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# Localization of Pectic Galactan in Tomato Cell Walls Using a Monoclonal Antibody Specific to (1→4)-β-D-Galactan[1]

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**To develop antibody probes for the neutral side chains of pectins, antisera were generated to a pectic galactan isolated from tomato (*Lycopersicon esculentum*) pericarp cell walls and to a (1→4)-β-galactotetraose-bovine serum albumin neoglycoprotein. The use of these two antisera in immunochemical assays and immunolocalization studies indicated that they had very similar specificities. A monoclonal antibody (LM5) was isolated and characterized subsequent to immunization with the neoglycoprotein. Hapten inhibition studies revealed that the antibody specifically recognized more than three contiguous units of (1→4)-β-galactosyl residues. The anti-galactan antibody was used to immunolocalize the galactan side chains of pectin in tomato fruit pericarp and tomato petiole cell walls. Although the LM5 epitope occurs in most cell walls of the tomato fruit, it was absent from both the locular gel and the epidermal and subepidermal cells. Furthermore, in contrast to other anti-pectin antibodies, LM5 did not label the cell wall thickenings of tomato petiole collenchyma.**

Pectins are major components of higher-plant primary cell walls. They have been shown to play an important role in cell adhesion (Vennigerholz and Walles, 1987) and are thought to carry out physiological roles by modulating cell wall pH, determining cell wall porosity, and acting as signaling molecules (Campbell and Labavitch, 1991; Carpita and Gibeaut, 1993).

Several pectic polysaccharides have been isolated and characterized. Homogalacturonan is composed of (1→4)-α-D-galactosyluronic acid residues, which can be methyl esterified and/or acetylated (O'Neill et al., 1990). In the absence of methyl esterification, the carboxylic acid groups of the galactosyluronic acid residues can associate with divalent calcium ions linking homogalacturonan chains together to form a gel (Jarvis, 1984). Rhamnogalacturonan I is a pectic polymer with a backbone of alternating (1→4)-α-D-GalA-(1→2)-α-L-Rha residues (Lau et al., 1985). The rhamnose residues can form attachment points at the C-4 position for neutral sugar side chains with the majority of these side chains composed predominantly of (1→4)-β-D-galactosyl- and or (1→5)-α-L-arabinosyl-linked residues (McNeil et al., 1982). Other glycosyl residues such as Xyl, Glc, and glucosyluronic acid may also be present (An et al., 1994). Within rhamnogalacturonan I, sparsely and densely galactosylated areas exist (De Vries et al., 1982). The exact structure, size, and arrangement of these (1→4)-β-galactan-rich neutral sugar side chains is not well understood. Recent studies involving hydrolysis of these side chains (Guillon and Thibault, 1989) and techniques such as high-performance anion-exchange chromatography (Schols et al., 1994, 1995) have begun to elucidate fine structure. Rhamnogalacturonan II is perhaps the most complex pectic polysaccharide, containing a wide and unusual range of sugar residues and glycosyl linkages, although, again, the exact structure and distribution of the side chains is unknown (Whitcombe et al., 1995). The functional role of the neutral side chains and their location within plant cell walls still remain unclear.

Pectic polysaccharides play an important role in the texture of fruits and vegetables. Marked changes in cell wall structure occur in ripening tomatoes (*Lycopersicon esculentum*) and in many other fruit. In the tomato, cell walls of the locular tissue are weakened and the placental gel is liquified (Van Buren, 1991). In the pericarp tissue the middle lamella is disrupted, resulting in a loss of intercellular adhesion and cell separation (Ben-Arie et al., 1979). These events almost certainly reflect alterations in the nature of the wall polysaccharides. Some of the most apparent changes occur in the pectic fraction (Gross and Wallner, 1979; Gross, 1984; Seymour et al., 1990) and include an increase in soluble polyuronide, a reduction in polyuronide molecular weight, and a pronounced loss of galactosyl residues. These galactosyl residues are commonly (1→4)-β-linked and appear to be lost from the Gal-rich side chains attached to the rhamnogalacturonan backbone (Seymour et al., 1990; Redgwell et al., 1992).

Polyuronide degradation is generally attributed to the action of PG, which appears in many fruits near the onset of ripening (Tucker et al., 1980). The loss of galactosyl residues does not appear to be related to PG action; tomato fruit in which PG activity is significantly reduced by the incorporation of an antisense PG transgene still exhibit a loss of Gal (Carrington et al., 1993). Transgenic tomatoes with reduced PG activity still soften but are more resistant to cracking and mechanical damage during the latter stages of ripening (Gray et al., 1994) and also produce tomato

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Abbreviations: GAL4, (1→4)-β-galactotetraose; HRP, horseradish peroxidase; PG, polygalacturonase; TG, tomato galactan.

paste with a higher viscosity than normal fruit. This suggests that PG is not responsible for the initial stages of fruit softening. The role of the loss of cell wall galactosyl residues, which appears to result from the action of a galactanase, is unknown. Galactanase activity is significantly reduced in the nonsoftening mutants of tomato (Carey et al., 1995), and substitution of polysaccharides with side chains is known to affect their gelling capacity and other physicochemical properties (Hwang and Kokini, 1991; Hwang et al., 1993). The structure-function relationships of these cell wall polymers is poorly understood and requires further investigation.

