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INVESTIGATION OF THE HETEROGENEITY OF XYLOGLUCANS FROM THE CELL WALLS OF APPLE

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ABSTRACT

Cell-wall material from parenchymatous tissues of apple was sequentially extracted with 50mM NaOH at 1°, M KOH at 1° and 20°, and 4M KOH at 20°, to leave a residue of α-cellulose. From the 4M KOH-soluble fraction, a crude xyloglucan was isolated by anion-exchange chromatography, and further resolved into seven xyloglucans by borate anion-exchange chromatography. The relative amounts of the xyloglucans, in order of elution, were 2.7:1.3:29.7:1.0:3.2:1.2:10.3. The structural features of five of the xyloglucans were determined by methylation analysis. These results show that apple xyloglucans exhibit heterogeneity.

INTRODUCTION

In auxin-induced growth of primary cell-walls of dicotyledons, xyloglucans are the main hemicellulosic polysaccharides involved[1–3]. Treatment of epicotyl tissues of peas and beans with auxin induced development of endo-(1→4)-β-D-glucanase activities[4] which resulted in the release of buffer-soluble xyloglucans having molecular weights much lower than those of the insoluble xyloglucans[5]. Further, treatment of bean epicotyl segments with auxin caused a decrease in the amount of 24% KOH-soluble xyloglucan and an increase in the 4% KOH-soluble xyloglucan, but the total amount of the two xyloglucans remained constant[6]. These results suggested that the xyloglucans of parenchymatous tissues might exhibit heterogeneity and this has been investigated using cell walls from apple parenchyma.

RESULTS AND DISCUSSION

Isolation and sequential extraction of cell-wall material (CWM). — The cell-

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wall material used in this study was virtually free from contamination with cytoplasmic proteins and starch. Depectination of the CWM was effected by extraction with cold 50mM NaOH to minimise degradation of the pectins by β-elimination and also hydrolysis of labile glycosidic linkages, *e.g.*, arabinofuranosyl and fucopyranosyl end-groups. This precaution was taken in order to eliminate the generation of multiple forms of xyloglucans from a common precursor.

Extraction of the depectinated material with M KOH at 0°, and then at 20–22°, solubilised the bulk of the hemicellulosic polymers which contain[7] appreciable amounts of pectic–hemicellulose and pectic–hemicellulose–protein–polyphenol complexes. Complexes containing xyloglucans were present in the cold and room temperature M KOH-soluble fractions. The carbohydrate composition (Table I) of the polymers recovered from the supernatant solutions and precipitates of the M KOH extracts showed that, whereas the polymers from the supernatant solutions were relatively rich in carbohydrate, the precipitates contained the bulk of the

TABLE I

MONOSACCHARIDE COMPOSITION OF POLYSACCHARIDES EXTRACTED FROM APPLE CELL-WALL MATERIAL DURING SEQUENTIAL EXTRACTION WITH ALKALI

Fraction	*Yield (%)*	*Monosaccharide composition*[a] *(μg/mg of dry wt.)*						
		Deoxy-hexose	*Ara*	*Xyl*	*Man*	*Gal*	*Glc*	*Uronic acid*
50mM NaOH, at 1°, soluble[c]	10.2	5	39	3	2	18	12	369[b]
M KOH, at 1°, soluble	1.4	33	74	245	62	77	258	60
insoluble[d]	1.4	4	3	25	7	5	26	32
at 20–22°, soluble	1.2	35	82	142	30	67	194	59
insoluble	2.2	4	23	8	7	6	17	31
4M KOH, at 20–22°, soluble	6.9	55	108	199	45	103	355	55
insoluble	0.1	5	73	15	4	6	36	59
Final residue	76.5	22	155	57	19	55	428	200

[a]After Saeman hydrolysis. [b]This value is probably too low, because of insolubility and incomplete hydrolysis. [c]In supernatant solution after dialysis. [d]Precipitate formed during dialysis.

