Hemicellulosic complexes from the cell walls of runner bean (*Phaseolus coccineus*)

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The 1 M-KOH extract from the depectinated cell walls of parenchymatous tissues of mature runner bean (*Phaseolus coccineus*) on neutralization, dialysis and concentration gave insoluble (hemicellulose A) and soluble (hemicellulose B) carbohydrate complexes in the weight ratio 2:1. Both fractions contained polysaccharide, protein and polyphenolic material. The structural features of the carbohydrates were examined by methylation analysis. Hemicellulose A contained mainly pectic arabinogalactan, with lesser amounts of arabinoxylan and glucan. Sequential fractionation of hemicellulose B by anion-exchange and hydroxyapatite chromatography gave a range of polysaccharide–protein–polyphenolic complexes. The main polysaccharides in these complexes were (acidic) arabinoxylans, galactans, arabinogalactans 1 and 2 and xyloglucans. The proteins contained small amounts of hydroxyproline, but were rich in aspartic acid and glutamic acid. Attempts to determine the nature of the polyphenolic material were unsuccessful. The structural features of the polysaccharide–protein–polyphenolic complexes are discussed in relation to the structure of the cell walls of parenchymatous tissues.

The primary cell walls of suspension-cultured and parenchymatous tissues of dicotyledonous plants contain a range of glycoproteins (Selvendran, 1975a; Lamport & Catt, 1981). Extraction of the depectinated primary cell walls of mature runner-bean (*Phaseolus coccineus*) parenchyma with acidified chlorite releases hydroxyproline-rich glycoproteins (Selvendran, 1975a; O'Neill & Selvendran, 1980a), which appear to be held within the wall matrix through isodityrosine and similar phenolic cross-links (Fry, 1982). However, treatment of depectinated cell walls from runner beans with aqueous alkali solubilized proteoglycans containing relatively small amounts of hydroxyproline, and the bulk of these are present in the 1 M-KOH-soluble fraction (Selvendran, 1975a).

Similar complexes have been isolated from broad-bean (*Vicia faba*) (Pusztai *et al.*, 1971) and French-bean (*Phaseolus vulgaris*) leaves (Brown & Kimmins, 1973; Brown *et al.*, 1975) and partially characterized. However, only limited data are available on the composition and structure of these hydroxyproline-poor proteoglycans from higher-

plant cell walls (Selvendran, 1975a; Selvendran & O'Neill, 1982; Stevens & Selvendran, 1984a).

In the present paper the isolation and partial characterization of polysaccharide–protein–polyphenol complexes from the 1 M-KOH-soluble extract of the cell walls of parenchymatous tissues of *Phaseolus coccineus* are reported.

Experimental

Cell-wall preparation

Cell-wall material (CWM) of parenchymatous tissues of mature runner beans was prepared by sequential extraction of the ball-milled fresh tissue as previously described (Selvendran, 1975b; Ring & Selvendran, 1978). Fresh tissue (100 g) gave about 1 g (dry weight) of cell-wall preparation.

Fractionation of CWM

CWM (1 g/100 ml) was depectinated with hot water and ammonium oxalate as previously described (O'Neill & Selvendran, 1983). The depectinated CWM was extracted (1 g/100 ml) with 1 M-KOH containing 10 mM-NaBH₄ at 20°C, for 2 h under argon, filtered through sintered glass, and the filtrate adjusted to pH 5.5 with acetic acid. The precipitate (hemicellulose A) that formed on

Abbreviations used: CWM, cell-wall material; m.s., mass spectrometry.

concentration and dialysis was separated by centrifugation. Both the precipitate and soluble extract (hemicellulose B) were exhaustively dialysed against distilled water and freeze-dried.

Ion-exchange chromatography

Hemicellulose B (300 mg) was dispersed in 15 ml of distilled water for 15 h and filtered to remove insoluble material (about 7 mg). The filtrate was fractionated on a column (40 cm $\times$ 2.5 cm) containing DEAE-Sephadex (Cl⁻ form) by elution with (i) 300 ml of water, (ii) a 0–0.55 M-NaCl gradient (total volume 600 ml) and (iii) 0.6 M-NaCl containing 0.1 M-NaOH (300 ml) at a flow rate of 20 ml/h. Fractions (6 ml) were collected and monitored for A_{275} . Portions (200 μ l) were assayed for total carbohydrate by the phenol/H₂SO₄ method (Dubois *et al.*, 1956). Appropriate fractions were pooled, dialysed and freeze-dried.

