The Amounts and Rates of Export of Polysaccharides Found Within the Membrane System of Maize Root Cells

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1. Maize seedling roots were incubated *in vivo* with D-[U-¹⁴C]glucose for 2, 5, 10, 15, 30 and 45 min. The total incorporation of radioactivity into polysaccharide components in isolated fractions was investigated, and the pattern of incorporation into different polysaccharide components within the rough endoplasmic reticulum, Golgi apparatus and exported material was analysed. 2. The membrane compartments reached a saturation value of radioactivity in polysaccharide components by 30 min incubation. Radioactivity in exported polysaccharide continued to increase after that time. The latter was formed and maintained by a steady-state turnover of polysaccharide synthesis and transport from the membrane system. 3. If the only access of the slime polysaccharide to the cell surface is via dictyosome-derived vesicles, the amount of slime components in the Golgi apparatus would have to be displaced every 0.3 min in order to maintain the observed rates of increase in slime. This is in contrast with a displacement time of about 2.5 min that is necessary for polysaccharide components in the Golgi apparatus to produce the observed increase in cell-wall material. The activity of the membrane system in the production of maize root slime is 8 times as great as that of the membrane system involved in cell-wall synthesis. 4. If the amount of polysaccharide material in the Golgi apparatus is maintained only by inflow of polymeric material from the rough endoplasmic reticulum the total amount of slime components in the rough endoplasmic reticulum would have to be displaced every 7 min to maintain a constant amount in the Golgi apparatus. If the endoplasmic reticulum contributed directly to the cell surface in the synthesis of cell-wall material, displacement times necessary to maintain the observed rate of polymer production would be very slow.

The intracellular membrane of plant cells is composed of nuclear membrane, endoplasmic reticulum, cisternae and vesicles of the Golgi apparatus and plasmalemma (Northcote, 1971). The involvement of this system in polysaccharide synthesis has been investigated by using a large number of experimental techniques and types of plant tissue (O'Brien, 1972). Previous evidence has suggested that the endoplasmic reticulum, Golgi apparatus and plasmalemma may be involved in the synthesis and transport of particular polysaccharides of plant cells. The synthesis of β -(1 $\rightarrow$ 4)-glucan of cellulose microfibrils in higher-plant cell-wall synthesis could take place at the plasmalemma (Preston & Goodman, 1967; Northcote & Lewis, 1968; Roberts & Northcote, 1970; Bowles & Northcote, 1972; Kiermayer & Dabberstein, 1973). However, the cellulosic scales of *Pleurochrysis* are synthesized within the secretory vesicles of the Golgi apparatus, before the incorporation of the dictyosome-derived membrane with the cell membrane (Herth *et al.*,

1972). The Golgi-apparatus cisternae and vesicles are most likely involved in the synthesis and transport of highly hydrated polysaccharides in specialized slime-producing areas of roots and other tissues (Northcote & Pickett-Heaps, 1966; Schnepf, 1966; Schwab *et al.*, 1969; Fraser & Gunning, 1973). The possible function of the endoplasmic reticulum in cell-wall polysaccharide synthesis and transport, in comparison with that of the Golgi apparatus, is uncertain. Electron-microscope observations coupled with radioautographic investigations (Pickett-Heaps, 1966, 1967*a,b*) into the comparative involvement of the two membrane compartments are inconclusive when there are no parallel chase experiments or analyses of cell-wall incorporation. Fractionation studies after incubation *in vivo* with radioactive metabolites are also difficult to interpret, since in general the rough endoplasmic reticulum is not isolated (Harris & Northcote, 1971; Mertz & Nordin, 1971; Jilka *et al.*, 1972).

In a previous investigation (Bowles & Northcote, 1972), results indicated that the total incorporation of radioactivity from D-[U-¹⁴C]glucose into polysaccharide components was much higher in an

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endoplasmic-reticulum fraction than in a dictyosome fraction of maize roots, but the specific radioactivity of the Golgi apparatus was 100% greater than that of the rough endoplasmic reticulum. In the present study we investigate the entry of radioactivity into polysaccharide components of the separate membrane fractions and exported material, during a time-course of incorporation of radioactive precursor by maize roots *in vivo*. The results indicate that radioactive incorporation in the membrane fractions reaches a saturation value, whereas there is a simultaneous continued incorporation of radioactivity into exportable polysaccharide throughout the time-course. This allows the calculation of the amounts of the polysaccharide components in the rough endoplasmic reticulum and Golgi apparatus, and of rates of turnover of the polysaccharide components within the compartments in order to produce the observed rates of increase of material into exported polysaccharide.

