high levels of unesterified pectin
compared to other cell types in the meristematic region of the root. This abundance of relatively unesterified pectin is a characteristic of other non-dividing or stationary cells and may play an important role in the rheological properties of the walls of these cells (McCann et al., 1994).

**Pectin distribution in other roots as indicated by monoclonal antibodies**

The most detailed description of the distribution of pectin epitopes in roots is that of Knox et al. (1990). The distribution of JIM 5 and JIM 7 epitopes in oat and eleven other angiosperm species was characterized in detail. JIM 7 labels cells of the stele and cortex of oat while JIM 5 preferentially labels the intercellular spaces (including three-way junctions) of the cortex. Neither antibody labels the epidermis. This tissue-specific labelling pattern was observed from the earliest developmental stages (near the initials) to the latest when cell elongation and differentiation had occurred. Root cap cells showed no appreciable labelling. Unlike oat, the stele and cortex of maize is labelled with JIM 5, but its epidermis and root cap were, like oat, unlabelled by either JIM 5 or JIM 7. Similar labelling patterns were observed among members of the Chenopodiaceae (a dicot family) in which the epidermis failed to label with either antibody.

While Chenopodiaceae exhibit a labelling pattern that resembles that of the Poaceae, other dicots exhibit abundant labelling of all cells in all tissues. These include members of the Apiaceae, Brassicaceae, Solanaceae, and Fabaceae. *Arabidopsis* therefore exhibits a pattern that is typical for members of the Brassicaceae. If the labelling pattern is conserved across members of this family and since the size of the quiescent centre is positively correlated with root diameter (Clowes, 1984), it is predicted that the zone of continuous-JIM 5 labelling should be larger in members of this family with thicker roots. Anti-PGA/RG1 labels three-way junctions in the root proper and all walls in the root cap of *Trifolium* indicating that these regions are rich in unesterified pectin (Lynch and Staehelin, 1992). This antibody labels root cap of oat only sparsely, supporting earlier assertions that such molecules were not abundant among the grasses (Lynch and Staehelin, 1995).

Since roots exhibit species-specific or family-specific patterns of anti-pectin labelling, it is possible that such labelling patterns might be used in systematic analyses. The anti-PGA/RG1 labelling pattern described by Knox et al. (1990) suggests that this antibody might be useful at higher taxonomic levels, i.e. to distinguish between monocots and dicots or to distinguish between grasses and non-grasses. Detailed examination of the Poaceae and their sister group, the Joinvilleaceae might be instructive in this respect. A number of phylogenies place Joinvilleaceae and the Poaceae as sister groups and a detailed phylogeny based on DNA and morphological characters places the bamboos at the base of the Poaceae clade with the Pooidae (of which oat is a member) constituting an early radiation within this group while maize is a member of a distinct group known as the PACC clade (Clark et al., 1995). Examination of anti-pectin labelling patterns within this phylogenetically well-defined group might indicate if such patterns are of systematic interest of if they are evolutionarily too labile.

*The pectic composition of the cell walls in the quiescent centre is characteristic of that found in other non-dividing cell types*

The quiescent centre is a small population of non-dividing cells found as a tier between the vascular initials above and the columella initials below, in the root meristems of most higher plants (Clowes, 1954, 1956, 1961). The size of this population varies from species to species and has been shown to be allometrically scaled with root diameter (Clowes, 1984). Median longitudinal sections labelled with the JIM 5 antibody revealed that all walls of the quiescent
centre cells contain a relatively uniform distribution of non-methyl esterified pectin compared to surrounding, dividing cells. This suggests that the walls of the quiescent centre are structurally different from those of neighbouring cells in the meristem. A uniform distribution of unesterified pectin is one such trait of older (non-dividing) cells. While there are no reports of cell wall differentiation between cells in the quiescent centre and neighbouring cells it seems likely that such differences might also exist in maize since it is possible to isolate surgically the quiescent centre by applying pressure to the root (Feldman, 1976). The ability to isolate the quiescent centre in such a manner suggests that the constituent cells adhere more strongly to one another than to neighbouring ‘non-quiescent centre’ cells. Unesterified pectic polysaccharides have the potential to cross-link tightly via Ca\(^{+}\) cross-bridges and, if present in the middle lamella, mediate cell adhesion. Therefore unesterified pectin might be expected to be present in relatively high amounts in this region of the maize root. McCann et al. (1994) have shown that the decrease in esterification observed in cultured tobacco cells is due to the de-esterification of non-methyl esterified pectins. McCann et al. (1994) have shown that the decrease in esterification observed in cultured tobacco cells is due to the de-esterification of non-methyl esterified pectins. McCann et al. (1994) have shown that the decrease in esterification observed in cultured tobacco cells is due to the esterification of methyl esters in dividing cells of the Arabidopsis root is required to allow the growth of the cell wall during this phase of development.

