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Differential patterns of arabinosylation by membranes of suspension-cultured cells of *Phaseolus vulgaris* (French bean) after subculture or elicitation

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Suspension-cultured cells of *Phaseolus vulgaris* (French bean) incorporated [1-^{3}H] arabinose *in vivo* into high-M_r polymers that could be separated into glycoprotein and polysaccharide. Microsomal membranes from suspension-cultured cells of beans incorporated arabinose from UDP-β-L-arabinose *in vitro* into both polysaccharide and glycoprotein. The enzyme involved in arabinan synthesis, arabinan synthase, appeared to be immunologically distinct from the protein:arabinosyltransferase system. Both these activities are inducible, but behave differently with either plant-growth-regulator or fungal-elicitor treatments. After subculture of cells entering the stationary growth phase the arabinan synthase activity reaches much higher values than does that of the protein transferase system during the initial period of cell division and growth, whereas after elicitation at the same growth stage, all the increased incorporation of arabinose occurs into glycoprotein of M_r higher than 200000 and to a greater extent into a specific glycoprotein of M_r 42500. Preliminary characterization of these glycoproteins prepared under non-reducing conditions and after acid and alkaline hydrolysis suggests that the high-M_r glycoprotein material is similar to arabinogalactan protein, whereas the lower-M_r material may be a hydroxyproline-rich protein existing as a dimer and that specifically increases during the hypersensitive response of the cells to the fungal elicitor from *Colletotrichum lindemuthianum*.

L-Arabinose is found in the primary cell wall of higher plants in both polysaccharide and glycoprotein. Arabinan, probably mostly α1→3- and α1→5-linked is a constituent of neutral pectin, and at least three types of hydroxyproline-rich glycoproteins are arabinosylated. Insoluble hydroxyproline-rich glycoproteins associated with α-cellulose are probably formed by oxidative cross-linking of soluble glycoprotein monomer by isodityrosine residues (Fry, 1982; Cooper & Varner, 1983). The soluble monomers are probably lectin-like bacterial agglutinins and have been well characterized (Allen *et al.*, 1978; Muray & Northcote, 1978; Ashford *et al.*, 1982; Leach *et al.*, 1982*a*,*b*; Selvendran & O'Neill, 1982; Matsumoto *et al.*, 1983). These glycoproteins have been implicated in a role in disease resistance (Esquerré-Tugayé & Lamport, 1979; Esquerré-Tugayé *et al.*, 1979, 1980; Stuart & Varner, 1980; Leach *et al.*, 1982*a*,*b*). Also found are distinct arabinogalactan proteins composed largely of carbohydrate (Clarke *et al.*, 1979; Fincher *et al.*, 1983) in which arabinofuranose residues are linked on to galactose side chains of the main galactan backbone (Clarke *et al.*, 1979; Lamport & Catt, 1980) and that may be secreted by suspension-cultured cells (Keegstra *et al.*, 1973; Pope & Lamport, 1974; van Holst *et al.*, 1981).

Studies of the incorporation of radioactive precursors *in vivo* indicate that the synthesis of arabinan (Kawasaki, 1981; Bolwell & Northcote, 1983*a*) and the glycoprotein (Chrispeels, 1969; Pope, 1977) occurs within the endomembrane system before subsequent transfer to the cell wall. Particulate enzyme systems capable of transfer of L-arabinose from UDP-arabinose to the respective endogenous acceptor have been demonstrated for polysaccharide (Bolwell & Northcote, 1981, 1983*a*) and for glycoprotein (Karr, 1972; Gardiner & Chrispeels, 1975; Owens & Northcote, 1981). In suspension-cultured cells of *Phaseolus vulgaris*, synthesis of arabinan is initiated at subculture and

is associated with cell division and subsequent primary growth (Bolwell & Northcote, 1983*b*). In the present study the control of incorporation of arabinose into polysaccharide and glycoprotein after subculture has been compared with treatment with a fungal elicitor from *Colletotrichum lindemuthianum* (Dixon & Lamb, 1979). These stimuli give rise to different responses, leading to increased arabinan synthesis with some incorporation into mainly high-M_r glycoprotein after growth-regulator treatment, and incorporation into a glycoprotein of M_r 42500 specifically increased after elicitation.

Materials and methods

Derivation and maintenance of cell suspension cultures of French bean (*Phaseolus vulgaris*; c.v. Canadian Wonder) was described previously (Bolwell & Northcote, 1983*b*). UDP-β-L-[1-^{3}H]arabinopyranose and non-radioactive UDP-β-L-arabinopyranose were synthesized as described previously (Bolwell & Northcote, 1981).

