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Inhibition of the Synthesis of Cell Wall Polysaccharides in Oat Coleoptile Segments by Jasmonic Acid: Relevance to Its Growth Inhibition

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Abstract. The inhibitory mode of action of jasmonic acid (JA) on the growth of etiolated oat (*Avena sativa* L. cv. Victory) coleoptile segments was studied in relation to the synthesis of cell wall polysaccharides using [^{14}C]glucose. Exogenously applied JA significantly inhibited indoleacetic acid (IAA)-induced elongation of oat coleoptile segments and prevented the increase of the total amounts of cell wall polysaccharides in both the noncellulosic and cellulosic fractions during coleoptile growth. JA had no effect on neutral sugar compositions of hemicellulosic polysaccharides but substantially inhibited the IAA-stimulated incorporation of [^{14}C]glucose into noncellulosic and cellulosic polysaccharides. JA-induced inhibition of growth was completely prevented by pretreating segments with 30 mM sucrose for 4 h before the addition of IAA. The endogenous levels of UDP-sugars, which are key intermediates for the synthesis of cell wall polysaccharides, were not reduced significantly by JA. Although these observations suggest that the inhibitory mode of action of JA associated with the growth of oat coleoptile segments is relevant to sugar metabolism during cell wall polysaccharide synthesis, the precise site of inhibition remains to be investigated.

JA and its related compounds are considered to be putative plant hormones based on the reasons for their being widely distributed in the plant kingdom: ABA-like physiologic activities with low concentrations and the interaction with other plant hormones (see reviews of Parthier 1991, Hamberg and Gardner 1992, Sembdner and Parthier 1993, Ueda et al. 1994a).

In our previous paper (Ueda et al. 1994b) we reported that JA substantially inhibited the growth of etiolated oat (*Avena sativa* L. cv. Victory) coleoptile segments in the presence and absence of IAA. JA did not affect oxygen consumption of the segments, osmotic potential of cell sap, and IAA-induced cell wall loosening as determined by a decrease in the minimum stress relaxation time (T_0). On the contrary, JA prevented an increase in the total amounts of cell wall polysaccharides in both the noncellulosic and cellulosic fractions during coleoptile growth. Moreover, JA-induced inhibition of oat coleoptile segment growth was partially recovered by the simultaneous addition of sucrose to the test solution. From the evidence described above, we suggested that the action mechanism of JA in the inhibition of oat coleoptile segment growth is associated with the sugar metabolism required for cell wall synthesis (Labavitch 1981, Taiz 1984), which is one of the important factors for continuous growth during the effect of IAA on promoting cell elongation (Baker and Ray 1965, Wada et al. 1968, Loescher and Nevins 1972, Sakurai and Masuda 1978, Nishitani and Masuda 1978).

Strenuous efforts to learn the mode of action of JA on the inhibition of oat coleoptile segment growth have been undertaken using [^{14}C]glucose. In this paper we demonstrate that JA effectively inhibits the incorporation of radioactivity into cell wall polysaccharides in both the noncellulosic and cel-

Abbreviations: JA, jasmonic acid; ABA, abscisic acid; IAA, indoleacetic acid; T_0, minimum stress relaxation time; TFA, trifluoroacetic acid; TCA, trichloroacetic acid; HPLC, high-performance liquid chromatography; EtOAc, ethyl acetate; TLC, thin-layer chromatography; JA-Me, methyl jasmonate; GLC-SIM, gas-liquid chromatography-selected ion monitoring.

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lulosic fractions, with almost no change in the level of UDP-sugars prior to the inhibition of IAA-induced elongation of oat coleoptile segments.

Materials and Methods

Plants

Oat (*A. sativa* L. cv. Victory) seeds were germinated and grown under weak red light for 2 days followed by additional growth in the dark for 2 days. After floating on distilled water for several h, 10-mm segments were prepared, as described previously (Ueda et al. 1994b). The segments were incubated with a test solution of 10 mM potassium phosphate buffer (pH 6.5) containing 10^{-5} M JA and/or IAA at 25°C in the dark. For the experiment described in Figure 2, the segments were pretreated with 10^{-5} M JA solution containing 30 mM sucrose for 4 h before the addition of 10^{-5} M IAA. Even after the addition of IAA, JA and sucrose were applied continuously to the test medium until the end of the incubation. All of the experiments except the incubation of the segments were made under a dim green light at 25°C. After the incubation, the segments were fixed in boiling methanol for the determination of cell wall polysaccharides. For the determination of UDP-sugars and the contents of conjugated JA in the segments, the segments were stored at −80°C before extraction.