Monoclonal antibodies are excellent tools for localizing pectic components of plant cell walls (Liners et al., 1989; Knox et al., 1990; Puhlmann et al., 1994; Steffan et al., 1995; Williams et al., 1996). To investigate the role of the Gal-rich neutral side chains of pectin in tomato cell walls and to enhance knowledge of their structure-function relationships, we have generated a monoclonal antibody specific to (1→4)-β-galactan, and this has been used as a probe to localize the neutral side chains within cell walls of tomato.

## MATERIALS AND METHODS

Tomatoes (*Lycopersicon esculentum* cvs Ailsa Craig and Solairo) were grown under glass at 18°C (day) and 16°C (night). Fruit were harvested at the mature, green stage (approximately 35 d postanthesis) and when red-ripe (50–55 d postanthesis). Ailsa Craig tomatoes were used for all experiments except tissue printing. Higher-quality tissue prints were obtained with Solairo fruit, although the actual result was the same with both cultivars.

### Polysaccharides

The isolation of a Gal-rich polysaccharide from tomato cell walls was carried out according to the method of Pressey (1983). The pectin-degrading enzymes PG and pectin methylesterase were purified from ripe tomato fruit (Tucker et al., 1980, 1982) and used to digest tomato cell wall material, prepared according to the protocol described by Seymour et al. (1987). The products of the digest were separated on a Bio-Gel A-15 m (Bio-Rad) column (2.6 × 67 cm) equilibrated with 0.15 M NaCl. The flow rate was maintained at 0.5 mL/min, and 80 × 5.3 mL fractions were collected. The fractions were analyzed for total sugars by the phenol-sulfuric acid method (Dubois et al., 1956) and for uronic acids according to Blumenkrantz and Asboe-Hansen (1973). The column yielded two peaks rich in neutral sugars. Fractions within each peak were pooled and the peaks designated as TG1 and TG2. The polysaccharide fractions were then further purified by chromatography on a DEAE-Sephadex column (2.6 × 20 cm) equilibrated with 50 mM sodium phosphate buffer, pH 6.5. The flow rate was maintained at 2 mL/min, and 80 × 9.8 mL fractions were collected. Material that had bound to the column was eluted with increasing (0–0.5 M) NaCl concentrations. This separated TG2 into two discrete sugar peaks. The three polysaccharides (TG1, TG2a, and TG2b) were precipitated with ethanol and collected by centrifugation. TG1 was used for the generation of antibodies. GC and uronic acid analysis revealed the composition of this galactan to be 69% Gal, 11% Ara, 8% Rha, 6% Glc, 1% Xyl, and 5% uronic acid. Similar proportions were found in TG2a and TG2b. $^{13}$C-NMR indicated that the galactosyl residues in all three galactans were (1→4)-β-linked.

Citrus pectin, apple pectin, polygalacturonic acid, Gal, locust bean gum, gum arabic, and larchwood arabinogalactan were purchased from Sigma. The lupin galactan and GAL4 were obtained from Megazyme (Warriewood, New South Wales, Australia). The (1→4)-β-galactobiose and (1→3)-α-(1→4)-β-(1→3)-α-galactotetraose were purchased from Dextra (Reading, Berkshire, UK). The (1→3)-β-galactobiose and (1→6)-β-galactobiose were kindly provided by Dr. J.-F. Valdor (Department of Biochemistry and Molecular Biology, University of Leeds). The (1→4)-β-galactotriose was a gift from Dr. H. Schols (Department of Food Science, Wageningen Agricultural University, The Netherlands).

### Preparation of a GAL4 Protein Conjugate

The GAL4 was coupled to BSA by Dextra Laboratories Ltd. A short, three-atom spacer arm was used to conjugate BSA to the GAL4, and the product (GAL4-BSA) was analyzed by matrix-assisted laser desorption time-of-flight MS, which indicated 7 to 19 sugar residues/mol of protein.

### Generation of Anti-(1→4)-β-Galactan Antibodies

TG1 plus an equivalent amount of methylated BSA was used to immunize one set of male Wistar rats, and the GAL4-BSA neoglycoprotein was used to immunize another set of male Wistar rats, according to standard immunization schedules. Methylated BSA was included in the TG1 immunogen preparation to increase the likelihood of an immune response, since acidic polysaccharides are rarely immunogenic (Vreeland, 1970). Tail bleeds were taken from each of the animals before and after immunizations, and antisera were prepared. The sera were screened for the desired antibody reactivities using an ELISA (see below).

