Planta (1981) 152:93–100

Planta
© Springer-Verlag 1981

The polysaccharide structure of potato cell walls: Chemical fractionation

M.C. Jarvis[1,*], M.A. Hall[2], D.R. Threlfall[1], and J. Friend[1]

[1] Department of Plant Biology, University of Hull, Hull HU6 7RX, England, and

[2] Department of Botany and Microbiology, University College of Wales, Aberystwyth, SY 23 3DA, U.K.

Abstract. Cell walls of potato tubers were fractionated by successive extraction with various reagents. A slightly degraded pectic fraction with 77% galacturonic acid was extracted in hot, oxalate-citrate buffer at pH 4. A further, major pectic fraction with 38% galacturonic acid was extracted in cold 0.1 M Na_2CO_3 with little apparent degradation. These two pectic fractions together made up 52% of the cell wall. Most of the oxalate-citrate fraction could alternatively be extracted with cold acetate-N,N′,N′-tetraacetic acid (CDTA) buffer, a non-degradative extractant which nevertheless removed essentially all the calcium ions. This fraction was therefore probably held only by calcium binding, and the remainder of the pectins by covalent bonds. Electrophoresis showed that both pectic fractions contained a range of molecular types differing in composition, with a high arabinose: galactose ratio as well as much galacturonic acid in the most extractable fractions. From methylation data, the main side-chains were 1,4′-linked galactans and 1,5′-linked arabinans, with smaller quantities of covalently attached xyloglucan. Extraction with NaOH-borate removed a small hemicellulose fraction and some cellulose. The main hemicelluloses were apparently a galactoxyloglucan, a mannan or glucomannan and an arabinogalactan.

Key words: Cell wall – Polysaccharide separation – *Solanum* (cell wall).

Introduction

The structures proposed for plant cell walls have been greatly influenced by the methods used to examine them. For many years the pectic and hemicellulosic groups of polysaccharides were distinguished by their extractability with Ca^{2+}-chelating solvents and with alkali. These extractants degrade the polysaccharides which they extract (Albersheim et al. 1960; Lindberg 1962). One cannot therefore tell whether the disruption of non-covalently bonded chain aggregates is all that is required to bring the polysaccharides into solution, or whether simultaneous degradation is necessary to break up a covalently-bonded network of polymers.

More recently, purified glycanases have been used to release plant cell wall fragments into solution so that they can be characterised. Degradation is still involved, but in this case it is known which covalent bonds are broken although the effect on non-covalent bonding is not always clear. The use of this approach has led to much new knowledge about cell wall polymers and their interconnections (Talmadge et al. 1973; Knee 1975; Gilkes and Hall 1977; Darvill et al. 1977 and 1978). However because the polymers are fragmented their chain length and solution properties cannot be determined, nor is it usually possible to decide whether different fragments are parts of the same molecule or are derived from different regions in the wall.

In this paper we shall describe the fractionation of potato (*Solanum tuberosum* L.) cell walls by sequential extraction with various solvents, degradation being monitored chromatographically; and the characterisation of the component polysaccharides by electrophoresis, molecular-sieve chromatography and methylation analysis. One of our intentions was to make a critical comparison of the two approaches, so that the limitations of the older cell wall research, and the information that can nevertheless be derived from it, might be more readily appraised.

Materials and methods

Materials. Deionised, glass-distilled water and reagent-grade buffer components containing $<30\ \mu g\ g^{-1}$ Ca were used for all experiments involving pectic polysaccharides.

* *Present address:* Agricultural Chemistry, Institute of Chemistry, Glasgow University, Glasgow G12 8QQ, U.K.

Abbreviations: GLC = gas-liquid chromatography; MS = mass spectrometry; V_0 = void volume; MW = weight-average molecular weight; DMSO = dimethylsulphoxide; EDTA = ethylenediamine tetraacetic acid; TFA = trifluoroacetic acid; CDTA = N,N′,N′-tetraacetic acid

0032-0935/81/0152/0093/$01.60

Dialysis tubing (Visking Gallenkamp, London, England) was washed inside with three changes of distilled water immediately before use, and still released traces of material interfering in the colorimetric carbohydrate assays; appropriate corrections were made.

The α-amylase preparation used was Sigma crystalline, type 11-A from *Bacillus subtilis*.

Cell wall preparation. The medullary regions of potato tubers were chilled in ice and homogenised with 5 l kg^{-1} ice-water slurry containing 5 g l^{-1} sodium deoxycholate and 10 g l^{-1} *n*-octanol, for 3 min in a Waring Blender at maximum speed. The homogenate was washed on a 120 mesh (B.S.) sieve with 40 l kg^{-1} ice-cold distilled water. The crude cell walls were suspended in 650 cm^3 kg^{-1} distilled water, homogenised for 2 min with an 'Ultra-turrax' top-drive homogeniser, and washed with 8 l kg^{-1} of distilled water. The homogenisation and sieving were repeated until no more starch grains were released. Lipids were removed by stirring with 800 cm^3 kg^{-1} chloroform-methanol (1:1 v:v), and the cell walls filtered off and freeze-dried. Volumes are relative to the fresh weight of tissue used.

Soluble polysaccharides lost during the preparation of the cell walls amounted to 0.3–0.5% of their final weight. The starch content of the cell wall preparations depended on the degree of homogenisation, but attempts to disintegrate the cells further by dry ball-milling led to degradation of pectins. Most of the work to be described was carried out on a cell wall preparation containing 21% starch.

