The interaction of xylosyltransferase and glucuronyltransferase involved in glucuronoxylan synthesis in pea (*Pisum sativum*) epicotyls

Elias A.-H. BAYDOUN,* Keith W. WALDRON† and Christopher T. BRETT‡

*Department of Biology, American University of Beirut, Beirut, Lebanon, †Food Research Institute, Colney Lane, Norwich NR4 7UA, U.K., and ‡Department of Botany, University of Glasgow, Glasgow G12 8QQ, U.K.

A particulate enzyme preparation from etiolated pea (*Pisum sativum*) epicotyls was found to incorporate xylose from UDP-D-xylose into β -(1→4)-xylan. The ability of this xylan to act as an acceptor for incorporation of [¹⁴C]glucuronic acid from UDP-D-[¹⁴C]glucuronic acid in a subsequent incubation was very limited, even though glucuronic acid incorporation was greatly prolonged when UDP-D-xylose was present in the same incubation as UDP-D-[¹⁴C]glucuronic acid. This indicated that glucuronic acid could not be added to preformed xylan. However, the presence of UDP-D-glucuronic acid inhibited incorporation of [¹⁴C]xylose from UDP-D-[¹⁴C]xylose into β -(1→4)-xylan, and neither *S*-adenosylmethionine nor acetyl-CoA stimulated either the xylosyltransferase or the glucuronyltransferase.

INTRODUCTION

Glucuronoxylans are the main hemicelluloses of secondary walls of dicotyledonous plants, and hence are a major constituent of hardwoods. They contain a backbone of β -(1→4)-linked xylose residues, to about 10% of which 4-*O*-methylglucuronic acid residues are attached by α -(1→2)-linkages (Timell, 1964). About half the xylose residues are acetylated on C-2 or C-3, and some arabinose residues may also be present as side chains (Aspinall, 1980).

Primary walls of dicotyledonous plants contain small amounts of a glucuronarabinoxylan, which is similar to the glucuronoxylan of secondary walls except that it contains a higher proportion of arabinose residues (Darvill *et al.*, 1980). In monocotyledonous plants arabinoxylans containing only a small proportion of glucuronic acid form a major part of the hemicellulose, and glucuronarabinoxylans are present in small amounts in gymnosperm cell walls (Northcote, 1972).

The biosynthesis of this group of polysaccharides has been investigated in several different systems. Bailey & Hassid (1966) obtained a particulate preparation from immature corn (*Zea mays*) cob that incorporated D-[¹⁴C]-xylose from UDP-D-[¹⁴C]xylose into β -(1→4)-xylan. Similar activity has been found in sycamore (*Acer pseudoplatanus*) and poplar (*Populus robusta*) (Dalesandro & Northcote, 1981a,b) and in bean (*Phaseolus vulgaris*) (Bolwell & Northcote, 1981). Glucuronyltransferase activity has been found in particulate preparations from corn cobs (Kauss, 1967) and pea epicotyls (Waldron & Brett, 1983). In each case transfer was from UDP-D-glucuronic acid, but in corn cobs the glucuronic acid was linked chiefly to galactose, whereas in peas it was linked to xylan. The methylation of glucuronic acid has been studied in particulate preparations from corn cob (Kauss & Hassid, 1967) and mung bean (Kauss, 1969), and has been shown to occur at the macromolecular level.

Thus information is available concerning most of the individual enzymes involved in glucuronoxylan biosynthesis, though the incorporation of acetyl and arabinosyl groups remains to be explored. Little is known, however, about the way in which these enzymes interact to form the complete polysaccharide. Waldron & Brett (1983) established that sustained incorporation of [¹⁴C]glucuronic acid from UDP-D-[¹⁴C]glucuronic acid into glucuronoxylan only occurred in the presence of UDP-D-xylose, and it was suggested that xylan was being synthesized that acted as an acceptor for the further incorporation of glucuronic acid. It is not known, however, whether glucuronic acid must be added while the xylan is being elongated, or whether glucuronic acid can be added to preformed xylan. These two possibilities are particular cases of two models of heteropolysaccharide synthesis, termed 'precise' and 'imprecise' respectively, that have been proposed by Waldron & Brett (1985).

In the present paper we report the presence of a UDP-D-xylose: β -(1→4)-xylan xylosyltransferase in etiolated pea epicotyl tissue, and describe experiments to test whether or not glucuronic acid can be added to preformed xylan. The effects of *S*-adenosylmethionine and acetyl-CoA, the likely donors of methyl and acetyl groups respectively, on the xylosyltransferase and glucuronyltransferase are also described.

