Plant Physiol. (1995) 107: 1129–1138

Characterization of the Cell-Wall Polysaccharides of *Arabidopsis thaliana* Leaves[1]

Earl Zablackis, Jing Huang, Bernd Müller[2], Alan G. Darvill, and Peter Albersheim*

Complex Carbohydrate Research Center and Department of Biochemistry and Molecular Biology, University of Georgia, 220 Riverbend Road, Athens, Georgia 30602–4712

The cell-wall polysaccharides of *Arabidopsis thaliana* leaves have been isolated, purified, and characterized. The primary cell walls of all higher plants that have been studied contain cellulose, the three pectic polysaccharides homogalacturonan, rhamnogalacturonan I, and rhamnogalacturonan II, the two hemicelluloses xyloglucan and glucuronoarabinoxylan, and structural glycoproteins. The cell walls of *Arabidopsis* leaves contain each of these components and no others that we could detect, and these cell walls are remarkable in that they are particularly rich in phosphate buffer-soluble polysaccharides (34% of the wall). The pectic polysaccharides of the purified cell walls consist of rhamnogalacturonan I (11%), rhamnogalacturonan II (8%), and homogalacturonan (23%). Xyloglucan (XG) accounts for 20% of the wall, and the oligosaccharide fragments generated from XG by *endo*glucanase consist of the typical subunits of other higher plant XGs. Glucuronoarabinoxylan (4%), cellulose (14%), and protein (14%) account for the remainder of the wall. Except for the phosphate buffer-soluble pectic polysaccharides, the polysaccharides of *Arabidopsis* leaf cell walls occur in proportions similar to those of other plants. The structures of the *Arabidopsis* cell-wall polysaccharides are typical of those of many other plants.

The cells of plants are surrounded by a hydrated wall composed of complex carbohydrates, glycoproteins, and phenolics (Darvill et al., 1980a; Fry, 1988). The primary cell walls of all plants, those walls that surround growing plant cells, are composed of cellulose, pectins, hemicelluloses, and in most cases glycoproteins (Darvill et al., 1980a). Many primary cell walls of cereals also contain mixed-linked β-glucans. Cellulose, a linear polymer of β-1,4-linked-D-glucopyranosyl residues, is the major load-bearing polymer in the wall (Franz and Blaschek, 1990). The pectins are a group of three polysaccharides, each of which contains a high proportion of 1,4-linked α-D-galactosyluronic acid residues (York et al., 1985b). The three pectic polysaccharides are homogalacturonan, RG-I, and RG-II (O'Neill et al., 1990). The hemicellulosic polysaccharides are functionally defined as those polysaccharides that form strong hydrogen-bonded complexes with cellulose fibers (York et al., 1985b). XG and AX are the hemicelluloses of the primary cell walls of higher plants.

Homogalacturonan, which is a chain of partially methylesterified 1,4-linked α-D-galactosyluronic acid residues, has a propensity to form gels, a property that may reflect its function in wall structure (Morris, 1986). RG-I is partially solubilized from cell walls by treatment with EPG. The backbone of RG-I consists of a linear diglycosyl repeating unit [-α-D-galactopyranosyluronic acid-(1,2)-α-L-rhamnopyranosyl-(1,4)-] (Lau et al., 1985). Approximately one-half of the 2-linked rhamnosyl residues of RG-I are substituted at C-4 with structurally complex oligoglycosyl side chains containing branched and linear arabinosyl and galactosyl residues and to a lesser extent terminal fucosyl and glucosyluronic acid residues (McNeil et al., 1982; Lau et al., 1987; O'Neill et al., 1990). RG-II is a highly substituted α-1,4-linked homogalacturonan that has a total of approximately 30 glycosyl residues. The 30 glycosyl residues of RG-II encompass 11 different sugars, including the following unusual or unusually modified sugars, which are present in cell walls only in RG-II: 2-*O*-methylxylose, 2-*O*-methylfucose, Kdo, 3-*C*-carboxy-5-deoxy-L-xylose (aceric acid), Dha, and apiose (York et al., 1985a; Stevenson et al., 1988a, 1988b). The backbone of RG-II consists of at least nine 1,4-linked α-D-galactosyluronic acid residues. Four different, structurally characterized oligoglycosyl side chains are attached to the RG-II backbone.

XGs are composed of a branched, 4-linked β-D-glucan backbone. In many plants, approximately 75% of the backbone glucosyl residues are substituted at O-6 with an α-D-xylosyl residue (Bauer et al., 1973). Selected xylosyl residues are themselves substituted at C-2 with α-L-fucopyranosyl-(1,2)-β-D-galactopyranosyl or β-D-galactopyranosyl units. The β-D-galactosyl residues are often substituted with one or two *O*-acetyl groups (York et al., 1988). XGs are thought to be load-bearing structures in the cell wall because of their ability to noncovalently cross-link cellulose microfibrils (Bauer et al., 1973; Hayashi and Maclachlan, 1984). The dynamic nature of this cross-linking

[1] Supported in part by U.S. Department of Energy (DOE)-funded (DE-FG09–93ER20097) Center for Plant and Microbial Complex Carbohydrates and DOE grant DE-FG09–93ER20115 (to A.G.D.). This is No. XXXIX in the series entitled Structure of Plant Cell Walls.

[2] Present address: SmithKline Beecham Fink GmbH, Benzstrasse 25, 71083 Herrenberg, Germany.

* Corresponding author; e-mail palbersh@mond1.ccrc.uga.edu; fax 1–706–542–4412.

Abbreviations: AX, arabinoxylan; Dha, 3-deoxy-D-*lyxo*-2-heptulosaric acid; EG, *endo*glucanase; EPG, *endo*polygalacturonase; GAX, glucuronoarabinoxylan; Kdo, 3-deoxy-D-*manno*-octulosonic acid; RG-I, rhamnogalacturonan I; RG-II, rhamnogalacturonan II; XG, xyloglucan.

Downloaded on January 16, 2021. - Published by https://plantphysiol.org
Copyright (c) 2020 American Society of Plant Biologists. All rights reserved.

has been hypothesized to be a major factor in controlling the rate of cell-wall expansion, thereby regulating plant growth. Furthermore, specific XG-derived, Fuc-containing oligosaccharides have been shown to have growth-inhibiting and -promoting activity (York et al., 1984; McDougall and Fry, 1991; Aldington and Fry, 1993).

GAX is an acidic hemicellulosic polysaccharide with a backbone consisting of 4-linked-β-D-xylosyl residues. In many dicotyledonous plants, approximately 25% of the backbone xylosyl residues are substituted at O-2 with either arabinosyl, glucosyluronic acid, or 4-*O*-methyl glucosyluronic acid residues (Darvill et al., 1980b). GAX and AX are found as the major hemicelluloses of Gramineae cell walls.