Removal of starch from extracted polysaccharide fractions. Starch-containing fractions were incubated at room temperature at pH 5.5 with 100 μg cm^{-3} *Bacillus subtilis* α-amylase. After incubation, normally for 2 h, the digests had a negative I_2/KI reaction for starch. The starch fragments were removed by molecular-sieve chromatography in deionised, distilled water on a 73 cm · 1.7 cm column of Bio-Gel P-30. Separation was normally complete, but with NaOH extracts low-MW glucose- and mannose-containing polymers overlapped with the starch fragments. The appearance of these low-MW hemicellulose fragments may possibly be attributed to degradation by a contaminating enzyme or enzymes in the α-amylase preparation used. After our experiments were completed Huber and Nevins (1977) showed that *B. subtilis* α-amylase preparations of the type used by us, and by many other groups, contain a β-glucanase contaminant attacking certain hemicellulosic glucans. This enzyme is not known to degrade mannose-containing polymers, however, and no 1, 4′-β-D-mannanase activity was detected in our preparations. Neukom and Markwalder (1975) have made similar observations.

In the extraction time-course experiments, precipitation of polysaccharides other than starch with ethanol (2 vol) was substituted for the Bio-Gel P-30 chromatography step. The results were corrected for the 5% of residual starch oligomers which ethanol precipitation did not remove.

Molecular-sieve chromatography and electrophoresis. Molecular-sieve chromatography was normally carried out in trimethylsilylated glass columns (60 · 1.8 cm) packed with Bio-Gel A-15 m (Bio-Rad Inc. Richmond, USA), and eluted at 8 cm^3 h^{-1} with acetate-EDTA buffer (0.1 M acetate and 0.02 M EDTA, pH 6.5). Pectins were equilibrated in this buffer before application to the column. Timed fractions were collected and assayed colorimetrically.

Pectic fractions to be subjected to electrophoresis were first de-esterified with 0.1 M Na_2CO_3 for 18 h at 2° C. The basic electrophoresis techniques have been described elsewhere (Jarvis et al. 1977). However, some modifications were introduced. Analytical electrophoresis was carried out on 20 cm trimethylsilylated glass-fibre strips with the ends wrapped in cellophane film to reduce evaporative flow. The conditions used were: buffer, 0.1 M acetate/0.02 M EDTA, pH 6.5; potential gradient 3.1 V cm^{-1}; running time 4 h; location by dipping in 1-naphthol-H_2SO_4-EtOH. Preparative column electrophoresis was carried out on a 110 cm^3 urea density gradient with pH buffering in the vicinity of the electrodes, the cathode being at the top for ease of sample application. The column composition is shown in Table 1. Separation conditions were as follows: potential gradient 18.3 V cm^{-1}; running time, 12 h. Fractions of 5.5 cm^3 volume were collected.

Table 1. Composition of electrode and gradient buffers for preparative column electrophoresis

	Volume cm^3	Buffer concentration	pH	% urea
Cathode electrolyte	25	0.5 M	4.0	0
Spacer	10	0.03 M	5.0	12
Sample	10	0.03 M	5.0	18
Density gradient	70	0.03 M	5.0	25 → 50
Anode electrolyte	25	0.5 M	6.5	50

Carbohydrate determination. Colorimetric methods, hydrolysis conditions and GLC of neutral monosaccharides have been described elsewhere (Mankarios et al. 1979). Monosaccharide identifications, including uronic acids, were confirmed by paper chromatography in ethyl acetate-acetic-acid-pyridine-water (50:12:18:10 by vol) (Jarvis and Duncan 1974).

Methylation analysis. Polysaccharides (1–10 mg) were dried over P_2O_5 and stirred in dry DMSO (2 cm^3) at 35° C overnight. They were then methylated by the Hakomori method, broadly as described by Lindberg (1972); however the potassium salt of the dimethylsulphinyl anion was used and the addition of MeI was extended over 4 h (W.D. Bauer, personal communication). The methylated polysaccharides were hydrolysed with 1 M TFA for 4 h at 100 °C and the partially methylated monosaccharides converted to acetylated alditols by the method used for the unmethylated sugars (Mankarios et al. 1979), except that $NaBD_4$ was used for reduction and all evaporations were carried out at room temperature. The partially methylated alditol acetates were separated by GLC on the OV-275/Reoplex-400 column used for alditol acetate analysis, but temperature programmed 120° C→220° C at 2° min^{-1}.

In GLC-MS (Darvill et al. 1975; Gilkes and Hall 1977) a column of 3% OV-275 on Gas-Chrom Q was used. Molar proportions of partially methylated alditol acetates were estimated using the effective carbon response factors of Sweet et al. (1975).

Calcium determination. Calcium was determined by atomic absorption spectrophotometry, both in extracts and in the residues after extraction. The latter (2–10 mg of cell wall material) were wet-ashed in 2 cm^3 of 50% perchloric acid prior to analysis.

