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Soluble Cell Wall Polysaccharides Released from Pea Stems by Centrifugation[1]

I. EFFECT OF AUXIN

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ABSTRACT

The metabolism of polysaccharides by pea stem segments treated with and without auxin was investigated using a centrifugation technique for removing solution from the free space of the cell wall. Glucose is the predominant sugar in both the ethanol-soluble and ethanol-insoluble fractions of the cell wall solution extracted with water. In the water-soluble, ethanol-insoluble polysaccharides, arabinose, xylose, galactose, and glucose make up 9.5, 23.8, 23.9, and 39.9%, respectively, of the neutral sugars, while rhamnose, fucose, and mannose are present at concentrations between 0.5 and 2.0%.

Auxin treatment enhances the levels of xylose and glucose in ethanol-insoluble polysaccharides relative to controls, and this difference can be detected within 30 minutes of auxin treatment. Cellulose-binding experiments show that the enhanced levels of xylose and glucose are in a polymer having the cellulose-binding properties of xyloglucan. ^{3}H-glucose labeling experiments confirm the auxin-enhanced metabolism of the xyloglucan fraction; however, increased labeling of arabinose is also observed in auxin-treated sections. Auxin treatment also causes a marked increase in the level of uronic acids centrifuged from pea internode sections. Thus, after 3 hours of incubation in indoleacetic acid, the level of uronic acids in the ethanol-insoluble polysaccharides which can be recovered by centrifugation is increased 2- to 3-fold over sections incubated in water. These auxin-enhanced changes in xylose, glucose, and uronic acids are correlated with enhanced rates of section growth.

Incubation of excised pea internode sections in acidic buffers also enhances the rate of xyloglucan and polyuronide metabolism. This acid-enhanced metabolism of xyloglucan and polyuronide is inhibited by low temperature, suggesting that it is enzyme-mediated.

Extraction of the cell wall solution with $CaCl_2$ increases the yield of all neutral sugars. Arabinose and mannose are increased 4- and 3-fold, respectively, and xylose and glucose by about 20%, while galactose levels are 40% higher in cell wall solution extracted with $CaCl_2$ than in that extracted with water. Although calcium increases the amount of neutral sugars extracted, it does not affect the auxin-induced changes in neutral sugars. Extraction of the cell wall solution with ethyleneglycol-bis-(β-aminoethyl ether)-N,N'tetraacetic acid enhances the yield of uronic acids and also increases the difference due to auxin treatment.

The cell wall has become the focus of research into the mechanism whereby plant hormones stimulate cell expansion. Heyn (7) was the first to show that auxin caused an increase in the extensibility of the cell wall, and there have been many demonstrations of this phenomenon in a wide variety of plant tissues (3, 4, 17). It is now generally accepted that growth of both auxin- and gibberellin-treated tissue is affected by changes in the cell wall which lead to enhanced extensibility.

Investigations of changes in the cell wall which accompany hormone-induced growth have been stimulated by significant advances in our understanding of the chemistry of the cell wall. Albersheim *et al.* (1, 9, 18) have described the component polysaccharides of the cell wall of dicots and proposed a structural model of the wall based on their interpretation of the interaction of cellulose, hemicellulose, and pectic polysaccharides. In this model, considerable importance was attached to the role which the hemicellulosic polymer xyloglucan could play in cell wall extensibility. Keegstra *et al.* (9) proposed that cell wall loosening could be achieved by cleavage of hydrogen bonds between cellulose and xyloglucan. This hypothesis was especially attractive in the light of the acid growth hypothesis of auxin action (16). Thus, the extensibility of the cell wall would be governed by protons pumped from the protoplast into the cell wall in response to auxin treatment. Valent and Albersheim (20) have shown that although hydrogen bonding between xyloglucan and cellulose occurs in the cell wall, the breaking of these bonds by H^+ released from the cell is not the mechanism whereby cell wall loosening occurs.

The importance of xyloglucan metabolism in auxin-induced extensibility and growth has been demonstrated by Labavitch and Ray (10, 11) and Gilkes and Hall (6). Labavitch and Ray (11) found that in auxin-treated pea internode sections, elongation is associated with enhanced metabolism of H_2O-soluble, ethanol-insoluble xyloglucan. Because xyloglucan is H_2O-soluble, it might be inferred that hydrogen bonds to cellulose are broken, but if there is more xyloglucan than there are cellulose binding sites in the wall, then some of the xyloglucan may not be bound to cellulose. Bauer *et al.* (1) have stated that there is roughly enough xyloglucan to encapsulate the cellulose in the wall. When and if this xyloglucan is linked to pectic polysaccharides, the covalent bond must also be cleaved (13). A similar release of H_2O-soluble xyloglucan was observed when pea stem sections were incubated in acidic buffers at pH 4.0 (8). From these data, Jacobs and Ray (8) postulated that the effect of H^+ was to activate an enzyme with an acidic pH optimum.

