Plant Physiol. (1990) 93, 998–1004
0032-0889/90/93/0998/07/$01.00/0

Received for publication September 11, 1989
Accepted March 12, 1990

Sugar Composition and Molecular Weight Distribution of Cell Wall Polysaccharides in Outer and Inner Tissues from Segments of Dark Grown Squash (*Cucurbita maxima* Duch.) Hypocotyls[1]

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ABSTRACT

The elongation growth of stem segments is determined by the outer cell layers (epidermis and collenchyma). We measured the sugar composition and molecular weight distribution of pectin and hemicellulose fractions obtained from inner and outer tissues of squash (*Cucurbita maxima* Duch.) hypocotyls. In addition, we studied the changes in these parameters after a 9 hour period of incubation of the segments. The results show that outer tissues have higher molecular weight pectin and hemicellulose compared to inner tissues (2–3 times higher). Incubation results in a 13 to 25% decrease in the amount of pectin and hemicellulose in inner tissues and an increase of 11 to 32% in the outer tissues. This increase in the outer tissues is accompanied by a decrease in the molecular weight of some of the components. These results clearly show that cell wall metabolism during elongation growth differs markedly in inner and outer tissues, and that future studies on the effect of auxin need to take these differences into account.

The role of the outer cell layers (essentially epidermis) of plant stem tissues in the regulation of auxin-induced growth has been studied using pea (8, 10, 13, 23, 26) and maize (7). These studies demonstrated that auxin changes the mechanical properties of epidermal cell walls but not those of the inner tissues, suggesting that auxin-induced growth is regulated by the epidermal cell walls. These studies also show that there is a difference in the mechanical properties of cell walls of the epidermis and the inner tissues.

Recently, we reported (24) that [^{14}C]glucose was incorporated more into cell walls of outer tissues (multiseriate epidermis) of squash hypocotyl segments than into those of inner tissues. Furthermore, using sections of pea stem, Kutschera and Briggs (9) demonstrated that cell wall synthesis was more active in epidermis than in inner tissues. They also showed (11) that the dry weight of epidermis from pea stem segments did not change during incubation, while that of the cortical cylinder decreased. These results imply the possibility that the cell walls of outer and inner tissues have a different chemical structure and metabolism.

Changes in the chemical compositions and mol wt of cell wall polysaccharides during growth have been studied in many plant systems (6, 12, 14, 16, 17, 19, 22, 25). In most studies, whole stem tissues were used to determine the changes in chemical composition of the cell wall. If stem growth is regulated by the outer (epidermal) tissues, changes in chemical composition of the cell walls in outer tissues would be related to stem growth. Not only the chemical composition of cell wall polysaccharides, but also the physicochemical properties including mol wt are closely associated with the cell elongation process (2, 22). However, neither the change of cell wall composition nor the mol wt of cell wall polysaccharides of outer and inner tissues during growth have, as yet, been determined. In the present study, we characterized the cell wall polysaccharides of outer and inner tissues from squash hypocotyl segments, and studied the changes in sugar composition and mol wt distribution as a first step in investigating the role of cell walls of outer tissues in stem growth.

MATERIALS AND METHODS

Plant Materials

Squash (*Cucurbita maxima* Duch. cv Houkou-Aokawaa-maguri, Takayama Seed Co., Shichikushimomidori-machi, Kitaku, Kyoto, Japan) seedlings were grown for 36 h in the dark as previously described (18, 24). Uniform seedlings which had 3 to 4 cm long hypocotyls were selected, and 10 mm long hypocotyl segments were excised from the region 5 mm below the cotyledonary node using a double-bladed cutter under dim green light (0.02–0.04 μmol/m^2/s). Excised segments were incubated for 9 h at 26 °C in the dark in a 9 cm Petri dish which had 9 mL of 10 mM K-citrate buffer (pH 6.8). After incubation, segments were separated into outer and inner tissues by peeling with forceps. To measure the thickness of the outer tissues that were removed, cross-sections were cut by hand from half-peeled hypocotyl segments. The boundary between the intact and peeled tissues was observed under a microscope (Olympus, Tokyo) with an ocular micrometer.