Results

Extraction of pectic polysaccharides with calcium-chelating solvents. Potato cell walls (100 mg) were stirred vigorously under reflux with 50 cm^3 of 0.1 M oxalate 0.35 M citrate buffer, pH 4.0. Aliquots (5 cm^3) were withdrawn and centrifuged, and starch was removed enzymically from the supernatant. Ex-

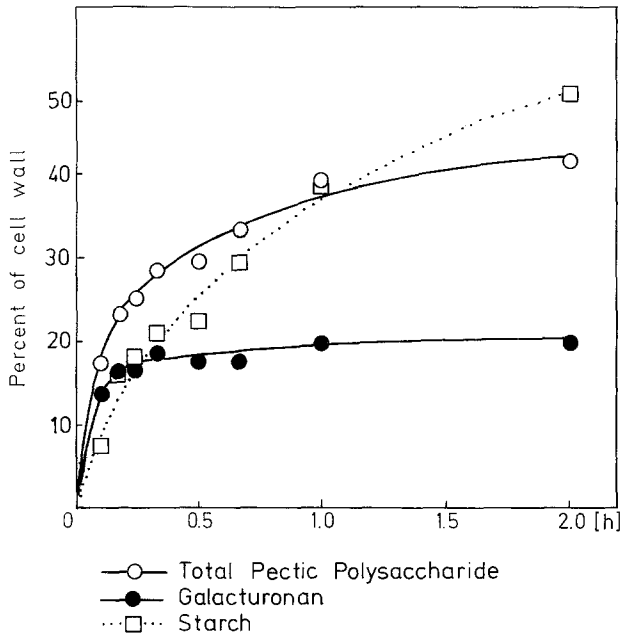

Fig. 1. Progress of extraction of pectic polysaccharides with oxalate-citrate buffer, pH 4, under reflux. In this and subsequent figures —○— represents total polysaccharide after removal of starch, and —●— represents uronic acid

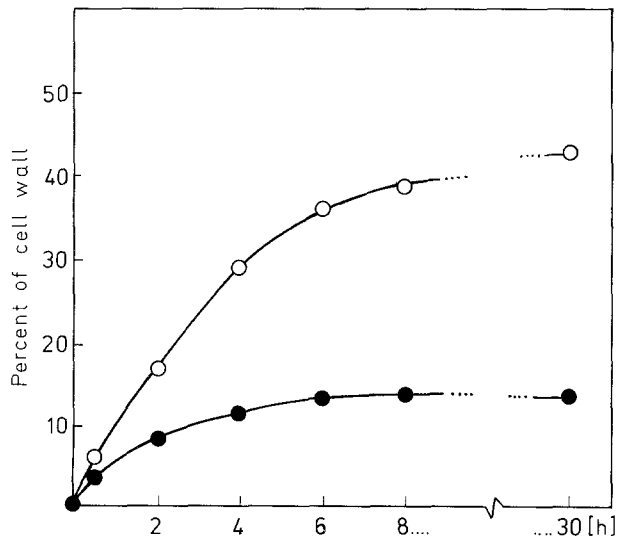

Fig. 2. Progress of extraction of pectic polysaccharides from the oxalate-citrate residue with 0.1 M Na_2CO_3

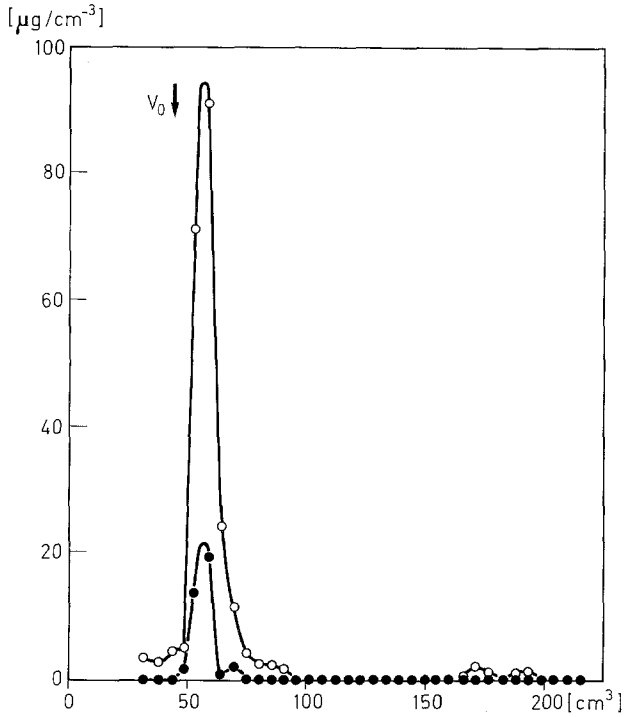

Fig. 3. Molecular-sieve chromatography of the Na_2CO_3-extracted pectic fraction on Bio-Gel A-15 m

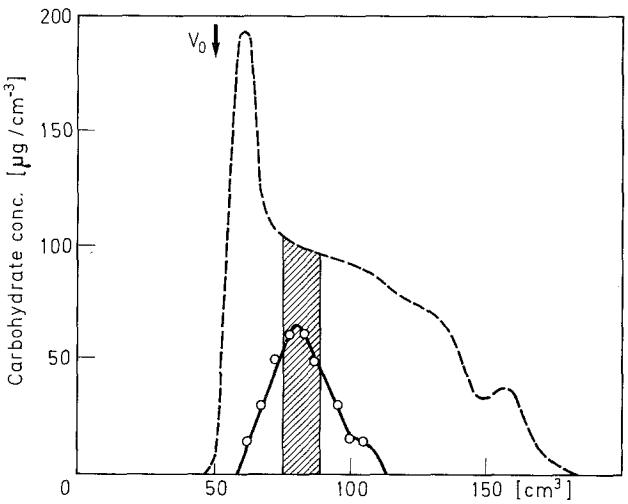

Fig. 4. Rechromatography of the oxalate-citrate fraction on Bio-Gel A-15 m. (- - -) original elution pattern; ▨ fraction removed for rechromatography; (—○—) elution pattern of rechromatographed fraction

traction of pectins was rapid during the first 15 min approximately, and slower thereafter (Fig. 1). In subsequent experiments the extraction time was standardised at 15 min. Molecular-sieve chromatography on Bio-Gel A-15 m (see Fig. 4) showed that the extracted polysaccharides were polydisperse but of high MW. The residue was washed with hot oxalate-citrate buffer and distilled water, and freeze-dried.

