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Changes in Enzymic Activities of Nucleoside Diphosphate Sugar Interconversions during Differentiation of Cambium to Xylem in Pine and Fir

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A protein fraction [precipitate obtained between 40 and 65% $(NH_4)_2SO_4$ satn.] prepared from cambial cells, differentiating xylem cells and differentiated xylem cells of pine and fir trees contained all the enzymes required for the nucleoside diphosphate sugar interconversions. By using UDP-D-[U-^{14}C]glucose or UDP-D-[U-^{14}C]galactose, UDP-D-[U-^{14}C]glucuronic acid and UDP-D-[U-^{14}C]xylose as substrates, the activities of UDP-D-galactose 4-epimerase (EC 5.1.3.2), UDP-D-glucuronate 4-epimerase (EC 5.1.3.6), UDP-D-xylose 4-epimerase (EC 5.1.3.5), UDP-D-glucose dehydrogenase (EC 1.1.1.22) and UDP-D-glucuronate decarboxylase (EC 4.1.1.35) were measured at different stages of cell-wall development. The specific activities and the activities per cell of these enzymes varied during differentiation of cambium to xylem according to the type of polysaccharide synthesized. Variations were also found between the two species investigated. These data, compared with those obtained in our previous work on angiosperms [see the preceding paper, Dalessandro & Northcote (1977) *Biochem. J.* **162**, 267–279], suggest that some control of polysaccharide synthesis operates at the level of the formation of the precursors of pectin and hemicellulose syntheses.

In gymnosperms the main metabolic pathways which lead to uridine diphosphate sugar formation and interconversion are the same as those described in the preceding paper for angiosperms (Dalessandro & Northcote, 1977). The UDP-sugars act as glycosyl donors during the biosynthesis of pectic substances and hemicelluloses of the cell wall. In addition, GDP-D-mannose and GDP-D-glucose (Elbein & Hassid, 1966; Elbein, 1969) serve as mannosyl and glucosyl donors in the formation of galactoglucomannans in gymnosperms.

The polysaccharides that occur in the hemicellulose of the gymnosperms are different from those of angiosperms (Timell, 1964, 1965; Northcote, 1972). *O*-Acetyl-(4-*O*-methylglucurono)xylans (Aspinall, 1959) are the main polysaccharides of this fraction in angiosperms, whereas arabinogalactans (Bouveng & Lindberg, 1958; Bouveng, 1959; Aspinall & Wood, 1963), glucomannans (Aspinall *et al.*, 1962; Timell, 1965), galactoglucomannans (Timell, 1965) and smaller amounts of arabino-(4-*O*-methylglucurono)-xylans (Garegg & Lindberg, 1960; Timell, 1965) usually make up the bulk of the material in gymnosperms. In these plants the hemicellulose components vary considerably, even in the same species (Timell, 1965). Hemicellulose deposition, unlike that of pectic substances, continues in increased amounts during secondary thickening (Northcote, 1963). It can be predicted, therefore, that the activities of the enzymes directly involved in the biosynthesis of UDP-D-galactose and UDP-L-arabinose should be higher in gymnosperms than angiosperms. Further, the unidirectional flux, from UDP-D-glucose to UDP-D-glucuronic acid and subsequently to UDP-D-xylose, catalysed by the enzymes UDP-D-glucose dehydrogenase and UDP-D-glucuronate decarboxylase should be less important in gymnosperms, since the requirement for xylans is much less than in angiosperms, where hemicellulose is formed almost exclusively of xylans.

We have measured the activities of the enzymes of UDP-sugar interconversion [UDP-D-galactose 4-epimerase (EC 5.1.3.2), UDP-D-glucuronate 4-epimerase (EC 5.1.3.6), UDP-D-xylose 4-epimerase (EC 5.1.3.5), UDP-D-glucose dehydrogenase (EC 1.1.1.22), UDP-D-glucuronate decarboxylase (EC 4.1.1.35)] in cambial cells, differentiating xylem cells and differentiated xylem cells isolated from pine (*Pinus silvestris*) and fir (*Abies grandis*).

