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Auxin-induced changes in the molecular weight of hemicellulosic polysaccharides of the *Avena* coleoptile cell wall

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The average molecular weight of the water soluble hemicelluloses (hemicellulose B) of the *Avena* coleoptile cell wall was determined by gel permeation chromatography (GPC) and viscometry. Analysis of the neutral sugar composition of hemicellulose B eluted from a GPC column (Sepharose 4B) indicated that it consists of β-glucan with a high molecular weight and arabinoxylan with a low molecular weight. A kinetic study of the effect of auxin on the molecular distribution of hemicellulose B demonstrated that auxin decreased the β-glucan content of the hemicellulose as early as the first hour incubation, but not the arabinoxylan content, when it stimulated the extension of the coleoptile segments. Calculation of the weight-average molecular weight from the chromatograms suggested that auxin decreased the molecular weight of hemicellulose B; this was also confirmed by viscometry. Thus, auxin may cause cell wall loosening, leading to cell extension, through its effect on β-glucan degradation or through the decrease in the molecular weight of hemicellulose B.

Key words: Auxin — *Avena* — Cell wall — Extension — Hemicellulose — Wall loosening.

It is accepted that auxin causes cell wall loosening when it stimulates cell extension (*3, 12*). The physical and chemical aspects of the mechanism of auxin-induced cell wall loosening, leading to cell extension, have been investigated. Yamamoto et al. (*22*) developed a stress-relaxation analysis for the measurement of the mechanical properties of the cell wall as affected by auxin. One parameter is specifically affected by auxin treatment; this parameter, designated as the minimum stress-relaxation time (T_0), decreases as early as the 15th minute of auxin treatment (*23*). The minimum stress-relaxation time is defined as η/G, where η is the viscosity of a dash-pot and G is the elastic modulas of a spring element in a Maxwell viscoelastic model. From rheological evidence, they assumed that auxin decreased the viscosity of the model, and that this decrease is due to a decrease in the average molecular weight of the polysaccharide components of the cell wall (*23*). Moreover, Kawamura et al. (*7*) demonstrated that changes in the hemicellulosic polysaccharide content are correlated with changes in T_0 values.

The stimulation of the turnover of the hemicelluloses of the cell wall by auxin

Abbreviations: IAA, indole-3-acetic acid; GPC, gel permeation chromatography; GLC, gas liquid chromatography; Ara, arabinose; Xyl, xylose; Gal, galactose; Glc, glucose.

has been reported in several studies (*6, 10, 11, 19*). We reported that the glucose content of the hemicellulosic components of the *Avena* coleoptile cell wall was decreased specifically by auxin and that this decrease was inhibited by a β-glucanase inhibitior, nojirimycin, which also inhibited auxin-induced cell extension and cell wall loosening (*14, 15*). These results suggested that the degradation of polysaccharides, including glucose, contribute to the decrease in the average molecular weight of the hemicelluloses, that is involved in cell wall loosening. Therefore, we determined the molecular distribution of the hemicelluloses from the *Avena* coleoptile cell wall as affected by auxin.

Materials and methods

Plant materials

Oat seedlings (*Avena sativa* L. cv. Victory) were grown as reported previously (*14*). Coleoptiles of four-day-old oat seedlings with their first leaves removed were incubated for 6 hr in distilled water in the dark. Coleoptile segments (1.4 cm-long) were excised from the segments with a double-bladed cutter. The segments (100–200) were floated in a petri dish (9 cm in diameter) with 20 ml of test solution containing K-citrate buffer (10 mM, pH 6.5) with or without 10^{-5} M IAA. At the end of incubation the segments were killed in boiling methanol, then stored in fresh methanol until use.

Extraction of the hemicelluloses

The extraction procedure for the hemicellulose B of the *Avena* coleoptile cell wall is summarized in Fig. 1. Segments that had been stored in methanol were rehydrated for 20 min then homogenized with a glass mortar and pestle. The homogenate was centrifuged, and the pellet was washed three times each with water, acetone and a methanol-chloroform mixture (1:1, v/v), then dried at 37°C. Dried cell wall materials were treated with 200 ppm pronase (Kaken Kagaku Co., Ltd.)