[1] Supported in part by Grants-in-Aid from the Ministry of Education, Science and Culture of Japan.

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Cell Wall Fractionation

Fractionation of cell walls of outer and inner tissues from the segments was conducted as previously described (24, 25). Separated outer and inner tissues were fixed for 10 min in 15 mL of boiling methanol. Next, tissues were rehydrated for 30 min at room temperature with several changes of water, then homogenized in water with a mortar and pestle. The homogenate was centrifuged for 10 min at 1000*g*, and the residue was washed three times with deionized water. The washed residue was washed three times with acetone, and then with a methanol:chloroform mixture (1:1, v/v). The washed residue was dried overnight at 40 °C and then treated with 2 units/mL porcine pancreatic α-amylase (type I-A, Sigma, St. Louis) in 100 mM sodium-acetate buffer (pH 6.5) for 2 h at 37 °C. After the residue had been washed three times with deionized water, it was treated for 18 h at 37 °C with 200 μg/mL pronase (Actinase, Kaken Kagaku Co., Tokyo) in 50 mM sodium-phosphate buffer (pH 6.5) containing 5% ethanol. The pronase solution was preincubated for 2 h at 37 °C to degrade contaminant glycanases.

Pectic substances were extracted from the cell walls by three treatments with 50 mM EDTA in 50 mM sodium-phosphate buffer (pH 6.8) at 95 °C. Next, hemicellulose was extracted for 18 h with 17.5% NaOH containing 0.02% $NaBH_4$. The hemicellulosic fraction was neutralized with glacial acetic acid in an ice-cold water bath. The pectic and neutralized hemicellulosic fractions were dialyzed in a Visking cellulose tubing (18/32) against deionized water for 18 h at 25 °C ± 2 °C. The dialyzed hemicellulosic fraction was centrifuged for 20 min at 10,000*g*. The precipitate and the supernatant were designated as HA[3] and HB fraction, respectively, and the alkali-insoluble fraction after extraction of hemicellulose as cellulose. Total sugar content of each fraction was determined by the phenol-sulfuric acid method (4) and UA contents by the carbazole method (5). The neutral sugar compositions of pectin and HB fraction were determined by the method of Albersheim *et al.* (1).

[3] Abbreviations: HA, hemicellulose A; HB, hemicellulose B; UA, uronic acid; RGAG, rhamnogalacturonans with arabinogalactan side chains.

Gel Permeation Chromatography of Pectin and HB Fraction

The fractionated pectin and HB fractions were lyophilized. The lyophilized samples were dissolved in 0.5 mL of 50 mM K-phosphate buffer (pH 7.2), and an aliquot (100–200 μL) was introduced into an HPLC system (model-302, Gilson, Middleton, WI) equipped with a refractive index detector (RID-300, Japan Spectroscopic Co., Ltd., Tokyo). The gel permeation column was a TSK-GEL 5000 PW column (Toyo Soda Co., Ltd., Tokyo). The sample was eluted with 50 mM K-phosphate buffer (pH 7.2) at a flow rate of 1 mL/min. Fractions were collected with a fraction collector (model-201, Gilson) at one minute intervals. Dextrans (Sigma, St. Louis, MO) of 10, 40, 70, 120, and 500 kD were used as molecular mass markers. The UA content in each fraction was determined by the carbazole method, and the neutral sugar composition was analyzed by GLC (1).