Materials and Methods

Chemicals and radiochemicals

The various chemicals and radiochemicals used have been described by Dalessandro & Northcote (1977).

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Trees

Trees, pine (*Pinus silvestris*) (35–37 years old) and fir (*Abies grandis*) (28–30 years old) about 20–24m tall and 350–400mm diam., were cut during August and September 1975 from Thetford forest at Mildenhall, Suffolk, U.K. They were cut into four logs about 700mm long starting from the basal part of the trunk. The material was transferred to a cold-room for further manipulation.

Preparation of cambial cells, differentiating xylem cells and differentiated xylem cells

The three kinds of cells were obtained by using the scraping techniques described in the preceding paper (Dalessandro & Northcote, 1977).

Enzyme preparation

Preparations of crude extract, $MnCl_2$ treatment and fractions of the extract prepared from cambial cells, differentiating and differentiated xylem cells were made as described in the preceding paper (Dalessandro & Northcote, 1977). The protein fraction precipitated between 40 and 65% $(NH_4)_2SO_4$ saturation was used for all enzymic assays.

Analytical methods

Paper chromatography, electrophoresis, radioactivity-counting procedure, detection methods, protein determination and procedures for the counting of cells were similar to those described in the preceding paper (Dalessandro & Northcote, 1977).

Enzyme assays

UDP-D-galactose 4-epimerase, UDP-D-xylose 4-epimerase, UDP-D-glucose dehydrogenase and UDP-D-glucuronate decarboxylase were assayed radiochemically as described in the preceding paper (Dalessandro & Northcote, 1977).

UDP-D-glucuronate 4-epimerase (EC 5.1.3.6)

Reaction mixtures contained UDP-D-[U-^{14}C]-glucuronic acid (0.432nmol), UDP-D-glucuronic acid (25nmol) and enzyme [protein precipitated between 40 and 65% $(NH_4)_2SO_4$ satn.] in 0.2M-sodium phosphate buffer, pH7.0, in a total volume of 20μl. The reactions, carried out in Durham tubes at 37°C for 10min, were terminated by directly spotting samples (10μl) on Whatman no. 1 paper and drying them in a stream of cold air. The reaction products (UDP-sugars) were separated by paper electrophoresis at pH2.0, for 20min at 4kV. The electrophoretograms were cut into strips (40mm×10mm) and their radioactivities were counted (Harris & Northcote, 1970). Paper strips containing the radioactive material that ran at the region of UDP-sugar markers were removed from the scintillant fluid, washed with toluene and benzene, dried under reduced pressure and eluted with glass-distilled water. The eluates were evaporated to dryness at 40°C under reduced pressure and the dry residues were hydrolysed with 0.05M-H_2SO_4 for 15min at 100°C. The hydrolysates were neutralized with Amberlite IR 45 resin (CO_3^{2-} form) and eluted with several changes of water. The eluates were evaporated to dryness and the dry residues were dissolved in 20μl of water. Each sample (10μl) was spotted on Whatman no. 1 paper and run chromatographically for 25h. The chromatograms were cut into strips (40mm×10mm) and counted for radioactivity. The radioactive material corresponding to the uronic acid markers was removed from the scintillation fluid, washed, dried, eluted with water and evaporated to dryness. The dry residues containing uronic acids were dissolved in 20μl of water and spotted on Whatman no. 1 paper, which was run electrophoretically at pH3.5 for 50min at 4.0kV. The radioactivity in the region corresponding to the markers (galacturonic acid and glucuronic acid) was counted. One unit of the enzyme activity was defined as the amount of enzyme that resulted in the formation of 1nmol of product in 1min.