Two fusions were performed with one-half of a spleen from each set of rats. Lymphocytes were fused with the IR983F rat myeloma line (Bazin, 1982) based on the method of Liddel and Cryer (1991). The fusion of spleen cells from the TG1-immunized rat resulted only in the generation of low-affinity antibodies, which were not pursued. Hybridoma supernatants from the GAL4-BSA fusion were screened for the presence of anti-(1→4)-β-galactan antibodies on an ELISA. A number of positive antibody-secreting cell lines were identified from the neoglycoprotein fusion. These cell lines were rescreened on an immunodot-binding assay to ascertain that the antibodies were not directed against BSA. Hybridomas were cloned by limiting dilution, and the cell lines were expanded and cryopreserved. Cell supernatants were collected and stored at 4°C. One monoclonal antibody (LM5, an IgG2c) was selected for characterization and use in further studies. In certain instances, for purposes of comparison, the anti-homogalacturonan

monoclonal antibodies JIM5 and JIM7 (Knox et al., 1990) were also used.

### ELISA

ELISAs were performed in 96-well microtiter plates (NUNC, Maxisorb, Paisley, Scotland, UK) coated with 2 μg of antigen/mL PBS, 100 μL/well, and left overnight at 4°C. Unbound antigen was washed out of the wells with water, and a blocking solution of PBS containing 3% milk protein was added (200 μL/well). After 1 h at room temperature, plates were washed and 100 μL/well of hybridoma supernatant was added. Two hours later, plates were washed again and a secondary antibody (rabbit anti-rat IgG coupled to HRP, obtained from Sigma) was added, diluted 1:5000 (100 μL/well). After a further 1 to 2 h, plates were washed thoroughly and antibody binding was detected by addition of HRP substrate (18 mL of water, 2 mL of 1 M sodium acetate buffer, pH 6.0, 200 μL of tetramethylbenzidene, and 20 μL of 6% $H_2O_2$) at 150 μL/well. The reaction was stopped with 2 M $H_2SO_4$ (30 μL/well), and the $A_{450}$ was read on a microtiter plate reader.

For the competitive inhibition ELISAs, a range of potential hapten and polysaccharide inhibitors of antibody binding (1 mg/mL solutions) were serially diluted 1:10 (in PBS/3% milk protein) down the ELISA plate prior to the addition of antibody. Fifty microliters of the anti-(1→4)-β-galactan (LM5, diluted to provide 90% of maximal binding) was then added to each well. All other steps in the ELISA were carried out as described above. Fifty percent inhibition of LM5 binding was determined by plotting inhibitor concentrations against absorbances. Values from controls with no inhibitor were taken as 0% inhibition of antibody binding, and values from controls with no antibody represented 100% inhibition.

The effects of acid and arabinofuranosidase treatments on LM5 labeling were also determined with an ELISA. The coated ELISA plates were incubated with the enzyme α-L-arabinofuranosidase (Megazyme), 5 units/mL in 50 mM sodium acetate, pH 4.0, 100 μL/well, for 1 h prior to addition of the primary antibody. Alternatively, plates were incubated with 4 M trifluoroacetic acid, 100 μL/well, for 1 h. All other steps were carried out as detailed above.

### Immunodot Binding Assay

Immunodot binding assays were carried out by the application of 10 μg (1 μL of 10 mg/mL solution) of citrus pectin, apple pectin, TG1, TG2a, TG2b, GAL4-BSA, BSA, lupin galactan, gum arabic, polygalacturonic acid, larchwood arabinogalactan, and locust bean gum onto strips of nitrocellulose. PBS/1% milk protein was used to block sites on the nitrocellulose. After 1 h the primary antibody (antisera [1/2000] or monoclonal antibody [1/20]) was added and left for a further 1 to 2 h. The nitrocellulose was then washed (with water), and rabbit anti-rat IgG linked to HRP (diluted 1:2000) was added. After 1 to 2 h, the nitrocellulose was washed extensively and antibody binding was detected by addition of the peroxidase substrate (25 mL of water, 5 mL of methanol containing 5 mg/mL 4-chloro-1-naphthol, and 30 μL of 6% $H_2O_2$). The reaction was stopped by washing the nitrocellulose with water.

### Tissue Printing

Cut surfaces of mature, green tomato fruit were pressed firmly onto a piece of nitrocellulose (for approximately 10 s). When the prints had dried, the nitrocellulose was blocked with PBS/1% milk protein for 1 h. The primary antibody was then added (LM5 and JIM5, diluted 1:10) and left for 1 to 2 h. After washing, the rabbit anti-rat IgG-HRP secondary antibody was added (diluted 1:2000). After a further 1 to 2 h, the nitrocellulose paper was washed, and antibody binding was detected as for the immunodot binding assay. The results were the same with cv Ailsa Craig and cv Solairo fruit.

