

Growth and cell wall changes in azuki bean epicotyls II. Changes in wall polysaccharides during auxin- induced growth of excised segments

Kazuhiko Nishitani¹, Hiroh Shibaoka² and Yoshio Masuda¹

¹ Department of Biology, Faculty of Science, Osaka City University, Sumiyoshi-ku, Osaka 558, Japan
and ² Department of Biology, Faculty of Science, Tokyo Metropolitan University, Fukazawa,
Setagaya-ku, Tokyo 158, Japan

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Changes in cell wall polysaccharides and mechanical properties of the cell wall were examined during IAA-induced elongation growth of excised azuki bean epicotyl segments under different growth conditions. Sucrose promoted IAA-induced cell elongation, but had very little effect on IAA-induced cell wall loosening. In the absence of sucrose, the amount of galactose in the cell wall decreased during the incubation period. IAA enhanced the decrease in the galactose level. In the presence of sucrose, on the other hand, IAA induced increases in the amounts of cellulose, galactose and xylose in non-cellulosic polysaccharides. These IAA-induced increases were not observed in the presence of mannitol at concentrations higher than 0.1 M, although cell wall loosening was induced by IAA even in the presence of 0.2 M mannitol.

Key words: Azuki bean epicotyl — Cell wall extension — Cell wall polysaccharides — IAA.

Auxin induces cell wall loosening and thus cell wall extension in excised plant parts (16). Biochemical processes underlying cell wall loosening have been investigated to elucidate the mechanism of cell extension. In monocots, such as *Avena* and barley, degradation of noncellulosic β -glucan is closely correlated with auxin-induced cell wall loosening (13, 16, 21, 22). In dicots, quantitative analysis of cell wall compositions has revealed active turnover of galactose and deposition of xylose in the cell wall during cell extension and maturation (7, 17-19, 27).

In a previous paper, we suggested that polysaccharides composed of galactose and xylose play some roles in the cell wall loosening and stiffening of intact azuki bean epicotyls (19). This paper describes changes in cell wall sugar compositions during the IAA-induced elongation of excised azuki bean epicotyl segments, with particular attention being paid to the effects of sucrose and mannitol on both the cell wall loosening and cell wall sugar compositions.

Materials and methods

Azuki bean (*Vigna angularis*) seedlings were grown in light as reported previously

Abbreviations: IAA, indole-3-acetic acid; T_0 , minimum stress-relaxation time.

(19). Epicotyls of 6-day-old seedlings were selected for uniformity of length (8–10 cm). From the epicotyls, 10-mm segments were excised between 5 and 15 mm below the first leaves. After these segments were washed in water for 2 hr, twenty segments each were incubated in a petri dish containing 4 ml of test solution at 25°C in light (ca. 2500 lux). The test solution consisted of 10 mM potassium phosphate buffer solution (pH 6.0) with or without 0.1 mM IAA in the presence or absence of various concentrations of mannitol and/or sucrose. After incubation, segment length was measured microscopically, then the segments were immediately killed in boiling methanol, treated with 200 ppm Pronase-P (Kaken Kagaku Co. Ltd.) for 18 hr at 37°C, and stored at –20°C until use.

Sugar compositions of the cell wall were analyzed as reported previously (19). Briefly, pronase-treated segments were crushed between glass plates, then washed with water, acetone and a methanol-chloroform mixture (1 : 1, v/v). The dried material was treated with 10 units of pancreatic α -amylase (Sigma Chemical Co.) for 3 hr at 37°C to remove starch, rinsed with water then acetone, and air-dried. Noncellulosic polysaccharides of the dried material were hydrolyzed with 2 N trifluoroacetic acid at 120°C, then the sugars liberated were reduced with sodium borohydride and acetylated for 3 hr with acetic anhydride at 120°C (3, 12). The amount of each alditol acetate was determined using a gas chromatograph (Hitachi Model 163) equipped with a microcomputer (Hitachi Model 834). The residue which remained insoluble on hydrolysis with 2 N trifluoroacetic acid was rinsed with water then acetone and air-dried. The dried residue was dissolved in 72% sulfuric acid (v/v). The amount of sugar in the solution was determined colorimetrically by a phenol-sulfuric acid method (6), the content being defined as α -cellulose content.