Calcium extraction by the oxalate-citrate buffer could not be measured due to the precipitation of

calcium oxalate. However extraction for 18 h at 17° C under toluene with 0.05 M acetate- 0.05 M 1,2-diaminocyclohexane N,N,N′,N′-tetraacetic acid (CDTA) at pH 6.5 solubilised 99.84% of the calcium and a pectic fraction amounting to 12.8% of the cell wall, with 74% uronic acid.

Much of the pectic polysaccharide remained in the cell walls after extraction with these reagents. The initial uronic acid content was 282 mg g^{-1}, of which only 148 mg g^{-1} was removed by oxalate-citrate in 15 min. A further 30 min extraction with boiling acetate-EDTA buffer, pH 6.5, removed an additional 63 mg g^{-1} of uronic acid and all but 0.13% of the calcium; but even though degradation must have been severe under these conditions 71 mg g^{-1} of uronic acid remained attached to the wall. The nature of the pectic polysaccharides not removed by oxalate-citrate buffer was therefore investigated further.

Mild alkaline extraction of the oxalate-citrate residue. In preliminary attempts to de-esterify the residual pectic fraction in situ, it was found that 0.1 M Na_2CO_3 at low temperatures brought substantial amounts of pectic polysaccharide into solution. The progress of the extraction was therefore followed by stirring 50 mg of oxalate-citrate residue with 12 cm^3 of 0.1 M Na_2CO_3 under nitrogen and assaying 2 cm^3 aliquots (Fig. 2). Extraction was complete in about 24 h under these conditions, whereas citrus pectin or the oxalate-citrate extract in solution was de-esterified in 4 h when assessed by electrophoresis on trimethylsilylated glass fibre paper.

In a separate experiment using the same Na_2CO_3 extraction conditions, each aliquot was divided into two portions. The first was filtered immediately and the supernatant neutralised to pH 6 with acetic acid. The second was neutralised to pH 6 and then stirred for 30 min at room temperature prior to filtration, to determine whether or not the extracted polysaccharides would re-adsorb to the residue under neutral conditions. No significant re-adsorption occurred.

The extracted polysaccharides eluted from Bio-Gel A-15 m as a narrow peak close to the void volume (Fig. 3). Na_2CO_3 still removed this pectic fraction when cold acetate-CDTA was substituted for hot oxalate citrate in the previous step.

Degradation of pectins during extraction. A high-MW portion of the oxalate-citrate extract, eluting between 1.45 V_0 and 1.80 V_0 from the Bio-Gel A-15 m column, was dialysed, freeze dried, subjected to a further 15 min reflux in the same oxalate-citrate buffer, and chromatographed again. The main peak was at the same elution volume as before but there was an appreciable low-MW 'tail', amounting to 10% of the carbohydrate applied and having a uronic acid content of 70%. The very high MW of the Na_2CO_3-extracted pectin, however, was not affected by the incubation time in Na_2CO_3. This implies that oxalate-citrate under reflux is slightly degradative and cold Na_2CO_3 is not.

This method of detecting depolymerisation is sensitive but depends on the absence of aggregation between pectin chains during chromatography. (see Anderson et al. 1969). The separations were reproducible only if EDTA was present in the eluting buffer. Two further experiments showed that aggregation was not occurring:

(a) Two fractions of the oxalate-citrate extract, eluting at intermediate positions, were collected and dialysed against deionised glass distilled water. They were then freeze dried, redissolved in buffer and applied individually to the column. Each fraction eluted as a peak somewhat broader than before but at the same volume (Fig. 4).

(b) A clear solution of 2.5 mg of the Na_2CO_3-extracted pectic fraction in 5 ml of acetate-EDTA buffer, pH 6.5, was heated for 5 min at 100° C and applied immediately to the column. Since all methyl ester groups had been removed in the preparation of the Na_2CO_3 extract, this treatment would be expected to disrupt any Ca^{2+}-dependent aggregation without depolymerising the rhamnogalacturonan chains. However, it had no effect on the elution volume, and the separation pattern was identical with that in Fig. 3.

Table 2. Polysaccharides in different wall fractions

Fraction	Per cent of dry, starch free cell wall
Oxalate-citrate extract	19.2
Na_2CO_3 extract	35.7
NaOH extract	9.5
Cellulose (gravimetric)	29.4

Other constituents; methyl esters 1.0%, protein 3.6%

Table 3. Mol percent composition of major cell wall polysaccharide fractions

	Oxalate-citrate extract	Na_2CO_3 extract	NaOH extract
Galacturonic acid	77.3	37.6	1.0
Rhamnose	2.2	4.1	0.3
Galactose	14.6	47.8	22.3
Arabinose	2.8	5.8	3.8
Glucose	0.9	1.0	61.4
Xylose	0.5	0.4	6.6
Mannose	1.3	2.0	4.6
Fucose	0.3	0.5	0.1
Apiose	0.1	0.8	–