Arabidopsis thaliana is used widely in molecular biology. The relative ease with which *Arabidopsis* mutants can be obtained has led to mutants with altered cell-wall glycosyl compositions (Reiter et al., 1993). Before characterizing the structural alterations in these mutants, we decided to determine the compositions and structures of the wall polysaccharides of wild-type plants. In this paper, we describe the isolation and characterization of *Arabidopsis* leaf cell-wall polysaccharides and compare the *Arabidopsis* polysaccharides with those of the walls of suspension-cultured sycamore cells.

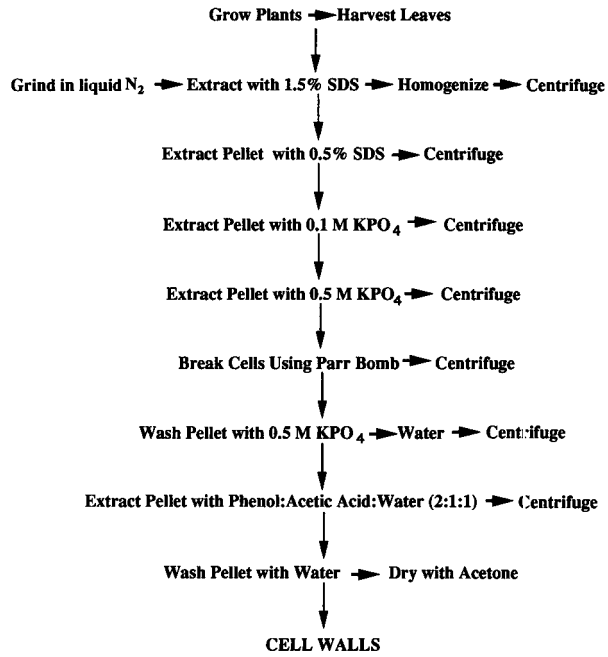


Figure 1. Scheme followed for the preparation of cell walls from leaves of *Arabidopsis*.

MATERIALS AND METHODS

Plant Growth and Isolation of Cell Walls

Seeds of wild-type *Arabidopsis thaliana* (Columbia type, Somerville lab) were obtained from Christopher Somerville, Clint Chappel, and Wolf-Dieter Reiter (Michigan State University, East Lansing). Plants were grown under a 12:12 h light:dark regime at 22°C for 4 weeks. The plants were placed in the dark for 24 h prior to harvesting. The leaves were processed and cell walls isolated as illustrated in the scheme shown in Figure 1. The individual wall polysaccharides and/or their oligosaccharide subunits were isolated as shown in Figure 2. The procedures used are described below.

Extraction of Cell-Wall Polysaccharides

Portions of the pectic material were solubilized from the walls by each of the following sequential treatments: phosphate buffer, EPG, NaOH, EPG, and Na_2CO_3. Residual starch and pectic material were removed by suspending cell walls (10 g L^{-1}) in buffer (0.1 M potassium phosphate, pH 7) containing 0.02% thimerosal and 5000 units of α-amylase (type IIA from *Bacillus* sp., Sigma) per g of cell wall. The reaction was carried out in polypropylene bottles placed in a shaking water bath at 30°C for 24 h. After 24 h the enzyme reaction mixture was centrifuged at 8000*g* for 20 min, the supernatant was removed and stored at 4°C, the pellet was suspended in buffer containing 0.02% thimerosal with an additional 5000 units of α-amylase per g of cell wall added, and the digestion was continued for an additional 24 h. The sample was then centrifuged as above, and the pellet was washed once with buffer and once with water and recovered by centrifugation. The supernatants and washes were combined in 1000 mol wt cutoff dialysis tubing, dialyzed against water at 4°C, concentrated using a Rotovapor, and then lyophilized. The pellet was washed with acetone and air dried. Extraction of the cell walls with potassium phosphate buffer in the absence of α-amylase extracted the same components from the walls (data not shown). No polygalacturonase activity was detectable in the α-amylase preparation.

Following treatment with α-amylase, the walls were treated with EPG as described by Lerouge et al. (1993). The walls were then de-esterified by suspending the samples in water, adjusting to pH 12 with cold 0.1 M NaOH, and stirring 4 h at 4°C while maintaining the pH at 12 by addition, as needed, of 0.1 M NaOH. The cell-wall suspension was filtered through glass-fiber filters, and the wall residue was washed extensively with water. The washes and NaOH-solubilized material were combined in 1000 mol wt cutoff dialysis tubing, dialyzed against water, concentrated using a Rotovapor, and then lyophilized. The walls were then re-treated with EPG and the extract was recovered as above.

Na_2CO_3 extraction of the walls after the second EPG treatment and purification of the Na_2CO_3-solubilized material were carried out as described by Ishii et al. (1989).

RG-I, RG-II, and oligogalacturonides were isolated from the EPG-, NaOH-, and potassium phosphate buffer-solubilized material as described by Marfà et al. (1991). RG-I isolated in the various extracts of *Arabidopsis* leaves had rhamnosyl:galactosyluronic acid ratios between 1:2 and 1:5 rather than 1:1 as was obtained elsewhere (O'Neill et al., 1990), suggesting that the *Arabidopsis* RG-I contained some 4-linked galactosyluronic acid residues resistant to EPG. It is known that methyl-esterified galactosyluronic acid residues are resistant to cleavage by EPG (McNeil et al., 1980).

Downloaded on January 16, 2021. - Published by https://plantphysiol.org
Copyright (c) 2020 American Society of Plant Biologists. All rights reserved.

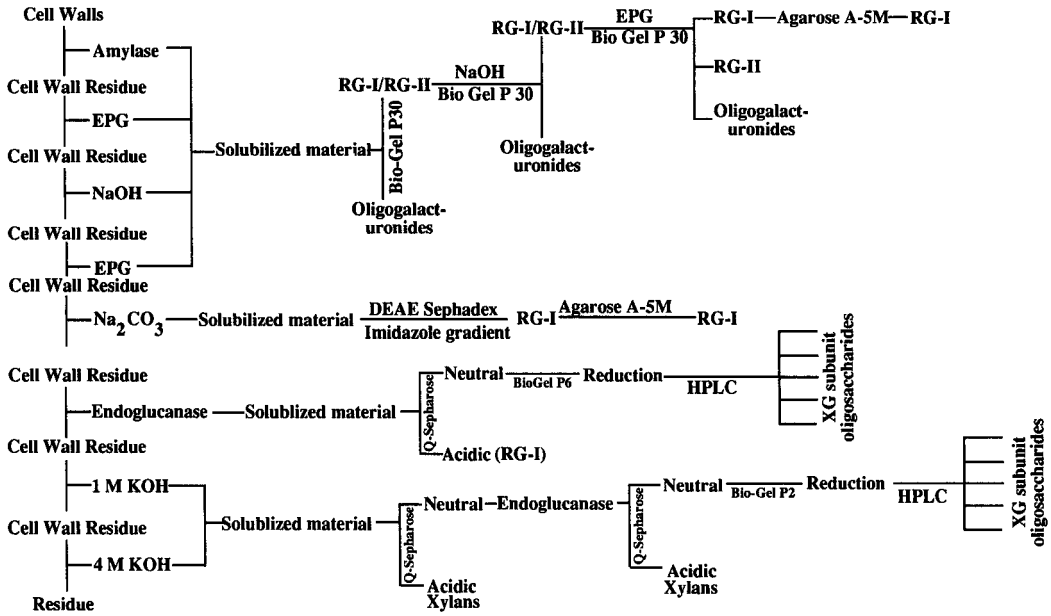


Figure 2. Scheme followed for the preparation and purification of polysaccharides from leaves of *Arabidopsis*.

