Biochem. J. (1983) **213**, 115–122
Printed in Great Britain

A glucuronyltransferase involved in glucuronoxylan synthesis in pea (*Pisum sativum*) epicotyls

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(*Received 22 December 1982/Accepted 4 March 1983*)

A particulate enzyme preparation made from epicotyls of 1-week-old etiolated pea (*Pisum sativum*) seedlings was shown to incorporate glucuronic acid from UDP-D-[U-^{14}C]glucuronic acid into a hemicellulosic polysaccharide. Optimum conditions for the incorporation include the presence of Mn^{2+} ions at between 4 and 10 mmol/litre and a pH between 5 and 6. UDP-D-xylose at 1 mmol/litre allows incorporation to continue for at least 8 h. In its absence, the reaction stops within 30 min. Analysis of the product by partial and total acid hydrolysis, followed by paper chromatography or electrophoresis, indicates that the polysaccharide produced is a glucuronoxylan.

Glucuronic acid is an important constituent of hemicelluloses in the cell walls of most higher plants. It is most commonly found in the form of 4-*O*-methylglucuronic acid side chains linked to the xylan backbone of glucuronoxylans.

In the dicotyledonous angiosperms, xylans are the main hemicellulose of secondary walls. They constitute between 20 and 30% of the dry weight of woody tissue (Aspinall, 1980). The backbone of the xylan consists of β1→4-linked xylose residues in a long, largely unbranched, chain. About one in ten xylose residues has an α1→2-linked 4-*O*-methylglucuronic acid side chain (Timell, 1964), and these side chains are distributed apparently randomly along the main xylan chain (Northcote, 1969). In addition, about 50% of the xylose units are acetylated on C-3 and/or C-2. Occasional arabinose side chains may also be present (Aspinall, 1980).

Glucuronoxylans are quantitatively most abundant in the secondary walls of dicotyledonous plants. However, they may also be present in lower amounts in the primary walls of some monocotyledonous plants (Burke *et al.*, 1974; Wada & Ray, 1978), and they are components of cell walls in gymnosperms (Timell, 1965).

In spite of the importance of glucuronoxylans, little work has been carried out to elucidate their mechanism of biosynthesis. Synthesis of the xylan chain has been studied in immature corn (*Zea mays*) cobs by Bailey & Hassid (1966) and in woody dicots [sycamore (*Acer pseudoplatanus*) and poplar (*Populus robusta*)] by Dalessandro & Northcote (1981), but the only analysis of the incorporation of glucuronic acid into hemicelluloses is the work of Kauss (1967) and Kauss & Hassid (1967) on immature corn cobs. They showed that UDP-D-glucuronic acid could be used as the donor of glucuronic acid, and that methylation from *S*-adenosylmethionine occurred at the macromolecular level. However, only a relatively small amount of the glucuronic acid incorporated was shown to be linked to xylose, and the evidence indicated that the majority was linked to galactose. In dicotyledonous plants, glucuronic acid has been shown to be incorporated from UDP-D-glucuronic acid into polysaccharide in a few cases (Villemez *et al.*, 1968; Villemez & Clark, 1969), but no detailed analysis of incorporation into glucuronoxylan has been carried out.

Since glucuronic acid is present in polysaccharides other than glucuronoxylan in some tissues (Northcote, 1969), and since it might also be present in glycoproteins, incorporation of glucuronic acid into a general 'hemicellulose' fraction may not necessarily involve glucuronoxylan synthesis. It is necessary to provide more definite evidence that glucuronic acid has indeed been incorporated into glucuronoxylan. This evidence may be provided by a structural analysis of the product. Additional evidence may be supplied by showing an interaction between the two sugar-nucleotide substrates needed for glucuronoxylan synthesis, UDP-D-glucuronic acid and UDP-D-xylose. This second approach is also a step in the direction of the complete synthesis of the glucuronoxylan molecule *in vitro*; a similar approach has been adopted in studies on the synthesis of xyloglucan (Ray, 1980) and glucomannan (Heller & Villemez, 1972).

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In the present paper we report the results of experiments *in vitro* concerning the incorporation of glucuronic acid from UDP-D-[^{14}C]glucuronic acid into hemicellulose by a particulate enzyme preparation from etiolated pea seedlings. Structural analysis indicates that the product is glucuronoxylan, and this conclusion is supported by the observation that incorporation from UDP-D-[^{14}C]-glucuronic acid is stimulated by UDP-D-xylose.

