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Structural features of cell wall polysaccharides from the cotyledons of mung bean *Vigna radiata*

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Abstract

The first analysis is reported of the cell wall of a food legume by methods that minimise the degradation of pectic polysaccharides. The cell walls of mung bean cotyledons (*Vigna radiata*, var. T77) were extracted in sequence with 0.05 M *trans*-1,2-cyclohexanediamine-*N*,*N*,*N*′,*N*′-tetraacetate, sodium salt (CDTA) at 20°C, 0.05 M Na_2CO_3 at 1 and 20°C, 0.5 M KOH at 1°C, 1 M KOH at 1 and 20°C, 4 M KOH at 20°C, and 4 M KOH containing 4% of boric acid at 20°C. The isolated polymers were fractionated by anion-exchange chromatography. The CDTA-solubilised pectic polysaccharides were less branched than those solubilised by Na_2CO_3; less than 10% of the cell wall was solubilised by these reagents. 0.5 and 1 M KOH released only 3.2% by weight of the cell wall; the extracts consisted of mixtures of xyloglucans and pectic polysaccharides. The bulk of the extractable pectic polysaccharides were only released by 4 M KOH. They were highly branched arabinorhamnogalacturonans and contained significant amounts of terminal xylopyranosyl residues. Preliminary evidence is given to show that at least some of the xylopyranosyl residues are attached to the 3-position of 1,4-linked galactopyranuronosyl residues of the backbone. Some of the pectic polysaccharides were associated with small amounts of xyloglucan even after refractionation by anion-exchange chromatography, suggesting that they may be covalently attached in a complex. Little neutral arabinan was detected. High levels of arabinans, released from the walls of other legume-cotyledons by earlier workers, were probably artefacts caused by β-elimination under their extraction conditions. In this

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study the wall residue after extraction contained mainly cellulose and pectic polysaccharides, mostly the latter. Thus the majority of the pectic polysaccharides in mung bean cotyledon cell walls are tightly bound and cannot be released by calcium chelators or by mild base hydrolysis.

Keywords: Mung bean; *Vigna radiata*; Legume; Cell wall; Pectins

1. Introduction

Legume seeds form an important part of the diet of many human populations and their major cytoplasmic constituents (e.g., protein, starch, lipids) have in consequence received close study. In contrast, legume seed cell walls remain relatively uninvestigated. The few existing studies used analytical procedures now known to extensively degrade cell wall polysaccharides. For example, neutral arabinans have been reported as constituents of the cell wall of soya bean, broad bean, and pea [1]. It is likely that the bulk of these polysaccharides arose from breakdown of pectic polysaccharides which are known to be highly esterified and hence liable to β-elimination [2]. Likewise, the high soluble-fibre content of some legume seeds suggested by certain fibre analyses [3,4] probably results from pectic breakdown under the conditions used [5]. To clarify these points, and to continue our investigation of the influence of cell wall composition and structure on the chemistry and properties of dietary fibre, there was a clear need to fractionate a legume seed cell wall by methods which minimise degradation. We now report on the large scale extraction of mung bean (*Vigna radiata*) cotyledons and on the composition and structural features of their major cell wall polymers.

2. Results and discussion

Isolation of cell wall material (CWM).—Frozen mung bean cotyledons were blended in aqueous 1.5% sodium dodecyl sulphate (SDS). Dialysis of the extract yielded 6.7% by weight of the unsoaked fresh material. Carbohydrate analysis (see Table 1) showed that mannose, glucose, and arabinose were the major constituents of the non-starch polysaccharide (although most of the mannose probably arose from lectin-glycoprotein [6]); the extract represented only 0.8% of the total yield of cell wall polysaccharide material (CWPM). The residue was ball-milled in 0.5% SDS for 11 h at 2°C to completely disrupt the tissue structure. Carbohydrate analysis of the solubilised, dialysed non-starch polysaccharide material (2.1% by weight of unsoaked fresh material) indicated that it contained 26.8% of total CWPM. The residue was extracted twice with 2:1:1 (w/v/v) phenol–acetic acid–water (PAW) to remove the intracellular protein completely; the dialysed extract contained very little non-starch polysaccharide (0.1% of total CWPM). Extraction of the residue with 90% dimethyl sulfoxide removed the remaining starch; carbohydrate analysis of the solubilised material suggested that it contained some non-starch polysaccharides (2.3% by weight of total CWPM). The final yield of CWM after dialysis was 1.5% by weight of the fresh material. It was white and free from starch as judged by the absence of

Table 1
Sugar composition of cell wall material and of material solubilised during purification of cell wall material from mung bean cotyledons

Fraction [a]	Anhydro sugars (μg/mg dry wt)									Yield of CWPM (%) [c]
	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA [b]	Total	
1.5% SDS	0.5		1.3	0.2	2.6	0.6	2.5 (2.5) [d]		7.7	0.8
0.5% SDS	1.1		68.8	6.0	13.1	15.7	171.2 (148.0)	39.0	314.9	26.8
PAW	0.4	0.4	2.4	1.9	4.9	1.0	67.6 (67.0)	7.2	85.8	0.1
Me_2SO	0.4		8.8	4.3			692.0 (692.0)	22.4	727.9	2.3
CWM	13.0	4.9	362.9	38.2	3.3	35.7	154.6	153.8	766.4	70.0

[a] See Experimental section for conditions of extraction. [b] Total uronic acid. [c] Yield as a percentage by weight of total cell wall polysaccharide material (CWPM). To calculate the CWPM content of the fractions, it was assumed that the mannose and glucose arose from lectin-glycoprotein and starch, respectively. The weight of CWPM in each extract was then calculated by multiplying the total weight of the remaining sugars per unit weight of dialysed material by its total weight. The total yield of CWPM was obtained by adding together the fraction yields of CWPM and the final weight of CWM; the fraction yields of CWPM were expressed as a percentage of this total. [d] Yields in brackets are from 1 M H_2SO_4 hydrolysis alone (see Experimental).

a colour reaction with iodine and the small amount (13 μg/mg) of glucose released by hydrolysis with 1 M sulfuric acid. Its composition is given in Table 1.