MATERIALS AND METHODS

Plant material

Peas (*Pisum sativum* var. Alaska; obtained from Sinclair McGill, Ayr, U.K.) were soaked overnight in water at room temperature and grown on damp vermiculite for 7 days at 22 °C in continuous darkness.

Particulate enzyme preparation

The procedure was similar to that of Waldron & Brett (1983). Epicotyls (6–9 cm long) were cut off, the hooks

‡ To whom correspondence should be addressed.

were excised and discarded, and the remainder of the tissue was cooled on ice. All subsequent operations were carried out at 0–4 °C. Epicotyl tissue (50 g) was homogenized in 150 ml of 10 mM-Tris/HCl buffer, pH 7.5, in a Polytron blender (two bursts of 5 s at speed 5). The homogenate was strained through four crossed layers of muslin, and the filtrate was centrifuged at 97 000 *g* for 30 min in a Sorval OTD-65B ultracentrifuge with a Sorvall AH 629 swing-out rotor. Each pellet (which was derived from 10 g of tissue) was resuspended in 0.5 ml of 50 mM-Tris/HCl buffer, pH 7.5, in a glass tissue homogenizer (Gallenkamp, Loughborough, Leics., U.K.) to give the particulate enzyme preparation, which was used within 10 min.

Incubation of enzyme preparation with sugar nucleotides

Standard incubations (variations are explained in the Results section) were carried out at 25 °C in a total volume of 100 μ l. The standard incubation medium for measurement of xylose incorporation contained UDP-D-[U-¹⁴C]xylose (8.3 kBq; made up to 1 mM by addition of non-radioactive UDP-D-xylose), MnCl₂ (10 mM) and enzyme preparation (50 μ l). The standard incubation medium for measurement of glucuronic acid incorporation contained UDP-D-[U-¹⁴C]glucuronic acid (460 Bq; 0.5 μ M), MnCl₂ (10 mM) and particulate enzyme preparation (50 μ l). Incubations were terminated by the addition of 1 ml of 70 % (v/v) ethanol. All incubations were carried out in duplicate, and results in Figures and Tables are shown as the means $\pm$ the difference between the results in each incubation and the mean.

Measurement of incorporation of radioactivity into polysaccharide

Terminated incubation mixtures were centrifuged at 10 000 *g* for 3 min in an Eppendorf Microfuge. The pellets were washed three times in 1 ml of 70 % (v/v) ethanol, with resuspension and centrifuging as above each time. The pellets were then extracted twice for 15 min at 100 °C with 0.5 ml of 50 mM-EDTA/50 mM-sodium phosphate buffer, pH 6.8, and finally washed once with 1 ml of water. The radioactivity in the pellets was determined by suspending them in 0.4 ml of water, adding 4 ml of Unisolve I (Koch-Light Ltd., Havershill, Sussex, U.K.) and counting the radioactivity in a Packard model 3380 liquid-scintillation spectrometer.

Acid hydrolysis

This was carried out in 0.5 ml of 0.1 M-trifluoroacetic acid for 1 h at 120 °C in a sealed tube. The acid was then removed by rotary evaporation. The products were analysed by descending paper chromatography on Whatman no. 1 paper in ethyl acetate/pyridine/water (8:2:1, by vol.) for 18 h. The dried chromatogram was cut into 1 cm-wide strips and placed in 1 ml of toluene scintillant (Harris & Northcote, 1970) for determination of radioactivity. Marker sugars were detected by the method of Trevelyan *et al.* (1950).

Incubation with enzymes

Incubations with xylanase were carried out at 5 mg/ml in 0.5 ml of 10 mM-sodium acetate buffer, pH 4.5, at 25 °C for 18 h. Incubations with xyloglucanase were carried out in 1 ml of 50 mM-sodium acetate buffer, pH 4.5, at 25 °C for 3 h. In each case the incubations were terminated by boiling for 5 min.

