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Differential Effect of Auxin on Molecular Weight Distributions of Xyloglucans in Cell Walls of Outer and Inner Tissues from Segments of Dark Grown Squash (*Cucurbita maxima* Duch.) Hypocotyls[1]

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ABSTRACT

Effects of indole-3-acetic acid (IAA) on the mechanical properties of cell walls and structures of cell wall polysaccharides in outer and inner tissues of segments of dark grown squash (*Cucurbita maxima* Duch.) hypocotyls were investigated. IAA induced the elongation of unpeeled, intact segments, but had no effect on the elongation of peeled segments. IAA induced the cell wall loosening in outer tissues as studied by the stress-relaxation analysis but not in inner tissues. IAA-induced changes in the net sugar content of cell wall fractions in outer and inner tissues were very small. Extracted hemicellulosic xyloglucans derived from outer tissues had a molecular weight about two times as large as in inner tissues, and the molecular weight of xyloglucans in both outer and inner tissues decreased during incubation. IAA substantially accelerated the depolymerization of xyloglucans in outer tissues, while it prevented that in inner tissues. These results suggest that IAA-induced growth in intact segments is due to the cell wall loosening in outer tissues, and that IAA-accelerated depolymerization of hemicellulosic xyloglucans in outer tissues is involved in the cell wall loosening processes.

Auxin induces cell elongation in plant stem tissues through promotion of cell wall loosening (18, 25). Roles of epidermis of stem tissues in auxin-induced elongation growth have been reported in monocots and dicots (13, 19). These studies suggest that auxin changes the mechanical properties of epidermal cell walls but not those of inner tissues, suggesting that auxin-induced growth of stem tissues is mediated by the changes in mechanical properties of epidermal cell walls.

Changes in the chemical structure of cell wall polysaccharides underlie the processes of cell wall loosening leading to cell elongation (15, 25). Specific biochemical modifications of cell walls, such as adjustment in mol wt and quantities of wall polysaccharides, are likely to be involved in mediation of the physical processes of cell wall loosening. A process is evaluated by a stress-relaxation method (18, 21, 23). Auxin-induced degradation of cell wall polysaccharides, such as β-glucans and xyloglucans, during auxin-induced elongation has been demonstrated in monocots and dicots (10, 21, 22, 24, 30). These studies indicate that the changes in mol wt of those polysaccharides are associated with the cell wall loosening processes. Using pea epicotyl segments, Labavitch and Ray (16) and Terry *et al.* (26) showed that auxin promoted the liberation of xyloglucans from cell walls when auxin promoted the elongation of segments. Furthermore, auxin-induced decrease in average mol wt of xyloglucans in hemicelluloses and cell wall loosening occurred even when elongation growth was osmotically suppressed (21). Recently, Hoson and Masuda (8, 9) demonstrated that antibodies and lectins specific for xyloglucans inhibited both auxin-induced cell wall loosening and degradation of xyloglucans of azuki bean epicotyl segments.

Our previous report (28) showed that the cell wall polysaccharides in outer and inner tissues from squash hypocotyl segments exhibited different chemical structures and difference in polymer metabolism were suggested. However, the changes in cell wall structures of outer and inner tissues during auxin-induced growth of stem segments have not been studied. In the present investigation, we examined the effect of auxin on mechanical properties and on composition of cell walls. A focus on the mol wt distribution of hemicellulosic xyloglucans derived from outer and inner tissues of squash hypocotyl segments served to clarify the role of outer tissues in auxin responses.

MATERIALS AND METHODS

Plant Materials

Squash (*Cucurbita maxima* Duch. cv Houkou-Aokawaa-maguri, Takayama Seed Co., Kyoto, Japan) seedlings were grown in the dark as previously described (27). Seedlings which had 3 to 4 cm long hypocotyl were selected and 10 mm long hypocotyl segments were excised from the region 5 mm below the cotyledonary node. Excised segments were immediately incubated for various periods at 26°C in the dark in a Petri dish containing 10 mM K-citrate buffer (pH 6.8) with or without 10^{-5} M IAA. After incubation, the segment length was measured using a binocular microscope, then segments were separated into outer and inner tissues by peeling with

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forceps. All manipulations were conducted under dim green light. Microscopic observation showed that the peeled tissues contained one layer of epidermis and a few layers of collenchyma cells. Our previous results showed that the outer tissues peeled before and after 9 h of incubation had the same tissue composition (28). In the present study, we confirmed that the outer tissues from control and IAA-treated segments incubated for 6 h were detached at the same layer of parenchyma cells adjacent to the collenchyma cell layers. Furthermore, the percentages of fresh weight of outer tissues to whole segments of control and IAA-treated segments were 21.4 ± 0.5% and 20.6 ± 0.5%, respectively. The results clearly indicated that the outer tissues separated from control and IAA-treated segments had the same tissue composition.