TABLE II

MONOSACCHARIDE COMPOSITION OF FRACTIONS OBTAINED FROM CHROMATOGRAPHY OF THE 4M KOH-SOLUBLE POLYMERS ON DEAE-SEPHADEX

Fraction	*Yield (%)*[a]	*Monosaccharide composition*[b] *(μg/mg of dry wt.)*						
		Deoxy-hexose	*Ara*	*Xyl*	*Man*	*Gal*	*Glc*	*Uronic acid*
"Neutral" fraction	77.6	60	71	223	54	104	407	27
Major acid fraction	10.1	55	341	77	7	101	93	200
Minor acid fraction	0.7	29	284	73	7	95	132	95

[a]Percentage of weight of material applied to column. [b]After Saeman hydrolysis.

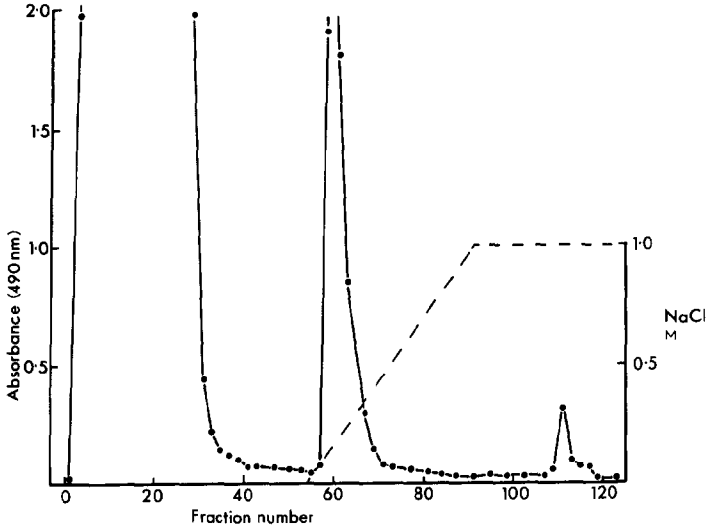

Fig. 1. Fractionation of the 4M KOH-soluble fraction of apple cell-wall material on DEAE-Sephadex: —●—, total carbohydrate; — — —, solvent gradient. For details, see text.

polyphenolic material. The compositions of the precipitates are comparable to those of the corresponding components from cabbage[8] and apple[7] cell-walls which were eluted rather slowly from the anion-exchange columns. The 4M KOH extract contained the bulk of the xyloglucans. The supernatant solution, when chromatographed on DEAE-Sephadex, afforded xyloglucans (~78%, not retained on the column) and two acidic fractions (~11%) (Fig. 1), which were probably pectic–hemicellulose–polyphenol complexes (Table II).

Fractionation of the xyloglucans. — Only one major xyloglucan peak was obtained[7] when a short anion-exchange column was used. However, when the xyloglucan fraction (500 mg) was chromatographed on a relatively large column (32 × 2.5 cm), it was resolved into seven components (Fig. 2, and XG1–7, Table III). The carbohydrate composition of the fractions, particularly the relative amounts of xylose and glucose, suggested that each fraction contained xyloglucans. Chromatography of the major xyloglucan (XG3), as the borate complex on DEAE-Sepharose (borate form), failed to effect further resolution. It is noteworthy that, whereas XG1–3 had relatively low contents of mannose, the later fractions, particularly XG4 and XG6, contained significant amounts of mannose.

Methylation analysis. — Methylation analysis of the fractions XG1 and XG3–6 revealed the main glycosidic linkages (Table IV). As reported previously[7,8], there were discrepancies between branch points and end groups, particularly the low recoveries of terminal xylosyl groups, which are reflected in the elevated values for the (1→4)- and (1→4),(1→6)-linked glucosyl residues. The major glycosidic linkages of XG1 and XG3 are comparable to those of a typical xyloglucan, in that there were relatively large amounts of (1→4)- and (1→4),(1→6)-linked glucosyl

