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The location of arabinosyl:hydroxyproline transferase in the membrane system of potato tissue culture cells

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Incubation of a particulate preparation from potato tissue culture cells with UDP-β-L-[1-^{3}H]arabinose yielded a glycoprotein fraction containing labelled material with the characteristics of hydroxyproline arabinosides. The sugar–protein linkage was resistant to hot alkaline hydrolysis, and the hydrolytic products showed similar electrophoretic and chromatographic behaviour to authentic hydroxyproline–arabinosides prepared from potato tissue culture cell walls. Incorporation of arabinose into glycoprotein was stimulated by the addition of de-arabinosylated potato lectin. The product of the incubation co-migrated with native potato lectin on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. The subcellular distribution of the arabinosyltransferase was investigated by fractionating potato tissue culture membranes on a discontinuous sucrose gradient in the presence or absence of Mg^{2+}. Under both fractionation conditions the highest specific activity of the enzyme was found in the Golgi-enriched fraction. The results are discussed in relation to the synthesis of the hydroxyproline-rich glycoprotein component of plant cell walls.

Plant cell walls contain a protein rich in hydroxyproline (Lamport & Northcote, 1960; Dougall & Shimbayashi, 1960). The hydroxyproline residues are *O*-glycosidically linked to arabinose oligosaccharides usually containing 3 or 4 sugar moieties (Lamport, 1969; Heath & Northcote, 1971). The arabinose residues are β-linked in the furanose form (Akiyama & Katō, 1977). Additionally, the serine residues are substituted with single galactose residues (Lamport *et al.*, 1973; Cho & Chrispeels, 1976) probably as the α-anomer (O'Neill & Selvendran, 1980). The glycoprotein is associated with the α-cellulose fraction of the cell wall (Heath & Northcote, 1971; Selvendran, 1975) and can only be extracted in a partially degraded form. Recently, however, a relatively mild procedure, involving sodium chlorite/acetic acid treatment, has been used to obtain a largely unmodified hydroxyproline-rich glycoprotein from bean cell walls (O'Neill & Selvendran, 1980). Chemically, the insoluble wall glycoprotein closely resembles potato lectin, a soluble glycoprotein that has been isolated and purified from potato tubers (Allen & Neuberger, 1973; Allen *et al.*, 1978; Muray & Northcote, 1978; Owens & Northcote, 1980). These hydroxyproline-rich glycoproteins are distinct from the arabinogalactan–proteins secreted by plant cells in suspension culture (Keegstra *et al.*, 1973; Pope & Lamport, 1974) and found in seed extracts of a large number of plant species (Jermyn & Yeow, 1975) in which the hydroxyproline is linked via galactose to a large arabinogalactan (Fincher *et al.*, 1974; Anderson *et al.*, 1977; Pope, 1977).

Labelling studies *in vivo* indicate that the cell-wall glycoprotein is synthesized in the cytoplasm in association with membranes and subsequently transferred into the cell wall (Chrispeels, 1969; Pope, 1977). The identity of the membrane components involved appears to be uncertain. Cell fractionation (Dashek, 1970) and autoradiographic studies (Roberts & Northcote, 1972) on sycamore suspension cultured cells implicated smooth-membrane vesicles in wall protein synthesis. These were possibly derived from the endoplasmic reticulum. A particulate enzyme system, which may be responsible for the arabinosylation of a cytoplasmic precursor of cell-wall glycoprotein, has been demonstrated in sycamore tissue culture cells (Karr, 1972). Gardiner & Chrispeels (1975) have shown that in carrot root tissue this enzyme activity may be associated with the Golgi apparatus; however, the products of the enzyme reaction were not characterized. We have investigated the enzymes that may be involved in the synthesis of cell-wall protein and in the present paper we report the occurrence of an arabinosyl:hydroxyprolinetransferase in the

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membrane system of potato tissue culture cells and provide evidence of its subcellular localization.

Materials and methods

Growth of tissue

Suspension cultures of potato callus (*Solanum tuberosum* cv. King Edward) were grown in approx. 150ml of PRL 4 medium (Gamborg, 1966) supplemented with 2,4-dichlorophenoxyacetic acid (6mg/litre) in 500ml conical flasks. The flasks were continuously agitated on an orbital shaker (100rev./min) in the dark at 26°C. Approximately half the cells in each flask were subcultured every 6–7 days.