Hydroxyapatite chromatography

Hydroxyapatite chromatography was performed on a column (20 cm $\times$ 0.9 cm) of hydroxyapatite (Bio-Gel HTP; Bio-Rad, Richmond, CA, U.S.A.) at a flow rate of 20 ml/h. Samples (10–30 mg) were dissolved in 50 mM-phosphate buffer, pH 7.0, and eluted from the column with (i) 50 mM-phosphate buffer (50 ml), (ii) a 50–500 mM-sodium phosphate buffer gradient (total volume 100 ml) and (iii) 500 mM-phosphate buffer containing 1 M-NaCl (50 ml). Fractions (1 ml) were collected and monitored for A_{275} . Portions (25 μ l) were assayed for total carbohydrate by the Dubois *et al.* (1956) method. Appropriate fractions were pooled, dialysed and freeze-dried.

Monosaccharide and amino acid analysis

Neutral sugars were analysed as their alditol acetates by g.l.c. after hydrolysis with 1 M-H₂SO₄ for 2.5 h at 100°C (Selvendran *et al.*, 1979). Uronic acid was determined colorimetrically (Blumenkrantz & Asboe-Hansen, 1973), with D-galacturonic acid as standard. Amino acids were released by hydrolysis with 6 M-HCl for 24 h at 110°C and analysed as their heptafluorobutyl-n-propyl derivatives by g.l.c. (March, 1975).

Phenolic content

An estimate of the phenolic content of water-soluble material was obtained from u.v. absorption at 275 nm with tyrosine as standard. Values were corrected for the presence of aromatic amino acids. The phenolic content of some of the fractions was also estimated by the acetyl bromide method (Morrison, 1972).

Characterization of bound phenolics

(a) *Hydrolysis.* Cell-wall preparations (20–50 mg)

were hydrolysed with either 2 M-HCl or 2 M-NaOH and the hydrolysates adjusted to pH 2.5. The ethyl acetate-soluble products were examined by t.l.c. on 0.25 mm-thick silica-gel G with, as solvents, (A) butanol/acetic acid/water (4:1:5, by vol., upper phase) or (B) toluene/acetic acid/water (10:7:3, by vol., upper phase). The plates were examined for fluorescence under long-wavelength u.v. light, for amino acids by spraying with ninhydrin (Consden *et al.*, 1944) and for reducing sugars after spraying with aniline hydrogen phthalate (Partridge, 1949).

(b) *Nitrobenzene and cupric oxide oxidation.* Portions (50–70 mg) of hemicelluloses A and B were oxidized with alkaline nitrobenzene or cupric oxide as described by Hartley (1971). The ethyl acetate-soluble reaction products were examined by g.l.c. on a column (2.7 m $\times$ 2 mm) containing 5% (w/w) CDMS (cyclohexanedimethanol succinate) at 210°C (Hartley, 1971) or by t.l.c. with solvent B.

Methylation analysis

Samples (1–5 mg) were methylated as previously described (Ring & Selvendran, 1978; O'Neill & Selvendran, 1980b) and the partially methylated alditol acetates separated by g.l.c. on a WCOT OV-225 capillary column (25 m $\times$ 0.25 mm). Combined g.l.c.-m.s. was performed on an AEI MS30 mass spectrometer using a wide-bore (25 m $\times$ 0.5 mm) WCOT OV-225 capillary column (O'Neill & Selvendran, 1983).

Results and discussion

Analysis of the 1 M-KOH-soluble material

The compositions of the cell walls and fractions obtained by sequential extraction with hot water, hot oxalate, 1 M- and 4 M-KOH at 20°C have been reported (O'Neill & Selvendran, 1983). Extraction of the depectinated cell walls with 1 M-KOH solubilized about 5% of the material which, on dialysis and concentration, gave hemicellulose A (insoluble) and hemicellulose B (soluble) in a ratio of about 2:1. Although both fractions have similar protein content and amino acid composition, they differ substantially in total carbohydrate and monosaccharide composition (Table 1, columns *a* and *b*). The insoluble fraction was rich in arabinose and galactose, with lesser amounts of other neutral sugars, uronic acid and about 20% (w/w) protein. By using the acetyl bromide method, a phenolic content of about 15% (w/w) was found. These results, in conjunction with those of methylation analysis (Table 3, column *a*), are consistent with the major carbohydrate being derived from a pectic arabinogalactan in association with smaller amounts of arabinoxylan- and glucan-like polymers. No attempt was, however, made to resolve the complex further.

