Effect of IAA on Growth and Soluble Cell Wall Polysaccharides Centrifuged from Pine Hypocotyl Sections

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ABSTRACT

Auxin-induced elongation and cell wall polysaccharide metabolism were studied in excised hypocotyl sections of ponderosa pine (Pinus ponderosa) seedlings. Sections excised from hypocotyls of ponderosa pine elongate in response to the addition of auxin. The neutral sugar composition of the extracellular solution removed from hypocotyl sections by centrifugation was examined. In cell wall solution from freshly excised sections, glucose, galactose, xylose, and arabinose make up more than 90% of the neutral sugars, while rhamnose, fucose, and mannose are relatively minor components. The neutral sugar composition of the polysaccharides of the pine cell wall solution is both qualitatively and quantitatively similar to that of pea. Following auxin treatment of pine hypocotyls, the neutral sugar composition of the cell wall changes; glucose, xylose, rhamnose, and fucose increase by nearly 2-fold relative to controls in buffer without auxin. These changes in neutral sugars in response to auxin treatment are similar to those found in pea, with the exception that in pea, rhamnose levels decline in response to auxin treatment.

Considerable attention has been focused on the role of the cell wall in the regulation of extension growth in angiosperms (5, 19). For auxin-controlled extension, there is overwhelming evidence that elongation is associated with changes in the mechanical properties of the cell wall of both dicots and monocots (5), and there is growing documentation that changes in cell wall metabolism accompany growth (7, 10, 12, 13). Despite the progress which has been made in our understanding of auxin-induced growth, our knowledge is limited to angiosperms. Although some aspects of gymnosperm development have been well studied (see reviews 16, 18), there is a paucity of information concerning the effects of auxin on growth and cell wall metabolism in this class of plants. Since xyleogluca has been reported in the cell walls of two pine species (4, 16) and it has been shown that xyleoglucan metabolism is related to IAA-induced growth (10, 11, 19), we have examined the possibility that IAA-enhanced growth in conifers is related to xyleoglucan turnover.

In this paper we report on the effects of IAA on growth and cell wall polysaccharide metabolism in excised sections of ponderosa pine hypocotyls. Cell wall metabolism was followed using a centrifugation technique to remove the solution from the free space of excised hypocotyl sections (22, 23) and gas chromatography to analyze the sugar components of the polysaccharides of this cell wall solution.

MATERIALS AND METHODS

Seeds of ponderosa pine (Pinus ponderosa, Pacific Southwest Forest and Range Experiment Station, Berkeley, CA) were surface sterilized in 5% Clorox for 30 min, rinsed with H2O and immersed in an aerated solution of 0.05% (w/v) Truban (5-ethoxy-3-trichloro-methyl-1,2,4-thiadiazole, Mallinkrodt, St. Louis, MO) for 2 d. Seeds were sown in moist vermiculite, placed in a cold room (4°C) for 1 week, and transferred to a dark chamber at 26°C. When the hypocotyls were 5 to 7 cm long (approximately 12 d), 1 cm-long sections were excised from the hypocotyls 2 to 3 mm below the cotyledons. Peas (Pisum sativum L. cv. Alaska) were grown and sections excised from the dark-grown seedlings as described previously (22). Excised hypocotyl and pea stem sections were vacuum infiltrated with ice-water and centrifuged as previously described (22). The H2O was replaced with 0.5 mM K2HPO4 containing 0.5 mM CaCl2 and 1% (w/v) sucrose with or without 17 μM IAA for the appropriate time. After the initial 30-min rinse period (time 0) or incubation in buffer sucrose + IAA, sections were vacuum infiltrated with ice-water and centrifuged as previously described (22). The infiltration and centrifugation were repeated twice and the resulting cell wall solutions combined. The cell wall solution was boiled for 5 min, dried under vacuum at 50°C, and made to 80% (v/v) ethanol. The precipitate was collected by filtration on a glass fiber filter (Whatman GF/A), hydrolyzed in 2N trifluoroacetic acid containing myo-inositol and acetylated as described by Albersheim et al. (1). Gas chromatography was performed under conditions similar to those described by Albersheim et al. (1) with linear temperature programming. Growth of excised sections was monitored by measuring the enlarged images of sections projected onto a flat surface (20).

All results reported are the average of three experiments, each using approximately 6 g tissue for each treatment.

RESULTS AND DISCUSSION

Excised sections of ponderosa pine hypocotyl elongated in response to the addition of IAA (Fig. 1). After 24 h incubation in IAA, hypocotyl sections increased in length by nearly 70% while those incubated in H2O elongated only 20%. Although auxins have been shown to stimulate radial growth of pine stems (3, 11, 14) and to promote cambial division (6), there have been a limited number of studies on the effects of auxin on elongation in this genus (17, 18). Our results support the observations of Hashizume (8, 9) who demonstrated that IAA promoted the elongation of excised hypocotyl sections of Pinus densiflora, Pinus thunbergii, and Pinus nigra. The demonstration of a mechanism for auxin
Fig. 1. The effect of IAA on elongation of 1-cm sections excised from hypocotyls of dark-grown ponderosa pine seedlings. The range in section lengths for the 8, 16, and 24-h periods was ±0.6, 0.9, and 2.3 mm, respectively.

Fig. 2. Gas chromatograms of the alditol acetate derivatives of the neutral sugars centrifuged from 1-cm sections excised from dark-grown pine hypocotyls and pea stems incubated in IAA (17 μM) for 8 h (pine) and 3 h (pea).

biosynthesis (11) together with observations of polar auxin transport (24) and auxin-enhanced growth in excised hypocotyl sections strongly implicate auxin in the control of elongation in Pinus seedlings.

