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Carbohydrate Research 295 (1996) 195–208

CARBOHYDRATE RESEARCH

Chemical and structural features of kiwifruit cell walls: Comparison of fruit and suspension-cultured cells

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Received 19 April 1996; accepted 3 September 1996

Abstract

Chemical and structural features were compared in cell-wall polysaccharides isolated from intact kiwifruit (*Actinidia deliciosa*) and suspension-cultured cells initiated from the same fruit. Morphological features of the cells and whole fruit tissue were also examined by electron microscopy. Cell-wall material (CWM) from cultured cells contained less cellulose, more protein, and had a higher degree of acetylation than cell-wall material from intact fruit. Whereas galactose was the major neutral sugar in the pectic polysaccharides of intact fruit, arabinose was predominant in cultured cells. The arabinose occurred as terminal and 5-substituted residues, presumably as neutral side-chains attached to the galacturonan backbone which was more highly branched in the cultured cells. The most significant structural difference in the hemicelluloses occurred in a galactoglucomannan purified from the 4 M KOH-soluble fraction. The backbone of the galactoglucomannan of the cultured cells was more branched than that in the intact fruit, with 84% of the 4-linked mannosyl residues substituted at O-6 with either single galactosyl or galactosyl-(1 → 2)-galactosyl groups. In intact fruit ~ 30% of the mannosyl residues were substituted at O-6. Rhamnogalacturonan II (RG-II) was partially purified from CWM and shown to possess a very similar composition of glycosyl residues in both intact fruit and cells. However, the primary cell walls of the cultured cells contained twice the amount of RG-II found in the cell walls of intact fruit. © 1996 Elsevier Science Ltd.

Keywords: Kiwifruit; Cell wall; Cultured cells; Polysaccharides

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PII S0008-6215(96)00234-0

1. Introduction

Suspension-cultured cells have been used to investigate many aspects of the physiology and metabolism of higher plants during growth and development. However, chemical studies of cell-wall structure using cultured cells have been performed mostly on non-fruit tissues. Suspension cultures of *Acer pseudoplatanus* in particular have been used to investigate the structure of cell-wall and extracellular polysaccharides and the molecular structure of cell walls [1,2]. Suspension-cultured cells of blackberry (*Rubus fruticosus*) were used by Cartier et al. [3] to characterise a cell-wall and extracellular galactoglucomannan (GGM). For the most part, however, cultured fruit cells have been used for physiological studies and to monitor changes associated with senescence and ethylene-related phenomena [4,5]. For structural investigations, the advantage in using cultured fruit cells instead of intact fruit is that the cells provide a convenient and continuous source of primary walls uncontaminated with secondary walls. Cells also allow near uniform radioactive labelling of cell-wall constituents, a near impossibility with intact fruit.

We have established a line of suspension-cultured cells from the outer pericarp of kiwifruit (*Actinidia deliciosa* [A.Chev.] C.F. Liang et A.R. Ferguson var. *deliciosa* cv. Hayward) which we intend to use as a source of cell-wall-associated enzymes and for elucidating chemical features of the fruit cell wall. It is not known to what extent the chemistry of the cell wall is altered by the differences in environment and physiological state which exist between cells in suspension culture and the parent fruit tissue. As a prelude to further investigations using cultured fruit cells we compared the composition and structural features of cell-wall polysaccharides isolated from kiwifruit fruit and from a cell-suspension culture derived from the same fruit.

2. Results and discussion

Cell-suspension cultures.—Samples of cells were taken at time 0, 4, 8, 12, and 18 days after sub-culturing and their growth characteristics measured by flow cytometry. The doubling time for cell number was 4 days up until 12 days. G_2 values (indicating dividing cells still joined by the developing cell plate) after 4, 8, 12, and 18 days were 19.2, 18.2, 19.3, and 11.7%, respectively. As the growth rate diminished, the number of dead cells increased to 33% after 18 days but the monosaccharide composition of cell-wall material (CWM) prepared from cells at each sampling time was very similar (data not shown).

Light and electron microscopy.—The suspension-cultured cells consisted of clumps which could easily be separated by gentle agitation into smaller groups of cells. These groups ranged in size from 2–3 cells to more than 20 (Fig. 1A). Individual isolated cells were rarely observed. On average the whole fruit cells were 2–3 times larger than the suspension-cultured cells. The latter were within a size range from 25×20 μm to 80×70 μm (mean $45 \pm 5 \times 37 \pm 4$ μm). Mature pericarp tissue also has two ranges of cell size, the smaller with diameters of 100–200 μm and the larger up to 1 mm [6].