Using a technique to isolate H_2O-soluble components from the free space of pea internode sections by centrifugation, Terry and Bonner (19) have also detected the release of a H_2O-soluble, ethanol-insoluble xyloglucan in response to auxin treatment. This technique allows for the removal of solubilized materials, including polysaccharides, from the cell wall without the introduction of

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cytoplasmic contaminants (19). In this paper we report a detailed investigation of the sugars and uronic acids removed from the cell wall of auxin- and nonauxin-treated pea internode sections by this centrifugation technique. Our results confirm in detail the observations of Terry and Bonner (19), and extend their findings to other components of the cell wall.

MATERIALS AND METHODS

Seven-day-old, dark-grown pea (*Pisum sativum* L. cv Alaska) seedlings were harvested and sections (1.2 cm) excised 1 cm below the hook as described previously (19). One hundred freshly harvested sections were packed into a plastic syringe barrel and washed for 15 min in ice-cold H_2O or incubated at room temperature for 1 h with the lower 3 mm of the sections immersed in H_2O. After the 1-h incubation, the barrels of sections were connected to a pump that first circulated 175 ml of H_2O past the sections for 30 min then 175 ml of the appropriate medium for various times at 180 ml min^{-1} (19).

Following the appropriate incubation, sections were infiltrated with ice-cold H_2O for 3 min at atmospheric pressure followed by 3 min under vacuum and then centrifuged at 1,000*g* for 5 min. This sequence was repeated twice. After three consecutive infiltrations and centrifugations, some sections were further infiltrated with 5 mM Hepes (pH 3.5), 50 mM EGTA[4] (pH 8), or 50 mM $CaCl_2$ for 3 min with vacuum and 15 min without vacuum and centrifuged. This was repeated once. The solution centrifuged from 1 barrel of 100 sections weighing approximately 3 g was filtered through a glass fiber filter (Whatman, GF/A), boiled for 5 min, dried at 47 C, dissolved in 0.5 ml of H_2O, made to 80% (v/v) ethanol by addition of 95% ethanol, and the precipitate collected by centrifugation. Neutral sugars present in the precipitate and supernatant were determined by gas chromatography after hydrolysis and alditol acetate derivatization as previously described (19). Polysaccharide precipitates were also analyzed for uronic acids using the method of Blumenkrantz and Asboe-Hansen (2).

The technique of McNeil *et al.* (12) was modified to study the cellulose binding capacity of polysaccharides. Approximately 0.5 mg of ethanol-insoluble polysaccharide was dissolved in 1.5 ml H_2O containing 30 mg of Whatman CC-41 cellulose, previously washed overnight with 4.4 M NaOH containing 26 mM $NaBH_4$ and rinsed to neutrality with H_2O, and stirred overnight at 3 C. The cellulose with adhering polysaccharide was removed from solution by centrifugation for 15 min at 1,500*g* at 25 C. The pellet was resuspended in 3.5 ml of 4.4 M NaOH containing 26 mM $NaBH_4$ and stirred overnight at 3 C. The cellulose was recovered from solution by centrifugation, the supernatant retained, and the pellet resuspended in 2 ml 4.4 M NaOH and centrifuged immediately. The resulting pellet was washed a second time with 2 ml NaOH and centrifuged. The supernatants were combined and filtered through a glass fiber filter, adjusted to pH 5 with glacial acetic acid, dialyzed against H_2O overnight at 3 C, and lyophilized. The supernatant from the cellulose-binding assay was filtered through a glass fiber filter, lyophilized, and the neutral sugars determined after hydrolysis and derivatization.

Labeling of cell wall polysaccharides with [^{3}H]glucose followed a procedure similar to that of Labavitch and Ray (10). Thirty-five 1-cm sections were incubated in 5.5-cm Petri dishes with shaking at 120 rpm in H_2O for 1 h at 25 C and then transferred to 4 ml of H_2O containing 30 μCi [^{3}H]glucose (18 μCi/mmol, Lot #927-253, New England Nuclear) an additional hour. Sections were removed from the label, washed with H_2O, and incubated for 2 h in 50 mM glucose followed by 2 h in H_2O, all at 25 C. Sections were then treated with 17 μM IAA or H_2O for 3 h, packed into plastic syringe barrels and centrifuged to remove the cell wall solution. The ethanol-insoluble polysaccharides from the cell wall solution were hydrolyzed and prepared for GC as described previously (19). To determine radioactivity in individual sugars, the alditol acetates were separated on a 1.83 m × 0.64 cm column of 3% SP2340 on 100 to 120 Supelcoport (Supelco Inc., Bellefonte, PA) with a 9:1 effluent splitter in a Hewlett Packard 5710A gas chromatograph. Separated alditol acetates were collected by condensation of the effluent vapor in scintillation vials cooled with Dry Ice. Radioactivity was determined after addition of a Triton X-114 based scintillation fluor in a Beckman LS-150 scintillation spectrometer.