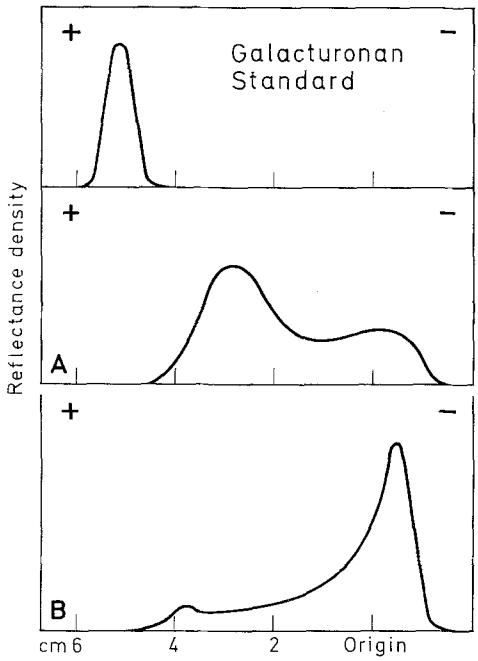

Fig. 5A, B. Densitometer scans of electrophoretograms of pectic fractions, separated on trimethylsilylated glass fibre paper and visualised with 1-napthol-H_2SO_4. **A** oxalate-citrate extracted fraction, after de-esterification. **B** Na_2CO_3-extracted fraction

Extraction of hemicelluloses. The residue after oxalate-citrate and Na_2CO_3 extraction of 500 mg of cell walls was stirred for 18 h at room temperature under nitrogen with 100 cm^3 of 24% (w:v) NaOH containing 4% (w:v) boric acid, in a polypropylene vessel. The supernatant was neutralised to pH 6.8 by cautious addition of acetic acid, with constant cooling. Starch was removed enzymically from the dialysed supernatant, with the loss of about 9% of the hemicellulosic polysaccharides.

The compact residue after hemicellulose extraction was hydrolysed with 2 M TFA for 2 h at 100° C and the supernatant analysed for carbohydrate, to check that the extraction of non-cellulosic polysaccharides had been reasonably complete. This appeared to be the case, the amount brought into solution (including some amylose) being only 1.06% of the cell wall weight.

The amounts of polysaccharide removed in the course of the various extractions are shown in Table 2.

Composition of the extracted polysaccharides. The sugar residues present in the various polysaccharide fractions are shown in Table 3. All the uronic acid residues in the cell wall appeared to be galacturonic acid; glucuronic acid was not detected by paper chromatography, although the sensitivity of the method is not high.

The release of neutral monosaccharides during

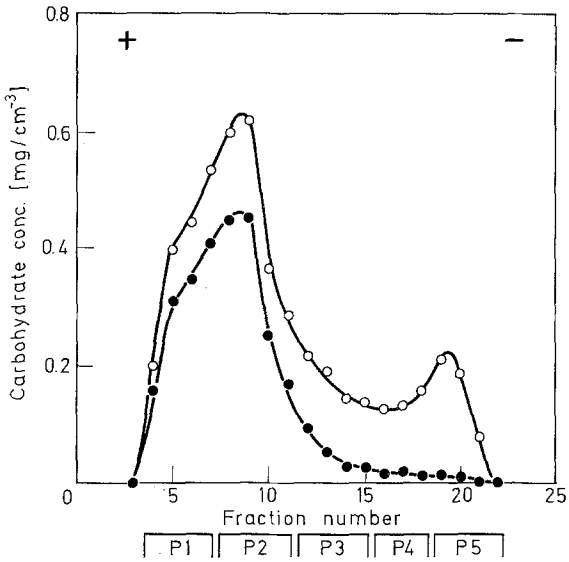

Fig. 6. Preparative column electrophoresis of the de-esterified oxalate-citrate extracted pectic fraction on urea density gradient

Table 4. Mol percent composition of fractions from electrophoresis of oxalate-citrate extracted polysaccharides

Pool	P-1	P-2	P-3	P-4	P-5
Fraction nos.	4–7	8–11	12–15	16–18	19–22
Galacturonic acid	78.3	72.7	28.3	10.0	2.0
Rhamnose	2.4	5.0	8.2	4.6	6.4
Galactose	9.8	14.0	49.4	76.8	78.3
Arabinose	3.5	6.3	10.3	6.5	7.1
Glucose	2.0	1.4	2.7	1.1	5.1
Xylose	1.6	0.1	0.5	0.4	0.3
Mannose	2.0	trace	0.2	0.4	6.7
Fucose	0.4	0.3	0.4	trace	trace

acid hydrolysis of the two main pectic fractions was followed over 32 h, to allow accurate estimation of the rhamnose content and calibration of other TFA hydrolyses for degradative losses (Mankarios et al. 1979). All but a trace of the rhamnose was released very slowly. The arabinose was released very rapidly, and the galactose more slowly in a sigmoidal fashion. This is consistent with galacturonosylrhamnose units, a peripheral position for arabinose and long chains of β-galactopyranosyl units.

The two major pectic fractions were subjected to electrophoresis on trimethylsilylated glass-fibre paper (Fig. 5). Both fractions were clearly polydisperse, and there was some overlap between them. The oxalate-citrate extracted fraction was separated by preparative column electrophoresis on a urea density gradient (Fig. 6), and the 8 cm^3 fractions were pooled into 5 groups, P1–P5 (Table 4). P1 and P2, with the highest galacturonic acid contents, contained the most rhamnose in relation to the other neutral sugars, and the highest ratio of arabinose to galactose (1:2.8 in P1; 1:11.0 in P5).

