Pectin Distribution at the Surface of Potato Parenchyma Cells in Relation to Cell−Cell Adhesion

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The crispness of fruits and vegetables is dependent, predominantly, on the maintenance of cell adhesion. There is a growing body of evidence to suggest that cell adhesion in plants is controlled at the edge of cell faces rather than across the entire cell surface. The aim of the current study has been to exploit antibody-labeling techniques in conjunction with methods that induce cell separation to explore the distribution of highly esterified and weakly esterified pectic polysaccharides on the cell surface. Potato parenchyma tissue was subjected to cooking and chemical treatments, which induced softening through cell separation. Scanning electron microscopy (SEM) revealed characteristic patterns on the surface of these separated cells, which outlined the imprint of neighboring cells. Monoclonal antibodies, JIM5 and JIM7, were used to locate weakly esterified and highly esterified pectin by silver-enhanced immunogold SEM. The edge-of-face structures labeled strongly with JIM5 but not JIM7, indicating that they contained polygalacturonic acid of low ester content. In addition, adhesion of the middle lamella to the face of the primary wall was found to differ from adhesion at the edge of each cell face. This, in conjunction with the antibody-labeling observations, complements previous transmission electron microscopy studies and is consistent with the edge-of-face regions having a specialist role in cell adhesion.

Keywords: *Antibody (JIM5, JIM7); cell wall, cell adhesion, pectic polysaccharide; potato; Solanum tuberosum; texture*

INTRODUCTION

The textural characteristics of edible plant organs depend on the characteristics of, and interactions between, the different levels of structure (Figure 1) (*1*). Of particular importance are the molecular mechanisms by which cells adhere to each other. It is the nature of cell adhesion, in conjunction with the mechanical properties of the cell walls, that provides the basis for mechanical strength within plant tissues and organs (*2*, *3*).

In most plant tissues, mechanical disruption involves rupture of the plant cell wall. Usually, the forces required to fracture the walls are less than those required to break the bonds involved in cell adhesion. However, physiological events (e.g., ripening of fruits and abscission) or physical treatments (e.g., thermal softening of vegetables) reduce adhesive strength, resulting in cell separation and tissue softening (*2*, *4*).

Cell adhesion manifests itself in the later stages of cell division. A highly localized region of the parent cell wall is controllably degraded. This facilitates connection of the newly formed middle lamella between the daughter cells and the middle lamella surrounding the parent cell (*5*, *6*). Subsequently, intercellular space formation initiates at the central point of contact between the two daughter cells and the adjacent cell. It then spreads along the middle lamella and terminates at electron-dense intrawall structures, which are consequently located in the corners of the predetermined intercellular spaces (*5*). Continuation of intercellular space formation would result in cell separation (*5*, *6*). Kolloffel and

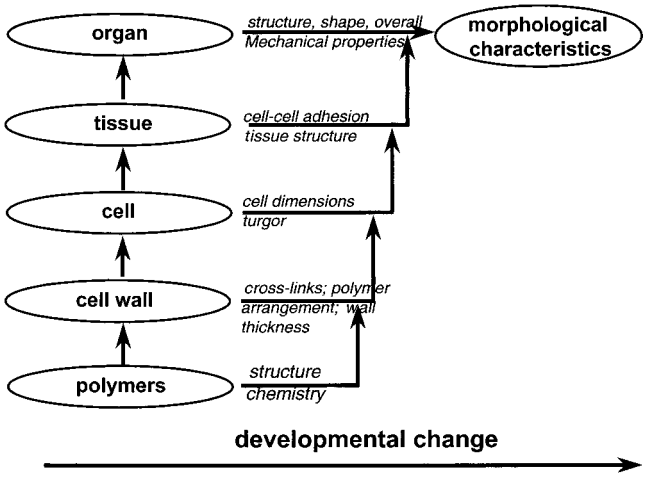

Figure 1. Hierarchy of structures that contribute to the morphological and mechanical properties of plants.

Linssen (*5*) speculated that the electron-dense regions represent sections of more or less continuous arcs along the edges of the intercellular space system and therefore along the edges of cell faces (*1*). It is possible that components at the edges of the cell faces play a key role in the maintenance of cell adhesion. There are several lines of evidence to support this hypothesis:

(a) Immunogold Labeling of Pectic Polysaccharides (7). Work of Knox et al. (*8*) in carrot root, Liners and Van-Cutsem (*9*) in suspension-cultured carrot cells, and Roy et al. (*10*) in ripe cherry tomato using monoclonal antibodies (mAbs) has strongly indicated that the "dark-staining" regions in the corners of intercellular spaces are enriched with weakly esterified homogalacturonan

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sequences. In contrast, highly esterified pectins are not differentially concentrated in these regions. Complementary studies using secondary ion mass spectrometry (SIMS) have demonstrated that calcium, which cross-links relatively unesterified pectic polymers (*11*), may also be concentrated in the corners of intercellular spaces (*12*), thus explaining why increasing the calcium content in many climacteric fruit tissues retards the rate of ripening-related softening (*13*). In conjunction with the observation that many primary-walled cells can be separated by chelators of calcium (*2*, *14*), the above evidence supports the hypothesis that cell adhesion at the face edges can involve calcium cross-linking of the localized weakly esterified pectins.