Unless otherwise stated, the results reported are all an average of 3 to 5 experiments of 200 to 400 pea stems per treatment.

RESULTS

The cell wall solution centrifuged from freshly harvested pea internode sections washed in ice water for 15 min was fractionated into ethanol-insoluble and ethanol-soluble polysaccharides (Table I). Glucose is the predominant neutral sugar in both fractions; in the ethanol-soluble fraction, glucose makes up 80% of the total neutral sugars, and in the ethanol-insoluble fraction, it comprises 40% of the neutral sugars. Xylose, galactose, and arabinose are prominent in the insoluble fraction but each makes up only 3 to 4% of the soluble fraction (Table I). Mannose, on the other hand, comprises 8% of the neutral sugars of the soluble fraction but only 2% of the insoluble fraction (Table I). Fucose and rhamnose are present in only trace amounts in the ethanol-insoluble fraction and each makes up less than 1.5% of the soluble fraction (Table I).

Incubation of sections in rapidly flowing medium with or without IAA for 3 h causes a change in the neutral sugar composition of the ethanol-insoluble polysaccharides centrifuged from the sections (Table II). In both auxin- and H_2O-treated sections, incubation in rapidly flowing buffer causes a reduction in the level of neutral sugars in the solution centrifuged from sections (compare Tables I, II, and IV). The effect of IAA is to increase the level of all neutral sugars except galactose relative to the untreated tissue. Thus, xylose and glucose levels are 41 and 37% higher in IAA-treated sections while rhamnose, fucose, and arabinose levels are 15, 65, and 17% higher, respectively (Table II). In all experiments, there was a stoichiometric increase in the level of xylose and glucose (Tables II–VII). Significantly, when xyloglucan levels were determined by cellulose binding, greater than 99% of the xylose and 88% of the glucose present in the H_2O-soluble, ethanol-insoluble fraction centrifuged from internode sections were removed from solution by binding to cellulose (Fig. 1 and Table II). A fraction of the bound xyloglucan is held tenaciously by cellulose since only about 68% of the xylose and 40% of the glucose were recovered (Table II). Galactose and arabinose are also bound to cellulose but to a lesser extent (about 10 and 5%, respectively) than xylose and glucose (Table II). Small amounts (less than 10%) of rhamnose and fucose are also bound to cellulose in the presence of IAA (Table II).

Changes in the neutral sugar and polyuronide composition of ethanol-insoluble polysaccharides centrifuged from pea internode sections can be detected within 30 min of IAA treatment (Table III). By 1 h of IAA treatment, xylose and glucose levels in polymers have reached 18 and 17% above controls and by 3 h are more than 50% above non-IAA-treated tissue (Table III). The stimulation of uronic acid metabolism by IAA is greater than that observed for neutral sugars. Thus, after 3 h incubation, IAA-enhanced stimulation of uronide levels is 160% (Table III). Auxin treatment does not enhance the levels of galactose while the levels of mannose are too low to make changes in its level meaningful (Table III).

The IAA-induced changes in xylose and glucose in the H_2O-soluble, alcohol-insoluble polysaccharides centrifuged from the

[4] Abbreviation: EGTA, Ethyleneglycol-bis-(β-aminoethyl ether)-*N,N'* tetraacetic acid.

Table I. *Neutral Sugar Composition of the Total Cell Wall Solution and the Ethanol-Insoluble and -Soluble Fractions of the Cell Wall Solution Centrifuged from Pea Internode Sections*

The freshly harvested sections were washed in ice water for 15 min before centrifugation.

Fraction		Neutral Sugar						
	Units	Rha	Fuc	Ara	Xyl	Man	Gal	Glc
Total	μg[a]	3.1	2.8	36.2	85.0	17.7	84.0	244.7
	%[b]	0.65	0.6	7.6	18.0	3.7	17.7	51.7
Ethanol-insoluble	μg	1.2	1.5	31.6	79.0	6.5	79.3	132.4
	%	0.4	0.5	9.5	23.8	2.0	23.9	39.9
Ethanol-soluble	μg	1.9	1.3	4.6	6.0	11.2	3.7	112.3
	%	1.3	0.9	3.3	4.3	7.9	2.6	79.6

[a] μg per 100 sections.
[b] % total neutral sugars.