Fractionation and Determination of Noncellulosic and Cellulosic Polysaccharides

Fractionation of trifluoroacetic acid (TFA)-soluble (noncellulosic) and TFA-insoluble (cellulosic) polysaccharides of oat coleoptile segments was carried out as described previously (Ueda et al. 1994b). Liberated sugars were reduced with sodium borohydride and acetylated with acetic anhydride (Albersheim et al. 1967). The amount of acetylated sugars was determined with a Hitachi model 163 gas-liquid chromatograph fitted with hydrogen flame ionizing detractor. The sugar contents of the cell wall polysaccharides in both the noncellulosic and cellulosic fractions were determined by the phenol sulfonic acid method with glucose as a standard (Dubois et al. 1956).

Incorporation of [^{14}C]Glucose into Cell Wall Polysaccharides

Thirty segments of oat coleoptiles were incubated in 3 ml of 10 mM potassium phosphate buffer (pH 6.5) containing 74 KBq of [^{14}C]glucose (specific activity 11.2 GBq/mmol) in the presence and absence of 10^{-5} M JA. After the incubation, segments were washed thoroughly with distilled water and then fixed with boiling methanol. According to the same procedure reported previously (Ueda et al. 1994b), TFA-soluble and -insoluble fractions were prepared as noncellulosic and cellulosic polysaccharide fractions. The radioactivity of each fraction was determined with a Packerd 460 liquid scintillation spectroscope using Scintisol EX-H (Wako Pure Chemical Industries). The inhibitory effect of JA on the incorporation of labeled glucose into each fraction was expressed as a percentage of the values stimulated by IAA.

Extraction, Purification, and Determination of UDP-sugars

The analysis of UDP-sugars in oat coleoptile segments was carried out according to Inouhe's method with modifications (Inouhe et al. 1987a). Sixty 10-mm segments of oat coleoptiles were homogenized with a mortar and pestle in 1 ml of 20% methanol solution containing 8% trichloroacetic acid (TCA) at 0°C. Homogenized materials were washed twice with the same solution. Homogenized materials and wash solutions were combined and then centrifuged. The supernatants were sufficiently washed several times with water-saturated diethyl ether to remove TCA. Aqueous residue containing UDP-sugars was frozen at −20°C and then lyophilized. The lyophilized material was dissolved in a small amount of distilled water and then centrifuged to remove impurities. UDP-sugar was determined using HPLC (Shimadzu model) with a Partisil 10-SAX anion exchange column (Whatman), according to the method already reported (Inouhe et al. 1987a).

Determination of JA and Its Conjugates in Oat Coleoptile Segments during Incubation with JA in the Presence and Absence of Sucrose

About 1,500 segments of oat coleoptiles that had been treated with 10^{-5} M JA in the presence and absence of 30 mM sucrose were harvested after a 4-h incubation at 25°C in the dark. Each segment was divided into half longitudinally with a razor blade. To remove JA not incorporated into coleoptiles but remaining bound to the surface of the segments, the divided segments were washed with *n*-hexane several times. Segments were homogenized with 80% ethanol using a glass homogenizer and extracted at 4°C in the dark. This procedure was repeated twice with fresh ethanol. Extracts were combined and evaporated in vacuo to give an aqueous residue. This aqueous solution was adjusted to pH 3 with HCl and then partitioned with ethyl acetate (EtOAc) three times (Yokota et al. 1980).

An EtOAc-soluble acidic fraction was concentrated to dryness and then purified with silica gel thin-layer chromatography (TLC) procedure using *n*-hexane:EtOAc:acetic acid (10:2:1,v/v/v) as the solvent system. The zone corresponding to R_f value of an authentic sample of JA was scraped off and then eluted with EtOAc.

The other half of the aqueous residue obtained after partition against EtOAc was hydrolyzed with 1 M NaOH at 60°C for 1 h and partitioned against EtOAc, followed by the purification using the TLC system as described above.