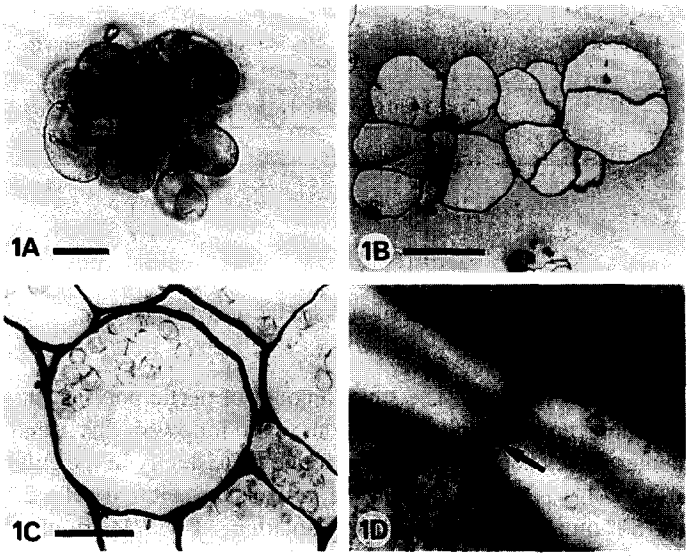

Fig. 1. (A) Unfixed suspension cells, light microscopy; (B) fixed suspension cells showing thin cell walls and small cell size, light microscopy; (C) fixed outer pericarp tissue showing thick cell walls and larger cell size, same magnification as 1B, light microscopy; (D) detail of cell wall between two cells in suspension culture, an isolated plasmodesmata is arrowed, transmission electron microscopy. ▬ A, B, C 100 μm, D 0.1 μm).

Both cultured and whole fruit cells contained large vacuoles with cytoplasm close to the cell wall (Fig. 1B). Cell walls in tissue-cultured cells had a smaller arithmetic-mean thickness than those in fruit pericarp tissue (average thickness 0.4 ± 0.04 μm compared to 1.1 ± 0.1 μm in unripe pericarp tissue, Fig. 1(B, C). Chloroplasts and starch grains which were numerous in whole fruit cells were rarely observed in cultured cells. A few cultured cells contained small starch granules (less than 1 μm in diameter). Transmission electron microscopy showed isolated plasmodesmata connecting cells within groups of the cultured cells (Fig. 1D), indicating a permanent connection. However, their frequency was markedly less than in cells of whole fruit and plasmodesmatal pit fields (as found in normal fruit tissue [6]) were absent.

Isolation and composition of CWM and solubilised polymers.—CWM was prepared from the cells and fruit by a procedure designed to minimise both solubilisation and inadvertent degradation of polysaccharides during extraction [7]. Phenol–acetic acid–water (PAW) used to inactivate endogenous enzymes during CWM isolation solubilised only a small amount of material from both the fruit and cells (Table 1). The Me_2SO-soluble fraction, however, was ~ 100-fold higher in the fruit compared to the cells. This result was expected, as unripe kiwifruit are very high in starch [7]. The composition of the Me_2SO-soluble fraction confirmed the presence of starch, as over 90% of the fraction was present as glucose (Table 2). Cultured cells, which were grown on medium containing 4% of D-glucose, contained relatively small amounts of starch.

CWM from cells contained less galactose and more arabinose, rhamnose, and fucose than CWM from fruit (Table 2). The ratio of galactose to arabinose was 7:1 in the CWM

Table 1
Yield of CWM, PAW-soluble, PAW-precipitate, and Me_2SO-soluble fractions from fruit and suspension cultured cells

Fraction	Yield (g/100 g fwt)	
	Cells	Fruit [a]
CWM	1.2	1.6
PAW-soluble	0.07	0.1
PAW-precipitate	0.7	0.8
Me_2SO	0.07	5.0

[a] At harvest, the fruit soluble-solids concentration was 4.2% (mean of 10 fruits).

of the fruit and 0.8:1 in the cells. The small amount of pectic polysaccharides contained in the PAW-soluble and precipitate fractions also followed this trend. In order to determine whether these changes were to be found consistently in the cultured cells over a long period, CWM was prepared from the suspension-cultured cells 2 years (subcultured at least 50 times) after the initial isolation of CWM from the cells. The composition of all fractions was nearly identical to the first CWM preparation.