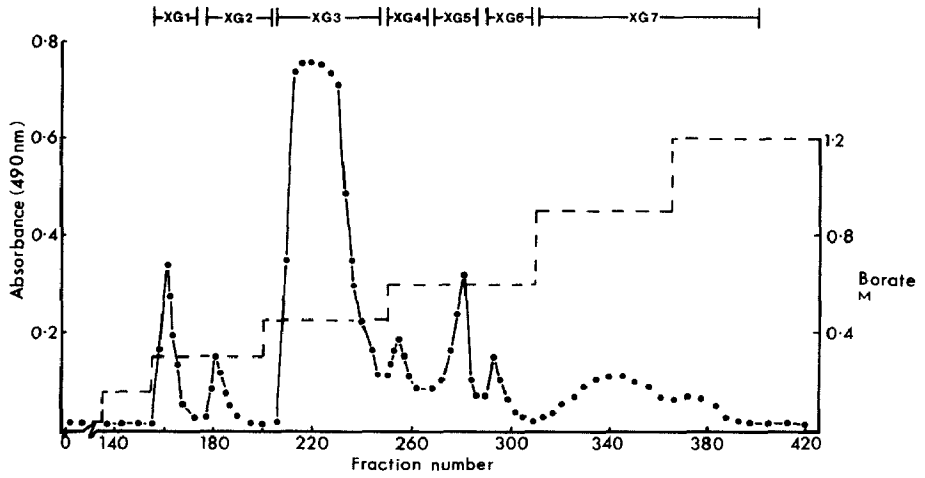

Fig. 2. Fractionation of xyloglucans from apple cell-walls on DEAE-Sephacel: —●—, total carbohydrate; — — —, solvent concentration. For details, see text.

TABLE III

MONOSACCHARIDE COMPOSITION OF XYLOGLUCAN FRACTIONS OBTAINED BY RE-CHROMATOGRAPHY OF THE NEUTRAL FRACTION[a] ON DEAE-SEPHACEL

Fraction	*Yield (%)*[b]	*Monosaccharide composition*[c] *(μg/mg of dry wt.)*						
		Fuc	*Ara*	*Xyl*	*Man*	*Gal*	*Glc*	*Uronic acid*
XG1	4.1	72	37	269	3	101	495	8
XG2	1.9	56	72	224	8	81	431	14
XG3	44.5	68	9	272	13	98	451	12
XG4	1.5	51	11	201	104	80	365	11
XG5	4.8	51	13	195	61	84	247	18
XG6	1.8	48	15	147	261	97	361	26
XG7	15.4	45[d]	144	127	74	110	265	66

[a]Neutral fraction from chromatography on DEAE-Sephadex (Table I). [b]Percentage of weight of material applied to column. [c]After Saeman hydrolysis. [d]May contain a small proportion of rhamnose.

residues and terminal xylosyl groups, and significant amounts of (1→2)-linked xylosyl residues and terminal galactosyl, terminal fucosyl, and terminal arabinosyl groups.

In addition to the above linkages, fractions XG4–6 contain significant amounts of (1→4)-linked mannosyl residues which could have come from substituted glucomannans that were co-eluted with the xyloglucans[9,10]. However, the possibility that they are an integral part of the xyloglucan backbone should not be ruled out.

Thus, the primary cell-walls of parenchymatous tissues of apples contain a range of xyloglucans, some of which may have (1→4)-linked mannosyl residues in

TABLE IV

PARTIALLY METHYLATED ALDITOL ACETATES FROM XYLOGLUCAN FRACTIONS

Alditol acetates	*Relative mol %*				
	XG1	*XG3*	*XG4*	*XG5*	*XG6*
2,3,4-Me_3Fuc[a]	2.9	3.0	—	0.7	0.8
2,3,5-Me_3Ara	1.3	1.0	3.0	0.4	0.8
2,3-Me_2Ara	1.2	0.5	—	—	—
2,5-Me_2Ara	0.6	—	—	—	—
3,5-Me_2Ara	0.6	—	—	—	—
2,3,4-Me_3Xyl	11.6	10.9	9.1	7.7	7.0
2,3-Me_2Xyl	0.5	—	Tr	1.4	1.5
3,4-Me_2Xyl	3.6	4.8	8.7	3.8	2.2
2,3,4,6-Me_4Gal	5.0	6.1	3.3	8.2	5.2
2,3,4-Me_3Gal	—	1.7	—	—	2.1
2,3,6-Me_3Man	—	2.1	22.2	13.6	27.6
2,3-Me_2Man	—	—	—	2.0	3.9
3,6-Me_2Man/Glc	—	—	—	1.8	—
2,3,6-Me_3Glc	24.9	22.4	17.4	22.8	23.8
2,3-Me_2Glc	47.8	47.5	36.3	37.6	25.1

[a]2,3,4-Me_3Fuc = 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylfucitol, *etc.*

the backbone. Because the conditions used for depectination were mild, it is unlikely that the xyloglucans present in relatively small amounts were products of hydrolysis of the major xyloglucans.