Results

The protein fraction precipitated between 40 and 65% $(NH_4)_2SO_4$ satn., obtained from cambial cells, differentiating xylem cells and differentiated xylem cells of pine and fir, catalysed the formation of UDP-D-[U-^{14}C]galactose, UDP-D-[U-^{14}C]glucuronic acid, UDP-D-[U-^{14}C]galacturonic acid, UDP-D-[U-^{14}C]-xylose, UDP-L-[U-^{14}C]arabinose and a [^{14}C]polyglucan when UDP-D-[U-^{14}C]glucose was used as substrate in the presence of NAD^+. This suggested the presence in the enzymic preparations of UDP-D-galactose 4-epimerase, UDP-D-glucuronate 4-epimerase, UDP-D-xylose 4-epimerase, UDP-D-glucose dehydrogenase, UDP-D-glucuronate decarboxylase and an enzyme system catalysing polyglucan formation. The activities of these enzymes were measured by using a direct substrate, the results were very reproducible and at least three assays were performed per enzyme per source. The reaction products were clearly identified by paper electrophoresis and paper chromatography. All assays were carried out at optimal pH and temperature. The protein precipitated between 40 and 65% $(NH_4)_2SO_4$ saturation retained full activity for at least 1 month when stored frozen at −16°C. All enzyme activities were linear functions of protein concentration and time over periods of at least 10min. The total conversion of substrate into product was not allowed to rise above 15% during the assays.

Table 1. *UDP-D-galactose 4-epimerase activity during differentiation in pine and fir*

The reaction mixtures contained 50nmol of UDP-D-galactose, 0.408nmol of UDP-D-[U-^{14}C]galactose (105700c.p.m.) and 5.0μg of enzyme [protein precipitated between 40 and 65% $(NH_4)_2SO_4$ satn.] in 0.2M-glycine/NaOH buffer, pH9.0, in a total volume of 20μl. Reaction time at 30°C was 5min; 1nmol of substrate was equivalent to 2097c.p.m. Unchanged radioactive substrate and product were determined as described in the text.

Enzyme source	UDP-D-[U-^{14}C]glucose formed in 5min (c.p.m.)
Pine	
Cambial cells	17040
Differentiating xylem cells	13730
Differentiated xylem cells	12930
Fir	
Cambial cells	20010
Differentiating xylem cells	32590
Differentiated xylem cells	14990

UDP-D-galactose 4-epimerase

Table 1 shows the activity of UDP-D-galactose 4-epimerase in cambial cells, differentiating xylem cells and differentiated xylem cells in pine and fir. In pine, as differentiation proceeded from cambium to xylem, the activity of the enzyme decreased, whereas in fir it was greatest in differentiating xylem cells.

Effect of substrate concentration on UDP-D-galactose 4-epimerase

UDP-D-galactose 4-epimerase isolated from cambial cells of pine catalysed the reversible reaction between UDP-D-galactose and UDP-D-glucose. With either UDP-D-[U-^{14}C]galactose or with UDP-D-[U-^{14}C]glucose as substrate, the enzyme gave a normal hyperbolic relationship between concentration of substrate and velocity of reaction. The Lineweaver–Burk (1934) plots for UDP-D-[U-^{14}C]galactose and UDP-D-[U-^{14}C]glucose were determined. The incubation mixture contained 9μg or 6.75μg of enzyme [protein precipitated between 40 and 65% $(NH_4)_2SO_4$ satn.] extracted from cambial cells of pine and UDP-D-[U-^{14}C]galactose or UDP-D-[U-^{14}C]glucose (0.27–5mM), in 0.2M-glycine/NaOH buffer, pH9.0, in a total volume of 20μl. Reaction time at 30°C was 5min. The apparent K_m values for UDP-D-galactose and UDP-D-glucose were calculated to be 1.25mM and 6.25mM respectively.

It has been reported that UDP-D-galactose 4-epimerase from *Saccharomyces cerevisiae* (Ray & Bhaduri, 1975) showed a sigmoidal plot of the reaction velocity versus substrate concentration when low concentrations of UDP-D-glucose were used as substrate. Glucose 6-phosphate (1.6 and 3.2mM) decreased the sigmoid nature of the curve. We have found that UDP-D-galactose 4-epimerase isolated from the cambium of pine did not show these properties (Fig. 1).