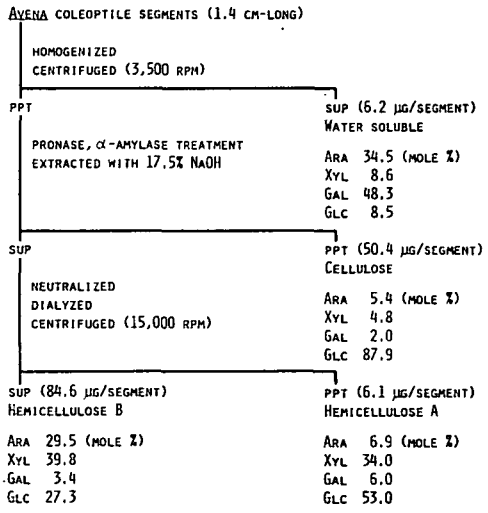

Fig. 1. *Fractionation of cell wall polysaccharides from* Avena *coleoptile segments and the neutral sugar content and composition of each fraction.* For details, see **Materials and methods.**

for 18 hr at 37°C. After three washes with water, the cell wall materials were treated with hog-pancreatic α-amylase (Sigma Chemical Co., Ltd.) for 2 hr at room temperature. The hemicellulose fraction then was extracted for 24 hr with 10 ml of 17.5% NaOH plus 0.1% $NaBH_4$ (*21*). The procedure for the extraction of the pectic substances was omitted, since these substances are only a few percent of the total weight of the *Avena* coleoptile cell wall (*1*, *5*, *17*). The alkali-soluble fraction was neutralized with acetic acid, then dialyzed against distilled water for 24 hr. After dialysis, the solution was centrifuged at 15,000 rpm for 30 min. The precipitate was the hemicellulose A fraction, and the supernatant was the hemicellulose B fraction that was lyophilized.

Molecular distribution of the hemicellulose B on Sepharose 4B

Five milligrams of the powder of the hemicellulose B were dissolved in 1 ml of acetate buffer (50 mM, pH 5.0) and chromatographed on Sepharose 4B (Pharmacia Fine Chemicals) with the same buffer solution as the eluant. The solution (ca. 2.2 ml) eluted from the column was collected in a fraction collector (LKB, Model 2112 Redirac).

An appreciable amount of precipitate was found in the hemicellulose B solution a few weeks after the lyophylized powder was dissolved in the acetate buffer. The precipitate was composed of glucose, which suggests that β-glucan with a high molecular weight was aggregated. Thus, the hemicellulose B solution was chromatographed on Sepharose 4B immediately after the lyophilized powder was dissolved in the acetate buffer.

Determination of neutral sugar contents and composition of the fractionated sample

The total sugar content of the solution in each fraction tube eluted from the GPC column was determined by the phenol-sulfuric acid method, then each remaining solution was dried under a stream of filtered air at 50°C and subjected to GLC to determine the sugar composition. The GLC method was similar to that reported previously (*14*).

The hemicellulose A and alkali-insoluble fraction (cellulose) were suspended in 0.1 ml of 72% H_2SO_4 and allowed to stand for 1 hr at room temperature, then the suspension was diluted to 2 N H_2SO_4 by the addition of distilled water, and hydrolyzed for 1 hr at 121°C. The sugar content of the solution of the alkali-insoluble, water soluble and hemicellulose B fractions was determined by the phenol-sulfuric acid method.

Determination of the intrinsic viscosity of the hemicellulose B

After lyophilization, about 5 mg of the hemicellulose B powder was dissolved in 1 ml of distilled water. An Ostwald type viscosimeter was used to determine the intrinsic viscosity. The times needed for 1 ml of the sample solution at different concentrations and of distilled water to flow through a capillary tube of the viscosimeter were measured (η and η_0, respectively). The relative viscosity η/η_0 for different concentrations of the sample solutions was calculated; the intrinsic viscosity was obtained by extrapolating the value $(\ln \eta/\eta_0)/c$ at zero concentration.

Results

The fractionation procedure, neutral sugar content, and the composition of each fraction extracted from the cell wall polysaccharides of *Avena* coleoptiles are summarized in Fig. 1. The water soluble fraction, in which the sugar content was 6.2 μg/segment, is rich in arabinose and galactose. The total sugar content of cellulose is 50.4 μg/segment, which means that the percentage of cellulose to the total weight of the polysaccharides is 34.2%. Small amounts of arabinose, xylose and galactose in the cellulose fraction were regarded as contaminants from the hemicelluloses.

