Biosynthesis of Cellulose in Vitro from Guanosine Diphosphate d-Glucose with Enzymic Preparations from Phaseolus aureus and Lupinus albus*

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SUMMARY

The incorporation of d-glucose-14C from guanosine diphosphate d-glucose-14C into a β-1,4-linked polymer (cellulose) is catalyzed by a particulate preparation from Phaseolus aureus and Lupinus albus. The particulate enzyme preparations from both plants can be solubilized by extraction with digitonin; the solubilized proteins are also active in synthesizing β-(1 → 4)-glucan as the original particulate preparations. The reaction reaches half the maximal incorporation in less than 1 min.

Upon degradation of the polymer, β-(1 → 4)-radioactive cellohexitols are formed. However, a small proportion of mannose-14C is also obtained, indicating the presence of an epimerase which interconverts d-glucose and d-mannose. A glycolipid, δ, apparently, not involved as an intermediate in the biosynthesis of the β-(1 → 4)-glucan from GDP-d-glucose.

It was also shown that a soluble enzyme preparation from peas and extracts from mung bean seedlings or leaves of spinach, buckwheat, mustard, and parsley contain pyrophosphorylase or pyrophosphorylases capable of forming 14C-labeled GDP-d-glucose from GTP and α-d-glucose 1-phosphate (4).

On the basis of these results, the formation of cellulose in plants was postulated to take place by the following two reactions.

\[
\text{GTP} + \alpha-\alpha\text{glucose 1-phosphate} \xrightarrow{\text{pyrophosphorylase}} \ \text{GDP-d-glucose} + \text{PP}_i \\
\text{acceptor-(β-1,4-d-glucose)}_n + n(\text{GDP}) \\
\]

From the results in this laboratory it appeared that, when GDP-d-glucose is used as substrate, enzymic plant preparations produce a glucan consisting only of β-(1 → 4)-glucosyl linkages, while under similar conditions a β-(1 → 3)-linked glucan is synthesized from UDP-d-glucose (5).

However, Brummond and Gibbons (6) reported that a particulate enzyme preparation from Lupinus albus is capable of incorporating d-glucose-14C from UDP-d-glucose-14C into a heterogeneous product “consisting of polysaccharides of sizes ranging from water soluble to insoluble in alkali.” The alkali-insoluble fraction (about 7%) appeared to be a polymer consisting of d-glucose residues combined by β-(1 → 4)-glucosidic linkages.

Villemez, Franz, and Hassid (7) described a modified particulate preparation from Phaseolus aureus which synthesized an alkali-insoluble glucan from UDP-d-glucose containing both β-(1 → 4)- and β-(1 → 3)-linkages. This glucan was similar to the alkali-insoluble glucan reported by Ordin and Hall (8) that was produced from UDP-d-glucose with a particulate enzyme preparation from Avena sativa which appeared to contain similar mixed linkages.

However, all of these investigators are in agreement that, when GDP-d-glucose is used as substrate, an alkali-insoluble glucan is formed which contains exclusively β-(1 → 4)-glucosidic linkages.

There were, however, minor dissimilarities in the experimental conditions of the enzyme preparations used by Brummond and Gibbons (6) and by Villemez et al. (7) in comparison with those originally used by Barber, Elbein, and Hassid (2).

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The apparent inconsistencies in the results of the various groups of workers who claim to have obtained synthesis of β-(1 → 4)-glucose-linked polysaccharides from UDP-n-glucose as substrate have already been investigated (9). It was found that the conditions modified from those used by Barber et al. (2) for biosynthesis of cellulose are not responsible for the production of a glucan from UDP-n-glucose containing β-(1 → 4)-linkages. Careful repetition of the work under the respective conditions did not substantiate the results of these investigators (6, 7). UDP-n-glucose produced a glucan consisting entirely of β-(1 → 3)-glucosyl linkages (9).

The present paper reports further studies pertaining to the type of linkage in the polymer synthesized from GDP-D-glucose by particulate enzyme preparations and with active digitonin-solubilized extracts from *P. aureus* and *L. albus*. The kinetics of the reaction at various substrate concentrations and the effect of alkali treatment on the chemical structure of the polymer were also investigated.