### Immunofluorescence Microscopy

Small sections of tomato petiole tissue and pericarp tissue from unripe and ripe fruit were cut from cv Ailsa Craig tomatoes. The material was fixed with 4% paraformaldehyde in 0.2 M phosphate buffer (pH 7.4), dehydrated in a graded ethanol-water series from 70 to 100%, and embedded in LR White acrylic resin (London Resin Co., Basingstoke, UK).

Sections (0.5–1 μm thick) were cut from the resin-embedded tissue on an Ultracut microtome (Reichert-Jung, Vienna, Austria) and collected on multi-well slides (ICN). Sections were blocked with 25 μL of PBS/1% milk protein. A 25-μL droplet of monoclonal antibody (diluted 1:20) was then placed onto each well of the multi-well slide and left for 2 h. Sections were washed and the secondary antibody was added (rabbit anti-rat IgG coupled to fluorescein isothiocyanate, obtained from Sigma, diluted 1:100). After a further 2 h, sections were washed again before adding Citifluor AF1, an anti-fade reagent (Agar Scientific, Stansted, Essex, UK). Sections were viewed on a microscope with epifluorescence, and micrographs were taken with black-and-white print film (HP5 400 Ilford, Mabberley, Cheshire, UK). In certain cases antibodies were incubated in the presence of antigens at 1 mg/mL.

### Immunogold Labeling for Electron Microscopy

Sections (50–100 nm thick) were cut from the resin-embedded material, placed onto nickel grids, and blocked with 25 μL of PBS/2% BSA for 10 min. The grids were then transferred to 25-μL droplets of the antibody (diluted 1:10) and left for 1 h. Grids were washed thoroughly with distilled water and transferred to 25-μL droplets of secondary antibody (goat anti-rat IgG coupled to 20-nm gold particles, diluted 1:40, obtained from BioCell [Cardiff, UK]). After 1 h, grids were again washed with distilled water. Sections were left unstained or stained with 4% uranyl acetate (1 min), then washed and left to dry. The sections were viewed on a CM10 transmission electron microscope (Philips, Eindhoven, The Netherlands).

## RESULTS

### Characterization of Anti-(1→4)-β-Galactan Antibodies

Antisera generated following immunization with the tomato galactan (aTG) and the GAL4-BSA neoglycoprotein (aGal4) had similar binding specificities against a range of polysaccharides when screened on an immunodot binding assay (Fig. 1). The aGal4 serum appeared to have a higher affinity for the polysaccharides dotted onto the nitrocellulose than did aTG. The major difference in specificity was that aTG interacted with gum arabic, whereas aGal4 did not.

The monoclonal antibody to (1→4)-β-galactan, LM5, was generated from a fusion following immunization with GAL4-BSA. The specificity of LM5 was determined using the immunodot binding assay, which revealed a positive reaction between the LM5 monoclonal antibody and a number of polysaccharides, all of which contained (1→4)-β-galactosyl residues (Fig. 1). The antibody interacted strongly with the neoglycoprotein, but this interaction was specific to the GAL4 portion of the conjugate only, since no binding was observed with BSA alone. LM5 reacted with the (1→4)-β-galactans from lupin, of which Gal constitutes 91%, and from tomato, which are composed of >60% Gal. The dot blots showed that LM5 reacted weakly against citrus pectin, apple pectin, and polygalacturonic acid, and very weakly with locust bean gum (Fig. 1).

In addition, an indirect competitive inhibition ELISA revealed that BSA was ineffective as an inhibitor, whereas the (1→4)-β-galactotetrasaccharide did inhibit antibody binding (58 μg/mL resulted in 50% inhibition of LM5 binding). The (1→4)-β-galactans from lupin and tomato also acted as inhibitors of antibody binding (0.7 μg/mL lupin galactan and 12 μg/mL TG1 resulted in 50% inhibition of LM5 binding). (1→3)-α-(1→4)-β-(1→3)-α-Galactotetraose, (1→4)-β-galactotriose, (1→4)-β-galactobiose, (1→3)-β-galactobiose, (1→6)-β-galactobiose, and Gal were all ineffective inhibitors of LM5 binding up to concentrations of 500 μg/mL. Furthermore, a (1→4)-α-galacturonic acid hexasaccharide and polysaccharides such as larchwood arabinogalactan, gum arabic, and xyloglucan were also completely ineffective as inhibitors of LM5 binding at equivalent concentrations.