To analyze the mechanical properties of the cell wall, the epidermis of live segments was peeled off with forceps in three strips, then killed in boiling methanol, treated with 200 ppm Pronase-P for 18 hr at 37°C, and stored in methanol until use. Stress-relaxation analysis of the epidermis was carried out as described previously (19). A rehydrated strip of epidermis was stretched with an Instron tensile tester (Model TM-M) equipped with a HITAC-10 II minicomputer. T_0 , the minimum stress-relaxation time representing the degree of cell wall loosening (28, 29), was calculated from an equation based on a Maxwell viscoelastic model according to the method developed by Yamamoto et al. (28, 29). The mechanical properties of the epidermal cell wall, represented by T_0 , have been shown to regulate the auxin-induced elongation of stem segments (14, 25).

Results

Fig. 1A and B show the effects of IAA and sucrose on the elongation growth of azuki bean segments. The same result has already been reported by Shibaoka (24). IAA-induced elongation was enhanced as the concentration of added sucrose increased (Fig. 1A). The time-course effect of sucrose at the concentration of 50 mM on the IAA-induced elongation is shown in Fig. 1B.

The mechanical property of the epidermal cell wall was measured in order to examine the effects of IAA and sucrose on cell wall loosening of azuki bean segments. IAA induced a decrease in the T_0 value of the epidermal cell wall, indicating that it caused cell wall loosening (Fig. 2). While sucrose showed a promotive effect

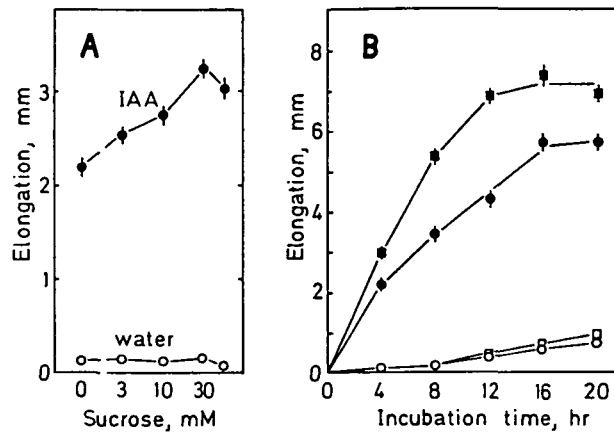


Fig. 1. A. Effects of sucrose concentrations on IAA-induced elongation of azuki bean epicotyl segments. Segments (10 mm long) were incubated for 6 hr in light in 10 mM potassium phosphate buffer solution (pH 6.0) containing various concentrations of sucrose in the presence or absence of 0.1 mM IAA. The data are mean values with standard errors ($n = 20$). B. Time-course effect of sucrose on IAA-induced cell elongation. Segments were incubated for various period in 10 mM potassium phosphate buffer solution (pH 6.0) with or without 0.1 mM IAA and/or 50 mM sucrose. The data are mean values with standard errors ($n = 20$). \circ : water, \square : sucrose, \bullet : IAA, \blacksquare : sucrose+IAA.

on the IAA-induced elongation of the segment (Fig. 1), it showed very little effect on the IAA-induced cell wall loosening represented by the T_0 value.

Fig. 3 shows that IAA-induced changes in the amounts of cellulose and non-cellulosic neutral sugars depend on the concentration of sucrose. The time-course