**Experimental Procedure**

**Materials and Methods**

GDP-D-glucose, uniformly labeled in the D-glucose moiety, 194 µCi per µmole, was purchased from International Chemical and Nuclear Corporation, Burbank, California. Samples of uniformly labeled, chemically synthesized GDP-D-glucose, uniformly labeled and GDP-α-mannose-14C were kindly provided by Dr. A. Filbin. Cellohexitosides were prepared by acetolysis of cellulose (10). Radioactive emissions, unless otherwise specified, were counted in vials in Liquifluor (New England Nuclear) in a Nuclear-Chicago model Mark I liquid scintillation counter.

**Solvents Used for Separation of Hydrolysis Products**

The degradation products were separated by paper chromatography on Whatman No. 1 paper using 1-propanol-ethyl acetate-water, 7:1:2 (Solvent 1), 1-butanol-pyridine-water, 6:4:3 (Solvent 2), ethyl acetate-pyridine water, 8:2:1 (Solvent 3), and ethyl acetate-acetic acid-formic acid-water, 18:3:1:4 (Solvent 4).

**Enzyme Preparations**

The procedures used for the preparation of the particulate enzymes were essentially those described by Barber et al. (2) and by Brummond and Gibbons (6), with minor modifications.

Barber et al. (2) grew the *P. aureus* seedlings for 3 to 4 days. The particulate enzyme, obtained by grinding in Tris buffer (0.1 M, pH 7.5), was centrifuged at 20,000 × *g* and assayed in Tris buffer with low concentrations of 2.5 × 10^-4 M of sugar nucleotide. The insoluble polymer was isolated by heating the separated particles and residual sand. The 1,000 × *g* supernatant liquid was free of radioactivity, four washings of water by centrifugation until all soluble radioactivity had been removed. The radioactivity in the residual polymer was determined by counting in a Nuclear-Chicago gas flow counter, model C-110B.

The method of Brummond and Gibbons (6) for the preparation of an active particulate enzyme from *L. albus* was slightly modified by centrifuging the pulp at 1,000 × *g* to precipitate coarse particles and residual sand. The 1,000 × *g* supernatant liquid was then recentrifuged at 20,000 × *g* for 30 min, and the pellet was resuspended in Tris buffer (0.05 M, pH 8.0) and centrifuged again. (Brummond and Gibbons used a pellet which was obtained by centrifugation between 10,000 and 140,000 × *g* for 2 hours.) The resulting pellet was resuspended again in 0.5 ml of 0.05 M Tris buffer, pH 8.0, for each 30 g of seedlings, and was used as the particulate enzyme.

**Digitonin Treatment of Particles**

The particles prepared by Barber et al. (2) were suspended in Tris-HCl buffer (0.1 M, pH 7.5) and stirred gently with an equal volume of digitonin (1.6% solution in 0.05 M sucrose in water) for 20 min at 5°C. The mixture was diluted with 3 volumes of 0.4 M sucrose in the same Tris-HCl buffer and centrifuged at 34,000 × *g* to give "digitonin residue" and "solubilized enzyme." Both the digitonin residue and the solubilized enzyme proved to be active for the synthesis of cellulose polymer when GDP-D-glucose was used as substrate.

**Partial Acid Hydrolysis of Polymer**

Partial degradation of the polymer was accomplished by acid hydrolysis and acetolysis.

**Acid Hydrolysis**—The polymer was suspended in concentrated hydrochloric acid to which an equal volume of fuming HCl (saturated aqueous solution of HCl at 0°C) was added. The mixture was left for 2 to 3 hours at room temperature (5°C). The HCl was removed by evaporation under reduced pressure several times with water at an external temperature below 40°C, and the resulting oligosaccharides were separated by paper chromatography in Solvent 1.

**Acetolysis**—The acetolysis was carried out with minor modifications according to the procedure of Wolfrom and Thompson (10). The dry material was dissolved in a mixture of glacial acetic acid, acetic anhydride, and concentrated sulfuric acid. The acetolysis was allowed to proceed at room temperature for 90 to 100 hours, and after the mixture was cooled to 4°C it was neutralized with NaHCO₃. The mixture was allowed to remain at room temperature for 1 to 2 hours and extracted with chloroform. The chloroform extract was dried with anhydrous CaCl₂ and reduced in a vacuum to dryness. The acetylated oligosaccharides were deacetylated with freshly prepared sodium methoxide and separated by paper chromatography in Solvent 1.