Either of the eluted fractions was methylated with etherial diazomethane. The contents of methyl jasmonate (JA-Me) in both fractions were estimated using gas liquid chromatography-mass spectrometry selected ion monitoring system (GLC-SIM) according to the same procedure reported previously (Ueda et al. 1991).

Results

Inhibition of IAA-induced Elongation of Oat Coleoptile Segments by JA and the Prevention by the Simultaneous Addition of Sucrose

JA substantially inhibited IAA-induced elongation of oat coleoptile segments after a lag period of 2-h incubation in the dark (Fig. 1). As the JA-induced inhibition had previously been shown to be partially antagonized by the simultaneous addition of sucrose (Ueda et al. 1994b), the effect of pretreating the coleoptiles with sucrose was examined. As shown in Figure 2, the JA-induced inhibition of

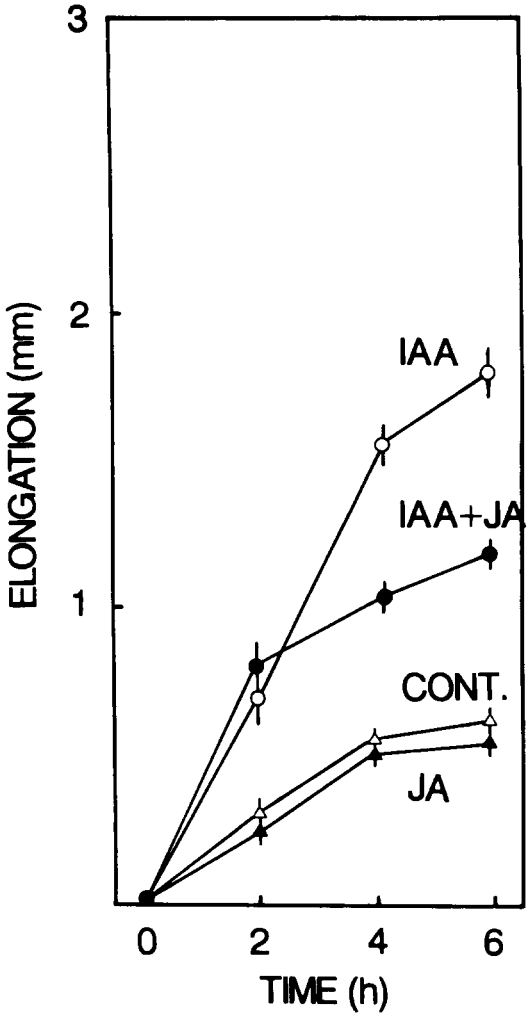

Fig. 1. Effects of JA on the IAA-induced elongation of oat coleoptile segments. Coleoptile segments (10-mm long) were incubated with 10^{-5} M and JA and 10^{-5} M IAA. Data are mean values with standard error (n = 15). *Cont.*, control.

