A NEW INTERMEDIATE IN PLANT CELL WALL SYNTHESIS

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SUMMARY

The synthesis of a soluble uridylic acid containing polysaccharide and of the insoluble pollen tube wall have been studied. The time course of incorporation of arabinose into the soluble and wall fractions and the disappearance of label from arabinose in the soluble fraction and its corresponding increase in the pollen tube wall in pulse-chase experiments, suggest that the soluble polysaccharide is a large precursor subunit of some component of the cell wall.

A uridine phosphate-polysaccharide complex is found in the cytoplasm of the pollen tube of Tradescantia. This complex has been characterized and described (10). It sediments in sucrose gradients with a major peak at about 16 S and a minor peak at about 4 S. When pollen tubes are labeled with ^{14}C-sucrose and the polysaccharide isolated from the cytoplasm, the principal labeled sugar is arabinose, with lesser amounts of label in galactose and some other minor unidentified sugars. Acid hydrolysis of the cytoplasmic polysaccharide releases 5'-UMP (10).

This communication describes the kinetics of formation of the cytoplasmic polysaccharide, and its incorporation into the insoluble pollen tube wall. Preliminary results have been briefly reported (7).

MATERIALS AND METHODS

Methods for collection of pollen of Tradescantia paludosa and growth conditions have been described (9). Sucrose-^{14}C(U)

(346 mc/mM) and myoinositol-2-^{3}H (both from New England Nuclear), were used for labeling. The labeling conditions are described in the legends to the figures in the text. Normal pollen growth medium contains 5% sucrose. For the pulse part of pulse-chase experiments, pollen was grown in a medium in which the 5% sucrose was replaced with 2% pentaerythritol. For lily pollen tubes pentaerythritol has been reported to be an adequate osmotic substitute for sucrose (4). A concentration of 2% pentaerythritol was found to be the optimum for Tradescantia pollen, although even at this concentration a small fraction of the tubes burst.

To isolate cytoplasmic and wall fractions, the pollen tubes were extracted with a 1% SDS (sodium dodecyl sulfate) solution in water. After cooling to precipitate out the SDS the extract was treated with 10 µg/ml of DNase and 100 µg/ml of pancreatic ribonuclease for 45 min. at room temperature to partly hydrolyze the DNA and RNA. This was followed by a treatment with 100 µg/ml of pronase. The suspension was centrifuged at 20,000 g for 10 min. to isolate the insoluble wall fraction from the soluble cytoplasmic fraction. The wall residue was extracted twice more with 1% SDS and the supernatants combined. Both fractions were made $>$ 70% in ethanol and kept at -20°C overnight. The pellets obtained after centrifugation at 20,000 g for 15 min. were washed twice with ethanol and used for further analysis of sugars. This procedure removed all traces of ^{14}C-sucrose contamination. SDS is not necessary for the extraction of the soluble polysaccharide. Homogenization of the pollen in a hypotonic Tris-HCl (pH 7.5) buffer is adequate (10). SDS was used to obtain wall fractions entirely free of cytoplasmic contamination.

The fractions were hydrolyzed in 2N trifluoro-acetic acid in sealed capillary tubes at 121°C for 40 min (1), and analyzed

by paper chromatography (Whatman No. 1) in an ethyl acetate - pyridine -H_2O (8:2:1) solvent system. Standard sugar spots were located with alkaline $AgNO_3$ reagent. The chromatograms were cut into strips of 0.5 or 1.0 cm and counted in an Ansitron scintillation spectrometer with a toluene scintillation fluid (Liquifluor, New England Nuclear Corp.).

RESULTS

Figure 1 shows chromatograms of the hydrolyzates of soluble cytoplasmic and insoluble wall polysaccharide fractions from pollen labeled continuously during the first 10 min of growth with ^{14}C-sucrose. The cytoplasmic polysaccharide contains labeled arabinose and galactose, but practically no labeled glucose. On the other hand, the insoluble wall contains a large