Treatment of cultures

After 8 days in CMD medium [B5 medium of Gamborg *et al.* (1968) supplemented with 2,4-dichlorophenoxyacetic acid (2mg/litre), 20% (v/v) deproteinized coconut milk and 2% (w/v) sucrose] cells were subjected to growth-factor treatment by subculture into CMD medium (Bolwell & Northcote, 1983*b*) or elicitor treatment (Dixon & Lamb, 1979) by addition of a preparation from *Colletotrichum lindemuthianum* (equivalent to 30μg of glucose/ml) prepared by the method of Anderson-Prouty & Albersheim (1975). Cells were harvested by filtration. Incorporation of L-[1-^{3}H]arabinose into cells 48h after subculture into CMD medium has been described by Bolwell & Northcote (1983*a*).

Preparation of membranes

Cells were homogenized in 50mM-potassium phosphate buffer, pH7.2, containing 1mM-dithiothreitol, 0.4M-sucrose and 10mM-$MgCl_2$ (1g of tissue/ml of buffer) at 4°C in a pestle and mortar. The slurry was filtered through muslin and centrifuged at 1000*g* for 15min. The supernatant was then centrifuged at 15000*g* for 10min to sediment most of the larger organelles. The supernatant was then subjected to centrifugation at 200000*g* for 1h. The final microsomal pellet was resuspended in homogenization medium.

Analysis of membranes prepared from cells incubated with L-[1-^{3}H]arabinose

Fractionation of membrane prepared from cells incubated with 50μCi of L-[1-^{3}H]arabinose was described previously (Bolwell & Northcote, 1983*a*). Material insoluble in 10% (w/v) trichloroacetic acid was prepared for analysis by repeated washing in acetone. The pellet was dissolved in 50mM-potassium phosphate buffer, pH7.2, before analysis on CsCl gradients as described by Green & Northcote (1978).

Incorporation of L-[1-^{3}H]arabinose from UDP-β-L-[1-^{3}H]arabinose into membranes

Standard assays for the arabinan synthase activity and the fractionation of the products into lipid-soluble, water-soluble and trichloroacetic acid-insoluble products were described previously (Bolwell & Northcote, 1981), as was characterization of polysaccharide and putative lipid products (Bolwell & Northcote, 1981, 1983*a*). Material insoluble in 10% trichloroacetic acid was resuspended in acetone, then centrifuged. All the radioactivity was recovered in the pellet, which was then boiled for 20min in 0.05M-Tris/HCl buffer, pH6.8, containing 100mM-dithiothreitol, 10% (v/v) glycerol, 2% (w/v) sodium dodecyl sulphate and 0.006% Bromophenol Blue. Samples were then subjected to electrophoresis on 10% (w/v)-polyacrylamide slab gels using the system of Laemmli (1970). After staining the gel in Coomassie Blue, tracks were scanned at 638nm in an MSE densitometer (MPS type 940.800). Tracks were then cut into 10mm × 2mm slices and solubilized by incubating with 100μl of 100-volume H_2O_2 (30%, w/v) at 60°C for 24h before counting for radioactivity. Alternatively radioactivity was visually detected by fluorography by the method of Laskey & Mills (1975).

Analysis of glycoprotein

After incubation of membranes prepared from cells 12h after elicitation with UDP-β-L-[1-^{3}H]arabinose for 30min under standard conditions, they were immediately solubilized in 50mM-Tris/HCl (pH7.2)/2% sodium dodecyl sulphate at 37°C for 1h. The sample was then fractionated on a column (1.5cm × 100cm) of Sephacryl S-300 equilibrated in 50mM-Tris/HCl, pH7.2, containing 0.1% sodium dodecyl sulphate. Radioactive fractions were analysed after both acid and alkaline hydrolysis. Strong-acid hydrolysis was performed for sugar analysis with 3% (w/v) H_2SO_4 at 120°C for 1h. The hydrolysate was neutralized with solid $BaCO_3$ and sugars separated by ascending t.l.c. (on Polygram SILG silica-gel plates in ethyl acetate/pyridine/acetic acid/water, 6:3:1:1, by vol). Neutral sugar markers were run in parallel and detected with aniline hydrogen phthalate (Wilson, 1959). Amino acids were analysed after acid hydrolysis in 6M-HCl at 105°C for 24h. The presence of hydroxyproline was detected colorimetric-