The cellulose, protein, acetyl, and methyl ester contents of the CWMs were also determined. Values were the average of three separate determinations. CWM from fruit contained 28.2, 1.6, and 0.5% of cellulose, acetyl, and protein, respectively. Comparable values for the cells were 21.3, 2.3, and 2.7%. The methyl ester content was similar in both fruit and cells (48–51%). The increased acetyl content in the cells compared to the fruit CWM may be an important consideration when contemplating the use of enzymic

Table 2
Non-cellulosic monosaccharide composition of CWM, PAW-soluble, PAW-precipitate, and Me_2SO-soluble fractions isolated from fruit and suspension-cultured cells

Fraction	Sugar (mol%)								Total (μg/mg)
	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	GalA	
CWM									
Fruit	2.7	0.3	4.0	6.3	0.6	29.2	3.1	53.7	563.5
Cells	4.1	1.1	16.9	6.2	0.7	13.3	2.3	55.4	446.6
PAW-soluble									
Fruit	0.7	1.9	12.3	19.4	4.5	24.4	19.9	16.7	653.6
Cells	0.6	0.7	41.6	4.1	11.2	19.4	15.0	7.2	538.7
PAW-precipitate									
Fruit	2.0	tr [a]	3.0	5.0	1.1	22.1	35.0	31.9	395.2
Cells	2.1	1.4	29.2	tr	12.3	26.4	14.5	14.1	44.1
Me_2SO									
Fruit	tr	tr	0.8	0.8	0.4	0.7	92.8	4.5	1000
Cells	1.2	1.3	8.9	8.6	11.8	19.8	33.3	15.2	530.6

[a] tr = Trace.

Table 3
Monosaccharide composition of solubilized cell-wall polysaccharides from fruit and suspension-cultured cells

Fraction	% CWM	Sugar (mol%)								Total (μg/mg)
		Rha	Fuc	Ara	Xyl	Man	Gal	Glc	GalA	
H_2O										
Fruit	5.0	2.3	tr [a]	2.7	3.3	0.8	37.4	2.5	51.1	690.9
Cells	1.3	3.7	tr	10.8	3.0	0.9	7.8	2.6	71.3	512.0
CDTA										
Fruit	10.9	1.0	0.1	1.2	0.5	0.1	3.9	13.2	80.0	728.1
Cells	15.2	2.3	tr	10.3	1.0	0.2	3.8	0.9	81.6	602.2
Na_2CO_3										
Fruit	23.2	1.6	tr	1.9	0.4	tr	11.2	1.6	83.4	813.1
Cells	17.2	4.3	tr	14.2	1.6	0.4	6.5	1.2	71.8	463.3
KOH										
Fruit	15.6	1.3	0.7	1.9	29.0	8.1	12.8	32.2	14.0	712.5
Cells	20.4	2.3	1.6	9.5	16.8	8.8	15.0	27.3	18.7	524.0
Residue										
Fruit	45.1	4.6	tr	4.0	2.5	2.1	33.2	22.0	31.7	245.3
Cells	46.0	5.3	1.2	18.6	7.7	0.5	18.2	11.6	36.9	345.3

[a] tr = Trace.

hydrolysates as a source of polysaccharide fragments for structural analysis, as *O*-acetylation is known to alter the susceptibility of polysaccharides to glycanases [8]. The CWM from the cells contained five times as much protein as the fruit. As PAW is a very effective extractant for protein, some of this protein in the CWM is likely to be native to the wall and not merely cytoplasmic protein which has bound to the CWM during homogenisation of the tissue. The amino acid composition of the protein in both the suspension-cultured cells and the intact fruit was similar to that previously reported for CWM from the outer pericarp of intact fruit [9]. However, the protein from the CWM of the cultured cells contained three times the amount of hydroxyproline found in the intact fruit (3.5 mol% compared to 1.2 mol%). Hydroxyproline-rich proteins are associated with extensin-type proteins which are intimately associated with the α-cellulose of some plants [10]. The elevated level of hydroxyproline may indicate an increased synthesis of extensin-type proteins in the cell walls of the cultured cells.

The capacity of the CWMs to swell in water was measured [11]. The CWM from cultured cells swelled four times as much as CWM from fruit.

Yield and composition of cell-wall fractions.—The yield and carbohydrate composition of cell-wall fractions was determined in order to characterise in more detail differences between the cells and fruit. Chemical fractionation of CWM by sequential extraction with solutions of cyclohexane-*trans*-1,2-diaminetetraacetate (CDTA), Na_2CO_3, and KOH was based on published procedures [9]. The percentage of CWM represented by each fraction is given in Table 3. CDTA-soluble pectin, which reportedly includes middle lamella pectin [12], was higher in the cells than the fruit. Presumably some of the middle lamella pectin in the cultured cells sloughed off into the medium

Table 4
Monosaccharide composition of components separated on DEAE-Sepharose of the Na_2CO_3-soluble fraction from CWM of fruit and suspension-cultured cells and from the polysaccharides in the culture medium