Therefore, the RG-I was re-treated with NaOH and EPG and then treated with *exo*polygalacturonase as described by Lerouge et al. (1993). The products of the reaction were separated by gel permeation chromatography using a Bio-Gel P-30 column (2.5 × 35 cm) that had been equilibrated with 0.05 M sodium acetate, pH 5.2. The products were eluted from the column with 0.05 M sodium acetate and 5-mL fractions were collected. The RG-I that eluted from the column had a rhamnosyl:galactosyluronic acid ratio of approximately 1:1.

After Na_2CO_3 extraction, the walls were treated sequentially with EG, 1 M KOH, and 4 M KOH to solubilize hemicelluloses, xylan, and XG. The residual walls (1 g) remaining after Na_2CO_3 extraction were incubated (24 h, ambient temperature) in buffer (100 mL, 50 mM acetic acid-sodium acetate, 0.02% thimerosal, pH 5.2) containing *endo*-(1→4)-β-glucanase (60 units) from *Trichoderma reesei* (Megazyme Australia, Inc., Warriewood, Australia). The wall suspension was then passed through a glass-fiber filter. The filtrate was stored at 4°C, the walls were resuspended in buffer, another 60 units of the EG was added, and the suspension was incubated at ambient temperature for an additional 24 h. The wall suspension was then filtered as above and washed extensively with water. The EG-solubilized material and washes were combined, neutralized, and then separated into neutral and acidic fractions by passing the solution over a column of Q-Sepharose (2.5 × 45 cm) that had been equilibrated with 10 mM imidazole buffer, pH 7. The neutral fraction was concentrated to 10 mL on a Rotovapor, desalted by passing the concentrate over a column of Sephadex G-10 (2.5 × 17 cm), and then fractionated by gel permeation chromatography (see below). The bound acidic fraction was eluted from the column with 2 M imidazole, placed in 1000 mol wt cutoff dialysis tubing, dialyzed against water, concentrated using a Rotovapor, and lyophilized.

The KOH extractions were carried out as follows. EG residual walls (1 g) were dispersed in 1 M KOH (200 mL containing 1 mg mL^{-1} $NaBH_4$) and stirred for 2 h at 4°C. The wall suspension was then passed through a glass fiber filter and the walls were washed extensively with water. The 1 M KOH-solubilized material and washes were combined, neutralized with acetic acid, dialyzed, and lyophilized. The walls were resuspended in 4 M KOH (200 mL containing 1 mg mL^{-1} $NaBH_4$) and stirred for 2 h at ambient temperature. The 4 M KOH-solubilized material was treated as above and the wall residue dried with acetone.

The 1 and 4 M KOH extracts were each separated into neutral and acidic fractions by ion-exchange chromatography. The extracts (80–100 mg) were separately dissolved in 10 mM imidazole buffer (5–10 mL) and passed over a column of Q-Sepharose (2.5 × 45 cm) that had been equilibrated with 10 mM imidazole buffer, pH 7. The neutral fractions were concentrated on a Rotovapor, desalted by passing the concentrate over a column of Sephadex G-10 (2.5 × 17 cm), and lyophilized. The bound acidic fraction was eluted from the column with 2 M imidazole buffer, pH 7, placed in 1000 mol wt cutoff dialysis tubing, dialyzed against water, concentrated using a Rotovapor, and lyophilized.

XG oligosaccharide subunits were generated and purified from the neutral fractions of the EG and 4 M KOH extracts as follows. The neutral EG extract was separated into oligosaccharide size classes by Bio-Gel P-6 chromatography (1.5 × 85 cm column equilibrated with 10 mM imidazole buffer, pH 7). Four size-fractions were collected, concentrated to 10 mL, desalted by passing each concentrate over a column of Sephadex G-10 (2.5 × 17 cm), and lyophilized. The neutral component of the 4 M KOH extract (21 mg) was incubated (48 h, ambient temperature) in buffer (22 mL, 50 mM HOAc-NaOAc, 0.02% thimerosal, pH

Downloaded on January 16, 2021. - Published by https://plantphysiol.org
Copyright (c) 2020 American Society of Plant Biologists. All rights reserved.

5.2) containing *endo*-(1→4)-β-glucanase (1 unit) from *T. reesei* (Megazyme Australia, Inc.). The pH of the enzyme reaction mixture was adjusted to 7.8 and the solution passed over a Q-Sepharose column (1.5 × 10 cm) previously equilibrated with 10 mM imidazole buffer, pH 7. The neutral fraction was concentrated using a Rotovapor, desalted by passing the concentrate over a column of Sephadex G-10 (2.5 × 17 cm), and lyophilized. The neutral fraction of the *endo*-(1→4)-β-glucanase-re-treated 4 M KOH extract was separated into size classes by high-resolution Bio-Gel P-2 chromatography (York et al., 1990).

XG oligosaccharides from the EG and 4 M KOH extracts were converted to their corresponding oligoglycosyl alditols by reduction with $NaBH_4$ (York et al., 1993) and then lyophilized. The reduced XG oligoglycosyl alditols (1–10 mg) were dissolved in water (300 μL) and separated by reversed-phase HPLC using a Spherisorb 5 ODS (1) column (Phenomenex, Torrance, CA). The separation was accomplished by isocratic elution in 10% methanol (York et al., 1990).

Analytical Methods

Protein content was determined colorimetrically using the method of Bradford (1976). Hyp content and amino acid composition were determined as described by Lamport and Miller (1971) and Kieliszewski et al. (1992), respectively. Cellulose content was determined by the method of Updegraff (1969). Neutral sugar, uronic acid, and Kdo concentrations were determined by the following methods, respectively: anthrone method with Glc as the standard (Dische, 1962), metahydroxydiphenyl method with galacturonic acid as the standard (Blumenkrantz and Asboe-Hansen, 1973), and the modified thiobarbituric acid assay (York et al., 1985a).

Neutral glycosyl-residue compositions were determined by GLC of their alditol acetate derivatives. Combined neutral and acidic glycosyl-residue compositions were determined by GLC of their trimethylsilyl methyl-ester methylglycoside derivatives (York et al., 1985b).

