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A xylosyltransferase involved in the synthesis of a protein-associated xyloglucan in suspension-cultured dwarf-French-bean (*Phaseolus vulgaris*) cells and its interaction with a glucosyltransferase

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A particulate enzyme preparation made from suspension-cultured dwarf-French-bean (*Phaseolus vulgaris*) cv. Canadian Wonder cells was shown to incorporate xylose from UDP-D-[^{14}C]xylose into polysaccharide. The reaction was dependent upon the presence of UDP-D-glucose and was stimulated, and apparently protected, by GDP-D-glucose and GDP-D-mannose, though neither was able to replace UDP-D-glucose as a glycosyl donor. The product of the reaction was identified as xyloglucan by analysis of products of enzyme breakdown and acid hydrolysis. M_r determination after proteinase K digestion indicated that the nascent xyloglucan is closely associated with protein. Preincubation of the enzyme with UDP-D-glucose stimulated incorporation from UDP-D-[^{14}C]xylose, suggesting an 'imprecise' mechanism of biosynthesis, as defined by Waldron & Brett [(1985) in Biochemistry of Plant Cell Walls (Brett, C. T. & Hillman, J. R., eds.) (SEB Semin. Ser. **28**), pp. 79–97, Cambridge University Press, Cambridge].

INTRODUCTION

Xyloglucans are major structural hemicellulosic polysaccharides that occur in seeds, primary cell walls of plants and in the extracellular medium of suspension-cultured plant cells (O'Neill & Selvendran, 1983). They possess a β1-4-glucan backbone with α-xylose residues attached to the 6-position of β-glucose residues. Additional fucose-galactose residues are present attached to xylose in some, but not all, species. It has been proposed that in pea (*Pisum sativum*) xyloglucan, two subunits make up the molecule, a nonasaccharide with glucose, xylose, galactose and fucose in the ratio 4:3:1:1, and a heptasaccharide with glucose and xylose in the ratio 4:3, with units distributed in alternating sequence. In other tissues, however, the structure may be less regular (Ruperez *et al.*, 1985; O'Neill & Selvendran, 1986).

Xyloglucan is closely bound to cellulose microfibrils and apparently has an important structural function (Bauer *et al.*, 1973). However, the metabolism of xyloglucan is likely to be regulated by different processes from that of cellulose, since it is subject to turnover during growth (Hayashi & Maclachlan, 1984).

Investigations of xyloglucan biosynthesis have involved the use of particulate enzyme preparations from immature pea tissue and from suspension-cultured soybean (*Glycine max*) cells. Ray (1980) examined a UDP-xylose:xyloglucan xylosyltransferase associated with pea Golgi membranes whose action is stimulated by UDP-D-glucose. He suggested that at least two enzymes were involved: a β1-4-glucan synthetase, forming the backbone, and a xylosyltransferase, transferring xylose units on to the glucan core. Xylose transfer was stimulated by UDP-D-glucose when the latter was present only in a pre-incubation, suggesting that the β1-4-glucan backbone is being synthesized independently of the side chains.

Hayashi & Matsuda (1981*a*) investigated the incorporation of xylose from UDP-D-xylose by a particulate enzyme preparation from suspension-cultured soybean cells. They found incorporation was dependent upon the presence of UDP-D-glucose and a bivalent cation. They suggested that at least two enzymes were involved and that glucose transfer is dependent on the presence of UDP-D-xylose: a finding in contrast with the conclusions made by Ray (1980). Hayashi & Matsuda (1981*b*) proposed that the xyloglucan synthase complex transfers glucose and xylose alternately.

The mechanisms of cell-wall-polysaccharide biosynthesis are far from clear, but two possible models have been discussed by Waldron & Brett (1985). In the first model, 'imprecise' synthesis would occur, involving enzymes specific enough to define residues and linkages, but unable to give any further regularity to the product. Side chains would be irregularly attached and of variable length. The backbone would be synthesized independently of the side chains. In the second model, 'precise' synthesis would involve synthesis of identical repeating subunits linked in a precise manner, but of undefined number. It is expected that the backbone of such a 'precisely' synthesized molecule would be unable to grow without concomitant addition of side chains and non-glycosyl substituents.