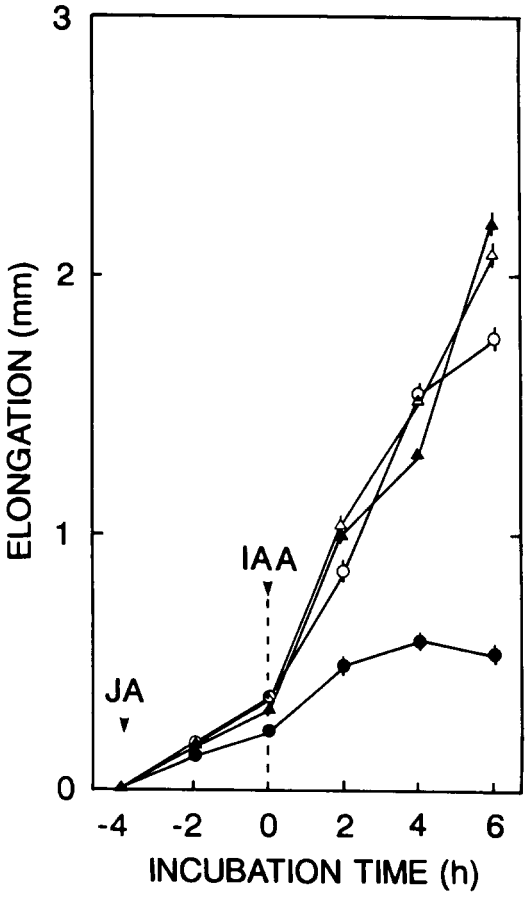

Fig. 2. Effects on growth of pretreating oat coleoptile segments with JA in the presence of sucrose before the addition of IAA. Oat coleoptile segments were incubated with 10^{-5} M JA either in the presence or in the absence of 30 mM sucrose for 4 h at 25 °C in the dark before the addition of 10^{-5} M IAA. JA and sucrose were used throughout the incubation. Data are mean values with standard error (n = 15). ○, control; ●, 10^{-5} M JA; △, 30 mM sucrose; ▲, 10^{-5} M JA + 30 mM sucrose.

growth of segments was completely reversed by the simultaneous application of 30 mM sucrose added to the test solution 4 h before the addition of IAA.

Effect of JA on the Total Amounts of Cell Wall Polysaccharides in Both the Noncellulosic Fraction and Cellulosic Fractions, and Neutral Sugar Compositions of Hemicellulosic Polysaccharides

In our previous paper (Ueda et al. 1994b), the total amounts of cell wall polysaccharides in both the noncellulosic and cellulosic fractions increased during coleoptile growth. IAA stimulated the increase in both polysaccharides fractions, whereas JA was inhibitory. In this experiment, JA at a concentration of 10^{-5} M also inhibited the increase of cell wall polysaccharides in both the noncellulosic and cellulosic fractions during coleoptile growth (Fig. 3).

As shown in Figure 4, neutral sugar compositions of hemicellulosic polysaccharides of oat coleoptile segments in 2- or 4-h incubations were not affected by 10^{-5} M JA in the presence and absence of IAA.

Determination of the Contents of Conjugated JA in the Segments Treated with JA in the Presence and Absence of Sucrose

The endogenous levels of JA and conjugated JA in oat coleoptile segments treated with 10^{-5} M JA in the presence and absence of 30 mM sucrose for 4 h were estimated by GLC-SIM analysis. As shown in