Materials and methods

Chemicals

UDP-D-[U-^{14}C]glucuronic acid (925 kBq · ml^{-1}, 9.77 GBq · mmol^{-1}) was purchased from Amersham International. Non-radioactively labelled sugar nucleotides were purchased from Sigma. Peas (*Pisum sativum* var. Alaska) were supplied by Sinclair McGill (Ayr, Scotland) and arrived predressed in 'Furnasan Slurry'.

Particulate enzyme preparation

Peas were soaked for 6 h in water and grown in darkness on damp tissue paper for 7 days at 22°C. The etiolated seedlings were harvested, the hook and seed leaves removed, and the epicotyls cut into 2 cm lengths. All further operations in the preparation of the enzyme were carried out at between 0 and 4°C. Epicotyl tissue (25 g) was homogenized in 100 ml of Tris/HCl (10 mmol/litre), pH 7.5 (two bursts of 5 s at speed 5 on a Polytron blender), and the homogenate was then strained through four crossed layers of muslin. The filtrate was centrifuged for 30 min at 97000 ***g*** in a Sorvall OTD-65B ultracentrifuge with a Sorvall AH627 swing-out rotor. The pellets were each resuspended in 0.5 ml of Tris/HCl (50 mmol/litre), pH 7.5, to make the particulate enzyme preparation. This was kept on ice and used within 20 min.

Incubations

The standard incubation mixture (variations are explained in the Results section) consisted of 50 μl of particulate enzyme preparation, $MnCl_2$ (10 mmol/litre) and UDP-D-[U-^{14}C]glucuronic acid (0.5 μmol/litre; 27750 d.p.m.) in a total volume of 100 μl. Incubations were carried out at 25°C and were terminated by addition of 1 ml of 70% (v/v) ethanol. During the preparation of labelled hemicellulose for analysis, the incubation medium was scaled up by a factor of 10.

Measurement of incorporation of radioactivity into polysaccharide

The terminated incubation mixtures were centrifuged at 10000 ***g*** for 30 s in an Eppendorf Microfuge. The pellets were washed three times in 0.5 ml of 70% (v/v) ethanol to remove unchanged UDP-D-[^{14}C]glucuronic acid and any low-molecular-weight radioactive products. Measurement of total incorporation of radioactivity into 70% (v/v)-ethanol-insoluble material was achieved by drying the pellets under reduced pressure and counting on a scintillation spectrometer.

Extraction of 'pectin' from the 70% (v/v)-ethanol-insoluble material was by a method adapted from that described by Stoddart *et al.* (1967). Each pellet was resuspended in 0.5 ml of buffer containing 0.05 mol of EDTA/litre and 0.05 mmol of sodium phosphate/litre, pH 6.8. The mixture was heated to 100°C in a boiling-water bath for 15 min, centrifuged at 10000 ***g***, and the supernatant removed and stored. The same procedure was repeated on the residue, and the supernatant from the second extraction was added to that from the first. 'Pectin' was measured by counting the radioactivity in the supernatant.

Remaining insoluble material was washed three times in the EDTA/sodium phosphate buffer used above. Hemicellulose was extracted by a method adapted from that described by Boffey & Northcote (1975); the pectin-extracted pellets were vortex-mixed with 1 ml of 24% (w/v) KOH and left for 48 h at room temperature. Alkali-insoluble material was removed by centrifugation at 10000 ***g*** for 5 min, and the supernatant was adjusted to pH 4.5 by addition of 1 ml of acetic acid, and adjusted to 73% (v/v) ethanol by addition of 6 ml of 96% (v/v) ethanol. A small quantity of pure cellulose powder (Macherey, Nagel and Co., 516 Düren, Germany) was added to help in centrifuging down the hemicellulose precipitated over the next 48 h. At the end of this time the precipitates were centrifuged at 4000 ***g*** for 15 min. The supernatants were decanted off and the precipitates washed twice in 70% (v/v) ethanol before counting for radioactivity.

Hydrolysis of polysaccharides

Partial acid hydrolysis was carried out in 0.4 ml of trifluoroacetic acid (2 mol/litre) at 100°C in sealed Reacti-vials [Pierce and Warriner (U.K.) Ltd., Chester, U.K.] for 1 h or more. Total acid hydrolysis was by the same procedure, except that the sealed vessels were autoclaved at 120°C. After hydrolysis, the non-hydrolysed material was centrifuged down at 10000 ***g*** for 5 min and the hydrolysate was evaporated to dryness under reduced pressure overnight at room temperature.