Fraction	Amount (mg)	Sugar (mol%)								Total (μg/mg)
		Rha	Fuc	Ara	Xyl	Man	Gal	Glc	GalA	
Fruit										
Buffer	25.0	2.1	tr [a]	3.0	0.6	0.3	23.8	1.9	68.4	875.5
0.25 M	–	–	–	–	–	–	–	–	–	–
0.5 M	68.4	1.4	tr	1.7	0.2	tr	6.4	0.5	89.7	811.4
Cells										
Buffer	11.1	6.5	tr	22.9	2.6	2.4	9.2	3.1	53.4	456.9
0.25 M	4.2	4.9	tr	39.2	4.3	3.0	14.5	3.2	30.9	312.0
0.5 M	42.8	4.8	tr	10.1	1.1	0.6	4.7	0.8	77.8	620.8
Medium										
Buffer	33.5	0.3	3.4	10.9	18.4	3.4	18.2	27.6	17.7	726.6
0.25 M	38.1	1.5	tr	21.4	tr	0.3	32.9	tr	44.0	762.4
0.5 M	21.5	2.1	tr	6.0	tr	tr	5.3	tr	86.5	658.0

[a] tr = Trace.

during growth. If this was so, the increased CDTA-soluble pectin must represent primary cell-wall polysaccharide held in the primary wall by Ca^{2+}-bridges.

The water-, CDTA-, Na_2CO_3-soluble, and residue fractions of the cells each contained higher proportions of arabinose and rhamnose than equivalent fractions of the fruit (Table 3), suggesting that the pectic polysaccharides of the cells were either more highly branched or had longer side-chains than the fruit pectic polysaccharides. The KOH-soluble fraction of the cells also contained increased proportions of arabinose and rhamnose but, as this fraction is known to contain a mixture of hemicellulosic and pectic polysaccharides [13], it was not possible to attribute the increased arabinose in the fraction to a particular polysaccharide.

Purification of pectic polysaccharides and hemicelluloses for methylation analysis.—(a) Na_2CO_3-soluble fraction. In order to establish whether arabinose in the pectic polysaccharide fractions was covalently linked to the galacturonan backbone or whether it existed as a separate arabinan, the Na_2CO_3-soluble fractions were separated on DEAE-Sepharose by sequential elution with 0.05, 0.25, and 0.5 M imidazole/HCl buffer. Polysaccharide recoveries and the sugar compositions are given in Table 4. Despite high levels of uronic acid a moderate amount of each Na_2CO_3-soluble fraction was not retained on the DEAE-Sepharose and was eluted with the 0.05 M imidazole/HCl buffer. However, the largest amount of each fraction was retained on the anion exchanger and was recovered in the 0.5 M buffer. The sugar composition of the 0.5 M fraction from the cells approximated that of the original Na_2CO_3-soluble fraction (Table 3) and therefore established that most, if not all, of the extra arabinose in the fraction was covalently linked to the pectin. The relative proportions of arabinose, galactose, and uronic acid varied markedly in the buffer, 0.25 and 0.5 M fractions, demonstrating the considerable heterogeneity of the pectic polysaccharides in the cell wall of the cultured

Table 5
Glycosyl-linkage analysis of the carboxyl-reduced 0.5 M DEAE Sepharose fraction of Na_2CO_3-soluble polysaccharides isolated from CWM of fruit and suspension-cultured cells

Linkage	Composition (mol%)	
	Fruit	Cells
T-Rha	tr [a]	0.8
2-Rha	1.4	3.4
2,4-Rha	tr	2.1
T-Ara	1.2	6.6
5-Ara	0.7	3.9
T-Fuc	tr	1.0
4-Man	0.6	1.4
T-Gal	0.3	2.7
4-Gal	7.8	6.2
3,4-Gal	0.5	1.2
2,4-Gal	0.2	0.5
4,6-Gal	0.3	0.2
T-GalA [b]	1.8	2.1
4-GalA	82.3	67.2
3,4-GalA	0.8	1.8
2,4-GalA	0.8	1.0

[a] tr = Trace.
[b] Analysed as 6,6-dideuterioglycosyl residues.

cells. A similar diversity in pectin structure has been shown to exist in the cell walls of intact kiwifruit [8]. The 0.5 M fraction was subjected to gel-permeation chromatography on Sepharose CL-2B. The elution profiles were broad (K_{av} 0.2–0.8) indicating considerable heterogeneity over a similar molecular weight range for the Na_2CO_3-soluble pectin from both cells and fruit (data not shown).

The 0.5 M fraction of the Na_2CO_3-soluble polymers was carboxyl-reduced, methylated, converted into partially methylated alditol acetates, and examined by GLC–MS. The fraction from the cells contained increased proportions of both terminal and 5-substituted arabinosyl residues compared to the fruit (Table 5). The increased rhamnose was accommodated as both 2- and 2,4-substituted rhamnosyl residues indicating that the galacturonosyl backbone of the cell-derived pectin had a higher proportion of rhamnosyl residues than that of the fruit fraction and that some of the rhamnosyl residues were additional branch points in the pectic backbone.