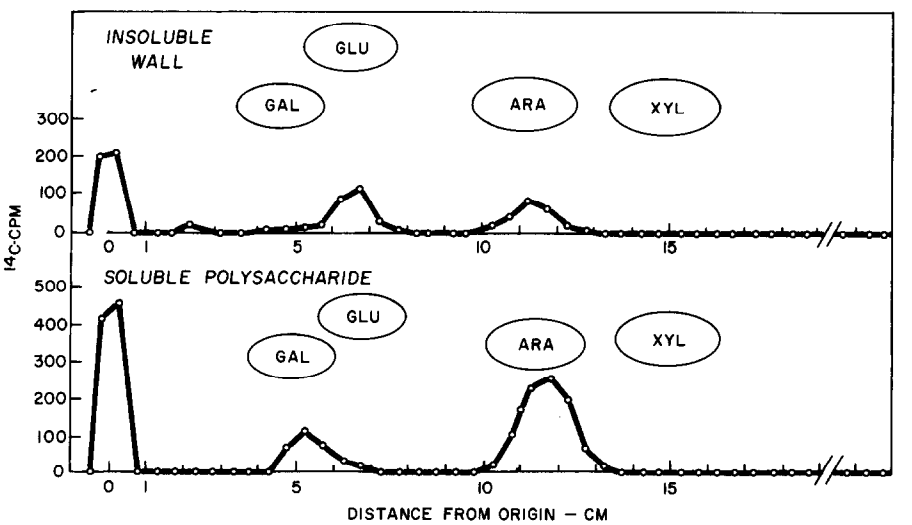

Figure 1. Distribution of radioactivity in chromatograms of TFA hydrolyzates of insoluble wall and soluble polysaccharide fractions. Pollen was grown for 10 min in growth medium with 5% sucrose containing in addition 12 μc/ml of ^{14}C-sucrose. The radioactivity in the soluble and wall fractions was determined as described under Materials and Methods. (GAL = galactose; GLU = glucose, ARA = arabinose; XYL = xylose).

amount of labeled arabinose and glucose but very little labeled galactose. The arabinose/galactose ratio is about 2 for the soluble material and about 9 for the wall. The large amount of labeled glucose present in the wall fraction could be due to the synthesis of other polysaccharide components of the wall, and, or, to the contamination of the wall fraction by starch grains.

Inositol has been shown to be a specific percursor of cell wall D-galacturonsyl and L-arabinosyl residues in pear pollen (16) and lily pollen (6). Accordingly ^{3}H-myo-inositol was tried out as a lable to see if the large amount of label in glucose polysaccharides in the wall could be eliminated. ^{14}C-sucrose was found to be a much more effective label as radioactivity was found in arabinose within two minutes, whereas, with inositol as label the arabinose residues were not labeled to any significant

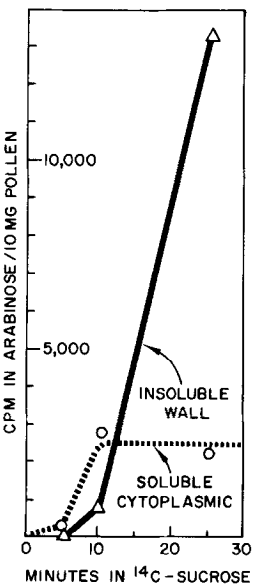

Figure 2. Time course of incorporation of radioactivity into arabinose of the cytoplasmic and wall fractions. Pollen was grown continuously in pollen medium with 5% sucrose containing in addition 12 µc/ml of ^{14}C-sucrose. At different times samples were taken and the radioactivity in the arabinose of the soluble and wall fractions determined as described under Materials and Methods.

extent for over 30 min. Hence sucrose was used as the label in all the experiments reported.

In the experiments described in Figures 2 and 3 the radioactivity in the arabinose of the polysaccharide fractions has been used as a measure of their synthesis. This is reasonable since arabinose is the principal labeled sugar in the soluble polysaccharide and is also present in large amounts in the wall.

In Figure 2 are shown the kinetics of formation of the cytoplasmic and wall fractions. The soluble fraction is synthesized soon after the start of incubation of pollen grains in the medium. Within two minutes, label is incorporated into the soluble material, and only after a lag of about 5 min, the time when pollen tubes are first beginning to be seen, does one see incorporation of the label into the arabinose of the wall. The kinetics of label incorporation into the soluble material and the insoluble pollen tube wall seem to follow a precursor-