The significant amount of non-starch polysaccharide material released during ball-milling with aqueous 0.5% SDS was further investigated as the bulk of it would have arisen from the cell wall. The extract was treated with alpha-amylase and pullanase to remove starch; the sugar composition of the residue is given in Table 2. The residue was fractionated by anion-exchange chromatography using DEAE Trisacryl M. Two acidic fractions were obtained (see Table 2) which together represented a 65% recovery of the material applied to the column. The ratio of rhamnose to uronic acid in these fractions was high (1:5.5 and 1:6.5 respectively), both when compared with the material applied to the column and with

Table 2
Sugar composition of material solubilised in 0.5% SDS extract and in fractions after anion-exchange chromatography

Fraction [a]	Recovery (%)	Anhydro sugars (μg/mg)								
		Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA [b]	Total
0.5% SDS	100	1.1		68.8	6.0	13.1	15.7	171.2	39.0	314.9
0.5% SDS (enzyme treated)	60	2.2		145.9	13.6	31.1	35.3	23.8	45.0	296.9
After anion-exchange chromatography										
0.12 M NaCl	42	23.7		467.7	36.9	3.1	109.9	15.0	131.4	787.7
0.25 M NaCl	31	27.2		328.4	34.2	0.2	84.8	10.2	165.5	650.5

[a] See Experimental section for conditions of extraction. [b] Total uronic acid.

Table 3
Sugar composition of extracts after sequential extraction of cell wall material from mung bean cotyledons

Fraction [a]	Recovery (%)	Anhydro sugars (μg/mg dry wt)								
		Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA [b]	Total
CWM	100	13.0	4.9	362.9	38.2	3.3	35.7	154.6 (13.1) [c]	153.8	766.4
CDTA-1	3.9	14.2	0.4	205.6	31.2	5.2	15.6	11.4	270.9	554.5
CDTA-2	0.7	10.2	1.0	156.4	19.6	4.8	17.2	11.8	470.5	691.5
Na_2CO_3 (1°C)	3.9	19.5		363.4	37.6	4.4	34.1	12.1	177.8	648.9
Na_2CO_3 (20°C)	1.4	24.7		414.1	45.0	10.2	38.8	27.3	200.9	761.6
0.5 M KOH	1.0	9.6	10.3	284.1	97.6	5.3	43.4	149.0	193.0	792.3
1 M KOH (1°C)	1.5	4.8	26.0	144.2	160.7	7.2	75.7	214.4	163.0	796.0
1 M KOH (20°C)	0.7	9.5	20.0	266.3	99.0	5.3	71.1	170.4	94.3	735.9
4 M KOH	25.7	16.4	8.5	539.2	59.3	21.2	116.4	153.9	83.2	998.1
4 M KOH + H_3BO_3	8.8	19.9	6.1	550.4	57.9	4.3	50.5	45.0	163.0	897.1
Wall residue	37.1	7.7	2.8	272.2	26.6	9.1	18.7	234.0 (24.5)	145.7	716.8

[a] See Experimental section for conditions of extraction. [b] Total uronic acid. [c] Yields in brackets are from 1 M sulfuric acid hydrolysis alone (see Experimental).

the materials extracted subsequently by other reagents (see below). Their overall composition suggested that they were highly branched pectic polysaccharides [7]. The presence of arabinogalactan–proteins (AGPs) [7] could not be ruled out; their occurrence might account for the low carbohydrate recovery of the fractions (22.2 and 35.0%, respectively). The increase in the rhamnose to uronic acid ratio in the recovered fractions suggested that the mixture also contained rhamnogalacturonans rich in galacturonic acid, with a low degree of esterification, which were irreversibly retained by the column.

Sequential extraction of CWM.—The cell wall was extracted in sequence with 0.05 M *trans*-1,2-cyclohexanediamine-*N*,*N*,*N*′,*N*′-tetraacetate, sodium salt (CDTA; twice), 0.05 M Na_2CO_3 at 1 and 20°C, 0.5 M KOH at 1°C, 1 M KOH at 1 and 20°C, 4 M KOH at 20°C, and 4 M KOH at 20°C containing 4% of boric acid, to leave an α-cellulose residue (see Experimental). The merits of this procedure have been discussed [8]. Table 3 contains data on the various fractions. The apparent sugar content of the CDTA fractions was rather low. This may have been due to the incomplete removal of CDTA in the dialysis step [8,9] (see below), and also to precipitation of material during acid hydrolysis [8]. The latter effect may also have lowered the carbohydrate recoveries of the Na_2CO_3-solubilised fractions. CDTA-1 contained the bulk of the calcium chelator-soluble pectic polysaccharides; the small amount of additional material solubilised in CDTA-2 was richer in uronic acid. Both fractions contained arabinose as the major neutral sugar but there were significant amounts of galactose and, unusually, of xylose. The total yield of CDTA-extracted material was 4.6% of total CWM. The compositions of the Na_2CO_3 (1°C) and Na_2CO_3 (20°C) extracts were broadly similar although the bulk of the material was in the 1°C fraction. The total yields of Na_2CO_3- and CDTA-solubilised materials were similar. Both Na_2CO_3 extracts contained approximately twice as much arabinose, galactose, and rhamnose and half as much uronic acid as the CDTA fractions. This suggested that the Na_2CO_3 extracts contained more highly branched pectic polysaccharides, possibly held in the wall by sugar-ester linkages [7,9]. Their xylose content was again unusually high, and their glucose content

was too low to be attributed to a xyloglucan component [8]. The total yield of Na_2CO_3-extracted material was 5.3% of total CWM.

Carbohydrate analysis of the 0.5 M KOH extract suggested that it contained highly branched pectic polysaccharides as major components; the presence of fucose, xylose, and glucose suggested xyloglucans as additional constituents [7]. Carbohydrate analysis of the 1 M 1°C and 1 M 20°C KOH fractions revealed increasing amounts of xylose. The yields of these three KOH extractions were similar and only 3% of total CWM overall. Extraction with 4 M KOH gave by far the highest yield of any extract at 25.7% of total CWM. Carbohydrate analysis showed the extracted material to be virtually all carbohydrate of high arabinose content. It suggested that 75% by weight of the material was branched pectic polysaccharide of low uronic acid content and and that the remainder was xyloglucan together with a trace of glucomannan [7]. These figures were calculated on the assumption that all rhamnose, arabinose, galactose, and uronic acid arose from pectic polysaccharides and that xylose, glucose, and fucose arose from xyloglucans [7]. The ratios of arabinose–uronic acid–rhamnose were 33:5:1. Thus the ratio of rhamnose to uronic acid was exceptionally high in this extract, and similar in this respect to the pectic polysaccharides solubilised by 0.5% SDS during ball-milling (see above). Both the amount of material extracted and its high pectic content stand in stark contrast to the results of 4 M KOH extraction of other plant tissues which have xyloglucans as their major hemicellulosic component and which have been examined by the present protocol. Yields of 4 M KOH-soluble material from the parenchyma of onion bulbs [10], potato tubers [11], and runner bean pods [12] have been reported as 7.7, 2.4, and 5.2% of total CWM, respectively, and the percentage content of pectic polysaccharide and xyloglucan, estimated in the same way as above (and ignoring the presence of any arabinogalactans), at 29 and 52%, 35 and 58%, and 24 and 46%, respectively.