Table II. *Effect of IAA on the Cellulose Binding of Ethanol-Insoluble Sugars of the Cell Wall Solution Centrifuged from Pea Internode Sections*

Treatment[a]	Sugars					
	Rha	Fuc	Ara	Xyl	Gal	Glc
	μg/100 sections					
Total ethanol-insoluble sugars						
+ IAA	1.5	5.6	34	58	71	115
− IAA	1.3	3.4	29	41	68	84
+ IAA/− IAA	1.15	1.65	1.17	1.41	1.04	1.37
Cellulose binding assay						
Not bound to cellulose						
+ IAA	ND[b]	ND[b]	19	Tr[c]	42	7
− IAA	ND[b]	ND[b]	16	Tr[c]	37	6
+ IAA/− IAA			1.19		1.14	1.17
Bound to cellulose						
+ IAA	0.1	0.7	1.9	25	6.5	78
− IAA	Tr[c]	Tr[c]	1.6	17	7.2	57
+ IAA/− IAA			1.19	1.47	0.90	1.37

[a] Sections incubated 3 h ± 17 μM IAA prior to centrifugation.
[b] Not determined.
[c] Tr, trace; less than 0.1 μg/100 sections.

cell wall correlate well with IAA-stimulated growth of the internode section (Fig. 2). Growth monitored by changes in fresh weight can be detected within 30 min of IAA treatment as can changes in the levels of both xylose and glucose.

The labeling of sugars in ethanol-insoluble polysaccharides which can be centrifuged from the cell wall was followed using procedures similar to those described by Labavitch and Ray (10). Labeled glucose was incorporated by sections and the turnover of this label was monitored in the presence or absence of IAA (Table IV). Auxin enhances the level of xyloglucan which can be centrifuged from the wall, but arabinose and galactose levels decrease slightly (Table IV). When radioactivity in neutral sugars is measured, however, arabinose, xylose, and glucose each show enhanced levels of labeling in the presence of auxin. The specific activities (cpm/μg sugar) of xylose, galactose, and glucose do not change appreciably with auxin treatment while that of arabinose increases by 46% with IAA (Table IV).

Incubation of sections in buffered medium at pH 4.5 causes a 27 to 72% increase in neutral sugars and uronic acid centrifuged from sections relative to incubation at pH 6.6 (Table V). If sections are incubated in IAA at pH 4.5, there is an enhancement of the levels of all neutral sugars and uronic acids by about 18 to 25% (Table V).

Since extraction of cell wall polymers has been shown to be enhanced by the presence of salts, especially $CaCl_2$ (5), internode sections were infiltrated with $CaCl_2$ and KH_2PO_4 following incubation in either H_2O or IAA. When $CaCl_2$ (50 mM) or phosphate buffer (50 mM KH_2PO_4, pH 6.0) is used to infiltrate internode sections prior to centrifugation, the level of neutral sugars extracted increases relative to infiltration with H_2O (Table VI). With $CaCl_2$, there is a 4-fold increase in the level of extractable arabinose and a 3-fold increase in mannose. Xylose and glucose levels are increased only 20% while the level of galactose is raised by 40% over H_2O-infiltrated tissue. With infiltration in phosphate buffer there is also an increase in the extraction of neutral sugars, but the changes in arabinose and mannose are considerably smaller than those seen with $CaCl_2$ (Table VI). Although $CaCl_2$ causes a marked increase in the extraction of extracellular sugars, it does not affect IAA-induced changes in neutral sugars (Table VII). Although the level of arabinose is increased by 4-fold in $CaCl_2$-extracted sections, IAA does not influence this amount (Table VII). Xylose, glucose, and uronic acid levels, on the other hand, increase by greater than 50% with calcium after treatment with IAA.