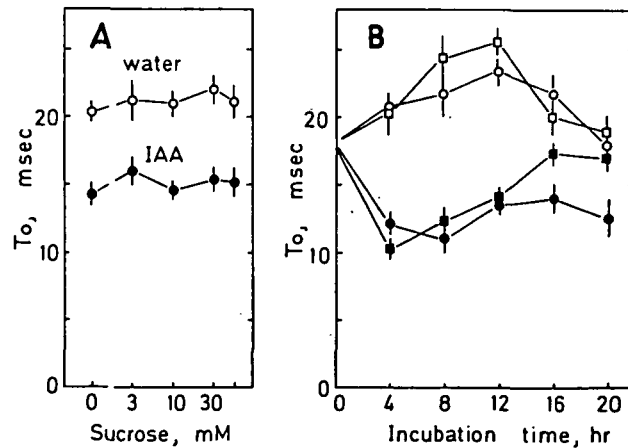


Fig. 2. A. Effects of sucrose concentrations on IAA-induced cell wall loosening. Segments were incubated for 6 hr under the same conditions as in Fig. 1A. After incubation, the epidermis of the segment was peeled off with forceps then subjected to stress-relaxation analysis. The data are means with standard errors ($n = 20$). B. Time-course effect of sucrose on IAA-induced cell wall loosening. Segments were incubated for various period under the same conditions as in Fig. 1B. After incubation, the epidermis of the segment was peeled off with forceps then subjected to stress-relaxation analysis. The data are mean values with standard errors ($n = 20$). \circ : water, \square : sucrose, \bullet : IAA, \blacksquare : sucrose+IAA.

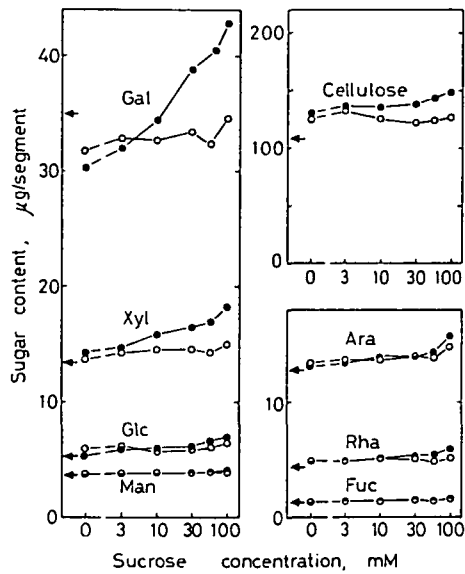


Fig. 3. Effects of sucrose concentrations on the amounts of cellulose and noncellulosic neutral sugars of azuki bean epicotyl segments. Segments (10 mm long) were incubated for 6 hr in 10 mM potassium phosphate buffer solution (pH 6.0) containing various concentrations of sucrose in the presence (—●—) or absence (—○—) of 0.1 mM IAA. After incubation, the segments were killed in boiling methanol then their cell wall compositions were analyzed. Contents of cellulose (upper right) and noncellulosic neutral sugars (left and lower right) in each segment were determined by a phenol sulfuric acid method and gas chromatography, respectively (see **Materials and methods**). Arrows indicate initial values of individual sugar contents.

of IAA-induced changes in the cell wall sugar compositions in the two conditions are shown in Fig. 4. In the presence of sucrose, the amount of individual noncellulosic neutral sugars in the wall increased during the incubation period. IAA caused a considerable increase in the amounts of galactose and xylose. The effect of IAA was detected within 4 hr after the initiation of incubation. In the absence of sucrose, all the neutral sugar levels, except for galactose, remained constant during the incu-

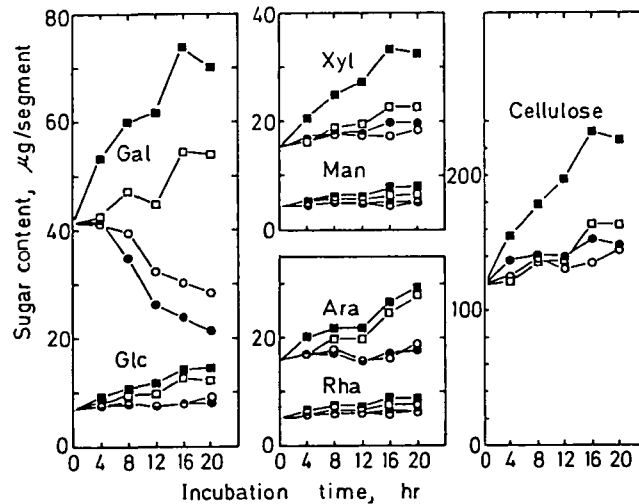


Fig. 4. Time course of IAA-induced changes in sugar compositions of azuki bean epicotyl segments. Segments (10 mm long) were incubated in 10 mM potassium phosphate buffer solution (pH 6.0) with or without 0.1 mM IAA and/or 50 mM sucrose for various periods. After incubation, the segments were killed in boiling methanol then their cell wall compositions were analyzed (see **Materials and methods**). ○: water, □: sucrose, ●: IAA, ■: sucrose + IAA.