Glycosyl-linkage compositions were determined using a modification of the Hakomori procedure (Hakomori, 1964). Oligo- and polysaccharides were per-*O*-methylated with butyl-lithium (2 M solution in cyclohexane; Aldrich, Milwaukee, WI) and iodomethane (Kvernheim, 1987), and the resulting products were isolated using Sep-Pak C_{18} cartridges (An et al., 1994). Uronic acid-containing oligo- and polysaccharides were methylated as above, carboxyl reduced with Superdeuteride (1 M Li-triethylborodeuteride in tetrahydrofuran, Aldrich) as described by York et al. (1985b), and remethylated as above. The glycosyl-linkage compositions were then determined by GC-MS of the partially methylated, partially acetylated alditol acetate derivatives (York et al., 1985b).

Oligogalacturonides were separated on an analytical CarboPac-I anion-exchange column installed in a Dionex (Sunnyvale, CA) BioLC system. The oligogalacturonides were detected using pulsed high-performance anion-exchange chromatography with pulsed amperometric detection as described by O'Neill et al. (1990). The components were compared to standard oligogalacturonides by their retention times.

XG oligosaccharide subunit structures were determined by ^{1}H-NMR spectroscopy. Hydroxyl protons of the XG oligoglycosyl alditols obtained by treatment of the walls with EG or KOH were exchanged with deuterons and the samples dissolved in distilled water. The 500-MHz ^{1}H-NMR spectra were recorded under standard conditions (York et al., 1993).

RESULTS AND DISCUSSION

Arabidopsis Cell Walls Are Composed of Protein and the Standard Six Wall Polysaccharides

Cell walls were isolated from the leaves of *Arabidopsis* and analyzed for protein and carbohydrate content. The *Arabidopsis* leaf cell walls are relatively rich in proteins that have an amino acid composition similar to those of a variety of other plant tissues (Kieliszewski et al., 1992). The purified walls contain 136 μg of protein containing only 1.7 μg Hyp per mg of cell wall prior to subjecting the walls to various extraction procedures. The cellulose-rich residue that remains after subjecting the walls to all of the extraction procedures described in this paper contains 20 μg protein and 1.3 μg Hyp per mg. Thus, approximately 99% of the total wall protein was removed by the extraction procedures. The intact walls (Table I) contain only 0.6 mol% Hyp. Thus, the *Arabidopsis* leaf cell walls are not as rich in Hyp as are, for example, the walls of suspension-cultured sycamore cells, which contain 12 mol% Hyp (Lamport and Northcote, 1960).

The carbohydrate components of the *Arabidopsis* cell walls were isolated, purified, and chemically characterized. Only six polysaccharides are present in the walls of *Arabi-*

Table I. *Amino acid compositions of Arabidopsis leaf cell walls and suspension-cultured sycamore cells*

Amino Acid	*Arabidopsis*		Sycamore[a]
	Intact	Residue[b]	Intact
		mol %	
Hyp	0.6	4.4	12.9
Asx	9.2	7.7	8.5
Thr	5.4	3.8	3.8
Ser	5.8	4.3	9.4
Glx	11.5	8.2	8.2
Pro	4.8	6.8	4.5
Gly	9.5	16.5	3.2
Ala	9.9	8.6	3.6
Val	6.5	7.1	6.2
Cys	n.d.[c]	n.d.	n.d.
Met	0.8	0.8	1.5
Ile	5.4	5.2	3.8
Leu	12.4	10.3	5.7
Tyr	2.3	1.5	5.1
Phe	3.8	5.2	4.4
Lys	5.4	4.5	13
His	1.3	0	3.4
Arg	4.8	5	3.3

[a] From Lamport and Northcote, 1960. [b] Cell-wall residue after extraction of polysaccharides. [c] n.d., Not determined.

Downloaded on January 16, 2021. - Published by https://plantphysiol.org
Copyright (c) 2020 American Society of Plant Biologists. All rights reserved.

Table II. *Percentage (by weight) of each extract accounted for by wall polysaccharides from Arabidopsis leaves*

Extract	Percentage of Wall Solubilized	Percentage of Extract Accounted for by					
		RG-I	RG-II	Homogalacturonan	Xylan	XG	Cellulose
Phosphate	34	11	11	37	0	0	0
EPG[a]	18	22	9	39	0	0	0
NaOH and carbonate	8	12	30	37	0	0	0
EG	5	40	0	0	4	40	16[b]
KOH	10	0	0	0	40	50	2[b]
Residue	20	n.d.[c]	n.d.	n.d.	n.d.	n.d.	63
% of Wall	95	11	8	23[d]	4	20[e]	14

[a] Cell walls contain 14% protein prior to EPG extraction. [b] Cellobiose. [c] n.d., Not determined. [d] Value is probably low because of loss of mono-, di-, and trimers of galacturonic acid during dialysis of oligogalacturonides. [e] Estimated value, see text.

dopsis; these are homogalacturonan, RG-I, RG-II, GAX, XG, and cellulose, the same polysaccharides that are found in the primary cell walls of all other higher plants studied (Table II; Fig. 2). The primary walls of some Gramineae contain, in addition to the six polysaccharides described here, a mixed-linked (β-1,3 and 1,4) glucan. No evidence of any other polysaccharide was found.

The relative amounts of the six polysaccharides differ in different plants and different tissues of individual plants. Even if we take this into consideration, *Arabidopsis* leaves are particularly rich in phosphate buffer-soluble pectic polysaccharides (20%). The distribution and percentages of the various polysaccharides solubilized from *Arabidopsis* leaf cell walls by each of the extraction procedures used are summarized in Table II.

Arabidopsis Cell Walls Are Rich in Phosphate Buffer-Soluble Pectic Polysaccharides

A problem facing those who study cell walls is to decide which polymers removed during purification of the walls actually originate from the wall and not, for example, from the cytoplasm. This is particularly pertinent to *Arabidopsis* wall studies, because an important fraction of the purified walls is extracted by phosphate buffer. In our studies of *Arabidopsis*, we have followed the widely accepted convention of not including, as wall components, any polymers extracted during preparation of the walls.

Cold water-soluble pectic polysaccharides have been obtained from a variety of cell walls (Jansen et al., 1960; Jensen and Ashton, 1960; Barbier and Thibault, 1982; Stevens and Selvendran, 1984; Redgwell and Selvendran, 1986). The amount of cold water-soluble pectic polysaccharides solubilized from different plant cell walls varies from less than 1% to greater than 10% of the wall. *Arabidopsis* leaf cell walls are rich in soluble pectic polysaccharides with 34% of the walls solubilized by phosphate buffer, which includes pectic polysaccharides that account for 20% of the walls. The phosphate buffer-soluble extract contains approximately one-half of all of the pectic material recovered from *Arabidopsis* leaf cell walls. Evidently much of the pectic polymers of *Arabidopsis* leaf cells are relatively weakly held in the wall.