Because calcium has been implicated in the cross-linking of pectic polysaccharide chains in the cell wall (13, 15), the effect of the calcium chelator EGTA on the extractibility of the polysaccharides was examined. When internode sections previously infiltrated with H_2O and centrifuged are infiltrated with 50 mM EGTA to remove calcium from the wall and especially from between polyuronides, and recentrifuged, the level of neutral sugars is only about 30% of that recovered by the initial H_2O infiltration, and

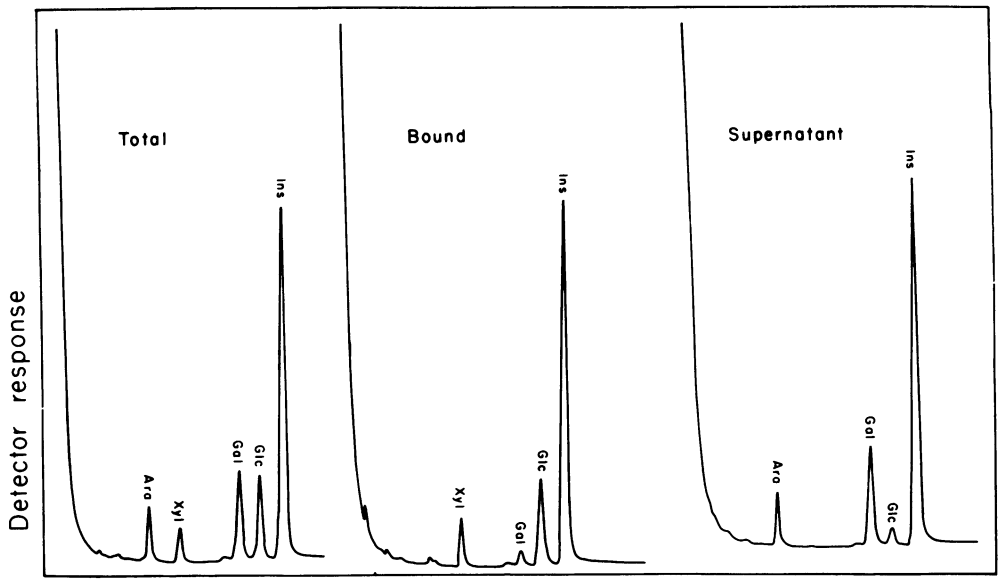

FIG. 1. Gas chromatograms of alditol acetate derivatives of the neutral sugars centrifuged from 1.2-cm pea internode sections incubated in IAA for 3 h (Total). The cellulose-binding assay was performed on the ethanol-insoluble, water-soluble polysaccharides. (Bound), bound to cellulose; (Supernatant), not bound to cellulose.

Table III. *Effect of Incubation Time in IAA on Neutral Sugar and Uronic Acid Composition of Ethanol-Insoluble Fraction of the Cell Wall Solution Centrifuged from Pea Internode Sections*

Time	IAA[a]	Δ Fresh Wt[b]	Sugar[c]					
			Ara	Xyl	Man	Gal	Glc	UA
h								
0.5	+	0.8	27.0	52.0	9.4	52.8	143	78.8
	−	0.63	27.0	47.0	9.7	52.3	133	48.5
	+/−	1.27	1.00	1.11	0.97	1.01	1.08	1.62
1	+	0.782	21.0	49.4	5.2	52.6	105.3	ND[d]
	−	0.378	21.2	41.8	6.1	52.2	90.1	ND
	+/−	2.07	1.00	1.18	0.85	1.01	1.17	ND
2	+	1.455	21.4	47.7	7.6	55.1	105.3	ND
	−	0.650	20.3	33.8	5.6	60.5	71.3	ND
	+/−	2.24	1.05	1.41	1.36	0.91	1.48	ND
3	+	1.93	22.5	45.8	4.8	59.8	95.4	54.2
	−	0.8	23.5	29.9	4.5	57.7	63.0	20.8
	+/−	2.41	0.96	1.53	1.07	1.04	1.51	2.61

[a] Sections incubated ± 17 μM IAA.
[b] Fresh weight (g) of 100 sections.
[c] Neutral sugars and uronic acids, μg/100 sections.
[d] Not determined.

the effect of IAA on the levels of these sugars is unchanged (Table VIII). Extraction with EGTA, however, results in elevated levels of uronic acids and an enhancement of the effect of IAA (Table VIII). If sections previously infiltrated in H_2O and centrifuged are infiltrated in an acidic buffer (Hepes, 50 mM, pH 3.5, 0 C), there is no further release of uronic acids while the level of neutral sugars is from 10 to 20% of that centrifuged from H_2O-infiltrated sections (Table VIII).