Carbohydrate analysis of the 4 M KOH + borate extract suggested it to be again a mixture of highly branched pectic polysaccharides (ca. 80% by weight), (fucogalacto)xyloglucan ($< 10\%$; the xylose content exceeded the glucose content which shows that much of the xylose was a pectic component), and traces of glucomannan. The pectic polysaccharides were inferred to be slightly less branched than those of the 4 M KOH extract with ratios of arabinose–uronic acid–rhamnose of 28:8:1. The yield of material was 8.8% of total CWM. In contrast to the 4 M KOH extracts, a preponderance of pectic polysaccharides was also observed in the 4 M KOH + borate extracts of potato tuber [11] and runner bean pod parenchyma [12] where pectic polysaccharides and xyloglucans represented approximately 61 and 25%, and 30 and 17% (this latter extract contained much glycoprotein), respectively, of the extracted material. However, the total yield of the latter was only 0.6% of total CWM in each case and the pectic polysaccharides were more highly branched; the respective arabinose–uronic acid–rhamnose ratios were 14:8:1 and 10:5:1.

Carbohydrate analysis of the cell wall residue (37% by weight of total cell wall material) suggested that it contained 25% by weight of cellulose and 44% by weight of highly branched pectic polysaccharides (arabinose–uronic acid–rhamnose ratios 35:19:1). This contrasts with onion and potato parenchyma where the relative amounts of cellulose and pectic polysaccharides in the wall residues were 50 and 30%, and 42 and 53%, respectively.

Thus the overall yield of pectic polysaccharides from mung bean cotyledons is high. Summing the pectic components of the various fractions suggested that pectic polysaccha-

Table 4
Carbohydrate composition of fractions from CDTA-soluble and Na_2CO_3-soluble extracts after anion-exchange chromatography

Fraction [a]		Recovery from column (%)	Anhydro sugars (μg/mg dry wt)								
			Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA [b]	Total
CDTA-1		(47.0) [c]									
0.125 M NaCl	(C1A)	3.7	26.3	3.0	356.7	57.7	11.9	59.0	25.1	459.3	999.0
0.25 M NaCl	(C1B)	27.2	6.5	12.1	156.0	21.6	6.9	15.8	15.1	640.8	874.8
0.25 M NaCl	(C1C)	13.1	11.8	2.5	108.5	13.1	11.4	13.4	24.1	375.0	559.8
0.5 M NaCl	(C1D)	1.2	9.7		23.2		8.7	2.2	21.3	104.3	174.8
1 M NaCl	(C1E)	1.6	6.4	1.8	24.2	6.4	12.4	6.4	19.8	76.9	154.3
CDTA-2		(48.6)									
0.25 M NaCl	(C2A)	34.4	7.1	0.8	179.0	15.7	3.2	16.0	7.9	379.7	685.6
0.5 M NaCl	(C2B)	14.2	6.7	0.7	371.1	60.3	11.8	32.3	83.8	435.3	999.9
Na_2CO_3 (1°C)		(53.5)									
0.125 M NaCl	(N1A)	8.1	6.5	1.4	465.1	5.5	4.0	44.6	10.4	111.9	649.4
0.25 M NaCl	(N1B)	45.4	8.9	2.6	350.6	31.6	3.6	23.1	6.9	407.2	834.0
Na_2CO_3 (22°C)	(N2A)	(52.2)									
0.125 M NaCl	(N2B)	3.0	24.3	3.8	531.9	16.9	28.2	102.7	72.1	218.0	997.9
0.25 M NaCl	(N2C)	46.4	22.8	4.3	591.9	64.3	8.9	34.2	31.8	240.9	999.1
0.5 M NaCl		3.0	7.1	4.1	318.9	36.2	13.8	17.7	62.7	243.8	704.3

[a] See Experimental section for conditions of extraction. [b] Total uronic acid. [c] Total recovery from column.

rides were approximately 50% by weight of the total cell wall material or 73% of the total cell wall carbohydrate content of the assorted extracts. A calculation based on the sugar analysis of pure CWM was in good agreement, suggesting a figure of 57% of CWM dry weight, or 73% of CWM total sugar content; this showed that the assumptions made in the calculations were valid.

Anion-exchange chromatography.—In previous studies [10–12], extracts of material were dialysed and freeze-dried. Although all extracts of onion [10] so prepared were freely soluble in water, the solubilities of certain of the extracts from potato [11] and runner bean [12] were low. To avoid these problems the various mung bean fractions were dialysed but were not evaporated to dryness or freeze-dried. Rather they were concentrated by evaporation to a volume suitable for direct application to the anion-exchange column. Extracts were fractionated on a DEAE Trisacryl column as previously described [10]; the sugar compositions and recoveries of the various fractions are given in Tables 4 and 5. The recoveries of the CDTA- and Na_2CO_3-solubilised materials were ca. 50%. The low recovery of certain CDTA- and Na_2CO_3-solubilised materials has been observed in potato [11] and runner bean [12] although recoveries of all fractions of onion were high (70–90%). Possible causes of the effect together with the benefits of the present anion-exchange medium have been discussed [10]. The recoveries of KOH-solubilised material in the present case were typically greater than 85%. Recoveries from anion-exchange chromatography of KOH extracts of potato [11] and runner bean [12] were also high; this was attributed to their high neutral polysaccharide content. In contrast, acidic pectic polysaccharides preponderated in the present case.

Table 5
Carbohydrate composition of KOH-soluble fractions after anion-exchange chromatography