RG-I

RG-I is a pectic polysaccharide solubilized from cell walls with EPG. The glycosyl-residue and glycosyl-linkage compositions of RG-I isolated from *Arabidopsis* leaf cell walls are compared in Table III to those of RG-I isolated

Table III. *Glycosyl-residue compositions and glycosyl-linkage compositions of Arabidopsis leaf and suspension-cultured sycamore cell RG-I released by EPG*

Glycosyl	*Arabidopsis*	Sycamore
	mol %	
Residue		
Ara	14	32
Rha	27	16
Fuc	1	2
Xyl	5	2
Gal	17	31
Glc	1	0
GalA	34	17
GlcA	1	1
Rha/GalA	0.79	0.94
Linkage		
T-Rha	2	2
2-	10	8
2,4-	3	8
2,3,4-	4	tr
T-Fuc	2	1
2,3-	1	0
T-Xyl*p*	4	2
T-Ara*f*	8	10
5-	6	11
3,5-	3	4
T-Gal	5	6
3-	4	3
4-	2	8
6-	6	8
2,4	tr	tr
T-GalA	1	2
4-	21	15

Downloaded on January 16, 2021. - Published by https://plantphysiol.org
Copyright (c) 2020 American Society of Plant Biologists. All rights reserved.

from suspension-cultured sycamore cells. The glycosyl linkages of *Arabidopsis* RG-I and sycamore RG-I are qualitatively the same.

The ratio of 2-linked to 2,4-linked rhamnosyl residues in RG-I is indicative of the degree of branching of the molecule's linear backbone, because the side chains of RG-I are attached to O-4 of the 2-linked rhamnosyl residues. *Arabidopsis* RG-I has fewer 2,4-linked rhamnosyl residues than 2-linked rhamnosyl residues in comparison with sycamore RG-I, indicating that *Arabidopsis* RG-I has fewer branches. Further evidence of less branching in *Arabidopsis* RG-I is provided by the smaller proportion of galactosyl and arabinosyl residues, which are only present in the side chains (Table III).

RG-I is also present in the EG and Na_2CO_3 extracts of *Arabidopsis* leaf cell walls. The presence of RG-I in the acidic fraction of the EG extract was verified by glycosyl-linkage composition analysis (Table IV). Although the EG extract accounts for only 5% of the *Arabidopsis* cell walls, RG-I accounts for 40% of the EG extract. The amount of RG-I solubilized from *Arabidopsis* leaf cell walls by Na_2CO_3 is similar to that extracted by Na_2CO_3 from sycamore cell walls (Ishii et al., 1989). The glycosyl-residue and glycosyl-linkage compositions of RG-I solubilized from *Arabidopsis* leaf cell walls by Na_2CO_3 are similar to those of RG-I isolated by the other extraction procedures (data not shown), including the ratio of 2-linked to 2,4-linked rhamnosyl residues (1:1). In contrast, RG-I solubilized from suspension-cultured sycamore cells by Na_2CO_3 has a 1:11 ratio of 2-linked to 2,4-linked rhamnosyl residues, indicating a much more highly branched molecule. Thus, *Arabidopsis* cell walls may not have the highly branched component of RG-I that is present in sycamore cells.

Table IV. *Glycosyl-linkage compositions of the acidic fractions from EG- and KOH-solubilized polysaccharides from Arabidopsis cell walls*

Glycosyl Linkage	EG	1 M KOH	4 M KOH	PS[a]
	mol %			
T-Rha	2		tr[b]	RG-I, RG-II
2-	12		6	RG-I, RG-II
3-	1	1	1	
2,4-	9	tr	7	RG-I
T-Fuc	2		2	RG-I, RG-II, XG
T-Xyl*p*	4	10	4	RG-I, RG-II, XG, GAX
4-		36	9	GAX
2,4-		19	2	GAX
4-Man		2		
T-Ara*f*	6	2	7	RG-I, RG-II, GAX
2-		3		GAX
3-		1	3	
5-	5	tr	7	RG-I
2,5-			3	
3,5-			3	RG-I
T-Ara*p*		9	tr	RG-II
T-Gal	10	1	7	RG-I, RG-II, XG
3-	3		4	RG-I
4-	7		7	RG-I
6-	4	1	4	RG-I
2,4-			2	RG-I, RG-II
T-Glc	4	tr	tr	
4-	2		2	XG
6-	2			XG
4,6-		1	4	XG
4-GalA	25	2	10	RG-I, RG-II
2,4-	3		tr	RG-II
T-GlcA		9	tr	GAX
2-		3	1	[c]

[a] Polysaccharide(s) that contain the indicated linkage. [b] tr, Trace. [c] Product of undermethylation.

RG-II

RG-II is a highly substituted 4-linked homogalacturonan that is distinguished by the presence of several unusual or unusually modified glycosyl residues. The glycosyl-residue and glycosyl-linkage compositions of *Arabidopsis* RG-II are similar to those of sycamore RG-II (Table V). Indeed, *Arabidopsis* RG-II contains all of the unusual sugars unique to RG-II. Our analysis might be taken as indicating that *Arabidopsis* RG-II contains fewer apiosyl and aceryl acid residues than does sycamore RG-II. These difficult to analyze, branched glycosyl residues are the attachment points of the RG-II side chains to the homogalacturonan backbone. Since the amount of the other glycosyl-residue components of the RG-II side chains are not lower than their counterparts in sycamore, the apparent low abundance of apiosyl and aceryl acid residues is in all likelihood the result of underestimation because of difficulties in quantifying these acid-labile residues.

Several glycosyl residues in the *Arabidopsis* RG-II analyses, detected by methylation analysis at 3 mol% or less, are not components of sycamore RG-II. Each of these glycosyl residues (2,4- and 3,4-linked rhamnosyl, 5-linked arabinofuranosyl, and 3- and 4-linked galactosyl) are major components of RG-I. The analysis of *Arabidopsis* RG-II also indicated higher than expected amounts of 2-linked rhamnosyl and 4-linked galactosyluronic acid residues, which are also abundant glycosyl residues of RG-I. Thus, the *Arabidopsis* RG-II preparation was likely contaminated with approximately 25% RG-I. If we consider the underestimation of apiosyl and aceryl acid residues and the contamination by RG-I, the glycosyl-residue and glycosyl-linkage compositions of *Arabidopsis* RG-II are typical of those of the other plants that have been analyzed.

Homogalacturonan

Homogalacturonan, as evidenced by unsubstituted, 4-linked galactosyluronic acid residues, is present in the phosphate buffer, EPG, NaOH, and Na_2CO_3 extracts of *Arabidopsis* leaf cell walls and accounts for at least 23% of the cell wall (Table II; Fig. 3). Many of the galactosyluronic acid residues in the homogalacturonan in all of the other plants so far studied are methyl esterified. Methyl-esterified galactosyluronic acid residues are resistant to EPG hydrolysis.

