Formation of UDP-Xylose and Xyloglucan in Soybean Golgi Membranes

Received for publication December 2, 1987 and in revised form February 9, 1988

TAKAHISA HAYASHI1, TORU KOYAMA, AND KAZuo MATSUDA2
Department of Agricultural Chemistry, Tohoku University, Sendai 980, Japan

ABSTRACT

Soybean (Glycine max) membranes co-equilibrating with Golgi vesicles in linear sucrose gradients contained UDP-glucuronate carboxy-lyase and xyloglucan synthase activities. Digitonin solubilized and increased the activity of the membrane-bound UDP-glucuronate carboxy-lyase. UDP-xylose did not inhibit the transport of UDP-glucuronate into the lumen of Golgi vesicles but repressed the decarboxylation of the translocated UDP-glucuronate. The results suggest that UDP-glucuronate is transported into the vesicles by a specific carrier and decarboxylated to UDP-xylose within the lumen. On incubation of UDP-[14C]glucuronate with Golgi membranes in the presence of UDP-glucose, [14C]xylose-labeled xyloglucan was formed. Although the Km value of UDP-glucuronate for the decarboxylation was 240 micromolar, the affinity of UDP-glucuronate for xyloglucan formation (31 micromolar) was similar to that of UDP-xylose (28 micromolar), suggesting a high turnover of UDP-xylose. The biosynthesis of UDP-xylose from UDP-glucuronate probably occurs in Golgi membranes, where xyloglucan subsequently forms from UDP-xylose and UDP-glucose.

Sugar nucleotides are known to function as substrates for the biosynthesis of polysaccharides in higher plants. Among UDP-sugars, UDP-xylose has been found only in trace amounts in the cellular pool (9, 15, 25). Nevertheless, the primary cell walls of dicot plants contain a considerable amount of xylose in xyloglucan (28). A mechanism must therefore be envisioned which supplies a sufficient amount of UDP-xylose to the biosynthetic system of xyloglucan.

In a previous paper (13), we demonstrated that a particulate enzyme system from soybean cells catalyzes the transfer of the xylosyl residue from UDP-xylose and of the glucosyl residue from UDP-glucose to xyloglucan. Xyloglucan synthesized in vitro in the absence of GDP-fucose (5) is composed of heptasaccharides (glucose/xylose, 4:3) and pentasaccharides (glucose/xylose, 3:2), and the elongation of the chain proceeds through the transformation of pentasaccharide into heptasaccharide (16). The incorporation of xylose into the polysaccharide was enhanced by the presence of UDP-glucose, presumably because the addition of xylose and glucose residues to the polysaccharide is concurrent (14). In suspension-cultured soybean cells, the concentration of UDP-xylose (2.5 μM) is far lower than that of UDP-glucose (103 μM) (15). In order to achieve efficient elongation of their xyloglucan chains, it is possible that plant cells may possess a precise compartmentation of the UDP-xylose biosynthetic pathway which is such that the cellular pool of this sugar nucleotide is considerably higher at site of polysaccharide synthesis.

Golgi membranes are known to contain xyloglucan glucosyltransferase (14, 23), and xylosyltransferase activities (4, 14, 23). When GDP-fucose is provided to the microsomes, the last step in the completion of the xyloglucan structure is the synthesis of the nonasaccharide subunit by galactosylation and fucosylation, and this reaction occurs in dictysomes before transport of the polysaccharide to the cell wall by secretory vesicles (4). It is therefore expected that UDP-xylose is either efficiently transported to the dictysomes or that the biosynthesis of this sugar nucleotide occurs as part of the xyloglucan synthase system in the membranes.

UDP-xylose is derived from UDP-glucose by the action of UDP-glucose dehydrogenase (EC 1.1.1.22) (27) and UDP-glucuronate carboxy-lyase (EC 4.1.1.35) (1). A particulate fraction from mung bean seedlings (8) contains a UDP-glucuronate carboxy-lyase activity which can be solubilized with 0.5% digitonin. Furthermore, John et al. (18) reported that the UDP-glucuronate carboxy-lyase of chondrocyte cells is part of a multienzyme complex of glycosyltransferases involved in mucopolysaccharide biosynthesis. It is thus possible that in plants a UDP-glucuronate carboxy-lyase could be part of the dictysome-associated xyloglucan glycosyltransferases complex. The present communication examines the localization of UDP-xylose biosynthesis in soybean cells and the in vitro formation of xyloglucan from UDP-glucuronate-derived UDP-xylose.