RESULTS

Anatomical Study of Peeled Outer Tissues

The outer tissues of dark-grown squash hypocotyls were easily removed with forceps, but microscopic observation revealed that the removed outer tissues contained one layer of epidermis and a few layers of collenchyma cells. To confirm that the outer tissues removed at 0 h had the same composition as the outer tissues after 9 h of incubation, the thickness of epidermis (one layer) and of the collenchyma cell layers was measured (Table I). The geometry of the parenchyma cells just under the collenchyma tissue was also determined, since the outer tissues were always detached by splitting the layer of parenchyma cells in two equal halves. The outer tissues removed at 9 h of incubation were slightly thicker than those at 0 h, mainly because of the swelling of collenchyma

Table I. *Anatomical Measurement of Tissue Thickness of Epidermis, Collenchyma, and Parenchyma of Dark-Grown Squash Hypocotyls*

Hypocotyl segments (1 cm in length) were incubated for 9 h in 10 mM K-citrate buffer (pH 6.8). Cross-sections were cut from half-peeled segments incubated for 0 and 9 h. The boundary between the intact and removed tissues was observed under a microscope to measure the thickness of epidermis, collenchyma, and parenchyma cells just under the collenchyma. Since the outer tissues were always detached from the inner tissues by splitting the layer of parenchyma cells just under the collenchyma, thickness of the outer tissues was calculated by summing the thickness of the epidermis, the collenchyma, and half that of the parenchyma cells. Data are means ± SE (n = 15).

h	Thickness			Outer Tissue (a + b+ c/2)
	Epidermis[a] (a)	Collenchyma (b)	Parenchyma cell (c)	
		μm		
0	19.3 ± 0.7	82.0 ± 4.2 (3.8 ± 0.2)[b]	39.9 ± 1.5	121.3 ± 4.5
9	18.3 ± 0.7	96.0 ± 2.6 (4.3 ± 0.2)	41.0 ± 1.6	134.8 ± 3.1

[a] Epidermis consisted of one layer of epidermal cells. [b] Figures in parentheses are number of cell layers.

Table II. *Geometry of Cross-Section of Dark-Grown Squash Hypocotyls*

Hypocotyl segments (1 cm in length) were incubated for 0 and 9 h in 10 mM K-citrate buffer (pH 6.8). Cross-sections were cut from the segments. Long (a) and short (b) axes of the sections were measured under microscope, since the section was ellipsoid. Total area of cross-section was calculated by (a × b) × $\pi/4$. Cross-section area of removed outer tissues was calculated from the dimension and the data in Table I as follows: Total area − (a − 2 × thickness of outer tissues) × (b − 2 × thickness of outer tissues) × $\pi/4$. Data are means ± SE (n = 15).

	Long Axis	Short Axis	Total Area	Area of Outer Tissue
	mm		*mm²*	*mm²*
0	3.28 ± 0.05[a]	2.54 ± 0.04	6.56 ± 0.18	1.08 (16.5)
9	3.50 ± 0.06	2.77 ± 0.07	7.62 ± 0.27	1.28 (16.8)

[a] Figures in parentheses are percentage of outer tissues area to total cross-section area of the hypocotyl.

tissue after 9 h. The results clearly indicate that the outer tissues removed at 0 and 9 h have the same tissue composition.

The cross-section of a squash hypocotyl is ellipsoid, and the long and short axes were measured to calculate the total cross-section area of the hypocotyls (Table II). The hypocotyl swelled during the incubation. The cross-sectional area of the outer tissues was calculated using the data in Table I. The percentages of cross-sectional area of outer tissues to the total cross-sectional area at 0 and 9 h were 16.5 and 16.8%, respectively. These values were closely comparable to the percentages of fresh weight of removed outer tissues to that of intact hypocotyls (17.1%). These data also indicate that the outer tissues removed from the segments incubated for 0 and 9 h have an almost identical tissue composition.