(b) Phenolic Cross-Links. The role of the edges of cell faces in cell adhesion was highlighted by work on Chinese water chestnuts (CWC) (*15*). The parenchyma of these tissues fails to undergo cell separation during thermal treatments, remaining firm and crisp (*1*). This research implicated diferulic acid cross-links between wall polymers in conferring thermal stability of cell adhesion (*16*). Fluorescence microscopy demonstrated that these cross-links were particularly concentrated at the edges of the cell faces (*15*). Their role in cell adhesion has been supported by studies on wall peroxidases (also located in these regions) (*17*), the activity of which can enhance cell adhesion (*18*).

(c) Mathematical Modeling. A third line of evidence may be obtained from a mathematical consideration of cellular structures. Cell adhesion has to withstand the tendency for turgor pressure to distend the plant cell to a spherical shape. Turgor-generated cell separation stress will concentrate at the corner of tricellular junctions and intercellular spaces (*19*), precisely where electron-dense material (*5*) and ferulate cross-links (*1*) have been located.

The aim of the present study has been to investigate the distribution of pectic polysaccharides at the surface of separated cells using JIM5 and JIM7 mAbs in conjunction with scanning electron microscopy (SEM). The study provides striking information on cell-wall-surface architecture during thermal and chemical induction of cell separation. In addition, it has indicated that the middle lamella is more strongly attached to the edge of the cell faces than to the face itself and provides further evidence for the role of the edge of cell faces in cell adhesion.

MATERIALS AND METHODS

Plant Material. Potatoes (*Solanum tuberosum* cv. Cara*)* were obtained from a local supplier. Samples were cut from the center portion of each tuber to avoid inclusion of cells from the vascular ring.

Preparation of Potato Strips. Sheets of potato tissue (1 mm thick) were cut from tubers using a Baker and Nixon slicer. From these, individual slices (1 × 4 × 40 mm) were prepared using a purpose-made precision instrument and immersed immediately in methanol. Strips were placed into sealed tubes containing fresh methanol and heated at 65 °C for 10 min to destroy enzyme activity (*20*, *21*). Strips were stored in methanol for 1, 6, and 12 months.

Mechanical Testing. All specimens were rehydrated for 1 h in distilled water prior to testing. Single-edge notches of 1.5 mm length were cut into all strips halfway along their length and perpendicular to the tensile axis using a purpose-made jig. The ends were glued to stainless steel plates with adhesive (Cyanolit 223-F, Eurobond, Sittingbourne, U.K.). All plates were clamped at a distance of 29 mm apart, to ensure minimum additional forces to each sample upon loading to the testing apparatus. The dimensions of the sample when attached to the plates were 29 mm long and 1 mm thick with a cross-sectional area of 2.5 mm^2 remaining at the failure zone after notching. These dimensions comply with the recommendations of the British Standards Institution (BSi) testing method, BS 2782 : Method 320E : 1976. The BSi sample dimensions for tensile testing of plastics were used because there is no available standard method for testing biological materials. Tensile tests were performed using a universal testing machine (Stable Microsystems TA-XT2, Godalming, U.K.) (TA). Samples were pulled to failure at a test speed of 0.5 mm/s, with the force being recorded through a 5 kg load transducer. All tests were performed with 10 replicates. Data were presented as a force−displacement curve.

The strength and failure strain that characterize each specimen were calculated from the force−deflection curve as

$$\text{strength} = F_{max}/A$$

where F_{max} is the force at failure (maximum force) and A is the un-notched cross-sectional area.

A can be expressed as $A = tw$, where t is the strip thickness and w is the un-notched strip width.

$$\text{failure strain} = (L_{max} - L)/L$$

where L_{max} is the length at which failure occurred and L is the initial length.

Thermal Treatment of Potato Tissues. Samples (8 mm diameter) were cut from potato tubers with a stainless steel cork borer and immersed in water at 100 °C for 1 min. They were then cooled rapidly by immersion in water at 4 °C and then snapped across into two halves.

SEM. The fracture surfaces of methanol-stored and thermally treated potato samples were analyzed by SEM before and after immunocytochemical labeling. Nonlabeled tissues were fixed in 30 g L^{-1} glutaraldehyde in 0.05 M cacodylate buffer (pH 7.2) overnight, dehydrated in an ethanol series, transferred to acetone, and critical point dried using liquid carbon dioxide. They were then mounted, fractured surface uppermost, onto aluminum pin stubs using silver conducting paint and coated with a layer of gold, ∼25 nm thick.

Samples were examined and photographed in a Leica Cambridge Stereoscan 360 SEM using the secondary electron detector and occasionally the backscatter electron detector.