MATERIALS AND METHODS

Materials. UDP-[U-14C]glucuronate (307 Ci/mol), UDP-[U-14C]xylose (269 Ci/mol), and Aquasol were obtained from New England Nuclear. Nonradioactive UDP-sugars were from Sigma, and Avicel was from Asahikasei. An endoglucanase of Trichoderma viride cellulase preparation was obtained from Seikagaku Kogyo, Japan. The Aspergillus oryzae enzyme preparation was a generous gift from Prof. A. Endo (Tokyo University of Agriculture and Technology, Japan). This enzyme mixture is capable of hydrolyzing noncellulosic polysaccharides into monosaccharides and isoromoverose (6-O-α-D-xylopyranosyl-D-glucose), in which all of the xylosyl residues of xyloglucan are recovered (12).

Soybean Membrane Preparations. Soybean seedlings (Glycine max) were grown in darkness for 6 d. and 1-cm segments were excised from the elongating region of hypocotyls. The tissue (50 g) was chopped at 2°C with razor blades in the presence of 50 ml of 50 mM Hepes/KOH (pH 7.0), 0.4 mM sucrose, 10 mM KCl, 1 mM MgCl2, 1 mM EDTA, and 1 mM DTT. The homogenate was filtered through nylon and centrifuged at 1000g for 10 min to remove cell-wall debris and nuclei.

The separation of organelles by sucrose gradient centrifugation was done according to the method described by Ray (23). The 1,000g supernate (about 9 ml per gradient) was layered onto 20 ml linear, 20 to 55% (w/v) sucrose gradients made up in 50 mM
Hepes/KOH (pH 7.0), 0.1 mM MgCl₂, 1 mM EDTA, and 1 mM DTT in 2.5 x 8.8 cm Ultra-Clear tubes. The gradients were centrifuged 3 h at 130,000g in the SW 28 rotor of a Beckman ultracentrifuge (model L5). Each gradient was divided into 22 fractions (1.7 ml), and densities were determined by refractometry. The fractions containing 14 to 36% (v/w) sucrose possessed a major peak of turbidity and were pooled and diluted with half a volume of homogenization buffer. Membranes from these fractions were pelleted by centrifugation for 30 min at 100,000g and resuspended in 100 mM Hepes/KOH (pH 7.0) and 0.4 mM sucrose. NADH-Cyt c reductase activity was determined by the procedure of Lord (19), latent IDPase activity by the procedure of Green (10), and Cyt c oxidase activity by the method of Moore and Proudlow (21).

**Assay for UDP-Glucose Dehydrogenase.** UDP-glucose dehydrogenase activities were determined by the procedure of Strominger and Mapson (27). The reaction mixture contained 100 mM glycine/NaOH (pH 8.7), 1 mM NAD, and soybean enzyme in a total volume of 0.5 ml. The reaction was started by the addition of 1 mM UDP-glucose, and the increase in absorbance at 340 nm due to NADH production was followed in a Hitachi double-beam spectrophotometer (model 200-10) fitted with the appropriate blank. Incubation was carried out at 25°C in a 0.5 ml, 1 cm light path cuvette. One unit of enzyme activity was defined as the amount of enzyme required to give an increase in optical density of 0.001/min under standard conditions.

**Assay for UDP-Glucuronate Carboxy-Lyase.** UDP-glucuronate carboxy-lyase activities were measured by the procedure of John et al. (17). The incubation mixture contained 1 mM UDP-[¹⁴C]glucuronate (9.8 x 10⁵ cpm/μmol), 1 mM NAD, 100 mM Hepes/KOH (pH 7.0), and soybean membranes in a total volume of 0.1 ml. The incubation was carried out for 20 min at 25°C. Into each assay tube was placed a smaller tube containing 0.2 ml of a mixture of ethanolamine and 2-methoxyethanol, 1:2 (v/v), and the larger tube was stoppered with a serum stopper with rubber septum. The reaction was terminated by introducing 0.2 ml of 2 M HCl with a needle through the stopper into the reaction mixture. The reaction mixtures were kept at 25°C for an additional 2 h. Trapped [¹⁴C]CO₂ in the ethanolamine/2-methoxyethanol tube was then determined in a liquid scintillation counter using a scintillator fluid containing 5.5 g/L of 2,5-di-phenyloxazole in 2-methoxyethanol/toluene, 1:2 (v/v). Enzyme assays were found to be proportional to the protein concentration employed. One unit of enzyme activity corresponds to 1 pmol of CO₂/min under standard conditions.