Separation of oligosaccharides produced by acid hydrolysis

This was performed by descending chromatography on Whatman no. 1 paper for 30 h in Solvent I (ethyl acetate/formic acid/acetic acid/water, 18:1:3:4, by vol.) (Ray & Rottenberg, 1964).

Marker sugars were detected by the method of Trevelyan *et al.* (1950).

Separation of monosaccharides

This was also performed by descending chromatography on Whatman no. 1 paper. The solvent (Solvent II) was ethyl acetate/pyridine/water (8:2:1, by vol.). Marker sugars were detected as described above.

Thin-layer electrophoresis

This was carried out on plastic sheets pre-coated with silica-gel G (Camlab, Cambridge, U.K.) at 300V for 4h. The buffer consisted of pyridine/acetic acid/water (1:10:89, by vol.). Marker sugars were located by spraying the plates with methanolic 0.5% α-naphthol, allowing to dry, spraying with 10% (v/v) H_2SO_4 in methanol, and drying at 70°C.

Elution of samples from paper-chromatographic strips

If the strips had been saturated with scintillation fluid for scintillation counting, the scintillant was first washed out of the strips by three washes with sulphur-free toluene. The remaining toluene was then allowed to evaporate. The strips were agitated in water (3ml/strip) for 5min. The water was then decanted and centrifuged at 4000*g* for 10min to remove paper particles. The strips were washed again in the same way, and the washings treated as above. The washings were combined and dried by rotary evaporation at 40°C.

Reduction of glucuronic acid in material eluted from chromatograms

The dried, eluted material was resuspended in redistilled methanol (1ml) which was then adjusted to 1mmol/litre with respect to HCl by addition of concentrated HCl. After heating to 100°C for 2h in a sealed Reacti-vial, the products were evaporated to dryness under reduced pressure. $NaBH_4$ reduction was then carried out for 1h at room temperature by addition of $NaBH_4$ (50μl of a 1mmol/litre solution). The reduction was terminated by addition of 200μl of acetic acid, and the sample was evaporated under reduced pressure to remove methyl borate and acetic acid. The products were hydrolysed for 2h in trifluoroacetic acid (2mol/litre) at 100°C in a sealed Reacti-vial. After being evaporated to dryness under reduced pressure, the hydrolysate was redissolved in water and analysed by paper chromatography in Solvent II.

Estimation of radioactivity

Pellets of 70% (v/v)-ethanol-insoluble material produced in incubations, or of precipitated hemicellulose, were dried under reduced pressure overnight at room temperature. The material was then vortex-mixed with 6 or 12ml of scintillation fluid/water (10:1, v/v). The scintillation fluid used was either Packard liquid-scintillation cocktail, type 299, or Triton/toluene scintillant (Brett, 1981).

Pectin extracts (1ml) were mixed with 10ml of one of the above scintillation fluids.

Strips of chromatography paper or of silica gel on its plastic backing were placed in a scintillation vial with 1ml of the scintillation fluid described by Harris & Northcote (1970).

All samples were counted for radioactivity in a Packard liquid-scintillation spectrometer, model 3380.

Results

Except where stated, all incubations were performed in triplicate. Results are shown as means ± S.D.

Incorporation of ^{14}C from UDP-D-[^{14}C]glucuronic acid into hemicellulose and the effect of UDP-D-xylose

Time courses of standard incubations (see the Materials and methods section) were performed in the presence and in the absence of UDP-D-xylose (1mol/litre), and the products were extracted for hemicellulose (Fig. 1*a*). Incorporation of radioactivity into hemicellulose stopped after 30min unless UDP-D-xylose (1mmol/litre) was present, in which case the incorporation continued for at least 8h (Fig. 1*b*). No other sugar nucleotide tested had this same stimulatory effect (Table 1), and further experiments (Fig. 1*c*) showed that UDP-D-xylose, when added to a final concentration of 1mmol/litre after 75min of incubation, still had the ability to increase incorporation. The effect of changing the UDP-D-xylose concentration is shown in Fig. 2.

The concentration of UDP-D-glucuronic acid was shown to influence the effect of UDP-D-xylose (Fig. 3*a*). As the concentration of UDP-D-glucuronic acid was increased to the millimolar level, the increase in incorporation of radioactivity into hemicellulose brought about by UDP-D-xylose (1mmol/litre) was lowered considerably. The effect of UDP-D-glucuronic acid concentration on incorporation is shown in Fig. 3(*b*).