The water soluble hemicellulose (hemicellulose B) consists of arabinose, xylose and glucose and is the major component of the hemicelluloses of the *Avena* coleoptile cell wall. Hemicellulose A is rich in xylose and glucose. Because of the insolubility of this fraction, molecular distribution was not investigated.

The molecular distribution of the hemicellulose B was determined by GPC on Sepharose 4B (Fig. 2). The weight-average molecular weight was calculated as 1.44 million from the chromatogram. There seem to be two peaks for polysaccharide molecules on the chromatogram. Each fraction eluted from the Sepharose 4B column was subjected to GLC analysis to determine the neutral sugar composition (Fig. 3.) The peak that eluted faster is a polysaccharide consisting of glucose. The other peak consists of arabinose and xylose. Galactose is distributed in all the fractions.

Changes in the molecular distribution of the hemicellulose during auxin-induced

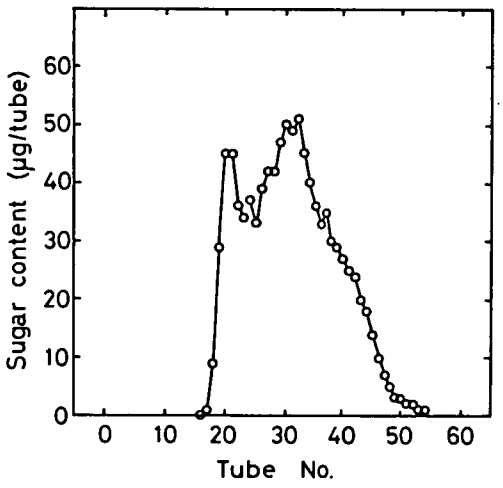

Fig. 2.

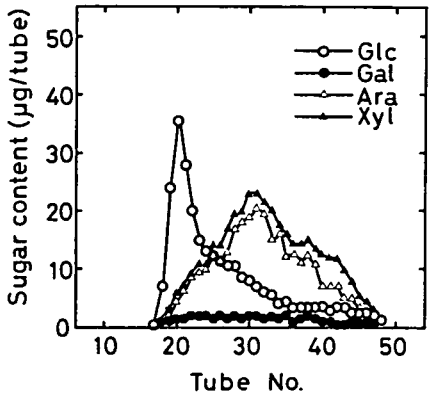

Fig. 3.

Fig. 2. *Molecular distribution pattern of hemicellulose B on Sepharose 4B.* Hemicellulose B from the *Avena* coleoptile cell wall was chromatographed on Sepharose 4B with acetate buffer (50 mM, pH 5.0) as the eluant. The sugar content was determined by the phenol-sulfuric acid method. The total sugar content shown in this chromatogram was 1 mg of hemicellulose B.

Fig. 3. *Neutral sugar composition of the hemicellulose B chromatographed on Sepharose 4B.* The fractionated solution eluted from a Sepharose 4B column (Fig. 2) was subjected to GLC analysis to determine the sugar composition.