**Assay for Xyloglucan Formation from UDP-Xylose.** Xyloglucan 6-α-xylosyltransferase activity was determined as previously described (13). The incubation mixture contained 2 μM UDP-[¹⁴C]xylose (5.4 x 10⁴ cpm/μmol), 2 mM UDP-glucose, 100 mM Hepes/KOH (pH 6.8), 10 mM MnCl₂, and soybean membranes in a total volume of 50 μl. Incubation was carried out for 20 min at 25°C. The reaction was terminated by heating in a boiling water bath for 5 min. Fifty μl of carrier soybean xyloglucan solution (containing 100 μg of xyloglucan) and 1 ml of 95% ethanol were added to the mixture, and the whole solution was mixed well and centrifuged. The supernatant was carefully removed, and the precipitate was washed with three 0.5-ml portions of 70% ethanol. The precipitate was suspended in 1 ml of 50 mM potassium acetate buffer (pH 5.0) containing 1 mg of A. oryzae enzyme preparation, covered with a few drops of toluene to prevent bacterial growth, and incubated for 12 h at 40°C. The reaction was stopped by heating in a boiling water bath for 5 min. The mixture was then deionized with Amberlite IR-120 (H⁻) and concentrated to dryness in vacuo. The residue was dissolved in 0.5 ml of water and chromatographed on Whatman No. 3MM filter paper with solvent A. The area corresponding to isopimemerovose on the paper chromatogram was excised and counted in 5 ml of toluene scintillator. Enzyme activity was expressed as 1 pmol of xylose incorporated into isopimemerovose/20 min under standard conditions.

**Transport Assay of Sugar Nucleotides.** Transport of sugar nucleotides was measured as reported by Sommers and Hirschberg (26). The specific activity of the sugar nucleotides was adjusted to 200,000 cpm/ml and 1 μM in an assay volume of 1 ml in HKM buffer (10 mM Hepes/KOH, 150 mM KCl, 1 mM MgCl₂ [pH 7.0]) containing 1 mg of Golgi protein. Following incubation of the reaction mixture for 5 min at 25°C, the reaction was stopped by cooling the samples on ice followed by centrifugation in a Ti 50 rotor at 100,000g for 30 min at 4°C. The pellets were surface-washed three times, each with 1 ml of ice-cold HKM buffer. Pellets were then suspended in 70% ethanol (2 ml), sonicated for 1 h in an ultrasonic bath at room temperature, and the mixture put on ice for 15 min. An aliquot of the supernatant was removed for determination of soluble radioactivity/mg protein in pellets. For determination of ethanol-insoluble radioactivity, the sonicated pellets were filtered on Whatman GF/C glass fiber filters, washed three times with ice-cold 66% ethanol, and the filters were counted in Aquasol.

**General Methods.** Paper chromatography of UDP-sugars was done on Whatman No. 3MM filter paper with ethanol/1 M sodium acetate (pH 7.0), 7:3 (solvent A) and ethanol/butan-2-one/0.5 M morpholinium tetraborate in 0.1 M EDTA (pH 8.6), 7:2:3 (solvent B) (6). Paper chromatography of sugars was performed with n-propanol/ethyl acetate/water, 3:2:1 (solvent C) or with n-butanol/pyridine/water, 6:4:3 (solvent D). Reducing sugars were detected on the chromatograms by treatment with alkaline silver nitrate (24). Protein was determined by a modification of the Lowry assay for membrane proteins (20). Radioactivity on the paper was located with an Aloka thin-layer chromatogram scanner type JTC-201 and the label in eluted regions of chromatograms was determined with a Packard Tri-Carb model 3385 liquid scintillation spectrometer.

**RESULTS AND DISCUSSION**

**Enzyme Distribution in Soybean Membranes.** Since xyloglucan synthesis is maximal during the formation of the primary cell walls, we investigated the elongating region of soybean hypocotyls. As shown in Table I, the activity of xyloglucan xyosyltransferase was associated entirely with the 100,000g sediment of soybean extracts. The UDP-glucose dehydrogenase activity was observed only in the supernatants. This distribution is similar to that reported in peas (27), suggesting that plant UDP-glucose dehydrogenase is a soluble enzyme. Most of the UDP-glucuronate carboxy-lyase activity was soluble (70% of total activity), but when membranes were prepared by homogenization with sand or with liquid nitrogen, the UDP-glucuronate carboxy-lyase activity in the 100,000g sediment became equal to that of the supernatant. This suggests that the enhanced activity is due to an increased exposure of membrane-associated enzymes to the substrate.