ally by the method of Kivirikko (1963) or by ascending t.l.c. in butanol/acetic acid/water (4:1:1, by vol.). Hydroxyproline and proline markers were run in parallel and detected with ninhydrin. Alkaline hydrolysis was accomplished by heating the sample with saturated $Ba(OH)_2$ in a sealed tube at 105°C for 6h. The hydrolysates were neutralized with H_2SO_4, centrifuged, evaporated to dryness *in vacuo* and dissolved in water. The samples were investigated by paper electrophoresis at pH 2 and 5kV for 45min or descending paper chromatography in butanol/acetic acid/water (15:3:5, by vol.) for 20h. Hydroxyproline arabinoside markers prepared by the method of Muray & Northcote (1978) from potato tuber or bean cell walls were run in parallel and stained with ninhydrin/isatin reagent (Kolor & Roberts, 1957). Radioactive spots were detected in 3cm × 1cm strips by liquid-scintillation counting (Harris & Northcote, 1970).

Fractions were also analysed for protein by absorbance at 260/280nm and for total carbohydrate by the phenol/H_2SO_4 assay (Dubois *et al.*, 1956). In samples without detergent present, protein was routinely determined by the method of Read & Northcote (1981).

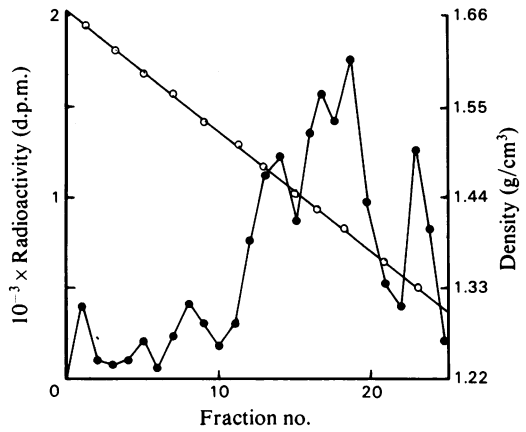

Fig. 1. *Distribution of radioactivity in CsCl density gradients after centrifugation of polymer labelled with radioactive arabinose*

Membranes prepared from cells incubated with 50μCi of L-[1-^{3}H]arabinose were prepared for analysis after removal of lipid and polysaccharide. Radioactive polymer insoluble in 10% trichloroacetic acid was centrifuged in a CsCl density gradient for 48h at 200000*g*, and fractions were collected and their density (○) and radioactivity determined (●). The initial density was 1.44g/cm^3.

Results

Demonstration of arabinosylated glycoprotein in membranes from cells incubated with L-[*1-*^{3}*H*]*arabinose in vivo*

Fractionation of membranes prepared from cells 48h after subculture into CMD medium that had been incubated with [1-^{3}H]arabinose for 1h *in vivo* showed that about 10% of the total incorporation was into material insoluble in 10% trichloroacetic acid. This material remaining after extraction of lipid and polysaccharide from the radioactive membranes was subjected to centrifugation on a CsCl gradient (initial density 1.44g/cm^3). The distribution of radioactivity is shown in Fig. 1. All the radioactivity was recovered polydispersively in the range of density expected for glycoprotein components (Fincher & Stone, 1974) with peaks at 1.35, 1.40, 1.45 and 1.47g/cm^3. Densities of pure protein and polysaccharide would be expected to be 1.33 and 1.60 respectively.

Incorporation of L-[*1-*^{3}*H*]*arabinose from UDP-β-*L-[*1-*^{3}*H*]*arabinose by isolated membranes*

Membranes showing maximum arabinan synthase activity were prepared from cells 48h after subculture in CMD medium. Fig. 2 shows a time course of incorporation into material of solubility characteristic of polysaccharide, monosaccharide and oligosaccharide lipid and glycoprotein. Incorporation into polysaccharide was linear up to 30min, whereas incorporation into other fractions was saturated after 5–10min, suggesting limitations in the amount of endogenous acceptor. The characterization of products soluble in chloroform/methanol (3:2, by vol.) and chloroform/methanol/water (10:10:3, by vol.) and therefore putatively lipid-bound and soluble in 10% trichloroacetic acid (polysaccharide) has been described previously (Bolwell & Northcote, 1981, 1983*a*). The material insoluble in 10% trichloroacetic acid was subjected to polyacrylamide-gel electrophoresis, and the distribution of radioactivity is shown in Fig. 3(*a*). Extended heating was required to solubilize the product completely. Of the material that entered the gel, one protein of M_r 42500 was specifically labelled, whereas material of M_r >200000 was also labelled, but ran polydispersively and stained poorly with Coomassie Blue.