In terms of these two models, the biosynthesis system proposed by Ray (1980) for pea xyloglucan appears to be 'imprecise', since the backbone can be synthesized independently of the side chains. On the other hand, the system proposed by Hayashi & Matsuda (1981*a*,*b*) for soybean xyloglucan appears to be 'precise'. Since it is unlikely that the same polysaccharide would be synthesized by completely different mechanisms in such

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closely related plants, the present study was undertaken to clarify the matter. We have studied xyloglucan synthesis in suspension-cultured dwarf-French-bean cells (*Phaseolus vulgaris*) and have found that the glucan backbone can be synthesized independently of side-chain addition, indicating an 'imprecise' biosynthesis mechanism.

MATERIALS AND METHODS

Chemicals and plant material

UDP-D-[U^{14}C]xylose (35.2 GBq·mmol^{-1}) was purchased from New England Nuclear, Stevenage, Herts., U.K. UDP-D-[U-^{14}C]glucose (11.1 GBq·mmol^{-1}), D-[U-^{14}C]glucose (10 GBq·mmol^{-1}) and D-[U-^{14}C]xylose (2.81 GBq·mmol^{-1}) were purchased from Amersham International, Amersham, Bucks., U.K. Non-radioactively labelled sugar nucleotides, proteinase K and driselase (a crude basidiomycete powder containing laminarinase, xylanase and cellulase; batch no. 74F-058-) were purchased from Sigma Chemical Company, Poole, Dorset, U.K. Dwarf French beans (*Phaseolus vulgaris*, cv. Canadian Wonder) were supplied by Sinclair McGill (Ayr, Scotland, U.K.) and arrived pre-treated with 'Lindane Thiram'. [1-^{3}H]Xylα1-6Glc (isoprimeverose) was generously given by Dr. S. C. Fry, Department of Botany, University of Edinburgh, Edinburgh, Scotland, U.K., and purified tamarind (*Tamarindus indica*) xyloglucan was kindly given by Dr. J. S. G. Reid, Department of Biological Science, University of Stirling, Stirling, Scotland, U.K.

Media

Callus and suspension bean cultures were grown on the B5 medium of Gamborg *et al.* (1968), supplemented with 2% (w/v) sucrose, 2,4-dichlorophenoxyacetic acid (2 mg/l) and deproteinized 20% (v/v) coconut milk, adjusted to pH 5.5 before autoclaving at 120 °C for 20 min. Solid media contained 1% agar, which was added before autoclaving.

Tissue culture

Seeds of *Phaseolus vulgaris* were surface-sterilized in a buffered solution of sodium hypochlorite (Milton; Richardson–Vicks, Egham, Surrey, U.K.) for 8 h and, after washing in distilled water, were then placed on sterile-water agar to germinate. When hypocotyls were 60–100 mm long, 5 mm sections were excised and placed on agar medium at 25 °C in the dark. The callus which formed after 3–4 weeks growth was removed from the explant and transferred to fresh medium. Thereafter portions of approx. 1 g were transferred to fresh medium every 4–6 weeks. After about 8 weeks, friable callus was transferred into liquid medium in 500 ml Erlenmeyer suspension-culture flasks, rotating at 100 rev./min at 25 °C. Subculturing was initially carried out after 3–4 weeks, after which the interval decreased to every 4–5 days.

Particulate enzyme preparation

Suspension-cultured tissue (harvested 3–6 days after subculturing) was filtered through four layers of muslin. The filtered tissue was weighed, then ground with acid-washed sand in a mortar with an equal volume of 100 mM-Tris, adjusted to pH 7.5 with HCl. After filtering through muslin a second time, the filtrate was centrifuged for 30 min at 97000 *g*. The pellet was resuspended in 0.5 ml of Tris/HCl, pH 7.5, in a glass homogenizer. All procedures were carried out at 0–4 °C.

Incubations

The standard incubation mixture (variations are explained in the Results section) contained 50 μl of enzyme preparation, 10 mM-$MnCl_2$, 21 μM-UDP-D-[^{14}C]-xylose and 2 mM-UDP-D-glucose in a total volume of 100 μl. Incubations were carried out at 25 °C and were terminated by addition of 96% (v/v) ethanol (1 ml).

Preparation of incubated material for the determination of radioactivity

The terminated mixtures were washed five times in 70% (v/v) ethanol, then four times in water to remove any low-M_r radioactive material.