Cell Wall Fractionation

Fractionation of cell walls was conducted as previously described (27, 28). Separated outer and inner tissues were fixed in boiling methanol. Methanol-fixed tissues were rehydrated with water, then homogenized in water with a mortar and pestles. The residue obtained by centrifugation was washed with water, acetone, and a methanol:chloroform mixture (1:1, v/v) and air-dried. The dried material was treated with 2 units/mL porcine pancreatic α-amylase (type I-A, Sigma) to remove starch, then with 200 μg/mL pronase (Actinase, Kaken Kagaku Co., Tokyo) to remove proteins.

Next, pectic substances were extracted from the cell walls by three treatments with 50 mM EDTA at 95°C. Then hemicellulose was extracted for 18 h with 17.5% NaOH containing 0.02% $NaBH_4$. The alkali-insoluble fraction after extraction of hemicellulose was designated as cellulose fraction. The alkali-extracted hemicellulosic fraction was neutralized with glacial acetic acid. The pectic and neutralized hemicellulosic fractions were dialyzed against deionized water. After the dialysis, hemicellulosic fraction was separated into two fractions by centrifugation (10,000 *g* for 20 min). The precipitate and the supernatant were designated as HA[2] and HB fraction, respectively. The total sugar content of each fraction was determined by the phenol-sulfuric acid method (4) and UA contents by the carbazole method (6). The neutral sugar compositions of pectin and HB fraction were determined by the method of Albersheim *et al.* (1).

Gel Permeation Chromatography of HB Fraction

The fractionated hemicellulose B fraction was lyophilized, then samples were dissolved in 0.5 mL of 50 mM K-phosphate buffer (pH 7.2), and an aliquot (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 0.5 min intervals. The elution pattern of hemicellulose B substances was monitored by refractive index. The xyloglucan content in each fraction was determined by the iodine staining method (12) with a slight modification (21). The mass-average mol wt of xyloglucans was calculated from the equation reported by Nishitani and Masuda (21). Dextrans (mol wt of 10,000, 40,000, 70,000, 120,000, and 500,000, Sigma) were used as molecular mass markers.

Measurement of Mechanical Properties of the Cell Wall

At the end of the incubation, the segments were separated into outer and inner tissues by peeling, and the peeled outer and inner tissues were fixed for 10 min in boiling methanol. Next, both tissues were rehydrated for 40 min at room temperature with several changes of deionized water before measurement of the mechanical properties of cell walls according to the method of Yamamoto *et al.* (31). The stress-relaxation