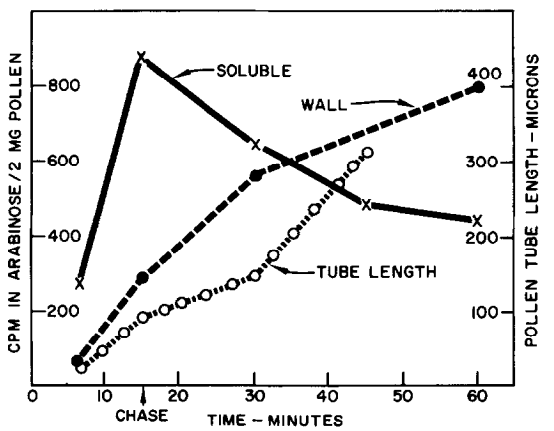

Figure 3. Pulse-chase experiment. Pollen was grown for 15 min in 2% pentaerythritol medium containing 1 μc/ml of ^{14}C-sucrose. An excess of 5% unlabeled sucrose medium was then added and the pollen was gently sedimented. The pellet was resuspended in fresh 5% sucrose medium for the chase. Aliquots were taken at the times shown and the radioactivity in the arabinose of the soluble and wall fractions determined as described under Materials and Methods.

product relationship. This experiment has been repeated three times and the kinetics follow a similar pattern.

In Figure 3 are shown the results of a pulse-chase experiment in which pollen was grown for 15 min in a 2% pentaerythritol medium containing 1 μc/ml of ^{14}C-sucrose. At the end of this time, the tubes were pelleted down and the medium replaced with 5% sucrose-containing medium for the chase. The radioactivity in the arabinose in the soluble fraction is seen to decrease very rapidly during the chase, whereas, that in the insoluble wall material continues to increase, although at a continuously decreasing rate. This experiment has been repeated twice with similar results.

In the experiments described here label has been added before the start of germination. The soluble polysaccharide, however, is synthesized not only during germination, but also at later times as shown by experiments in which labeling has been done at various times after germination.

When ^{3}H-uridine labeled cytoplasmic polysaccharide is hydrolyzed in 1N HCl for 15 min at 100°C, 5'-UMP is released (10). To see whether UDP is present, hydrolysis with 0.01M HCl for 10 min at 100°C was attempted. This treatment should release 5'-UDP from a UDP-sugar molecule (2). With 0.01M HCl hydrolysis, however, very little label is released. If one increases the acid concentration, although one obtains some radioactivity overlapping the region where UDP should travel on a chromatogram, there is a diffuse area where radioactivity is found. With increasing acid concentration or time of hydrolysis the label is finally all found in 5'-UMP. If UDP is at all present, it would appear that the linkage of UDP to a polysaccharide is more resistant to acid hydrolysis than the linkage of UDP to a sugar molecule, and as

the acid concentration is increased if any UDP is released it is very rapidly hydrolyzed to 5' UMP.

DISCUSSION

The data on the time course of incorporation of arabinose into the soluble and wall fractions (Fig. 2) and the disappearance of label from arabinose in the soluble fraction and its corresponding increase in the pollen tube wall (Fig. 3) suggest that the soluble polysaccharide is a precursor to some component of the pollen tube wall, possibly a neutral component. The increase in the arabinose/galactose ratio from about 2 for the soluble material to about 9 for the wall would indicate that some of the galactose is lost during the process of polymerization of the precursor into the cell wall. The significance of this is not at all clear at the present time.

The role of nucleotide diphosphate sugars in the synthesis of polysaccharides is well documented (5, 11). It is not known, however, how these polysaccharides which apparently are synthesized in the cytoplasm are put together to form the complex plant cell wall (7). Wall precursor material appears to be carried in vesicles as polysaccharide material and after fusion of the vesicles the precursor material is in some unknown manner further polymerized to form the wall (3, 12, 13, 15). If the wall precursor polysaccharide is carried in vesicles, some mechanism, enzymatic or other, must exist at the site of cell wall synthesis to further polymerize the polysaccharides into the cell wall.

My working hypothesis at the present time is that the 16 S cytoplasmic material is a UDP-polysaccharide complex. It is synthesized in the pollen cytoplasm and then transported to the pollen tube tip region where several such molecules are further polymerized, the UDP active ends providing the necessary energy,

to form at lease one component of the cell wall. Such a mechanism for the biosynthesis of large polysaccharides has not yet been described, although in bacterial wall polysaccharide synthesis, reasonably large precursors are involved (14).

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