Enzymic hydrolysis of polysaccharides

Driselase (50 mg) was dissolved in 50 mM-acetate buffer (1 ml) adjusted to pH 4.5 with NaOH. This was centrifuged for 5 min at 700 *g* to remove inert carrier material. Polysaccharide pellets were dried overnight under reduced pressure to remove ethanol, vortex-mixed with 1 ml of driselase solution and incubated overnight at 25 °C. Incubations were terminated by boiling for 5 min, and the supernatant was removed and dried under reduced pressure at 40 °C.

Laminarinase (10 mg) was dissolved in 50 mM-acetate buffer, pH 4.5 (1 ml). Polysaccharide pellets were prepared as above and incubated with enzyme solution (1 ml) for 5 h or overnight at 25 °C. Non-radioactively labelled samples (10 mg) were incubated in the same way.

Acid hydrolysis of polysaccharides

Vacuum-dried pellets were dissolved in 1 ml of trifluoroacetic acid (2 M) and autoclaved in sealed Reacti-Vials (Pierce and Warriner, Chester, U.K.) for 1 h or more at 120 °C. The hydrolysate was dried under reduced pressure at 40 °C.

Borohydride reduction of radioactive xylose and glucose for use as chromatographic markers

Samples of D-[^{14}C]glucose (0.75 mM, 7.4 KBq) and D-[^{14}C]xylose (2.6 mM, 7.4 KBq) were reduced in 200 mM-$NaBH_4$ in 1 M-NH_3 (0.5 ml) at 15–20 °C, then neutralized by the addition of acetic acid dropwise until effervescence ceased. Boric acid was removed by four evaporation cycles with 10 ml of redistilled methanol at 40 °C under reduced pressure.

Borohydride reduction and acid hydrolysis of radioactively labelled disaccharides

For identification of their reducing end group, radioactively labelled disaccharides were eluted from paper chromatograms, reduced as described above and hydrolysed in 3% (w/v) H_2SO_4 (1 ml) at 120 °C for 1 h. The solution was neutralized with Amberlite IR 45 ion-exchange resin (HCO_3^- form), then dried under reduced pressure at 40 °C.

Cadoxen solubilization of polysaccharide and determination of M_r by using Sepharose CL-6B and CL-2B

To solubilize polysaccharide, the dried pellet was

mixed with 0.25 ml of Cadoxen (Wood & McCrae, 1978) and sonicated for 3–4 days at room temperature. The M_r of the polysaccharide was then estimated by running the solubilized samples down Sepharose CL-6B and CL-2B gel-filtration columns (50 cm × 1 cm) in 50% (w/v) cadoxen solution. Dinitrophenyl-lysine and Blue Dextran were used as markers. Fractions (0.5 ml) were counted for radioactivity.

Chromatography

Descending paper chromatography was carried out on Whatman no. 1 paper in the following solvents: (A) butan-1-ol/pyridine/water (6:4:3, by vol.) (Brett & Leloir, 1977); (B) ethyl acetate/pyridine/water (8:2:1, by vol.); (C) butan-2-one/acetic acid/aq. satd. boric acid (9:1:1, by vol.) (Rees & Reynolds, 1958). Marker sugars were detected as described by Trevelyan *et al.* (1950) or, if radioactive, by liquid-scintillation counting.

Cellulose-binding assay

Radioactively labelled samples from incubations with UDP-D-[^{14}C]xylose were partially solubilized by three successive incubations (30 min each) with driselase (1 ml). This process solubilized 48% of the radioactivity. Supernatants were pooled, rotary-evaporated to dryness, dissolved in 400 μl of water and then added to 100 mg of cellulose powder (Machery–Nagel) with 2 ml of acetone to give an 83% (v/v) acetone solution. This was stirred for 30 min and then left to settle for 2 h. After centrifuging at 700 *g* for 5 min the supernatant was dried on to a chromatography-paper strip and its radioactivity was measured. The cellulose powder was then extracted for 30 min with 24% KOH (0.5 ml) and centrifuged as described above. The supernatant was neutralized with 2 drops of acetic acid, and its radioactivity was measured.