The pectic polysaccharides present in the above-mentioned extracts were treated with NaOH to de-esterify the galactosyluronic acid residues, and then the polysaccharr-

Downloaded on January 16, 2021. - Published by https://plantphysiol.org
Copyright (c) 2020 American Society of Plant Biologists. All rights reserved.

Table V. *Glycosyl-residue and glycosyl-linkage compositions of Arabidopsis leaf and sycamore RG-II*

Glycosyl	*Arabidopsis*	Sycamore
	mol %	
Residue		
Ara	17	10
Rha	10	12
Fuc	3	4
2Me-Fuc	1	4
Xyl	3	2
2Me-Xyl	2	5
Gal	9	9
Glc	2	2
GalA	44	31
GlcA	2	3
Api	3	6
Ace	1	3
Kdo	3	4
Dha	n.d.[a]	4
Linkage		
T-Rha	3	7
2-[b]	7	tr[c]
3-	4	6
2,4-[b]	3	0
3,4-[b]	3	0
2,3,4-	5	5
T-Fuc	4	5
3,4-	4	5
T-Xyl*p*	7	5
T-Ara*f*	5	6
5-[b]	3	0
T-Ara*p*	2	5
T-Gal	7	5
3-[b]	3	0
4-[b]	4	0
2,4-	2	6
T-GalA	7	10
4-[b]	13	9
2,4-	4	5
3,4-	3	7
2-GlcA	4	6

[a] n.d., Not determined. [b] RG-I contaminants. [c] tr, Trace.

ides were re-treated with EPG. This procedure generated mono-, di-, and trigalacturonides, providing evidence for the presence of methyl-esterified 4-linked galactosyluronic acid residues in *Arabidopsis* leaf cell walls.

The oligogalacturonides generated by EPG treatment of the cell walls before they were de-esterified ranged in size from degree of polymerization 1 to 12, similar to the size range of oligogalacturonides isolated by this procedure from suspension-cultured sycamore cells (Marfà et al., 1991). The sizes of the EPG-generated oligogalacturonides were determined by comparison, after de-esterification, of their high-performance anion-exchange chromatography with pulsed amperometric detection retention times with those of oligogalacturonide standards. The isolation from *Arabidopsis* leaf cell walls of EPG-generated oligosaccharides composed of unbranched sequences of 4-linked galactosyluronic acid residues establishes the presence of homogalacturonan in these walls.

GAX

GAX accounts for about 5% of the cell walls of dicots (Darvill et al., 1980b; Fry, 1988). This acidic hemicellulose is extracted from cell walls by treatment with base. *Arabidopsis* cell walls are no exception. Approximately one-half of the KOH-solubilized material is acidic in nature. Glycosyl-linkage composition analyses of the acidic fractions of the KOH-solubilized material showed that these fractions contain glycosyl residues characteristic of GAX, i.e. 4-linked xylosyl, T- and 2-linked arabinosyl, and T-glucosyluronic acid residues (Table IV). The acidic fractions of the KOH-solubilized material account for approximately 4% of the *Arabidopsis* leaf cell wall. GAX was not further purified from these fractions.

XG

The typical dicot cell wall contains about 20% XG. The EG and KOH extracts of *Arabidopsis* leaf cell walls contain glycosyl-residue and glycosyl-linkage compositions characteristic of XG (Table VI). The recovered XG accounted for only 7% of *Arabidopsis* leaf cell walls. However, the

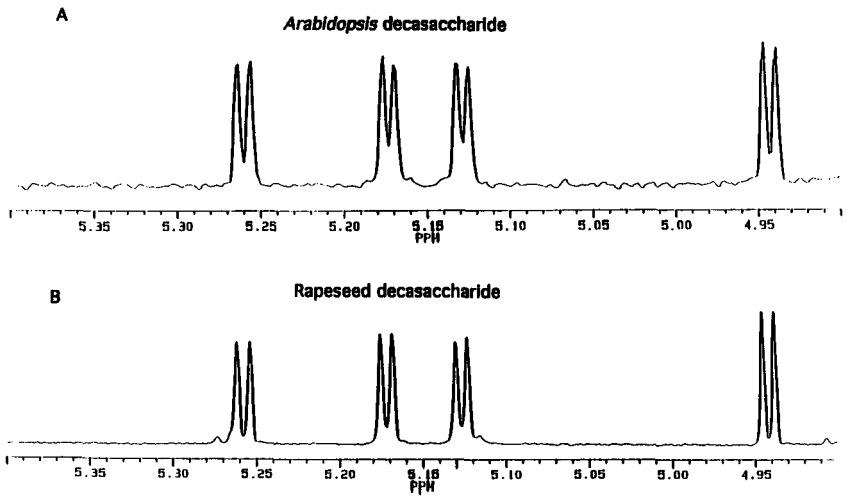


Figure 3. The α-anomeric regions of the ^{1}H-NMR spectra of reduced XG decasaccharide (i.e. nonaglycosyl alditol: XLFGol) isolated from *Arabidopsis* (A) and rapeseed (B).

Downloaded on January 16, 2021. - Published by https://plantphysiol.org
Copyright (c) 2020 American Society of Plant Biologists. All rights reserved.

low recovery of XG from *Arabidopsis* leaf cell walls relative to other dicot cell walls is undoubtedly due to the observed precipitation of XG during dialysis of the 4 M KOH extract. Glycosyl-residue composition analysis of the precipitate formed during dialysis of the neutral fraction of the 4 M KOH extract showed that it contains primarily xylosyl and glucosyl residues, which are the primary glycosyl residues of XG. Therefore, methylation analysis was performed on unextracted cell walls to obtain a more accurate approximation of the amount of XG present in *Arabidopsis* leaf cell walls. The amount of the glycosyl residues in the unextracted cell walls that can be attributed to XG (T- and 2-linked xylosyl, T-fucosyl, and 4,6-glucosyl) indicates that *Arabidopsis* leaf cell walls contain approximately 20% XG, a value typical of other dicots.

A small amount of glycosyl residues that could be from XG are found in the acidic fraction of the EG extract (Table IV). The existence of XG associated with pectic polymers has been reported (Selvendran, 1985), although the nature of the association between XG and pectic polymers has not been determined.