(b) KOH-soluble fraction. The bulk of polysaccharide contained in the 4 M KOH-soluble fraction consisted of xyloglucan and GGM. Amounts of pectic polysaccharides solubilised by 4 M KOH were removed by precipitation with hexadecyltrimethylammonium bromide (CTAB). The xyloglucan was then separated from the GGM by a combination of $Ba(OH)_2$ precipitation and gel-permeation chromatography, and each hemicellulose subjected to compositional and methylation analysis.

Table 6
Monosaccharide composition of xyloglucan and galactoglucomannan polysaccharides purified from fruit and suspension-cultured cells

Fraction	Sugar (mol%)							Total (μg/mg)
	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	
Xyloglucan								
Fruit	0.1	1.2	0.9	42.1	0.4	3.9	51.4	809.6
Cells	0.2	4.2	2.6	36.2	0.6	8.0	48.2	823.6
Galactoglucomannan								
Fruit	0.3	tr [a]	1.2	0.9	38.0	19.8	39.9	769.9
Cells	0.3	0.5	7.3	3.1	27.4	27.7	33.7	756.1

[a] tr = Trace.

Monosaccharide composition showed that the xyloglucan from the cells contained higher proportions of fucose, galactose, and arabinose than the xyloglucan from fruit (Table 6). The xyloglucan was subjected to methylation analysis. The overall recoveries of methylated alditol acetates were in good agreement with sugar values obtained by direct analysis.

The increased proportion of arabinose and fucose in xyloglucan from cells was found as terminal sugar derivatives (Table 7). Terminal sugars are not all linked directly to the glucan backbone of xyloglucan but are interrupted by interchain 2-substituted xylosyl and 2-substituted galactosyl derivatives, with 2-substituted xylose as the major contributor. Most of the additional galactose appeared as 2-substituted galactosyl residues,

Table 7
Glycosyl-linkage analysis of xyloglucan and galactoglucomannan purified from CWM isolated from fruit and suspension-cultured cells

Linkage	Composition (mol%)			
	Xyloglucan		Galactoglucomannan	
	Fruit	Cells	Fruit	Cells
T-Ara	1.1	1.7	0.5	3.3
5-Ara	–	–	tr [a]	1.3
T-Fuc	0.9	4.0	0.2	1.0
T-Xyl	25.4	27.0	0.6	1.5
2-Xyl	19.0	12.7	–	–
T-Gal	2.8	3.6	16.1	24.4
2-Gal	1.6	6.2	6.8	7.0
T-Man	0.9	0.7	–	–
4-Man	0.4	0.5	27.9	4.0
4,6-Man	–	–	12.2	22.1
T-Glc	tr	tr	1.4	0.8
4-Glc	21.2	15.6	33.1	31.9
4,6-Glc	26.4	28.0	1.3	2.8

[a] tr = Trace.

indicating that an increased proportion of interchain sugar derivatives may occur in the side-chains of cell-derived xyloglucan.

Sugar compositions showed that GGM from the cells, compared to the fruit, contained increased proportions of arabinose, xylose, and galactose but decreased proportions of mannose and glucose (Table 6). The ratio of mannose to galactose was 2:1 in the GGM of fruit whereas in the cells it was 1:1.

Methylation analysis showed that the GGM from kiwifruit cells possessed proportions of the major glycosyl linkages very similar to those reported for a GGM from suspension-cultured cells of tobacco (*Nicotiana tabcum*) [14] (Table 7). Tobacco GGM was built up of (1 → 4)-linked alternating D-glucopyranosyl and D-mannopyranosyl residues, with ~ 83% of the mannosyl residues substituted at O-6 by D-galactopyranosyl or 2-*O*-D-galactopyranosyl-D-galactopyranosyl side-chains. In kiwifruit cells the degree of branching was nearly identical, with ~ 84% of the 4-linked mannosyl residues substituted at O-6, with side chains of galactosyl and *O*-galactosyl-(1 → 2)-galactosyl groups. In contrast, in fruit, ~ 30% of the mannosyl residues were substituted at O-6. The increased proportion of arabinose in the GGM of the cells occurred mostly as terminal residues. Whether they are structural components of the GGM or contaminants derived from small amounts of pectin not removed by the CTAB precipitation is not known.