Table 1. *Effect of different sugar nucleotides at 1mmol/litre on the incorporation of radioactivity from UDP-D-[U-^{14}C]glucuronic acid into hemicellulose in 2h*

Sugar nucleotide	Incorporation (d.p.m.)
None	848 ± 20
UDP-D-Glc	662 ± 16
GDP-D-Glc	1152 ± 37
GDP-D-Man	1019 ± 32
UDP-D-Gal	537 ± 25
UDP-D-Xyl	1982 ± 76

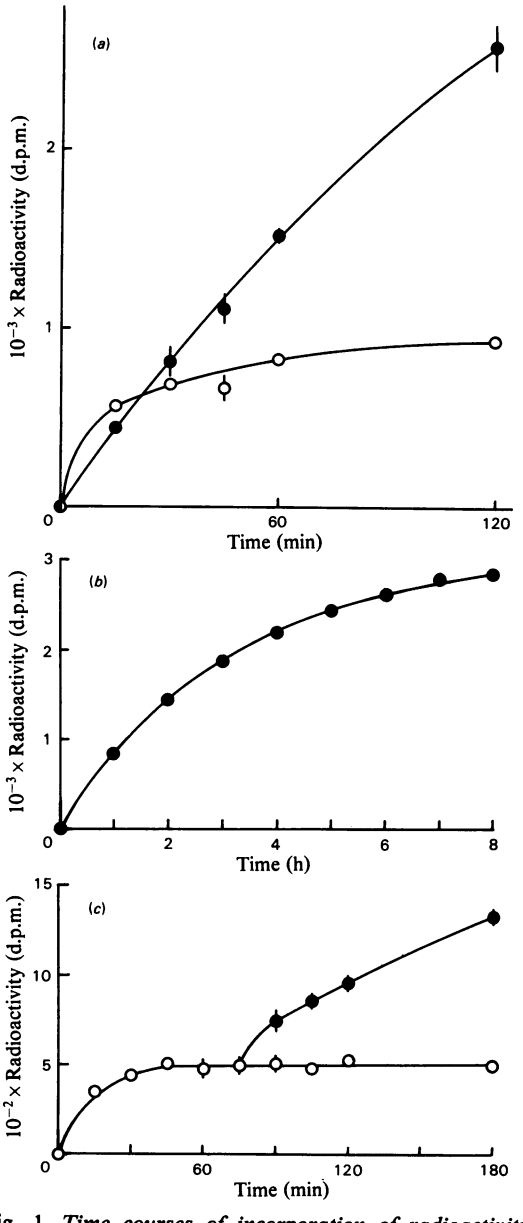

Fig. 1. *Time courses of incorporation of radioactivity from UDP-D-[U-^{14}C]glucuronic aicd into hemicellulose*
(*a*) Time courses (2h) in the presence (●) or absence (○) of UDP-D-xylose (1 mmol/litre). (*b*) An 8h time course in the presence of UDP-D-xylose (1 mmol/litre). (*c*) Time courses showing the effect of adding UDP-D-xylose (1 mmol/litre) after 75 min of incubation. ○, No addition; ●, UDP-D-xylose added.

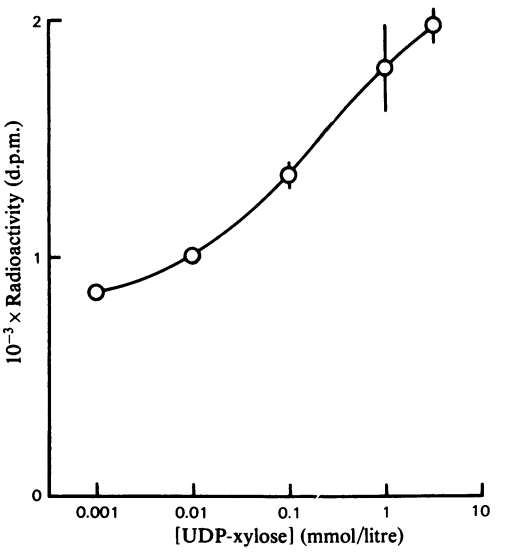

Fig. 2. *Effect of UDP-D-xylose concentration on incorporation of radioactivity from UDP-D-[U-^{14}C]glucuronic acid into hemicellulose during 2 h incubations*

Table 2. *Effect of bivalent cations (10 mmol/litre) on incorporation of radioactivity from UDP-D-[U-^{14}C]glucuronic acid into hemicullulose in the presence and absence of UDP-D-xylose (1 mmol/litre)*
Incubations were of 2h duration.