Transmission Electron Microscopy (TEM). Blocks of potato tissue, ∼1.5 mm^3, were cut from the inner cortex of raw potato and fixed in 30 g L^{-1} glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) for 2 h. Tissue blocks were washed three times in buffer and then postfixed in 10 g L^{-1} aqueous osmium tetraoxide for 2 h. The samples were then dehydrated through an ethanol series, transferred to acetone, and then infiltrated and embedded in Spurr resin. Sections ∼75 nm thick were cut using a diamond knife, collected on copper grids, and stained sequentially with uranyl acetate (saturated in 500 mL L^{-1} ethanol) and Reynold's lead citrate. Sections were examined and photographed in a JEOL 1200 EX/B TEM.

Immunogold Labeling of Pectins. Monoclonal antibodies (mAbs) JIM5 and JIM7 were kindly provided by Prof. K. Roberts (JIC, Norwich. U.K.). Specimens were fixed in 10 g L^{-1} glutaraldehyde in 0.05 M cacodylate buffer (pH 7.2) and stored in buffer until required. Prior to the labeling procedure, the samples were washed four times in phosphate-buffered saline containing Tween 20 (PBST: 0.14 M NaCl, 0.0015 M KH_2PO_4, 0.0027 M KCl, 0.5 mL L^{-1} Tween 20 and 0.9 mL L^{-1} Kathon, pH 7.4). To reduce nonspecific binding, all samples were then incubated for 4 h in PBST (adjusted to pH 8.2 to reduce binding of the colloidal gold to positively charged tissue components) containing 1% bovine serum albumin (BSA; fraction V, Sigma). The samples were then incubated overnight at 4 °C in the primary antibodies, rat mAb cell culture supernatant JIM5 or JIM7, diluted 1:10 in PBST−BSA. Control material was incubated in PBST−BSA alone. After five washings in PBST−BSA, all samples were incubated

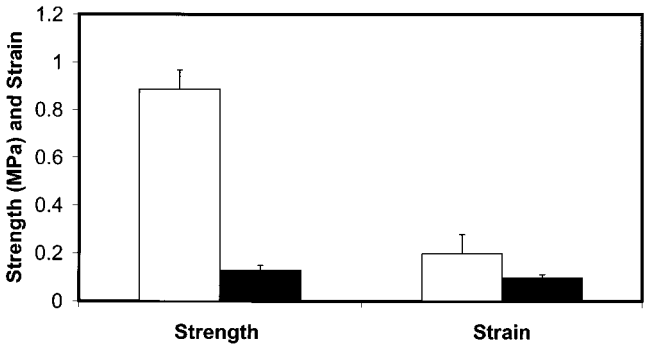

Figure 2. Strength and failure strain of potato tissue after storage in methanol for 1 (white bars) and 6 (black bars) months. Standard deviations shown.

overnight at 4 °C in secondary antibody, goat anti-rat with 5 nm colloidal gold label (British Biocell International, Cardiff, U.K.), diluted 1:50 with PBST−BSA.

To increase the size of the gold particles for SEM, the samples were incubated at room temperature for 15 min in a 1:1 mixture of enhancer and initiator from a silver-enhancing kit (British Biocell International) and then washed in distilled water. The samples were then dehydrated in ethanol, critical point dried, and examined by SEM as described above.

RESULTS

Induction of Cell Separation. Visualization of the cell surface was carried out after treatments that softened potato parenchyma tissues through the weakening of forces involved in cell adhesion. The first method resulted from a novel observation made during the prolonged storage of tissue strips in alcohol. The second method involved thermal softening of potato tissue (*4*).

Mechanical Properties of Potato Stored in Methanol and Then Rehydrated. Preliminary experiments had indicated that prolonged storage of potato tissue strips in methanol followed by rehydration resulted in tissue softening (*22*). Precise tensile tests were used to measure changes in the mechanical properties of potato parenchyma as a function of storage in methanol. All measurements of mechanical properties of methanol-treated potato parenchyma were carried out on tissues that had been rehydrated. Force and displacement values were recorded from the first major failure events of each sample and used to calculate strength and failure strain.

There was a dramatic decrease in the strength of potato parenchyma with increasing time of storage in methanol (Figure 2). The strength of potato strips stored in methanol for 6 months was <20% of that of strips stored in methanol for 1 month and failed at half the strain. Examination by SEM of these fracture surfaces shows that potato strips stored in methanol for 1 month failed through cell breakage (Figure 3a), whereas potato strips stored in methanol for 6 months failed due to cell separation (Figure 3b−d). These micrographs reveal a characteristic imprint on the surface of the potato cells, mapping the edges of the cell faces of previously neighboring cells. The edges of adjacent faces form a pattern similar to the fluorescence pattern observed when parenchyma cells of CWC are separated by treatment with hot dilute alkali (Figure 4) (*15*).