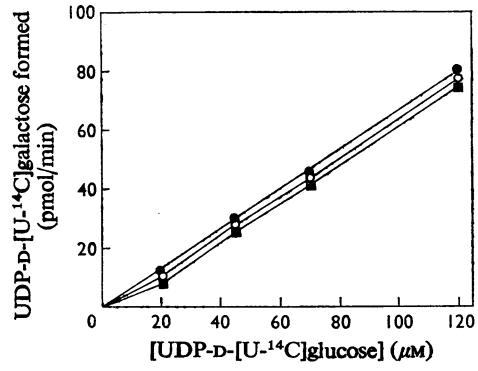


Fig. 1. *Kinetics of UDP-D-galactose 4-epimerase with low concentrations of UDP-D-[U-^{14}C]glucose as substrate in the presence or absence of glucose 6-phosphate*

The reaction mixture contained 9.0μg of enzyme [protein precipitated between 40 and 65% $(NH_4)_2SO_4$ satn.] extracted from cambial cells of pine, UDP-D-[U-^{14}C]glucose (20–120μM) and glucose 6-phosphate (○, 1.6, 3.2mM), in 0.2M-glycine/NaOH buffer, pH9.0, in a total volume of 20μl. Reaction time at 30°C was 5min. The amount of UDP-D-[U-^{14}C]-galactose formed was determined as described in the text. Glucose 6-phosphate concentration (mM): ●, 0; ○, 1.6; ■, 3.2.

UDP-D-glucuronate 4-epimerase

Both in pine and in fir, UDP-D-glucuronate 4-epimerase activity decreased during the differentiation of cambial cells to xylem (Table 2).

UDP-D-xylose 4-epimerase

Table 3 shows the comparison between the activity of UDP-D-xylose 4-epimerase in pine and fir. In pine the activity decreased from cambium to xylem, whereas in fir it was slightly greater in xylem than in cambium. In general the activity was greater in fir than in pine.

Effect of metal ions, NAD$^+$ and NADH on the 4-epimerase activities

The activities of UDP-D-galactose 4-epimerase, UDP-glucuronate 4-epimerase and UDP-D-xylose 4-epimerase were not dependent on the addition of Ca^{2+}, Mg^{2+}, NAD$^+$ and NADH at concentrations between 0.001 and 1mM.

Table 2. *UDP-D-glucuronate 4-epimerase activity during differentiation in pine and fir*

The reaction mixtures contained 25nmol of UDP-D-glucuronic acid, 0.432nmol of UDP-D-[U-^{14}C]glucuronic acid (135000c.p.m.) and 50μg of enzyme [protein precipitated between 40 and 65% $(NH_4)_2SO_4$ satn.] in 0.2M-sodium phosphate buffer, pH7.0, in a total volume of 20μl. Reaction time at 37°C was 10min; 1nmol of substrate was equivalent to 5308c.p.m. The amount of UDP-D-[U-^{14}C]galacturonic acid formed in 10min was determined by measurement of radioactivity as described in the text.

Enzyme source	UDP-D-[U-^{14}C]galacturonic acid formed in 10min (c.p.m.)
Pine	
Cambial cells	4550
Differentiating xylem cells	4040
Differentiated xylem cells	3240
Fir	
Cambial cells	4680
Differentiating xylem cells	2810
Differentiated xylem cells	1740

Table 3. *UDP-D-xylose 4-epimerase activity during differentiation in pine and fir*

The reaction mixtures contained 10nmol of UDP-D-xylose, 0.312nmol of UDP-D-[U-^{14}C]xylose (60000 c.p.m.) and 25μg of enzyme [protein precipitated between 40 and 65% $(NH_4)_2SO_4$ satn.] in 0.2M-sodium phosphate buffer, pH8.0, in a total volume of 20μl. Reaction time at 30°C was 5min; 1nmol of substrate was equivalent to 5818c.p.m. Unchanged radioactive substrate and product were determined as described in the text.