Table 1. Composition of hemicellulose A and hemicellulose B fractions obtained after ion-exchange chromatography
Composition (% w/w, or mol%) or yield (%)

Component	Fraction	Hemicellulose		Hemicellulose B DEAE-Sephadex fractions				
		(a) A	(b) B	(c) D-1	(d) D-2	(e) D-3	(f) D-4	(g) D-5
Neutral sugar (% w/w)		22.2	40.8	51.0	26.0	22.0	25.6	11.1
Uronic acid (% w/w)		8.8	10.1	8.5	20.0	22.0	7.5	8.5
Protein (% w/w)		19.9	20.6	20.3	19.4	28.9	39.7	29.2
Phenolic* (% w/w)		15.0	14.3	13.0	10.0	13.0	9.0	10.0
Yield (%) from ion-exchange		—	—	16.0	11.3	34.2	13.1	25.4
Monosaccharide (mol%)								
6-Deoxyhexose		4.6	3.7	—	—	Trace	5.2	5.8
Arabinose		42.5	26.8	20.6	12.6	23.9	23.0	24.2
Xylose		8.2	34.7	12.0	74.2	57.9	32.5	41.2
Galactose		27.9	24.8	44.0	7.0	11.7	35.7	19.8
Glucose		16.8	10.0	23.4	6.2	6.5	3.6	11.0
Amino acid (mol%)								
Ala		5.2	8.2	8.2	9.0	8.8	8.8	7.8
Gly		4.4	8.8	10.3	11.5	9.9	9.8	8.5
Val		5.9	6.7	6.7	6.3	6.6	6.4	5.9
Thr		3.6	4.5	5.7	4.8	4.7	4.5	4.1
Ser		5.3	7.9	9.7	8.5	8.0	8.0	7.0
Leu		9.8	9.1	7.2	9.5	9.1	9.6	10.4
Ile		4.1	3.6	2.1	3.2	4.7	4.8	4.8
Pro		5.2	6.4	7.8	9.5	5.8	5.3	4.8
Hyp		4.5	4.5	9.2	2.6	2.6	1.9	1.5
Asp		9.2	10.3	8.2	10.0	11.3	11.4	12.2
Phe		5.7	3.9	3.6	3.7	4.0	4.0	4.1
Glu		12.9	12.7	9.7	11.6	13.9	14.4	16.3
Lys		9.8	8.2	7.2	5.8	6.6	6.1	6.7
Tyr		4.9	2.4	1.5	1.1	1.8	1.6	1.1
Arg		5.2	1.5	1.0	1.1	1.1	1.3	2.2
His		4.1	1.2	2.6	1.6	1.5	2.1	2.6

* Determined by the acetyl bromide method (Morrison, 1972).

The soluble fraction contained appreciable amounts of xylose, arabinose and galactose, with some glucose, 6-deoxyhexose and uronic acid (Table 1, column *b*). Significant amounts of protein (20.6%) and polyphenolic material (14.3%) were also present. These results suggested that hemicellulose B contained carbohydrate in association with proteins and/or polyphenolics. Further evidence on the nature of the polysaccharides and their association with proteins and polyphenolics was obtained by column chromatography and methylation analysis.

Ion-exchange chromatography of hemicellulose B

Chromatography of hemicellulose B on DEAE-Sephadex gave a neutral (D1) and four acidic (D2–D5) fractions (Fig. 1) in the ratio 1.0:0.7:2.1:0.8:1.6 (total recovery, 60% of applied material). All the fractions were found to differ

Fig. 1. Fractionation of the 1M-KOH soluble (hemicellulose B) material from *P. coccineus* cell walls on DEAE-Sephadex (Cl^- form)
○, Total carbohydrate (A_{490}); □, A_{275} ; ----, Cl^- gradient. See the Experimental section for details.

in monosaccharide, amino acid and phenolic content (Table 1, columns *c-g*).

Although all the fractions contained protein (20–40%), only D1 contained an appreciable amount of hydroxyproline (about 9 mol%). It is also significant that all the fractions are rich (17.9–28.5 mol%) in aspartic acid and glutamic acid, which are present in small amounts in the hydroxyproline-rich glycoproteins of runner beans (O'Neill & Selvendran, 1980a).

Hydroxyapatite chromatography

Further fractionation of the components from anion-exchange chromatography was achieved on hydroxyapatite (Fig. 2). Fractions D1 and D2 were eluted as single components with 50 mM-phosphate buffer. However, fractions D3–D5 were resolved into two components, the first being eluted with 50 mM-phosphate buffer and the second being eluted between 0.25 and 0.30 M-phosphate buffer. In all cases u.v.-absorbing and polysaccharide-containing material co-chromatographed, suggesting the presence of proteoglycan complexes.