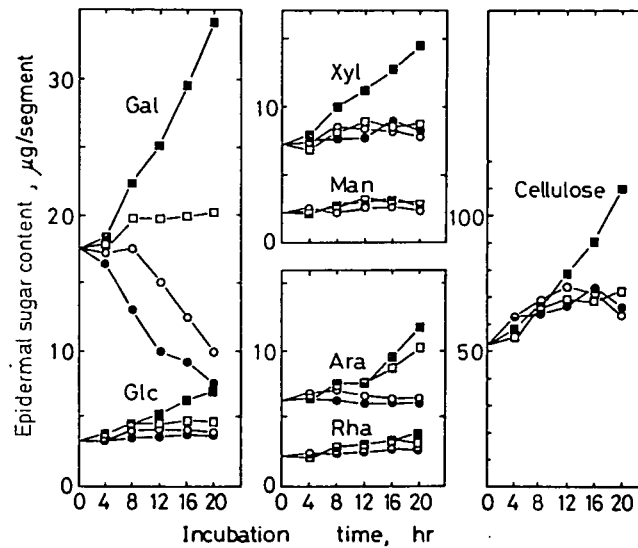


Fig. 5. Time course of IAA-induced changes in sugar compositions of epidermal cell walls of azuki bean epicotyl segments. Segments (10 mm long) were incubated in 10 mM potassium phosphate buffer solution (pH 6.0) with or without 0.1 mM IAA and/or 50 mM sucrose for various periods. After incubation, the epidermis of the segments was carefully peeled off with forceps. The epidermis recovered was killed in boiling methanol then its cell wall composition was analyzed (see Materials and methods). The symbols are the same as in Fig. 4.

bation period in the IAA-treated as well as non-treated segments. Under these conditions, the amount of galactose decreased and the decrease was enhanced by IAA, with a latent period of about 4 hr. With respect to cellulose, IAA caused it to increase considerably in the presence of sucrose. In the absence of sucrose, a very slight effect of IAA on its increase was observed in the early phase of the incubation period.

Mechanical properties of the epidermal cell wall have been considered to be responsible for the elongation growth of the whole segment (14, 25). Fig. 5 shows changes in sugar compositions of the epidermal cell wall during auxin-induced cell elongation. The epidermal cell wall included one half of the total polysaccharides of the whole stem segment (cf. Fig. 4 and 5). Changes in its sugar compositions were similar to those observed in the whole stem segment: a considerable increase in the amounts of galactose, xylose and cellulose was induced by IAA in the presence of 50 mM sucrose, while in its absence, IAA enhanced a decrease in the galactose level.

When segments are incubated in the presence of mannitol, which inhibits osmotic water absorption, IAA causes cell wall loosening without any apparent extension growth; cell wall loosening can be distinguished from cell wall extension using such osmoticum (5, 15). Fig. 6 shows the effects of IAA on cell wall extension and loosening in various concentrations of mannitol solution. When segments were incubated in the presence of 0.1–0.2 M mannitol the T_0 values were lower in the control segments. In *Avena* coleoptile segments, higher T_0 values were observed in segments incubated in the presence of mannitol (21). These effects of mannitol on