Chemicals and enzymes

UDP-D-[U-¹⁴C]xylose was obtained from Du Pont, New England Nuclear, Stevenage, Herts., U.K. UDP-D-[¹⁴C]glucuronic acid was obtained from Amersham International, Amersham, Bucks., U.K. Non-radioactive sugar nucleotides were obtained from Sigma Chemical Co., Poole, Dorset, U.K. Purified xyloglucanase (Edwards *et al.*, 1986) was generously given by Dr. J. S. G. Reid, Department of Biology, University of Stirling, Stirling, U.K.; it was homogeneous, as judged by SDS/polyacrylamide-gel electrophoresis, and inactive against a wide variety of glucans and other polysaccharides. Purified xylanase was prepared as described by Gibson & McCleary (1987); this enzyme gave one major (xylanase) band on SDS/polyacrylamide-gel electrophoresis, with only trace amounts of other protein, and was devoid of cellulase, pectinase, proteinase and xyloglucanase activity.

RESULTS

Properties of the xylosyltransferase

Under the standard incubation conditions for incorporation of radioactivity from UDP-D-[¹⁴C]xylose, incorporation continued at a steadily decreasing rate for at least 4 h (Fig. 1). The result of varying the concentration of UDP-D-xylose is shown in Fig. 2. These data give a non-linear double-reciprocal plot, in contrast with the results obtained for xylan synthases from bean (Bolwell & Northcote, 1981) and sycamore (Dalessandro & Northcote, 1981a). Analyses of the radioactive products showed that more than one product is formed, which may partly explain the non-linearity. With a low concentration (3 μ M) of UDP-D-xylose total acid hydrolysis followed by paper chromatography showed that most (78 %) of the radioactive product consisted of [¹⁴C]arabinose, and only 22 % was present as [¹⁴C]xylose.

Fig. 1. Time course of incorporation of radioactivity from UDP-D-[¹⁴C]xylose into polysaccharide

For experimental details see the text.

Fig. 2. Effect of concentration of UDP-D-xylose on the incorporation of xylose into polysaccharide

For experimental details see the text. Incubations were for 30 min.

Table 1. Effect of UDP-D-glucose and UDP-D-glucuronic acid on the incorporation of [14 C]xylose from UDP-D-[14 C]-xylose into polysaccharide

All sugar nucleotides were present at 1 mM, and standard incubation conditions were used for 30 min or 4 h.

Addition	Incorporation (Bq)	
	After 30 min	After 4 h
None (control)	6.98 $\pm$ 0.35	23.88 $\pm$ 0.02
UDP-D-glucose	22.16 $\pm$ 0.50	33.71 $\pm$ 0.14
UDP-D-glucuronic acid	5.50 $\pm$ 0.04	19.16 $\pm$ 0.66

In contrast, a higher concentration (1 mM) gave a polysaccharide product containing mostly (84 %) [14 C]xylose, with only 16 % arabinose. It is likely that at the lower concentration much of the xylose was epimerized to arabinose, probably at the sugar nucleotide stage. In view of this a UDP-D-xylose concentration of 1 mM was used in most of the subsequent experiments.

Nature of the [14 C]xylose-containing product formed from UDP-D-[14 C]xylose (1 mM)

Incubation of the radioactive product with β -(1 $\rightarrow$ 4)-xylanase solubilized 88 % of the radioactive product. The solubilized material contained radioactive xylose and short oligosaccharides. This enzyme does not solubilize xyloglucan, so the product appears to be β -(1 $\rightarrow$ 4)-xylan rather than xyloglucan. In support of this conclusion, it was found that purified xyloglucanase did not solubilize the radioactive product. However, if non-radioactive UDP-D-glucose was added to the incubation medium, incorporation of radioactivity from UDP-D-[14 C]xylose was greatly increased (Table 1). It appears,

therefore, that the enzyme preparation is able to synthesize xyloglucan, but only if exogenous UDP-D-glucose is supplied. A similar result was obtained with an enzyme preparation from suspension-cultured French-bean cells (Campbell *et al.*, 1988).

Interaction between xylosyltransferase and glucuronyltransferase

It has already been established that the sustained activity of the glucuronyltransferase that incorporates glucuronic acid into glucuronoxylan is dependent on the presence of exogenous UDP-D-xylose (Waldron & Brett, 1983). This was interpreted as a requirement for new xylan backbones to be formed, to which glucuronic acid side chains could then be added. In order to determine whether glucuronic acid could be added to preformed xylan, or whether backbone elongation and side-chain addition are tightly coupled, the following experiment was carried out. Membrane pellets were prepared in the normal way and then resuspended and incubated for 1 h in a medium (1 ml) containing non-radioactive UDP-D-xylose (1 mM) (incubation 1). At the end of the incubation the medium was diluted to 38 ml and centrifuged at the same speed (97000 *g*) and for the same period (30 min) as in the preparation of the original membrane pellets. The pellets from the second centrifugation were resuspended and used for incubations with UDP-D-[14 C]glucuronic acid in the presence or in the absence of UDP-D-xylose (1 mM) (incubation 2). Controls were also carried out in which incubation 1 contained no UDP-D-xylose.

