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CHANGES IN ENZYMIC ACTIVITIES OF UDP-D-GLUCURONATE DECARBOXYLASE AND UDP-D-XYLOSE 4-EPIMERASE DURING CELL DIVISION AND XYLEM DIFFERENTIATION IN CULTURED EXPLANTS OF JERUSALEM ARTICHOKE

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Key Word Index—*Helianthus tuberosus*; Compositae; Jerusalem artichoke; pith parenchyma explants; cell division tracheids; hormones; nucleoside diphosphate sugar enzymes; enzyme induction.

Abstract—The time course of the specific activities of UDP-D-glucuronate decarboxylase (E.C. 4.1.1.35) and UDP-D-xylose 4-epimerase (E.C. 5.1.3.5) have been determined, by using an enzymic preparation (protein precipitated between 40–65% $(NH_4)_2SO_4$ saturation), from cultured explants of Jerusalem artichoke treated and untreated with plant growth hormones. The cells of the explants were induced to divide and then some differentiation into tracheids took place over a period of 14 days. The changes in the specific activities of the two enzymes were correlated with the formation of the cell plate, primary growth and secondary thickening of the wall.

INTRODUCTION

Cytodifferentiation in higher plants has been extensively studied with particular attention to the induction of xylem differentiation in tissue culture experiments [1–6]. Recent reviews on xylogenesis indicate that auxin, cytokinins, gibberellins, ethylene and cAMP could induce or modify this process during all stages of cytodifferentiation [7–13].

Explants of pith parenchyma cells of Jerusalem artichoke (*Helianthus tuberosus* L.) and Romaine lettuce (*Lactuca sativa* L.) can be made to divide and differentiate into tracheary elements when cultured for 3 or 4 days *in vitro* under precisely defined hormonal, nutritional and environmental conditions [14–18]. An advantage of studying the Jerusalem artichoke system is that there is much information available about its growth and metabolism [19–21].

The differentiation of tracheary elements is characterized by the thickening of the secondary walls, lignification and death of the cells. It normally takes place after cell division [15, 16, 22, 23]. This transition from primary to secondary growth is accompanied by an alteration in the type of polysaccharide synthesized and incorporated into the cell wall. Consequently there are different requirements for the nucleoside diphosphate sugar precursors utilized for such biosyntheses [24, 25].

We have attempted to measure the activities of the enzymes which bring about the interconversions of the nucleoside diphosphate sugars during three stages of cell differentiation: division, elongation and secondary wall thickening. This we have done by using three different types of plant tissue. In order to investigate the changes during cell wall thickening we used the developing cambial cells of trees of angiosperms and gymnosperms as they differentiated into xylem [26, 27], for the changes that occurred during elongation we used the growing cells of the third internode of etiolated pea seedlings [28]. In this paper we report the time course of the specific activities of UDP-D-glucuronate decarboxylase (E.C. 4.1.1.35) [29, 30] and UDP-D-xylose 4-epimerase (E.C. 5.1.3.5) [31] in a system in which the cells were induced primarily to divide and then to differentiate to a much lesser extent into tracheids in the presence of hormonal stimuli.

RESULTS

Dark-cultured explants of storage pith parenchyma cells of Jerusalem artichoke did not divide and differentiate on a growth-medium containing mineral salts, vitamins and sucrose in the presence or absence of gibberellic acid (GA_3), over a period of at least three weeks in culture. However, when the explants were cultured on a growth-medium enriched with either indoleacetic acid (IAA 5 mg/l.) or indoleacetic acid (IAA 5 mg/l.) plus gibberelic acid (GA_3 1 mg/l.), rapid and vigorous cell division resulted. This was followed by cytodifferentiation of tracheary elements that were induced after a 48-hr lag-period. The IAA + GA_3 treatment in comparison with the IAA treatment gave almost the same amount of cell division but slightly more tracheary element formation. The callus grew as a hard and compact mass confined to the top side of the outer border of the explants.

Time course of cell division, tracheary element formation and variations in fresh weight of cultured explants of Jerusalem artichoke

Figure 1 shows quantitative data during the induction of cell division, tracheary element formation and