Sugar Contents of Cell Wall Fractions of Outer and Inner Tissues

Table III shows the sugar contents of cell wall fractions of outer and inner tissues from squash hypocotyl segments before and after 9 h of incubation. The segment elongation was 1.1 ± 0.04 mm (n = 35) after 9 h of incubation. Total sugar content of cell walls of inner tissues (521 μg/hypocotyl segment) was slightly higher than that of the outer ones (438 μg/hypocotyl segment) at 0 h. The fresh weight of inner tissues (68 mg/hypocotyl segment) was 5 times greater than that of outer ones (14 mg/hypocotyl segment), indicating that the density of cell walls of outer tissues (31.3 μg/mg fresh weight) is 4 times higher than that of inner ones (7.7 μg/mg fresh weight). This is not surprising since outer tissues consist mostly of collenchyma cells. The cell wall polysaccharides of both outer and inner tissues consisted of pectin, hemicellulose and cellulose in the ratio of 5:3:4 at 0 h. The neutral sugar contents in all the fractions of outer tissues significantly increased after 9 h of incubation (22% for pectin, 34% for HB, and 14% for cellulose). Thus, the total sugar contents of the cell wall in outer tissues significantly increased (68 μg increase per segment) after 9 h. In contrast, the sugar contents of most fractions in inner tissues substantially decreased after 9 h (−20 and −28% for pectic neutral sugars and UA, respectively, −13% for neutral sugars of HB, −27% for HA, and −25% for cellulose; 116 μg decrease per segment). The changes in sugar contents of each cell wall fraction of outer and inner tissues after 9 h were very notable, but summation of sugar contents in outer and inner tissues for each fraction obscured their characteristic changes.

Sugar Components of Pectin and HB Fraction of Outer and Inner Tissues

Table IV shows the contents of neutral sugar constituents of pectin of outer and inner tissues at 0 and 9 h. Gal was the

Table III. *Sugar Contents of Cell Wall Fractions of Outer and Inner Tissues from Squash Hypocotyl Segments*

Hypocotyl segments (10 mm in length) were incubated for 9 h in 10 mM K-citrate buffer (pH 6.8). Data are means ± SE (n = 3).

Fraction	Sugar Content[a]				Total (Outer + Inner)	
	Outer tissues		Inner tissues			
	0 h	9 h	0 h	9 h	0 h	9 h
	μg/tissue					
Pectin						
NS	81 ± 2	99 ± 2 (a)[b]	97 ± 1	78 ± 1 (b)	178 ± 2	177 ± 2
UA	107 ± 4	109 ± 3	117 ± 2	84 ± 5 (b)	224 ± 4	193 ± 6 (b)
HB						
NS	89 ± 1	119 ± 1 (a)	93 ± 1	82 ± 2 (b)	182 ± 1	201 ± 2 (a)
UA	8 ± 1	9 ± 1	11 ± 1	9 ± 1	19 ± 1	18 ± 1
HA						
NS	13 ± 3	10 ± 2	33 ± 3	24 ± 1 (b)	46 ± 4	34 ± 2 (b)
Cellulose						
NS	140 ± 2	160 ± 3 (a)	170 ± 3	128 ± 4 (b)	310 ± 4	288 ± 5 (b)
Total	438 ± 6	506 ± 5 (a)	521 ± 5	405 ± 7 (b)	959 ± 7	911 ± 9 (b)

[a] Sugar content of outer or inner tissue obtained from one hypocotyl segment (1 cm in length). [b] (a), significant increase from 0 h ($P < 0.05$); (b), significant decrease from 0 h ($P < 0.05$).

Table IV. *Neutral Sugar Compositions of Pectic Fraction of Cell Walls of Outer and Inner Tissues from Squash Hypocotyl Segments*

Hypocotyl segments (10 mm in length) were incubated for 9 h in 10 mM K-citrate buffer (pH 6.8). Pectin was extracted from the cell wall materials with hot EDTA. Lyophilized powder of pectic substances was hydrolyzed for 1.5 h with 2 M trifluoroacetic acid. Neutral sugar compositions of hydrolysate were determined by GLC. Data are means ± SE (n = 3).