The results of this experiment (Fig. 3) show that the

Fig. 3. Effect of preincubation (1 h) with UDP-D-xylose (1mM) on incorporation of [14 C]glucuronic acid from UDP-D-[14 C]glucuronic acid into polysaccharide

UDP-D-[14 C]glucuronic acid was present in incubation 2, but not incubation 1, in all cases. For full experimental details see the text. $\circ$ and $\bullet$, Incubation 1 with UDP-D-xylose; $\triangle$ and $\blacktriangle$, incubation 1 without UDP-D-xylose. $\circ$ and $\triangle$, Incubation 2 with UDP-D-xylose (1 mM); $\bullet$ and $\blacktriangle$, incubation 2 without UDP-D-xylose. The Figure shows the results of a typical experiment; the experiment was carried out three times, with essentially the same results each time.

presence of UDP-D-xylose in incubation 1 causes very little stimulation of incorporation from UDP-D-[14 C]-glucuronic acid in incubation 2. After 1 h of incubation 2, some stimulation had occurred, but there is very little incorporation of radioactivity thereafter. In contrast, the presence of UDP-D-xylose (1 mM) together with UDP-[14 C]glucuronic acid in incubation 2 permitted incorporation of radioactivity from UDP-[14 C]glucuronic acid to continue at a high rate for at least 4 h, as it does when incubation 1 is omitted altogether (Waldron & Brett, 1983).

These results suggest that only a very limited amount of glucuronic acid can be added to preformed xylan. However, a number of other explanations could be proposed, and these were explored in turn.

The first possible explanation is that no β -(1 $\rightarrow$ 4)-xylan was formed during incubation 1. This possibility can be discounted, since incubation 1 was identical with the incubations with UDP-D-[14 C]xylose already described above, except that the UDP-D-xylose was non-radioactive and the incubation was scaled up by a factor of 10-fold.

A second possible explanation is that the xylan formed in incubation 1 was subsequently degraded during the centrifugation step and during incubation 2. To test this, the following experiment was carried out. Membrane pellets were resuspended in 0.5 ml of buffer as normal, and three pairs of standard incubations were carried out for 1 h, each with UDP-D-[14 C]xylose (830 Bq; 1 mM). One pair of incubations was terminated immediately by addition of 70% (v/v) ethanol. The other two pairs of incubations were each diluted to 38 ml and centrifuged at 97000 *g* for 30 min. The supernatants were discarded, and 70% (v/v) ethanol was added to each of one pair of pellets. Each of the final pair of pellets was resuspended in 50 μ l of 50 mM-Tris/HCl buffer, pH 7.5, and incubated for 2 h in a medium containing non-radioactive UDP-D-glucuronic acid (0.5 mM) and MnCl₂ (10 mM) in a total volume of 100 μ l, before termination by addition of 70% (v/v) ethanol. Finally, radioactivity present in polysaccharide in each of the pellets was determined in the normal way.

The results of this experiment (Table 2) show that about 32% of the radioactive polysaccharide present at the end of the first incubation was lost during the centrifugation step, and a further 5% during the second incubation. Some of these losses are likely to have been due to incomplete recovery of membrane in the centrifugation step. A parallel experiment in which the UDP-D-[14 C]xylose in the first incubation was present at 1 μ M rather than 1 mM, and in which [14 C] arabinose will have predominated in the product, gave similar results (37% and 1% losses for the centrifugation and second incubation respectively), which is consistent with the losses being in large part due to incomplete recovery of particulate material rather than to polymer breakdown. Thus there may have been relatively little polymer breakdown, and the losses from all causes did not exceed 37%.

A third possible explanation for the failure of pre-incubation with UDP-D-xylose to stimulate subsequent incorporation of radioactivity from UDP-D-[14 C]-glucuronic acid is that UDP-D-xylose might be required, not only as xylose donor, but also as an allosteric activator of the glucuronyltransferase. To determine if this was so, the initial rate of glucuronic acid

Table 2. Effect of dilution, re-centrifugation and re-incubation on the [14 C]polysaccharide present at the end of an incubation of the particulate enzyme preparation with UDP-D-[14 C]xylose

Standard incubations were carried out with UDP-D-[14 C]-xylose for 1 h, and the radioactivity in polysaccharide was measured directly, or after dilution and centrifugation at 97000 *g* for 30 min, or after dilution, centrifugation and incubation for a further 2 h in the presence of UDP-D-glucuronic acid.