Determination of radioactivity

Pellets were suspended in 0.5 ml H_2O and vortex-mixed with 5 ml of Unisolve 1 (Koch–Light, Haverhill, Suffolk, U.K.). Alternatively, if samples were to be retained for further analysis, they were washed twice with ethanol, then vortex-mixed with 2 ml of toluene scintillant (Harris & Northcote, 1970). Strips of chromatography paper were placed in scintillation vials with 1 ml of toluene scintillant as described above. Radioactivity was determined on a Packard liquid-scintillation spectrometer (model 3380).

RESULTS AND DISCUSSION

Properties of the xylosyltransferase

The xylosyltransferase was found to be almost inactive unless UDP-D-glucose was present in the incubation (Table 1). The optimum concentration of UDP-D-glucose for incorporation of radioactivity from 21 μM-UDP-D-[^{14}C]xylose was found to be 2 mM (Table 2). The reaction was stimulated by bivalent cations, Mn^{2+} being the most effective (Table 3). The optimum concentration of Mn^{2+} was approx. 10 mM, and the enzyme showed a broad pH optimum with a maximum at about pH 7 (results not shown).

Characterization of the product

Acid hydrolysis of the product formed from UDP-D-[^{14}C]xylose in the presence of 2 mM-UDP-D-glucose, followed by paper chromatography in solvent B, showed that most of the radioactivity was present as xylose (94%), together with a small amount of arabinose (6%). Digestion of the product with driselase, followed by paper chromatography in solvent A, gave a single peak of radioactivity co-chromatographing with isoprimeverose (Xylα1-6Glc). This material was eluted from the chromatogram and subjected to acid hydrolysis followed by chromatography in solvent B; all the radioactivity co-chromatographed with xylose. Another sample of the product of the driselase digestion was reduced with sodium borohydride and then hydrolysed with acid and

Table 1. Effect of UDP-D-xylose concentration on incorporation of radioactivity from UDP-D-[^{14}C]xylose, in the presence and absence of UDP-D-glucose

Incubations were of 15 min duration under standard incubation conditions.

[UDP-D-xylose] (μM)	Radioactivity incorporated (d.p.m.) Without UDP-D-glucose	With UDP-D-glucose
0.8	227±43	1428±82
2.8	184±10	1305±80
21	112±20	1050±84
201	40±15	36±5

Table 2. Effect of UDP-D-glucose concentration on incorporation of radioactivity from UDP-D-[^{14}C]xylose

Incubations were of 15 min duration under standard incubation conditions.

[UDP-D-glucose] (mM)	Radioactivity incorporated (d.p.m.)
0	291±50
0.02	1278±25
0.2	1181±105
2	2668±225
5	2028±150

Table 3. Effect of bivalent cations on the incorporation of radioactivity from UDP-D-[^{14}C]xylose

Standard incubation conditions were used, with cations present at 10 mM and with an incubation time of 15 min.

Bivalent cation	Radioactivity incorporated (d.p.m.)
Ca^{2+}	480±14
Co^{2+}	664±69
Cu^{2+}	123±12
Mg^{2+}	606±70
Mn^{2+}	890±42

chromatographed with solvent C, which separates sugars from corresponding sugar alditols. The radioactivity still co-chromatographed with xylose, indicating that xylose occupied the non-reducing position in the disaccharide produced by driselase.

In a parallel experiment, the product was labelled with [^{14}C]glucose by incubation of the membrane preparation with UDP-D-[^{14}C]glucose and non-radioactive UDP-D-xylose; incubation of the product with driselase again produced a radioactive disaccharide which co-chromatographed with isoprimeverose in solvent A. Acid hydrolysis, followed by chromatography in solvent B, gave rise to [^{14}C]glucose, whereas borohydride reduction followed by acid hydrolysis and chromatography in solvent C produced only [^{14}C]glucitol. Hence glucose occupied the reducing end of the disaccharide produced by driselase digestion.

These results confirm that the product of driselase digestion was isoprimeverose, and this in turn identifies the product of the incubation of the membranes with UDP-D-[^{14}C]xylose and UDP-D-glucose as xyloglucan, since isoprimeverose is a characteristic structural unit of xyloglucan (Hayashi *et al.*, 1981).