UDP-β-L-[1-^{3}H]arabinose was synthesized enzymically from L-[1-^{3}H]arabinose using crude preparations of arabinokinase and UDP-arabinose pyrophosphorylase extracted from mung-bean seedlings by a modification of the methods of Neufeld *et al.* (1957, 1960). L-[1-^{3}H]Arabinose (sp. radioactivity 8Ci/mmol) was obtained from The Radiochemical Centre (Amersham, Bucks., U.K.).

Preparation and characterization of membrane fractions

Cells (6–7 days after subculturing; 25–30g fresh weight) were filtered through muslin, washed with water and homogenized at 4°C in a ground-glass homogenizer in 50mM-Tris/HCl buffer, pH7.4, containing 8% (w/w) sucrose, 20mM-2-mercaptoethanol, 1mM-EDTA and 0.1mM-$MgCl_2$. In experiments designed to maintain the attachment of ribosomes to membranes, the homogenization medium contained 1mM-$MgCl_2$ in place of EDTA. The homogenate was filtered through muslin and centrifuged at 2000*g* for 15min. The supernatant was layered on to a 10ml cushion of 60% (w/w) sucrose and centrifuged at 100000*g* for 60min in a Beckman ultracentrifuge at 4°C with an SW27 rotor. The supernatant (soluble fraction) was removed and the particulate material (membrane fraction) at the interface collected, mixed with an equal volume of homogenization medium and layered on to a discontinuous sucrose gradient. The gradient was prepared in 18ml cellulose nitrate tubes by layering in succession, 3ml of 45% and 4ml each of 39, 34 and 28% (w/w) sucrose. The sucrose solutions contained all the components of the homogenization medium. The gradient was centrifuged at 100000*g* for 3h at 4°C. Using a peristaltic pump, particulate material at each sucrose interface was collected, diluted with 50mM-Tris/HCl buffer, pH7.4, and pelleted at 100000*g* for 30min at 4°C. Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as a standard. Enzyme assays were performed at 25°C using a Beckman model 25 recording spectrophotometer. NADH:cytochrome *c* reductase activity was measured by the method of Shore & Maclachlan (1975), but antimycin A (25μg/ml) was added to decrease the activity of the mitochondrial enzyme (Lord *et al.*, 1973). Latent IDPase was assayed after leaving the membranes at 4°C for 48h (Shore & Maclachlan, 1975). Succinate dehydrogenase was estimated by the method of King (1967).

Arabinosyltransferase assay

Incubations were carried out at 25°C for 1h in a total volume of 100μl. The incubation mixture contained 50mM-Tris/HCl, pH6.5, 0.05μCi of UDP-β-L-[1-^{3}H]arabinose, 20mM-2-mercaptoethanol, 5mM-AMP, 10mM-$MgCl_2$, 5mM-$MnCl_2$, 0.2% (v/v) Triton X-100 and 100–200μg of protein (membrane fractions). The reaction was stopped by the addition of 1ml of 15% (w/v) trichloroacetic acid and proteins were precipitated overnight at 4°C. Precipitates were collected by filtration on to glass-fibre discs (Whatman GF/C), washed with 10% (w/v) trichloroacetic acid and dried. The discs were then successively extracted with chloroform/methanol (3:2, v/v) and chloroform/methanol/water (10:10:3, by vol.).