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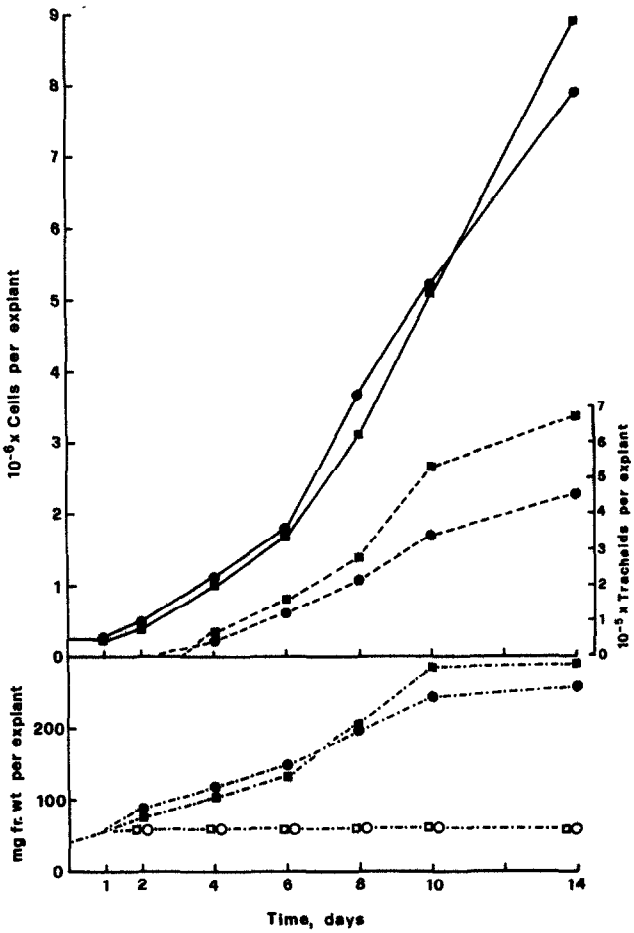

Fig. 1. Time course of cell division, tracheary element formation and variations in fresh weight of cultured explants of Jerusalem artichoke. Explants were cultured in the dark at 27° for 1, 2, 4, 6, 8, 10 and 14 days on a 1% Bacto-agar medium containing Murashige and Skoog's medium [42], sucrose, auxin (IAA 5 mg/l.) and gibberellin (GA_3 1 mg/l.) as indicated. The final fresh weight (mg), total cell number and tracheid number were means of 15 explants for each treatment. The inital cell number was 246000 ± 15800. The initial fresh weight was 42 ± 2 mg. IAA (5 mg/l.) treated explants, ●; IAA (5 mg/l.) + GA_3 (1 mg/l.) treated explants, ■; GA_3 (1 mg/l.) treated explants, □; Control, ○.

variations in fresh weight of explants cultured under different hormonal conditions over a period of 14-day incubation. In IAA and IAA + GA_3 treated explants, the first division appeared after a 28-hr lag-period. The formation of tracheary elements was observed approximately 20 hr later. During the time course, the total number of cells and tracheids and the fresh weight per explant, increased in a very similar way. Throughout growth, IAA + GA_3 treatment produced a higher percentage of cells differentiated as tracheary elements than those treated with IAA alone. Figure 2 shows that the total number of cells and tracheids per g fresh weight increased sharply in IAA and IAA + GA_3 treated explants after an initial lag-phase whereas the total number of cells remained constant in the control and GA_3 treated explants.

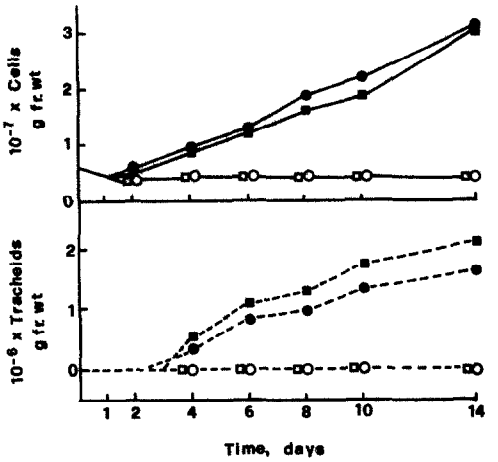

Fig. 2. Time course of the total number of cells and tracheids per g fresh weight in cultured explants of Jerusalem artichoke. Explants were cultured in the dark at 27° for 1, 2, 4, 6, 8, 10 and 14 days on a 1% Bacto-agar medium containing Murashige and Skoog's medium [42], 3% sucrose, auxin (IAA 5 mg/l.) and gibberellin (GA_3 1 mg/l.) as indicated. The initial cell number per g fresh weight was 5900000 ± 120000. IAA (5 mg/l.) treated explants, ●; IAA (5 mg/l.) + GA_3 (1 mg/l.) treated explants, ■; GA_3 (1 mg/l.) treated explants, □; Control, ○.