The heterogeneity of the xyloglucans suggests that these polysaccharides are probably actively metabolised. Recently, the occurrence of multiple forms of xyloglucans in suspension-cultured *Rosa glauca* cells was reported[11].

EXPERIMENTAL

General. — Methyl sulphoxide was vacuum-distilled over CaH_2 and stored over molecular sieve 3A. DEAE-Sephadex, DEAE-Sephacel, and DEAE-Sepharose were purchased from Pharmacia.

Neutral sugars were released by a modified Saeman-hydrolysis procedure[12] or by hydrolysis with M H_2SO_4, and analysed[13] as their alditol acetates by g.l.c. Uronic acid was determined colorimetrically by a modification[13] of the method of Blumenkrantz and Asboe-Hansen[14]. I.r. spectra were obtained with a Perkin–Elmer 297 spectrophotometer, using KBr discs.

Preparation of cell-wall material (CWM). — CWM of apple (var. Cox's Orange Pippin) was from the batch prepared and used by Stevens and Selvendran[7].

Sequential extraction of CWM. — CWM (32 g) was extracted with 50mM NaOH (2.5 L), containing 5mM EDTA[15] and 10mM $NaBH_4$, for 2 h and, again, for 1 h at 1° to remove the bulk of the pectic substances. The depectinated residue was then extracted sequentially[7] with M KOH at 1° and at 20–22°, and with 4M KOH at

20–22°. The pH of the extracts was adjusted to 4.5 with acetic acid and they were dialysed against H_2O. Precipitates which formed during dialysis were separated by centrifugation and freeze-dried. The supernatant solutions were concentrated under reduced pressure and frozen. A sample from each supernatant solution was freeze-dried for analysis.

Isolation of xyloglucans. — (*a*) A solution of a portion (~800 mg) of the 4M KOH-soluble material in potassium phosphate buffer (10mM, pH 6.4, 65 mL) was eluted from a column (15 × 1 cm) of DEAE-Sephadex (Cl^- form), initially with the potassium phosphate buffer (70 mL) and then with this buffer in a linear gradient of NaCl (0→M 120 mL). Fractions (2 mL) were collected and monitored for carbohydrate by reaction with phenol–sulphuric acid[16]. Appropriate fractions were combined, dialysed and, except for the minor acid component, concentrated to a small volume. The minor acidic component and samples of the "neutral" and major acidic components were freeze-dried for analysis. Yields (mg), calculated from the freeze-dried samples, were "neutral" component 621, major acidic component 80, and minor acidic component 6.

(*b*) A solution of a portion (~500 mg) of the "neutral" component in sodium borate (30mM, pH 8.0, 50 mL) was eluted from a column (32 × 2.5 cm) of DEAE-Sephacel (borate form) with sodium borate, with stepwise increases of concentration (0.15M) up to 0.6M, and by 0.3M up to 1.2M. Fractions (5 mL) were collected, monitored, appropriately combined, acidified to pH 4.5, dialysed, concentrated, and frozen. Samples were freeze-dried for analysis.

(*c*) A solution of a portion (~50 mg) of the main component in sodium borate (30 mM, pH 8.0, 3 mL) was eluted from a column (32 × 1 cm) of DEAE-Sepharose (borate form) with 30mM sodium borate (50 mL) and then with a linear gradient of sodium borate (30mM→1.2M, 140 mL). Fractions (2 mL) were collected, monitored, and processed as in (*b*).

Methylation analysis. — Polysaccharides were methylated by a modification of the Hakomori method, and then converted into partially methylated alditol acetates which were separated by g.l.c. on OV-225 and ECNSS-M columns and examined[17] by g.l.c.–m.s. (OV-225 column)[17]. The ECNSS-M column was used to separate the derivatives of (1→2)- and (1→4)-linked xylose from the terminal galactose derivative, which co-elute on OV-225. Methylated fractions showed negligible i.r. absorption for hydroxyl.

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