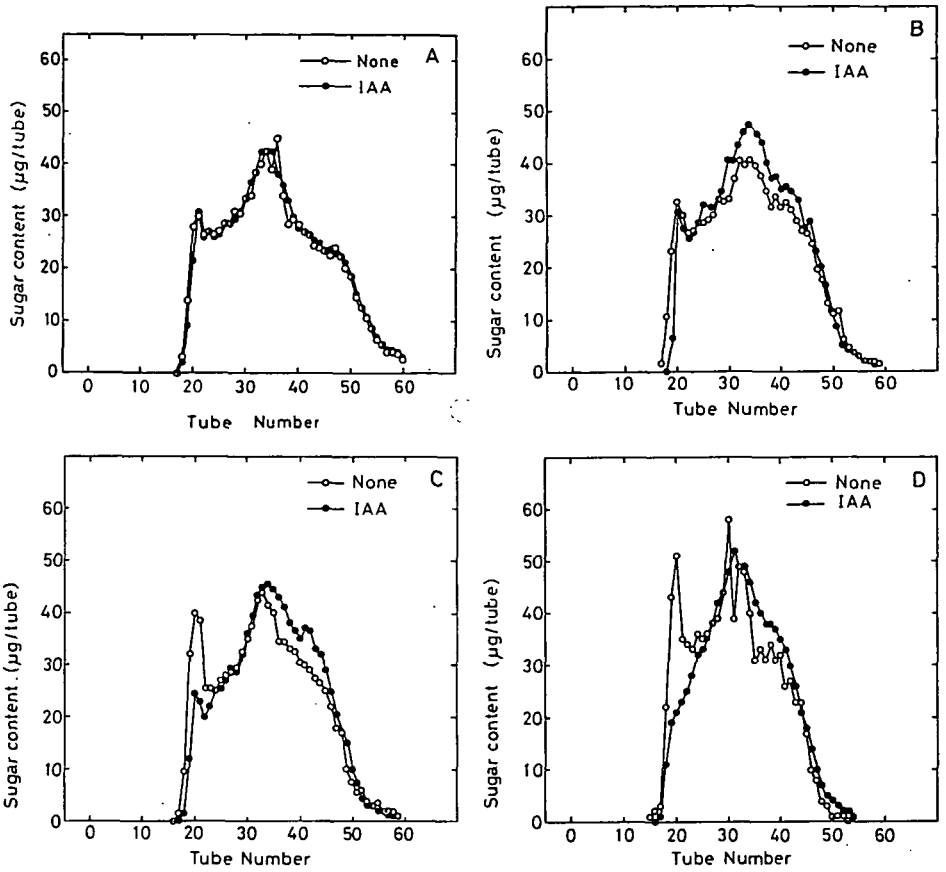

Fig. 4. *Changes in the molecular distribution of hemicellulose B chromatographed on Sepharose 4B during auxin-induced extension of* Avena *coleoptile segments.* Coleoptile segments (1.4 cm-long) were treated with or without 10^{-5} M IAA for 1 (A), 2 (B), 4 (C) and 6 hr (D). On completion of the growth experiment, the segments were immediately killed in boiling methanol for 5 min. The hemicellulose B of the coleoptile cell wall was extracted as described in **Materials and methods**. The himicellulose B was chromatographed on Sepharose 4B with acetate buffer (50 mM, pH 5.0) as the eluant. The sugar content was determined by phenol sulfuric acid method. The total sugar content shown in these chromatograms was 1 mg of hemicellulose B.

extension of the coleoptile segments is shown in Fig. 4. The area of the first peak on the chromatogram was decreased by auxin during incubation. Apparently the decrease began after 1 hr of auxin treatment. Out of five experiments, three showed that auxin caused the decrease in the first hour of incubation, but we found no effect of auxin before the first hour of incubation.

The neutral sugar composition of the hemicellulose B chromatographed on Sepharose 4B is shown in Fig. 5; coleoptile segments had been treated with or without IAA for 6 hr. It was clear that only the polysaccharide composed of glucose disappeared in response to auxin for 6 hr. Calculation of the weight-average molecular weights of the data shown in Fig. 4 indicates that auxin decreased the weight-

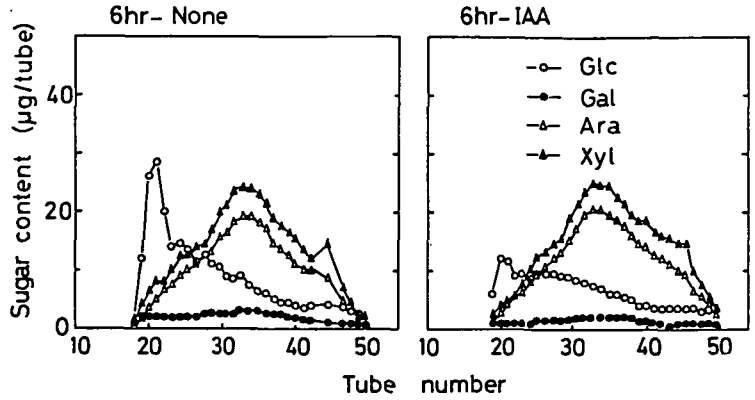

Fig. 5. *Neutral sugar composition of the hemicellulose B chromatographed on Sepharose 4B.* Coleoptile segments (1.4 cm-long) were treated for 6 hr with or without 10^{-5} M IAA. The hemicellulose B was extracted as described in **Materials and methods.** The fractionated solution eluted from the Sepharose 4B column (Fig. 4-D) was subjected to GLC analysis to determine the sugar composition.

average molecular weights of the hemicellulose B. The effect of auxin on the decrease in the weight-average molecular weights is shown in Fig. 6.