Sugar	Sugar Content[a]			
	Outer tissue		Inner tissue	
	0 h	9 h	0 h	9 h
	μg/tissue			
Rha	8.5 ± 0.3	13.8 ± 0.3 (a)[b]	10.0 ± 0.3	10.4 ± 0.2
Ara	7.8 ± 0.3	10.7 ± 0.1 (a)	8.1 ± 0.1	8.5 ± 0.2
Xyl	0.9 ± 0.1	1.3 ± 0.1 (a)	2.0 ± 0.1	1.7 ± 0.1
Man	1.1 ± 0.1	7.1 ± 0.1 (a)	1.0 ± 0.1	7.4 ± 0.3 (a)
Gal	51.0 ± 2.0	55.2 ± 1.0	43.0 ± 0.5	28.5 ± 0.4 (b)
Glc	11.6 ± 1.0	10.6 ± 1.0	33.1 ± 0.9	21.8 ± 0.8 (b)

[a] Sugar content of outer or inner tissue obtained from one hypocotyl segment (1 cm in length). [b] (a), significant increase from 0 h ($P < 0.05$); (b), significant decrease from 0 h ($P < 0.05$).

major pectic neutral constituents in outer tissues, while both Gal and Glc were the major in inner tissues. Gal and Glc contents of outer tissues were unchanged 9 h of incubation and the other sugar contents increased during the 9 h incubation. In contrast, in inner tissues, Gal and Glc contents decreased during the 9 h incubation.

Table V shows the contents of neutral sugars of the HB fraction. The major sugar components were Xyl, Man, Gal, and Glc in outer and inner tissues. The contents of Fuc, Xyl, and Man were significantly higher in outer tissues at 0 h, while those of Rha, Gal, and Glc were significantly higher in inner tissues at 0 h. The contents of every sugar residues of outer tissues significantly increased during the 9 h incubation. On the other hand, in inner tissues, the major components (Xyl, Man, Gal, and Glc) were decreased during the 9 h incubation, while the minor components (Rha, Fuc, and Ara) increased.

Mol Wt Distribution of Pectic and HB Polysaccharides of Outer and Inner Tissues

Figure 1 shows the elution patterns of constituents of pectic polysaccharides from outer and inner tissues at 0 h. Rhamnogalacturonans are considered as the major pectic polysaccharides in dicots, and in most cases they contain Ara and Gal residues (3, 21); the elution patterns of Rha, Ara, Gal, and UA are shown in Figure 1. Pectic polysaccharides of both outer and inner tissues were separated into two types of polysaccharides that eluted in low (about 13 kD) and high (about 550–1500 kD) molecular mass regions. Most UA eluted in low molecular mass regions, while neutral sugars

Table V. *Neutral Sugar Compositions of HB Fraction of Cell Walls of Outer and Inner Tissues from Squash Hypocotyl Segments*

Hypocotyl segments (10 mm in length) were incubated for 9 h in 10 mM K-citrate buffer (pH 6.8). Hemicellulose was extracted with 17.5% NaOH from the cell wall materials which had been treated with hot EDTA. Hemicellulose fraction was neutralized with glacial acetic acid. The neutralized solution was centrifuged for 20 min at 10,000g. Supernatant (HB) was subjected to GLC analysis to determine the sugar composition. Data are means ± SE (n = 3).

Sugar	Sugar Content			
	Outer tissue		Inner tissue	
	0 h	9 h	0 h	9 h
	μg/tissue			
Rha	0.8 ± 0.1	1.8 ± 0.1 (a)[b]	1.4 ± 0.1	2.7 ± 0.2 (a)
Fuc	1.8 ± 0.1	4.2 ± 0.1 (a)	1.0 ± 0.1	1.4 ± 0.1 (a)
Ara	0.8 ± 0.1	2.1 ± 0.1 (a)	1.0 ± 0.1	2.1 ± 0.2 (a)
Xyl	20.1 ± 0.4	28.9 ± 0.2 (a)	17.1 ± 0.1	15.1 ± 0.9
Man	11.7 ± 0.1	15.8 ± 0.1 (a)	8.7 ± 0.2	6.2 ± 0.2 (b)
Gal	19.7 ± 0.1	26.2 ± 0.1 (a)	23.5 ± 0.5	17.7 ± 0.9 (b)
Glc	33.8 ± 0.7	40.3 ± 0.5 (a)	40.3 ± 0.3	36.4 ± 1.1 (b)

[a] Sugar content of outer or inner tissue obtained from one hypocotyl segment (1 cm in length). [b] (a), significant increase from 0 h ($P < 0.05$); (b), significant decrease from 0 h ($P < 0.05$).