Fraction [a]		Recovery from column (%)	Anhydro sugars (μg/mg dry wt)								
			Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA [b]	Total
0.5 M KOH (1°C)		(88.8) [c]									
Neutral	(K1A)	33.6	2.3	22.0	50.4	198.0	4.1	41.3	212.5	58.8	589.4
0.25 M NaCl	(K1B)	55.2	9.2	3.5	371.4	52.9	4.0	36.3	29.0	274.3	780.6
1 M KOH (1°C)		(85.0)									
Neutral	(K2A)	57.3	4.6	51.3	58.2	217.2	9.8	99.9	350.0	3.5	794.8
0.125 M NaCl	(K2B)	6.2	7.2	5.5	220.5	19.8	7.0	39.7	47.9	224.9	572.5
0.25 M NaCl	(K2C)	18.0	11.0	6.7	322.4	51.5	7.3	49.3	45.9	224.3	718.4
0.5 M NaCl	(K2D)	3.5	5.2	2.6	90.2	18.4	6.3	13.1	35.0	209.3	380.1
1 M KOH (22°C)		(90.0)									
Neutral	(K3A)	42.5	6.0	34.6	124.2	162.6	31.7	75.9	333.5	15.6	784.1
0.125 M NaCl	(K3B)	11.7	12.2	6.1	520.2	20.1	5.9	97.7	72.3	10.0	734.5
0.25 M NaCl	(K3C)	35.8	11.4	6.7	504.2	61.6	5.2	45.7	59.6	304.0	998.4
4 M KOH ($+NaBH_4$)		(86.6)									
Neutral (A)	(K4A)	8.7	0.6	40.2	103.4	185.7	14.7	90.3	316.4	45.0	796.3
Neutral (B)	(K4B)	18.0	7.7	15.6	510.0	70.5	7.6	72.1	110.5	112.9	906.9
Gradient											
0–0.1 M NaCl	(K4C)	30.4	11.3	4.9	649.4	43.4	10.5	84.8	49.0	145.7	999.0
0.1–0.2 M NaCl	(K4D)	29.5	11.0	5.7	596.8	62.6	10.6	34.4	36.4	136.4	893.9
4 M KOH ($+H_3BO_4$)			(89.8)								
Neutral	(K5A)	43.2	12.5	94.9	522.4	60.5	4.7	45.2	53.2	181.8	975.2
0.125 M NaCl	(K5B)	14.1	11.5	4.5	428.7	27.7	9.4	41.5	41.7	180.0	745.0
0.25 M NaCl	(K5C)	32.5	12.0	4.2	511.0	57.2	4.1	35.4	29.5	277.8	931.2

[a] See Experimental section for conditions of extraction. [b] Total uronic acid. [c] Total recovery from column.

Fractionation of CDTA- and Na_2CO_3-solubilised material.—The CDTA- and Na_2CO_3-soluble fractions were all acidic; no material was eluted by buffer alone. The fractions selected corresponded to well-resolved peaks and are described in Table 4. The sugar contents of the acidic fractions C1C, C1D, and C1E from CDTA-1 were low. This was almost certainly due to CDTA contamination. In olive [9], the presence of CDTA in late-running anion-exchange chromatography peaks has been demonstrated by NMR; exhaustive dialysis failed to remove all the chelating agent. Mort et al. [13] have found it impossible, by dialysis, to remove all the CDTA from pectic polysaccharides extracted from suspension-cultured cotton cells. In contrast, dialysis-purified CDTA extracts of onion [10] had a high carbohydrate content and were thus not contaminated significantly by CDTA. This suggests that the fine structure of the extracted pectic polysaccharide influences the ease of CDTA removal. It is likely that residual calcium ions can complex the carboxyl groups of both the CDTA and the pectic polysaccharides, resulting in an association of the chelator with the polymer [9]. The number and distribution of free carboxyl groups on the pectic polysaccharide would influence the tenacity of this interaction and would explain the variable ease

of CDTA removal. In addition the complex would be negatively charged and would be eluted in a late-running fraction, as observed [9].

The uronic acid content of all the CDTA fractions was high at 44–73%; in all cases it comprised over 50% of the total of the putative pectic components. The two major fractions (C1B and C2A) had arabinose–uronic acid–rhamnose ratios of 24:98:1 and 25:53:1, respectively. By contrast the Na_2CO_3 extracts all had arabinose as their major pectic component; the arabinose–uronic acid–rhamnose ratios of the two major fractions (N1B and N2B) were 39:46:1 and 26:11:1, respectively. This suggested that the Na_2CO_3-extracted materials were more highly branched than the CDTA-extracted materials and had a lower proportion of uronic acid. These features are in broad agreement with those found in earlier studies of other systems and their significance has been discussed [10]. All fractions contained small but significant amounts of fucose, xylose, glucose, and mannose. In the major fractions C1B, C2A, N1B, and N2B glucose, constituted 1.7, 1.2, 0.8, and 3.2% by weight, respectively, of the total carbohydrate content; it was present in the minor, more acidic 0.5 M and 1 M NaCl fractions in greater amounts (8.4–12.8%). Mannose was present in all fractions in the range 0.4–2.8% by weight of total carbohydrate content, with the exception of the 0.5 M and 1 M NaCl CDTA-1 fractions where it was more predominant (8.7 and 12.4%, respectively, by weight).

Fractionation of 0.5, 1, and 4 M KOH-solubilised material.—As already noted, the recoveries of these fractions from the anion-exchange column were high at over 85%. The fractions selected from the 0.5 and 1 M KOH extracts corresponded to well-resolved peaks and are described in Table 5. The 4 M KOH elution profile is shown in Fig. 1. Carbohydrate analysis showed that all fractions contained small but significant amounts of mannose. From the relative amounts of glucose, xylose, galactose, and fucose, the neutral fractions of the 0.5 M, 1 M (1°C), 1 M (20°C), and 4 M KOH extracts were inferred to contain fucogalactoxyloglucans together with small amounts of pectic polysaccharides and perhaps some xylans. Apart from K4B, all these neutral fractions had carbohydrate contents in the range 59–79%: the nature of the material unaccounted for was not investigated but was presumed to be proteinaceous and phenolic. The fraction K5A from the 4 M KOH + borate extract was unlike the other neutral fractions as it was composed almost entirely of carbohydrate with pectic polysaccharides as the major component. There was also evidence of small amounts of xyloglucan. K3B, eluted with 0.125 M NaCl, appeared, uniquely, to be mainly non-artefactual (see above) neutral arabinogalactan. Carbohydrate analysis of the other fractions eluted by NaCl suggested that they all contained highly branched pectic polysaccharides and possibly small amounts of xyloglucan. The non-carbohydrate content of the fractions was variable; at the extremes K2D was only 38% carbohydrate whereas K4C was composed entirely of carbohydrate. Apart from the minor fraction K2D which was highly acidic, there was a trend from comparable amounts of arabinose and uronic acid in the acidic 0.5 M and 1 M, 1°C KOH fractions, through to arabinose–uronic acid ratios of 3–4:1 in the 4 M KOH and 4 M KOH + borate acidic extracts. The ratio of rhamnose–uronic acid also followed this trend.