Inasmuch as it is generally agreed that auxin-induced growth is accompanied by changes in the properties of the cell wall (5), we investigated the metabolism of cell wall polysaccharides in ponderosa pine using a centrifugation technique for removing H2O-soluble polysaccharides from the free space of the sections (22, 23). Because incubation of pine hypocotyl sections in 17 μM IAA for 8 h elicits a growth response equal in magnitude to that obtained when pea internode sections are incubated in the same solution for 3 h (data not shown), these incubation times were selected for a comparison of the effects of IAA on polysaccharide metabolism. The neutral sugar composition of the H2O-soluble, alcohol-insoluble polysaccharides removed from freshly excised hypocotyl sections of ponderosa pine is shown in Figure 2 and Table I. Glucose, xylose, galactose, and arabinose made up greater than 90% of the total neutral sugars of the H2O-soluble polysaccharides (Table I). The neutral sugar composition of the cell wall solution centrifuged from pine seedlings was similar to that obtained from peas (Fig. 2, Table I). In peas, glucose, galactose, xylose and arabinose made up more than 90% of the neutral sugars; however, although glucose was the predominant neutral sugar, galactose, and xylose occurred in nearly the same proportions (21.1 and 19.4%, respectively). The proportions of mannose, fucose, and rhamnose were similar in pine and pea cell wall solutions, but rhamnose made up a more significant fraction of the neutral sugars in the polysaccharides of pine (1.7%) than of pea (0.3%) (Table I). The water-soluble uronides were not assayed due to insufficient material.

Our data on the neutral sugar composition of the cell wall polysaccharides of pea internode and pine hypocotyl sections do not agree with those reported by Burke et al. (4) for suspension-cultured sycamore and Douglas fir. There are striking similarities, however, between the neutral sugar composition these authors report for isolated cell walls and that of our soluble material. Burke et al. (4) showed that glucose, galactose, xylose, and arabinose were the predominant neutral sugars found in the cell wall, and that mannose, rhamnose, and fucose occurred as minor components accounting for less than 10% (4).

Changes in the neutral sugar composition of the polysaccharides centrifuged from pine and pea sections were followed after incubation in auxin. After 8 h incubation in IAA, the ethanol-insoluble polysaccharides centrifuged from pine showed more (nearly 2-fold) rhamnose, fucose, xylose, and glucose than controls incubated in the absence of IAA (Table II). There was also more mannose and galactose (12 and 25%, respectively) in the cell wall solution of sections incubated in the presence of IAA, but the levels of arabinose in +IAA and −IAA treatments were about the same (Table II). In comparison to controls incubated in the absence of IAA, the cell wall solution centrifuged from pea internode sections after 3 h incubation in IAA showed enhanced levels of fucose, xylose, and glucose, but the levels of rhamnose, arabinose, mannose, and galactose were lower than in sections incubated in the absence of IAA (Table III).

Our previous experiments with peas (23) also document the effect of auxin on xylose and glucose, and we have demonstrated by cellulose binding that most of the xylose and a large fraction of the glucose are in a polymer having the properties of xyloglucan. We do not know whether the xylose and glucose isolated from pine bind to cellulose; however, the comparable and greater
amounts of xylose and glucose in +IAA compared to −IAA-treated pine tissue suggest that xyloglucan is an important cell wall polymer in this species also. Little et al. (15) have reported that the major hemicellulose of Pinus radiata is a fuco-galacto-xyloglucan, and Burke et al. (4) have reported the presence of a galactosylxyloglucan polymer in Douglas fir cell walls. These workers suggested that this polymer was structurally similar to sycamore xyloglucan except that the xylosyl residues possessed galactose attached at C-2 as well as having terminal galactosyl residues. Another or other glucans may also be present in pine, since the amount of glucose after 8 h incubation in IAA was less than in freshly harvested sections, whereas the amount of xylose increased in the presence of IAA (Tables I and II).

Considerable importance has been attached to the xyloglucan found in dicot cell walls, since Albersheim and his colleagues (2, 16, 21) ascribed an important structural role for this polymer. The importance of xyloglucan was further demonstrated by Labavitch and Ray (12, 13) and Jacobs and Ray (10), who showed that auxin (12, 13) and low pH (10) treatments would release a water-soluble xyloglucan from the cell wall of peas. Our results with the centrifugation technique have confirmed these observations, and the data presented in this paper indicate that in ponderosa pine auxin treatment leads to enhanced levels of xylose and glucose as well as rhamnose, fucose, and galactose.

**LITERATURE CITED**


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**Table II. Effect of IAA on the Neutral Sugars of the Alcohol-Insoluble Polysaccharides of the Cell Wall Solution Centrifuged from Excised Hypocotyl Sections of Pine**

Data are average of three experiments, each using approximately 6 g tissue. The +IAA/−IAA values varied by less than 5% among experiments. The largest variation in total amounts was in xylose, which varied by 17% among the three +IAA experiments.

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* Excised hypocotyl sections were incubated in 17 µM IAA or buffer for 8 h.

**Table III. Effect of IAA on the Neutral Sugars of the Alcohol-Insoluble Polysaccharides of the Cell Wall Solution Centrifuged from Excised Internode Sections of Pea**

Data are average of three experiments, each using approximately 6 g tissue. The +IAA/−IAA values varied by less than 5% among experiments. The largest variation in total amounts was in glucose, which varied by 7% among the three +IAA experiments.

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* Excised internode sections were incubated in 17 µM IAA or buffer for 3 h.