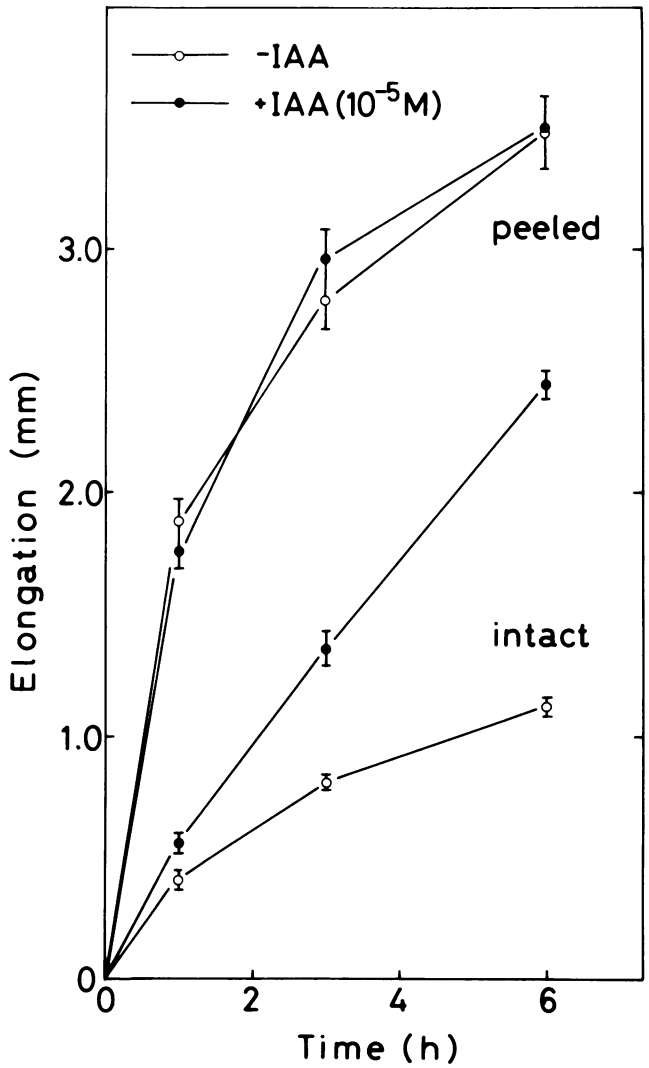

Figure 1. Effect of IAA on elongation of peeled and intact (unpeeled) squash hypocotyl segments. Intact and peeled segments (10 mm in length) were incubated in 10 mM K-citrate buffer (pH 6.8) in the presence or absence of 10^{-5} M IAA. Data are means ± SE (n = 20).

[2] Abbreviations: HA, hemicellulose A; HB, hemicellulose B; UA, uronic acid; Rha, rhamnose; T_0, minimum stress-relaxation time.

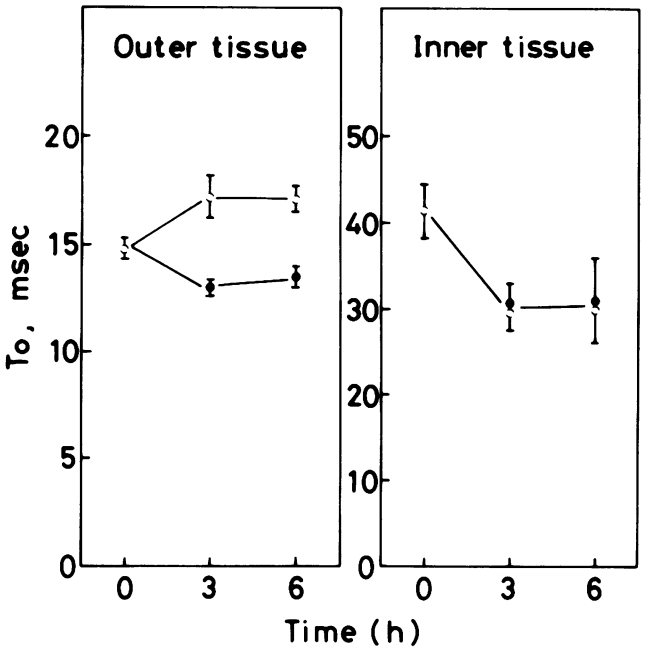

Figure 2. Effect of IAA on the T_0 of cell walls of outer and inner tissues from squash hypocotyl segments. Segments (10 mm in length) were incubated in 10 mM K-citrate buffer (pH 6.8) in the presence (●) or absence (○) of 10^{-5} M IAA. At the end of the incubation, segments were separated into outer and inner tissues. Both tissues were fixed in boiling methanol, and then subjected to stress relaxation analysis. Data are means ± SE (n = 18).

parameter, T_0, was calculated from the equation reported by Fujihara *et al.* (5) with a microcomputer (9801-UV, NEC, Tokyo).

RESULTS

Effects of IAA on the Elongation and Mechanical Properties of Cell Walls

IAA differentially influenced growth of peeled and unpeeled (intact) squash hypocotyl segments (Fig. 1). IAA at 10^{-5} M induced the elongation of unpeeled segments within 1 h of IAA application, and peeled segments elongated more rapidly than unpeeled segments. However, IAA did not promote the elongation of peeled segments. The influence of IAA was also examined in the T_0 of cell walls prepared from outer and inner tissues (Fig. 2). Unpeeled segments treated in the presence or absence of IAA, were then immediately separated into outer and inner tissues to measure T_0. IAA significantly decreased the T_0 value of cell walls in outer tissues but it did not affect inner tissues.