Refractionation of the major acidic 4 M KOH extracts K4C and K4D.—The two largest fractions obtained by KOH extraction, K4C and K4D (7.8 and 7.6% by weight of total CWM, were refractionated by anion-exchange chromatography (see Table 6 and Fig. 1). Both fractions displayed considerable heterogeneity. All the fractions from K4C were more

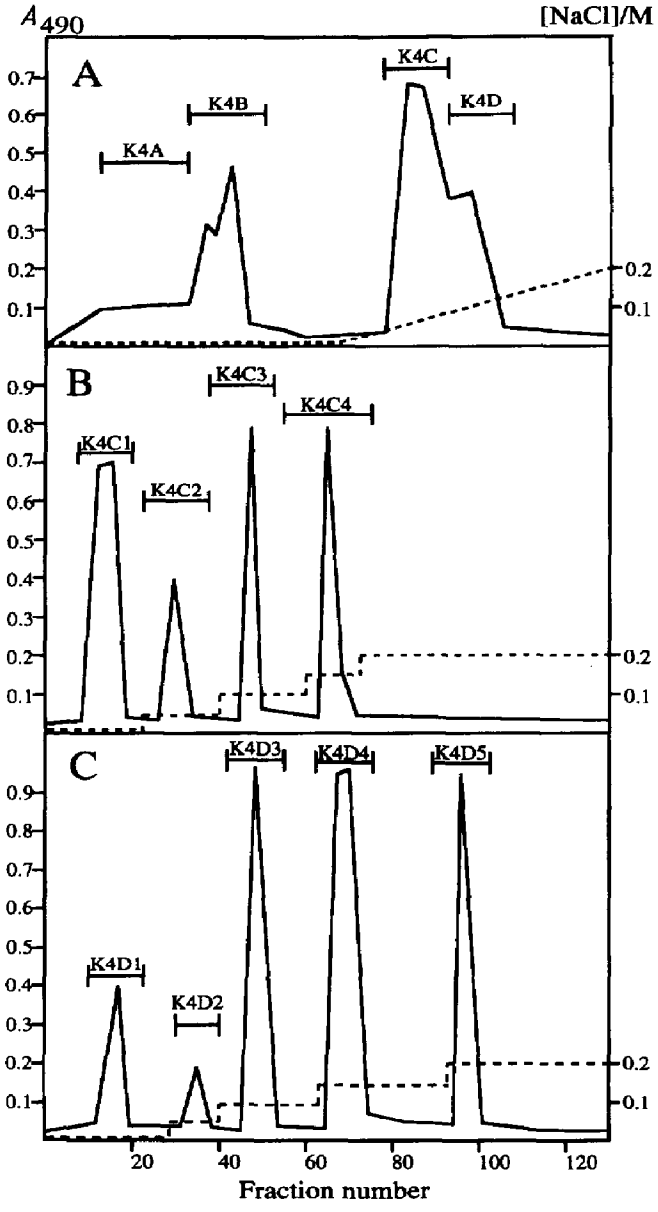

Fig. 1. Anion-exchange chromatography of the polymers extracted with 4 M KOH. Fractions were analysed for their total sugar content. **A**, Material initially extracted with 4 M KOH; **B**, refractionation of fraction K4C; **C**, refractionation of fraction K4D.

than 99% carbohydrate; the fractions from K4D contained 85–91% carbohydrate. The elution profiles of K4C and, in particular, K4D were curious; they contained material that eluted at salt concentrations lower than those originally required to elute them. The causes of this behaviour were not investigated. The original gradient might have co-eluted material whose mutual association or conformation had been changed by the removal of earlier eluted material. The gradient used for the refractionation study was more gradual, and might have allowed separation rather than co-elution. Whatever the explanation, the refractionation

Table 6
Carbohydrate composition of two gradients fractions obtained from 4 M KOH after refractionation by anion-exchange chromatography

Fraction [a]		Recovery from column (%)	Anhydro sugars (μg/mg dry wt)								
			Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA [b]	Total
Gradient											
0–0.1 M NaCl	(K4C)	(92.3) [c]	11.3	4.9	649.4	43.4	10.5	84.8	49.0	145.7	999.0
Neutral	(K4C1)	44.0	8.8	4.7	659.4	34.8	3.1	68.7	22.9	196.8	999.0
0.05 M NaCl	(K4C2)	6.6	10.8	5.1	740.6	20.5	8.1	94.4	18.2	101.9	999.6
0.1 M NaCl	(K4C3)	20.4	12.9	5.0	591.6	68.7	22.3	98.9	100.2	100.3	999.9
0.15 M NaCl	(K4C4)	21.3	12.7	4.6	672.0	50.2	8.3	62.1	40.0	147.2	997.1
Gradient											
0.1–0.2 M NaCl	(K4D)	(84.9)	11.0	5.7	596.8	62.6	10.6	34.4	36.4	136.4	893.9
Neutral	(K4D1)	19.4	13.2	8.3	723.7	65.0	7.5	35.1	32.8	15.4	871.0
0.05 M NaCl	(K4D2)	4.1	8.1	4.1	685.6	30.5	9.6	60.5	46.7	8.8	853.9
0.1 M NaCl	(K4D3)	19.7	9.6	4.8	632.5	39.0	15.9	36.5	56.7	114.9	909.9
0.15 M NaCl	(K4D4)	31.7	11.0	4.6	526.3	63.7	10.0	27.7	28.1	216.8	888.2
0.025 M NaCl	(K4D5)	14.0	13.0	6.5	426.7	113.2	9.7	13.9	17.8	314.0	914.6

[a] See Experimental section for conditions of extraction. [b] Total uronic acid. [c] Total recovery from column.

revealed the considerable heterogeneity of the 4 M KOH extract. All fractions had small but significant mannose contents. Their carbohydrate analyses suggested that they might be xylose-containing pectic polysaccharides complexed to small amounts of (fucogalacto)xyloglucan. Evidence of similar, presumably covalently linked complexes has been found in suspension-cultured sycamore cells [14], and in 1 M KOH-solubilised material from onion [10], cabbage [15], apple [16], and runner bean [17]. K4D1 and K4D2 were exceptional in that they contained extremely low levels of uronic acid, yet were rich in arabinose.