Bivalent cation	Incorporation (d.p.m.) Without UDP-D-xylose	With UDP-D-xylose
Mn^{2+}	671 ± 12	1356 ± 26
Ni^{2+}	379 ± 24	231 ± 21
Ca^{2+}	290 ± 24	331 ± 10
Mg^{2+}	283 ± 24	407 ± 6
Co^{2+}	625 ± 69	630 ± 18
None	192 ± 22	142 ± 11

Optimum pH for the incorporation of radioactivity from UDP-D-[^{14}C]glucuronic acid into the hemicellulose fraction was between pH 5 and 6. At pH 7.5, at which standard incubations were carried out, the incorporation was about 80% of the maximum observed. Some bivalent cations could stimulate the incorporation (Table 2), and because of this, Mn^{2+} (which is more common in plant cells than Co^{2+} or Ni^{2+} ions) was included in the standard incubations. High concentrations of Mn^{2+} inhibit the incorporation (Table 3); the greatest stimulation occurred at Mn^{2+} concentrations between 4 and 10 mmol/litre, and at the concentration used in standard incubations (10 mmol/litre) the incorporation was 87% of the maximum observed.

Analysis of radioactivity incorporated into hemicellulose from UDP-D-[^{14}C]glucuronic acid in the presence of UDP-D-xylose (1 mmol/litre)

Total acid hydrolysis of a hemicellulose sample containing 6920 d.p.m. was carried out for 180 min

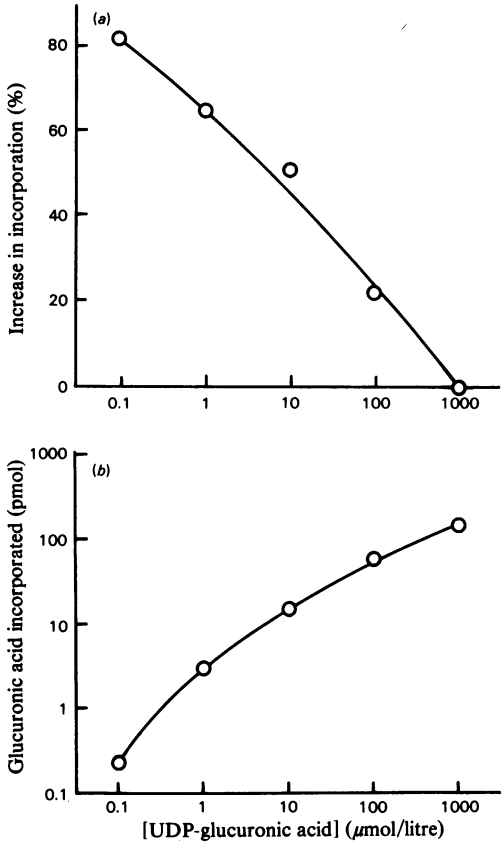

Fig. 3. *Effect of UDP-D-glucuronic acid concentration on incorporation of glucuronic acid from UDP-D-glucuronic acid into hemicellulose during 2 h incubations*
(*a*) Percentage increase in incorporation caused by the presence of UDP-D-xylose (1 mmol/litre). (*b*) Total incorporation of glucuronic acid.

Table 3. *Effect of Mn^{2+} concentration on the incorporation of radioactivity from UDP-D-[U-^{14}C]glucuronic acid into hemicellulose during 2 h incubations in the presence and absence of UDP-D-xylose (1 mmol/litre)*

	Incorporation (d.p.m.)	
[Mn^{2+}] (mmol/litre)	Without UDP-D-xylose	With UDP-D-xylose
1	457 ± 18	587 ± 35
10	835 ± 17	1958 ± 137
100	28 ± 1	78 ± 11
1000	6 ± 1	7 ± 3

at 120°C (see the Materials and methods section). Non-hydrolysed material was removed by centrifugation at 10000 *g*, and the pellet accounted for 140 d.p.m. The hydrolysate was evaporated to dryness under reduced pressure to remove tri-