Effect of IAA on the Sugar Compositions

Table I shows the sugar content of cell wall fractions of outer and inner tissues of initial segments and the segments treated for 6 h in the presence or absence of 10^{-5} M IAA. In outer tissues, sugar contents of pectin, HB and cellulose fractions were significantly increased after 6 h incubation irrespective of IAA treatment. In inner tissues, uronic acid content in the pectic fraction and neutral sugar contents of HA and cellulose fraction significantly decreased irrespective of IAA treatment. Neutral sugar contents of pectin and HB fractions in inner tissues remained at the initial levels. Thus, the total sugar content of cell walls in outer tissues (422 μg/hypocotyl segment at 0 h) increased about 20% after 6 h,

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

Hypocotyl segments (10 mm in length) were incubated for 6 h in 10 mM K-citrate buffer (pH 6.8) in the presence or absence of 10^{-5} M IAA. After incubation, segments were separated into outer and inner tissues by peeling with forceps. Data are means ± SE (n = 3).

Fraction		Sugar Content					
		Outer tissues			Inner tissues		
		Initial	−IAA	+IAA	Initial	−IAA	+IAA
		μg/tissue					
Pectin	NS	75 ± 1	92 ± 1[a]	91 ± 3[a]	81 ± 2	81 ± 1	83 ± 1
	UA	87 ± 3	111 ± 2[a]	111 ± 1[a]	106 ± 1	96 ± 3[a]	101 ± 1[a]
HB	NS	97 ± 2	120 ± 2[a]	128 ± 1[a,b]	119 ± 2	117 ± 6	114 ± 1
	UA	7 ± 1	8 ± 1	8 ± 1	11 ± 1	10 ± 1	9 ± 1
HA	NS	6 ± 1	4 ± 1	5 ± 1	36 ± 1	15 ± 2[a]	12 ± 1[a]
Cellulose	NS	150 ± 3	173 ± 6[a]	176 ± 4[a]	222 ± 3	175 ± 5[a]	178 ± 3[a]
Total		422 ± 5	508 ± 7[a]	519 ± 5[a]	575 ± 5	494 ± 9[a]	497 ± 4[a]

[a] Significant difference from the initial level at 5% level. [b] Significant difference between ± IAA treatment at 5% level. NS, neutral sugar; UA, uronic acid; HB, hemicellulose B; HA, hemicellulose A.

Table II. *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 6 h in 10 mM K-citrate buffer (pH 6.8) in the presence or absence of 10^{-5} M IAA. Pectin was extracted from the cell wall materials with hot EDTA. Extracted pectic substances were hydrolyzed for 1.5 h with 2 N trifluoroacetic acid. Neutral sugar compositions of hydrolysate were determined by GLC. Data are means ± SE (n = 3).

Sugar	Sugar Content					
	Outer tissues			Inner tissues		
	Initial	−IAA	+IAA	Initial	−IAA	+IAA
	μg/tissues					
Rha	10.5 ± 0.5	13.4 ± 0.2[a]	13.5 ± 0.9[a]	10.7 ± 0.3	10.5 ± 0.6	11.1 ± 0.3
Ara	8.7 ± 0.5	10.1 ± 0.2	10.0 ± 0.4	8.9 ± 0.4	8.1 ± 0.3	8.6 ± 0.1
Xyl	1.5 ± 0.2	1.3 ± 0.1	1.3 ± 0.1	1.8 ± 0.1	1.9 ± 0.1	1.9 ± 0.1
Man	1.7 ± 0.1	1.8 ± 0.1	1.7 ± 0.1	1.4 ± 0.1	1.2 ± 0.1	1.1 ± 0.1
Gal	44.4 ± 1.4	57.4 ± 1.2[a]	57.0 ± 0.9[a]	34.7 ± 0.6	33.5 ± 0.4	34.5 ± 0.2
Glc	7.7 ± 0.4	7.2 ± 0.1	6.3 ± 0.2[a,b]	22.9 ± 0.5	25.1 ± 1.9	24.8 ± 0.5

[a] Significant difference from the initial level at 5% level. [b]Significant difference betwen ±IAA treatment at 5% level.