Amino acid analysis of selected fractions.—The amino acid compositions of the unfractionated cell wall (CWM), the 0.5 M KOH extract, the 4 M KOH + borate extract, and the α-cellulose residue were determined. The overall level of protein in the CWM was low which made the accurate determination of amino acids difficult. The proportions of amino acids by weight in the 0.5 M KOH and 4 M KOH + borate extracts were low at 138 and 22 μg/mg, respectively. Hydroxyproline (frequently a major component of cell wall glycoproteins [7]) represented only 5.1% of the amino acid content of the 0.5 M KOH extract and was virtually absent from the 4 M KOH + borate fraction. The 0.5 M KOH extract had points of similarity with the 1 M KOH extract of potato [11] (where no preliminary 0.5 M KOH extraction was undertaken), although the latter had only 35 μg/mg protein. Both extracts had aspartate as their most abundant amino acid (10.7 and 13.2 mol% of total amino acid for mung bean and potato, respectively) and high levels of glycine (10.1 and 9.3 mol%), valine (7.1 and 7.6 mol%), and glutamate (10.6 and 8.5 mol%); hydroxyproline was not a major component of either system (5.1 and 2.7 mol%). Unlike potato [11] and runner bean [12,18] the 4 M KOH + borate fraction had a low protein content; in particular, hydroxyproline-rich glycoproteins were absent. Only 18 nmol/mg of glutamate (0.2% by weight) and a trace of histidine were detected in the α-cellulose residue. Very low levels

Table 7
Partially methylated alditol acetates derived from selected fractions of the CDTA- and Na_2CO_3-soluble extracts and from the wall residue

Alditol acetate	Relative mol %					
	C1B	C1B [a]	N1B	N1B [a]	Wall residue	Wall residue [a]
3,4-Me_2-Rha	2.3	1.8	2.9	2.6	1.5	2.0
3-Me-Rha	1.0	0.6	1.6	0.6	0.6	0.6
2,3,4-Me_3-Fuc	0.5	0.5			0.4	
2,3,5-Me_3-Ara	16.2	24.6	17.8	23.4	17.7	18.5
2,3-Me_2-Ara	35.7	34.6	51.6	52.0	33.9	36.2
2-Me-Ara	5.6	3.8	2.6	3.2	2.8	2.8
3-Me-Ara	14.0	7.7	11.1	6.6	9.7	7.6
Arabinitol	4.7	2.9	2.6	1.4	2.6	1.3
2,3,4-Me_3-Xyl	1.9	3.4	3.0	2.5	2.2	3.2
3,4-Me_2-Xyl					0.3	0.3
2,3-Me_2-Xyl		1.0			0.3	1.1
2,3,4,6-Me_4Gal	trace	1.3	2.4	2.4	0.6	0.6
2,3,6-Me_3-Gal	5.8	2.0	3.6	1.3	1.7	1.1
2,3,6-Me_3-Glc	3.1	5.0			23.3	15.4
2,3-Me_2-Glc	3.8	3.2			1.9	1.9
Glucitol	5.3		0.7		0.4	
2,3,4-Me_3-GalA						0.7
2,3-Me_2GalA		6.2		3.7		5.2
2-Me-GalA		1.8		0.3		2.1

[a] Carboxyl reduced with $LiAlD_4$ after methylation (see Experimental).

of glycoprotein were also found in the α-cellulosic component of potato [11] where glutamate was also the major component. Both results contrast with the high levels of hydroxyproline-rich glycoproteins found in α-cellulose residue of runner bean parenchyma [12,18].

Methylation analysis of selected CDTA- and Na_2CO_3-solubilised materials.—The two largest fractions from anion-exchange chromatography of the CDTA-1 and Na_2CO_3 (1°C) extracts, C1B and N1B, were subjected to methylation analysis (see Table 7). C1B was first de-esterified to minimise β-elimination [8]. In each case a portion of the methylated material was carboxyl-reduced before its conversion into partially methylated alditol acetates (PMAAs). Comparison of the data from the carboxyl-reduced samples with the methylation analysis data was instructive. The recoveries of galacturonic acid were low. This is often the case for pectic polysaccharides [8,9]. It may be due to non-methylation of regions of polygalacturonic acid, β-elimination of the polymer, and loss of the resulting fragments on dialysis (β-elimination of non-esterified pectic polysaccharides under Hakomori conditions has been demonstrated unequivocally in certain instances [18]), and/or under-reduction of the carboxyl groups necessary for the detection of galacturonic acid as a PMAA. We shall discuss the relative importance of these factors in a future paper on the methylation linkage-analysis of pectic polysaccharides [19]. In addition, when uronic acid was excluded from the data, the sugar analyses suggested that the fractions contained less arabinose and more xylose than did the linkage analyses. Thus C1B contained 69.2 and

80.0 mol% of arabinose and 9.6 and 4.8 mol% of xylose, and N1B 84.1 and 90.0 mol% of arabinose and 7.5 and 2.5 mol% of xylose, respectively. Xylosyl residues, and in particular terminal xylopyranosyl residues, are often underestimated in linkage analysis [20], and the same unknown mechanism would seem to operate here, depressing the xylose and inflating the arabinose totals. However, despite this apparent specific underestimation, overall terminal sugar totals slightly exceeded, or were comparable with branch-point totals — the ratios for C1B and N1B were 0.80 and 0.88, respectively. This suggests that the residues to which the the terminal xylosyl residues were attached were also underestimated by linkage analysis. Aspinall et al. [21] have reported terminal xylopyranosyl residues attached to the 3-position of some of the galacturonosyl residues of soya bean pectic polysaccharides. This was inferred from the isolation of the pseudoaldobiouronic acid Xyl*p*-(1→3)-Gal*p*A obtained by partial acid hydrolysis and enzymatic degradation. In the present case, 1,3,4-linked galacturonosyl residues would presumably be underestimated by linkage analysis in the same way as 1,4-linked galacturonosyl residues (see above). Support for the presence of terminal xylopyranosyl residues attached to the 3-position of 1,4-linked galacturonosyl residues as a general feature of pectic polysaccharides has come from other recent studies [22]. Preliminary partial acidolysis and methylation analysis data for N1B strongly suggest that the terminal xylopyranosyl residues are attached to position 3 of the 1,3,4-linked galacturonosyl residues [23]. The methylation analyses suggest that both fractions contain rhamnogalacturonans [7] bearing short arabinan sidechains, and that C1B also contained a small amount of complexed xyloglucan. The ratio of terminal arabinofuranosyl residues to mid-chain arabinofuranosyl residues suggested average sidechain dp values of 4–5 for both C1B and N1B; a value of 3 has been reported for the Na_2CO_3-soluble pectins of onion [24].