DISCUSSION

Our results extend the findings of Terry and Bonner (19) on the carbohydrates centrifuged from pea internode sections. The data show that H_2O-soluble, ethanol-insoluble sugars make up 70% of the carbohydrates centrifuged from the cell wall and that three neutral sugars, namely xylose, galactose, and glucose, make up nearly 90% of the neutral sugar composition of these polymers. All of the xylose and greater than 90% of the glucose of the H_2O-soluble, ethanol-insoluble fraction bind to cellulose showing that the predominant polymer in the cell wall solution is a polymer having properties similar to xyloglucan (12, 13). The material which binds to cellulose has a higher ratio of glucose to xylose than normally found in xyloglucan (1), indicating that another glucan may bind to the cellulose or to the xyloglucan. Incubation of sections in H_2O for up to 3 h causes a decline in the level of all neutral sugars; however, the decline in the levels of xylose and glucose can be arrested by incubation in IAA. Auxin does not influence the levels of arabinose and galactose in the H_2O-soluble fraction but always causes a marked increase in the level of uronic

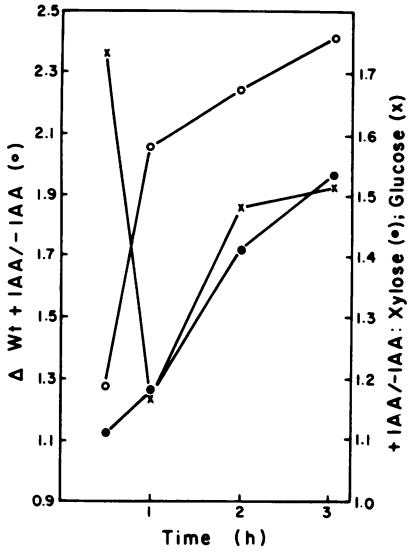

FIG. 2. Correlation between IAA-induced growth, expressed as change in weight, and the xylose and glucose composition of the ethanol-insoluble fraction of the cell wall solution centrifuged from excised pea internode sections.

Table IV. *Effect of IAA on Metabolism of Labeled, Ethanol-Insoluble Sugars of the Cell Wall Solution Centrifuged from Pea Stems*

Thirty-five sections (1 cm) incubated 1 h in 30 μCi[^{3}H]glucose in 4 ml H_2O, 2 h in 50 mM glucose, 2 h in H_2O, then 3 h ± 17 μM IAA.

Treatment		Neutral Sugar			
	Units	Ara	Xyl	Gal	Glc
+ IAA	μg[a]	8.7	10.8	26.4	46.5
	cpm[b]	204	140	425	248
	Specific activity[c]	23.4	13	16	5.3
− IAA	μg[a]	10.5	9.0	26.5	38.5
	cpm[b]	168	108	420	204
	Specific activity[c]	16	12	15.8	5.3
+ IAA/ − IAA	μg[a]	0.83	1.20	1.00	1.21
	cpm[b]	1.21	1.30	1.01	1.22
	Specific activity[c]	1.46	1.08	1.01	1.00

[a] μg g^{-1} fresh wt.
[b] cpm g^{-1} fresh wt.
[c] cpm μg^{-1} neutral sugar.

acids. The auxin-induced increase in xyloglucan and uronic acids is paralleled by an increase in section growth. Changes in xylose and glucose and uronic acid can be detected within 30 min of auxin treatment, an observation which lends credence to the suggestion that the appearance of xyloglucan and polyuronide polymers is related to the rapid growth response.

Xyloglucan is widely recognized as an important component of the hemicellulose fraction of the cell walls of dicotyledonous plants (13), although there remains controversy over the role which this polymer might play in the structure of the cell wall. Albersheim and colleagues (1, 9, 13, 18) have argued that xyloglucan is an important constituent of the hemicellulose component of the cell because of its unique capacity to hydrogen-bond to cellulose. Preston (15) has recently questioned whether xyloglucan can hydrogen-bond to cellulose, and he has suggested that a pure xylan rather than xyloglucan binds to cellulose microfibrils in the

Table V. *Effect of pH and IAA on the Ethanol-Insoluble Sugar Composition of the Cell Wall Solution Centrifuged from Pea Internode Sections*

Sections were incubated for 1 h at pH 4.5 or 6.6 without IAA or at pH 4.5 ± 17 μM IAA.

Treatment	Sugars				
	Ara	Xyl	Gal	Glc	UA
	μg/100 sections				
pH 4.5	25	51	70	151	112
pH 6.6	17	37	55	112	65
pH 4.5/pH 6.6	1.47	1.38	1.27	1.35	1.72
pH 4.5 − IAA	29	48	65	107	84
pH 4.5 + IAA	35	60	78	134	99
+ IAA/− IAA	1.21	1.25	1.20	1.25	1.18

Table VI. *Effect of H_2O, $CaCl_2$ and KH_2PO_4 Extraction on the Ethanol-Insoluble Sugar Composition of the Cell Wall Solution Centrifuged from Pea Internode Sections*

Sections were incubated 3 h in 17 μM IAA prior to extraction 3 times with either H_2O, or 50 mM $CaCl_2$ or 50 mM KH_2PO_4 (pH 6.0).