**Oxidation of Oligosaccharides**

The oligosaccharides obtained from the hydrolysis of the glucans were oxidized with lead tetraacetate according to the method of Charlon and Perlin (13) as modified by Felmgold, Neufeld, and Hassid (5). The reacting D-glucose unit of a β-(1 → 4)-linked disaccharide (celllobiose) on oxidation produces erythrose, while the reducing moiety of laminaribiose, in which the D-glucose units are combined through a β-(1 → 3)-linkage, yields D-arabinose.
The particulate enzyme was prepared from P. aureus seedlings as described in the text. Each mixture contained 10 μl of enzyme (0.033 mg of protein), 0.25 μmole of MgCl₂, 2.5 μmoles of Tris-HCL buffer, pH 7.5, and GDP-d-glucose-¹⁴C (concentration indicated below) in a final volume of 50 μl. The tubes were incubated at room temperature for 1 min and the reaction was terminated by placing the tubes in a boiling water bath for 2 min. Each reaction mixture was applied to Schleicher and Schuell analytical filter paper, 589 Orange Ribbon paper and chromatographed for 36 hours in ethyl alcohol-1.0 M ammonium acetate, pH 3.8 (211). The chromatograms were cut into paper strips (5 × 0.5 cm) and counted in toluene with Liquid fluor in a liquid scintillation counter. The radioactive peak at the origin of the chromatogram was taken as the amount of polymer synthesized. The values reported in parentheses for the radioactivity incorporated into the polymer were obtained by treating a duplicate set of reaction mixtures with 5 ml of 2% NaOH in a boiling water bath for 5 min, followed by filtration on Whatman glass fiber filter paper (2.4 GF/C). The residue on the filter paper was washed with 30 ml of water, dried, and counted in toluene in a liquid scintillation counter.

### TABLE I

<table>
<thead>
<tr>
<th>Concentration of GDP-d-glucose-¹⁴C</th>
<th>GDP-d-glucose-¹⁴C Added</th>
<th>Incorporated</th>
<th>d-Glucose-¹⁴C Incorporated per 0.033 mg of protein per min</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>cpm</td>
<td>μg/μl</td>
<td></td>
</tr>
<tr>
<td>6.8 × 10⁻⁷</td>
<td>2,000</td>
<td>1,200</td>
<td>4.1a</td>
</tr>
<tr>
<td>1.7 × 10⁻⁶</td>
<td>5,000</td>
<td>4,110</td>
<td>4.8a</td>
</tr>
<tr>
<td>3.4 × 10⁻⁶</td>
<td>10,000</td>
<td>4,180</td>
<td>5.6a</td>
</tr>
<tr>
<td>6.8 × 10⁻⁶</td>
<td>20,000</td>
<td>4,180</td>
<td>5.4a</td>
</tr>
<tr>
<td>1.0 × 10⁻⁵</td>
<td>54,000</td>
<td>4,180</td>
<td>19.2</td>
</tr>
<tr>
<td>1.5 × 10⁻⁴</td>
<td>81,000</td>
<td>4,180</td>
<td>22.1</td>
</tr>
<tr>
<td>2.0 × 10⁻⁴</td>
<td>108,000</td>
<td>4,180</td>
<td>33.1 (32.4)</td>
</tr>
<tr>
<td>2.5 × 10⁻⁴</td>
<td>135,000</td>
<td>404 (375)</td>
<td>37.2 (34.5)</td>
</tr>
<tr>
<td>3.0 × 10⁻⁴</td>
<td>162,000</td>
<td>525 (408)</td>
<td>48.3 (37.5)</td>
</tr>
</tbody>
</table>

* These values represent synthesis of polymer after 1 min as described in the legend of Fig. 1.

### RESULTS

#### Kinetics

The incorporation of radioactive d-glucose from GDP-d-glucose-¹⁴C into water-insoluble polymer was shown to be extremely rapid. The reaction reaches half the maximal incorporation in less than 1 min (Fig. 1); because of this, it is not possible to determine the initial velocity of the reaction and the reliable Kₘ for the enzyme. The amount of polymer synthesized from GDP-d-glucose-¹⁴C and the amounts of remaining substrates during the course of the reaction with four different initial substrate concentrations are also shown in Fig. 1.

A 10-fold increase in substrate concentration (6.8 × 10⁻⁷ M to 6.8 × 10⁻⁴ M) did not produce a significant increase in the incorporation of d-glucose into the polymer. No linear relationship was observed even when the substrate concentration was raised to 3.0 × 10⁻⁴ M (Table I).