Rhamnogalacturonan II (RG-II).—Partially purified RG-II was isolated from cells and fruit by incubating CWM with Driselase and subjecting the solubilised fragments to ion-exchange and gel-permeation chromatography. While most of the CWM is hydrolysed to monosaccharides or small oligosaccharides by Driselase action the overall structure of RG-II resists degradation, with the loss of only some terminal non-reducing sugars from the RG-II side-chains [15]. Following digestion the solubilised components were bound to QAE-Sephadex and the fraction enriched in RG-II was recovered in 1.0 M buffer. The subsequent gel-permeation profiles of this fraction, for both cell and fruit, were nearly identical, giving a single major peak at K_{av} 0.4 on Sephacryl S-100 (< 8 kDa). The glycosyl-residue compositions following acid hydrolysis were also similar (Table 8) and, except for a higher than expected rhamnose content and the presence of xylose (it is possible that some RG-I co-purified with RG-II), did not differ greatly from the glycosyl-residue composition of Pectinol RG-II [14]. Thus, the compositional differences between fruit and cells found in the bulk of the cell-wall pectins did not apply to the complex structure of RG II which seems likely to be highly conserved throughout the plant kingdom [16]. However, the amount of RG-II in the primary cell wall of suspension-cultured kiwifruit cells (6.0% w/w) was twice that found in intact fruit.

Medium-soluble pectic polysaccharides from cultured cells.—The medium-soluble polymers from the kiwifruit cell cultures were subjected to ion-exchange chromatography on DEAE-Sepharose and the composition of the retained and non-retained fractions determined (Table 4). Whereas the CDTA- and Na_2CO_3-soluble pectic polysaccharides isolated from CWM contained more arabinose than galactose, the 0.25 M fraction from the medium-soluble polymers contained more galactose than arabinose. In cell-suspension cultures the apoplast is the culture medium and it is thought that polysaccharides in the medium result in part from sloughing off of intact polymers from the primary wall

Table 8
Glycosyl-residue composition of Pectinol RG-II and partially purified RG-II released by Driselase from CWM of fruit and suspension-cultured cells

Monosaccharide	Amount (mol%)		
	Cultured cells	Intact fruit	Pectinol RG-II [a]
2-O-Me-Xyl	3.7	2.9	3.1
2-O-Me-Fuc	3.0	2.3	3.4
Api/Ara [b]	13.8	10.1	15.7
Rha	22.8	25.8	15.3
Fuc	2.9	2.9	2.2
Gal	8.0	6.6	11.3
Xyl	2.6	3.8	–
AceA [c]	–	–	4.4
Uronic acid	43.2	45.5	44.7

[a] Q. Guo [15].
[b] Arabinose and apiose could not be separated on the SP-2330 column.
[c] Aceric acid was not detectable as an alditol acetate.

[8]. The medium-soluble polymers should therefore resemble very closely those of the cell wall in composition. Why the medium-soluble pectic polysaccharides of kiwifruit cells are not more representative of the cell-wall pectin is not known. Because of the extensive heterogeneity of kiwifruit pectic polymers it was thought that there may have been a preferential solubilisation from the cell wall of smaller molecular weight polysaccharides which contain increased galactose. However, gel-permeation chromatography on Sepharose-2B gave almost identical profiles for the Na_2CO_3-soluble pectin and the 0.25 M fraction from the medium.

3. Conclusion

Cell walls of suspension-cultured kiwifruit cells possessed several chemical features which contrasted with those of the fruit from which they were derived. Major changes to the primary structure of the matrix polysaccharides in the cultured cells occurred in the greater proportion of arabinose in the pectic polysaccharides and the increased degree of branching of the matrix polysaccharides in general. This trend was reinforced in the cultured cells with the finding that RG-II, a complex and highly branched polysaccharide, was present in the walls of the cultured cells at twice the level found in intact fruit. These features did not change during several cell cycles or over a 2-year period in which the cells were sub-cultured at least 50 times. The increased branching of the matrix polysaccharides in the cultured cells may confer a more open structure on the primary wall. This finding was supported by the observation that CWM from cells swelled much more in aqueous suspension than did CWM from intact fruit. The cultured cells are in a state of rapid growth and hence will be metabolically more active than the cells of nearly mature fruit tissue. The reduced density of their cell walls may provide a more

permeable barrier for nutrients and metabolites to move between the cytosol and the medium. It remains to be determined why arabinose and not galactose was the preferred sugar in the increased number of neutral side-chains found in the pectic polysaccharides of the cultured cells.