Pretreatment of TG, immobilized on an ELISA plate, with acid conditions (likely to hydrolyze terminal arabinofuranose residues) or with the enzyme α-L-arabinofuranosidase had no effect on LM5 binding.

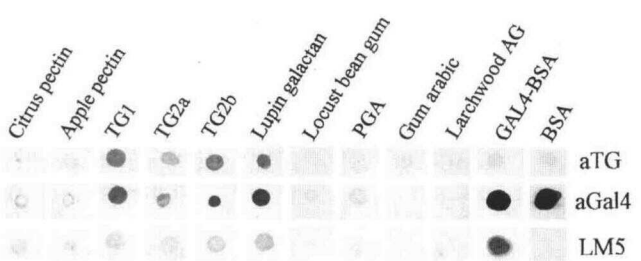


**Figure 1.** Immunodot binding assay of a range of polysaccharides with antisera aTG and aGal4 at a 2000-fold dilution and LM5 hybridoma supernatant at a 20-fold dilution. AG, Arabinogalactan; PGA, polygalacturonic acid.

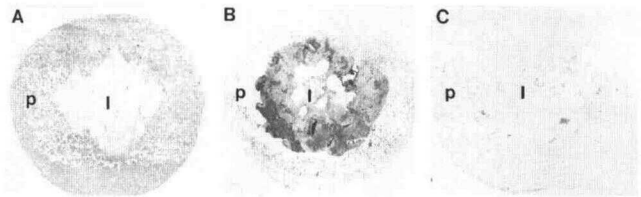


**Figure 2.** Tissue prints of mature, green Solairo tomato fruit probed with the monoclonal antibodies LM5 (A) and JIM5 (B). In the control print (C), the primary antibody is absent. p, Pericarp; l, locular gel.

### Immunolocalization Studies with the Anti-(1→4)-β-Galactan, LM5

Tissue printing is a useful technique for immunolocalization of many types of macromolecules (Varner and Ye, 1994). Tissue printing with LM5 revealed an abundance of the epitope in the pericarp of mature, green cv Solairo tomato fruit with little or no labeling in the locular tissue (Fig. 2). The anti-homogalacturonan antibody JIM5 (Knox et al., 1990) bound to pectin throughout the fruit, particularly in the locular jelly-like cavity surrounding the seeds. Tissue printing was carried out several times with two different tomato cultivars, Solairo and Ailsa Craig, both of which gave the same result. Although a direct comparison of anti-(1→4)-β-galactan-labeled tissue prints from unripe and ripe fruit might have revealed differences in the distribution or abundance of the LM5 epitope, tissue printing with ripe tomato fruit proved to be extremely difficult due to the high water content and softness of the fruit, which resulted in poor-quality tissue prints.

Immunofluorescently labeled sections of resin-embedded material revealed that the anti-(1→4)-β-galactan bound to tomato pericarp cell walls (Fig. 3, A and D). The epidermal cells were not labeled and the subepidermal cells reacted weakly with LM5. The intercellular spaces were generally not labeled, which was in contrast with the abundant labeling by the anti-homogalacturonan antibody JIM5, as shown in Figure 3B. In unripe tissue (Fig. 3A) the distribution of the LM5 epitope within the cell walls was variable, particularly in the smaller cells immediately beneath the epidermis. Here, the cell walls tend to be thicker compared with the larger, thinner-walled cells found away from the epidermis and toward the locular tissue. In certain areas of the cell wall, immunofluorescence in the middle lamella seemed particularly intense; in other areas labeling in this region appeared to be absent. There were also regions of the cell wall that had no label. Such regions were not found with JIM5 or JIM7, although in comparison with these antibodies cell wall labeling with LM5 was more abundant. The most striking difference between these three antibodies appeared to be labeling of the epidermal cells, which was absent with LM5 and most abundant with JIM7. In ripe tissue, the overall cell wall labeling with LM5 appeared more intense than in unripe tissue. There was, however, reduced labeling of the subepidermal cells, and the epidermis remained unlabeled (Fig. 3D). The epidermal cells are seen more clearly in sections of ripe fruit due to autofluorescence (Fig. 3F). The LM5 epitope did not appear to be as evenly distributed throughout the cell walls in ripe tissue with reduced label in