Immunogold Labeling of the Fracture Surfaces of Potato Stored in Methanol. The fracture surfaces of methanol-stored potato were immunoprobed to locate weakly esterified and highly esterified pectic polysaccharides with JIM5 and JIM7 mAbs. JIM5 reacts with

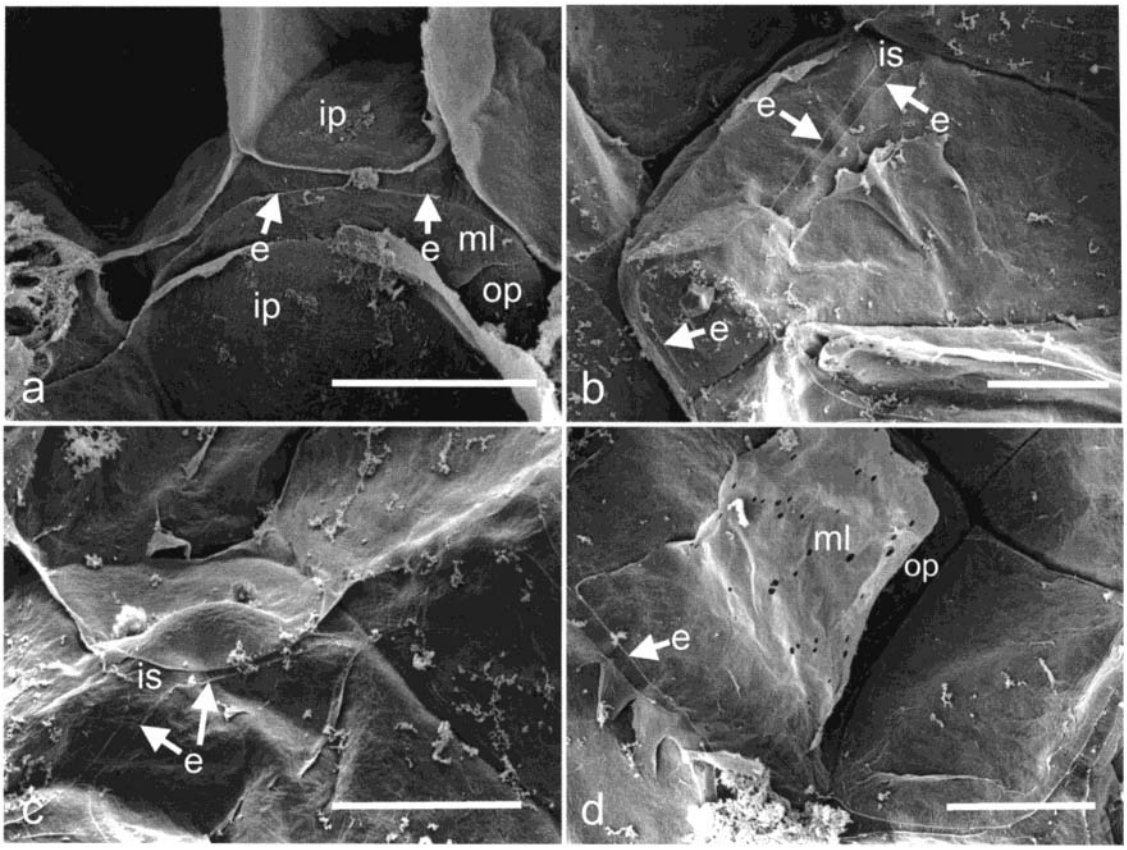

Figure 3. Fracture surface of potato tissue: (a) after storage in methanol for 1 month (cell breakage); (b−d) after storage in methanol for 6 months. Labels (for all figures): e, edge of face; ml, middle lamella; p, pit field; op, primary cell wall−outer surface; ip, primary cell wall−inner surface; is, intercellular space; c, cytoplasm. Bars = 50 μm.

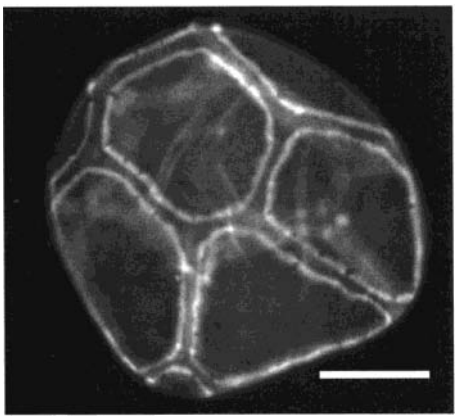

Figure 4. UV autofluorescence at pH 11 of CWC single cell after treatment in 50 mM Na_2CO_3 for 30 min at 100 °C, demonstrating "tramline" patterns at edges of cell faces. Bar = 20 μm.