Analysis of the product of the UDP-arabinose incubation

Precipitates after trichloroacetic acid addition were collected by centrifugation, washed successively with 10% (w/v) trichloroacetic acid, acetone, chloroform/methanol (3:2, v/v), chloroform/methanol/water (10:10:3, by vol.) and dried. The pellets were hydrolysed with saturated $Ba(OH)_2$ (1.5ml) in a sealed tube at 105°C for 6h. The hydrolysates were neutralized with H_2SO_4, rotary evaporated to dryness and dissolved in water. The presence of hydroxyproline–arabinosides in the sample was investigated by paper electrophoresis at pH2 (acetic acid/formic acid/water, 4:1:45, by vol.) and 5kV for 45min and descending paper chromatography in butanol/acetic acid/water (15:3:5, by vol.) for 20h. Hydroxyproline–arabinoside markers, prepared from depectinated potato tissue culture cell walls by alkaline hydrolysis (Muray & Northcote, 1978), were run in parallel and stained with ninhydrin-isatin reagent (Kolor & Roberts, 1957). Strong acid hydrolysis was performed in 3% (w/v) H_2SO_4 at 120°C and 103kPa for 1h. The hydrolysate was neutralized with solid $BaCO_3$ and sugars were separated by descending paper chromatography (on Whatman no. 1 paper, in ethyl acetate/pyridine/water, 8:2:1, by vol., for 20h). Neutral sugar markers were run in parallel and were detected by aniline hydrogen phthalate (Wilson, 1959).

Samples of the alkaline hydrolysate were mixed with hydroxyproline–arabinosides, made up to a total volume of 1ml and applied to a Sephadex G-25 (Pharmacia, Uppsala, Sweden) column

(1 cm × 16 cm). The column was eluted with 0.1 M-acetic acid and fractions (1.2 ml) were assayed for hydroxyproline by the method of Kivirikko (1963) and counted for radioactivity.

Samples were prepared for sodium dodecyl sulphate/polyacrylamide-gel electrophoresis by stopping the incubation with chloroform/methanol (3:2, v/v) and extracting in the same solvent for 18h at 4°C. Particulate material was washed successively with methanol (75%, v/v) chloroform/methanol/water (10:10:3, by vol.) and extracted with phenol/acetic acid/water (2:1:1, by vol.) for 18h at 4°C. Particulate material was removed by centrifugation and proteins were precipitated by the addition of 5 vol. of acetone and 0.02 vol. of ammonium formate (10%, w/v). Precipitates were collected by centrifugation and boiled for 20min in sample buffer consisting of 1 M-Tris/HCl, pH 6.8, 100 mM-dithiothreitol, 2% sodium dodecyl sulphate, 10% glycerol and 0.6% Bromophenol Blue. Samples were analysed on 15% slab gels using the system of Laemmli (1970). Marker tracks were cut out and stained with Coomassie Brilliant Blue R. Radioactive tracks were cut into 10 mm × 2 mm slices and dried. Gel segments were solubilized by incubating with 100 μl of H_2O_2 (100-volume) at 60°C for 24h, and counted for radioactivity.

Preparation of deglycosylated potato lectin

Potato lectin was purified from potato tubers by affinity chromatography on a fetuin–Sepharose matrix (Owens & Northcote, 1980). Arabinose residues were removed from the polypeptides by mild acid hydrolysis. Samples (5 mg) were hydrolysed in 30 mM-oxalic acid, pH 3, at 100°C for 3h. The hydrolysate was neutralized with NaOH, extensively dialysed against water and freeze-dried.

Measurement of radioactivity

Glass-fibre discs, paper chromatograms and electrophoretograms (cut up into strips) were counted for radioactivity in 0.5 ml of toluene-based scintillant (8.75 g of 2,5-diphenyloxazole and 0.125 g of 1,4-bis-(5-phenyloxazol-2-yl)benzene, in 2.5 litres of toluene) in a Searle mark III liquid-scintillation system model 6880. Aqueous samples were counted for radioactivity by adding 1 vol. to 10 vol. of Triton/toluene scintillant [6 g of 2,5-diphenyloxazole, 0.075 g of 1,4-bis-(5-phenyloxazol-2-yl)-benzene, 750 ml of Triton X-100 and 1.5 litres of toluene].