Changes in the patterns of arabinosylation after subculture into CMD medium or elicitation

Cells 8 days after subculture into CMD medium were subjected to either plant-growth-regulator treatment by a further subculture into CMD medium (Bolwell & Northcote, 1983*b*) or treatment with an elicitor preparation from *Colletotrichum lindemuthianum*. Significant differences were found in the patterns of arabinosylation by isolated

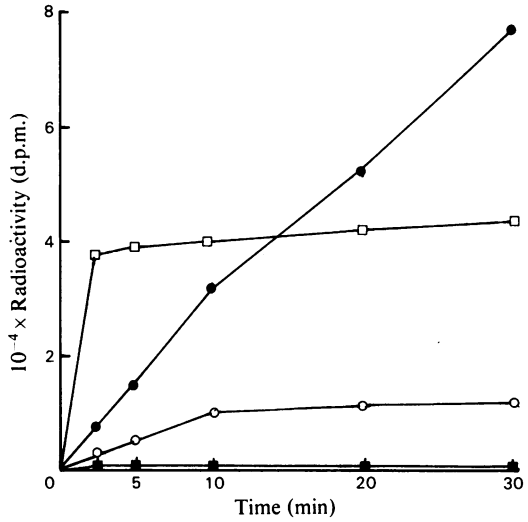

Fig. 2. *Time course of incorporation of* L-[*1*-^{3}*H*]*arabinose from UDP-β*-L-[*1*^{3}-*H*]*arabinose by isolated membranes into material of different solubilities*

Membranes isolated from cells 48h after subculture in CMD medium were incubated with 1.33μCi of UDP-β-L-[1-^{3}H]arabinose and sampled at various times. Each sample was subjected to sequential extraction into material soluble in: chloroform/methanol (3:2, v/v) (monosaccharide lipid, ■); 85% ethanol (low M_r, not shown); chloroform/methanol/water (10:10:3, by vol.) (oligosaccharide lipid, □) 10% trichloroacetic acid (arabinan; ●); and material insoluble in 10% trichloroacetic acid (glycoprotein; ○).

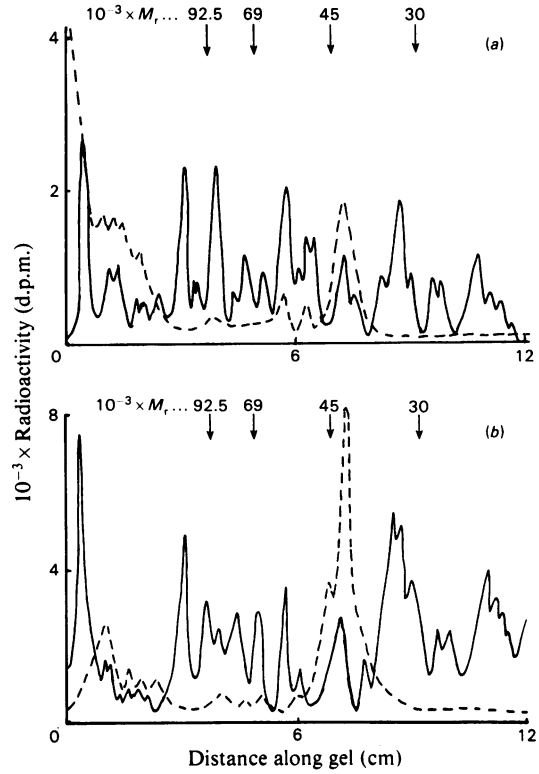

Fig. 3. *Electrophoretic profile of membranes incubated with UDP-β-*L-[*1*-^{3}*H*]*arabinose in vitro prepared from* (*a*) *cells 48h after subculture, and* (*b*) *cells 12h after elicitation and analysed on a sodium dodecyl sulphate/polyacrylamide gel*

Radioactive membranes were prepared for sodium dodecyl sulphate/polyacrylamide-gel electrophoresis and analysed on 10% gels. Profiles are shown for the analysis of 100μg of membrane protein for absorbance after Coomassie Blue staining (——), and radioactivity after gel slicing (----).