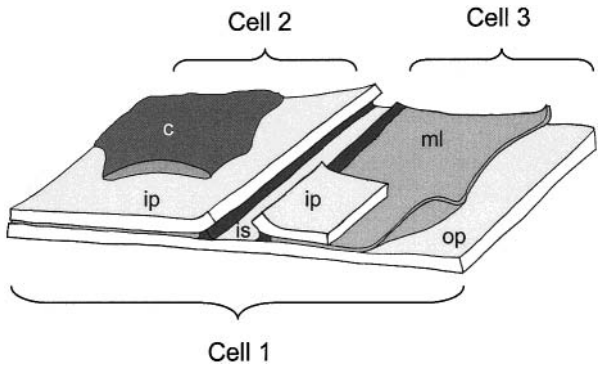

Figure 5. Schematic diagram illustrating spatial relationships between wall domains. Labels: same as for Figure 3.

pectins with a low degree of esterification (0−50%) (*8*), and JIM7 reacts with pectins with a high degree of esterification (35−90%) (*8*). Figure 5 illustrates the different fracture surfaces that were observed by SEM to aid interpretation of the following micrographs. Figures 6 and 7 show the labeling patterns obtained with these mAbs on the fracture surface of potato stored in methanol for 1 month and 1 year, respectively, followed by rehydration.

Potato stored in methanol for 1 month and then rehydrated failed due to cell breakage. The fracture surfaces of these samples consist of the inside of broken cells (Figure 6a) characterized by the presence of residual cytoplasm on the fracture surface. Areas of cell wall, which appear label-free, were probably initially covered with precipitated cytoplasmic components that were then detached through subsequent procedures. However, it is possible that this is due to microheterogeneity in wall composition or differences in cell type. Most cells fractured along the cell walls quite close to the edges of the faces. Both JIM5 and JIM7 exhibited similar labeling patterns (parts a and b, respectively, of Figure 6), suggesting pectins of both low and high degrees of esterification are evenly distributed across the inner surface of the cell walls. Control incubations were almost label-free (Figure 6c).

Cells stored in methanol for 1 year failed due to cell separation (Figure 7a). Patterns depicting the edges of cell faces were clearly visible on the surface of separated cells. JIM5 mAbs were distributed across the whole outer wall surface but were particularly concentrated on the face edge structures (Figure 7b), indicating a concentration of weakly esterified pectins in these locations. JIM7, which detects highly esterified pectins, also labeled over the whole cell surface but were not concentrated on the edges of cell faces (Figure 7c). Control incubations were again almost label-free (Figure

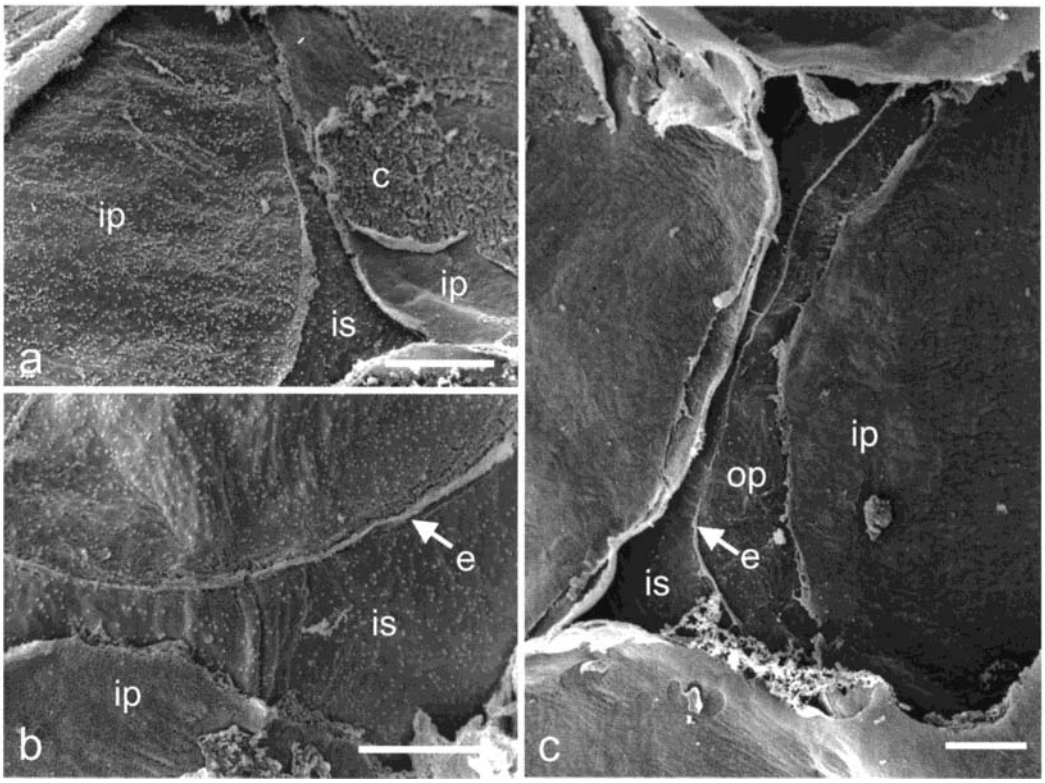

Figure 6. Fracture surface of potato stored in methanol for 1 month: (a) immunolabeled with mAb JIM5; (b) immunolabeled with mAb JIM7; (c) control. Labels: same as for Figure 3. Bars = 10 μm.