Results

Incorporation of arabinose from UDP-β-L-[1-^{3}H]-arabinose into hydroxyproline–arabinosides by isolated membranes

A total particulate fraction (100000 *g* pellet) was prepared from potato tissue culture cells and incubated with UDP-β-L-[1-^{3}H]arabinose. Incorporation of arabinose into glycoprotein was assayed by precipitation with trichloroacetic acid. To ensure the removal of any lipids, precipitates were extracted

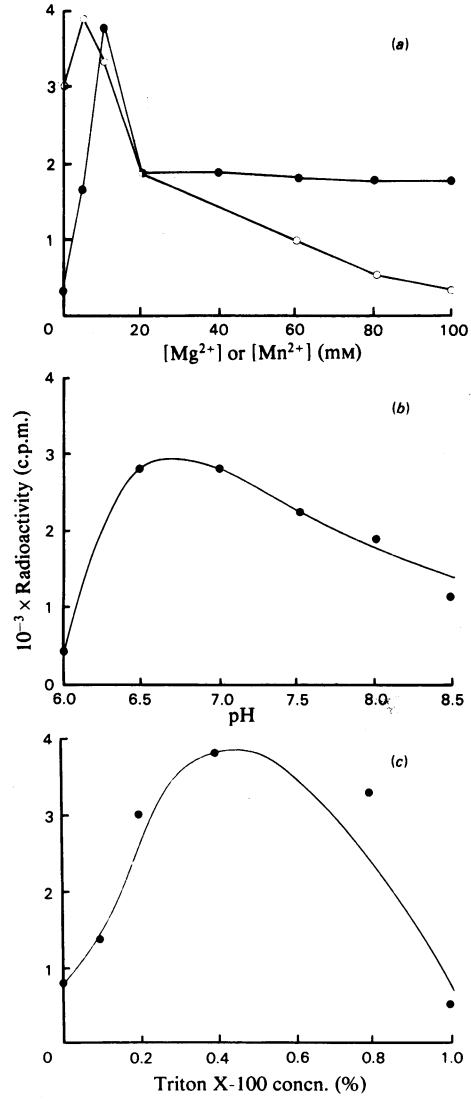

Fig. 1. *Conditions for the incorporation of arabinose by isolated membranes from UDP-β-L-[1-^{3}H]arabinose into precipitates after addition of trichloroacetic acid*

Isolated membranes (100000 *g* pellet) were incubated with UDP-β-L-[1-^{3}H]arabinose for 1h at 25°C. Proteins were precipitated by the addition of trichloroacetic acid (15%, w/v), collected by filtration on to glass-fibre discs and counted for radioactivity. The effect of varying (*a*) the Mg^{2+} (●) and Mn^{2+} (○) concentration, (*b*) the pH and (*c*) the concentration of Triton X-100 on incorporation into glycoprotein was determined.

with chloroform/methanol (3:2, v/v) and chloroform/methanol/water (10:10:3, by vol.). The precipitate was hydrolysed in saturated $Ba(OH)_2$ and the hydrolytic products were electrophoresed at pH2 or chromatographed in butanol/acetic acid/water (15:3:5, by vol.). In both separations a single major peak of radioactivity was detected. The peak coincided with hydroxyproline–arabinoside marker with a mobility of $R_{hydroxyproline}$ 0.36 on electrophoresis and 0.15 on chromatography. The peak of radioactivity was eluted with water, hydrolysed with strong acid and the sugars were separated by paper chromatography. The radioactivity coincided with arabinose only. The hydrolytic products were mixed with a crude mixture of hydroxyproline–arabinosides and passed through a column (1 cm × 16 cm) of Sephadex G-25. Radioactive material was eluted as a single peak after the void volume, coincident with a peak of hydroxyproline-containing material. Figs. 1(*a*)–1(*c*) show the optimum conditions for the incorporation of arabinose into glycoprotein. The enzyme activity required the addition of Mg^{2+} up to a concentration of 10mM, but showed negligible further stimulation by exogenous Mn^{2+} (in the presence of 10mM-Mg^{2+}) and was inhibited by concentrations greater than 5mM. The pH optimum, approx. 6.5, is in agreement with the result of Karr (1972). Incorporation was stimulated by the addition of Triton X-100 up to 0.4% (v/v), but was inhibited at higher concentrations.