To confirm this, we determined the intrinsic viscosity ([η]) of the hemicelluose B with an Ostwald type viscosimeter. Changes in the intrinsic viscosities of the hemicellulose B during auxin-induced extension are shown in Fig. 7. Auxin

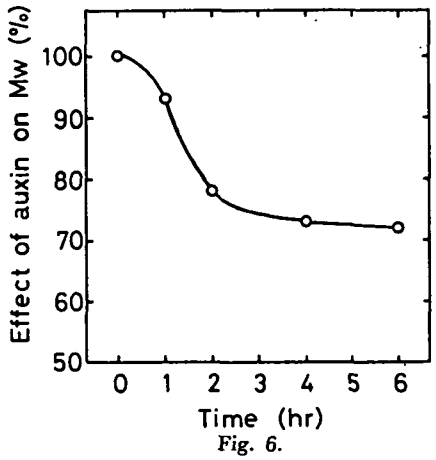

Fig. 6.

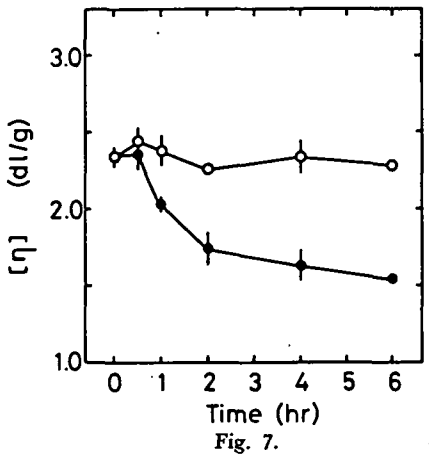

Fig. 7.

Fig. 6. *Effects of auxin on the weight-average molecular weight of hemicellulose B of the* Avena *coleoptile cell wall.* The weight-average molecular weight (Mw) was calculated from the data shown in Fig. 4. The Mw for the hemicellulose B from the cell wall of the initial segments was 1.44 million. The ordinate shows the percent of the Mw of hemicellulose B of auxin-treated coleoptiles to that of the control.

Fig. 7. *Changes in the intrinsic viscosity of hemicellulose B during auxin-induced extension of* Avena *coleoptile segments.* Coleoptile semgents (1.4 cm-long) were treated with or without 10^{-5} M IAA for different periods. The hemicellulose B of the coleoptile cell wall was extracted as described in **Materials and methods.** The intrinsic viscosity was measured with an Ostwald type viscosimeter. Vertical lines show standard errors.

decreased the intrinsic viscosity after 1 hr of incubation. Since the intrinsic viscosity is related to the average molecular weight (M) as $[\eta]=K\cdot M^{\alpha}$, where K and α are constants, auxin decreased the average molecular weight of the hemicellulose B, when it stimulated the extension of coleoptile segments.

Discussion

The hemicellulose B from the *Avena* coleoptile cell wall seems to consist of two kinds of polysaccharides, one a β-glucan with a molecular weight estimated at about 2 million and the other an arabinoxylan with a molecular weight estimated at about 0.6 million. The former has a higher molecular weight than the latter. Wada and Ray (*20*) also reported two polysaccharides, although their molecular weights were both lower than those we calculated. Although we did not investigate the uronic acid component of the hemicellulose B, glucuronic acids may be attached to arabinoxylans, as reported by Wada and Ray (*20*).

The results shown in Fig. 3 clearly demonstrated that auxin decreased the content of the polysaccharide with the higher molecular weight which was composed of glucose (Fig. 4). Since there has been much evidence that β-glucan is the component of the hemicellulose of *Avena* coleoptile cell wall (*2, 9, 13, 20*), we concluded that auxin caused β-glucan degradation when it stimulated extension of coleoptile segments.