To confirm the association of UDP-glucuronate carboxy-lyase with the particulate fraction, soybean extracts were subjected to isopycnic centrifugation and membranes were fractionated on a linear sucrose gradient (23). Markers were located in the following regions: NADH-Cyt c reductase (endoplasmic reticulum) at a density of 1.11 g/cm³; IDPase (Golgi) at a density of 1.15 g/cm³; Cyt c oxidase (mitochondria) at 1.18 g/cm³. The major protein peaks in the gradient were located in regions occupied by Golgi and mitochondria. Both xyloglucan synthase and UDP-glucuronate carboxy-lyase activities co-equilibrated with Golgi membrane fractions (Fig. 1). The results strongly suggest that UDP-glucuronate carboxy-lyase activity is associated with Golgi membrane vesicles.

Soybean membranes were also fractionated by the procedure...
Table I. Distribution of UDP-Glucose Dehydrogenase, UDP-Glucuronate Carboxylase, and Xyloglucan Synthase in the Homogenates of Soybean Hypocotyls

The homogenate obtained was centrifuged for 30 min at 100,000 g, and the resulting pellet was resuspended in 0.1 M Hepes/KOH buffer (pH 7.0) containing 0.4 M sucrose.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Volume</th>
<th>Protein*</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ml</td>
<td>mg</td>
<td>UDPG DH&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Homogenate</td>
<td>12.0</td>
<td>25</td>
<td>7300</td>
</tr>
<tr>
<td>100,000g supernatant</td>
<td>11.1</td>
<td>15</td>
<td>7450</td>
</tr>
<tr>
<td>100,000g pellet</td>
<td>0.8</td>
<td>9</td>
<td>40</td>
</tr>
</tbody>
</table>

* From 10 g tissue.  
<sup>b</sup> UDP-glucose dehydrogenase.  
<sup>c</sup> UDP-glucuronate carboxy-lyase.  
<sup>d</sup> Xyloglucan synthase.

Although glucan synthase II activity was observed in the 1.2/1.4 M sucrose interface.

Some Properties of Membrane-Associated UDP-Glucuronate Carboxy-Lyase. The activity of membrane-associated UDP-glucuronate carboxy-lyase was linear with time for at least 30 min. The enzyme had an optimum pH range from 6.0 to 7.5 and its apparent $K_m$ value, determined according to Lineweaver and Burk plots, was approximately 240 µM. By opposition, the $K_m$ value for the soluble enzyme was about 700 µM, indicating a higher affinity of the membrane-associated enzyme for UDP-glucuronate.

It has been reported that plant and animal UDP-glucuronate carboxy-lases are allosterically inhibited by UDP-xylose (17, 18). Likewise, in our system, UDP-xylose inhibited the membrane-associated UDP-glucuronate carboxy-lyase. Such feedback inhibition probably functions to control levels of UDP-xylose in soybean cells. When rates of the reaction in the presence of UDP-xylose (50 or 100 µM) were plotted against substrate concentration, the activity plots were sigmoidal, and 100 µM UDP-xylose inhibited approximately 60% of the enzyme activity. When Golgi membranes were incubated with digitonin (0.5%), the activity of UDP-glucuronate carboxy-lyase doubled (Table II). These results suggest that the stimulatory effect (22) of the detergent could be due to an increased accessibility of the substrate to the UDP-glucuronate carboxy-lyase site on the inner surface of the Golgi membrane. However, in the same detergent conditions, some inhibitory effect of xyloglucan synthase activity was observed. Since xyloglucan synthesis requires at least two enzyme activities, i.e. that of xyloglucan 4-β-glucosyltransferase and of 6-α-xylosyltransferase (13), it may be possible that detergents either break the multienzyme cooperation or diffuse primers and substrates. Finally, digitonin produced a complete solubilization of the membrane-associated UDP-glucuronate carboxy-lyase activity from soybean Golgi membrane preparations. However, xyloglucan synthase activity could not be extracted with detergents such as Triton, digitonin, cholate, and Zwittergents.