while that in inner tissues (575 μg/hypocotyl segment at 0 h) decreased about 14%. IAA slightly increased the neutral sugar contents of HB fraction in outer tissues after 6 h incubation, but it had little effect on the sugar content of other fractions of outer tissues nor did it influence any fractions of inner tissues. Tables II and III show the neutral sugar compositions of pectin and HB fractions of outer and inner tissues, respectively. In pectic fraction (Table II), Gal content in outer tissues substantially increased after 6 h in the presence or absence of IAA. The levels of other sugar components in outer tissues and those of all sugar components in inner tissues changed very little. IAA had only a small effect on the levels of sugar components of pectic fraction of outer and inner tissues during a 6 h incubation. By contrast, the level of several sugar components of HB fraction (Table III) in outer tissues significantly increased after 6 h (except Rha residue), whereas Xyl and Gal in inner tissues slightly decreased. IAA promoted the increases in Man, Gal, and Glc contents in outer tissues, while it promoted decreases in the Gal content in inner tissues after 6 h.

Effect of IAA on the Mol Wt Distribution of Xyloglucans

Figure 3 shows the elution patterns as detected by refractometry of HB substances derived from outer and inner tissues at 0 and 6 h of incubation in the presence or absence of IAA. In outer tissues, the elution patterns of initial and control (−IAA at 6 h) segments were nearly similar, while that of IAA-treated segments shifted slightly to a population of polymer with lower mol wt. This was especially apparent in the

Table III. *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 6 h in 10 mM K-citrate buffer (pH 6.8) in the presence or absence of 10^{-5} M IAA. Hemicellulose was extracted with 17.5% NaOH from 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 neutral sugar composition. Data are means ± SE (n = 3).

Sugar	Sugar Content					
	Outer tissues			Inner tissues		
	Initial	−IAA	+IAA	Initial	−IAA	+IAA
	μg/tissue					
Rha	1.7 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	3.8 ± 0.1	3.5 ± 0.1	3.4 ± 0.1[a]
Fuc	3.1 ± 0.1	3.7 ± 0.1[a]	4.2 ± 0.2[a]	2.7 ± 0.1	2.4 ± 0.1	2.4 ± 0.1
Ara	1.7 ± 0.1	2.1 ± 0.1[a]	2.3 ± 0.1[a]	2.4 ± 0.1	3.0 ± 0.1[a]	2.8 ± 0.1[a]
Xyl	24.3 ± 0.8	29.7 ± 0.5[a]	31.4 ± 0.7[a]	24.2 ± 0.6	21.9 ± 0.5[a]	21.0 ± 0.2[a]
Man	13.4 ± 0.2	17.1 ± 0.2[a]	18.0 ± 0.1[a,b]	11.3 ± 0.4	12.1 ± 0.7	11.5 ± 0.2
Gal	20.8 ± 0.3	25.5 ± 0.7[a]	27.9 ± 0.3[a,b]	29.2 ± 0.9	26.2 ± 0.3[a]	25.0 ± 0.3[a,b]
Glc	32.4 ± 0.9	39.8 ± 0.7[a]	42.0 ± 0.3[a,b]	45.5 ± 1.0	47.5 ± 4.3	47.7 ± 0.8

[a] Significant difference from the initial level at 5% level. [b] Significant difference between ±IAA treatment at 5% level.