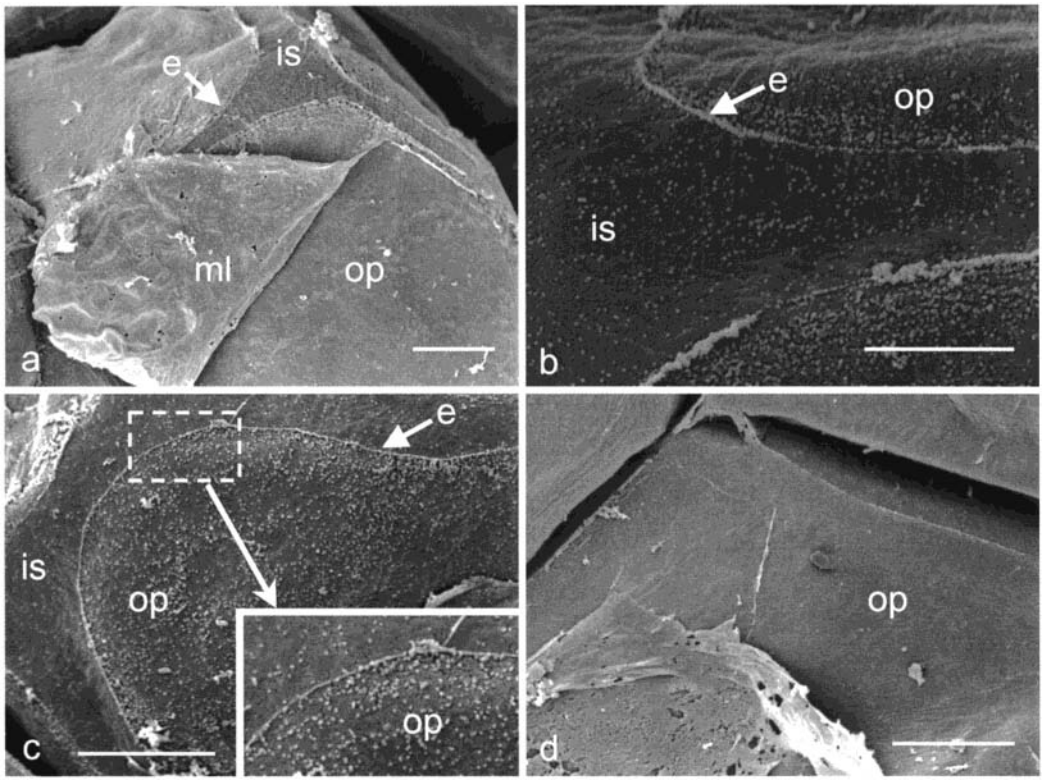

Figure 7. Fracture surfaces of potato stored in methanol for 1 year: (a) visualization of middle lamella; (b) immunolabeled with mAb JIM5; (c) immunolabeled with mAb JIM7 (see inset to show lack of binding of JIM7 at the edge of face); (d) control. Labels: same as for Figure 3. Bars = (a, c, d) 20 μm; (b) 10 μm.

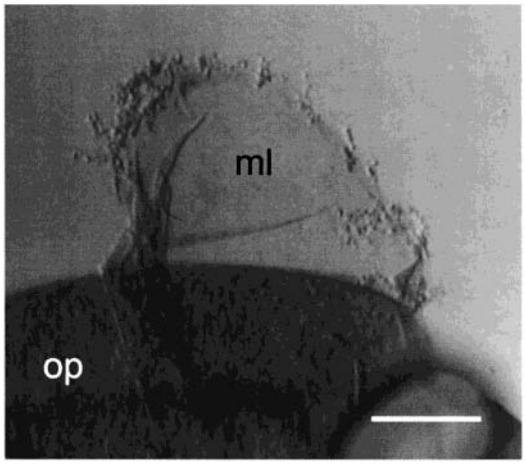

Figure 8. Flap of cell-wall material probably originating from the middle lamella on the surface of potato cells separated after treatment in methanol for 1 year. Labels: same as for Figure 3. Bar = 20 μm. Material was visualized by toluidine blue and viewed by light microscopy.

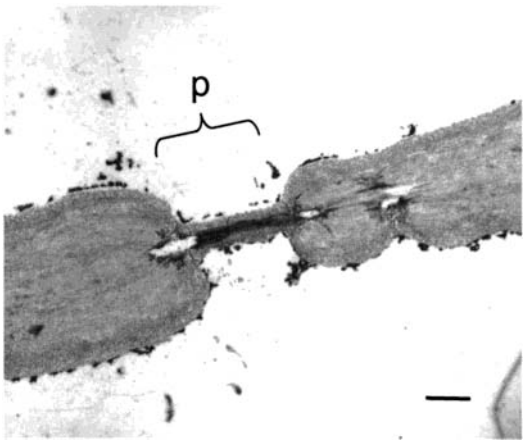

Figure 9. Transmission electron micrograph of untreated potato cell wall demonstrating dimensions of pit fields (p). Labels: same as for Figure 3. Bar = 1 μm.