Translocation of UDP-Glucuronate and UDP-Xylose across Golgi Vesicle Membranes. Low concentrations (1 µM) of either UDP-[<sup>14</sup>C]glucuronate (200,000 cpm) or UDP-[<sup>14</sup>C]xylose (200,000 cpm) was added to 1 µMrewided plasma membranes isolated from soybean hypocotyls in 1.5 ml of standard assay buffer. The rate of incorporation of UDP-glucuronate and UDP-xylose into xyloglucan was determined by thin-layer chromatography of resuspended membranes after 30 min at 30°C (16). UDP-Xylose was, however, not totally removed from the membranes after 30 min of incubation. It was decided to use a continuous sucrose gradient to separate these compounds. A sucrose gradient was prepared by mixing 2.1 ml of 0.8 M sucrose and 3.5 ml each of 1.0, 1.2, 1.4, and 1.5 M, and was centrifuged for 90 min at 100,000 g. Then the resuspended membranes were incubated with either UDP-glucuronate or UDP-xylose for 30 min at 30°C (16). UDP-Xylose was, however, not totally removed from the membranes after 30 min of incubation.

Table II. Effect of Digitonin on the Activities of UDP-Glucuronate Carboxy-Lyase and Xyloglucan Synthase

Enzyme activities were determined in the presence and absence of the detergent under the standard conditions.

<table>
<thead>
<tr>
<th>Digitonin</th>
<th>UDP-Glucuronate Carboxy-Lyase</th>
<th>Xyloglucan Synthase</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>units</td>
<td>pmol</td>
</tr>
<tr>
<td>None</td>
<td>326</td>
<td>2.42</td>
</tr>
<tr>
<td>0.05</td>
<td>523</td>
<td>2.24</td>
</tr>
<tr>
<td>0.1</td>
<td>576</td>
<td>1.87</td>
</tr>
<tr>
<td>0.5</td>
<td>612</td>
<td>1.55</td>
</tr>
</tbody>
</table>

Downloaded from October 23, 2017 - Published by www.plantphysiol.org  
Copyright © 1988 American Society of Plant Biologists. All rights reserved.
were incubated with Golgi membrane vesicles (1 mg of protein for 5 min. The membranes were then washed with buffer and extracted with 70% ethanol. The result showed that 11.2% of UDP-[14C]glucuronate and 8.9% of UDP-[14C]xylose (19,000 cpm), respectively, were translocated (Table III). Nontranslocated UDP-[14C]glucuronate was not transformed to UDP-xylose, indicating that decarboxylation of UDP-glucuronate does not occur outside vesicles. The translocated radioactive products from UDP-[14C]glucuronate were subjected to paper chromatography with solvent A and separated to UDP-glucuronate (15,000 cpm) and UDP-xylose (7,400 cpm), showing that 33% of the translocated UDP-glucuronate was decarboxylated to UDP-xylose. The area corresponding to UDP-xylose was excised and rechromatographed with solvent B. The radioactivity was further distributed in UDP-xylose and UDP-arabinose, suggesting the epimerization of UDP-xylose to UDP-arabinose. This was in agreement with previous observations that membrane preparations of mung bean (8) and pea (11) contain a UDP-arabinose 4-epimerase and, also, with the result that translocated UDP-[14C]xylose was epimerized to UDP-arabinose (data not shown).

When 1 μM UDP-[14C]glucuronate (200,000 cpm) plus 10 μM UDP-xylose was incubated with the vesicles, 10.9% of the label was transported into the vesicle lumen. This transport efficiency was similar to that observed in the absence of UDP-xylose. In the presence of UDP-xylose, however, only 10% of the translocated UDP-glucuronate was decarboxylated to UDP-xylose. This suggests that UDP-xylose does not inhibit the transport of UDP-glucuronate into Golgi vesicle lumen but represses the decarboxylation of the UDP-glucuronate translocated. Finally, when 1 μM UDP-[14C]xylose (200,000 cpm) plus 10 μM UDP-glucuronate was incubated with the vesicles, 9.0% of UDP-xylose was translocated, similar to the level observed in the absence of UDP-glucuronate. No UDP-glucuronate was formed from provided UDP-xylose. These results suggest that the sugar nucleotides are independently transported in the vesicle lumen by specific carriers (7), which might be a sugar nucleotide carrier protein (26), and that the UDP-glucuronate translocated is decarboxylated to UDP-xylose at the vesicle membranes.