In order to exclude the possibility that the xyloglucan contained a β1-3-glucan backbone, the linkage of the [^{14}C]xylose-labelled product was analysed by hydrolysis with a β1-3-glucanase preparation, laminarinase. The activity of this preparation was first tested against tamarind xyloglucan and against commercial laminarin, and hydrolysates were analysed by paper chromatography in solvent A. The laminarinase preparation hydrolyses the laminarin to yield a series including laminaritetrose, laminaritriose, laminaribiose and glucose, but it has no activity against tamarind xyloglucan. A [^{14}C]xylose-labelled product of the incubation of UDP-D-[^{14}C]xylose with membrane preparation was incubated with laminarinase for 5 h and for 24 h. The hydrolysates were analysed by chromatography in solvent A, and no evidence of breakdown was found. Hydrolysis of a [^{14}C]glucose-labelled product of an incubation with UDP-D-[^{14}C]glucose (2 mM, 925 Bq) and UDP-D-xylose (20 μM), on the other hand, yielded two peaks of radioactivity corresponding to glucose and laminaribiose.

These results indicate that the glucan backbone of the radioactive product of incubation of membrane preparation with UDP-D-glucose was not β1-3-linked, and at least some of the product of incubation with UDP-D-[^{14}C]glucose was β1-3-linked.

Further analysis of the [^{14}C]xylose-labelled polysaccharide was carried out by examining binding of the product to cellulose. Bauer *et al.* (1973) have presented evidence in support of the theory that hemicelluloses bind strongly to cellulose, an attachment mediated through hydrogen bonds, and reported that xyloglucan fragments became bound to Whatman cellulose in less than 5 min. Valent & Albersheim (1974) found that for a seven-sugar xyloglucan fragment, 45% binding occurred at 25 °C when the reaction was in 65% (v/v) acetone, and Aspinall *et al.* (1969) reported that, for xyloglucan and its digestion products, up to two-thirds of the bound radioactivity was charateristically desorbed by 24% KOH.

[^{14}C]Xylose-labelled pellets from 60 min incubations were pooled to give a sample containing 50000 d.p.m. This was subjected to partial driselase hydrolysis, and then assayed for binding to cellulose and release of bound product by KOH extraction. Of the radioactivity, 59% was found to bind to the cellulose powder in 83% (v/v) acetone, and 31% of the bound radioactivity was then released by KOH extraction. These results provide further evidence that the [^{14}C]xylose-labelled product is xyloglucan.

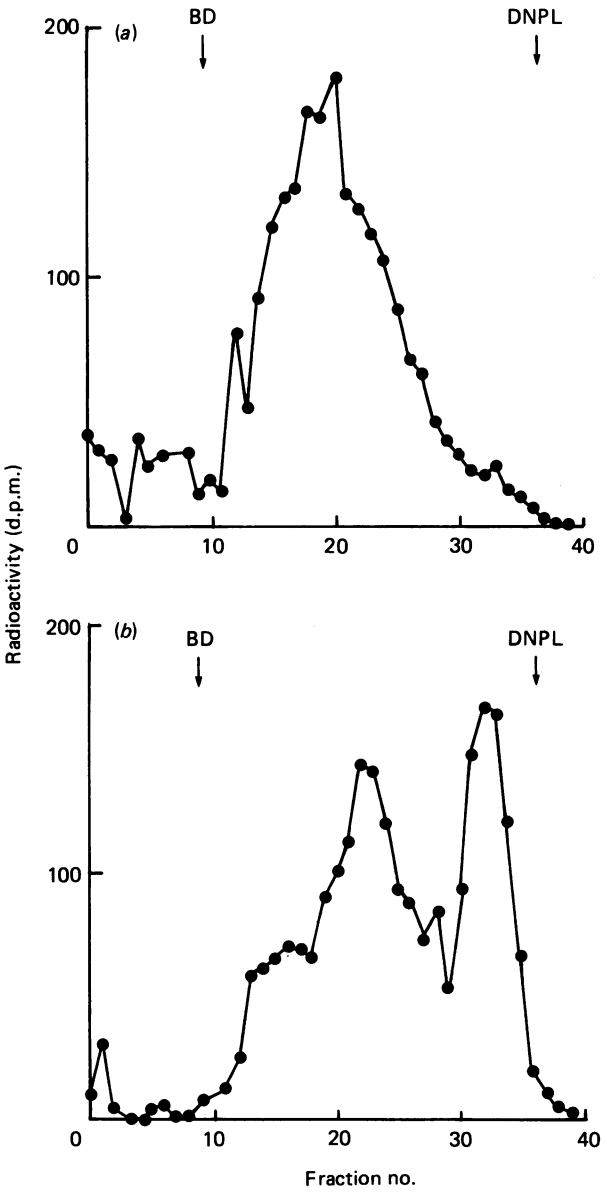

Fig. 1. Gel-filtration analysis of [^{14}C]xylose-labelled, Cadoxen-solubilized polysaccharide using Sepharose CL-2B, with Blue Dextran (BD) and dinitrophenyl-lysine (DNPL) as markers