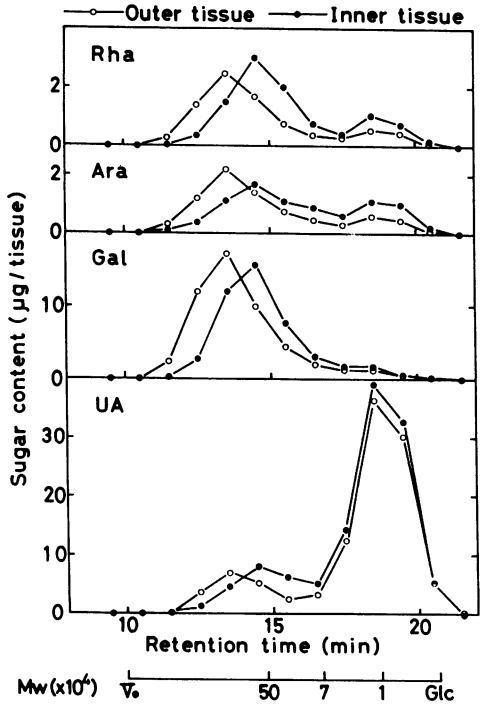

Figure 1. Molecular distributions of pectic components from outer and inner tissues from dark-grown squash hypocotyl segments. Lyophilized powder of pectin was dissolved in 0.5 mL of 50 mM K-phosphate buffer (pH 7.2). A portion of the sample solution (100–200 μL) was introduced to HPLC with a gel permeation column (TSK-GEL 5000 PW). The eluate was collected with a fraction collector for each fraction at 1 min intervals. Uronic acid (UA) contents were determined by a carbazole method, and neutral sugar compositions (Rha, Ara, and Gal) were determined by GLC. Dextrans of 10, 40, 70, 120, and 500 kD were used as molecular mass markers.

(Rha, Ara, and Gal) eluted mainly in high molecular mass regions. The sugar residues of outer tissues in high mol wt regions eluted earlier than those of inner tissues (molecular mass of outer tissues, about 1500 kD; molecular mass of inner tissues, about 550 kD). The sugar contents in high mol wt regions of outer and inner tissues were very similar. The low molecular mass polysaccharides of both the outer and inner tissues eluted at the same position with similar peak height. Rha and Ara contents in low molecular mass regions were slightly higher in inner tissues than in outer ones.

Figure 2 shows the elution patterns of HB polysaccharides from outer and inner tissues at 0 h. HB fraction had a complex sugar composition, suggesting that it consisted of several species of polysaccharides. The main peaks of Rha, Ara, Gal, and UA for the inner tissues eluted around 14 to 15 min (molecular mass about 550 kD). Peaks of Gal and UA of outer tissues eluted around 14 to 15 and 17 to 19 min (molecular mass about 13–33 kD). Fuc of outer tissues eluted earlier (15–16 min, mol wt about 220 kD) than that of inner tissues (16–17 min, molecular mass about 85 kD). The main peaks of Xyl eluted around 17 to 18 min (about 33 kD) for both tissues. The elution patterns of Man and Glc of both tissues were almost similar (molecular mass about 13 kD). The amounts of Rha, Gal, and UA in inner tissues eluted in high mol wt regions were much more than those of outer tissues.

Figure 3, A and B, shows the elution patterns as detected by refractometry of pectic substances and HB substances, respectively, of outer and inner tissues at 0 and 9 h of incubation. The large peaks at 20 min contained little sugar and refraction is due to the presence of salts. The most noticeable change is in the HB inner tissue sample where a high molecular mass peak substantially disappears.