**Xyloglucan Synthesis from UDP-[14C]Glucuronate.** Xyloglucan was directly synthesized from UDP-[14C]glucuronate in soybean Golgi membrane vesicles. Radioactive polysaccharides formed in the vesicles were digested with A. oryzae enzymes, and radioactive isoprimeverose (6-O-α-D xylopyranosyl-D-glucose) was identified by paper chromatography (12) (Fig. 2). The presence of radioactivity from UDP-[14C]xylose in this disaccharide indicates that xyloglucan has been synthesized via UDP-xylose from UDP-glucuronate. Pentasaccharide (glucose/xylose, 3:2) and heptasaccharide (glucose/xylose, 4:3) units were also obtained from the digest of the polysaccharides with T. viride endoglucanase (data not shown). The formation of relative amounts of the two oligosaccharide units could be controlled by the ratio of UDP-glucuronate to UDP-glucose (16).

The apparent $K_m$ value of UDP-glucuronate in the presence of 2 mM UDP-glucose was 31 μM, similar to that of UDP-xylose (28 μM) (13). The result shows the high turnover of UDP-xylose in Golgi membranes. The incorporation of xylose into xyloglucan from UDP-glucuronate was increased in the presence of UDP-glucose ($K_m = 24 μM$), as a certain amount of elongation of the

---

### Table III. Transport of UDP-Xylose (UDP-Xyl) and UDP-Glucuronate (UDP-GA) into Golgi Vesicles

<table>
<thead>
<tr>
<th>Added UDP-sugar</th>
<th>Radioactive Solute within Vesicles</th>
<th>Insoluble</th>
<th>Radioactivity Translocated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UDP-Xyl</td>
<td>UDP-GA</td>
<td>pmol/mg protein</td>
</tr>
<tr>
<td>1 μM UDP-[14C]glucuronate</td>
<td>37</td>
<td>75</td>
<td>1</td>
</tr>
<tr>
<td>1 μM UDP-[14C]glucuronate</td>
<td>10</td>
<td>98</td>
<td>0.5</td>
</tr>
<tr>
<td>10 μM UDP-xylose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 μM UDP-[14C]xylose</td>
<td>85</td>
<td>4</td>
<td>8.9</td>
</tr>
<tr>
<td>1 μM UDP-[14C]xylose</td>
<td>82</td>
<td>4</td>
<td>9.0</td>
</tr>
</tbody>
</table>

---

FIG. 2. Paper chromatography of A. oryzae enzyme hydrolysate of the 14C-polysaccharides formed from UDP-[14C]glucuronate with soybean Golgi membranes. The reaction mixture contained 20 μM UDP-[14C]glucuronate (6.1 × 10⁴ cpm/μmol), 2 mM UDP-glucose, 100 mM Hepes/KOH (pH 6.8), 10 mM MnCl₂, and Golgi membranes in a total volume of 50 μl. 14C-Polysaccharides formed were digested with A. oryzae enzymes and the hydrolysate was chromatographed on Whatman No. 3MM filter paper. After chromatography with solvent C (panel A), the area (bracket) corresponding to isoprimeverose from the chromatogram A was rechromatographed with solvent D (panel B). From right to left, the references of panel A are isoprimeverose, glucose, and xylose, and those of panel B are isoprimeverose, galactose, glucose, arabinose, and xylose.
ß-1,4-glucan backbone accompanies the transfer of xylosyl residues. The Ke value of UDP-glucose was also the same as that from UDP-xylene. John et al. (17) reported that UDP-glucuronate carboxy-lyases from wheat germ are activated by UDP-glucose and allosterically inhibited by UDP-xylene. These facts suggest that the enzyme system for UDP-xylene formation as well as xyloglucan synthesis is controlled through activation by UDP-glucose and inhibition by UDP-xylene in soybean cells.

We have demonstrated for the first time that soybean UDP-glucuronate carboxy-lyase is associated with Golgi membranes (Table I; Fig. 1), where xyloglucan synthesis occurs, and that xyloglucan has been synthesized via UDP-xylene from UDP-glucuronate (Fig. 2). Our results suggest that the carboxy-lyase might be part of a multienzyme complex of glycosyltransferases involved in xyloglucan biosynthesis. It is interesting to note that the soybean enzyme occurs as a membrane-associated form as well as a soluble form, while the enzymes from animals (3, 18) are particle-bound and those from fungi (2) are soluble.

Acknowledgment—We thank Dr. A. Camirand (Université de Montréal, Canada) for her valuable advice during the final preparation of this paper.

LITERATURE CITED