Analysis of the individual fractions again revealed differences in monosaccharide, amino acid and phenolic content (Table 2). Fractions D1/H1 and D2/H1 had essentially the same composition as the material before hydroxyapatite chromatography. The fractions which resolved into two components, particularly those eluted with 50 mM-phosphate buffer, had a higher carbohydrate content and were richer in xylose. Although the protein content of the fractions was similar, the proportion of acidic amino acids increased from 20.8 mol% in D1/H1 to 30.3 mol% in D5/H2.

The amino acid compositions of the fractions obtained from hydroxyapatite chromatography are comparable with those of the proteoglycans obtained by alkali extraction of French-bean leaves (Brown & Kimmins, 1973; Brown *et al.*, 1975). The hydroxyproline content is low (<4.0 mol%), with relatively high levels of aspartic acid, glutamic acid, alanine, glycine, serine and leucine. Furthermore, the proteoglycan complexes from runner bean and French bean (Brown & Kimmins, 1978) contain a range of polysaccharide or oligosaccharide moieties.

Glycosidic-linkage analysis

Methylation analysis of hemicelluloses A and B and the fractions obtained by sequential anion-exchange and hydroxyapatite chromatography of hemicellulose B revealed the main types of glycosidic linkages present (Table 3). The data are consistent with the occurrence of a range of polysaccharides such as pectic galactan and arabinogalactans 1 and 2, arabinoxylans and xyloglucans in various amounts in the complexes.

Fig. 2. Chromatography on hydroxyapatite of the fractions D1–D5 (a–e respectively) obtained from the DEAE-Sephadex column

○, Total carbohydrate (A_{490}); ■, A_{275} . See the Experimental section for details.

It should be emphasized, however, that the methylation-analysis data must be treated with caution, since in some fractions there is a discrepancy between the proportions of terminal and branch-point residues. Excess terminal residues may be attributed to the occurrence of highly glycosylated proteoglycans, whereas excess branch points may indicate some degree of undermethylation.

Table 2. Composition of fractions obtained after hydroxyapatite chromatography of DEAE-Sephadex fractions

		Composition (% _w , w/w, or mol%) or relative amount (%)								
Component	Fraction	...	D1	D2	D3		D4		D5	
			(a) H1	(b) H1	(c) H1	(d) H2	(e) H1	(f) H2	(g) H1	(h) H2
Neutral sugar (% _w , w/w)			64.2	45.7	30.9	14.9	14.7	6.5	14.6	4.1
Uronic acid (% _w , w/w)			6.9	20.9	20.5	22.6	9.6	4.7	9.4	4.3
Protein (% _w , w/w)			16.5	23.1	32.2	30.7	19.2	20.1	27.2	31.7
Phenolics* (% _w , w/w)			11.3	9.6	11.4	16.5	7.2	7.4	10.5	7.1
Relative amount (%)			100	100	75	25	70	30	60	40
Monosaccharide (mol%)										
6-Deoxyhexose			2.8	0.3	0.5	4.1	3.8	7.7	4.1	8.4
Arabinose			12.8	11.1	17.8	35.3	22.1	33.8	33.7	43.6
Xylose			13.3	69.3	68.0	44.7	51.8	23.9	39.5	18.1
Galactose			45.8	10.2	9.6	13.4	15.8	22.1	18.8	18.8
Glucose			25.3	9.1	4.1	2.5	6.5	12.5	5.9	11.1
Amino acid (mol%)										
Ala			11.6	9.0	9.3	8.9	9.1	10.2	8.5	10.0
Gly			14.0	11.7	11.1	10.2	11.3	7.1	11.5	10.0
Val			5.5	5.0	6.1	4.8	5.9	5.1	5.4	3.7
Thr			3.7	4.1	5.4	4.1	4.8	4.1	5.0	3.3
Ser			14.0	10.2	8.9	9.2	8.6	8.2	8.1	9.3
Leu			7.3	9.6	9.3	9.6	10.2	9.2	10.4	10.6
Ileu			3.0	12.3	4.3	2.1	4.3	3.1	3.8	2.3
Pro			4.3	6.2	6.4	6.1	5.9	4.6	6.2	5.0
Hyp			1.8	3.1	2.6	3.4	3.2	3.6	3.8	1.6
Asp			8.0	8.6	11.8	11.3	10.8	12.2	10.8	12.0
Phe			4.3	3.5	4.2	3.4	4.3	4.1	4.2	4.3
Glu			12.8	10.1	14.1	15.7	14.6	17.9	13.8	18.3
Lys			5.5	3.4	4.5	8.5	4.3	7.1	4.2	7.0
Tyr			1.2	1.4	1.3	1.4	1.1	2.0	2.3	1.0
Arg			2.4	1.3	0.6	1.0	1.1	1.0	1.2	1.0
His			0.6	0.5	0.3	0.3	0.5	0.5	0.8	0.3