Enzyme source	UDP-L-[U-^{14}C]arabinose formed in 5min (c.p.m.)
Pine	
Cambial cells	2890
Differentiating xylem cells	2360
Differentiated xylem cells	1640
Fir	
Cambial cells	8520
Differentiating xylem cells	7990
Differentiated xylem cells	9340

UDP-D-glucose dehydrogenase

Table 4 shows that the activity of UDP-D-glucose dehydrogenase decreased from cambium to xylem in pine, but it increased during the differentiation of cambial cells to sapwood in fir.

Radioactive polysaccharide formation in the presence of UDP-D-[U-^{14}C]glucose as substrate

Incubation of the enzyme fraction isolated from cambial cells, differentiating and differentiated xylem cells of pine and fir catalysed the transfer of [U-^{14}C]glucose from UDP-D-[U-^{14}C]glucose into a water-soluble radioactive polysaccharide which was formed of glucosyl residues. The linkages between the glucose units of this polysaccharide were not characterized. Table 5 shows the specific activities and the units of enzyme activity per cell of the enzyme system forming this polyglucan. In pine the activity increased from cambium to xylem, whereas it was more or less constant in fir. However, the units of enzyme activity per cell were greater in xylem.

Table 4. *UDP-D-glucose dehydrogenase activity during differentiation in pine and fir*

The reaction mixtures contained 50nmol of UDP-D-glucose, 0.4nmol of UDP-D-[U-^{14}C]glucose (125000 c.p.m.), 20nmol of NAD^+ and 50μg of enzyme [protein precipitated between 40 and 65% $(NH_4)_2SO_4$ satn.] in 0.2M-sodium phosphate buffer, pH8.0, in a total volume of 20μl. Reaction time at 30°C was 10min. 1nmol of substrate was equivalent to 2480c.p.m. The amount of UDP-D-[U-^{14}C]glucuronic acid formed in 10min was determined by measurement of radioactivity as described in the text.

Enzyme source	UDP-D-[U-^{14}C]glucuronic acid formed in 10min (c.p.m.)
Pine	
Cambial cells	2990
Differentiating xylem cells	1090
Differentiated xylem cells	1080
Fir	
Cambial cells	1430
Differentiating xylem cells	1740
Differentiated xylem cells	2800

UDP-D-glucuronate decarboxylase

The protein precipitated between 40 and 65% $(NH_4)_2SO_4$ satn. converted UDP-D-[U-^{14}C]glucuronic acid into a mixture of UDP-D-[U-^{14}C]xylose, UDP-L-[U-^{14}C]arabinose and UDP-D-[U-^{14}C]galacturonic acid. By measuring the appearance of these reaction products at short time-intervals, it was established that the formation of UDP-L-[U-^{14}C]-arabinose was due to UDP-xylose 4-epimerase and not to the presence of a UDP-D-galacturonate decarboxylase, as demonstrated in the prokaryotic organism *Ampullariella digitata* (Fan & Feingold, 1972). For this reason the activity of UDP-D-glucuronate decarboxylase was measured by taking into account the amount of UDP-L-[U-^{14}C]arabinose formed as a result of the enzymic 4-epimerization of UDP-D-[U-^{14}C]xylose.

Table 5. *Activity of the enzyme system catalyzing the formation of a polyglucan during differentiation in pine and fir*
The reaction mixtures contained 50nmol of UDP-D-glucose, 0.4nmol of UDP-D-[U-^{14}C]glucose (125000c.p.m.), 20nmol of NAD^+ and enzyme [protein precipitated between 40 and 65% $(NH_4)_2SO_4$ satn.] in 0.2M-sodium phosphate buffer, pH8.0, in a total volume of 20μl. Reaction time at 30°C was 10min; 1nmol of substrate was equivalent to 2480c.p.m. The amount of ^{14}C-labelled polysaccharide was determined as described in the text.