DISCUSSION

Sakurai *et al.* (19) demonstrated the presence of two types of pectic polysaccharides of different mol wt in the cell walls of squash hypocotyls. They found a high mol wt RGAG and low mol wt homogalacturonan with few neutral sugar residues. The present results confirmed their results and further demonstrated that the mol wt of RGAG in outer tissues is about three times as high as that in inner tissues, while homogalacturonans have similar mol wt in both tissues. RGAG was also found in HB as reported by Sakurai *et al.* (19), but the amount of RGAG per segment was much greater in the inner tissues than in outer tissues.

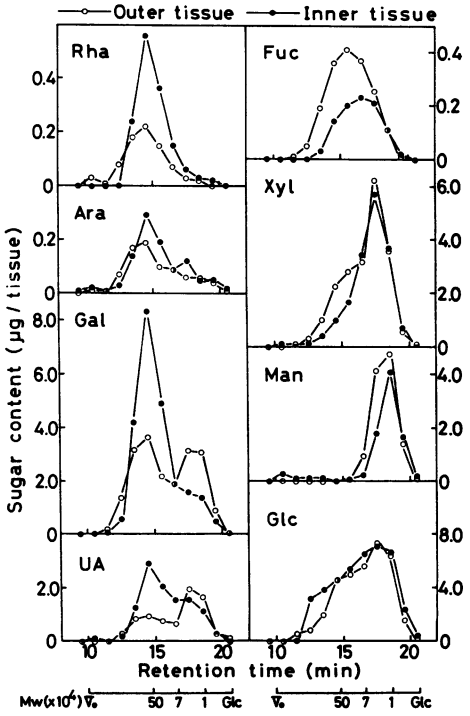

Figure 2. Molecular distributions of HB components from outer and inner tissues from dark-grown squash hypocotyl segments. Lyophilized powder of HB was dissolved in 0.5 mL of 50 mM K-phosphate buffer (pH 7.2). A portion of the sample solution (100–200 μL) was introduced to HPLC with a gel permeation column (TSK-GEL 5000 PW). The eluate was collected with a fraction collector for each fraction at 1 min intervals. Uronic acid (UA) contents were determined by a carbazole method, and neutral sugar compositions (Rha, Ara, Gal, Fuc, Xyl, Man, and Glc) were determined by GLC.

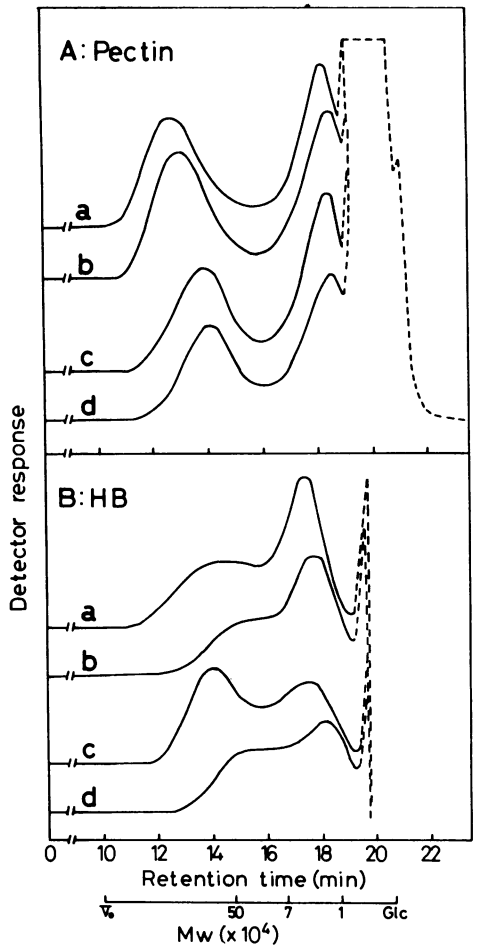

Figure 3. Elution profiles of pectin (A) and HB fractions (B) from outer and inner tissues of dark-grown squash hypocotyl segments before and after 9 h of incubation. Squash hypocotyl segments were excised and outer tissues were immediately removed from the segments (0 h), or after 9 h of incubation. Elution profiles by HPLC analysis were monitored by a refractive index detector. Elution profiles shown by broken lines were due to contaminant salts. a, Outer tissues at 0 h; b, outer tissues at 9 h; c, inner tissues at 0 h; d, inner tissues at 9 h.