7d). Control cells on the fracture surface presented a rather loose appearance, suggesting a general loss of cell adhesion throughout the tissue after storage in methanol for 1 year (Figure 7d).

Middle Lamella Localization. Of particular interest was the frequent observation of a flap of cell-wall material that was attached to the cell surface at the edges of the cell faces (Figures 3d and 7a). When partially detached from the lower cell surface, it was found to be perforated by circular holes of similar size. These were ~2 μm in diameter. The layer was rich in pectic polysaccharides as indicated by light microscopy and staining with toluidine blue (Figure 8). A detailed chemical characterization is currently being undertaken by FTIR spectroscopy of this layer; preliminary results confirm that this layer is composed predominantly of pectic polysaccharides, whereas FTIR readings from the cell surface beneath are consistent with cellulose and pectin patterns (Parker, Parker, Wilson, Smith, and Waldron, unpublished observations). These results strongly suggest that this layer is the middle lamella. It appears to be firmly attached to the edges of the cell faces but poorly attached to the face of the primary cell walls, allowing it to float free when torn. This suggests that the mode of adhesion of the middle lamella to the edge of the face is quite different from any adhesion throughout the rest of the cell face. This is consistent with the edge of the face being the predominant mode of adhesion between cells. The perforations in this layer

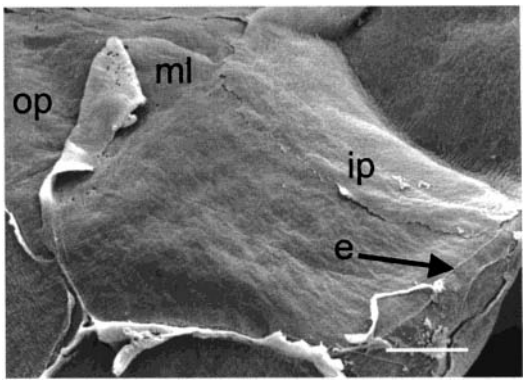

Figure 10. Fracture surface of thermally treated potato tissue showing middle lamella layer and primary walls. Labels: same as for Figure 3. Bar = 25 μm.

coincide with positions of pit fields and they have similar dimensions (Figure 9).

Thermal Treatments on Fresh Potato. Thermal softening of potatoes results in cell separation and softening (*1*). Cores of fresh potato tissue that were cooked for 1 min and then snapped failed mostly due to cell breakage (because heat had not penetrated through to the core). However, cells near the edge were influenced by the heat treatments and so were separated, again revealing face-edge structures (results not shown). Complementing the observations above, Figure 10 shows the middle lamella layer (with pit field perforations), sandwiched between two cells, the broken upper cell and the intact cell beneath.

Incubations with JIM5 resulted in substantial labeling of weakly esterified pectins across the cell surface (Figure 11a), especially concentrating on the edges of cell faces (Figure 11b). In contrast, highly esterified pectins detected by JIM7 were present in small numbers across the cell surface and were not concentrated on the face edges (Figure 11c,d). Control incubations were again almost label-free (results not shown).

DISCUSSION

In this study, two approaches (storage in methanol and thermal treatment) have been employed to induce cell separation during fracture of potato tissue. This has facilitated studies by SEM in conjunction with mAb labeling of wall polymers to investigate the distribution of those polymers on the cell surfaces.

The chemical mechanisms that lead to cell separation as a result of prolonged storage in methanol are not known. Heating plant tissues in methanol or other alcohols has often been used to inactivate endogenous enzymes during preparation of samples for studies on wall extensibility (*23*). (It is for this reason that we initially stored potato samples in methanol.) Here, we have reported that prolonged storage of potato tissues in methanol results in loss of strength and a corresponding increase in cell separation. This contrasts with the work of Cleland (*24*), who reported that sections of *Avena* coleoptile can be treated and stored similarly for up to a year without a change in their mechanical properties. Further studies will be required to elucidate the mechanism of action. It is interesting to speculate whether the methanol effect indicates the involvement of methanol-soluble components in the cell adhesion or if the changes in strength relate to a time-dependent rearrangement of wall polymers.

In contrast, thermally induced cell separation is likely to involve and possibly result from β-eliminative depolymerization of pectic polymers of the middle lamella (*2*, *4*, *25*). The very short thermal treatment used in this study resulted in samples showing a gradation of cells affected by heat treatment: cell separation at the surface of the core and cell breakage in the middle where the heat did not penetrate. These two treatments offer a unique opportunity to observe structures at the cell surface, which are usually solubilized away before cell separation occurs.