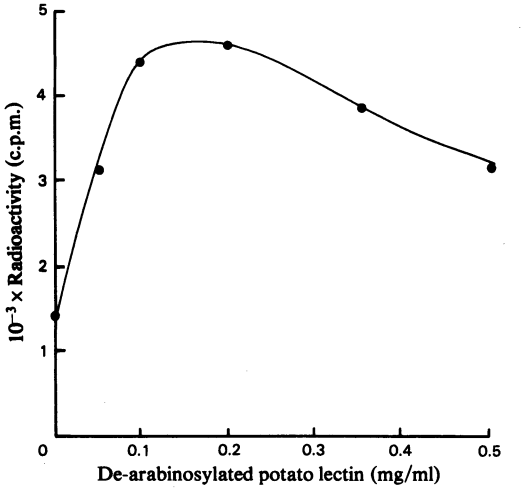

Fig. 2. *Effect of deglycosylated potato lectin on the arabinosyltransferase activity*

Isolated membranes (100000*g* pellet) were incubated with UDP-β-L-[1-^{3}H]arabinose and different concentrations of de-arabinosylated potato lectin for 1h at 25°C. Proteins were precipitated by the addition of trichloroacetic acid (15%, w/v) collected by filtration on to glass-fibre discs, extracted with lipid solvents and counted for radioactivity.

Effect of exogenous deglycosylated potato lectin on arabinosyltransferase activity

The complete removal of arabinose residues from potato lectin by mild acid hydrolysis was verified by strong acid hydrolysis of the polypeptide, followed by paper chromatography. The only sugar detectable was galactose, which is known to be *O*-glycosidically linked to serine residues in the native lectin (Allen *et al.*, 1978). Incorporation of arabinose into glycoprotein was stimulated by the addition of deglycosylated lectin up to a concentration of 0.2mg/ml (Fig. 2).

Polyacrylamide-gel electrophoresis of the radioactive product extracted by sodium dodecyl sulphate from an incubation with de-arabinosylated potato lectin (0.2mg/ml) gave a single radioactive peak (Fig. 3). This corresponded to a protein with an apparent mol.wt. of 89000 ± 10000 and coincided

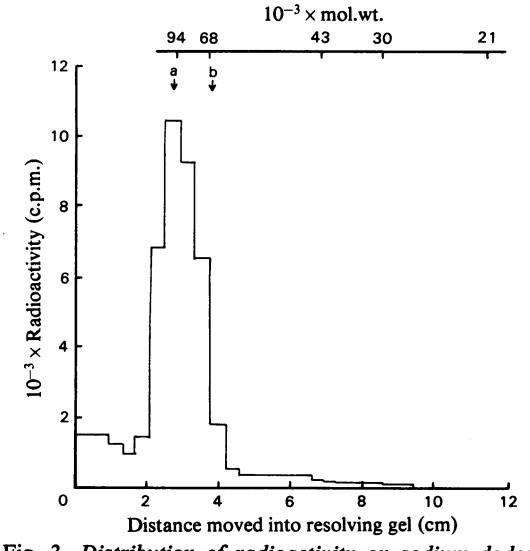

Fig. 3. *Distribution of radioactivity on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of glycoprotein labelled with [^{3}H]arabinose*

Isolated membranes (100000*g* pellet) were incubated with UDP-β-L-[1-^{3}H]arabinose and de-arabinosylated potato lectin (0.2mg/ml) for 1h at 25°C. Samples were prepared for sodium dodecyl sulphate/polyacrylamide-gel electrophoresis as described in the Materials and methods section. Marker proteins run in parallel were phosphorylase *b* (mol.wt. 94000), bovine serum albumin (68000), ovalbumin (43000), carbonic anhydrase (30000) and soya-bean trypsin inhibitor (21000). Arrows a and b indicate the positions of potato lectin and de-arabinosylated potato lectin respectively.

with the position of native potato lectin. This suggested that the de-arabinosylated lectin resembled the endogenous acceptor of the arabinose from UDP-L-arabinose and that the lectin had been re-glycosylated *in vitro*.

Distribution of marker activities in membrane fractions

The membrane-fractionation procedure was adapted from Baydoun & Northcote (1980) and avoids pelleting and resuspension of membranes to decrease the chances of their modification or destruction. Membranes were prepared in the presence of high Mg^{2+} concentration to maintain the attachment of ribosomes to the endoplasmic reticulum and in the presence of EDTA to effect their removal from the membranes. The membranes were separated into four fractions according to their positions on a discontinuous sucrose gradient; fraction 1, 8/28%; fraction 2, 28/34%; fraction 3, 34/39%; fraction 4, 39/45% sucrose interface. The results of the assays of the marker enzymes are summarized in Table 1.