Table 5. Products from methylation analysis of oxalate-citrate, Na_2CO_3 and NaOH-borate extracts. All values are expressed as mole percentages of the total neutral sugar derivatives identified, and were calculated using the effective carbon response factors of Sweet et al. (1975)

Monosaccharide	O-Methyl ether	Mode of linkage	Mol % in:		
			Oxalate-citrate extract	Na_2CO_3 extract	NaOH extract
Rhamnose	3,4-di	2-linked[a]	[1.4]	[2.0]	–
	3-	2,4-linked[b]	[2.4]	[2.1]	–
Galactose	2,3,4,6-tetra	terminal	3.9	2.7	7.9
	2,3,6-tri	4-linked	49.2	77.9	4.1
	2,4-di	3,6-linked[c]	8.1	–	3.9
Arabinose	2,3,5-tri	terminal *f*-	1.9	0.9	3.5
	2,3,4-tri	terminal *p*-[d]	0.6	0.5	[3.7]
	2,5-di	3-linked *f*-[a]	[1.4]	[2.0]	–
	2,3-di	5-linked *f*-	16.8	11.9	2.6
	3-	2,5-linked *f*	–	–	trace
Glucose	2,3,4,6-tetra	terminal[e]	–	–	[0.7]
	2,4,6-tri	3-linked[d]	[2.4]	[2.1]	–
	2,3,6-tri	4-linked	2.5	–	37.9
	2,3-di	4,6-linked[c]	4.3	0.9	15.8
	unmethylated	cellulose	–	–	5.2
Xylose	2,3,4-tri	terminal	1.1	–	0.8
	3,4-di	2-linked[f]	1.8	–	6.9
	2,3-di	4-linked[f]	trace	–	trace
	4-	2,3-linked	4.7	1.0	–
Mannose	2,3,4,6-tetra	terminal[e]	1.3	–	[0.7]
	2,3,6-tri	4-linked	–	–	6.5

[a] The derivatives from 2-linked rhamnose and 3-linked arabinofuranose were not resolved by GLC, but MS showed that both were present. The total is shown in brackets

[b] The same applies to 2,4-linked rhamnose and 3-linked glucose

[c] The 3,6-linked and 4,6-linked hexoses were not identified by GLC-MS but are assumed to be the galactose and glucose derivatives respectively

[d] The value for terminal arabinopyranose in the NaOH extract includes phthalate derived from the extraction vessel

[e] Terminal glucose and terminal mannose were not distinguished

[f] 2-linked and 4-linked xylose were distinguished by MS of their $NaBD_4$-reduced derivatives

The polysaccharide fractions extracted with oxalate-citrate buffer, Na_2CO_3 and NaOH were subjected to methylation analysis. The results shown in Table 5 are typical of 2–3 separate analyses performed on each fraction. Agreement was generally close, although there was some variation in the size of the peak corresponding to 2,3,6-tri-O-methyl galactose, a common problem with 4-linked β-galactan chains.

Discussion

Mature potato tubers contain 15–20% starch and 1–1.5% cell walls. We were unable to prepare starch-free cell walls without using a prolonged α-amylase treatment to degrade the starch grains. As the purity of commercial amylases was uncertain a different approach was adopted. The crude cell walls were wet-sieved, reducing the starch content to $<20\%$, and used in that form. When soluble wall fragments were released in subsequent experiments, contaminating starch was removed by a brief and mild α-amylase treatment in solution. It should be mentioned that after we completed our experiments Ring and Selvendran (1978) published details of an efficient non-enzymic method of removing starch from cell walls.

In the selective-extraction experiments we were attempting to answer the questions: what forces retain these polysaccharides in the cell wall, and how do the extractants effect their removal?

The short extraction with hot oxalate-citrate was intended to remove any pectic polysaccharides held only by Ca^{2+}- stabilised chain aggregates (Grant et al. 1973; Morris et al. 1977). It probably overestimates this fraction somewhat due to slight depolymerisation of pectins bound in other ways, as about 6% less polysaccharide was extracted by cold acetate-CDTA. Nevertheless it is evident that not all the pectins were bound covalently, in the manner suggested by Keegstra et al. (1973); about a quarter were

not. Prolonged extraction at 100° C apparently solubilised bound pectins only by depolymerising the galacturonan chains between their points of attachment. Complete extraction in this way is not to be expected.

Cold Na_2CO_3 proved to be a very efficient extractant for the pectins remaining after the removal of calcium ions. The extraction was irreversible and depolymerisation was not apparent. The most obvious interpretation is that the polysaccharides were attached by covalent alkali-labile cross-links. Phenolic esters might be suggested (see Friend 1976; Fry 1979), although other types of bonding are possible.

The use of these relatively non-degradative extractants allowed the pectic polysaccharides to be examined intact in approximately their native state, which is not possible when enzymes are used to fractionate cell walls. The CDTA extractant (developed after the rest of the work was complete) is particularly promising in this respect. The molecular size of the extracted polysaccharides was higher than has been generally recognised. A dextran eluting at the same point as the Na_2CO_3-extracted pectin fraction would be about $7 \cdot 10^6$, although the more extended pectin would be of somewhat lower MW (Smidsrød and Haug 1971). Electrophoresis showed that the pectins comprised a continuous range of molecular species, from the cold acetate-EDTA extract with 78% galacturonic acid, through the oxalate-citrate extracted fractions P5–P1, to the near-neutral components of the Na_2CO_3 extract. There was a parallel decrease in extractability, within as well as between the extraction steps, due perhaps to increasingly frequent cross-links. There was also a parallel decrease in the arabinose: galactose ratio, due partly to lengthening of the galactan side-chains (Jarvis et al. unpublished). On partial depolymerisation (Jarvis et al. 1977), the potato pectins tended to segregate into strongly and weakly acidic components. This suggests that the above variations in composition could have resulted from varying proportions of galacturonan blocks rich and poor in neutral side-chains and rhamnose units as proposed by Barrett and Northcote (1965). The pectins of the outer region of the potato cell wall are probably richer in unsubstituted galacturonic acid units than the remainder (McClendon 1964).