Enzyme source	Specific activity (nmol/min per mg of protein)	10^7 × Units of enzyme activity per cell
Pine		
Cambial cells	7.22	4.0
Differentiating xylem cells	8.00	8.2
Differentiated xylem cells	12.50	6.4
Fir		
Cambial cells	3.60	3.2
Differentiating xylem cells	3.55	3.8
Differentiated xylem cells	3.45	5.7

Table 6. *UDP-D-glucuronate decarboxylase activity during differentiation in pine and fir*
The reaction mixtures contained 25nmol of UDP-D-glucuronic acid, 0.432nmol of UDP-D-[U-^{14}C]glucuronic acid (135000c.p.m.) and 50μg of enzyme [protein precipitated between 40 and 65% $(NH_4)_2SO_4$ satn.] in 0.2M-sodium phosphate buffer, pH7.0, in a total volume of 20μl. Reaction time at 37°C was 10min. 1nmol of substrate was equivalent to 5308c.p.m. The amount of UDP-D-[U-^{14}C]xylose and UDP-L-[U-^{14}C]arabinose formed in 10min was determined by measurement of radioactivity as described in the text. UDP-D-[U-^{14}C]xylose and UDP-L-[U-^{14}C]-arabinose formed were corrected for the loss of C-6 from the [U-^{14}C]glucuronic acid.

Enzyme source	Product formed in 10min (c.p.m.)		
	UDP-D-[U-^{14}C]xylose	UDP-L-[U-^{14}C]arabinose	Total
Pine			
Cambial cells	9848	2060	11908
Differentiating xylem cells	9002	2237	11239
Differentiated xylem cells	4570	701	5271
Fir			
Cambial cells	20188	10586	30774
Differentiating xylem cells	16449	7287	23736
Differentiated xylem cells	6418	2279	8697

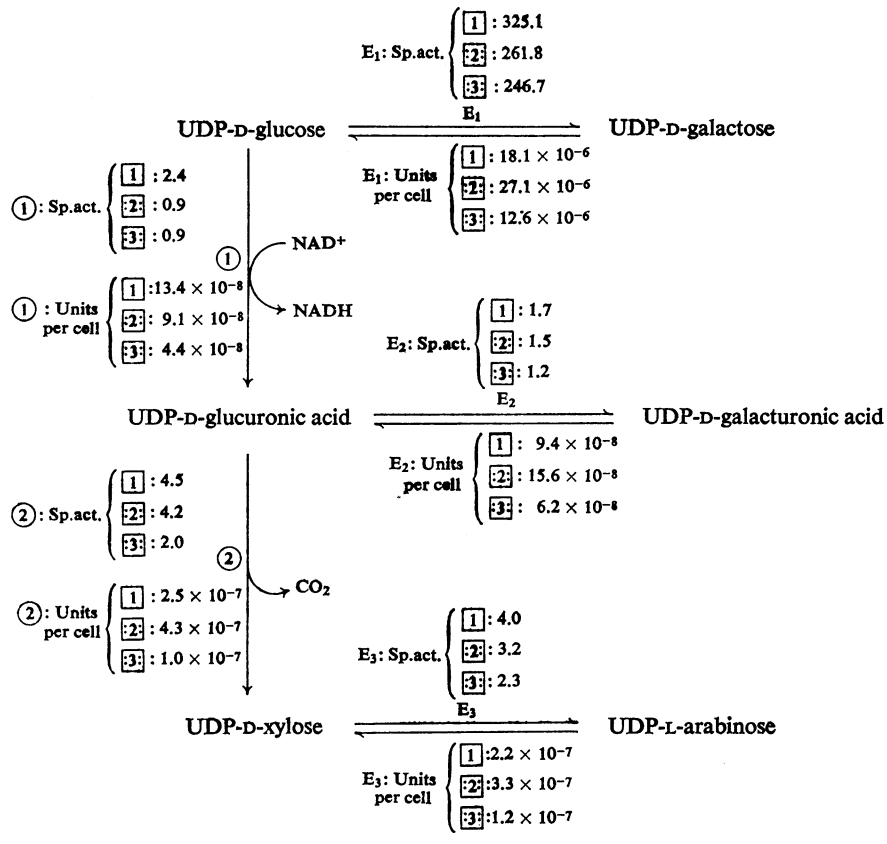
As shown in Table 6, the UDP-D-glucuronate decarboxylase activity decreased from cambium to xylem both in pine and in fir; the activity was greater in fir than in pine.