NADH:cytochrome *c* reductase has been used as a marker for the endoplasmic reticulum in plants (Lord *et al.*, 1973; Bowles & Kauss, 1976; Nagahashi & Beevers, 1978). In the presence of EDTA, the highest specific activity of this enzyme occurred in fraction 1. In the presence of Mg^{2+}, NADH:cytochrome *c* reductase assumed a broad distribution through the gradient with the highest specific activity in fraction 3. A similar shift has been observed in other tissues (Lehle *et al.*, 1978; Hopp *et al.*, 1979; Bollini & Chrispeels, 1979) and provides evidence for the localization of the endoplasmic reticulum.

Succinate dehydrogenase, which is located in mitochondrial membranes (Veegar & Zeylermarker, 1969), was found in fractions 3 and 4 and the 2000*g* pellet, with no activity detectable in the other fractions.

IDPase has been used as a specific marker for the Golgi apparatus (Ray *et al.*, 1969). Under both conditions of membrane preparation IDPase activity was found in all fractions, with the highest specific activity occurring in fraction 2. The IDPase activity in fraction 2 is probably associated with dictyosome membranes (Baydoun & Northcote, 1980). The high specific activity of the enzyme found in the supernatant probably represents non-specific phosphatases (Quail, 1979).

The fractionation procedure produced a separation of endoplasmic reticulum (fraction 1) and mitochondrial membranes (fractions 3 and 4) with particulate material at intermediate density (fraction 2) derived from the Golgi apparatus.

Distribution of arabinosyltransferase activity in membrane fractions

Membranes were fractionated in the presence of EDTA and subcellular fractions assayed for arabinosyltransferase activity with added de-arabinosylated lectin (0.2 mg/ml). The highest specific activity was found in fraction 2, identified with the Golgi apparatus (Table 2). The same result was obtained for membranes prepared in the presence of Mg^{2+}. No shift in activity from fractions 1 to 3 was observed, suggesting that there

Table 1. *Distribution of marker enzyme activities in membrane fractions prepared in the presence of either EDTA or Mg^{2+}*

Potato tissue culture cells were homogenized at 4°C in the presence of EDTA to effect the removal of ribosomes from the endoplasmic reticulum or in the presence of Mg^{2+} to maintain their attachment to the endoplasmic reticulum. The homogenate was filtered through muslin, and the filtrate was centrifuged at 2000*g* for 15 min. The supernatant was layered on to a 60% (w/w) sucrose cushion, and centrifuged at 100000*g* for 60 min. The supernatant from this centrifugation was collected and constitutes the soluble fraction. Particulate material at the 60% sucrose interface was collected and fractionated on a discontinuous sucrose gradient to yield four membrane fractions. Fractions were assayed for enzyme activities and protein as described in the Materials and methods section. Recoveries of marker enzymes were 75–80%. The relative specific activity is calculated as the ratio of the specific activity in the subcellular fraction to that in the homogenate.

		Relative specific activities					
		NADH:cytochrome *c* reductase		Succinate dehydrogenase		Inosine diphosphatase	
Fraction	Additions ...	EDTA	Mg^{2+}	EDTA	Mg^{2+}	EDTA	Mg^{2+}
2000*g* pellet		1.24	0.89	5.20	2.32	0.24	0.66
1. 8/28% interface		6.81	5.23	0	0	1.44	1.24
2. 28/34% interface		3.80	8.89	0	0	1.51	1.49
3. 34/39% interface		1.15	12.72	7.00	5.93	0.80	0.78
4. 39/45% interface		0.98	8.84	4.60	8.32	0.54	0.95
Supernatant		0.76	1.19	0	0	1.60	1.62

Table 2. *Distribution of arabinosyltransferase activity in subcellular fractions*
Potato tissue culture cells were homogenized at 4°C in the presence of EDTA. Subcellular membranes were fractionated on a discontinuous sucrose gradient as described in the Materials and methods section. Fractions were incubated with UDP-β-L-[1-^{3}H]arabinose and de-arabinosylated potato lectin (0.2mg/ml) for 1h at 25°C. Proteins were precipitated with trichloroacetic acid (15%, w/v), collected by filtration on to glass-fibre discs, extracted with lipid solvents and counted for radioactivity. The relative specific radioactivity is calculated as the ratio of the specific radioactivity (c.p.m./mg of protein per h) in the subcellular fraction to that in the homogenate.