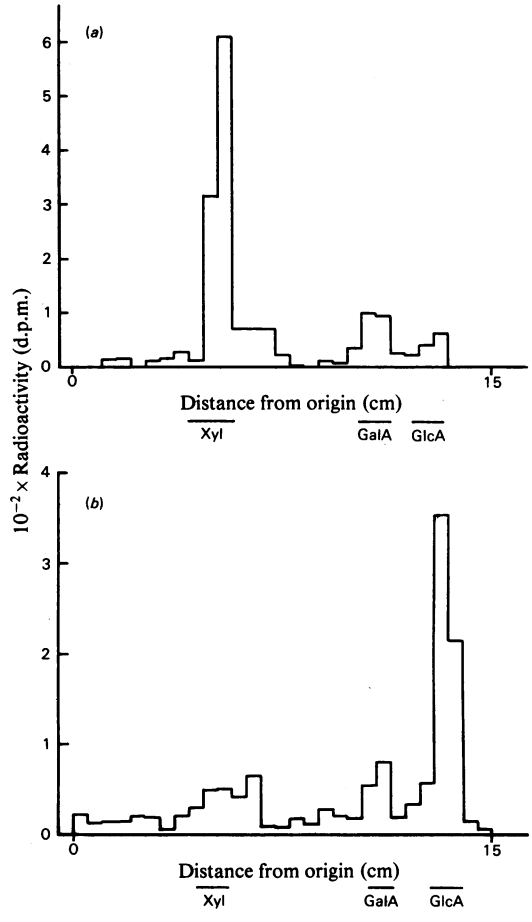

Fig. 4. *Thin-layer-electrophoretic analysis of hydrolysate of hemicellulose labelled with radioactivity incorporated from UDP-D-[U-^{14}C]glucuronic acid*
(*a*) Profile of total acid hydrolysate. (*b*) Profile of total acid hydrolysate that has been subjected to NaOH treatment.

fluoroacetic acid, and then redissolved in 50 μl of water. Non-soluble material was removed by centrifugation (30 d.p.m.) and the supernatant re-desiccated as above. After redissolving the material in 15 μl of water, two 5 μl aliquots of hydrolysate (A and B) were collected. B was evaporated to dryness, redissolved in NaOH (0.33 mol/litre), and left for 1 h at room temperature in order to hydrolyse any glucuronolactone produced during total acid hydrolysis. Both A and B were subsequently analysed by thin-layer electrophoresis (Fig. 4). The results indicate that the major product after NaOH treatment is glucuronic acid. Without NaOH treatment, the main product is glucuronolactone, which moves with neutral-sugar markers in this system. The low yield (64% in A and 52% in B) is probably due to

degradation of [^{14}C]glucuronic acid during the original hydrolysis, since when UDP-D-[^{14}C]glucuronic acid (27750d.p.m.) was subjected to the same experimental procedue, the final yield of [^{14}C]-glucuronic acid after the thin-layer electrophoresis was only about 50% of the quantity used at the start.

Additional identification of the incorporated label utilized a method adapted from that described by Lin & Hassid (1966). Glucuronic acid is known to be difficult to hydrolyse from polysaccharides (Kauss & Hassid, 1967), but if it is reduced to glucose the molecule can be easily freed by acid hydrolysis. Hemicellulose was subjected to partial acid hydrolysis (see the Materials and methods section) for 1 or 2h as described above. The hydrolysate, having been separated from non-hydrolysed material by centrifugation, evaporated to dryness under reduced pressure and redissolved in water, was analysed by paper chromatography in solvent I. Several peaks were seen on the resulting chromatogram (Fig. 5*a*) and, as time of hydrolysis is increased, the material in peak III increases substantially relative to that in peaks I, II and IV (Fig. 5*b*). Peak IV co-chromatographs with xylose in this chromatography system, but similar analysis of the partial acid hydrolysate using Solvent II shows no radioactivity running with xylose. Peak IV of Fig. 5 is probably the lactone form of glucuronic acid, which is produced under acid conditions and co-chromatographs with xylose in Solvent I.