Enzymic activity

A protein fraction (protein precipitated between 40–65% $(NH_4)_2SO_4$ saturation) obtained from cultured explants of Jerusalem artichoke treated and untreated with plant growth regulators converted UDP-D-[U-^{14}C]-glucuronic acid into UDP-D-[U-^{14}C]xylose and UDP-L-[U-^{14}C]arabinose. This was due to the presence of

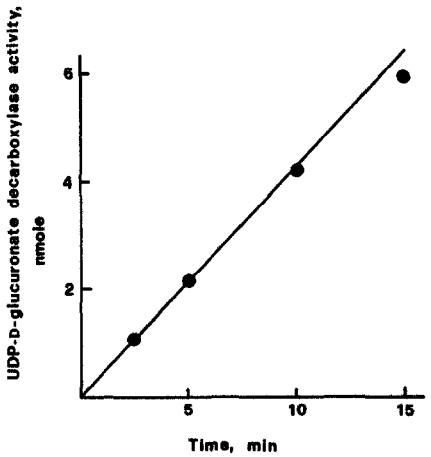

Fig. 3. Reaction rate of UDP-D-glucuronate decarboxylase as a function of time in cultured explants of Jerusalem artichoke. The reaction mixtures contained 50 nmoles of UDP-D-glucuronic acid, 0.432 nmoles of UDP-D-[U-^{14}C]glucuronic acid (135000 cpm) and 25 µg of enzyme (protein precipitated between 40–65% $(NH_4)_2SO_4$ saturation) extracted from cultured explants of Jerusalem artichoke treated with IAA (5 mg/l.), in 0.2 M NaPi buffer, pH 8, in a total volume of 20 µl at 37°. The amount of UDP-D-[U-^{14}C]xylose and UDP-L-[U-^{14}C]-arabinose formed was determined as described [26]. UDP-D-[U-^{14}C]xylose and UDP-L-[U-^{14}C]arabinose were corrected for the loss of the C-6 from [U-^{14}C]glucuronic acid. Enzyme from IAA (5 mg/l.) treated explants (2-day incubation), ●.

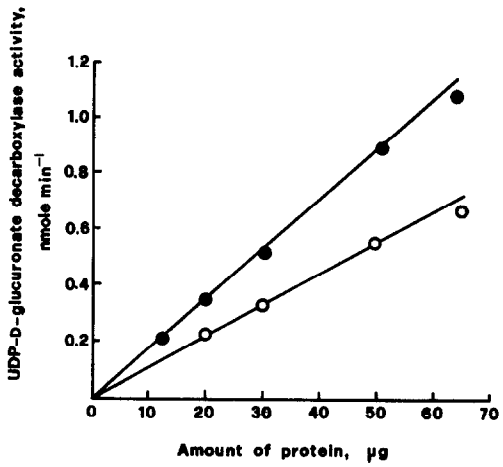

Fig. 4. Reaction rate of UDP-D-glucuronate decarboxylase as a function of protein concentration in cultured explants of Jerusalem artichoke. The reaction mixtures contained 50 nmoles of UDP-D-glucuronic acid, 0.432 nmoles of UDP-D-[U-^{14}C]-glucuronic acid (135000 cpm) and enzyme (protein precipitated between 40–65% $(NH_4)_2SO_4$ saturation) extracted from cultured explants of Jerusalem artichoke treated and untreated with IAA (5 mg/l.), in 0.2 M NaPi buffer, pH 8, in a total volume of 20 µl. Reaction time at 37° was 5 min. The amount of UDP-D-[U-^{14}C]xylose and UDP-L-[U-^{14}C]arabinose formed in 5 min was determined as described [26]. UDP-D-[U-^{14}C]xylose and UDP-L-[U-^{14}C]arabinose were corrected for the loss of the C-6 from the [U-^{14}C]glucuronic acid. Enzyme from IAA (5 mg/l.) treated explants (2-day incubation), ●; Enzyme from control (2-day incubation), ○.

UDP-D-glucuronate decarboxylase and UDP-D-xylose 4-epimerase in the enzymic system. The activity of these two enzymes was measured by using UDP-D-[U-^{14}C]-glucuronic acid as substrate for UDP-D-glucuronate decarboxylase and UDP-D-[U-^{14}C]xylose as substrate for UDP-D-xylose 4-epimerase.

Properties of UDP-D-glucuronate decarboxylase E.C. 4.1.1.35)

Reaction rate as a function of time and enzyme concentration. The enzyme activity varied linearly with time for at least 10 min (Fig. 3) and it was a linear function of protein concentration (Fig. 4).

Kinetics of UDP-D-glucuronate decarboxylase. The effect of UDP-D-[U-^{14}C]glucuronic acid concentration on the reaction rate is shown in Fig. 5. The apparent K_m value calculated by the method of Lineweaver and Burk [32] was 0.6 mM.

Effect of NAD$^+$, NADH, Ca^{2+}, Mg^{2+} and p-hydroxymercuribenzoate on reaction rate. NAD^+, NADH, Ca^{2+} and Mg^{2+} in the concentration range 0.001–1 mM had no effect on UDP-D-glucuronate decarboxylase activity. The enzymic activity was 95% inhibited by *p*-hydroxymercuribenzoate (1 mM). Cysteine did not remove this inhibition.

Effects of plant hormones on the isolated enzyme preparation. IAA (5 mg/l.), GA_3 (1 mg/l.) and zeatin (1 mg/l.) did not affect the reaction rate.