Xyloglucans exist commonly in hemicellulosic polysaccharides in monocots and dicots, and dicot xyloglucans usually have Gal and Fuc residues (2, 3). Since Fuc residues are only found in xyloglucans and not in other polysaccharides in dicots (3, 19, 20), the elution profile of Fuc may directly show the mol wt distribution of xyloglucans in squash hypocotyl cell wall (19). Average calculated molecular mass of xyloglucan based on the Fuc elution pattern was 455 kD for outer tissues and 210 kD for inner tissues, suggesting that outer tissues has higher molecular mass xyloglucans than inner tissues. The higher amount of Xyl detected as a shoulder peak of the elution profile in outer tissues also supports the above suggestion. A decrease in mol wt and degradation of xyloglucans were reported in several plant stem tissues, when exogenously applied auxin stimulated elongation growth of the stem tissues (6, 14). It is postulated that the changes in xyloglucans due to auxin action results in cell wall loosening which, in turn, allows cell elongation. The xyloglucans found in inner tissues of squash hypocotyls have a lower molecular mass; this implies that the cell wall of inner tissues is already loosened but the cell wall of outer tissues with its higher molecular mass xyloglucans restrains the elongation of inner tissues. In fact, hypocotyl segments without outer tissues grow 4 to 5 times faster than those with outer tissues (24). If epidermal tissues regulate the rate of auxin-induced cell elongation (7, 9, 23), auxin is probably involved in the depolymerization of xyloglucans in epidermal tissues. This possibility is currently being examined.

An analysis of changes in the sugar content of cell wall fractions during 9 h of incubation clearly showed that outer tissues and inner tissues synthesized different cell wall components. The sugar contents of most of the cell wall fractions of outer tissues significantly increased after 9 h of incubation, while those of inner tissues significantly decreased. Isotope studies with squash hypocotyl segments demonstrated that the incorporation of [^{14}C]Glc into the cell wall fractions of outer tissues was three times higher than that of inner tissues (24). These findings strongly suggest that cell wall synthesis is more active in outer tissues than in inner tissues in squash hypocotyls. The hypocotyl segments were incubated in the absence of an external supply of sugar, and the possibility exists that an active transport of sugar from inner to outer tissues promotes wall synthesis of outer tissues, and that the depletion of sugar in inner tissues reduces wall synthesis there. Therefore, supplement of sugars to the medium may inhibit the decrease in wall sugars of inner tissues.

Analysis of sugar components of pectin and HB revealed that outer tissues had a slightly different sugar compositions than inner tissues. The difference was more prominent after 9 h of incubation; Gal content in pectin and HB increased in outer tissues while they decreased in inner tissues. Using epicotyl segments of *Vigna angularis,* Nishitani *et al.* (14, 15) demonstrated that Gal content in the noncellulosic wall components decreased during incubation and IAA accelerated the decrease. We assume that the Gal decrease found in the whole tissue segment in the absence of IAA is due to the decrease in Gal in inner tissues. Therefore, it is necessary to confirm whether the IAA-induced decrease in Gal content occurs in outer tissues.

Synthesis and turnover of cell walls during IAA-induced elongation of plant cells have been extensively studied (12, 22), but most of the studies have not taken account of the difference in activity of wall synthesis between outer and inner tissues. Therefore, investigation of cell wall metabolism during hormone-induced growth of plant tissues should be done using separated tissues to clarify the effect of the hormone on the cell walls of each tissue.

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