CHARACTERIZATION OF INTERMEDIATES IN PLANT CELL WALL BIOSYNTHESIS[1]

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SUMMARY: Results are presented of a partial chemical characterization of membrane bound intermediates in the biosynthesis, from GDPM-^{14}C, of P. aureus cell wall polysaccharide. Two types of intermediates appear to participate: (1) a lipid derivative of a monosaccharide, similar to that which mediates M. lysodeiticus mannan biosynthesis; and (2) a low molecular weight protein derivative of an oligosaccharide.

Glycolipids are known to be intermediates in the synthesis of bacterial cell wall polysaccharides (1-3). An intermediate of similar lipid structure is involved in the synthesis of Micrococcus lysodeiticus mannan (4) and may be involved in mannan biosynthesis in yeast (5) and higher plants (6). We have reported evidence which indicated that particle bound material played a part in the synthesis of plant cell wall polysaccharides (7). The radioactive material that had the properties of an intermediate could be extracted with 45% phenol from P. aureus particulate preparations which had been reacted with radioactive sugar nucleotides. This communication provides information on the chemical nature of the radioactive compounds in the phenol extract which are formed from GDPM-^{14}C.

METHODS

The particulate enzyme system from Phaseolus aureus was prepared and reacted as described previously (7). The reactions were stopped by adding trichloroacetic acid to a final concentration of 5%. A few mg. of cellulose powder were added to serve as a carrier. The water soluble material was

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removed by 2 extractions with water at 0°, the insoluble material being collected by centrifugation. The water insoluble residue was then suspended in 0.5 ml of butanol-saturated water, and this was extracted twice with equal volumes of water-saturated butanol. The butanol phases (Component I) were combined, backwashed with water, and preserved at -20° for further analysis. The aqueous phase with the butanol insoluble residue was then extracted with two 0.5 ml portions of 90% phenol at 0°. The phenol phases were combined, washed with water, and dialyzed against 2 l of distilled water for 24-36 hours. The contents of the dialysis bag, which included a precipitate, were homogenized by sonic oscillation and lyophilized. The dried powder (Component II) was stored at -20° for further analysis. The radioactive material contained in the powder could be partially solubilized by stirring with aqueous solutions of surface active agents or concentrated urea solutions.

RESULTS AND DISCUSSION

We reported previously (7) that following dialysis of the 45% phenol extract from enzyme preparations which had been reacted with GDPM-^{14}C, a large amount of the water-insoluble radioactivity was found to be soluble in 1:1 chloroform-methanol. Additional experimentation revealed that this chloroform-methanol extractible material had chemical properties different from that material which remained insoluble. Also, kinetic and reversibility data suggested that the phenol extractible material contained more than one chemical component. The phenol extractible radioactive material formed from GDPM-^{14}C could be separated into two relatively homogeneous fractions by various chromatographic procedures. However, an easier method of fractionation was devised. This was based on the observation that one of the components (henceforth called I in this report) could be extracted from the water-insoluble portion of the original reaction mixture (providing the reaction mixture had not been dried prior to extraction) with 1:1 chloroform-methanol or with water-saturated butanol. The other component (henceforth called II in this report) which was insoluble in lipid solvents or water, could be extracted with 45% phenol, as before, but also

with the following aqueous solutions: 6M pyridinium acetate (pH 4.2), Triton X-100, Triton N-101, sodium dodecyl sulfate, sodium cholate, sodium deoxycholate Both components exhibited kinetic and other properties consistent with their being intermediates in plant cell wall polysaccharide biosynthesis.

Component I - This mannolipid has properties similar to material reported previously (4,6). The radioactive lipid appears to be a polyisoprenol phosphate derivative of mannose. The experimental results suggesting that this is the case are: (1) the material consists of one radioactive component as evidenced by a single peak of radioactivity upon column chromatography on Bio Sil A, DEAE-cellulose, or upon thin-layer chromatography on silica gel G and silica gel H. This Phaseolus aureus mannolipid has chromatographic properties identical to that of a sample of Micrococcus lysodeiticus mannolipid (4) provided by W. J. Lennarz, Johns Hopkins School of Medicine.

(2) Quantitative hydrolysis occurs at pH 2 when the solution is heated at 100° for 15 min., all of the radioactivity being water soluble following the reaction. Paper chromatography of this water-soluble radioactive material with 10:4:1 ethyl acetate-pyridine-water results in a single radioactive peak which has a chromatographic mobility identical to that of D-mannose. If this radioactive peak is eluted from the paper, reduced with sodium borohydride, and chromatographed on paper with 9:1:1 butan-2-one-acetic acid-water (saturated with boric acid), a single radioactive peak with a chromatographic mobility identical to that of D-mannitol results. Therefore, the only radioactive sugar moiety in the compound is D-mannose. Also, the mildness of the hydrolytic conditions precludes the possibility of a substantial hydrolysis of sugar-sugar glycosidic linkages. Therefore, the D-mannose moiety is linked to the lipophilic portion of the molecule as a monosaccharide.