(*a*) Incubated with 10 mM-Tris/HCl, pH 7.5 (0.5 ml) or (*b*) incubated with proteinase K (5 mg/ml) in above buffer (0.5 ml). Incubations were performed at 25 °C for 16 h, and were terminated by addition of 100% Cadoxen (0.5 ml).

In order to estimate the M_r of the xyloglucan product, it was dissolved in Cadoxen and passed through columns of Sepharose CL-6B and CL-2B in Cadoxen. It was excluded from Sepharose CL-6B, but was partially included in CL-2B, indicating an M_r of between 10^6 and

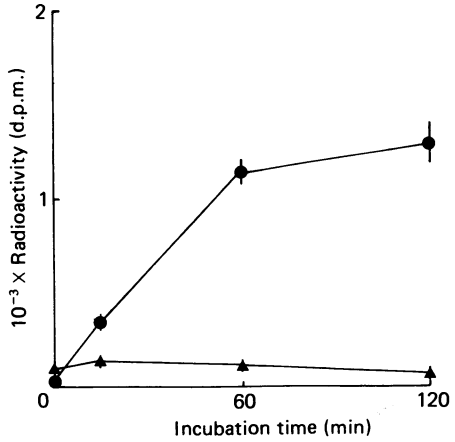

Fig. 2. Time course of incorporation of radioactivity from UDP-D-[^{14}C]xylose into xyloglucan under standard incubation conditions, in the presence (●) or absence (▲) of UDP-D-glucose (2 mM)

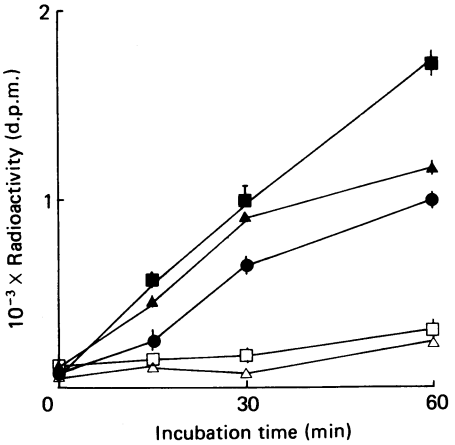

Fig. 3. Effect of GDP-D-mannose and GDP-D-glucose on incorporation of radioactivity from UDP-D-[^{14}C]xylose into xyloglucan in the presence and absence of UDP-D-glucose (2 mM):

(■) = GDP-D-glucose (2 mM) plus UDP-D-glucose; (□) = GDP-D-glucose (2 mM) minus UDP-D-glucose; (●) = standard incubation conditions; (▲) = GDP-D-mannose (2 mM) plus UDP-D-glucose, and (△) = GDP-D-mannose (2 mM) minus UDP-D-glucose.

10^7 (Fig. 1*a*). However incubation of the product with proteinase K before dissolution in Cadoxen resulted in a much lower M_r on passage through Sepharose CL-2B (Fig. 1*b*), even though proteinase K did not hydrolyse purified xyloglucan. This indicates that the nascent xyloglucan is closely associated with protein; since Cadoxen is likely to break most non-covalent bonds, the association is probably covalent, which suggests that xyloglucan may be synthesized on a protein 'primer'. This result would explain the inability of cellulose-derived oligosaccharides to act as acceptors for the xylosyl- and glucosyl-transferases in soybean (Hayashi & Matsuda, 1981*a*).

Interaction of the xylosyltransferase with a UDP-glucose:xyloglucan glucosyltransferase

As shown above, incubation of the membrane preparation with UDP-D-[^{14}C]glucose in the presence of UDP-D-xylose results in the incorporation of glucose units into xyloglucan. Other products are probably also formed, since high rates of incorporation are seen at 2 mM-UDP-D-glucose (9000 d.p.m. incorporated in a 15 min incorporation under standard incubation conditions, but with 55000 d.p.m. in UDP-D-[^{14}C]glucose and no radioactivity in UDP-D-xylose).