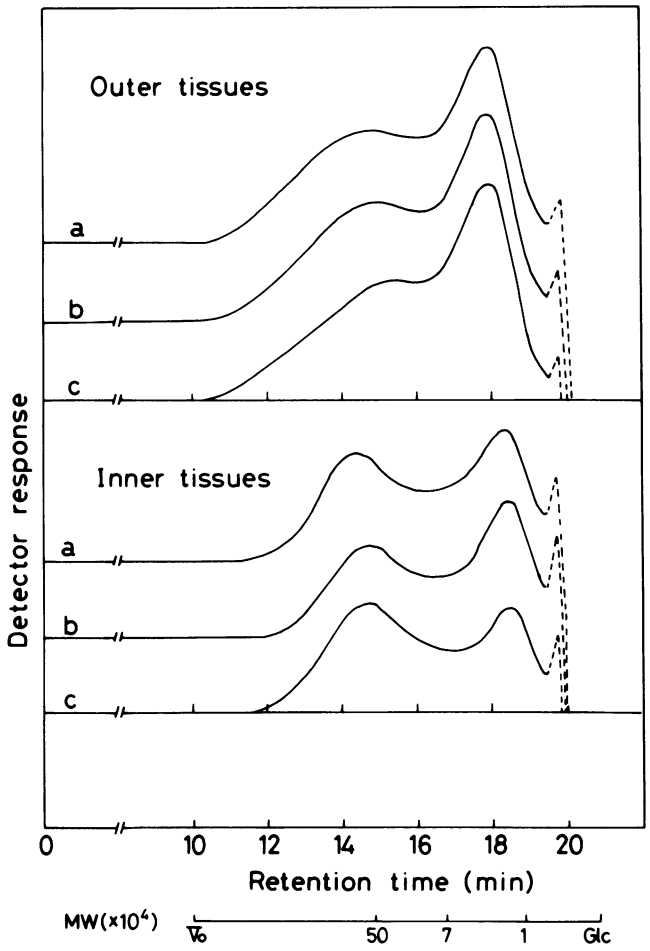

Figure 3. Elution profiles of HB fraction of outer and inner tissues from squash hypocotyl segments. Segments (10 mm in length) were incubated for 6 h in 10 mM K-citrate buffer (pH 6.8) in the presence or absence of 10^{-5} M IAA. At the end of the incubation, segments were separated into outer and inner tissues, and then subjected to cell wall fractionation. Lyophilized powder of HB was dissolved in 0.5 mL of 50 mM K-phosphate buffer (pH 7.2), and an aliquot (200 μL) was introduced to HPLC with a gel permeation column (TSK-GEL 5000 PW). Elution profiles by HPLC analysis were monitored by a refractive index detector. Elution profiles shown by broken lines were due to contaminant salts. a, initial (0 h); b, −IAA at 6 h; c, +IAA at 6 h.

shoulder peak (around 14.5 min). In inner tissues, 6 h of incubation in the absence of IAA caused a shift of the peak eluted from high mol wt regions to low mol wt regions. Elution pattern of IAA-treated segments was similar to that of initial segments.

Figure 4 shows the elution patterns of xyloglucans in HB fraction of outer and inner tissues detected by the iodine method. Xyloglucans in outer tissues harvested at 0 h eluted much earlier than those in inner tissues. The mass-average mol wt of xyloglucans in outer tissues was 2.3 times as high as that in inner tissues at 0 h (Table IV). In the absence of IAA, the mass-average mol wt of xyloglucans in outer and inner tissues decreased to 85 and 92% of the initial levels after 6 h incubation, respectively. IAA accelerated the decrease of mol wt of xyloglucans in outer tissues, while it prevented the decrease in inner tissues.

DISCUSSION

The important role of the outer tissues, especially epidermal tissues, in regulation of stem elongation has been demonstrated in pea (19) and maize (13). These studies demonstrated that segments of coleoptile and epicotyl with epidermis removed elongated more rapidly than intact (unpeeled) segments at the early stage of the treatment. The results indicate that in intact segment epidermis is subjected to strong tissue tension produced by the expanding force of the inner tissues and, thus, the elongation of the intact segments is regulated by the epidermis. Furthermore, auxin-induced growth was not observed in segments in the absence of the epidermis, and