Methylation analysis indicated that in both pectic fractions 1,4-linked galactans were the dominant neutral chain type, followed by 1,5-linked arabinans. From the electrophoretic data these were covalently attached to the rhamnogalacturonan, mainly through the rhamnose C-4 position. Both our graded acid hydrolysis results and those of Ring and Selvendran (1978) showed that the bonding sequence could not be galactan→arabinan→rhamnogalacturonan, but the question of whether the arabinan chains were attached to the galactans or directly to the rhamnogalacturonan will be dealt with in a succeeding paper. Potato pectins are clearly of the galactan-rich type like the pectins of lupin and soybean cotyledons (Hirst et al. 1947; Aspinall et al. 1967) and onion bulbs (Mankarios et al. 1980).

Methylation analysis of the oxalate-citrate extracted pectic fraction showed that 4- and 4,6-linked glucose; terminal, 2-linked and 2,3-linked xylose; and terminal and 3,6-linked galactose were present in quantities consistent with the presence of xyloglucan and highly branched galactan (or arabinogalactan; Clarke et al. 1979) chains as minor components of this fraction. From the monosaccharide composition of the acidic electrophoresis fractions P1 and P2 and from small-scale qualitative methylation analysis of these fractions some of the xyloglucan was covalently bound to the pectic molecule, probably by a glycosidic linkage as it survived the Na_2CO_3 de-esterification. Only a small fraction of the xyloglucan in the wall was bound in this way; it amounted to less than 4% of the oxalate-citrate extracted pectin, and did not prevent its extraction. It was barely detectable in the Na_2CO_3 extracted pectin, even though the latter was more firmly retained in the wall. The same is true of the 3,6-linked galactan. Our results therefore provide no firm evidence that these components function as covalent bridges between the pectins and other cell wall polysaccharides, in the manner suggested by Keegstra et al. (1973).

Polysaccharides that would now be called pectic, i.e. those with a rhamnogalacturonan core, have appeared in the hemicellulose fractions from cell walls of many species in the past, indicating that prior extraction of pectins with chelating agents had not been complete (e.g. Norman 1937; Aspinall et al. 1966; Knee 1973). These 'polyuronide hemicelluloses' must invariably have been heavily degraded by the strongly alkaline extraction conditions, but common features such as the high proportion of neutral side-chains can sometimes be discerned.

The NaOH-borate extract in our fractionation scheme contained little pectic material, but much 1,4′-glucan. The glucan was assumed to be β-linked from its resistance to α-amylase. Its extraction with alkali implies a low degree of crystallinity in the cellulose microfibrils, common in primary cell walls (Gordon et al. 1977). Apart from these polysaccharides, the remaining products of the methylation analysis were consistent with the presence of the following types of hemicellulose; a xyloglucan in which the xylose side-chains were heavily substituted on the 2-position with galactopyranose units as in the galactoxyloglucan of onions (Mankarios et al. 1980), a 1,4′-linked mannan or glucomannan, and an arabinogalactan of

the type in which the galactan chain is highly branched at the 3- and 6-positions (Clarke et al. 1979). The relationships between these polysaccharides in the intact cell wall, and in particular the relative importance of hydrogen bonding (Bauer et al. 1973) and covalent cross-linking (Lindberg 1962; Morrison 1977), in attaching them to the microfibrils, are not known. The elucidation of the alkali-labile bonds holding hemicelluloses in place will require techniques more selective than extraction with strong NaOH.

The work described here was financially supported by the Agricultural Research Council and the Science Research Council of the U.K., to whom we are grateful. We also wish to thank Mr. C.F.G. Jones and Miss K.A. Braithwaite for skilful technical assistance, Dr. A.H. Gordon for the gift of a derivative of apiose, and Dr. W.D. Bauer, Dr. R.R. Selvendran, and Dr. H.J. Duncan for useful discussions.

References

Albersheim, P., Neukom, H., Deuel, H. (1960) Splitting of pectin chain molecules in neutral solutions. Arch. Biochem. Biophys. **90**, 46–51

Anderson, D.M.W., Dea, I.C.M., Munro, A.C. (1969) Molecular-sieve chromatography with 'Sepharose 4B': the detection of artefacts of high molecular weight in acidic polysaccharides. Carbohydr. Res. **9**, 363–365

Aspinall, G.O., Cottrell, I.W., Egan, S.V., Morrison, I.M., Whyte, J.N.C. (1967) Polysaccharides of soy-beans. Part IV. Partial hydrolysis of the acidic polysaccharide complex from cotyledon meal. J. Chem. Soc. (C), 1017–1080

Aspinall, G.O., Hunt, K., Morrison, I.M. (1966) Polysaccharides of soybeans. Part II. Fractionation of hull cell-wall polysaccharides and the structure of a xylan. J. Chem. Soc. (C), 1945–1949

Barrett, A.J., Northcote, D.H. (1965). Apple fruit pectic substances. Biochem. J.**94**, 617–627

Bauer, W.D., Talmadge, K.W., 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

Clarke, A.E., Anderson, R.L., Stone, B.A. (1979) Form and function of arabinogalactans and arabinogalactan-proteins. Phytochemistry **18**, 521–540