* Determined from u.v. absorption at 275 nm.

Polysaccharide complexes of hemicellulose

The carbohydrate of D1/H1 appears to be derived from pectic galactan, xyloglucan and small amounts of xylan. The amount of xyloglucan present in this complex is about 5.0% of the xyloglucan that can be extracted with 4M-KOH (O'Neill & Selvendran, 1983). Fraction D1/H1 is probably a xyloglucan-pectic complex (with associated protein and polyphenolics) and is comparable with the xyloglucan-pectic complex isolated from suspension-cultured sycamore (*Acer pseudo-platanus*) cell walls by Bauer *et al.* (1973), although the presence of protein or polyphenolics was not reported. Evidence for the occurrence of small amounts of xyloglucan-pectic-protein-polyphenol complexes in cabbage (*Brassica oleracea*) cell walls has recently been provided (Stevens & Selvendran, 1984a).

The bulk of the carbohydrate in fraction D2/H1 is an acidic xylan in which about 16% of the backbone is substituted with side chains terminating in

xylose, glucuronic acid and possibly arabinose. The presence of small amounts of (1→4)-linked Galp residues and appreciable amounts of protein and polyphenolic material would be consistent with the occurrence of (acidic) xylan-pectic-protein-polyphenol complexes. Similar complexes have also been found in the cell walls of cabbage (Stevens & Selvendran, 1984a) and carrot (*Daucus carota*) (Stevens & Selvendran, 1984b).

The fractions D3/H1 and D3/H2 show differences in the proportions of glycosidic linkages present. Both contain (acidic) arabinoxylans with about 20% of the (1→4)-linked Xylp residues substituted through position 2 by side chains terminating in xylose, arabinose and possibly glucuronic acid. The overall compositions of these arabinoxylans are similar to those of the glucuronoarabinoxylans isolated from suspension-cultured sycamore (Davill *et al.*, 1980a), tobacco (*Nicotiana tabacum*) (Eda *et al.*, 1983) and immature cabbage leaf (Stevens & Selvendran, 1984a) cell walls. Fraction D3/H2 also contained appreciable quantities of (1→4)-linked

Table 3. *Methylation analysis of the hemicellulosic and hydroxyapatite fractions*

Linkage	Component ...	Composition (relative mol%)							
		Hemicellulose		D1	D2	D3		D4	D5
		(a) A	(b) B	(c) H1	(d) H1	(e) H1	(f) H2	(g) H1	(h) H1 (i) H2
T-6-deoxyhex*		2.5	1.4	1.3	Trace	—	—	—	1.3
2-6-deoxyhex		—	—	—	—	—	—	Trace	4.0
2,4-6-deoxyhex		—	—	—	—	—	—	Trace	1.3
T-Araf		13.5	5.6	2.4	2.0	4.7	8.3	5.0	10.3
T-Arap		—	2.8	—	3.3	3.1	3.8	3.1	1.3
2-Araf		16.0	6.5	4.2	2.1	3.1	11.1	3.1	14.8
3-Araf		9.0	3.7	1.7	1.1	1.2	5.4	Trace	6.3
5-Araf		7.0	3.5	1.5	Trace	1.5	2.4	3.1	3.3
2,5-Araf		—	—	—	—	—	—	Trace	1.2
T-Xylp		—	1.4	3.3	6.8	6.8	4.1	3.1	1.3
4-Xylp		9.7	29.9	6.6	62.4	54.3	39.2	40.9	14.6
2,4-Xylp		—	6.0	—	12.2	12.3	8.9	16.5	5.1
T-Galp		8.7	2.3	Trace	1.1	2.1	1.0	1.8	3.8
2-Galp		—	—	—	—	—	—	Trace	1.2
3-Galp		—	—	—	—	—	—	1.9	2.0
4-Galp		18.5	27.3	52.0	6.5	6.5	13.8	6.2	15.3
6-Galp		—	—	—	—	1.2	—	1.5	2.5
3,6-Galp		—	—	—	—	2.2	—	8.3	12.3
4,6-Galp		—	—	1.5	—	—	—	—	—
4-Glcp		15.1	8.2	15.3	—	1.0	2.0	—	6.0
4,6-Glcp		—	1.4	10.2	2.4	—	—	5.5	4.6

* T-6-deoxyhex is derived from the derivative 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methyl-6-deoxyhexitol (etc.).