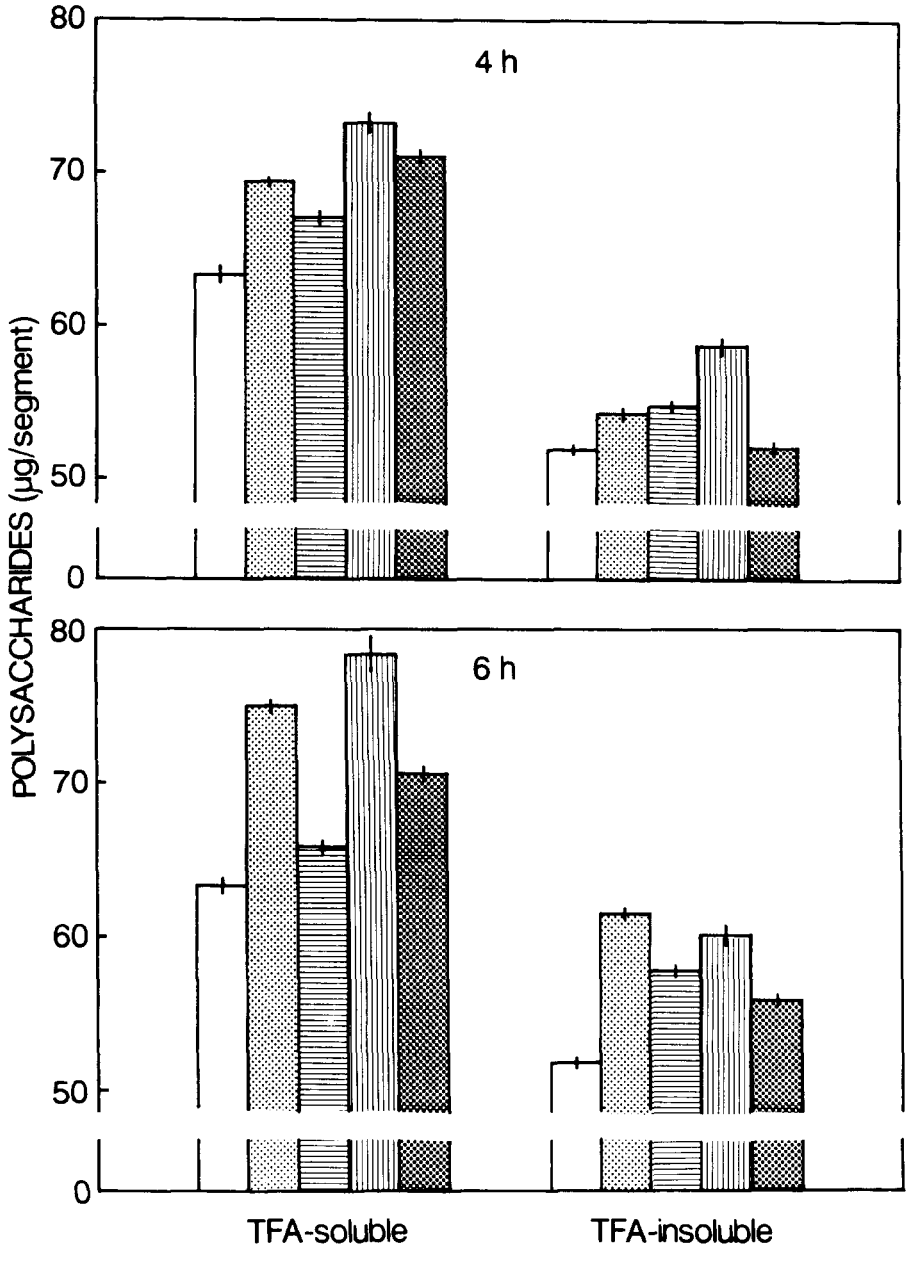

Fig. 3. Effects of JA on the sugar content in TFA-soluble and -insoluble fractions of oat coleoptile cell wall. Twenty coleoptile segments were incubated for 4 or 6 h in 10 mM potassium phosphate buffer (pH 6.5) containing 10^{-5} M JA and/or 10^{-5} M IAA. After TFA hydrolysis, the sugar content in TFA-soluble (noncellulosic) and TFA-insoluble (cellulosic) fractions was determined by the phenol-sulfuric acid method. *Bars* indicate the standard error of the mean (n = 3). □, initial; ⊡, control; ▤, 10^{-5} M JA; ▥, 10^{-5} M IAA; ▩, 10^{-5} M IAA + 10^{-5} M JA.

Table 1, the contents of JA conjugate in the segments were not affected by the addition of 30 mM sucrose. Moreover, a level of free JA sufficient to induce growth inhibition of the segments was found even in the segments treated with 30 mM sucrose.

Effect of JA on the Incorporation of [^{14}C]Glucose into Cell Wall Polysaccharides

Figure 5 shows the kinetics of the total incorporation of [^{14}C]glucose into oat coleoptile segments. IAA significantly stimulated the incorporation during coleoptile growth. JA slightly inhibited the IAA-stimulated incorporation into the segments.

Figure 6 shows the inhibitory effect of JA on IAA-induced stimulation of the incorporation into cell wall polysaccharides in both the noncellulosic and cellulosic fractions. After 2 h of the incubation, no inhibitory effect of JA on growth of oat coleoptile segments was observed. However, the incorporation of [^{14}C]glucose into the cellulosic fraction was already reduced by JA. After a 4-h incubation, JA had reduced extremely the incorporation of [^{14}C]glucose into cell wall polysaccharides in both the noncellulosic and cellulosic fractions. JA did not inhibit the uptake of labeled glucose into the