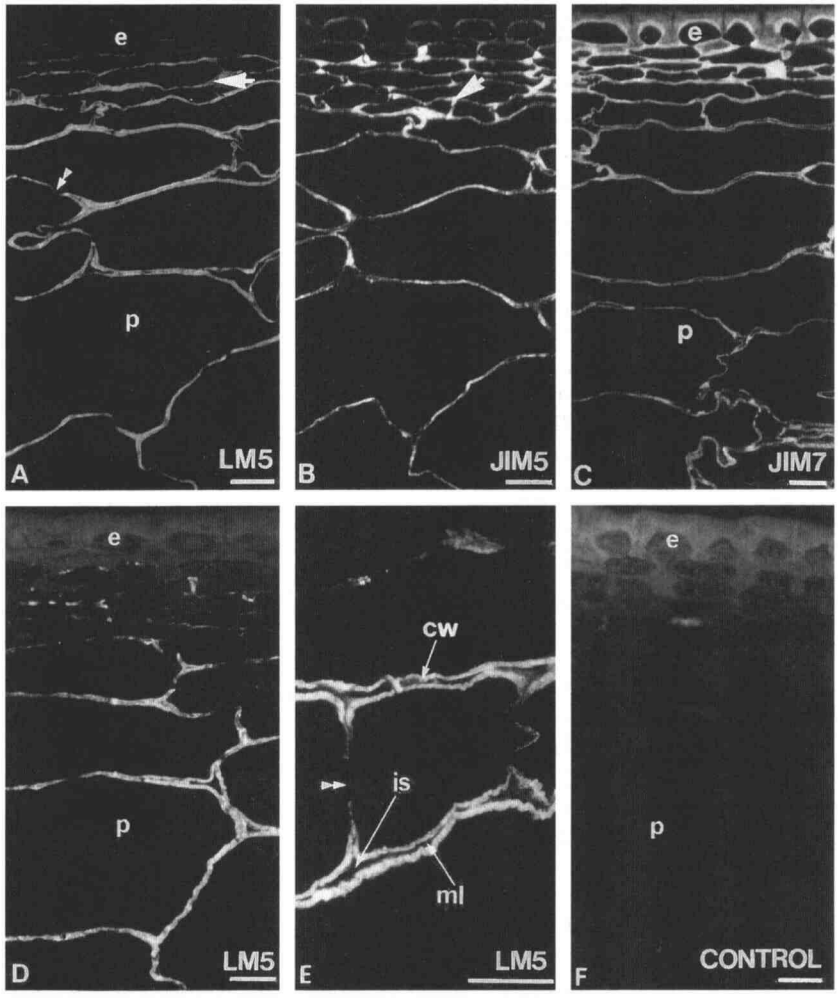


**Figure 3.** Indirect immunofluorescence of sections (approximately 1 $\mu$m in thickness) of unripe (A–C) and ripe (D–F) tomato pericarp tissue labeled with LM5 (A, D, and E), JIM5 (B), and JIM7 (C). The control image (F) demonstrates autofluorescence of the epidermis in ripe tomato fruit. The single arrowheads signify unlabeled (LM5) and labeled (JIM5) intercellular spaces, and double arrowheads denote unlabeled regions of cell walls. e, Epidermis; p, pericarp; cw, cell wall; is, intercellular space; ml, middle lamella. Bar = 100 $\mu$m.

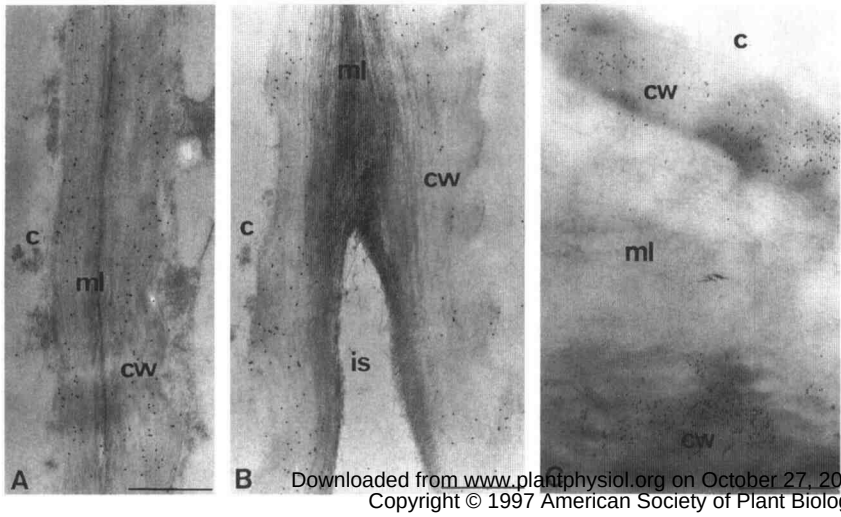


**Figure 4.** Immunogold-labeled sections of unripe (A and B) and ripe (C) tomato pericarp with LM5. Sections were stained after immunogold labeling with 4% uranyl acetate for 1 min (A and B) or left unstained (C). cw, Cell wall; ml, middle lamella; c, cytoplasm; is, intercellular space. Bar = 1 $\mu$m.

the middle lamella region (Fig. 3E). Labeling with LM5 in unripe and ripe tissue was abolished in the presence of the GAL4 or TG1, whereas BSA had no effect. Arabinofuranosidase and acid treatment of tomato pericarp sections also had no effect on LM5 labeling (data not shown).