Extraction	Sugars				
	Ara	Xyl	Man	Gal	Glc
	μg/100 sections				
H_2O	21.5	56.4	6.1	58.5	114.6
$CaCl_2$	82.6	68.8	19.1	84.3	140.9
$CaCl_2/H_2O$	3.84	1.22	3.13	1.44	1.23
KH_2PO_4	30.1	66.2	11.6	67.8	138.1
KH_2PO_4/H_2O	1.40	1.17	1.90	1.16	1.20

Table VII. *Effect of IAA on the Ethanol-Insoluble Sugar Composition of the Cell Wall Solution Centrifuged from Pea Internode Sections after Infiltration with 50 mM $CaCl_2$*

Sections were incubated for 3 h in 0.5 mM KH_2PO_4, 0.1 mM $CaCl_2$, 1% (w/v) sucrose, ± 17 μM IAA then extracted 3 times with 50 mM $CaCl_2$.

Treatment	Sugars					
	Ara	Xyl	Man	Gal	Glc	UA
	μg/100 sections					
+ IAA	106.3	62.4	11.2	65.8	122.3	50.0
− IAA	108.8	38.3	8.6	64.6	79.9	33.2
+ IAA/ − IAA	0.98	1.63	1.30	1.02	1.53	1.51

living wall. Nevertheless, a polymer rich in xylose and glucose which binds strongly to cellulose *in vitro* is the predominant polysaccharide in the solution centrifuged from the cell wall of pea stem segments.

Labavitch and Ray (10, 11) and Jacobs and Ray (8) have shown a positive correlation between xyloglucan and extension growth in pea internode sections. Labavitch and Ray (10) found that a xyloglucan from the H_2O-soluble fraction of the pea cell wall showed enhanced turnover in the presence of IAA, while Jacobs and Ray (8) also demonstrated an enhancement of xyloglucan metabolism in pea stem sections incubated at an acid pH. Our results demonstrate a similar correlation between xyloglucan metabolism and growth (Fig. 2, Table III). Because the centrifugation technique used in this study does not require tissue homogenization or other disruptive treatments, we believe that the data provide strong support for the cleavage of a xyloglucan fragment from the cell wall following auxin and low pH treatments and that the presence of H_2O-soluble xyloglucan in the cell wall solution is

Table VIII. *Effect of EGTA and Acidic Extraction Following H_2O-Extraction on the Ethanol-Insoluble Sugar Composition of the Cell Wall Solution Centrifuged from Pea Internode Sections Treated with or without IAA*

Sections were extracted 3 times with H_2O prior to extraction (2 times) with either EGTA (50 mM) or acid (5 mM Hepes, pH 3.5). Sections were incubated ± 17 μM IAA for 3 h prior to extraction.

Extraction	Treatment	Sugars				
		Ara	Xyl	Gal	Glc	UA
		μg/100 sections				
H_2O	+ IAA	39	57	50	108	58
	− IAA	39	38	44	73	28
	+ IAA/ − IAA	1.00	1.50	1.14	1.48	2.07
EGTA	+ IAA	12	12	16	34	90
	− IAA	11	10	14	28	32
	+ IAA/ − IAA	1.09	1.20	1.14	1.21	2.81
H_2O	+ IAA	35	50	52	117	56
	− IAA	31	39	48	89	18
	+ IAA/ − IAA	1.12	1.28	1.08	1.31	3.11
pH 3.5	+ IAA	3	5	6	19	0
	− IAA	7	7	6	21	0
	+ IAA/ − IAA	0.43	0.71	1.00	0.90	0

due to the activity of a specific cell wall degrading enzyme, especially since incubation at low pH for 30 min at 0 C has no effect on xyloglucan or polyuronide metabolism (Table VIII).

Auxin treatment also markedly enhances the level of uronic acids in H_2O-soluble, ethanol-insoluble polymers centrifuged from the cell wall (Table V). Indeed, the effect of auxin on polyuronides is substantially greater than its effect on xyloglucan-like polymers. Thus, IAA stimulates the release of xyloglucan-like polymers by as much as 50%, but it stimulates polyuronide levels in the cell wall solution by 150 to 200%. In the cell wall some of the pectic polymers are believed to be covalently linked to hemicellulose polymers, and their release requires chemical extraction or enzymic treatment (13). There is also the possibility that polyuronides are weakly hydrogen-bonded to hemicellulose components or that Ca^{2+} or Mg^{2+} cross bridges bond pectins to themselves or other acidic polysaccharides (13, 15). Since auxin causes a marked enhancement of uronic acid levels in ethanol-insoluble polymers, we presume that auxin enhances the level of an enzyme to cleave covalent linkages and release pectic and hemicellulosic polymers. It might be speculated that auxin-enhanced release of pectic polymers from the cell is related to the changes in wall extensibility known to be stimulated by auxin treatment. A role for these polymers in cell wall stiffening has been suggested by several investigators (13, 15), and their involvement in auxin-enhanced wall loosening has also been proposed (14).