Methylation analysis of the neutral and polysaccharide solubilised by 0.5, 1, and 4 M KOH.—The KOH-extracted fractions eluted from the anion-exchange column by buffer alone were subjected to methylation analysis (Table 8). The results strongly suggested that (fucogalacto)xyloglucans were the major components of fractions K1A, K2A, K3A, and K4A. 1,4,6-Linked glucopyranosyl, 1,4-linked glucopyranosyl, 1,2-linked xylopyranosyl, terminal xylopyranosyl, terminal galactopyranosyl, and terminal fucopyranosyl residues were all present and in proportions typical of a xyloglucan [7] although terminal xylopyranosyl residues were again underestimated (see above). Small but significant amounts of 1,4-linked xylopyranosyl residues and the virtual absence of 1,2,4-linked rhamnopyranosyl residues in these fractions suggested the presence of xylans [7]. The xylans may form part of a xylan–xyloglucan complex; evidence for the latter has also been found in cultures of poplar cells [25] and in runner bean cell walls [12]. K1A, K2A, K3A, and K4A also contained significant amounts (16.3–20.3 mol% of total neutral sugars) of arabinosyl residues. Terminally-, 1,5-, 1,2,5-, 1,3,5-, and 1,2,3,5-linked arabinofuranosyl residues were found. Although these residues usually form part of the sidechains of pectic polysaccharides, the virtual absence from the fractions of 1,2,4-linked rhamnopyranosyl residues, their normal point of attachment [7], precluded this. The arabinosyl residues may form part of free arabinan moieties [1], or of arabinans associated with xyloglucans.

Methylation analysis of K4B suggested that it contained pectic polysaccharides with arabinose and xylose as major components, together with small amounts of xyloglucan. Its composition was broadly similar to N1B except that the lower proportion of 1,5-linked

Table 8
Partially methylated alditol acetates derived from selected neutral fractions of the KOH-soluble extracts

Alditol acetates	Relative mol %					
	K1A	K2A	K3A	K4A	K4B	K4B [a]
3,4-Me_2-Rha		0.1			1.8	2.1
2,4-Me_2-Rha		0.1			trace	0.9
2,3,4-Me_3-Fuc	2.7	6.4	4.4	4.5	1.8	0.8
2,3,5-Me_3-Ara	4.2	4.7	4.3	3.9	22.7	24.0
3,5-Me_2-Ara	1.6	0.7	2.9			
2,3-Me_2-Ara	6.8	6.8	6.7	6.4	33.0	29.0
2-Me-Ara	1.3	0.7	0.9	0.9	3.9	4.9
3-Me-Ara	4.0	1.8	1.5	3.6	11.2	12.6
Arabinitol	1.0	2.0		1.0	4.3	5.1
2,3,4-Me_3-Xyl	7.4	7.9	7.7	8.8	2.3	2.5
3,4-Me_2-Xyl	5.7	6.6	6.0	5.6	1.2	0.1
2,3-Me_2-Xyl	20.2	3.2	3.8	1.2		
2,3,6-Me_3Man	2.2					
2,3-Me_2-Man		0.2		0.9		
2,3,4,6-Me_4Gal	2.5	5.8	3.9	4.3	2.4	2.4
3,4,6-Me_3-Gal	trace					
2,3,6-Me_3-Gal	3.3	4.8	8.2	6.0	7.4	3.6
2,3,4-Me_3-Gal		0.2		2.1		
2,3-Me_2-Gal		0.1				
2,3,4,6-Me_4-Glc						
2,3,6-Me_3-Glc	12.6	14.2	15.3	12.5	2.6	3.0
2,6-Me_2-Glc	1.5		1.3			
3,6-Me_2-Glc	0.6		0.8			
2,3-Me_2-Glc	21.0	32.1	30.9	28.5	4.7	4.5
3-Me-Glc	0.8		1.7			
Glucitol	0.6	0.3			0.6	
Hexitol hexaacetate		1.6		4.4		
2,3-Me_2-GalA						3.6
2-Me-GalA						0.9

[a] Carboxyl reduced with $LiAlD_4$ after methylation (see Experimental).

arabinofuranosyl residues suggested that it bore shorter sidechains; the average sidechain dp (calculated as above) was 3.2. Carboxyl-reduction of the material confirmed that some of the 1,4-linked galactopyranuronosyl residues were branched at C-3.

Methylation analysis of the refractionated acidic 4 M KOH-solubilised materials and of an acidic 4 M KOH + borate fraction.—Methylation analysis of K4C3 and K4C4 (Table 9) showed that they were essentially pectic polysaccharides of very similar composition to N1B and K4B with average sidechain lengths (see above) of 4 in each case; small amounts of xyloglucan were also present. Despite the anomalous behaviour during refractionation by anion-exchange chromatography, the compositions of K4D1 and K4D5 and K4C3 and K4C4 were similar. The 4 M KOH + borate fraction K5C was unique in this group in containing no 1,2,5-linked arabinofuranosyl residues. The composition of the pectic component was otherwise similar to those of K4C3 and K4C4. A small amount of xyloglucan was again indicated. More terminally-linked xylopyranosyl residues than 1,4,6-linked glu-

Table 9
Partially methylated alditol acetates derived from selected acidic fractions of the KOH-soluble extracts

Alditol acetates	Relative mol %						
	K4C3	K4C4	K4C4 [a]	K4D1	K4D5	K4D5 [a]	K5C
3,4-Me_2-Rha	2.4	2.6	1.9	2.0	1.3	1.5	1.6
3-Me-Rha	1.1	1.0	0.6	0.7	0.5	0.7	
2,3,5-Me_3-Ara	20.9	21.0	26.4	24.1	16.5	25.6	22.0
2,3-Me_2-Ara	39.1	40.4	41.6	36.9	51.0	38.8	44.5
2-Me-Ara	4.9	4.7	4.4	4.1	5.7	3.9	3.4
3-Me-Ara	13.1	12.9	7.9	11.3	1.6	11.1	
Arabinitol	5.5	4.5	4.2	4.0	6.5	2.9	2.6
2,3,4-Me_3Xyl	2.0	2.8	2.5	3.6	1.4	1.9	4.1
3,4-Me_2-Xyl	trace		0.1	0.5			0.8
2,3-Me_2-Xyl	0.6	0.5	0.2	1.9	0.3	0.4	2.8
2,3,4,6-Me_4-Gal	1.1	0.7	0.4	1.1	0.4	0.4	1.3
3,4,6-Me_3-Gal	trace						
2,3,6-Me_3-Gal	6.2	5.1	2.3	2.9	3.1	2.7	2.2
2,3-Me_2-Gal				2.9		0.2	
2,3,6-Me_3-Glc	0.8	1.3	1.7	0.9	8.4	6.0	1.4
2,3-Me_2-Glc	1.5	1.6		1.3	1.5	2.0	2.5
3-Me-Glc	0.5		1.7	2.9			
Glucitol		0.6				4.8	
2,3-Me_2-GalA			3.4			1.4	
2-Me-GalA			3.0			0.6	

[a] Carboxyl reduced with $LiAlD_4$ after methylation (see Experimental).

copyranosyl residues were present, which showed that some of the terminal sugar was a pectic component.