4. Experimental

General.—Polysaccharide fractions and CWM were hydrolysed with 2 M CF_3CO_2H for 1 h at 121 °C. Sugar mixtures were converted into alditol acetates [17] for GLC analysis. The column, SP-2330 fused silica (30 m × 0.32 mm), was maintained at 120° C for 2 min and then raised to 220 °C at 25 °C/min. The cellulose content was determined by the procedure of Updegraff [18]. The CF_3CO_2H-resistant material was dissolved in 72% H_2SO_4 and the carbohydrate content determined by the phenol–H_2SO_4 method [19] using D-glucose as a standard. Uronic acid was determined by the method of Blumenkrantz and Asboe-Hansen [20]. The degree of methylation and acetylation of CWMs was determined by the method of Voragen et al. [21]. Protein content was estimated by the Kjeldahl procedure. Amino acids were released by hydrolysis with 6 M HCl containing 1% phenol and analyzed by the method of Heinriksen and Meredith [22].

Preparation of cell-suspension culture.—Kiwifruit (40 fruit from a single vine) were harvested from the HortResearch Orchard at Kumeu on 24 February 1992 (4.2° Brix). At this time (~ 12 weeks after anthesis) cell division had nearly ceased and fruit size had reached 75% of its maximum [23]. A cell-suspension culture was initiated from callus of the outer pericarp tissue and grown in a modification of the medium of Pech et al. [24]. Inositol was omitted from the medium and 4% D-glucose was used as the carbohydrate source. A suspension of cells (15 mL) was subcultured every 14 days into a 250-mL flask containing fresh medium (50 mL). Cells were grown at 27 °C under low light on a rotary shaker (100 rpm).

Flow cytometry.—The growth rate and number of cells undergoing division was measured using flow cytometry as previously described [22]. Nuclei were analyzed using a Coulter Epics Profile II flow cytometer with a 15-mW argon laser at 488 nm. G_2 values were determined using the multicycle software package (Phoenix Flow systems).

Light and transmission electron microscopy.—Unfixed suspension-culture cells were suspended in 0.2 M phosphate buffer, gently agitated, and viewed directly using light microscopy. Cell diameters were measured using a video microscopy system and Optimas image analysis software.

Samples of suspension-culture cells and mature unripe kiwifruit pericarp were fixed in 2% paraformaldehyde, 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 1 h and washed in 0.1 M phosphate buffer with 0.2 M sucrose for a minimum of 1 h (3 changes) with centrifugation (2000*g* for 30–60 s) to concentrate the culture cells between each change. Each sample was subdivided into two portions, one portion was post-fixed in 1% osmium tetroxide in 0.1 M phosphate buffer with 0.2 M sucrose for 1 h and then washed in distilled water. The cell-culture samples were then encapsulated in alginate (after centrifugation the cell pellet was mixed with a 2% solution of sodium alginate and drops were solidified in 50 mM $CaCl_2$). All samples were subsequently

processed, embedded in LR white resin, and sectioned as previously described [6]. Light microscopy was performed on unosmicated material sectioned at 2 μm and stained for 3 min in aq 0.1% Toluidine Blue. Electron microscopy was performed on osmicated material sectioned at 100 nm and stained with aq 1% uranyl acetate followed by aq lead citrate [25]. Cell-wall thickness, as represented by the arithmetic-mean barrier thickness [26], was determined from light microscopy using steriological methods [6].

Isolation and fractionation of CWM.—(a) Fruit. CWM was prepared from the same fruit tissue used to initiate callus for the suspension-cultured cells. Outer pericarp (200 g) was cryo-milled in liquid N_2 and the frozen powder homogenised in PAW (400 mL). The slurry was centrifuged at 4000 *g* for 10 min and the supernatant solution filtered through Miracloth™. The residue was re-suspended in water and the supernatant solution recovered as before. The residue was extracted overnight in 90% Me_2SO to solubilise starch. The PAW- and Me_2SO-soluble fractions were recovered following dialysis and freeze-drying. The residue (CWM) was washed on a Miracloth filter with water and freeze-dried.

(b) Cells. Cells from 12-day suspension cultures (24 × 250-mL flasks) were filtered through Miracloth on a Buchner funnel and washed with water. The filtrate containing the culture medium was dialysed and the polymers recovered after freeze-drying. The cells (170 g) were cryo-milled in liquid N_2 and the PAW-soluble, Me_2SO-soluble, and CWM fractions isolated as described for fruit.

CWM from both fruit and cells (3 × 0.5 g) was sequentially extracted with water, 0.05 M CDTA, 0.05 M Na_2CO_3, and 4 M KOH, and the solubilised polymers and final residues were recovered as described previously [7].

Ion-exchange chromatography of polysaccharides.—The Na_2CO_3-soluble fractions from fruit and cells and the medium-soluble polymers (~ 100 mg from each fraction) from the cultured cells were dissolved in 100 mL of 0.05 M imidazole/HCl buffer (pH 6.5) and fractionated on a column (2.5 × 16 cm) of DEAE-Sepharose by stepwise sequential elution with 0.05, 0.25, and 0.5 M buffer.