The structure of XG subunit oligosaccharides is of interest because of the growth-promoting and growth-inhibiting properties of some of the subunits. Therefore, XG subunit oligosaccharides were generated by EG treatment, purified from the neutral fractions of the EG and KOH extracts, and structurally characterized. The glycosyl-residue and glycosyl-linkage compositions of the quantitatively predominant XG subunit oligosaccharides are presented in Table VI. The glycosyl-residue and glycosyl-linkage compositions of the XG subunit oligosaccharides from *Arabidopsis* are the same as those of the corresponding XG subunit oligosaccharides of other plants. ^{1}H-NMR spectroscopy verified that the *Arabidopsis* XG subunit oligosaccharides, when reduced to the corresponding oligoglycosyl alditols, have the structures XXXGol, XXLGol, XXFGol, and XLFGol, which are the same as those isolated from such plants as pea, sycamore, and rapeseed (Fig. 3; Table VI). We were unable to determine whether *Arabidopsis* XG is acetylated in muro (York et al., 1984), because all of our assays were carried out on Na_2CO_3- or KOH-extracted walls, treatments that would hydrolyze acetyl esters.

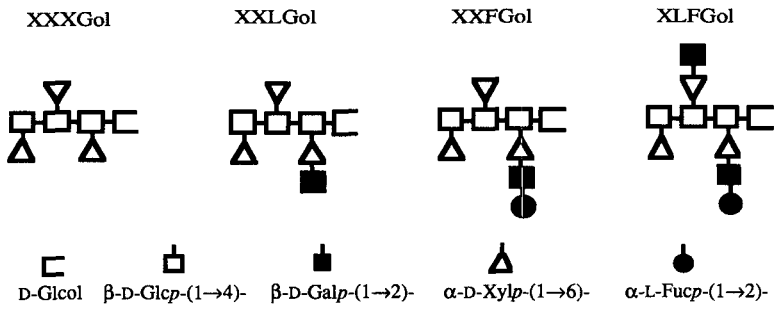
Cellulose

Cellulose, the load-bearing cell-wall polysaccharide that consists of long, linear chains of 4-linked glucosyl residues, is not solubilized by the extraction procedures used on the *Arabidopsis* cell walls (Fry, 1988). Twenty percent of the starting cell-wall material remained after the various chem-

Table VI. *Glycosyl-residue and glycosyl-linkage compositions of the XG subunit oligosaccharides from Arabidopsis leaf cell walls*

Numbers in parentheses are theoretical values.

Glycosyl	Heptasaccharide (XXXGol)	Octasaccharide (XXLGol)	Nonasaccharide (XXFGol)	Decasaccharide (XLFGol)
		mol %		
Residue				
Fuc			12 (11)	11 (10)
Xyl	47 (43)	37 (38)	35 (33)	33 (30)
Gal		17 (13)	11 (11)	19 (20)
Glc	53 (57)	45 (50)	42 (44)	37 (40)
Linkage				
T-Fuc			9 (11)	9 (10)
T-Xyl	37 (43)	25 (25)	18 (22)	9 (10)
2-		12 (13)	10 (11)	19 (20)
T-Gal		11 (13)		13 (10)
2-			9 (11)	9 (10)
4-Glc-ol	15 (14)	11 (13)	15 (11)	12 (10)
6-	18 (14)	11 (13)	12 (11)	11 (10)
4,6-	30 (29)	30 (25)	27 (22)	18 (20)



Downloaded on January 16, 2021. - Published by https://plantphysiol.org
Copyright (c) 2020 American Society of Plant Biologists. All rights reserved.

ical and enzymatic extractions. Cellulose, as evidenced by 4-linked glucosyl residues, accounts for 63 mol% of the wall residue. Additionally, 1% of the *Arabidopsis* leaf cell wall was recovered as cellobiose following *endo*-(1→4)-β-glucanase treatment and is assumed to originate from cellulose. Thus, cellulose accounts for approximately 14% of the *Arabidopsis* leaf cell wall.

The *Arabidopsis* cell-wall residue remaining after all of the extraction procedures contained minor amounts of rhamnosyl (3 mol%), mannosyl (11 mol%), arabinosyl (5 mol%), xylosyl (6 mol%), galactosyl (3 mol%), and galactosyluronic acid (8 mol%) residues, indicating that the residue contained some of the noncellulosic primary cell-wall polysaccharides that were incompletely extracted by the methods used, a phenomenon observed in studies of sycamore cell walls (Koller et al., 1991). The partial extraction of the wall polysaccharides probably reflects heterogeneity in the bonding of the polysaccharides to other components of the walls.

CONCLUSION

The results presented here show that the leaf cell walls of *Arabidopsis* contain the same polysaccharides found in the primary cell walls of many other plants. Except for the high proportion of phosphate buffer-soluble pectic polysaccharides, *Arabidopsis* leaf cell walls appear to be typical of other dicots. We are now in a position to characterize the cell walls of *Arabidopsis* mutants, e.g. the mutant deficient in cell-wall fucosyl residues recently reported by Reiter et al. (1993).

ACKNOWLEDGMENTS

We would like to thank Drs. C. Somerville (Carnegie Institute of Washington at Stanford University), C. Chappel (Purdue University), and W-D. Reiter (University of Connecticut) for seeds and leaf material of *A. thaliana* and Dr. M. Kieliszewski (Complex Carbohydrate Research Center) for the amino acid analyses.

Received July 13, 1994; accepted November 21, 1994.
Copyright Clearance Center: 0032–0889/95/107/1129/10.

LITERATURE CITED

Aldington S, Fry SC (1993) Oligosaccharins. Adv Bot Res **19:** 1–101

An J, O'Neill MA, Albersheim P, Darvill AG (1994) Isolation and structural characterization of β-D-glucosyluronic acid and 4-*O*-methyl β-D-glucosyluronic acid-containing oligosaccharides from the cell-wall pectic polysaccharide, rhamnogalacturonan I. Carbohydr Res **252:** 235–243

Barbier M, Thibault J-F (1982) Pectic substances of cherry fruits. Phytochemistry **21:** 111–115

Bauer WD, Talmadge KW, Keegstra K, Albersheim P (1973) The structure of plant cell walls. II. The hemicellulose of the walls of suspension-cultured sycamore cells. Plant Physiol **51:** 174–187

Blumenkrantz N, Asboe-Hansen G (1973) New method for quantitative determination of uronic acids. Anal Biochem **54:** 484–489

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal Biochem **72:** 248–254

Darvill AG, McNeil M, Albersheim P, Delmer DP (1980a) The primary cell walls of flowering plants. *In* NE Tolbert, ed, The Biochemistry of Plants, Vol I. Academic Press, New York, pp 91–162

Darvill JE, McNeil M, Darvill AG, Albersheim P (1980b) Structure of plant cell walls. XI. Glucuronoarabinoxylan, a second hemicellulose in the primary cell walls of suspension-cultured sycamore cells. Plant Physiol **66:** 1135–1139

Dische Z (1962) Color reactions of carbohydrates. *In* RL Whistler, ML Wolfrom, eds, Methods in Carbohydrate Chemistry, Vol 1. Academic Press, New York, pp 475–524

Franz G, Blaschek W (1990) Cellulose. *In* PM Dey, JB Harborne, eds, Methods in Plant Biochemistry, Vol 2. Academic Press, London, pp 291–322