When viewed with an electron microscope, the cell walls in unripe tissue were seen to be composed of fairly densely packed fibrils with the middle lamella visible as a more electron-dense region between the walls of adjacent cells (Fig. 4A). LM5 immunolabeled the cell walls of inner pericarp cells abundantly. The gold particles appeared to be generally distributed across the cell wall, although there were some indications of more densely and more sparsely labeled regions. The areas of expanded middle lamella at intercellular spaces were not labeled (Fig. 4B). In ripe fruit, the cell walls appeared to disintegrate and disperse, particularly in the middle lamella region. LM5 labeled the intact cell walls but not the disintegrating fibrillar material occurring in the greatly expanded middle lamella (Fig. 4C).

Since immunolocalization studies with LM5, JIM7, and JIM5 in tomato fruit indicated differences in the distribution of three structural elements of pectin within the cell wall, further investigations of primary cell walls from other regions of the tomato plant were carried out. Transverse sections of tomato petioles labeled with the anti-(1→4)-β-galactan and the anti-pectic homogalacturonan antibodies JIM5 and JIM7 revealed different binding patterns in the collenchyma tissue (Fig. 5). Collenchyma cells have an unevenly thickened primary cell wall. LM5 labeled the inner region of the primary cell wall only and not the thickened areas at cell junctions. Labeling was also mainly restricted to the tangential walls of the collenchyma cells with little or no labeling in the radial walls. In contrast, JIM5 labeling was predominantly localized within the regions of thickened primary cell wall with comparatively minor labeling in the tangential and radial walls. JIM7 labeling was much more abundant and evenly distributed throughout the cell walls. Attempts were made to confirm these findings with immunogold labeling experiments. However, LM5 labeling was very sparse due to the comparatively low abundance of the galactan in tomato petiole cell walls (in comparison with tomato pericarp).

## DISCUSSION

Highly specific antibodies have been generated by immunizing rats with a pectic galactan isolated from tomato pericarp cells (TG1) and with GAL4-BSA. The antisera isolated from TG1-immunized (aTG) and GAL4-BSA-immunized (aGal4) rats showed a very similar specificity for a variety of (1→4)-β-galactosyl-containing polysaccharides, indicating that both antisera recognized similar epitopes. The aGal4 serum did not react with gum arabic or larchwood arabinogalactan, both of which are composed typically of (1→3)(1→6)-β-linked galactosyl residues. The aTG serum did, however, react weakly with gum arabic, so there may exist a small proportion of anti-arabinosyl antibodies in aTG. The monoclonal antibody LM5 was isolated from a fusion following immunization with GAL4-BSA and was found to be specific for (1→4)-β-galactosyl residues. An indirect competitive ELISA revealed that more than three consecutive units of (1→4)-β-Gal are required for interaction with LM5, since (1→4)-β-galactotriose was ineffective as an inhibitor of antibody binding, as was (1→3)-α-(1→4)-β-(1→3)-α-galactotetraose. The only available hapten found to inhibit LM5 binding was the GAL4, which was as expected. The binding observed between LM5 and citrus pectin, apple pectin, polygalacturonic acid, and locust bean gum was likely to be a result of small amounts of Gal in these polysaccharides. Ara substitution of the tomato galactan did not appear to influence the binding of LM5.

Tissue printing studies with the anti-(1→4)-β-galactan demonstrated that the LM5 epitope was found predominantly in the pericarp tissue of mature, green tomato fruit. Since only soluble components are transferred to the nitrocellulose during tissue printing, it was possible that the LM5 epitope was present in the locular gel in an insoluble form. Immunofluorescent labeling of the gel with LM5 indicated that this was not the case (data not shown). In addition, studies by Huber and Lee (1986) and Cheng and