Methods

Growth of tissue and general method of incubation

Seedlings of the *Zea Mays* var. Caldera were grown under sterile conditions on filter paper (Harris & Northcote, 1970). For each experiment, seedlings were selected 48 h after sowing, and the seedling roots were incubated *in vivo* with D-[U-¹⁴C]glucose (specific radioactivity 320 mCi/mmol) from The Radiochemical Centre, Amersham, Bucks., U.K., diluted with sterile water (10 µl, 1 µCi per root). Seedling roots (20) were harvested after 2, 5, 10, 15, 30 and 45 min incubation.

Subfractionation procedures and analysis

Subcellular fractions were prepared, hydrolysed, and analysed as described by Bowles & Northcote (1972, Scheme 1). The soluble polymers analysed were of two types, that present in the supernatant

of the homogenization medium (S₂, Bowles & Northcote, 1972) and that present in the incubation medium of the roots (this consisted mainly of root slime). The individual sugar components of the smooth-membrane fraction (pellet 3 of Scheme 1; Bowles & Northcote, 1972) were not analysed. The pattern of radioactivity in the various monomers of the soluble polymers (S₂) was determined only after 30 and 45 min incubation. Neutralized hydrolysates of the subcellular fractions were run on paper electrophoresis at pH 2.0 (Bowles & Northcote, 1972). Total neutral sugar and acid compounds remained at the origin. The radioactivity of different samples was measured on strips (4 cm × 1 cm) cut from chromatograms and electrophoretograms (Bowles & Northcote, 1972; Harris & Northcote, 1970) on a Nuclear-Chicago Unilux I Scintillation spectrophotometer (Bowles & Northcote, 1972). The efficiency of counting was 50%.

Results

Total incorporation of radioactivity into the fractions

The total incorporation of radioactivity into the different isolated fractions is shown in Table 1. Incubation for 2 min in D-[U-¹⁴C]glucose was sufficient to result in the recovery of radioactivity in the rough endoplasmic reticulum, cell wall and soluble polymer fractions (S₂), and this increased as the time of incubation increased. However, by 30 min the radioactive incorporation into the three membrane fractions (i.e. the fractions enriched in dictyosomes, smooth membranes and rough endoplasmic reticulum) had reached a similar value to that found after 45 min and subsequently after 2 h (Bowles & Northcote, 1972). Incorporation of radioactivity into the exportable polysaccharides, those of the wall, slime and soluble material (Bowles & Northcote, 1972), did not reach a saturation value, and incorporation of radioactivity into these fractions continued to increase substantially after 30 min.

Table 1. Total amount of radioactivity incorporated from D-[U-¹⁴C]glucose into polysaccharide components of different subcellular fractions of maize roots, during a time-course of incubations *in vivo*

Roots were incubated with radioactive glucose for a number of time-points, and then homogenized. Subcellular fractions were obtained by differential centrifugation and discontinuous sucrose-density-gradient centrifugation.

Fraction	Time of incubation in D-[U- ¹⁴ C]glucose (min)	Radioactivity (c.p.m.)						
		...	2	5	10	15	30	45
Rough endoplasmic reticulum		300		1120	12280	30600	88300	94700
Smooth membrane		0		80	300	1060	1908	2222
Golgi apparatus		0		250	1220	2800	5200	4500
Cell wall		375		4270	13150	31140	59410	124400
Soluble materials (S ₂)		60		200	1370	4800	26200	34490

Table 2. *Relative amounts of radioactivity incorporated from D-[U-¹⁴C]glucose into the polysaccharide components of the rough endoplasmic reticulum fraction from maize roots, during a time-course of incubations in vivo*

Roots were incubated with radioactive glucose for a number of time-points, and then homogenized. The fractions were obtained by centrifugation for 2 h at 4°C and 10⁵g (r_{av} , 83 mm) or a supernatant (S₁) free from heavier membrane components.