Both modes of cell separation have revealed similar patterns of localization of highly esterified and weakly

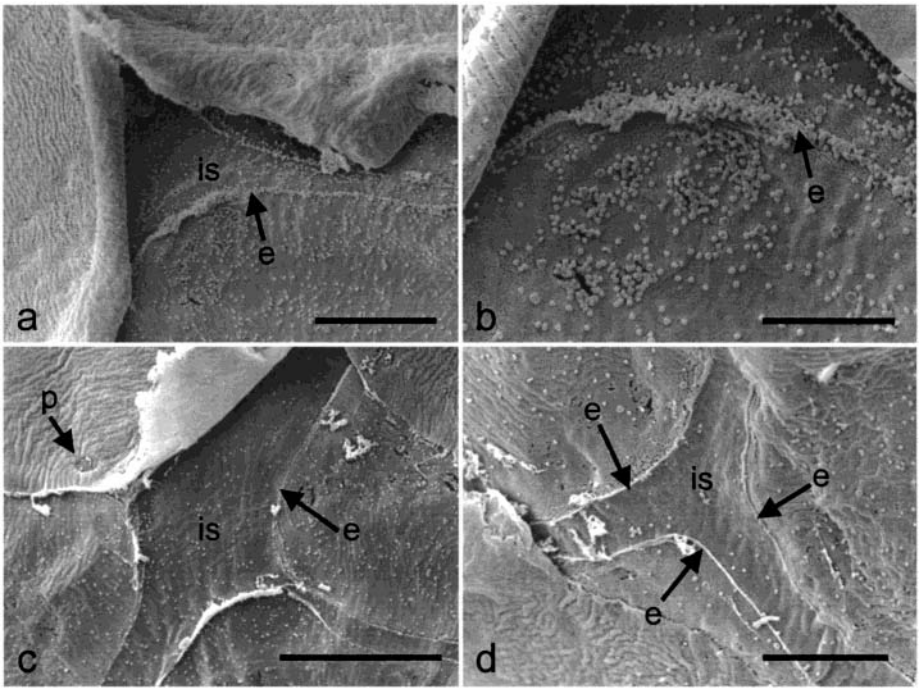

Figure 11. Fracture surfaces of thermally treated potato tissue (a, b) immunolabeled with mAb JIM5 [(b) is a magnified region of (a)]; (c, d) immunolabeled with mAb JIM7. Labels: same as for Figure 3. Bars = (a, c) 20 μm; (b, d) 10 μm.

esterified polygalacturonic acid on the surface of potato parenchyma cells. The distribution of different components in plant cell walls is likely to be related to their specific functions within the wall (*26*). By highlighting the concentration of weakly esterified epitopes at the edges of the cell faces, this study, in conjunction with the work of Knox (*8*), Liners and Van Cutsem (*9*), and Roy et al. (*10*), verifies the hypothesis of Kolloffel and Linssen (*5*) that the components at the corners of intercellular spaces form an arc along the edges of the cell faces. This is consistent with a role for weakly esterified pectic polysaccharides in cell adhesion (see Introduction).

Recently, JIM5 and JIM7 have been further characterized (*27*). JIM5 binds weakly to completely de-esterified pectin; the binding is greatly increased by the presence of methyl-esterified pectin up to ~40%. JIM7 binds to a range of esterified pectin from about 15 to 80%. In light of these findings, the results presented in this study are consistent with the presence of low-esterifed pectins at the edge of the cell face.

These results, in conjunction with other studies involving mAbs (cited above), do not preclude the involvement of the middle lamella pectic polysaccharides across the whole cell face from contributing to cell adhesion. However, our investigations show that the chemistry of adhesion of the middle lamella to the adjacent primary walls differs considerably with location. Of note is the observation that the intact middle lamella can become easily detached from the cell face but not from the edge of the faces after treatments to induce cell separation (Figures 3d, 7a, 8, and 10). Thus, bonds that join the edge of the middle lamella to the edge of the cell face, and those which cross-link the polymers within the middle lamella, appear to be more stable to the heat and methanol treatments compared to bonds that join the face of the middle lamella to the face of the primary walls.

CONCLUSIONS

This study has utilized immunocytochemical methods in combination with SEM to demonstrate the spatial distribution of pectic polysaccharides in situ on the cell surface of potato parenchyma tissue.

Domains at the corners of intercellular spaces correspond to the edges of adjacent cell faces and are enriched with weakly esterified polygalacturonic acid. Adhesion in these domains must be overcome during fruit ripening, cooking, or chemical treatments to allow cell separation.

The chemistry of adhesion of the middle lamella to the primary wall face is different from adhesion at the edge of each cell face. This, in conjunction with the above immunocytochemical observations, is consistent with a specialist role of the face-edge regions in cell adhesion. These may provide a focus for future biotechnological exploitation.

ACKNOWLEDGMENT

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