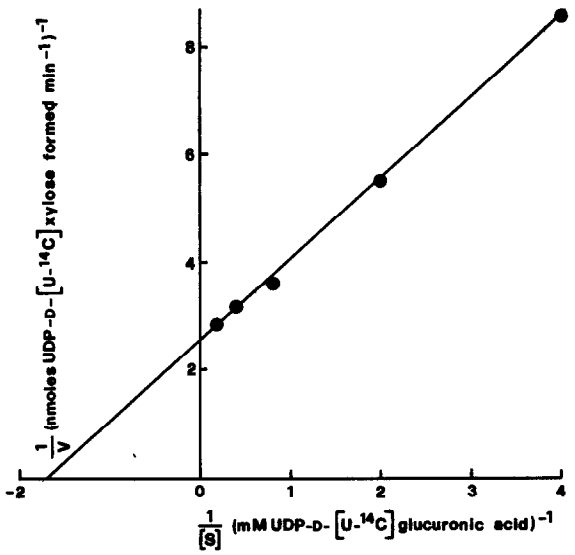

Fig. 5. Kinetics of UDP-D-glucuronate decarboxylase in cultured explants of Jerusalem artichoke. The reaction mixtures contained 25 µg of enzyme (protein precipitated between 40–65% $(NH_4)_2SO_4$ saturation) extracted from cultured explants of Jerusalem artichoke treated with IAA (5 mg/l.), and UDP-D-[U-^{14}C]glucuronic acid (1–20 mM), in 0.2 M NaPi buffer, pH 8, in a total volume of 20 µl. Reaction time at 37° was 5 min. The amount of UDP-D-[U-^{14}C]xylose and UDP-L-[U-^{14}C]-arabinose formed was determined as described [26]. UDP-D-[U-^{14}C]xylose and UDP-L-[U-^{14}C]arabinose were corrected for the loss of the C-6 from [U-^{14}C]glucuronic acid. Enzyme from IAA (5 mg/l.) treated explants (2-day incubation), ●.

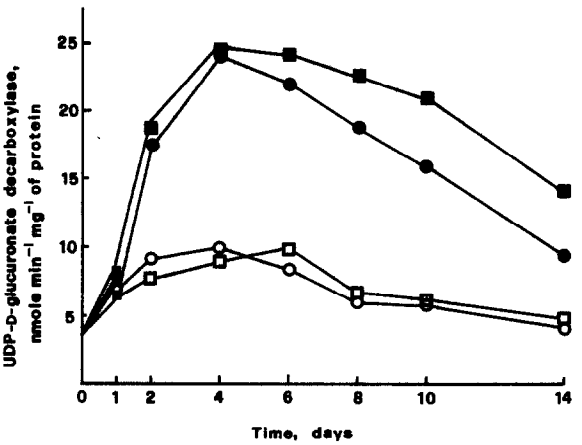

Fig. 6. Time course of the specific activity of UDP-D-glucuronate decarboxylase in cultured explants of Jerusalem artichoke. Specific activity (nmoles/min/mg protein). The reaction mixtures contained 50 nmoles of UDP-D-glucuronic acid. 0.432 nmoles of UDP-D-[U-^{14}C]glucuronic acid (135000 cpm) and enzyme (protein precipitated between 40–65% $(NH_4)_2SO_4$ saturation) extracted from cultured explants of Jerusalem artichoke treated and untreated with plant growth hormones as indicated, in 0.2 M NaPi buffer, pH 8, in a total volume of 20 µl. Reaction time at 37° was 5 min. The amount of UDP-D-[U-^{14}C]-xylose and UDP-L-[U-^{14}C]arabinose formed was determined as described [26]. UDP-D-[U-^{14}C]xylose and UDP-L-[U-^{14}C]-arabinose were corrected for the loss of the C-6 from [U-^{14}C]-glucuronic acid. Enzyme from IAA (5 mg/l.) treated explants, ●; Enzyme from IAA (5 mg/l.) + GA_3 (1 mg/l.) treated explants, ■; Enzyme from GA_3 (1 mg/l.) treated explants, □; Enzyme from control, ○.