Darvill, A.G., McNeil, M., Albersheim, P. (1978) Structure of plant cell walls. VIII. A new pectic polysaccharide. Plant Physiol. **62**, 418–422

Darvill, A.G., Roberts, D.P., Hall, M.A. (1975) Improved gas-liquid chromatographic separation of methylated acetylated alditols. J. Chromatogr. **115**, 319–324

Darvill, A.G., Smith, C.J., Hall, M.A. (1977) Auxin induced proton release, cell wall structure and elongation growth: a hypothesis. In: Regulation of cell membrane activities in plants, pp. 275–281, Marrè, E. and Ciferri, P., eds. Elsevier, Amsterdam

Friend, J. (1976) Lignification in infected tissue. In: Biochemical aspects of plant-parasite relationships, pp. 291–303. J. Friend, D.R. Threlfall eds. Academic Press, London

Fry, S.C. (1979) Phenolic components of the primary cell wall and their possible rôle in the hormonal regulation of growth. Planta **146**, 343–351

Gilkes, N.R., Hall, M.A. (1977) The hormonal control of cell wall turnover in *Pisum sativum* L. New Phytol. **78**, 1–15

Gordon, A.H., Hay, A.J., Dinsdale, D., Bacon, J.S.D. (1977). Polysaccharides and associated components of mesophyll cell-walls prepared from grasses. Carbohydr. Res. **57**, 235–248

Grant, G.T., Morris, E.R., Rees, D.A., Smith, P.J.C., Thom, D. (1973) Biological interactions between polysaccharides and divalent cations: the egg-box model. FEBS Lett. **32**, 195–198

Hirst, E.L., Jones, J.K.W., Walder, W.O. (1947) The pectic substances. Part VII. Constitution of galactan from *Lupinus albus*. J. Chem. Soc. 1225–1229

Huber, D.J., Nevins, D.J. (1977) Preparation and properties of a β-D-glucanase for the specific hydrolysis of β-D-glucans. Plant Physiol. **60**, 300–304

Jarvis, M.C., Duncan, H.J. (1974) Paper chromatography of plant sugars. J. Chromatogr. **92**, 454–456

Jarvis, M.C., Threlfall, D.R., Friend, J. (1977) Separation of macromolecular components of plant cell walls: electrophoretic methods. Phytochemistry **16**, 849–852

Keegstra, K., Talmadge, K.T., Bauer, W.D., Albersheim, P. (1973). The structure of plant cell walls. III. A model of the walls of suspension-cultured sycamore cells based on the interconnections of the macromolecular components. Plant Physiol. **51**, 188–196

Knee, M. (1973) Polysaccharides and glycoproteins of apple fruit cell walls. Phytochemistry **12**, 637–653

Knee, M., Fielding, A.H., Archer, S.A., Laborda, F. (1975) Enzymic analysis of cell wall structure in apple fruit cortical tissue. Phytochemistry **14**, 2213–2222

Lindberg, B. (1962) Recent advances in methods of isolating and purifying hemicelluloses. Pure Appl. Chem. **5**, 67–75

Lindberg, B. (1972). Methylation of polysaccharides. In: Methods in enzymology XXVIII B, pp. 178–195. V. Ginsburg, ed. Academic Press, New York

McClendon, J.H. (1964) Evidence for the pectic nature of the middle lamella of potato tuber cell walls based on chromatography of macerating enzymes. Am. J. Bot. **51**, 628–633

Mankarios, A.T., Hall, M.A., Jarvis, M.C., Threlfall, D.R., Friend, J. (1980) Cell wall polysaccharides from onions. Phytochemistry **19**, 1731–1733

Mankarios, A.T., Jones, C.F.G., Jarvis, M.C., Threlfall, D.R., Friend, J. (1979) Hydrolysis of plant polysaccharides and GLC analysis of their constituent neutral sugars. Phytochemistry **18**, 419–422

Morris, E.R., Rees, D.A., Thom, D., Welsh, E.J. (1977). Conformation and intermolecular interactions of carbohydrate chains. J. Supramol. Struct. **6**, 259–274

Morrison, I.M. (1977) Extraction of hemicelluloses from plant cell-walls with water after preliminary treatment with methanolic sodium methoxide. Carbohydr. Res. **57**, C4–C6

Neukom, H., Markwalder, H. (1975) Isolation and characterization of an arabinogalactan from wheat flour. Carbohydr. Res. **39**, 387–389

Norman, A.G. (1937) The biochemistry of cellulose, the polyuronides, lignin etc. Clarendon Press, Oxford

Ring, S.G., Selvendran, R.R. (1978) Purification and methylation analysis of cell wall material from *Solanum tuberosum*. Phytochemistry **17**, 745–752

Smidsrød, O., Haug, A. (1971) Estimation of the relative stiffness of the molecular chain in polyelectrolytes from measurements of viscosity at different ionic strengths. Biopolymers **10**, 1213–1227

Sweet, D.P., Shapiro, R.H., Albersheim, P. (1975) Quantitative analysis by various G.L.C. response-factor theories for partially methylated and partially ethylated alditol acetates. Carbohydr. Res. **40**, 217–225

Talmadge, K.T., Keegstra, K., Bauer, W.D., Albersheim, P. (1973) The structure of plant cell walls. I. The macromolecular components of the walls of suspension-cultured sycamore cells with a detailed analysis of the pectic polysaccharides. Plant Physiol. **51**, 158–173

Received 31 October 1979: accepted 2 February 1981