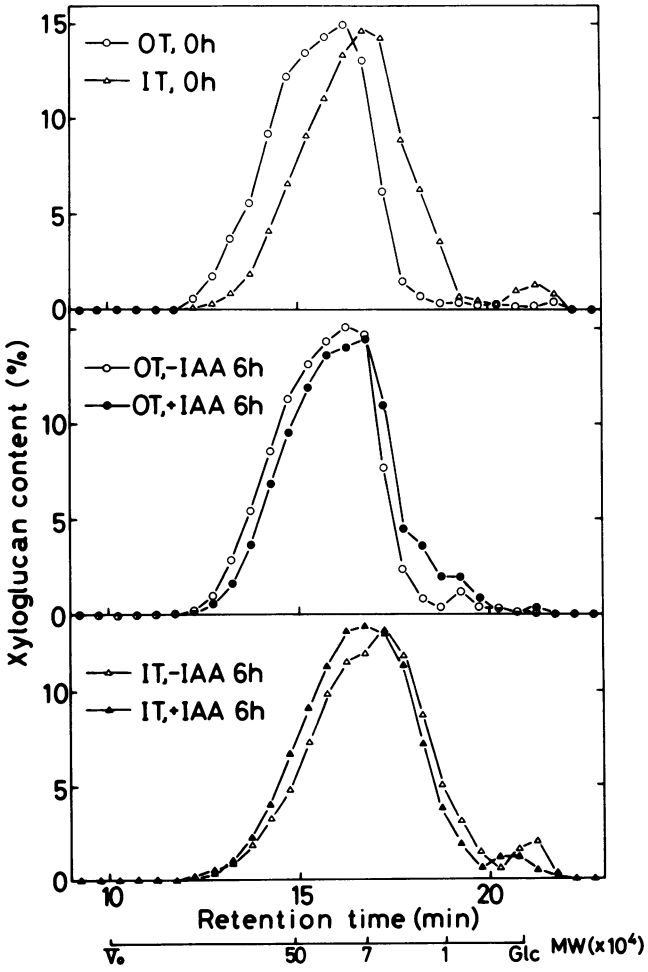

Figure 4. Elution profiles of xyloglucans in HB fraction of outer and inner tissues from squash hypocotyl segments. Lyophilized powder of HB was dissolved in 0.5 mL of 50 mM K-phosphate buffer (pH 7.2), and an aliquot (200 μL) was introduced to HPLC with a gel permeation column (TSK-GEL 5000 PW). The eluate was collected with a fraction collector for each fraction at 0.5 min intervals. The xyloglucan content in each fraction was determined by the iodine staining method. OT, Outer tissues; IT, inner tissues. Data are means ± SE (n = 3).

Table IV. *Mass-Average Molecular Mass of Xyloglucans in HB Fraction of Cell Walls of Outer and Inner Tissues from Squash Hypocotyl Segments*

Hypocotyl segments (10 mm in length) were incubated for 6 h in 10 mM K-citrate buffer (pH 6.8) in the presence or absence of 10^{-5} M IAA. Extracted hemicellulosic substances were fractionated into HB and HA fractions by centrifugation. Lyophilized powder of HB was dissolved in 50 mM K-phosphate buffer (pH 7.2), and subjected to gel permeation chromatography. Mass-average mol wt of xyloglucans was calculated from the elution profile of xyloglucans which was detected by the iodine staining method. Data are means ± SE ($n = 3$).

	Mass-Average Molecular Mass	
	Outer tissues	Inner tissues
	kD	
Initial	484 ± 7.0 (100%)	213 ± 2.0 (100%)
−IAA	410 ± 5.2 (85%)	195 ± 4.3 (92%)
+IAA	319 ± 1.0[a] (66%)	222 ± 1.0[a] (104%)

[a] Significant difference between ± IAA treatment at 5% level.

auxin caused an increase in cell wall extensibility only in epidermis, indicating that the growth-promoting action of auxin is mediated through a change in mechanical properties of cell walls of the epidermal tissues. Squash hypocotyl segments with outer tissues removed (peeled segment) elongated more rapidly than intact (unpeeled) segments, and IAA-induced growth promotion was found only in intact segments (Fig. 1). Furthermore, measurement of the mechanical properties of the cell walls (Fig. 2) clearly showed that IAA decreased the T_0 value in outer tissues but not in inner tissues, suggesting that IAA-induced cell wall loosening occurs only in outer tissues judged by a decrease in the T_0 value. Our present results obtained from squash hypocotyl segments confirmed the observations in other plant systems. The reason why the inner tissues which grew much faster (at least, first few hours) than the outer tissues, and showed higher T_0 values than do outer tissues, needs to be answered. It is, however, possible that the high tissue tension of inner tissues is relieved on the removal of outer tissues, and the relieved tension causes the burst growth of inner tissues even if the cell walls have high T_0 values. Note that even mature tissue can extend when the tissue is stretched by an additional force (29).