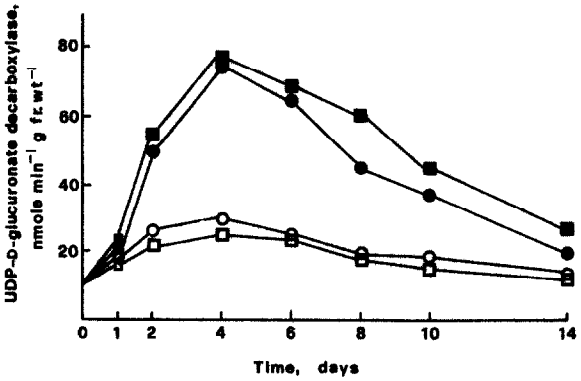

Fig. 7. Time course of the total activity per g fresh weight of UDP-D-glucuronate decarboxylase in cultured explants of Jerusalem artichoke. Total activity per g fresh weight (nmoles/min^{-1} g fr. wt^{-1}). The reaction mixtures contained 50 nmoles of UDP-D-glucuronic acid, 0.432 nmoles of UDP-D-[U-^{14}C]-glucuronic acid (135000 cpm) and enzyme (protein precipitated between 40–65% $(NH_4)_2SO_4$ saturation) extracted from cultured explants of Jerusalem artichoke treated and untreated with plant growth hormones as indicated, in 0.2 M NaPi buffer, pH 8, in a total volume of 20 µl. Reaction time at 37° was 5 min. The amount of UDP-D-[U-^{14}C]xylose and UDP-L-[U-^{14}C]-arabinose formed was determined as described [26]. UDP-D-[U-^{14}C]xylose and UDP-L-[U-^{14}C]arabinose were corrected for the loss of the C-6 from [U-^{14}C]glucuronic acid. Enzyme from IAA (5 mg/l.) treated explants, ●; Enzyme from IAA (5 mg/l.) + GA_3 (1 mg/l.) treated explants, ■; Enzyme from GA_3 (1 mg/l.) treated explants, □; Enzyme from control, ○.

Time course of UDP-D-glucuronate decarboxylase activity in cultured explants of Jerusalem artichoke

Changes in the specific activity (nmoles/min/mg of protein) and in the total activity per g fresh weight (nmoles/min/g fresh weight) of UDP-D-glucuronate decarboxylase in explants cultured under different hormonal conditions are shown in Figs. 6 and 7 respectively. Under all the experimental conditions, at the end of 24 hr, the enzymic activity had increased slightly to nearly the same level (initial lag-phase). A marked increase in the activity of the enzyme was then observed for IAA and IAA + GA_3 treated explants at the end of 48 hr (beginning of division phase). However, after the 6th day although cell division and tracheary element formation were occurring at the same rate, the enzyme activity in IAA and IAA + GA_3 treated explants started to decrease. In the control and GA_3 treated explants, where cell division and cytodifferentiation did not occur, there was a slight increase in the enzymic activity between the 2nd and the 4th day, followed by a decrease.

Properties of UDP-D-xylose 4-epimerase (E.C. 5.1.3.5)

Reaction rate as a function of time and enzyme concentration. The enzymic activity was a linear function of time (Fig. 8) and protein concentration (Fig. 9).

Kinetics of UDP-D-xylose 4-epimerase. The Lineweaver–Burk plot [32] showing the reciprocal of reaction rate versus the reciprocal of UDP-D-[U-^{14}C]-xylose concentration is presented in Fig. 10. The apparent K_m of UDP-D-xylose 4-epimerase for UDP-D-xylose was calculated to be 0.16 mM.

Effect of NAD^+, NADH, Ca^{2+}, Mg^{2+} and p-hydroxymercuribenzoate on reaction rate. NAD^+, NADH, Ca^{2+} and Mg^{2+} between 0.001 and 1 mM had no effect on reaction rate. *p*-Hydroxymercuribenzoate (1 mM) inhibited all the enzymic activity.

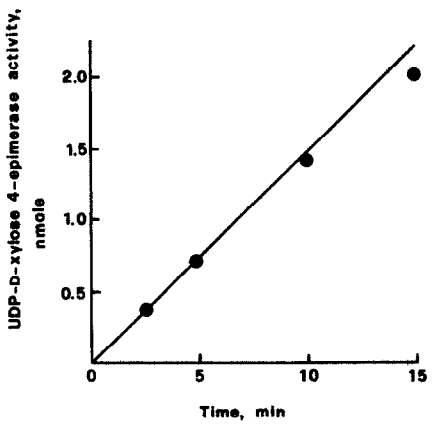

Fig. 8. Reaction rate of UDP-D-xylose 4-epimerase as a function of time in cultured explants of Jerusalem artichoke. The reaction mixtures contained 10 nmoles of UDP-D-xylose, 0.511 nmoles of UDP-D-[U-^{14}C]xylose (98300 cpm) and 52.4 µg of enzyme (protein precipitated between 40–65% $(NH_4)_2SO_4$ saturation) extracted from cultured explants of Jerusalem artichoke treated with IAA (5 mg/l.), in 0.2 M NaPi buffer, pH 8, in a total volume of 20 µl, at 30°. One nmole substrate was equivalent to 9350 cpm. The amount of UDP-L-[U-^{14}C]arabinose formed was determined as described [26]. Enzyme from IAA (5 mg/l.) treated explants (8-day incubation), ●.

Effect of plant hormones on the isolated enzyme preparation. IAA (5 mg/l.), GA_3 (1 mg/l.) and zeatin (1 mg/l.) had no effect on reaction rate.