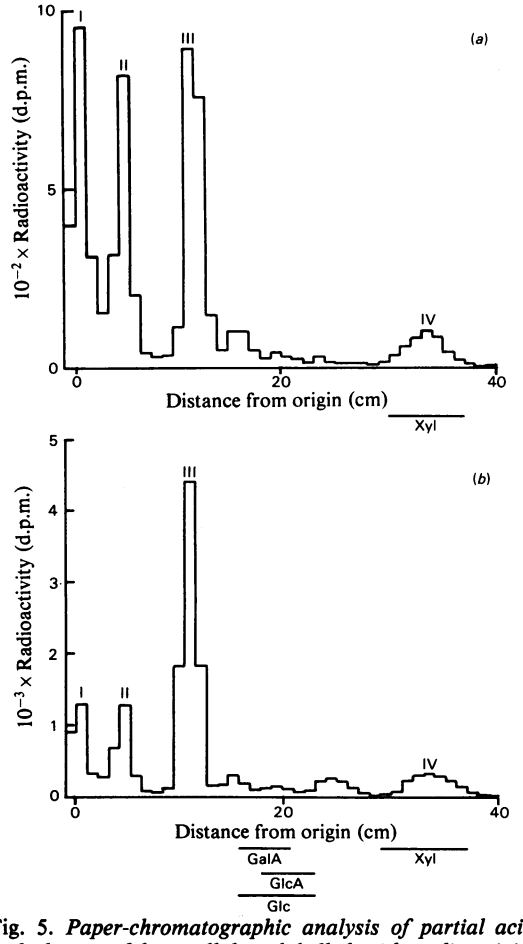

Fig. 5. *Paper-chromatographic analysis of partial acid hydrolysate of hemicellulose labelled with radioactivity from UDP-*D*-[U-*^{14}*C]glucuronic acid*

(*a*) Profile of a 1h hydrolysate. (*b*) Profile of a 2h hydrolysate.

The material in peak III of Fig. 5 has a similar R_{xylose} value to that reported for a disaccharide containing glucuronic acid linked α1→2 to xylose (Ray & Rottenberg, 1964) that is quite stable under acid conditions. When eluted and analysed by thin-layer electrophoresis this material runs almost as fast as galacturonic acid. To obtain further information about this compound, peak III was eluted, dried, esterified by treatment with methanolic HCl (1 mol/litre), and reduced in $NaBH_4$ (see the Materials and methods section). This procedure is able to convert mannuronic acid completely into mannitol (Lin & Hassid, 1966); it would therefore be expected to convert GlcAα1→2Xyl to Glcα1→2xylitol. The products were hydrolysed with trifluoroacetic acid, evaporated to dryness under reduced pressure and redissolved in water. The aqueous sample was analysed by paper chromatography in solvent II, a system in which neutral sugars are separated but uronic acids do not move from the origin. Half of the products co-chromatographed with glucose, which is likely to have been derived from glucuronic acid by reduction. The other half remained at the origin of the chromatogram, and elution of this, followed by chromatography in solvent I showed that it contained unreduced glucuronic acid, unchanged peak-III material, and a small amount of material that co-chromatographed with xylose (probably glucuronolactone).

Effect of proteinases

Digestion of hemicellulose by Proteinase K and Pronase had no effect on the ability of water to solubilize the incorporated radioactivity. This indicates that the hemicellulosic material was not glycoprotein.

Effect of freezing and preincubation on enzyme activity

If 97000*g* pellets were frozen either very quickly in liquid nitrogen, or slowly in air at −18°C, then the activity of the particulate enzyme preparation subsequently made from the pellets (in terms of its ability to incorporate radioactivity from UDP-D-[U-^{14}C]glucuronic acid into hemicellulose) was

Table 4. *Effect of preincubating the particulate enzyme at 25°C on incorporation of radioactivity from UDP-D-[U-^{14}C]glucuronic acid into hemicellulose in the presence and absence of UDP-D-xylose (1 mmol/litre)*
Incubations were 2h in duration.

Preincubation time (min)	Incorporation (d.p.m.) Without UDP-D-xylose	With UDP-D-xylose
0	851 ± 11	1597 ± 66
30	480 ± 14	1069 ± 83
60	428 ± 21	1035 ± 54

decreased by at least 80%. As a result, fresh enzyme preparations were made for all incubations.

The activity of freshly made particulate enzyme preparations was also shown to decrease quickly if stored at between 0 and 4°C. After storage for 8h the quantity of radioactivity incorporated into hemicellulose from UDP-D-[U-^{14}C]glucuronic acid in 15min is only 18% of the quantity incorporated during a 15min incubation without previous storage. If the particulate enzyme preparation was incubated at 25°C for different times before use, the final incorporation of radioactivity into hemicellulose was shown to decrease (Table 4). However, a significant amount of activity remained after 1h of preincubation.

Incorporation of radioactivity into α-cellulose

The α-cellulose left after KOH extraction was shown to have no radioactivity present unless UDP-D-xylose (1mmol/litre) had been present in the incubation. In the latter case only a small amount of radioactivity was present (less than 2% of the radioactivity incorporated into hemicellulose).

