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CELL-WALL POLYMERS OF THE LETTUCE EMBRYONIC AXIS

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Abstract—Cell-walls were isolated from the embryonic axes of lettuce fruits and subjected to sequential solvent extraction. Analysis of the pectic and hemicellulosic wall fractions by partial methylation analysis indicated that the major components are arabinogalactans, rhamnogalacturonans, and xyloglucans found typically in dicotyledonous plants.

INTRODUCTION

Phytochrome mediates a water potential decrease in the embryonic axes (radicle and hypocotyl) of lettuce (*Lactuca sativa* L.) seeds enabling these axes to overcome the osmotic restraint of an external medium or the mechanical restraint of seed layers enveloping the embryos [1, 2]. Phytochrome promotes this decreased water potential through changes in both the osmotic and pressure potentials and directly mediates changes in wall extensibility [3, 4]. Preliminary to a study of the phytochrome-directed changes in cell-wall structure, the fundamental composition of the cell-wall polymers of elongating embryonic axes was examined.

RESULTS AND DISCUSSION

Only *ca* 80 mg of cell walls were obtained for analysis as a result of the small size of lettuce embryonic axes; the walls were composed primarily of pectic substances (25%), hemicellulose (26%) and cellulose (31%) (Table 1). These values were close to those of cell walls from hypocotyls extracted similarly [5]. DMSO alone extracted only about 10% of total wall material, but it was used to facilitate extraction of polymers by other agents [6]. About 80% of the neutral sugar in the DMSO extract was glucose (Table 2). Two per cent EDTA extracted nearly 25% of the wall polymers; substantial amounts of neutral sugar were found in this fraction (Table 1), comprised of 40% arabinose, 25% galactose, and 15% rhamnose (Table 2). Glucose accounted for only 10% of the neutral sugar in this pectic fraction. Even though both TFA and H_2SO_4 hydrolysis yielded low amounts of uronic acid, the pectic material [EDTA + $(NH_4)_2$ oxalate-extractable] contained over 70% of the uronic acid recovered (Table 1). Recoveries of uronic acid-rich polymers coupled with the presence of large amounts of rhamnose (Tables 1 and 2) indicate the likelihood of rhamnogalacturonan polymers common to dicots [7]. Partial methylation analysis of this fraction showed the presence of 2,4-linked rhamnose and a trace of 2-linked rhamnose (Table 3) consistent with the composition of other dicot rhamnogalacturonans [8, 9]. The low yield of the rhamnogalacturonan derivatives is expected based on resistance of the GalA-(1 → 2)-linked rhamnose disaccharide to acid hydrolysis [10].

The C-4 of 2,4-linked rhamnose is likely to be the point of attachment of neutral side-chains of araban, galactan or arabinogalactan [10]. Consistent with this observation, large amounts of arabinose and galactose were found in the pectin of lettuce axes (Table 2) and the presence of *t*- and 5-linked arabinose and 3-, 6- and 3,6-linked galactose by partial methylation analysis (Table 3); these linkages are typical of arabinogalactans [11].

Although 2% $NaBH_4$ was found to extract polyuronates in one investigation [12], little uronic acid was obtained with this solution from the walls of lettuce axes (Table 1). Instead, substantial amounts of protein were extracted and over 60% of the neutral sugar fraction was composed of nearly equal amounts of arabinose and galactose (Tables 1 and 2). The 0.1 N KOH extract also contained substantial amounts of protein and the neutral sugar was enriched in arabinose and galactose.

Concentrated KOH (4 N) extracted about 26% of the wall material (Table 1); glucose and galactose were the most abundant neutral sugars. Even though xylose constituted only 11% of the total neutral sugar recovered from the wall, over 60% of the xylose was in this fraction (Table 2). Partial methylation analysis of the fraction showed large amounts of 4-hexose, 4,6-glucose, and *t*-xylose linkages indicating that xyloglucans are the major hemicellulosic polymers (Table 3). The *t*- and 5-arabinose links as well as 3-, 6-, and 3,6-linked galactose showed that arabinogalactan is present in this fraction as well.