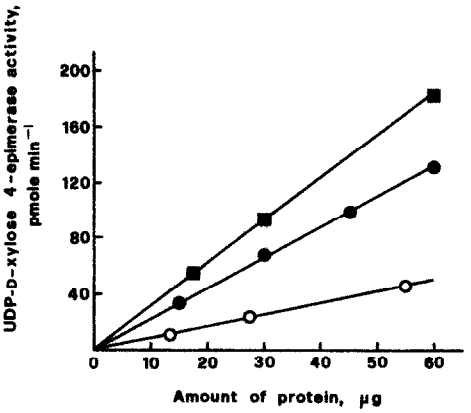

Fig. 9. Reaction rate of UDP-D-xylose 4-epimerase as a function of protein concentration in cultured explants of Jerusalem artichoke. The reaction mixtures contained 10 nmols of UDP-D-xylose, 0.511 nmoles of UDP-D-[U-^{14}C]xylose (98300 cpm) and enzyme (protein precipitated between 40–65% $(NH_4)_2SO_4$ saturation) extracted from cultured explants of Jerusalem artichoke treated and untreated with plant growth hormones as indicated, in 0.2 M NaPi buffer, pH 8. in a total volume of 20 µl. Reaction time at 30° was 5 min. One nmole substrate was equivalent to 9350 cpm. The amount of UDP-L-[U-^{14}C]-arabinose formed was determined as described [26]. Enzyme from IAA (5 mg/l.) treated explants (6-day incubation), ●; Enzyme from IAA (5 mg/l.) + GA_3 (1 mg/l.) treated explants (6-day incubation), ■; Enzyme from control (6-day incubation), ○.

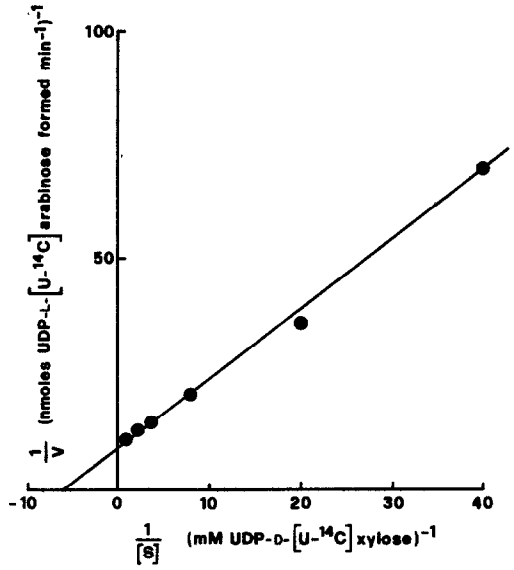

Fig. 10. Kinetics of UDP-D-xylose 4-epimerase with UDP-D-[U-^{14}C]xylose as substrate in cultured explants of Jerusalem artichoke. The reaction mixtures contained 75 µg of enzyme (protein precipitated between 40–65% $(NH_4)_2SO_4$ saturation) extracted from cultured explants of Jerusalem artichoke treated with IAA (5 mg/l.), and UDP-D-[U-^{14}C]xylose (0.025–1.25 mM), in 0.2 M NaPi buffer, pH 8, in a total volume of 20 µl. Reaction time at 30° was 5 min. The amount of UDP-L-[U-^{14}C]arabinose formed was determined as described [26]. Enzyme from IAA (5 mg/l.) treated explants (8-day incubation), ●.

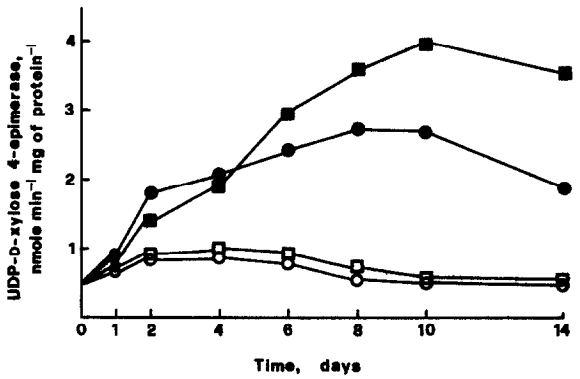

Fig. 11. Time course of the specific activity of UDP-D-xylose 4-epimerase in cultured explants of Jerusalem artichoke. Specific activity (nmoles/min/mg protein). The reaction mixtures contained 10 nmoles of UDP-D-xylose, 0.511 nmoles of UDP-D-[U-^{14}C]xylose (98300 cpm) and enzyme (protein precipitated between 40–65% $(NH_4)_2SO_4$ saturation) extracted from cultured explants of Jerusalem artichoke treated and untreated with plant growth hormones as indicated, in 0.2 M NaPi buffer, pH 8, in a total volume of 20 µl. Reaction time at 30° was 5 min. One nmole substrate was equivalent to 9350 cpm. The amount of UDP-L-[U-^{14}C]arabinose formed was determined as described [26]. Enzyme from IAA (5 mg/l.) treated explants, ●; Enzyme from IAA (5 mg/l.) + GA_3 (1 mg/l.) treated explants, ■; Enzyme from GA_3 (1 mg/l.) treated explants, □; Enzyme from control, O.