The determination of the intrinsic viscosity of the hemicelluloses also was evidence of the auxin-induced degradation of β-glucan. Since the dependency of the viscosity of the molecular weight of polydisperse polymers is due to polymers with high molecular weights (*4*), changes in the intrinsic viscosity caused by auxin appear to result from the degradation of the β-glucan with the high molecular weight. Fig. 8 also shows that the effect of auxin on the decrease in the weight-average molecular weight calculated from the gel chromatogram (Fig. 2 and 4) is comparable to that on the decrease in the intrinsic viscosity calculated from the data in Fig. 7.

According to Flory's theory (*4*), we calculated the effective radii as 37 nm for

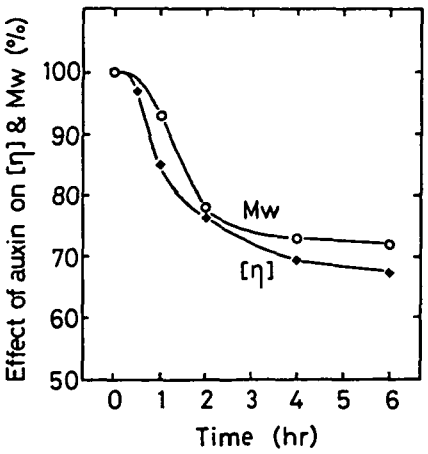

Fig. 8. *Effects of auxin on the weight-average molecular weight and the intrinsic viscosity of hemicellulose B of the* Avena *coleoptile cell wall.* The effect of auxin on the intrinsic viscosity (% to the control) was calculated from the data shown in Fig. 7.

β-glucan and 20 nm for arabinoxylan. It appears that the volume of a β-glucan molecule is 6.3 times as large as that of an arabinoxylan molecule, provided that these molecules assume random coil. Thus, β-glucan degradation contributes to the decrease in the viscosity of the matrix of the cell wall. This leads to cell wall loosening, since viscosity is manifested by the interaction between polymer molecules, and the larger the size of the polymer the more tenacious the interaction (*4*). Most probably auxin-induced cell wall loosening is caused by auxin producing a decrease in the viscosity of the cell wall matrix.

If auxin decreases the viscosity of the cell wall matrix, then physical parameters, which represent the viscous component of the cell wall in stress-relaxation analysis, must be changed by auxin. Of these parameters obtained by the stress-relaxation analysis, T_0 and viscosity $\left[\eta = \int_0^\infty H(\tau)d\tau\right]$ are the probable candidates. T_0, which is decreased by auxin as early as the 15th minute, is represented by η/G. Yamamoto et al. (*22*) suggested that auxin decreased η. However, the kinetics of the auxin-induced decrease in the molecular weight of the hemicellulose B that occurs after 1 hr of incubation is not definitely comparable to that of the auxin-induced decrease in T_0 values that occurs as early as the 15th minute of auxin treatment.

On the other hand, preliminary calculation of the parameter of viscosity showed that it was decreased by auxin as early as 1 hr of incubation; this suggests that β-glucan degradation contributes to the decrease in viscosity.

What role does β-glucan degradation play in auxin-induced extension? For the biphasic response of excised organ segments to auxin (*8*, *18*), we assume that the auxin-induced decrease in T_0 values is mainly due to hydrogen ions secreted by this action, but the decrease in viscosity, that results from β-glucan degradation is due to an action of auxin other than that on hydrogen ion secretion. It is unlikely that the hydrogen ion causes the decrease in viscosity, because it does not affect the glucan content of the hemicellulose of the *Avena* coleoptile cell wall (*14*). Apparently, the onset of β-glucan degradation caused by auxin coincides with that of the second phase of the biphasic response to auxin. Furthermore, Vanderhoff et al. (*18*) demonstrated that protein synthesis is involved in the second phase but not in the first one. β-Glucan degradation also was inhibited by cycloheximide (*16*). Thus, we conclude that auxin-induced extension in the first phase results from the action of the hydrogen ions secreted by auxin, but the extension in the second phase is caused by the decrease in the viscosity of the cell wall matrix that probably is due to β-glucan degradation of the cell wall polysaccharides.

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