membranes incubated with UDP-arabinose. After subculture there is an induction of arabinan synthase activity and consequently increased incorporation into polysaccharide, which was previously described (Bolwell & Northcote, 1983*b*, 1984) (Fig. 4*a*). Concomitant with this is an increased incorporation into endogenous glycoprotein, which on analysis by polyacrylamide-gel electrophoresis appears mainly to be into the high-M_r material, since the level of arabinosylation of the 42500-M_r protein changes little. In complete contrast, elicitation brings about increased membrane-bound arabinosyltransferase activity that is nearly all directed towards glycoprotein, as no incorporation into polysaccharide is induced (Fig. 4*b*). The increase in arabinosylation of glycoprotein is mainly confined to transfer on to the 42500-M_r glycoprotein, as revealed by the gel scans shown in Fig. 5.

Characterization of the protein:arabinosyltransferase activity

Membranes showing maximum arabinosylation of endogenous protein were prepared from cells 12h after elicitation and incubated for 30min with UDP-[1-^{3}H]arabinose. Polyacrylamide-gel electrophoresis of this material gave a similar distribution of radioactivity (Fig. 3*b*) to that found for membranes from cells 48h after subculture, but with an 8–10-fold increase in incorporation. Two species were labelled again: a polydispersive high-M_r material and the 42500-M_r protein. Addition of cell-wall material containing possible endogenous acceptor to the assay mixture gave increased incorporation into the high-M_r fraction only. This material, which was soluble in 5% trichloroacetic acid but stained poorly with Coomassie Blue, shares characteristics of arabinogalactan-protein (Chrispeels, 1969; van Holst *et al.*, 1981).

Membrane was also extracted under conditions milder than those used for analysis on polyacrylamide-gel electrophoresis and subjected to

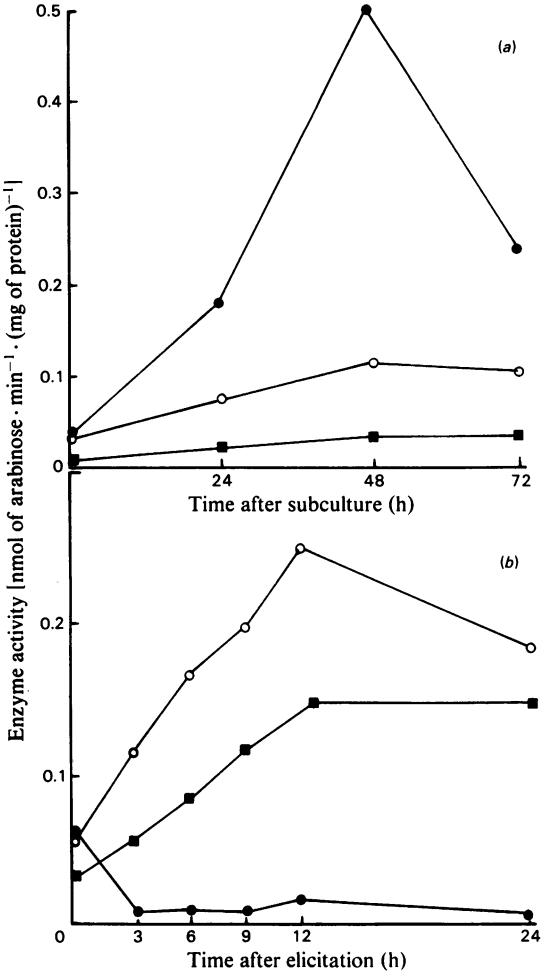

Fig. 4. *Changes in enzyme activities in cells (a) during cell division and growth after subculture into CMD medium and (b) after elicitation*

●, Arabinan synthase activity; ○, arabinose incorporated into total glycoprotein; ■, arabinose incorporation into glycoprotein of M_r 42000.

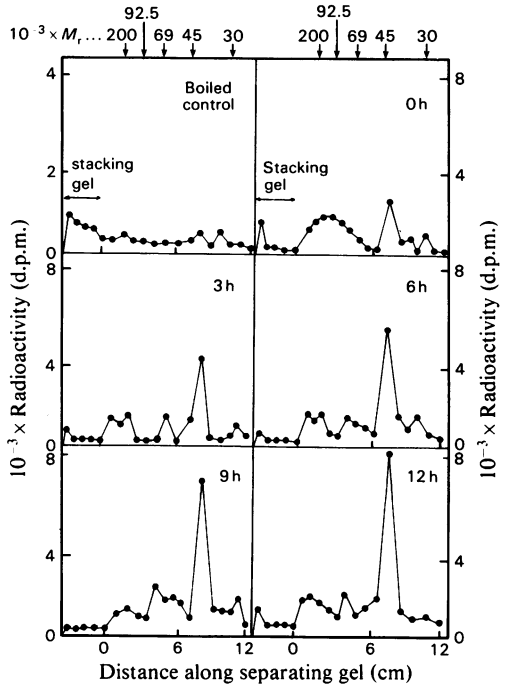

Fig. 5. *Electrophoretic profile of radioactivity incorporated by membranes in vitro after elicitation*