Specific activities and units of enzyme activities per cell of the enzymes of UDP-sugar interconversion during differentiation in pine

Specific activities (nmol/min per mg of protein) and units of enzyme activities per cell (nmol/min per cell) of UDP-D-galactose 4-epimerase, UDP-D-glucuronate 4-epimerase, UDP-D-xylose 4-epimerase, UDP-D-glucose dehydrogenase and UDP-D-glucuronate decarboxylase determined for cambial cells, differentiating xylem cells and differentiated xylem cells isolated from pine are shown in Scheme 1. The activity of all the enzymes decreased during the transition from cambium to sapwood. The units of enzyme activity per cell were always greatest in differentiating xylem cells and lowest in xylem, except for UDP-glucose dehydrogenase, in which the greatest activity was found in cambium.

Specific activities and units of enzyme activities per cell of the enzymes of UDP-sugar interconversion during differentiation in fir

Scheme 2 compares specific activities (nmol/min per mg of protein) and units of enzyme activities per cell (nmol/min per cell) of the enzymes of UDP-sugar interconversion measured during the differentiation of cambial cells to sapwood in fir. The activity of UDP-glucose dehydrogenase increased from cambium to xylem when measured either as specific activity or as units of enzyme activity per cell. But UDP-D-glucuronate decarboxylase and UDP-D-glucuronate 4-epimerase decreased sharply from cambium to xylem. The highest specific activity and units of enzyme activity per cell of UDP-D-galactose



KEY: [1], cambial cells; [2], differentiating xylem cells; [3], differentiated xylem cells

Scheme 1. *Specific activities and units of enzyme activities per cell of the enzymes of UDP-sugar interconversion during differentiation in pine*

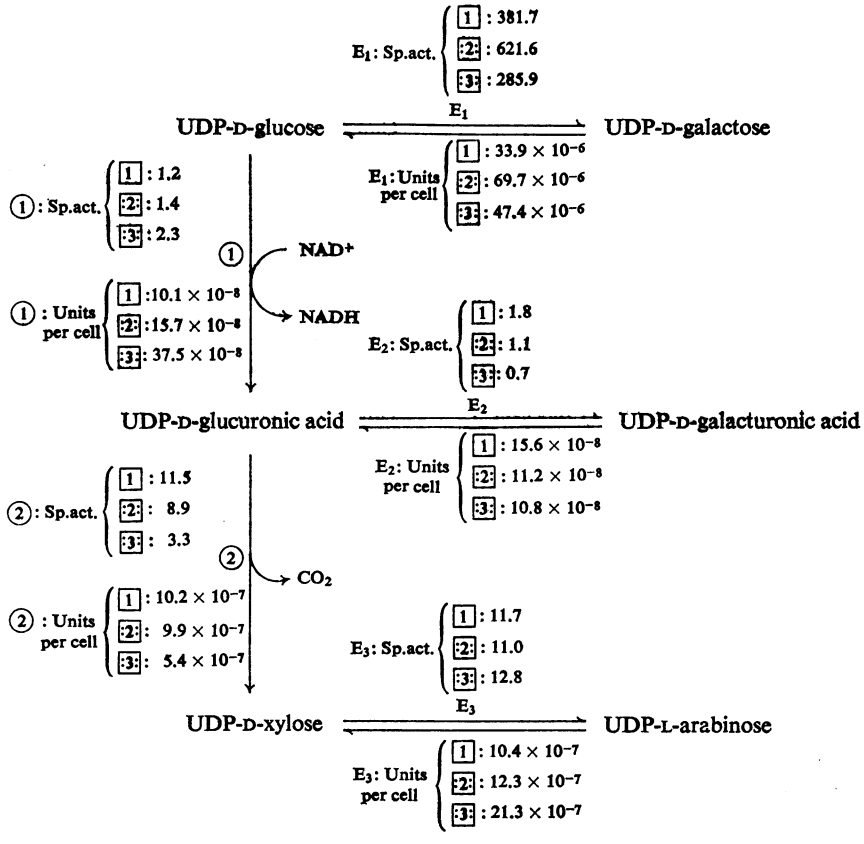
Sp. act., specific activity (nmol/min per mg of protein). Units per cell, units of enzyme activity per cell (nmol/min per cell). E_1, UDP-D-galactose 4-epimerase; E_2, UDP-D-glucuronate 4-epimerase; E_3, UDP-D-xylose 4-epimerase; ①, UDP-D-glucose dehydrogenase; ②, UDP-D-glucuronate decarboxylase.