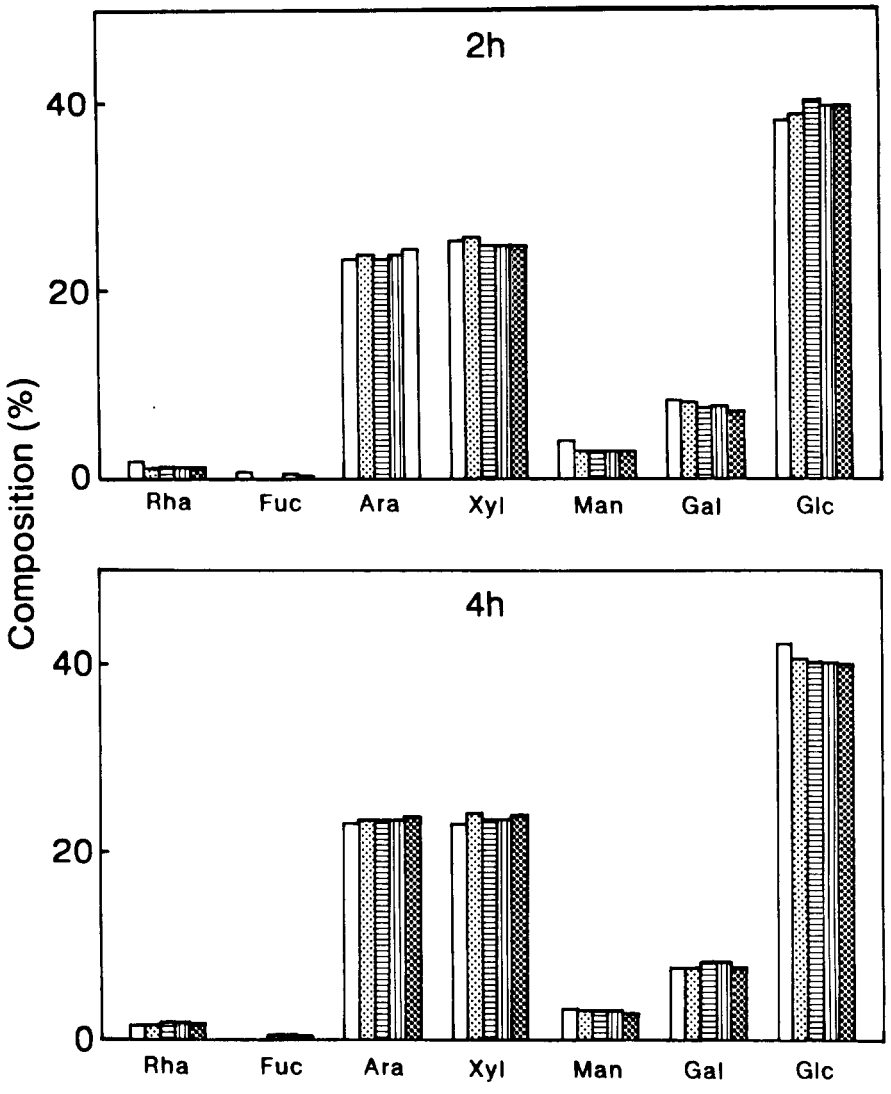

Fig. 4. Effects of JA on the composition of neutral sugars of matrix cell wall polysaccharides. Oat coleoptile segments were incubated with 10^{-5} M JA in the presence or absence of 10^{-5} M IAA at 25°C in the dark. Determination of the neutral sugar composition was made after 2 and 4 h of the incubation. *Bars* indicate the standard error of the mean (n = 3). □, initial; ⊡, control; ▤, 10^{-5} M JA; ▥, 10^{-5} M IAA; ▩, 10^{-5} M IAA + 10^{-5} M JA.

soluble fraction. On the contrary, JA had almost no effect on incorporation into cell wall polysaccharides in the absence of IAA (data not shown).

Effect of JA on the Levels of UDP-sugars and ATP of Oat Coleoptile Segments

Figure 7 shows the endogenous levels of UDP-sugars and ATP in oat coleoptile segments after 4 h of treatment in the dark. Neither the levels of UDP-sugars nor of ATP of oat coleoptile segments were reduced significantly by JA in the presence and absence of IAA.

Discussion

The cell walls of plants have fundamentally significant functions for plant growth and development. The quality and quantity of cell wall polysaccharides are among the important factors for cell elongation. The degradation and the synthesis of cell wall polysaccharides in relation to cell elongation induced by plant hormones have been studied extensively. When IAA induces cell elongation, two distinctive phases of the growth are involved (Vanderhoff et al. 1976). The first phase has been suggested to be due to the action of protons secreted in response to IAA, a phenomenon known as acid growth (Rayle 1973, Cleland and Rayle 1978, Vanderhoff and Dute 1981). The second phase is related to cell wall synthesis.

The observations in this and previous studies (Ueda et al. 1994b) indicated that JA inhibited substantially the increase of the total amounts of cell wall polysaccharides during oat coleoptile segment growth but had no effect on the composition of neutral sugars in hemicellulose. These results suggest that JA affects sugar metabolism in relation to the synthesis of cell wall polysaccharides.