It has been reported that biochemical modification of cell walls, such as changes in mol wt and quantities of cell wall polysaccharides, may represent the basis for the physical processes of cell wall loosening (18, 23, 25). Therefore, we assumed that the chemical changes in cell wall polysaccharides in outer tissues induced by IAA are involved in the wall loosening processes. Using squash hypocotyl segments, our previous results showed that the cell wall compositions in outer tissues are different from those in inner tissues (28). Therefore, we studied effects of IAA on sugar compositions of cell wall polysaccharides in outer and inner tissues. In the absence of IAA, total sugar content of cell walls in outer tissues significantly increased, and those in inner tissues significantly decreased after 6 h incubation (Table I). This result confirms our previous results (28). On the other hand, IAA-induced changes in cell wall sugar content were very small in both outer and inner tissues. Furthermore, analyses of neutral sugar composition (Tables II and III) also showed that there were no remarkable changes in neutral sugar compositions in pectin and HB fraction of both outer and inner tissues between control and IAA-treated samples. Little effect of auxin on changes in cell wall sugar contents was also found in epicotyl segments of *Vigna angularis* (20). These results imply that structural modifications of cell wall polysaccharides for wall loosening was not fully expressed in quantities of sugar components. Bulk quantities of polysaccharides already present in the cell wall probably mask a subtle, important change in quantities of some sugar components, as demonstrated by Kutschera and Briggs (14).

Xyloglucans are common in hemicellulosic polysaccharides in monocots and dicots (2, 3, 7). Degradation of xyloglucans during auxin-induced cell elongation was demonstrated in azuki bean epicotyl (21, 22), pea stem (16, 26), oat coleoptile (10), and *Pinus* hypocotyl (17). Furthermore, the degradation of xyloglucans induced by IAA was found within 30 min after the IAA application, and degradation occurred even when elongation growth of stem tissues was osmotically suppressed by mannitol (10, 21). Recently, using azuki bean epicotyl segments, Hoson and Masuda (8, 9) demonstrated that the antibodies and lectins specific for xyloglucans inhibited the IAA-induced elongation, cell wall loosening, and decrease in mol wt of xyloglucans. These findings clearly indicate that in dicot stems the degradation of xyloglucans induced by auxin is the most dominant process for cell wall loosening which leads the elongation growth of stem tissues. Xyloglucans have been shown to react with iodine to form an iodine-xyloglucan complex (12), and the iodine staining method has been proved to be specific for xyloglucans (11). Our previous report showed that the mass-average mol wt of xyloglucans in outer and inner tissues calculated based on the Fuc elution patterns were 455,000 and 210,000, respectively (28). These values have a good accordance with the present result (Table IV), suggesting that the elution pattern of Fuc residue directly show the mol wt distribution of xyloglucans in cell wall of squash hypocotyls. Although alkaline extraction for HB may alter mol wt distribution by cleaving phenolic substances bound to wall polysaccharides, present results clearly showed that IAA substantially accelerated the decrease in mass-average mol wt of xyloglucans in HB fraction in outer tissues (Table IV). Since the sugar content in HA faction was substantially smaller than that of the HB fraction in outer tissues (Table I), most of the hemicellulosic polysaccharides, such as xyloglucans, were contained probably in HB fraction. Therefore, we speculate that IAA primarily degrades the hemicellulosic xyloglucans in cell walls of outer tissues and this leads the cell wall loosening in outer tissues, resulting in the promotion of elongation growth of whole (intact) segments. However, one cannot exclude the possibility that IAA accelerates the synthesis of small mol wt of xyloglucans and/or suppresses the synthesis of high mol wt of xyloglucans. The mass-average mol wt of xyloglucans in inner tissues was about half as large as in outer tissues, and IAA prevented the decrease in mol wt of xyloglucans in inner tissues (Table IV). Contrary effects of IAA on changes in mol wt of xyloglucans in outer and inner tissues cannot be explained at present. Because cell walls in outer and inner tissues have different chemical structures (28) and activity in cell

wall synthesis (27), we assume that metabolism of xyloglucans in inner tissues are largely different from those in outer tissues. The existence of different types of xyloglucans in stem tissue was found in azuki bean epicotyls (22) and *Pinus* hypocotyls (17). In these reports, hemicellulosic polysaccharides were extracted from cell walls with different concentration of KOH solutions. Weak alkaline extracted fraction contained low mol wt xyloglucans, while strong alkaline fraction contained high mol wt xyloglucans. Therefore, we assume that two types of xyloglucans extracted with weak and strong alkaline may reflect xyloglucan molecules in outer and inner tissues. This possibility is being examined.

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