The effect of adding UDP-D-glucose (2 mM) to incubations containing 21 μM-UDP-D-[^{14}C]xylose is shown as a time course in Fig. 2. It was necessary, however, for UDP-D-glucose to be present from the start of the incubation, since delaying addition of UDP-D-glucose to 20 min or more after the start of the incubation with UDP-D-[^{14}C]xylose abolished the stimulatory effect of UDP-D-glucose. This was true even if fresh enzyme was added along with UDP-D-glucose at 20 min, implying that the UDP-D-[^{14}C]xylose was being rapidly degraded in the absence of UDP-D-glucose. However, adding UDP-D-[^{14}C]xylose 20 min after the start of an incubation containing UDP-D-glucose resulted in considerable incorporation of radioactivity (approx. 1800 d.p.m.); UDP-D-glucose therefore is not degraded as rapidly as UDP-D-[^{14}C]xylose, perhaps because of its higher concentration, or alternatively it is able to provide glucose for the synthesis of a glucan backbone to which xylose can later be added.

To clarify whether xylose can be added to a preformed glucan backbone, preincubation of the membrane preparation with or without UDP-D-glucose (2 mM) was carried out under standard incubation conditions, except for the absence of UDP-D-xylose, followed by dilution of the incubation medium with approx. 40 ml of fresh buffer. This was centrifuged at 4 °C and 97000 *g* for 30 min, then resuspended in fresh buffer (0.5 ml). Incubations were then carried out with UDP-D-[^{14}C]-xylose, in the presence and absence of UDP-D-glucose. No incorporation was observed, with or without UDP-D-glucose, indicating that enzyme inactivation had occurred, probably during the centrifugation step.

In a search for possible protecting agents for the enzymes, ATP, GTP, GDP-glucose and GDP-mannose were each included separately in incubation media at 2 mM. GDP-D-mannose and GDP-D-glucose appeared to exert a protective effect. In order to check that neither was able to replace UDP-D-glucose as a glycosyl donor, each was tested for its effects on incorporation from UDP-D-[^{14}C]xylose in standard incubations and found to have no effect. However, some stimulation by GDP-D-mannose and GDP-D-glucose was observed when UDP-D-glucose was also present, perhaps owing to a decrease in enzyme denaturation or substrate breakdown (Fig. 3). The protective action of GDP-D-glucose was confirmed by the observation that, when it was present at 2 mM in the incubation medium, stimulation of incorporation was seen on addition of UDP-D-glucose 20 min after the start of incubations (results not shown).

Since GDP-D-glucose appeared to have the greatest protective effect, it was included in the preincubation and incubation media in the presence and absence of UDP-D-glucose. Under these conditions, the presence of UDP-D-glucose in the preincubation medium greatly enhanced subsequent incorporation of radioactivity during in-

cubation with UDP-D-[^{14}C]xylose, whether or not UDP-D-glucose was present in the incubation mixture. Preincubation and incubation in the absence of UDP-D-glucose resulted in the incorporation of 20 d.p.m., whereas the presence of UDP-D-glucose in the preincubation, but not the incubation, gave an incorporation of 500 d.p.m. Inclusion of UDP-D-glucose in the incubation, but not the preincubation, resulted in the incorporation of 550 d.p.m., and inclusion of UDP-D-glucose in both preincubation and incubation media caused a further increase in incorporation to 950 d.p.m. A control in which the preincubation was carried out at 0 °C rather than at 25 °C gave no enhancement of incorporation of radioactivity in subsequent incubations with UDP-D-[^{14}C]xylose, showing that the UDP-D-glucose was effectively washed out of the membranes by the subsequent centrifugation process. Hence it can be concluded that glucose can be incorporated into xyloglucan in the absence of UDP-D-xylose and that the backbone can be elongated without simultaneous addition of side chains. This is in accord with the conclusions of Ray (1980), and suggests an 'imprecise' mechanism of synthesis. This is surprising in view of the apparent regularity of xyloglucans in primary walls (Hayashi & Maclachlan, 1984); it may be that only a few glucose units can be added without addition of xylose units, and that the xylosyltransferase is sufficiently specific to give rise to the repeating structure by addition of xylose residues in a regular pattern.

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