Galp residues, indicating the occurrence of arabinoxylan-pectic complexes.

Owing to insufficient material and the low carbohydrate content of fraction D4/H2, only D4/H1 was methylated. However, it would appear from their overall carbohydrate content that both fractions contain similar polysaccharides. The main carbohydrate component of fraction D4/H1 is a branched (acidic) arabinoxylan with about 30% of the backbone substituted through position 2. It also contained arabinogalactans 1 and 2, with the latter type being predominant. The presence of arabinogalactan 2 is inferred from the occurrence of (1→3)-, (1→6)- and (1→3,6)-Galp residues. This is in contrast with the earlier fractions, where arabinogalactan 1 was dominant. The presence of arabinogalactan 2 polysaccharides in these cell walls is of interest, as these polymers have not been isolated from a source known to contain only primary cell walls (Darvill *et al.*, 1980b).

The major carbohydrate component of fraction D5/H1 is also derived from an (acidic) arabinoxylan. However, it also contained a significant amount of arabinogalactan 1, with lesser amounts of arabinogalactan 2 and xyloglucan. Fraction D5/H2, by contrast, contains comparable amounts of arabinogalactan 2, (acidic) arabinoxylan and

xyloglucan, all of which may be derived from polysaccharide-protein-polyphenol complexes.

Occurrence of polyphenolic material

Extraction of depectinated cell walls of runner bean with 1 M-KOH, followed by t.l.c. or g.l.c. of the ethyl acetate-soluble material, failed to reveal detectable quantities of ester-bound phenolics. Examination of a cell-wall preparation from wheat (*Triticum aestivum*) bran by the same procedure demonstrated the presence of about 0.5% (w/w) esterified phenolic acids (Ring & Selvendran, 1980). Therefore the cell walls of runner bean probably contain considerably less than 0.5% (w/w) esterified simple phenolics. This contrasts with the cell walls of spinach (*Spinacia oleracea*), which contain 0.2–0.5% (w/w) esterified ferulic acid (Fry, 1979).

Spectrophotometric examination of hemicellulose B revealed strong absorption around 275 nm, which, when corrected for the presence of aromatic amino acids, corresponds to about 11.0% (w/w) of polyphenolic material. Examination of the fractions obtained by anion-exchange and hydroxyapatite chromatography gave phenolic contents in the range 7.0–16.4% (w/w). These values were confirmed, when sufficient material was available, by

the acetyl bromide method (Morrison, 1972), which does not suffer from interference by protein or carbohydrate.

The above data are consistent with the presence of bound phenolic residues in the hemicellulose B fractions. Owing to the alkaline conditions of extraction, it is unlikely that they are simple phenolics esterified to carbohydrate or protein. Partial acid or alkaline hydrolysis failed to reveal detectable quantities of phenolic material attached by cold-alkali-stable linkages such as ferulic acid attached to *N*-terminal amino acids (Van Sumere *et al.*, 1975). It is therefore possible that oxidatively coupled phenolics comparable with those recently found in spinach cell walls (Fry, 1984) are present.

Degradation of hemicelluloses A and B with alkaline nitrobenzene or cupric oxide failed to produce identifiable components. This may be attributed to the high levels of protein present in these fractions, which cause considerable interference with such procedures.

The results of the present investigation have shown that the 1M-KOH soluble hemicellulosic polymers from parenchymatous tissue of *P. coccineus* consist of a range of proteoglycan-polyphenol complexes. Since the cell-wall preparations used were virtually free of cytoplasmic contamination (Selvendran, 1975b), it is unlikely that the complexes are artefacts, but are native to the wall.

Although the function(s) of these complexes are unknown, the associated proteins are distinct from the hydroxyproline-rich wall glycoproteins, which have a structural role (Lamport & Catt, 1981). It is probable that some of the proteins in the complexes are cell-wall enzymes that have been immobilized, by phenolic and/or glycosidic cross-linkages, at specific sites in the cell wall. Other proteoglycan-polyphenolics may act as linking components within the wall matrix.

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