The extent of the reaction is proportional to the quantity of enzyme used (Fig. 2). Similar results were obtained with the particulate enzyme prepared from L. albus.
TABLE II
Effect of stepwise addition of enzyme on incorporation of glucose-\(^{14}\)C from GDP-D-glucose-\(^{14}\)C into water-insoluble polymer

The enzyme was prepared from \(P.\) aureus as described in the text. A total volume of 50 \(\mu\)l of solution containing \(10^{-4}\) \(\text{m}\) GDP-D-glucose-\(^{14}\)C (62,500 cpm) and 10 \(\mu\)l of enzyme (0.2 mg of protein) was incubated for 15 min, another 10 \(\mu\)l of enzyme were then added, and the mixture was incubated for 30 min longer. In the control, 10 \(\mu\)l of enzyme were incubated for 30 min under the same conditions. Radioactivity in the water-insoluble polymer was assayed as described in the text.

<table>
<thead>
<tr>
<th>GDP-D-glucose-(^{14})C added</th>
<th>GDP-D-glucose-(^{14})C incorporated with 20 (\mu)l of enzyme added stepwise; 10 (\mu)l of enzyme for 30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>cpm</td>
<td>cpm</td>
</tr>
<tr>
<td>62,500</td>
<td>422</td>
</tr>
<tr>
<td>202</td>
<td></td>
</tr>
</tbody>
</table>

FIG. 3. Degradation of water-insoluble polymer with fuming HCl. The enzyme from \(P.\) aureus was prepared as described in the text. The incubation mixture consisted of 50 \(\mu\)l of enzyme (1 mg of protein), 5.0 \(\mu\)moles of Tris-HCl buffer, pH 7.5, and 1 \(\mu\)m GDP-D-glucose-\(^{14}\)C (180,000 cpm) in a total volume of 100 \(\mu\)l. The mixture was allowed to react at room temperature for 30 min. The polymer was isolated as described in the text and then hydrolyzed with fuming HCl-concentrated HCl (1:1) for 2 hours at room temperature. The resulting oligosaccharides were separated by paper chromatography in Solvent 1.

FIG. 4. Degradation of water-insoluble polymer by acetolysis. The polymer was prepared with the particulate enzyme preparation from \(P.\) aureus as described in Fig. 3. The acetolysis of the water-insoluble polymer and deacetylation of the oligosaccharides were carried out as described in the text. The oligosaccharides were separated by paper chromatography in Solvent 1.

FIG. 5. Degradation by acetolysis of polymers resulting from incubation of GDP-D-glucose-\(^{14}\)C (1 \(\mu\)m, 50,000 cpm) with different enzyme systems. The enzymes were prepared as described in the text. The polymers were prepared and subjected to acetolysis as described in Fig. 3. The oligosaccharides were separated by paper chromatography in Solvent 1. •----•, water-insoluble polymer produced by \(P.\) aureus enzyme; •----•, water-insoluble polymer produced by \(L.\) albus enzyme; •----•, alkali-insoluble polymer produced by \(P.\) aureus enzyme.

The possibility of the presence of an inhibitor in the reaction was eliminated by incubating samples of \(10^{-4}\) \(\text{m}\) GDP-D-glucose with 10 \(\mu\)l of enzyme for 15 min (after which time there was no further incorporation of D-glucose into insoluble glucan), and then the addition of a further, equal, quantity of enzyme, followed by a second 15-min period of incubation. The incorporation of radioactivity in the polymer obtained was double the amount produced when 10 \(\mu\)l of enzyme were incubated under similar conditions for 30 min (Table II).

Chemical Structure of Polymers—It was found that the use of hydrochloric acid gave poor degradation of the polymers, even on prolonged treatment. Paper chromatography of the products (Solvent 1) revealed glucose, cellobiose, and cellotriose, but about 80% of the radioactivity resided in nonmigrating material (Fig. 3). Acetolysis (10), however, gave a more satisfactory degradation (Fig. 4); celldextrins up to cellotetraose could be identified chromatographically. The cellotetraose peak was identified by prolonged chromatography of the slower migrating material in Solvents 1 and 2. The minor amount of nonmigrating material (<25%) was degraded further by treatment with 1 \(N\) HCl for 15 min at 100°, which produced identifiable oligosaccharides. The spectrum of the oligosaccharides that appeared on chromatography was essentially the same as those obtained from the water-insoluble polymers synthesized by enzyme preparations from either \(P.\) aureus or \(L.\) albus (Fig. 5); it did not vary for substrate concentrations of \(10^{-4}\) \(\text{m}\) and \(10^{-5}\) \(\text{m}\). Treatment of the polymers with hot alkali removed approximately 10 to 15% of the radioactivity (counts per minute) but apparently did not affect the chemical structure, as shown by acetolysis and examination of the resulting oligosaccharides (Fig. 5). The peaks migrating similarly to glucose and cello-
TABLE III

Recrystallization of cellobiose-14C

To radioactive cellobiose isolated from the degradation of the polymer shown in Fig. 5, 20 mg of recrystallized cellobiose (Pfahns-tiel) were added as carrier and the mixture was recrystallized from ethanol-acetone.