These crude fractionations of the cell-wall polymers of the lettuce axis undoubtedly contained mixtures of distinct polymers, yet from analysis of the distribution of the neutral sugars and the partial methylation analyses, the results indicate that the major non-cellulosic polymers of the growing axis are

Table 1. Composition of fractions of cell walls of lettuce axes

Fraction	% recovered*	Constituent (mg/mg dry wt)				
		Neutral sugar	Uronic acid	Amino acid	Protein†	Total recovered
DMSO	9.1	0.310	0.010	0.105	0.06	0.425
2% EDTA	24.9	0.175	0.083	0.052	0.03	0.310
0.25% NH_4 oxalate	2.9	0.151	0.028	0.086	0.05	0.265
2% $NaBH_4$	4.8	0.148	0.008	0.235	0.17	0.391
0.1 N KOH	1.8	0.221	0.022	0.560	0.60	0.803
4 N KOH	25.5	0.360	0.012	0.164	0.16	0.536
Cellulose	30.8	ND	ND	ND		

*68.0 mg of purified wall material were extracted sequentially, and 62% of the starting material was recovered.

†Protein was determined only for comparison with the amino acid analysis, and these values are not included in determining total components recovered.

ND—not, determined.

Table 2. Neutral sugar composition of the polysaccharide fractions

Fraction	Sugar (μ mol/mg cell-wall)*						
	Fuc	Rha	Ara	Xyl	Man	Gal	Glc
DMSO	—	0.029	0.351	0.213	0.088	0.244	3.251
		(0.7)	(8.4)	(5.1)	(2.1)	(5.8)	(77.9)
2% EDTA	—	0.436	1.149	0.250	—	0.731	0.296
		(15.2)	(40.1)	(8.7)		(25.5)	(10.4)
2% $NaBH_4$	—	0.099	0.548	0.096	0.025	0.475	0.373
		(6.1)	(33.9)	(5.9)	(1.6)	(29.4)	(23.1)
0.1 N KOH	—	0.016	0.106	0.016	0.034	0.094	0.091
		(4.4)	(30.1)	(4.4)	(8.8)	(26.5)	(25.7)
4 N KOH	0.111	0.227	0.732	0.906	0.084	1.206	1.221
	(2.5)	(5.1)	(16.3)	(20.2)	(1.9)	(26.9)	(27.2)
Total from	0.111	0.807	2.886	1.481	0.231	2.75	5.232
fractions	(0.8)	(6.0)	(21.4)	(11.0)	(1.7)	(20.4)	(38.8)
Total cell wall	—	1.40	3.10	1.52	0.42	2.66	3.27
		(11.9)	(26.3)	(12.9)	(3.6)	(22.6)	(26.9)

*The values in parentheses are the mol % of sugar recovered for each fraction. Values for total cell wall represent sugars recovered upon TFA hydrolysis of purified but unfractionated lettuce cell walls.

rhamnogalacturonans, arabinogalactans, and xyloglucans. Thus the walls of the lettuce axis are representative of those of other dicots [10].

EXPERIMENTAL

Plant material. Waldemann's lettuce seeds (cv. Grand Rapids) were purchased from Pieter-Wheeler Seed Co., Gilroy, CA and stored desiccated at −20° to preserve photosensitivity of germination. Seeds were sown in 9-cm Petri dishes lined with filter paper saturated with deionized H_2O. Dishes of seeds were incubated in darkness at 23° for 7 hr and then given a 5-min treatment with red light sufficient to potentiate >90% germination of intact seeds. Embryos were isolated from the enveloping fruit coats under dim green safelight as described previously [3]; *ca* 100 embryos per treatment were transferred to 10 ml deionized water in 50-ml Erlermeyer flasks and incubated in darkness 30–32 hr at 20° in a reciprocating shaking water bath. After incubation, the embryos were rinsed with deionized H_2O, and the embryonic axes (each about 1-cm long) were excised from the cotyledons with a scalpel, frozen in liquid N_2, and stored at −20° until enough had been collected for isolation of cell-wall material.

Isolation of cell walls. The frozen embryonic axes of *ca* 10 000 embryos were homogenized in 3.0 ml of 0.05 M Tes, pH 7.0, and centrifuged at 1200 *g* for 15 min. The pellet was washed sequentially with 5 ml of 0.5 M KPi buffer, pH 7.0, (×1), deionized H_2O (×4), $CHCl_3$–MeOH (2:1) at 45° for 30 min (×2), and Me_2CO (×1). After air-drying, the grey-white pellet was stored over P_2O_5 *in vacuo*.