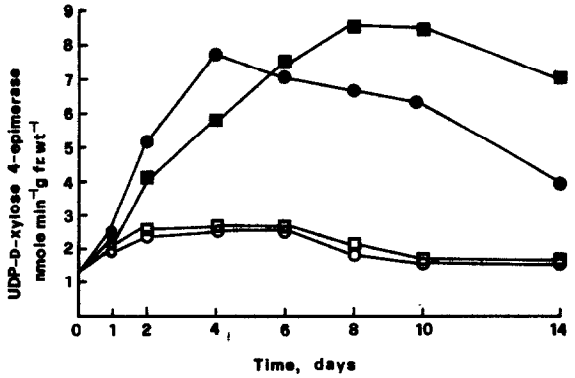

Fig. 12. Time course of the total activity per g fresh weight of UDP-D-xylose 4-epimerase in cultured explants of Jerusalem artichoke. Total activity per g fresh weight (nmoles/min/g fr. wt). The reaction mixtures contained 10 nmoles of UDP-D-xylose. 0.511 nmoles UDP-D-[U-^{14}C]xylose (98000 cpm) and enzyme (protein precipitated between 40–65% $(NH_4)_2SO_4$ saturation) extracted from cultured explants of Jerusalem artichoke treated and untreated with plant growth hormones as indicated, in 0.2 M NaPi buffer, pH 8, in a total volume of 20 µl. Reaction time at 30° was 5 min. One nmole substrate was equavalent to 9350 cpm. The amount of UDP-L-[U-^{14}C]arabinose formed was determined as described [26]. Enzyme from IAA (5 mg/l.) treated explants, ●; Enzyme from IAA (5 mg/l.) + GA_3 (1 mg/l.) treated explants, ■; Enzyme from GA_3 (1 mg/l.) treated explants, □; Enzyme from control, O.

Time course of UDP-D-xylose 4-epimerase activity in cultured explants of Jerusalem artichoke

Changes in the specific activity (nmoles/min/mg of protein) and in the total activity per g fresh weight (nmoles/min/g fresh weight) of UDP-D-xylose 4-epimerase during the time course are shown in Figs. 11 and 12. The enzymic activity of the dormant explants (zero time) increased after 24 hr of culture under all conditions of growth. Then the activity sharply increased in those explants treated with IAA and IAA + GA_3 until the 10th day when it declined till the end of the 14th day. IAA + GA_3 treated explants had an enzymic activity higher than that of those treated with IAA alone. Control and GA_3 treated explants showed little variation in the activity of UDP-D-xylose 4-epimerase during the time course.

DISCUSSION

Pith parenchyma cells of Jerusalem artichoke normally divide and differentiate into tracheary elements when cultured *in vitro* under a defined physical–chemical environment [16, 18, 33]. After an initial lag-phase (the first 24 hr), the resting cells which are induced to divide, do so with a high degree of synchrony at least during the first two division cycles [34]. Most of these cells continue to divide, but some expand and differentiate into tracheids. This transition from the differentiated quiescent parenchymatous cells to dividing cells (dedifferentiated state) and to the redifferentiation into tracheary elements is achieved and controlled by complex hormonal interactions. The induction of meristematic-type cells and the subsequent differentiation of some of them into tracheids is accompanied by an enhancement of the metabolic paths which lead to the biosynthesis of different cell wall polysaccharides during primary and secondary growth.

Pectic substances are only synthesized during the formation of cell plate, middle lamella and primary cell wall whereas the formation of secondary thickening is characterized by an increased synthesis of hemicelluloses and cellulose [24]. UDP-D-xylose 4-epimerase in addition to UDP-D-glucuronate and UDP-D-galactose 4-epimerases are the enzymes that form the UDP sugars of the galactose series which act as glycosyl donors of the polygalacturonic acid backbone, the arabinan, the galactan and the arabinogalactan polymers of pectic materials [25]. On the other hand, UDP-D-glucose dehydrogenase and UDP-D-glucuronate decarboxylase lead to the formation of UDP-D-glucuronic acid and UDP-D-xylose which are the precursors of xylans. These polymers represent the main hemicellulose fraction of angiosperms [25]. Therefore, UDP-D-glucuronate decarboxylase and UDP-D-xylose 4-epimerase are directly involved in the production of some of the precursors of hemicellulose and pectin. The specific enzymic activities of these two enzymes in cultured pith parenchyma explants treated and untreated with plant growth regulators increased during the initial lag-phase (the first 24 hr) which is a period of active metabolic change. This initial phase is induced by cutting out the explants and incubating them on a solid medium and the enhancement of enzyme activities was not significantly affected by treatment with hormones. However, with the beginning of cell division, induced by treatment with IAA and IAA + GA_3, the specific activity of UDP-D-xylose 4-epimerase and UDP-D-glucuronate decarboxylase increased sharply compared with that of the control and GA_3 treated explants where neither division nor differentiation occurred. UDP-D-xylose 4-epimerase continued to increase until the 10th day of incubation in actively dividing and differentiating cells and then slightly decreased. Little variation was observed in the activity of the epimerase in the control and GA_3 treated explants over 14 days.