Table 1. Levels of JA and conjugated JA in oat coleoptile segments.[a]

	JA (ng/segment)	
	Free form	Conjugated form
Control	8.92	1.28
30 mM sucrose	8.85	0.90

[a] Oat coleoptile segments were incubated with 30 mM sucrose in the presence or absence of 10^{-5} M JA for 4 h at 25°C in the dark. Measurements were made by GLC-SIM.

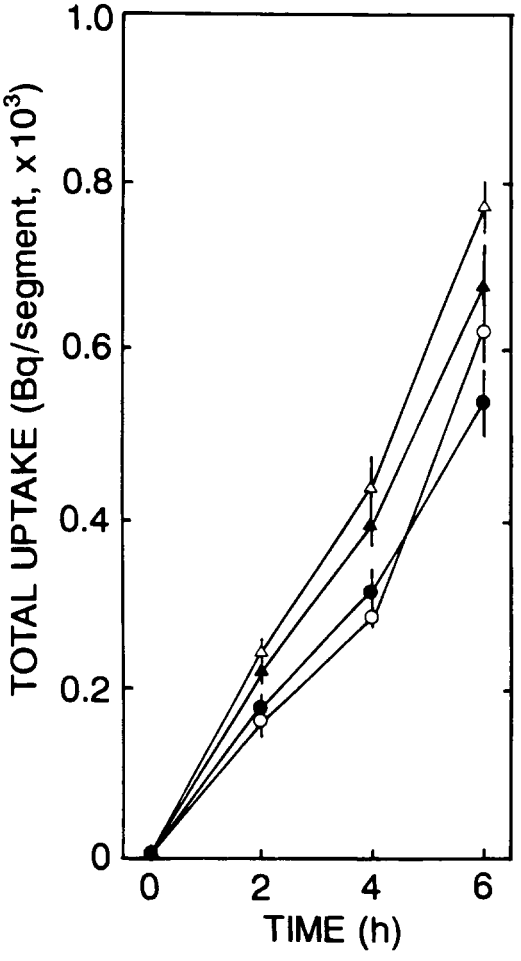

Fig. 5. Effects of JA on the incorporation of [^{14}C]glucose into oat coleoptile segments. Oat coleoptile segments were incubated with [^{14}C]glucose in the presence of 10^{-5} M JA and/or 10^{-5} IAA at 25°C in the dark. *Bars* indicate the standard error of the mean (n = 3). ○, control; △, 10^{-5} M IAA; ●, 10^{-5} M JA; ▲, 10^{-5} M IAA + 10^{-5} M JA.

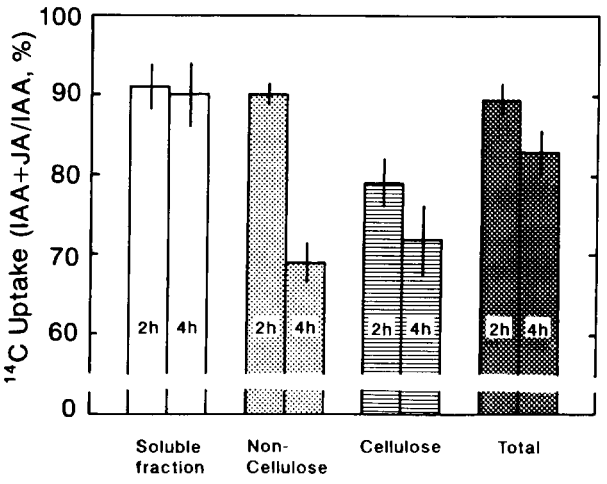

Fig. 6. Effects of JA on the incorporation of radiolabeled glucose into noncellulosic and cellulosic cell wall polysaccharide fractions in oat coleoptile segments stimulated by IAA. Oat coleoptile segments were incubated with [^{14}C]glucose in the presence of 10^{-5} M JA and/or 10^{-5} IAA at 25°C in the dark. *Bars* indicate the standard error of the mean (n = 3).