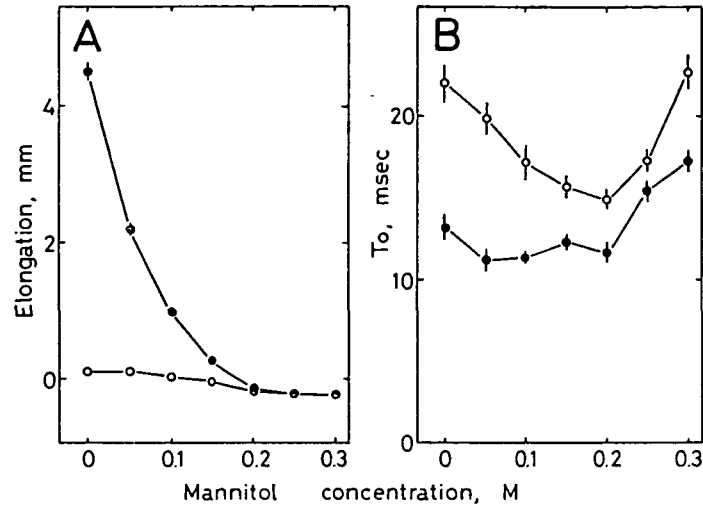


Fig. 6. Effects of water stress by mannitol on IAA-induced elongation and cell wall loosening. Segments (10 mm long) were incubated for 6 hr in 10 mM potassium phosphate buffer solution (pH 6.0) with (●) or without (○) 0.1 mM IAA in the presence of various concentrations of mannitol. After incubation, segment length was measured (A). Next, the epidermis was peeled off with forceps and subjected to stress-relaxation analysis (B). The data are mean values with standard errors ($n=20$).

the T_0 value of the cell wall remain unexplained. Although higher concentrations of mannitol completely depressed the IAA-induced elongation, cell wall loosening, which is represented by a decrease in T_0 , was induced by IAA even in the presence of 0.2 M mannitol.

Effects of mannitol concentrations on the increase in the amount of neutral sugars are shown in Fig. 7. The IAA-induced increase in the amounts of galactose, xylose and cellulose decreased as the mannitol concentration increased. The effects

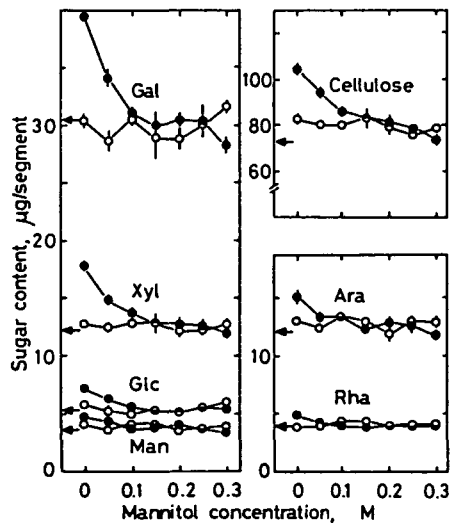


Fig. 7. Effects of water stress by mannitol on IAA-induced changes in sugar compositions of azuki bean epicotyl segments. Segments (10 mm long) were incubated for 6 hr in 10 mM potassium phosphate buffer solution (pH 6.0) with 50 mM sucrose with (●) or without (○) 0.1 mM IAA in the presence of various concentrations of mannitol. After incubation, the segments were killed in boiling methanol then their cell wall compositions were analyzed. The data are mean values with standard errors ($n=3$). Arrows indicate initial individual sugar contents.

of mannitol on both cell wall extension and cell wall synthesis more or less paralleled each other.

Discussion

In *Avena* coleoptile segments (4, 5) and pea (1) and azuki bean (9) epicotyl segments, incorporation of ^{14}C -labeled glucose into cell wall materials has been shown to be enhanced by IAA. In the present study, quantitative analysis of the amount of each component sugar of cell wall polysaccharides showed an increase in the net amount, when the segments were incubated in the presence of an adequate amount of sucrose (see Fig. 3, 4 and 5). Thus, IAA caused differential effects on the promotion of individual polysaccharide syntheses, i.e., it caused a substantial increase in the amounts of cellulose, galactose and xylose, but only slightly changed the amounts of other noncellulosic sugars such as glucose, mannose, arabinose, rhamnose and fucose. Similar increases in the net amounts of galactose, xylose and cellulose in the cell wall have been found in the corresponding region of growing intact azuki bean epicotyl (19).

Table 1 shows the percentage increases in individual component sugars in the growing region (between 5–15 mm below the apex) of intact epicotyls and excised segments incubated in the presence or absence of IAA and/or sucrose. In the growing region of the intact epicotyl, all the component sugars increased up to about 100% in 24 hr, indicating the occurrence of coordinated synthesis of the cell wall. Table 1 also indicates that the percentage increases in each component sugar in excised segments incubated in the presence of both 0.1 mM IAA and 50 mM sucrose were closest to those for intact epicotyls among the four treatment types.