Fractionation of cell-wall polymers. About 80 mg of wall material was recovered; 68 mg was extracted sequentially with 10 ml of anhydrous DMSO (Pierce), 24 hr, under N_2 and with constant stirring (×1); 2% Na_2EDTA, 1 hr, 100° (×2); 0.25% NH_4-oxalate, pH 4.5, 1 hr, 100° (×2); 1% $NaBH_4$, 24 hr, under N_2 and with constant stirring, 23°; 0.1 N KOH + 3 mg/ml $NaBH_4$, 24 hr, under N_2 and with constant stirring, 23°; 4 N KOH + 3 mg/ml $NaBH_4$, 24 hr, under N_2 and with constant stirring, 23° (×2). Unextracted polymers were recovered after each extraction by centrifugation at 20°

Table 3. Linkage composition of the pectic and hemicellulosic fractions

Sugar	Linkage	Pectic substances (EDTA-extractable) (mol% of recovered derivatives)	Hemicellulose (KOH-extractable)
Rha	2-	tr	1.9
	2, 4-	5.0	1.9
	total	5.0	3.8
Ara	*t*-	8.1	2.3
	2-	3.7	0.5
	5-	9.9	10.0
	total	21.7	12.8
Xyl	*t*-	16.3	7.8
	3,4-	n.d.	11.2
	total	16.3	19.1
Gal	3-	31.5	0.5
	6-	12.8	1.2
	3,6	12.7	3.7
	total	57.0	5.5
Glc (hexose)	3-	tr	1.0
	4-	tr	34.3
	4, 6-	tr	23.5
	total	—	58.8

n.d.—not detected; tr—trace.

for 15 min. Insoluble material remaining after the last extraction was suspended in H_2O, neutralized with HOAc, washed 3× in 10 ml of H_2O, and lyophilized. Supernatant liquids of the alkaline extractions were neutralized with HOAc, and all supernatants were dialysed for 18 hr against deionized H_2O. Dialysates of duplicate extracts were combined, and all were lyophilized. Dried material was stored over P_2O_5 *in vacuo*.

Characterization of the polymers. Recoveries of wall material in the fractions were assayed gravimetrically, and 0.5–3.0 mg samples hydrolysed with 2 ml 2 N TFA, 2 hr, 121°. Supernatants were cleared by centrifugation and dried under N_2. Hydrolysate residue was dissolved in 1.0 ml H_2O, and portions analysed for neutral sugar by the PhOH–H_2SO_4 method [13], uronic acids by the *m*-dihydroxyphenol method of ref. [14], and α-NH_2 by the method of ref. [16]. For comparison, uronic acids also were measured directly in the dry material by the carbazole method [15]. Both the *m*-hydroxyphenol and carbazole methods of uronic acid detection resulted in an uncharacterized interference reaction with the neutral sugars. This interference was subtracted by scanning the reaction mixture spectroscopically and integrating the area of absorption due to uronic acid. Neutral sugars in the remainder of the sample were derivatized to their respective alditol acetates [17] and separated by GC on 3% SP-2340 in a 2 m × 0.2 mm glass column temp. programmed 190–230° at 5°/min with injection and FID at 250°. N_2 carrier gas flow was 40 cm^3/min.

Additional 0.3–2.0 mg samples were suspended in 0.1 N NaOH, sonicated into solution, and analysed for protein by the Coomassie Blue assay (Bio-Rad) relative to bovine serum albumin standards.

Three to 5 mg of EDTA- or 4 N KOH-extracted polysaccharides were sonicated in 1.0 ml of anhydrous DMSO and methylated essentially according to Hakomori as described in ref. [18], except that the K-dimethyl sulfinyl anion was used. Partially methylated polysaccharides were dialysed overnight against H_2O; hydrolysis in 2 N TFA and preparation of alditol acetates was as described except that $NaBD_4$ was used as reducing agent for samples analysed by GC/MS.

Partially methylated alditol acetates were separated by GC on 3% OV-225 in a 2 m × 0.2 mm glass column temp. programmed 170–230° at 1°/min. Injector and FID were at 250°. Nitrogen carrier gas flow as 20 cm^3/min. EIMS was performed with a Finnigan 4000 GC/MS with a 6100 data system at 65 eV and a He flow at 20 cm^3/min. The frequency distributions of mass fragments were compared with values published by Jansson *et al.* [19].

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