	[14 C]Polysaccharide present	
	(Bq)	(%)
After incubation	7.00 $\pm$ 0.40	100
After incubation, dilution and re-centrifugation	4.78 $\pm$ 0.26	68
After incubation, dilution, re-centrifugation and re-incubation	4.38 $\pm$ 0.45	63

Fig. 4. Effect of UDP-D-xylose acid on incorporation of radioactivity from UDP-D-[14 C]glucuronic acid (0.5 μ M) into polysaccharide

For experimental details see the text. Concentrations of UDP-D-xylose used were: $\circ$, none; $\bullet$, 10 μ M; $\triangle$, 30 μ M; $\blacktriangle$, 100 μ M; $\square$, 300 μ M; $\blacksquare$, 1 mM.

incorporation was studied at a number of different concentrations of UDP-D-xylose. No stimulation of incorporation of radioactivity was observed (Fig. 4); instead, UDP-D-xylose inhibited the incorporation, the degree of inhibition increasing with UDP-D-xylose concentration and the apparent K_i being approx. 0.1 mM.

Thus it seems likely that the failure of preincubation with UDP-D-xylose to stimulate subsequent incorporation of glucuronic acid is due to a requirement for glucuronic acid to be added shortly after the incorpora-

Table 3. Effect of *S*-adenosylmethionine (1 mM) and acetyl-CoA (1 mM) on incorporation of [¹⁴C]xylose from UDP-D-[¹⁴C]-xylose (1 mM) and of [¹⁴C]glucuronic acid from UDP-D-[¹⁴C]glucuronic acid (0.5 μM) into polysaccharide

Incubations contained either UDP-D-[¹⁴C]xylose (830 Bq) or UDP-D-[¹⁴C]glucuronic acid (460 Bq) plus UDP-D-xylose (1 mM), and were for 1 h.

Addition	Incorporation (Bq)	
	From UDP-D-[¹⁴ C]xylose	From UDP-D-[¹⁴ C]glucuronic acid
None (control)	0.93 ± 0.02	3.48 ± 0.33
<i>S</i> -Adenosylmethionine	0.95 ± 0.00	3.88 ± 0.07
Acetyl-CoA	0.74 ± 0.09	2.74 ± 0.09
<i>S</i> -Adenosylmethionine + acetyl-CoA	0.76 ± 0.07	2.74 ± 0.15

tion of the xylose residue that acts as the point of glucuronic acid attachment. If this is the case, the xylose addition might be expected to be dependent in some degree on the presence of the donor for glucuronic acid, in order to prevent the formation of long stretches of non-glucuronidated xylan. However, UDP-D-glucuronic acid (1 mM) was found to decrease the incorporation of radioactivity from UDP-D-[¹⁴C]xylose after incubation periods of 30 min and 4 h (Table 1).

Effect of acetyl-CoA and *S*-adenosylmethionine on incorporation of radioactivity from UDP-D-[¹⁴C]xylose and UDP-D-[¹⁴C]glucuronic acid

The complete glucuronoxylan is made up not only of glucuronic acid and xylose residues, but also of arabinose, acetyl and methyl groups. The synthesis of the complete molecule therefore requires incorporation of all five entities. In view of the interactions observed between glucuronic acid incorporation and xylose incorporation, it was of interest to consider whether the presence of donors of the other components affected the incorporation of glucuronic acid and xylose. The presence of UDP-L-arabinose as a result of epimerization of UDP-D-xylose makes the effect of UDP-L-arabinose difficult to assess, but the effects of *S*-adenosyl-L-methionine and acetyl-CoA were tested. The results (Table 3) indicate that acetyl-CoA weakly inhibits both enzymes, whereas *S*-adenosyl-L-methionine has no effect or is slightly stimulatory.