Since JIM 7 labels cell walls uniformly throughout the root, i.e. cells at all stages of development exhibit similar labelling intensities, the changes in JIM 5 labelling are probably not the result of de-esterification of methyl esterified polysaccharides, but a consequence of de-esterification of non-methyl esterified pectins. McCann et al. (1994) have shown that the decrease in esterification observed in cultured tobacco cells is due to the de-esterification of unidentified, non-methyl esters. In this study the total esters were seen to decrease between the elongation and stationary phases while the methyl ester component remained unchanged.

**Significance of the temporal incorporation of pectic polysaccharide into the wall**

The results show that while esterified pectin is incorporated into the cell plate, the unesterified form only appears
Fig. 4. Cross-section of a root showing the central cells of the quiescent centre with a uniform pattern of labelling with antibody recognizing the relatively unesterified epitope, JIM 5, while the walls of actively dividing cells nearby (cortical/endodermal and epidermal cells) exhibit much less labelling. (A) Central cells (c) of the quiescent centre are uniformly heavily labelled with the JIM 5 antibody, bar = 2 μm. (B) Higher magnification showing the heavy JIM 5 labelling of walls of the central cells, bar = 500 nm. (C) JIM 5 labelling of cortical endodermal (i) and epidermal (e) cells in the same plane of section as (B) showing a dramatically decreased level of JIM 5 labelling. Bar = 500 nm.
Pectin in Arabidopsis roots

Later. Another pectic polysaccharide, RG-I, has been shown to be absent from the developing cell plate in the roots of *Trifolium pratense* only to appear later in the life of the cell (Moore and Staehelin, 1988). Unlabelled transverse walls are never found next to each other along a file suggesting that the non-esterified pectin begins to accumulate in the cell wall during the subsequent cell cycle. Since PGA is thought to be deposited in the wall in its esterified form it is likely that the action of pectin esterases convert the esterified PGA to the unesterified form after the cell plate has formed (Kauss and Hassid, 1967; Zhang and Staehelin, 1992; Levy and Staehelin, 1992). This process continues later in the development of the cell resulting in walls with a uniform distribution of unesterified pectin (Goldberg *et al.*, 1986). The uniform distribution of the relatively unesterified pectin may therefore result from the activity of pectin esterases in the wall over a period of time.

**Mechanical consequences of locating unesterified pectins at three-way junctions and at the root periphery**

Transverse sections of the root clearly illustrate the concentration of unesterified pectin in the three-way junctions between cells near the root tip. Immunological labelling indicates that the unesterified pectin is predominantly found in the middle lamella and is most strongly associated with its elaboration at the three-way junctions in a range of species (Moore and Staehelin, 1988; Knox *et al.*, 1990). When spaces have formed at the three-way junctions, label is clearly associated with the middle lamella next to the air space. While spaces are important in root physiology it is important for structural reasons...
that these spaces do not ‘spread’ along the line of weakness between each cell resulting in the separation of the cells from each other. As a group of cells expand there is a tendency for shape to change and for angled corners to become curved with the concomitant appearance of air spaces at three-way cell junctions. It is then important for these junctions to be reinforced to avoid cells separating from one another. The localization of unesterified pectin to these junctions may serve to strengthen these regions.

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References