Auxin-enhanced synthesis of the cell wall on the basis of the incorporation of labeled glucose has been reported to be diminished when plant tissues are incubated in the presence of mannitol, which is known to inhibit cell wall expansion (4, 5, 20). Data shown in Fig. 7 indicate that mannitol completely inhibits the IAA-induced increase in the amount of noncellulosic polysaccharides as well as that of cellulose in the azuki bean cell wall. These observations indicate that turgor pressure exerted

Table 1 Percentage increase in cell wall component sugars in growing intact epicotyls and excised segments

Treatment	% Increase in sugar content							Cellulose
	Noncellulosic neutral sugars							
	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	
Intact epicotyl ^a	113	112	79	175	133	80	126	132
Excised segment ^b								
Water	23	11	21	21	15	–31	28	22
IAA	26	29	12	29	15	–48	18	26
Sucrose	50	41	77	47	44	31	81	39
IAA + sucrose	69	99	87	113	76	70	113	92

^a The upper region of the epicotyl (between 5–15 mm below the apex) was marked with India ink then allowed to grow for 24 hr.

^b Segments excised from the upper region were incubated for 20 hr in 10 mM potassium phosphate buffer solution with or without 0.1 mM IAA and/or 50 mM sucrose.

on the cell wall is required for cell wall synthesis and that this synthesis occurs during the process of cell wall extension which is induced by auxin.

In addition to turgor pressure, auxin-induced cell wall synthesis also depends on the availability of substrate such as sucrose, because no increase in the amount of cell wall polysaccharides was observed without added sucrose (see Fig. 3, 4 and 5). A similar result has been obtained in experiments using radioisotopic methods (1, 4). However, the T_0 value, representing the magnitude of cell wall loosening (15, 28, 29), was independent of the supply of sucrose (see Fig. 2). These findings support the idea that cell wall synthesis is a secondary effect of auxin in the process of cell wall extension which follows cell wall loosening. IAA-induced cell wall extension without synthesis, which occurs under starved conditions, leads to cell wall thinning. Sucrose as a substrate reduces cell wall thinning by enhancing synthesis during extension. Thus, the promotive effect of sucrose on IAA-induced cell wall extension may be attributable to the sucrose-dependent cell wall synthesis. A similar effect of sucrose has been reported for *Avena* coleoptile segments (23).

Specific decrease in the amount of galactose suggests the involvement of degradative turnover of polysaccharides chiefly composed of galactose (see Fig. 4 and 5). According to the model of sycamore cell walls proposed by Keegstra et al. (2, 10), 1,4-linked galactan side chains are covalently attached to both rhamnogalacturonans and xyloglucans which are in turn attached to cellulose microfibrils by hydrogen bonding. In pea stem segments, auxin has been shown to cause liberation of xyloglucan presumably from the cell wall (8, 11, 12). On the basis of these facts, it has been proposed that the primary action of cell wall loosening involves the splitting of bonds interconnecting the cellulose microfibrils within the polysaccharide matrix (2, 12, 26). On the assumption that endoglycanase participates in the primary action of cell wall loosening, which would involve splitting of galactan chains (2), the action of the enzyme would result in exposure of the terminal ends of galactan chains. Exowise degradation of the galactan chain by galactosidases would lead to a decrease in the amount of the galactan. In fact, β -galactosidase activity is specifically correlated to auxin-induced elongation of excised segments as well as endogenous elongation of intact pea epicotyls (26).

In coleoptile segments of monocots such as *Avena* (13, 16, 21) and barley (22), IAA has been shown to induce degradation of noncellulosic β -glucan in the cell wall under conditions where exogenous substrate for cell wall synthesis was not available. The degradation of galactan in dicots is analogous to that of the β -glucan in monocots (Fig. 4 and 5). In general, noncellulosic β -glucans are rich in coleoptiles of cereal plants and galactans in dicots (16, 18). The difference in the cell wall polysaccharide composition between monocots and dicots may be the cause for the difference in the polysaccharides which are degraded during the IAA-induced elongation. Thus, the remarkable decrease in galactose amount, which is observed in azuki bean epicotyl, seems to reflect the action of the exogalactanase under starved conditions. In the presence of sucrose, degradation of the galactan seems to be overcome by active synthesis of the polysaccharide.

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