<table>
<thead>
<tr>
<th>No. crystallizations</th>
<th>Cellobiose precipitate</th>
<th>Evaporated mother liquor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>139</td>
<td>139</td>
</tr>
<tr>
<td>2</td>
<td>130</td>
<td>132</td>
</tr>
<tr>
<td>3</td>
<td>135</td>
<td>139</td>
</tr>
</tbody>
</table>

dextrins (glucose), \(n = 2\) to 4 which were eluted from the paper could not be distinguished from genuine markers in Solvent 2 and 4. The eluted material of the cellobiose peak co-crystallized with authentic cellobiose, and there was no decline in specific activity over three recrystallizations (Table III).

Lead tetraacetate oxidation (13) of the eluted oligosaccharides, followed by sodium borohydride reduction and complete acid hydrolysis, yielded the following results: the cellobiose peak (1100 cpm) produced glucose (507 cpm) and erythritol (264 cpm); the cellotriose peak (2700 cpm) gave glucose (1900 cpm) and erythritol (412 cpm); and the cellotetraose peak (2000 cpm) yielded glucose (1514 cpm) and erythritol (200 cpm).

Periodate oxidation of an eluted cellotriose peak (3705 cpm), followed by sodium borohydride reduction and subsequent acid hydrolysis with 1 N HCl, produced only glycerol (780 cpm) and erythritol (1540 cpm). There was no indication of arabinitol production from either of the oxidation procedures employed, thus showing the absence of any (1 \(\rightarrow\) 3)-glycosyl linkages.

Apart from the peaks thus identified as cellobiose, cellotriose, and cellotetraose, chromatography in Solvent 1 revealed several additional peaks in low proportion. The fastest of these peaks, migrating between glucose and cellobiose, had the same mobility in Solvents 1 and 2 as laminaribiose (D-glucosyl-\(\beta\)-(1 \(\rightarrow\) 3)-D-glucose). However, in Solvent 4, this peak was distinguishable from laminaribiose, with \(R_L = 1.1\) (\(R_L = R_{laminaribiose}\)). An additional small peak was obtained in Solvent 1, migrating between cellobiose and cellotriose. On the basis of chromatographic mobility, the faster intermediate peak was assumed to be a disaccharide and the slower a trisaccharide. An indication of the possible nature of these peaks was afforded by the observation that a small amount of radioactive mannose was liberated by acetylation of the polymer. The insoluble material produced by incubation of enzyme with GDP-D-glucose-14C in the presence of GDP-D-mannose or yeast cofactor gave increased quantities of "intermediate trisaccharide and disaccharide" on acetylation, with the production of an additional peak of \(R_L = 0.93\), as well as that of \(R_L = 1.1\), in Solvent 4. Finally, the polymer produced from GDP-D-glucose-14C in the presence of an equal amount of GDP-D-mannose-14C gave increased amounts of the peaks with \(R_L = 0.93\) and 1.1 in Solvent 4. Acid hydrolysis of the intermediate disaccharides (\(R_L = 1.1\) and \(R_L = 0.93\) in Solvent 4) and trisaccharide peaks gave glucose and mannose. The mannose had a much lower specific activity than the glucose, except in the case of polymers produced in the presence of GDP-D-mannose-14C. Sodium borohydride reduction followed by acid hydrolysis of the trisaccharide and the disaccharide of \(R_L = 1.1\) (Solvent 4) yielded mannitol at the reducing ends of the oligosaccharides, whereas glucitol was obtained from the di-
saccharide with $R_L = 0.93$. Thus it appears that the disaccharide of $R_L = 1.1$ is glucosyl mannosone, while that with $R_L = 0.93$ is mannosyl glucose. Partial acid hydrolysis of the mixed trissaccharide produced cellulose and the disaccharide with $R_L = 1.1$, indicating that the trissaccharide is $\beta-D$-glucosyl-(1 $\rightarrow$ 4)-D-glucosyl-D-mannose. In order to determine the particular linkage of the glucosyl moieties in the mannoe units, the disaccharide of $R_L = 1.1$ was subjected to selective oxidation. Periodate oxidation of the disaccharide of $R_L = 1.1$ produced from the polymer, which was synthesized from GDP-D-mannose-14C, followed by sodium borohydride reduction and acid hydrolysis, gave glycerol (318 cpm) and erythritol (400 cpm). Thus, the disaccharide with $R_L = 1.1$ is $D$-glucosyl-(1 $\rightarrow$ 4)-D-mannose and the disaccharide with $R_L = 0.93$ is $D$-mannosyl-D-glucose. Lack of material precluded the proof of the point of linkage between the mannose and glucose in the mannosyl glucose with $R_L = 0.93$.