These results suggest that the changes in the specific activity of the epimerase during the time course were correlated with the increased demand for UDP-L-arabinose which is one of the precursors required for pectin synthesis during the formation of cell plate, middle lamella and primary wall. Effects of auxin on net pectin synthesis have been reported [35–39]. Further, Rubery and Northcote [40] have demonstrated that 2',4'-dichlorophenoxyacetic acid increased the incorporation of radioactive arabinose into the pectins of sycamore callus, probably by increasing the activity of the transglycosylase which transfers L-arabinose from UDP-L-arabinose to the pectic arabinogalactan. Our results indicate that there is also an increase in the activity of the epimerase which produces the activated precursor of araban and/or arabinogalactan.

Although fully differentiated tracheids were observed approximately 20 hr after cell division had occurred, a considerable amount of tracheids per gram fresh weight was present at the end of the 4th day in IAA and IAA + GA_3 treated explants. The appearance of this specific type of cytodifferentiation was paralleled by the highest specific enzymic activity of UDP-D-glucuronate decarboxylase. In both sycamore and poplar during the differentiation of cambial cells to xylem cells a similar response was observed [26]. These data confirm the close correlation between UDP-D-glucuronate decarboxylase activity and secondary wall formation. However, it was also found that UDP-D-glucuronate decarboxylase activity started to decrease gradually from the 6th day in both IAA and IAA + GA_3 treated explants, although, the percentage of differentiation remained nearly constant. This could be explained when it is considered that tracheids are cells that undergo terminal differentiation with accompanying death during the normal course of their development. So that in any cell count of the number of tracheids present in an explant after 4 days an increasing percentage of these will be dead cells.

Wright and Bowles [41] have reported that, in cultured explants of cabbage and cos lettuce supplied with D-[U-^{14}C]-glucose, the IAA-zeatin treatment which induced division and tracheary element differentiation was accompanied by a remarkable increase in the amount of radioactivity in polysaccharides isolated from Golgi apparatus, endoplasmic reticulum and walls. Our data suggest that this enhancement in the synthetic capacities of the polysaccharide-synthesizing endomembrane system is also associated with an increased catalytic activity of the enzymes producing the activated precursors of pectin and hemicellulose syntheses.

EXPERIMENTAL

Chemicals and radiochemicals. The various chemicals and radio-chemicals used are described in ref. [26].

Preparation of cultured explants of Jerusalem artichoke. Jerusalem artichoke tubers (*Helianthus tuberosus* L.) were purchased from the local market and stored in sterile dry sand at 2°. Before use, the selected tubers were thoroughly washed in H_2O, soaked in a 10% NaClO soln for 20 min and rinsed repeatedly with H_2O. All further manipulations were done aseptically under a sterile air bench. The tubers were cut transversely 2 cm from the upper and basal end and then from each tuber one cylinder of storage pith parenchyma was taken with a 4 mm (i.d.) cork borer from the central core. The cylinders were successively cut into 1 mm thick explants and washed $\times 3$ in H_2O. The explants were dried on filter paper and transferred to Petri dishes, 10 explants per dish, containing 3% sucrose (w/v), 1% agar, Murashige and Skoog's medium [42] and plant growth regulators (1AA + GA_3) in various combinations as specified for each experiment. The media were adjusted to pH 5.7 and autoclaved at 103.4 N/m^2 at 120° for 10 min. The solutions of IAA and GA_3 were cold sterilized by Millipore filtration and added to the autoclaved medium before cooling. The Petri dishes, containing the explants, were cultured in the dark at 27 ± 1°. After varying periods of incubation the explants were used either for the enzyme extraction or for the counting of the cells (total number of the cells and number of tracheids for each explant).

Enzyme preparation. Preparations of crude extract, $MnCl_2$ treatment and fractions of the extract prepared from cultured explants of Jerusalem artichoke treated and untreated with plant hormones were made as previously described [26]. The protein fraction pptd between 40–65% $(NH_4)_2SO_4$ saturation was used for all enzymic assays. It retained full activity for at least a month when stored at −16°.

Analytical methods. PC, electrophoresis, radioactivity counting procedure, detection methods, protein estimation and procedures for the counting of cells were similar to those previously described [26].

Enzyme assays. UDP-D-glucuronate decarboxylase (E.C. 4.1.1.35) and UDP-D-xylose 4-epimerase (E.C. 5.1.3.5) were assayed radiochemically as previously described [26].

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