Membranes were prepared from cells at various times after elicitation and were incubated with UDP-β-L-[1-^{3}H]arabinose for 10min. After removal of lipid and polysaccharide, the material insoluble in 10% trichloroacetic acid was subjected to analysis on sodium dodecyl sulphate/10%-polyacrylamide-gels. The distribution of radioactivity (●) in gel slices is shown.

fractionation on Sephacyl S-300 in the presence of 0.1% sodium dodecyl sulphate. Three major peaks of radioactivity were found, at M_r 316000 and 84000, and in the low-M_r region (Fig. 6). Polyacrylamide-gel electrophoresis of the 84000-M_r protein showed that it was a dimer of the 42500-M_r protein.

These three products were analysed after acid and alkaline hydrolysis (Table 1). Hydroxyproline-containing material was eluted coincidently with the radioactivity for the 316000- and 84000-M_r proteins (Fig. 6). All the radioactivity was recovered as arabinose. Other non-radioactive sugars were detected (Table 1). Alkaline hydrolysis followed by electrophoresis or paper chromatography gave radioactive spots with R_{Hyp} characteristic of hydroxyproline (Hyp) arabinosides reported previously (Lamport, 1967; Heath & Northcote, 1971; Muray & Northcote, 1978) for the 84000-M_r material. Alkaline hydrolysis of the high-M_r material gave radioactive spots of much lower R_{Hyp}, suggesting a much longer oligosaccharide. The properties described tentatively identify the 84000-M_r protein as a hydroxyproline-rich arabinosylated glycoprotein similar to that from potato, and the 316000-M_r protein as an arabinogalactan-protein.

Consequences for the regulation of arabinose incorporation by membranes

The increase in arabinan synthase activity observed after subculture and during subsequent cell division and primary growth is probably due to synthesis of the enzyme *de novo* (Bolwell & Northcote, 1983*b*, 1984). The monoclonal antibody that inhibited the arabinan synthase (Bolwell & North-

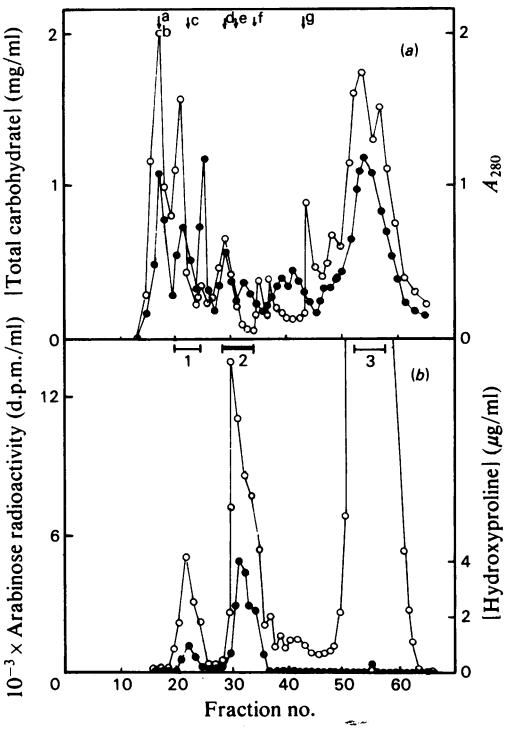

Fig. 6. *Gel filtration of radioactive membrane solubilized in sodium dodecyl sulphate on Sephacryl S-300*

Membrane prepared from cells 12h after elicitation was incubated with UDP-β-L-[1-^{3}H]arabinose for 30min and then solubilized in 50mM-Tris/HCl (pH7.2)/2% sodium dodecyl sulphate at 37°C for 1h. Samples (0.5ml) were then fractionated on a column (1.5cm × 100cm) of Sephacryl S-300 equilibrated in 50mM-Tris/HCl, pH7.2, containing 0.1% sodium dodecyl sulphate. Fractions (1.5ml) were collected and assayed for (*a*) neutral sugar (○) and A_{280} (●) and (*b*) radioactive arabinose (○) and, after acid hydrolysis, for hydroxyproline (●). The column was calibrated by using the following markers: a, Blue Dextran; b, urease (M_r 480000); c, phenylalanine ammonia-lyase (280000); d, β-galactosidase (116000); e, bovine serum albumin (68000); f, ovalbumin (44000); and g, ribonuclease (14000) in the absence of sodium dodecyl sulphate. Analysis of the numbered peaks is described in Table 1.