Since these results indicate the possible presence in the particulate preparations of an enzyme system which incorporates mannosone into the glucan, attempts were made to separate the glucan-synthesizing activity from that which incorporates mannosone. Storage of the particular enzyme for varying lengths of time at 5° caused considerable decline in enzymic activity but did not appear to affect significantly the chemical structure of the polymers produced on incubation with GDP-D-glucose (Fig. 6).

**Effect of Digitonin—**Active soluble preparations could be obtained by extraction of the particulate enzyme preparations from *P. aureus* and *L. albus* with digitonin. The polymers synthesized from GDP-D-glucose with the digitonin extracts and the digitonin-treated residual particles revealed a similarity in pattern to oligosaccharides obtained by acetolysis and by paper chromatography of decarboxylated oligosaccharides in Solvent 1 (Fig. 7). The enzymic activity toward GDP-D-glucose increased slightly per unit of protein present (Fig. 2), both in the digitonin extracts and in the residual digitonin-extracted particles. This finding appears to be in contrast to the observed large increase in activity when UDP-D-glucose-14C was used as a substrate (9).

**Search for Glycolipid Intermediate—**Particulate enzyme was incubated separately with GDP-D-glucose at $10^{-3} M$ and $10^{-4} M$ concentrations. The water-insoluble precipitates were extracted by hot alcohol and then with chloroform-methanol, 2:1, and the organic extracts were combined. After evaporation, the residues were dissolved in Liquifluor and the radioactivity was determined by liquid scintillation counter. There was negligible incorporation of radioactivity in the organic phase.

**DISCUSSION**

The inability to obtain a linear initial velocity-time graph does not appear to be due to lack of substrate in the reaction mixture. With initially low concentrations of $6.8 \times 10^{-7} M$ and $1.7 \times 10^{-6} M$ GDP-D-glucose-14C, no further synthesis of polymer occurs after 2 min, and the corresponding amounts of GDP-D-glucose-14C present in the reaction mixtures at that time are 12.5 and 16.5%, respectively. However, with higher initial substrate concentrations, such as $6.8 \times 10^{-6} M$, the synthesis of the polymer after 2 min proceeds at a very low rate, even though 22% of the initial amount of GDP-D-glucose added is still present in the reaction mixture at that time (Fig. 1).

The assumption of production of an inhibitor during the reaction would not explain the impediment of further incorporation of D-glucose into the insoluble polysaccharide, because when an equal quantity of enzyme was added stepwise to the reaction mixture, followed by a 15-min incubation period, the radioactivity in the polysaccharide was found to be double the amount of that which is obtained when one-half of the quantity of enzyme is incubated for 30 min (Table II).

The incorporation of D-mannose into the polymer is shown by the isolation of mixed glucose-mannose disaccharides and a mixed trissaccharide. The addition of GDP-D-mannose causes an increased incorporation of glucose-14C from GDP-D-glucose-14C, and the polymer produced is, apparently, of much longer chain length (1). Possibly, the presence of D-mannose is necessary to continue chain elongation, and this may be correlated with the fact that a pure cellulose is not usually found in the growing roots or shoots of higher plants (15), whereas a common component is mannosone.

The possibility of a glucolipid intermediate being involved in the biosynthesis of the polymer (16) could not be substantiated, since extraction of the water-insoluble material with organic solvents resulted in the solution of negligible amounts of radioactive material. It may be concluded that either a glucolipid is not formed in the system, or that it has a very high turnover rate, thus preventing the isolation of appreciable quantities of radioactive glucolipid. If the second possibility applied, the lack of isolation of glucolipid at $10^{-3} M$ substrate concentration, at which concentration very little insoluble glucan is formed, would indicate that the lipid, too, is formed optimally at very low substrate concentrations.

**Acknowledgment—**We thank Dr. Tin-Yin Liu for his technical assistance in some of the analytical work.

**REFERENCES**

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