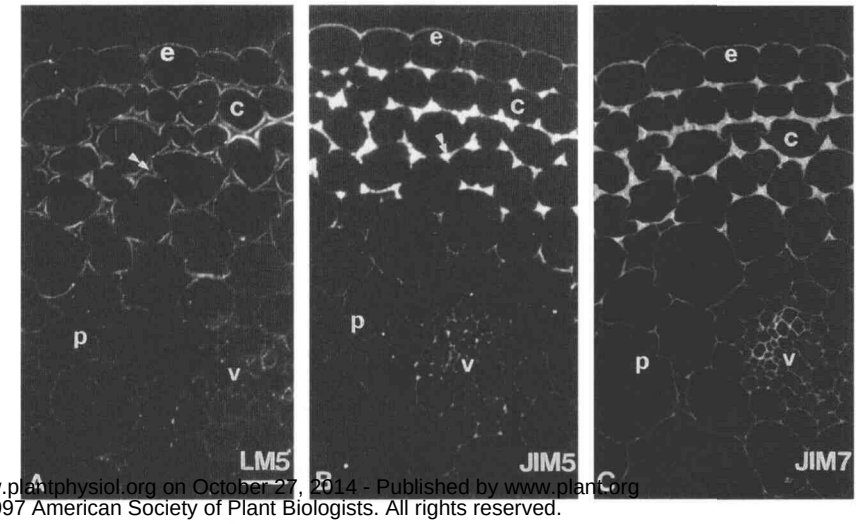


**Figure 5.** Indirect immunofluorescence of transverse sections of tomato petiole labeled with LM5 (A), JIM5 (B), and JIM7 (C). Double arrowhead denotes central regions of thickened collenchyma cell walls. e, Epidermis; c, collenchyma tissue; p, parenchyma tissue; v, vascular tissue. Bar = 100 μm.

Huber (1996) reported that a loss of galactosyl residues occurs in the locular tissue concomitantly with gel formation. JIM5, which recognizes the unesterified galacturonic acid backbone of pectin, binds throughout the tomato fruit, particularly in the locular gel that surrounds the seeds. These results demonstrate spatial differences in the distribution of structural elements of pectin between different regions of the fruit. It may also indicate a role for the neutral side chains of pectin in maintaining the firmer texture of the pericarp tissue.

Immunofluorescence and immunogold labeling confirmed that the LM5 (1→4)-β-galactan epitope occurred in the cell walls of tomato pericarp and revealed that it was absent from the outer pericarp (epidermal and subepidermal) cell walls. In unripe tomato pericarp the LM5 epitope was generally distributed throughout the cell walls, but no consistent pattern of labeling could be determined, with some regions of the cell wall and/or middle lamella labeled and others not labeled. In onion, the middle lamella has been reported to contain pectins with a lower proportion of neutral sugar side chains than pectins from the primary cell wall (Redgwell and Selvendran, 1986). However, in this study immunolabeling indicated that LM5 bound to many regions of the middle lamella. In ripe fruit, cell walls undergo extensive degradation. Degradation is most evident in the middle lamella region and the inner part of the primary cell wall, whereas the outer part maintains its fibrillar appearance (Ben-Arie et al., 1979). The LM5 epitope was still abundant in ripe fruit, although the disintegrating fibrillar material was generally not labeled. Thus, the major difference between unripe and ripe pericarp appeared to be a reduction in middle lamella labeling in ripe fruit, which may be the main site of galactosyl loss during ripening. However, the overall intensity of cell wall labeling with LM5 appeared to increase during ripening. This is not as expected but may reflect cell wall changes that make the epitope much more accessible.

Since immunolocalization studies with the three anti-pectin antibodies in tomato fruit revealed differences in the distribution of these three structural elements of pectin within the cell wall, additional investigations were carried out with further plant tissues. Localization of the neutral side chains in tomato petiole sections with the anti-(1→4)-β-galactan revealed a pattern of labeling in the collenchyma tissue that was different from labeling with JIM7 and JIM5. Labeling of the parenchyma tissue with these three antibodies was very similar, but the LM5 epitope was generally less abundant. Collenchyma is a specialized structural tissue that provides strength and support to areas of growth. The walls of collenchyma cells are much thicker than typical primary cell walls, but they maintain the ability to expand. The cell walls are unevenly thickened and are often thickened at junctions between three or more cells (Mauseth, 1988). In collenchyma cells, LM5 labeling was most abundant in the inner region of the tangential primary walls and notably absent from the central region of the thickened areas. In comparison, JIM5 labeled the thickened regions of the cell walls predominantly, and JIM7 labeling was present throughout the collenchyma cell walls. The patterns of pectin epitopes in these thickened areas suggest that distinct pectin domains have specific structural roles in collenchyma cell wall architecture. Indeed, Jarvis (1992) reported on a role for pectin in controlling the thickness of collenchyma cell walls.

The (1→4)-β-galactans are known to be some of the most flexible cell wall polymers (Ha et al., 1996), and the presence of side chains decreases the ability of pectin molecules to cross-link and form a coherent gel network (Hwang and Kokini, 1991). The absence of neutral galactan side chains, the abundance of acidic pectin epitopes in the epidermal cells of the tomato fruit, and collenchyma cell wall thickenings may indicate that extensive cross-linking of the homogalacturonan chains occurs at these locations, promoting rigidity and strength. Pectic galactans are degraded not only in ripening fruit but also in senescent flower petals and during hypocotyl extension growth (Labavitch and Ray, 1974; Nishitani and Masuda, 1980). The generation of the highly defined monoclonal antibody described here provides a powerful tool with which to follow the developmental dynamics and to increase our understanding of the functional role of galactan-rich side chains of pectin in plant cell walls.

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