Gel-permeation chromatography.—Three systems were used: a column (2.5 × 90 cm) of Sepharose CL-2B to separate pectic polysaccharides, Sephacryl S-300 (2.5 × 90 cm) for the 4 M KOH-soluble hemicellulose fraction, and Sephacryl S-100 High Resolution for products of Driselase digestion. Sepharose CL-2B and Sephacryl S-300 were equilibrated in 0.05 M acetate buffer (pH 6) containing 125 mM NaCl. Sephacryl S-100 was equilibrated in 1:1:23 pyridine–AcOH–H_2O. Polymers (5–10 mg) were dissolved in 1.0 mL of the buffer and eluted through the column at 10 mL/h; 2-mL fractions were collected and assayed for carbohydrate by the phenol–H_2SO_4 method [19].

Purification of xyloglucan and galactoglucomannan.—The following procedure, a modification of existing methods [27], was applied to 200 mg of the 4 M KOH-soluble fraction isolated from the CWM of the fruit and cells.

The KOH-soluble fraction was dissolved in water (20 mL), undissolved material removed by centrifugation, and aq 1% CTAB (25 mL) added to the supernatant solution while stirring. A solution (50 μL) of 2 M $(NH_4)_2SO_4$ was added to assist flocculation of the pectic polymers; samples were left overnight at 4 °C, then centrifuged. The supernatant solution was dialysed against aq 50% EtOH for 2 days and then against

water for a further 2 days, concentrated, and freeze-dried to give the pectin-free hemicellulose fraction (~ 70 mg).

The pectin-free hemicellulose fraction was dissolved in aq 5% NaOH (20 mL). The solution was filtered through glass-fibre paper and then aq 5% $Ba(OH)_2$ (20 mL) was added to precipitate the GGM. Xyloglucan remained in the solution. After 1 h the suspension was centrifuged, and the supernatant solution was neutralised with AcOH and dialysed to give partially purified xyloglucan. The residue (GGM) was washed with aq 5% $Ba(OH)_2$ (5 mL), dissolved in aq 5% NaOH (5 mL), and then neutralised with AcOH. The solution was dialysed (cut-off 3.5 kDa) and freeze-dried to give partially purified GGM.

Each of the partially purified hemicelluloses was subjected to gel-permeation chromatography on Sephacryl S-300 to remove small amounts of contaminating xyloglucan from the GGM, and GGM from the xyloglucan.

Carboxyl-reduction of glycosyluronic acid residues and methylation analysis.—Prior to methylation, carboxyl groups of the uronic acid residues of selected pectic polymers were reduced to the corresponding 6,6-dideuterioglycosyl residues [9]. Methylation of carboxyl-reduced pectic polysaccharides and hemicellulosic polysaccharides was performed using the method of Ciucanu and Kerek [28]. GLC–MS of the partially methylated alditol acetates was accomplished with a VG 70-SE mass spectrometer using an SP-2330 column maintained at 70 °C for 4 min, raised to 150 °C at 25 °C/min, and then to 220 °C at 4 °C/min. Identifications were based on peak retention times and comparison of their electron impact mass spectra with published spectra. The molar response factors reported by Sweet et al. [29] were used.

Isolation of modified RG-II.—Amounts (250 mg) of CWM were suspended in 1:1:98 pyridine–AcOH–H_2O (4 mL) containing 0.5% Driselase (Sigma, D-9515, from Basidiomycetes, partially purified as described by Fry [30]). A drop of toluene was added and the CWM suspensions were incubated at 37 °C for 24 h. Insoluble material was removed by centrifugation and each supernatant solution was dried down several times to remove buffer. The CWM hydrolysate was redissolved in pyridine/acetate buffer (0.05 M AcO^-) and eluted through a column (7.5 × 1.5 cm) of QAE-Sephadex (AcO^-) which had been equilibrated in 0.05 M pyridine/acetate buffer (pH 4.7). The hydrolysate was followed by 50 mL of 0.05, 0.1, and 0.8 M and 100 mL of 1.0 M pyridine/acetate buffer. Each fraction was dried down several times from water and freeze-dried.

The 1.0 M fraction was dissolved in 1:1:23 pyridine–AcOH–H_2O (1 mL) and subjected to gel-permeation chromatography on a column of Sephacryl S-100 High Resolution (1.5 × 97 cm, flow rate ~ 10 mL/h) equilibrated in the same buffer. Fractions (2 mL) were collected and 300-μL aliquots assayed for carbohydrate by the phenol–H_2SO_4 method [19].

Acknowledgements

We thank Ms. I.E.W. O'Brien for the flow cytometer measurements. This work was funded by The Foundation for Research Science and Technology (CO6406), The Swiss National Science Foundation, and The New Zealand Kiwifruit Marketing Board.

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