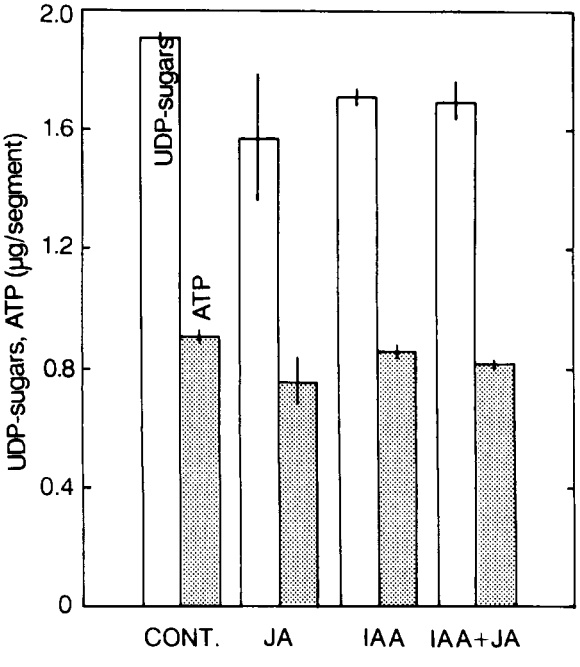

Fig. 7. Effects of JA on the levels of UDP-sugars and ATP in oat coleoptile segments. *Bars* indicate the standard error of the mean (n = 3). Analyses and determination of sugar nucleotides were performed as described in the Materials and Methods section.

The incorporation of [^{14}C]glucose into cell wall polysaccharides in both hemicellulosic and cellulosic fractions was reduced significantly by JA even during a 2-h incubation, a period during which JA-induced inhibition of growth of segments was not visible. Four h after the beginning of treatment, the incorporation of radioactivity into these polysaccharides fractions was reduced similarly to about 70% of IAA treatment. These results indicate that

the inhibition of the synthesis of cell wall polysaccharides of oat coleoptile segments by JA is considered not to be the consequence but the cause of the JA-induced inhibition of growth. In fact, the inhibitor of the synthesis of cellulosic polysaccharides, 2,6-dichlorobenzonitrile, inhibited IAA-induced elongation of coleoptile segments to a certain extent (data not shown).

UDP-sugars are very important key intermediates in the pathway of the synthesis of cell wall polysaccharides (Feingold and Avigad 1980), although the *myo*-inositol pathway is really engaged in some plant species (Loewus and Loewus 1983). The main components of UDP-sugars in oat coleoptiles have already been identified as being UDP-glucose (73%) and UDP-galactose (8%) (Inouhe et al. 1986). Treatment with galactose decreased the level of UDP-glucose and then inhibited IAA-induced cell elongation of oat coleoptile segments. Moreover, the simultaneous addition of sucrose with galactose resulted in the increase of the level of UDP-sugars in the segments, and thus galactose-induced growth inhibition was partially reversed (Inouhe et al. 1987a, 1987b). In the present study, however, JA did not reduce the level of UDP-sugars of oat coleoptile segments. A similar observation has already been made in the inhibitory effect of ABA on IAA-induced cell elongation and cell wall synthesis of the segments of squash hypocotyls (Wakabayashi et al. 1989, 1991). These authors suggested that the apparent inability of ABA to affect the level of UDP-sugars might be a result of the large pool size of sugar nucleotides in the hypocotyl segments. For a similar reason, JA did not influence the level of UDP-sugars in oat coleoptile segments.

The inhibitory effect of JA on IAA-induced elongation was eliminated completely by preincubating the segments with JA in the presence of sucrose before the addition of IAA. Glucose and fructose had an effect similar to that of sucrose, but other neutral sugars constituting cell wall polysaccharides did not (data not shown). These results strongly suggest that there are two possible mechanisms by which sucrose, glucose, or fructose could eliminate the inhibitory effect of JA on oat coleoptile growth. One possibility is the inactivation of JA by its conversion into a conjugated form with sugar moieties during the pretreatment with sucrose. Conjugated JA has been shown previously to be physiologically less active than free JA (Miersch et al. 1987). This explanation is not likely because the levels of the conjugated form of JA in oat coleoptile segments were almost same as those in the presence and in absence of sucrose. Moreover, considerably higher amounts of JA were present in the segments even in the pretreatment of sucrose. Another possibility is that sucrose interferes with the metabolism of non-cellulosic and cellulosic cell wall polysaccharides in JA-treated coleoptiles. Further studies using sucrose are required for the determination of the inhibitory site of JA in cell wall synthesis.

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