We have also shown that infiltration of sections with a Ca^{2+}-rich medium (50 mM Ca^{2+}) results in extraction of uronic acid-rich polymers (Table VII). Extraction of sections with EGTA but not with an acidic medium following water extraction results in the recovery of twice the amount of uronic acids extracted by H_2O alone (Table VIII). EGTA-extraction greatly enhances and Ca^{2+} slightly decreases the yield of uronic acids from auxin-treated tissue (Tables VII and VIII).

The cell wall solution centrifuged from freshly harvested sections or sections incubated in H_2O or IAA for up to 3 h also contains high levels of arabinose and galactose. Thus, these two neutral sugars make up 35 to 40% of the alcohol-insoluble polymers but only a small fraction of either binds to cellulose (Tables I and II). These observations are consistent with the reported neutral sugar composition of H_2O-soluble, alcohol-insoluble polymers in peas reported by Labavitch and Ray (10) and with the composition of xyloglucan for sycamore and bean tissue (13). When sections are extracted with $CaCl_2$, there is a 3-fold increase in the level of arabinose and mannose and only a 20 to 40% increase in xylose, galactose, and glucose. Calcium has been shown to remove hydroxyproline-rich protein from the cell wall (5), and since this cell wall protein has been shown to be linked to arabinose, the dramatic increase in this sugar caused by Ca^{2+}-extraction possibly represents its removal with the protein. When cell walls are extracted with 50 mM KH_2PO_4 under the same conditions as the extraction with 50 mM $CaCl_2$, the amount of arabinose is increased by only 40%. Although Ca^{2+} promotes the release of arabinose from internode sections, the level of this neutral sugar in Ca^{2+}-extracted polysaccharides is not affected by IAA treatment (Table VII).

Although auxin does not cause an increase in the levels of either arabinose or galactose during a 3-h incubation period, labeling experiments suggest that the turnover of arabinose differs from that of xylose, galactose, or glucose. Whereas the specific activities of xylose, galactose, and glucose do not change with IAA treatment, that of arabinose is increased by 46%. This suggests that there is no qualitative change in the metabolism of those polymers rich in xylose, galactose, and glucose, rather that auxin merely accelerates the rate at which the metabolism of the polymer occurs. The higher specific activity for arabinose, on the other hand, indicates that following IAA treatment a polymer whose arabinose content is different is being metabolized.

The effect of auxin on the neutral sugar composition of the H_2O-soluble, alcohol-insoluble polymers of the cell wall solution contrasts with the effect of low pH. Treatment of sections with a medium at pH 4.5 causes an elevation of the level of arabinose and galactose as well as xylose, glucose, and uronic acids. Incubation of sections treated at pH 4.5 with auxin causes a further stimulation of the level of arabinose, xylose, galactose, glucose, and uronic acids in the cell wall solution centrifuged from pea internode sections. Jacobs and Ray (8) have also shown that incubation of pea internode sections at pH 4.0 increases the level of H_2O-soluble xyloglucan. Their analysis of the H_2O-soluble polymers released in response to low pH shows a predominance of xylose and glucose in a neutral polymer and galactose, arabinose, and xylose in an acidic polymer (8).

Our analysis of the neutral sugar and uronic acid composition of the cell wall solution centrifuged from pea internode sections under various conditions of incubation and infiltration allows us to draw the following conclusions: (a) incubation of sections in auxin stimulates the release of a H_2O-soluble, ethanol-insoluble xyloglucan and a polymer rich in uronic acids; (b) arabinose and galactose levels do not change during incubation in IAA; (c) incubation at pH 4.5 enhances xyloglucan and uronic acid and arabinose and galactose levels. Extraction of sections with Ca^{2+} causes a 3-fold increase in arabinose and mannose; however, auxin does not significantly affect the level of these neutral sugars. Extraction of sections with EGTA following H_2O-infiltration causes the release of an amount of uronic acids equal to that obtained during the initial extraction with H_2O, and the levels of the EGTA-extractable pectic polymers are dramatically increased by IAA treatment.

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