Fry SC (1988) The growing plant cell wall: chemical and metabolic analysis. Longman Scientific & Technical, Essex, UK

Hakomori S (1964) A rapid permethylation of glycolipid and polysaccharide catalyzed by methylsulfinyl carbanion in dimethyl sulfoxide. J Biochem **55:** 205–208

Hayashi T, Maclachlan G (1984) Pea xyloglucan and cellulose. I. Macromolecular organization. Plant Physiol **75:** 596–604

Ishii T, Thomas J, Darvill A, Albersheim P (1989) Structure of plant cell walls. XXVI. The walls of suspension-cultured sycamore cells contain a family of rhamnogalacturonan-I-like pectic polysaccharides. Plant Physiol **89:** 421–428

Jansen EF, Jang R, Albersheim P, Bonner J (1960) Pectic metabolism of growing cell walls. Plant Physiol **35:** 87–97

Jensen WA, Ashton M (1960) Composition of developing primary wall in onion root tip cells. I. Quantitative analyses. Plant Physiol **35:** 313–323

Kieliszewski M, de Zacks R, Leykam JF, Lamport DTA (1992) A repetitive proline-rich protein from the gymnosperm Douglas fir is a hydroxyproline-rich glycoprotein. Plant Physiol **98:** 919–926

Koller A, O'Neill MA, Darvill AG, Albersheim P (1991) A comparison of the polysaccharides extracted from dried and non-dried walls of suspension-cultured sycamore cells. Phytochemistry **30:** 3903–3908

Kvernheim AL (1987) Methylation analysis of polysaccharides with butyllithium in dimethyl sulfoxide. Acta Chem Scand Ser B Org Chem Biochem *41:* 150–152

Lamport DTA, Miller DH (1971) Hydroxyproline arabinosides in the plant kingdom. Plant Physiol **48:** 454–456

Lamport DTA, Northcote DH (1960) Hydroxyproline in primary cell walls of higher plants. Nature **188:** 665–666

Lau JM, McNeil M, Darvill AG, Albersheim P (1985) Structure of the backbone of rhamnogalacturonan I, a pectic polysaccharide in the primary cell walls of plants. Carbohydr Res **137:** 111–125

Lau JM, McNeil M, Darvill AG, Albersheim P (1987) Treatment of rhamnogalacturonan I with lithium in ethylenediamine. Carbohydr Res **168:** 245–274

Lerouge P, O'Neill MA, Darvill AG, Albersheim P (1993) Structural characterization of endo-glycanase-generated oligoglycosyl side chains of rhamnogalacturonan I. Carbohydr Res **243:** 359–371

Marfà V, Golin DJ, Eberhard S, Mohnen D, Darvill A, Albersheim P (1991) Oligogalacturonides are able to induce flowers to form on tobacco explants. Plant J **1:** 217–225

McDougall GJ, Fry SC (1991) Xyloglucan nonasaccharide, a naturally-occurring oligosaccharin, arises *in vivo* by polysaccharide breakdown. J Plant Physiol **137:** 332–336

McNeil M, Darvill AG, Albersheim P (1980) Structure of plant cell walls. X. Rhamnogalacturonan I, a structurally complex pectic polysaccharide in the walls of suspension-cultured sycamore cells. Plant Physiol **66:** 1128–1134

McNeil M, Darvill AG, Albersheim P (1982) Structure of plant cell walls. XII. Identification of seven differently linked glycosyl residues attached to *O*-4 of the 2,4-linked L-rhamnosyl residues of rhamnogalacturonan I. Plant Physiol **70:** 1586–1591

Morris RR (1986) Molecular interaction in polysaccharide gelation. Br Polymer J **18:** 14–21

O'Neill M, Albersheim P, Darvill A (1990) The pectic polysaccharides of primary cell walls. *In* PM Dey, JB Harborne, eds, Methods in Plant Biochemistry, Vol 2. Academic Press, London, pp 415–441

Downloaded on January 16, 2021. - Published by https://plantphysiol.org
Copyright (c) 2020 American Society of Plant Biologists. All rights reserved.

Redgwell RJ, Selvendran RR (1986) Structural features of cell-wall polysaccharides of onion *Allium cepa*. Carbohydr Res **157:** 183–199

Reiter W-D, Chapple CCS, Somerville CR (1993) Altered growth and cell walls in a fucose-deficient mutant of *Arabidopsis*. Science **261:** 1032–1035

Selvendran RR (1985) Developments in the chemistry and biochemistry of pectic and hemicellulosic polymers. J Cell Sci (Suppl) **2:** 51–88

Stevens BJH, Selvendran RR (1984) Structural features of cell-wall polysaccharides of the carrot *Daucus carota*. Carbohydr Res **128:** 321–333

Stevenson TT, Darvill AG, Albersheim P (1988a) 3-Deoxy-D-lyxo-2-heptulosaric acid, a component of the plant cell-wall polysaccharide rhamnogalacturonan-II. Carbohydr Res **179:** 269–288

Stevenson TT, Darvill AG, Albersheim P (1988b) Structural features of the plant cell-wall polysaccharide rhamnogalacturonan-II. Carbohydr Res **182:** 207–226

Updegraff DM (1969) Semimicro determination of cellulose in biological materials. Anal Biochem **32:** 420–424

York WS, Darvill AG, Albersheim P (1984) Inhibition of 2,4-dichlorophenoxyacetic acid-stimulated elongation of pea stem segments by a xyloglucan oligosaccharide. Plant Physiol **75:** 295–297

York WS, Darvill AG, McNeil M, Albersheim P (1985a) 3-Deoxy-D-manno-2-octulosonic acid (KDO) is a component of rhamnogalacturonan II, a pectic polysaccharide in the primary cell walls of plants. Carbohydr Res **138:** 109–126

York WS, Darvill AG, McNeil M, Stevenson TT, Albersheim P (1985b) Isolation and characterization of plant cell walls and cell wall components. Methods Enzymol **118:** 3–40

York WS, Harvey LK, Guillen R, Albersheim P, Darvill AG (1993) Structural analysis of tamarind seed xyloglucan oligosaccharides using β-galactosidase digestion and spectroscopic methods. Carbohydr Res **248:** 285–301

York WS, Oates J, Van Halbeek H, Darvill AG, Albersheim P (1988) Location of the *O*-acetyl substituents on a nonasaccharide repeating unit of sycamore extracellular xyloglucan. Carbohydr Res **173:** 113–132

York WS, Van Halbeek H, Darvill AG, Albersheim P (1990) Structural analysis of xyloglucan oligosaccharides by ^{1}H-n.m.r. spectroscopy and fast-atom-bombardment mass spectrometry. Carbohydr Res **200:** 9–31

Downloaded on January 16, 2021. - Published by https://plantphysiol.org
Copyright (c) 2020 American Society of Plant Biologists. All rights reserved.