Sugar	Time of incubation in D-[U- ¹⁴ C]glucose (min)	...	Radioactivity (%)					
			2	5	10	15	30	45
Uronic acids			15.3	4.4	3.13	7.4	6.9	7.7
Galactose			2.6	12.2	13.2	16.0	16.4	18.0
Glucose			55.0	30.1	13.8	13.7	10.1	9.6
Mannose			3.4	0	0.8	1.3	1.4	2.0
Arabinose			8.8	16.2	21.0	19.6	21.4	20.8
Xylose			11.1	27.0	45.3	35.5	39.7	37.4
Fucose			3.4	9.6	2.1	5.5	3.3	3.5
Total radioactivity (c.p.m.)			300	1120	12280	30600	88300	94700

Table 3. *Relative amounts of radioactivity incorporated from D-[U-¹⁴C]glucose into the polysaccharide components of the dictyosome fraction from maize roots, during a time-course of incubations in vivo*

Roots were incubated with radioactive glucose for a number of time-points, and then homogenized. The fractions were obtained by a discontinuous sugar-density-gradient centrifugation. The particulate band present at the 0.5 M-/1.25 M-sucrose interface was centrifuged to give a pellet.

Sugar	Time of incubation in D-[U- ¹⁴ C]glucose (min)	Radioactivity (%)						
		...	2	5	10	15	30	45
Uronic acids			0	7.1	8.0	9.4	8.6	8.6
Galactose			0	13.4	11.4	15.9	14.7	16.5
Glucose			0	11.7	11.8	9.5	6.7	6.7
Mannose			0	2.9	1.5	0.9	1.0	0.8
Arabinose			0	16.8	17.5	18.5	20.3	20.5
Xylose			0	40.3	45.8	39.3	42.3	42.1
Fucose			0	7.5	2.6	4.7	4.7	2.7
Total radioactivity (c.p.m.)			0	250	1220	2800	5200	4500

Table 4. *Relative amounts of radioactivity incorporated from D-[U-¹⁴C]glucose into the polysaccharide components of the cell-wall fraction from maize roots, during a time-course of incubations in vivo*

Roots were incubated with radioactive glucose for a number of time-points and then homogenized. Cell-wall fractions were obtained by centrifugation of an initial homogenate for 30 min at 4°C and 4000g (r_{av} , 83 mm).

Sugar	Time of incubation in D-[U- ¹⁴ C]glucose (min)	Radioactivity (%)						
		...	2	5	10	15	30	45
Uronic acids			0	6.2	7.7	9.0	8.9	8.0
Galactose			30.9	12.5	17.5	15.5	15.9	15.8
Glucose			32.0	41.3	19.9	39.6	37.5	40.6
Mannose			0	0	1.2	1.5	1.7	1.8
Arabinose			0	8.5	13.2	9.1	9.8	10.0
Xylose			20.3	26.5	33.6	16.0	16.8	14.8
Fucose			16.5	4.8	6.5	8.1	8.2	8.3
Total radioactivity (c.p.m.)			375	4270	13150	31140	59410	124400

Distribution patterns of radioactive incorporation

The distribution pattern of radioactivity in polysaccharide components in the rough-endoplasmic-reticulum fraction (pellet 4 of Scheme 1, Bowles &

Northcote, 1972) during the time-course of incubation in radioactive precursor is shown in Table 2. The percentage pattern of incorporation changed during the time of incubation. By 15 min the pattern exhibited was the same as that found at saturation.

Table 5. *Relative amounts of radioactivity incorporated from D-[U-¹⁴C]glucose into the polysaccharide components of the total exportable polysaccharide (cell wall, root slime, soluble material) from maize roots after 30 and 45 min incubation*

Roots were incubated with radioactive glucose for 30 and 45 min and then homogenized. Exportable polysaccharide, composed of cell wall, root slime and soluble material was obtained by fractionation procedures.