cote, 1984) did not inhibit the arabinosylation of protein when membranes prepared from elicited cells were treated identically. The enzyme systems catalysing arabinan synthesis and transfer of arabinose on to protein are probably distinct. Regulation of the arabinosylation of the protein is much more complex, as it is dependent on the level of endogenous acceptor and the degree of arabinosylation already present and may involve intermediates such as lipid acceptors, although these have not yet been found in this system (Bolwell & Northcote, 1983*a*).

Table 2 shows that when the level of endogenous acceptor for the 42500-M_r protein is estimated from gel scans, the molar ratio of arabinose incorporation at saturation is similar for each time point. This suggests that it is feasible to compare initial rates of incorporation as an estimate of arabinosyltransferase activity with the caution that it is not known whether the endogenous acceptor is at saturation. Owens & Northcote (1981) found that, for the enzyme system in potato, endogenous acceptor alone was at about half-saturation, which is acceptable for comparative purposes. Initial-rate analysis of incorporation (Table 2) shows about a threefold induction of arabinosyltransferase activity and, together with the measurements of the level of the acceptor, suggest that both activities are induced concomitantly after elicitation.

Discussion

Transfer of arabinose residues to polymers that are subsequently found in the primary cell wall occurs mainly within the Golgi apparatus both for arabinan synthesis in bean (Bolwell & Northcote, 1984) and for transfer on to hydroxyproline-rich glycoproteins (Karr, 1972; Gardiner & Chrispeels, 1975; Owens & Northcote, 1981). Arabinosylation of endogenous protein acceptors has now been demonstrated in bean both *in vivo* and in isolated membranes. No evidence for intermediate transfers involving lipid intermediates has yet been found for both the polysaccharide or glycoprotein, although these may be required and have been demonstrated in pea (Hayashi & MacLachlan, 1984). The arabinan synthase and the protein:arabinosyltransferase appear to be distinct, since a monoclonal antibody that inhibits the synthase (Bolwell & Northcote, 1984) does not affect transfer on to protein.

Isolated membranes do not incorporate arabinose into polymer to any great extent when prepared from suspension-cultured cells entering a stationary growth phase. After subculture the action of plant growth regulators stimulates cell division and growth. Concomitant with these processes there is an increase in arabinan synthase activity, which is probably due to synthesis of the enzyme *de novo* (Bolwell & Northcote, 1983*b*, 1984). Qualitative control of the type of polysaccharide synthesized is dependent on the peaks of enzyme activity appearing at different times during subsequent development (Bolwell & Northcote, 1981, 1983*b*; Dalessandro & Northcote, 1981; Bolwell *et al.*, 1984).

Table 1. *Analysis of radioactive fractions prepared by gel filtration of solubilized membranes*
Pooled fractions were prepared by gel filtration as described in Fig. 6. The apparent M_r of fractions was determined by comparison with the elution of standards of known M_r. Hydroxyproline and sugar content was investigated after acid hydrolysis. Sugars were detected by t.l.c.; those indicated in parentheses were in low abundance. The R_{Hyp} of radioactive spots was determined after alkaline hydrolysis.

Fraction ...	1	2	3
M_r (av.)	316000	84000	1000
Total carbohydrate (μg/ml)	394.5	77.1	439.9
Arabinose (d.p.m./ml)	2820	9785	135128
Total protein (μg/ml)	16.0	26.8	0
Hydroxyproline (μg/ml)	0.4	2.4	trace
Non-radioactive sugars	Gal (Xyl, Glc)	Gal	Gal, Glc, Xyl, Rha
R_{Hyp} (electrophoresis)	0.26, 0.35, 0.54	0.43, 0.54, 0.66, 0.73	0.21
R_{Hyp} (chromatography)	0.13, 0.25, 0.48	0.17, 0.80, 0.96	0.51

Table 2. *Relative levels of the protein:arabinosylation components after elicitation*
Membrane preparations were incubated with UDP-[1-^{3}H]arabinose for either 5min, to give initial rates, or for 30min, to give total incorporations, and analysed.