(3) Partial acid hydrolysis, pH 2 for 1 min at 100°, results in the production of a small amount of a radioactive component that has the electrophoretic properties of D-mannose 1-phosphate. This implies that the D-mannose moiety is linked to the lipophilic portion of the molecule through at least one phosphate group.

(4) The mannolipid is stable to mild alkaline hydrolysis under conditions reported by Sher, Lennarz, and Sweeley (7). This suggests that the linkage between the D-mannose moiety and the lipophilic portion of the molecule is a phosphodiester, rather than a pyrophosphate, linkage.

(5) The enzymic reaction forming the mannolipid from GDPM-^{14}C is reversed quantitatively by 0.001 M GDP, but not by GMP. The formation of undecaprenol pyrophosphate saccharide derivatives (1-3) is reversed by nucleotide monophosphates, as opposed to that of Micrococcus undecaprenol phosphate mannose (8) which is reversed by GDP. The reversibility data is, therefore, consistent with the D-mannose moiety of the P. aureus mannolipid being linked via a phosphodiester linkage to the lipophilic moiety.

(6) Preliminary results of mass spectral analysis of the lipophilic moiety resulting from pH 2 hydrolysis of the partially purified mannolipid gives, in addition to other peaks, a pattern of fragments differing in mass by about 14 units, and another pattern, superimposed, differing in mass by about 70 units. The largest fragment visible in the spectra had a mass of about 600. Due to the small quantity of material available, and the resulting limitation on extent of purification, these results can only be suggestive. However, these preliminary data are consistent with the lipophilic portion of the P. aureus mannolipid being a long chain isoprenoid.

Component II - This material appears to be an oligosaccharide derivative of a low molecular weight membrane protein. The experimental evidence indicating that this is the case is: (1) Solubility data - insoluble in lipid solvents; insoluble in water or weak salt solutions; soluble in phenol, 6M pyridinium acetate (pH 4.2), 8M urea, aqueous solutions of Triton X-100, Triton N-101, sodium dodecylsulfate, sodium cholate, and sodium deoxycholate.

(2) Relatively rapid mobility in agarose gel electrophoresis in a buffer consisting of 20% dimethyl formamide in 0.01 M acetate/acetic acid, pH 4.0. Migration was towards the negative pole indicating a positively charged compound at pH 4.0.

(3) Treatment of Component II with Pronase, a bacterial protease, results in the production of radioactive, low molecular weight, water soluble compounds. This is a consistent product of proteolysis for a protein covalently linked to a radioactive saccharide. After mild acid hydrolysis of this mixture of water soluble compounds (0.04 N HCl, 30 min., 100°), paper chromatography (2:1:2 ethyl acetate-acetic acid-water) resulted in a single peak of radioactivity with a mobility comparable to cellotetrose.

(4) Complete hydrolysis of the radioactive portion of Component II with 1N HCl for one hour at 100° produces D-mannose as the only radioactive product.

(5) A determination of molecular weight, using the sodium dodecyl sulfate - acrylamide gel electrophoresis method (9-10), indicates a molecular weight of about 11,500 for component II.

When the P. aureus enzyme preparation is incubated with GDPG-^{14}C, only one type of intermediate is formed. There is no radioactive component with the properties of Component I formed from GDPG-^{14}C. However, an intermediate with all the properties of Component II, i.e. a low molecular weight glycoprotein, appears to mediate the polymerization of glucose from GDPG.

All sugar nucleotides that we have used as precursors in the Phaseolus aureus particulate enzyme system, i.e. UDP-xylose, UDP-arabinose, UDP-glucose, UDP-glucuronic acid, UDP-galactose, GDP-glucose, GDP-mannose, form phenol soluble compounds. For some of them, like GDPM and UDPG, compounds of types I and II are formed. For others, like GDPG and UDPGal, only type II compounds are formed. We have not examined the nature of these intermediates from the remaining sugar nucleotides yet.

The full implications of the role of these two components in the mechanism of plant cell wall formation must await further information. But, the functional analogy, suggested by the structural similarity of component I, to the glycolipids which are involved in bacterial cell wall biosynthesis, is impossible to avoid. Also, the presence of a membrane protein (Component II) as a carrier in the

polymerization process provides an attractive working hypothesis. It would obviate the necessity for a "primer" polysaccharide, and could also provide a source of orientation for the positioning of the completed polysaccharide molecule in the developing cell wall.

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