Discussion

Our results show that pea epicotyls contain a membrane-bound glucuronyltransferase that transfers glucuronic acid from UDP-glucuronic acid to a product insoluble in 70% ethanol. The following evidence indicates that this product is a glucuronoxylan.

The product is not a glycoprotein. This is shown by its resistance to digestion by two non-specific proteinases, Pronase and Proteinase K.

The effect of UDP-xylose on glucuronic acid incorporation from UDP-glucuronic acid suggests that the product is a glucuronoxylan. In the absence of UDP-xylose, the incorporation of glucuronic acid ceases after about 15min. In the presence of UDP-xylose, the incorporation of glucuronic acid is prolonged for at least 8h. These observations might be explained in the following ways. (1) UDP-xylose might be an allosteric activator of the glucuronyltransferase; the termination of the glucuronic acid incorporation after 15min would then have to be due to degradation of endogenous UDP-xylose by another enzyme in the membrane preparation. (2) UDP-xylose might protect the glucuronyltransferase from denaturation, which would occur within 15min of incubation in the absence of UDP-xylose. (3) UDP-xylose might be required as a second sugar donor for the formation of the product.

The first and second explanations were ruled out by preincubation of the enzyme for 1h before addition of UDP-glucuronic acid (Table 4). The preincubated enzyme was still able to incorporate glucuronic acid into the product, showing that neither degradation of an activator nor denaturation of the enzyme had occurred. Denaturation of the enzyme in the absence of UDP-xylose was further ruled out by the fact that UDP-xylose would cause incorporation of glucuronic acid to resume, even when added after 75min of incubation with UDP-glucuronic acid.

The third explanation, that UDP-xylose is a second sugar donor, seems the most likely one. This would imply that the product is a glucuronoxylan. The incorporation seen in the absence of added UDP-xylose could be due to a small amount of endogenous UDP-xylose present in the membrane preparation, or it could be due to the presence in the membrane preparation of a small amount of non-glucuronidated xylan which could act as an acceptor for a limited amount of glucuronic acid. Addition of UDP-xylose would bring about the formation of further xylan chains, each capable of accepting further glucuronic acid side chains.

Analysis of the [^{14}C]hemicellulose produced in the presence of UDP-xylose gave further evidence for the synthesis of glucuronoxylan. Paper-chromatographic analysis of partial acid hydrolysates (Fig. 5) gave rise to radioactive oligosaccharides, peaks II and III, which chromatograph with the same R_{xylose} values as GlcAα1→2Xylβ1→4Xyl (peak II) and GlcAα1→2Xyl (peak III) (Ray & Rottenberg, 1964).

The high temperature required for complete acid hydrolysis of the radioactively labelled hemicellulose to its constituent monosaccharides also indicates the presence of the GlcAα1→2Xyl linkage. A similar resistance to acid hydrolysis of 4-*O*-MeGlcAα1→2Xyl and 4-*O*-MeGlcAα1→2Xylβ1→4Xyl was observed by Kauss & Hassid (1967).

The only radioactive monosaccharides observed after total acid hydrolysis were glucuronic acid and glucuronolactone. Since glucuronolactone is formed from glucuronic acid under acid conditions, it is clear that the radioactivity was present in the glucuronoxylan as glucuronic acid residues, with no radioactive xylose. Presumably, therefore, no UDP-

glucuronic acid decarboxylase activity was present in the membrane preparation.

The slight, but reproducible, inhibition of the glucuronyltransferase by UDP-xylose, which is observed at short incubation times, may be due to competitive inhibition. This would not be surprising, since the UDP-glucuronic acid and UDP-xylose differ only in the presence of a carboxy group in the former, and UDP-xylose was present at a much higher concentration than was UDP-glucuronic acid. The decrease in the effect of UDP-xylose on the glucuronyltransferase at high UDP-glucuronic acid concentration might have been due to the reverse effect, namely competitive inhibition of the xylosyl transferase by UDP-glucuronic acid.

The effect of bivalent cations on the glucuronyltransferase is similar to the effect of these cations on the methylation of glucuronic acid residues in glucuronoxylan in corn cobs (Kauss & Hassid, 1967). It might be, therefore, that glucuronidation of xylans and methylation of the resulting glucuronoxylans are controlled in parallel in the plant cell by the levels of bivalent cations.

We thank the Science and Engineering Research Council (U.K.) for a Research Studentship granted to K. W. W., during the tenure of which this research was carried out.

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