	Incorporation				
		42kDa protein			
Time after elicitation (h)	Total glycoprotein (initial) [nmol·min^{-1}·(mg of membrane protein)$^{-1}$]	Initial [nmol·min^{-1}·(mg of membrane protein)$^{-1}$]	Total [nmol·(mg of membrane protein)$^{-1}$]	Protein* [μg·(mg of membrane protein)$^{-1}$]	Incorporation/protein [nmol·(mg of 42kDa protein)$^{-1}$]
0	0.25	0.12	0.65	29	22.4
3	0.46	0.23	1.23	47	26.1
6	0.56	0.28	1.24	50	24.8
9	0.44	0.24	1.36	69	19.7
12	0.67	0.43	2.42	86	28.1
24	0.47	0.29	1.42	61	23.2

* The level of the 42kDa protein was estimated from gel scans by comparison with the scans given by known standards of bovine serum albumin and run on the same gel.

Arabinogalactan protein and the hydroxyproline-rich glycoprotein are the major arabinose-containing structural proteins of the wall (Lamport, 1970). After subculture, isolated membranes have been found to be capable of incorporating arabinose into both a polydisperse high-M_r material and a distinct protein of M_r 42500 in addition to, but to a much lower extent than, the incorporation into arabinan. In contrast, if cells at an identical stage before subculture are treated with a fungal elicitor from *Colletotrichum lindemuthianum* (Dixon & Lamb, 1979), there is a totally different response in which no induction of arabinan synthase occurs, although there is a specific rapid increase in the arabinosylation of protein by membranes. In this response both the 42500-M_r core protein and the level of arabinosyltransferase activity appear to increase concomitantly. During this peroid after elicitation, which resembles the hypersensitive response, other enzymes, particularly those involved in phytoalexin production, also increase (Lawton *et al.*, 1983*a*,*b*).

A preliminary characterization of these glycoproteins has been carried out. For both glycoproteins there was an exact coincidence of hydroxyproline with radioactive arabinose in the fractions obtained by gel filtration. The high-M_r material is tentatively identified as an arabinogalactan protein on the basis that its M_r (av.) of about 316000, solubility in 5% trichloroacetic acid, staining properties, heterogeneity and the presence of hydroxyproline, arabinose and galactose is similar to the arabinogalactan-protein already described in bean (van Holst *et al.*, 1981). Alkaline hydrolysis also gave radioactive material, rich in carbohydrate, consistent with the structure of the hydroxyproline-linked galactan. Comparison of the lower-M_r material with hydroxyproline-rich glycoproteins, particularly potato lectin, showed similarities. The M_r of 84000 under non-reducing conditions was similar, and alkaline hydrolysis gave mobile arabinosides of mobility identical with that of hydroxyproline arabinosides. It is tentatively identified as a hydroxyproline-rich glycoprotein. The

hydroxyproline content, and probably therefore the level of the glycoprotein, increases in the cell walls of *Phaseolus vulgaris* infected by *Colletotrichum lindemuthianium* (Esquerré-Tugayé *et al.*, 1980). The 42500-M_r glycoprotein may represent the precursor of this material, which may prove to have agglutination properties and be important in disease resistance, as found for potato (Leach *et al.*, 1982*a*,*b*). Alternatively, this glycoprotein could represent a membrane-bound peroxidase that has a similar M_r and contains arabinose. However, no changes in peroxidase activity have been observed after elicitation (M. P. Robbins, personal communication), which circumstantially suggests that the 42500-M_r glycoprotein is not a form of this enzyme. A similar distribution of M_r has recently been found in pea membranes labelled with radioactive arabinose or xylose (Hayashi & MacLachlan, 1984).

During growth and differentiation the level of the enzymes involved in the interconversion of nucleotide-sugars do not change extensively, and the capacity for rapid changes in the direction of glycosylation is maintained by the availability of all nucleotide-sugars (Dalessandro & Northcote, 1977*a*,*b*). The present study shows that, in response to two distinct types of exogenous stimulation, the pattern of arabinosylation that is induced within the endomembrane system is very different. Treatment with plant growth regulators induces synthesis *de novo* of arabinan synthase involved in the production of polysaccharide required for cell-plate and primary-cell-wall production, whereas elicitation gives rise to an increase in activity of a distinct arabinosyltransferase involved in the glycosylation of a glycoprotein that may be of importance in disease resistance.

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