Methylation analysis of the α-cellulose residue.—Methylation analysis of the α-cellulose residue (Table 7) indicated that it contained pectic polysaccharides very similar in type to K4C3 and K4C4, and cellulose. Low levels of 1,4,6-linked glucopyranosyl residues suggested that small amounts of xyloglucan were also present.

3. Conclusions

The composition of the mung bean cotyledon cell wall is unlike any other cell wall so far examined. Inspection of the carbohydrate analyses of the various extracts (see above) suggests that the cell wall consists of approximately 55% by weight of pectic polysaccharides, 10% of xyloglucan, and 10% of cellulose. In contrast, other non-graminaceous cell walls which have been studied contained typically 10–20% less pectic polysaccharide and 15–20% more cellulose. In addition, the pectic polysaccharides of mung bean are less readily extractable; the calcium chelator CDTA and Na_2CO_3 (after de-esterification) liberated less than 10% by weight of CWM. The majority of the extractable materials were solubilised only by 4 M KOH; and the wall residue still contained 44% of pectic polysaccharides and only 25% of cellulose. Preliminary evidence suggests that the terminal xylosyl

residues found in all the pectic polysaccharides are bound to the 3-position of some galacturonosyl residues of the pectic backbone. Evidence for xyloglucan–pectic complexes and xylan–xyloglucan complexes, a feature of other tissues, was found in certain fractions purified by anion-exchange chromatography. Arabinans, reported as features of other legume cell walls, were only detected in trace amounts. This suggests that earlier reports of their occurrence was a result of extensive β-elimination of the pectic polysaccharides under the extraction conditions used [1]. Methylation analysis suggested that the arabinan side-chains of the pectic polysaccharides were short, with a typical average dp of 3–5.

4. Experimental

General methods.—Neutral sugars were released by Saeman and 1 M H_2SO_4 hydrolysis or by 1 M H_2SO_4 hydrolysis alone, and analysed as their alditol acetates [26] by GC. Uronic acids were determined colorimetrically by a modification [27] of the method of Blumenkrantz and Asboe-Hansen [27]. Amino acids were released by hydrolysis with 6 M HCl at 110°C for 24 h and measured as their *N*-heptafluorobutyryl propyl ester derivatives [28].

Preparation of CWM.— Mung bean seeds (*Vigna radiata*, var. T77) were soaked for 12 h at 25°C and decorticated manually. The cotyledons were blotted dry and frozen in liquid N_2. The cotyledons (700 g) were homogenised in 1.5% SDS (1400 mL) containing 5 mM $Na_2S_2O_5$ for 2 min. The homogenate was filtered through two layers of muslin cloth and a nylon cloth (postlip paper 150). The combined filtrate and washings were exhaustively dialysed against water and freeze-dried. The residue was washed with aq 0.5% SDS containing 3 mM $Na_2S_2O_5$ (2 × 1050 mL), suspended in the same solution, ball-milled (Pascall 1-L pots) at 1°C for 11 h at 60 rpm, and then centrifuged. The residue was washed twice with distilled water and centrifuged. The combined 0.5% SDS and water washings were exhaustively dialysed against water and freeze-dried. The residue was extracted with 2:2:1 (w/v/v) phenol–AcOH–water (PAW; 2 × 695 mL) and centrifuged; it was then washed twice with distilled water and centrifuged. The combined filtrate and washings were dialysed against aq 25% AcOH (2×) and water (1×) and freeze-dried. The residue was suspended in aq 90% Me_2SO (2666 mL), sonicated for 20 min at 20°C, and centrifuged. The residue was washed with water (200 mL), exhaustively dialysed at 1°C, and stored as a frozen suspension at −20°C. The combined washings were exhaustively dialysed against water and freeze-dried.

Sequential extraction of cell wall material.—*trans*-1,2-Cyclohexanediamine-*N*,*N*,*N*′,*N*′-tetraacetate, Na salt (CDTA) (1 M, pH 6.8, 25 mL) was added to a suspension of CWM (7 g in 475 mL) to a final concentration of 50 mM. The mixture was stirred at 20°C for 6 h, mixed with water, and centrifuged. The sediment was re-extracted with CDTA (50 mM, pH 6.8, 700 mL) for 2 h. The extracts with their respective washings (CDTA1 and CDTA 2) were filtered and dialysed. A suspension of the residue in 50 mM Na_2CO_3 (560 mL) containing 20 mM $NaBH_4$ was stirred for 20 h at 1°C. The extraction was repeated for 2 h at 20°C; it was then repeated with successive amounts (500 mL) of 0.5 M KOH at 1°C, 1 M KOH at 1°C and 20°C, 4 M KOH at 20°C, and 4 M KOH containing 20 mM H_3BO_3 at 20°C. The extractions were carried out under Ar and each solution contained 20 mM $NaBH_4$.

All the extracts were adjusted to pH 5 with AcOH, dialysed exhaustively, concentrated, and freeze-dried. The α-cellulose residue was acidified to pH 5 and frozen. Samples of the various residues taken during the extractions were dialysed and stored, in solution, at −20°C.

Treatment of 0.5% SDS-extracted material with alpha-amylase and pullanase.—Starch was removed from the 0.5% SDS fraction by treatment with alpha-amylase and pullanase as described by Selvendran and DuPont [29], except that gelatinisation at 80°C was omitted.

Anion-exchange chromatography.—The solutions of extracted polymer were concentrated, if necessary, so that addition of phosphate buffer (pH 6.5) at 1°C gave final concentrations of polymer and buffer of 1 mg/mL and 50 mM, respectively. The resultant solutions were stirred at 20°C for 6 h and centrifuged. The supernatant solutions were passed, in 50-mL batches, through a column (91 × 15 cm) of DEAE Trisacryl M (phosphate form). All fractions were eluted sequentially with 50 mM buffer (100 mL) and (except the 4 M KOH extract) 100 mL of 50 mM buffer containing 0.125, 0.25, 0.5, and 1 M NaCl. The 4 M KOH extract, after elution with buffer, was eluted with a 0–0.2 M NaCl gradient; the buffer concentration was maintained at 50 mM. Fractions (5 mL) were collected and assayed for carbohydrate by the phenol–H_2SO_4 method [30].

Methylation analysis.—The CDTA-extracted fractions were first de-esterified with 50 mM Na_2CO_3 for 16 h at 1°C, neutralised, dialysed, and freeze-dried. Polysaccharides were methylated by a modified [31] Hakomori [32] method using sodium methylsulfinylmethanide, and converted into alditol acetates [33] for analysis by GC [34] and GC–MS [12].

Carboxyl reduction with $LiAlD_4$.—Methylated material was carboxyl-reduced by the method of Lindberg [34].

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