DISCUSSION

The membrane preparation used in this and previous work (Waldron & Brett, 1983) was not fractionated by differential or density-gradient centrifugation since it was important not to lose any of the enzymes involved in glucuronoxylan synthesis. Hence the preparation would be expected to contain UDP-D-[¹⁴C]xylose 4-epimerase, and this enzyme is probably responsible for the production of UDP-L-[¹⁴C]arabinose from UDP-D-[¹⁴C]-

xylose. The polymer into which the arabinose is incorporated is not known; it could be glucurono-(arabino)xylan or arabinan or arabinogalactan. The fact that the ratio of [¹⁴C]xylose to [¹⁴C]arabinose in the polysaccharide product was much lower when micromolar rather than millimolar concentrations of UDP-D-[¹⁴C]xylose were used might be due to a relatively low K_m either for the epimerase or for the arabinosyltransferase. The arabinosyltransferase studied by Bolwell & Northcote (1981) in bean had an apparent K_m of less than half that of the xylosyltransferase in the same system.

The high [¹⁴C]xylose/[¹⁴C]arabinose ratio found when UDP-D-[¹⁴C]xylose (1 mM) was used permitted the xylosyltransferase to be investigated with this concentration of substrate. The product was found to be xylan rather than xyloglucan, though addition of UDP-D-glucose to the incubation medium resulted in the formation of xyloglucan as well as xylan, as found previously in pea by Ray (1980).

The xylosyltransferase was used to form xylan in the membranes *in situ*, and the ability of this xylan to act as an acceptor for subsequent transfer of glucuronic acid was tested. Only a small amount of such transfer was observed, relative to controls, and it had virtually ceased after 1 h of incubation with UDP-D-[¹⁴C]glucuronic acid. The increase in transfer over that observed after preincubation without UDP-D-xylose could have been due to a small amount of UDP-D-xylose carried over into the second incubation from the first, i.e. not washed out during the dilution and subsequent centrifugation. Alternatively, it may be that the most newly formed portions of the xylan chains were available for transfer of glucuronic acid, even though the bulk of the xylan formed during the preincubation was not able to accept glucuronic acid. The total amount of xylan that was formed in incubation 1 and that was still present in incubation 2 corresponded to 0.6 nmol of xylose, on the basis of data presented in Table 2. The glucuronic acid incorporated into glucuronoxylan after 1 h of incubation 2 was approx. 1.2 pmol where preincubation had taken place in the presence of UDP-D-xylose, and 0.7 pmol where UDP-D-xylose had been absent from the preincubation. Hence it was not an overall shortage of xylan that prevented further transfer of glucuronic acid into preformed xylan after 1 h of incubation 2, but rather a shortage of xylan that was available for glucuronic acid transfer.

The reasons for this very limited availability of preformed xylan for subsequent transfer of glucuronic acid are not clear. It is possible that the main bulk of the xylan adopts a three-dimensional configuration that is unfavourable for binding to the glucuronyltransferase; such a configuration might be favoured by binding to membrane proteins or other nascent cell-wall polysaccharides. Alternatively, the most recently synthesized part of the xylan chain may form part of a stable complex with the xylosyltransferase and glucuronyltransferase, preventing the glucuronyltransferase from moving to the internal regions of the xylan chain.

Though there appear to be constraints on the activity of the glucuronyltransferase, it is clear that other parts of the biosynthetic machinery for glucuronoxylan synthesis are less tightly constrained. No stimulation of xylose incorporation by UDP-D-glucuronic acid was observed; indeed, there was some inhibition, though this may have

been partly due to dilution of the UDP-D-[¹⁴C]xylose by non-radioactive UDP-D-xylose, derived by decarboxylation of UDP-D-glucuronic acid. Hence extensive lengths of xylan chain, which cannot be glucuronidated, arise *in vitro* in the absence of UDP-D-glucuronic acid. This situation is not known to occur *in vivo* in this tissue. Neither S-adenosylmethionine nor acetyl-CoA had any great effect on either the glucuronyltransferase or xylosyltransferase. In some of the incubations whose results are given in Table 3, all five of the likely donors for glucuronoxylan synthesis (UDP-D-glucuronic acid, UDP-D-xylose, UDP-L-arabinose, S-adenosylmethionine and acetyl-CoA) will have been present, since UDP-L-arabinose is apparently formed from UDP-D-xylose *in situ*. However, this 'full house' of substrates did not cause any significant change in glucuronyltransferase or xylosyltransferase activities.

The incorporation of glucuronic acid is therefore the only aspect of glucuronoxylan synthesis that shows signs of being 'precise', according to the models put forward by Waldron & Brett (1985). Other aspects appear to be 'imprecise', especially the incorporation of methyl groups, which is thought to occur at internal positions on the polymer chain (Kauss & Hassid, 1967). Studies of the arabinosyltransferase and acetyltransferases are needed to complete the picture.

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