Fraction	10^{-3} × Radioactivity in precipitates (c.p.m.)	Specific radioactivity	Relative specific radioactivity
Homogenate	1.82	26765	1
2000*g* pellet	0.89	13284	0.5
1. 8/28% interface	1.53	42500	1.6
2. 28/34% interface	3.0	63830	2.4
3. 34/39% interface	3.22	38795	1.4
4. 39/45% interface	1.17	15195	0.6
Supernatant	0.49	9423	0.4

is no significant arabinosyltransferase activity associated with the endoplasmic reticulum.

Discussion

The walls of potato tissue culture cells contain a hydroxyproline-rich glycoprotein in which the hydroxyproline residues are glycosylated with tri- and tetra-arabinosides (Muray & Northcote, 1978). An arabinosyl:hydroxyproline transferase has been identified in the membrane system of potato callus cells. This enzyme system may be responsible for the glycosylation of the cytoplasmic precursor of the cell-wall glycoprotein.

The hydroxyproline–arabinosides of the wall glycoprotein consist of several different linkages. Arabinose is β-linked to the 4-hydroxy group of hydroxyproline and the arabinose residues are interconnected by a mixture of β1–3 and β1–2 bonds (Akiyama & Katō, 1976, 1977; O'Neill & Selvendran, 1980). The arabinosyl:hydroxyproline transferase may therefore consist of a complex of several different enzymes. Alternatively, in view of the close steric similarity between the 4-*trans*-hydroxyproline and L-arabinofuranose rings, there may be a single transglycosylase that specifically adds arabinose residues on to secondary hydroxy groups attached to a ring structure, either proline or arabinose.

In both the sugar nucleotide and hydroxyproline–arabinoside, the arabinose residues are in the β-form. Since each transglycosylation event is usually accompanied by a change in configuration, this suggests that the sugars are added individually via an intermediate. In the synthesis of yeast mannoprotein (Sharma *et al.*, 1974; Bretthauer & Wu, 1975), and the glycosylation of oviduct microsomal proteins (Chen *et al.*, 1975), α-mannose residues in the glycoprotein are incorporated initially from GDP-α-D-mannose via dolichyl phosphate intermediates. By analogy we suggest that arabinosylation of peptidyl hydroxyproline may also involve an intermediate, possibly a polyprenyl phosphate monosaccharide.

Consistent with the role of the Golgi apparatus as the principal site of *O*-glycosylation in plant and animal tissues (Northcote, 1979), the arabinosyl:hydroxyproline transferase on the membranes co-sedimented with IDPase, a marker for the Golgi apparatus. There was no evidence for any of this arabinosyltransferase being present at the endoplasmic reticulum.

Potato lectin from which arabinose residues had been removed by mild acid hydrolysis acted as an effective acceptor for the arabinosyltransferase. The endogenous acceptor of the enzyme, which may represent the cytoplasmic precursor of the hydroxyproline-rich wall glycoprotein, must therefore resemble potato lectin.

We have shown that the Golgi apparatus is involved in the synthesis of the cell-wall glycoprotein of the callus tissue. This glycoprotein may therefore be incorporated directly into the cell wall from the endomembrane system. The glycoprotein containing hydroxyproline that is found in the cell wall of higher plants is insoluble. However, in the differentiated potato tuber an additional glycoprotein is found that is a soluble lectin. The lectin contains hydroxyproline and is chemically very similar to the glycoprotein of the wall. During the synthesis of the lectin, arabinosylation of the peptidyl hydroxyproline presumably takes place in the Golgi apparatus by the same or similar enzyme system to the one we have investigated in the present paper, which is probably concerned with the cell-wall glycoprotein (lectin is not formed by potato callus). If this is the case then during differentiation of the tuber, when both cell-wall glycoprotein and lectin are produced, which are almost identical chemically, at least two mechanisms for transport or for final localization of the glycoproteins must be in operation.

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