4-epimerase and UDP-D-xylose 4-epimerase were found in differentiating xylem cells and differentiated xylem cells respectively.

Discussion

The enzymic interconversion of the nucleoside diphosphate sugars shown in Schemes 1 and 2 presents two sections, one a route involving UDP-D-glucose dehydrogenase and UDP-D-glucuronate decarboxylase whereby UDP-D-glucose is converted into UDP-D-xylose, and a second section which involves three epimerases whereby UDP-D-glucose, UDP-D-glucuronic acid and UDP-D-xylose are converted into the corresponding epimers based on D-galactose.

The specific activities and the units of enzyme activities per cell of the enzymes responsible for the conversion of UDP-D-glucose into UDP-D-xylose in fir and in pine are very much lower compared with those that occur in sycamore and in poplar (Dalessandro & Northcote, 1977). In addition, although in sycamore there is approximately a threefold increase in the activities of the two enzymes during differentiation, there is a decrease in the activities during differentiation of the gymnosperm cambium (except for the very small increase of the extremely low activities of the dehydrogenase found during the differentiation of the cambium of fir). This difference between the gymnosperms and the angiosperms reflects the increase in xylan polymers that are deposited in the secondary thickenings of the angiosperms com-



KEY: [1], cambial cells; [2], differentiating xylem cells; [3], differentiated xylem cells

Scheme 2. *Specific activities and units of enzyme activities per cell of the enzymes of UDP-sugar interconversion during differentiation in fir*

Sp. act., specific activity (nmol/min per mg of protein). Units per cell, units of enzyme activity per cell (nmol/min per cell). E_1, UDP-D-galactose 4-epimerase; E_2, UDP-D-glucuronate 4-epimerase; E_3, UDP-D-xylose 4-epimerase; ①, UDP-D-glucose dehydrogenase; ②, UDP-D-glucuronate decarboxylase.

pared with the very low amounts of these polysaccharides found in the gymnosperm cell wall. It does suggest, therefore, that some control of the type and amount of polysaccharide that is formed is made by an adjustment of the amounts of the activities of the enzymes shown in Schemes 1 and 2.

These ideas are also further substantiated by a consideration of the epimerases. The activities of these enzymes are much higher in the gymnosperms compared with those of the angiosperms, and this is paralleled by the larger amounts of arabinogalactans and galactoglucomannans that are deposited into the cell walls of pine and fir compared with the smaller amounts of arabinogalactans that are deposited only initially into the pectic substances of the cell plate and primary walls of angiosperm tissue (Northcote, 1963).

The activities of these enzymes, although not responsible for the immediate regulation of polysaccharide synthesis, do vary to some extent during differentiation, and between tissue of various species. The variation indicates that the activities of the enzymes correspond to the type of polysaccharide synthesized, and that some preliminary control of synthesis is exerted at this stage. Nevertheless a pool of all the possible cell-wall polysaccharide precursors can be made available, and probably does occur in all the tissues of all the species, even when little or no polysaccharide is synthesized from some of the donors that can be made available.

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