Sugar	Time of incubation ¹ in D-[U- ¹⁴ C]- glucose (min)	Radioactivity (%)		Rate of increase in radioactivity (c.p.m./min)
		30	45	
Uronic acids		12.1	14.0	861
Galactose		15.6	16.2	723
Glucose		18.7	18.1	618
Mannose		1.0	1.2	91
Arabinose		18.5	17.1	439
Xylose		28.9	26.7	692
Fucose		5.3	6.6	477
Total radioactivity (c.p.m.)		239645	298195	3901

Table 3 shows the distribution pattern of radioactivity in the dictyosome fraction (pellet 2 of Scheme 1, Bowles & Northcote, 1972). The percentage pattern of incorporation of label was very similar throughout the time-course of incubation in the radioactive precursor; 40% of the label was present in xylose, both at 5 min and 45 min incubation time. Table 4 shows the distribution pattern of radioactivity in the cell-wall fraction (pellet 1 of Scheme 1; Bowles & Northcote, 1972) over the time-course. Radioactivity was found in galactose, glucose, xylose and fucose after 2 min incubation with labelled glucose. The relatively high proportion of label in fucose may indicate that slime polysaccharide was the main constituent of the fraction, and therefore it is possible that radioactivity in the wall fraction corresponded to that incorporated into the surface root-cap cell walls of maize roots. By 10 min the incorporation pattern more closely resembled the typical composition pattern of maize cell walls. From 15 to 45 min the percentage pattern of incorporation of label remained the same, although total incorporation into the fraction continued to increase.

Table 5 shows the distribution pattern of radioactivity in the exportable polysaccharides at 30 min and 45 min, i.e. at incubation times when the radioactivity in polysaccharide in the membrane fractions had reached saturation. The increase in radioactivity in each sugar in the exportable polysaccharides is shown in Table 5.

Discussion

In a previous investigation (Bowles & Northcote, 1972), maize seedling roots were incubated *in vivo*

with radioactive glucose for 2 h. Subcellular fractionation of the roots resulted in the isolation of fractions enriched in dictyosomes, smooth membranes and rough endoplasmic reticulum, and also exportable polysaccharides composed of cell walls, root slime and soluble polymers. The incorporation of radioactivity into the polysaccharide components of the rough microsomal fraction was shown to be high in comparison with that of the dictyosome fraction. However, calculations of relative specific radioactivities of each membrane fraction indicated that the Golgi apparatus showed a 100% increase in specific radioactivity compared with the rough endoplasmic reticulum, since maize root cells were shown to contain 40 times more rough endoplasmic reticulum than did the dictyosome membrane.

In the present investigation we have shown that after 30 min incubation of the maize seedling roots in radioactive glucose, the various membrane fractions prepared from the tissue became saturated with radioactivity. No further increase in total radioactivity of each of the sugars making up the polymeric material within the membranes was detected, even after a further period of 90 min. The amount of radioactive materials was calculated by assuming that the saturation value obtained for the radioactive incorporation indicated that the specific radioactivities of the radioactive sugars has become equivalent to that of the radioactive glucose supplied to the roots. It has also been assumed that the relative yields of the different fractions which were isolated were the same.

The wall material and the total soluble polysaccharides represent polysaccharides exported from the cell by the membrane system (Bowles & Northcote, 1972). The incorporation of radioactivity into these substances increased throughout the period of incubation from 0 to 45 min. It is known that the radioactivity of the membrane compartments was saturated after 30 min, so that the rate of incorporation of radioactivity of the materials into exportable polysaccharide between 30 and 45 min of the incubation represents the rate of incorporation from the polymeric material saturated with radioactivity within the membrane system into the polysaccharide exported from the cell. Calculation of the increases in radioactivity into the different monomers per min can therefore be used to give a measure of rate of export of sugars across the plasma-membrane into the exportable polysaccharide. The values of the amounts of polysaccharide components in the membrane fractions, and the rates of increase in the number of pmol of each sugar observed in the exportable polysaccharide, are shown in Table 6. The results have been obtained from a mixed cell population of whole root sections, including root-cap tissue. At the root cap, specific polysaccharides of known composition are secreted (Harris & Northcote,

Table 6. *Comparison of the amounts of polysaccharide components in the dictyosome and rough-endoplasmic-reticulum fractions, and the rate of increase (pmol/min) observed in the total exportable polysaccharide of maize roots*

An average amount of polysaccharide components was calculated from the radioactivity recorded for each sugar in a hydrolysate of the respective membrane fractions at saturation value of radioactivity, i.e. after incubation for 30 and 45 min. The rate of increase (pmol/min) of sugars into the total exportable polysaccharide was calculated from the entry of radioactivity into the polysaccharides when the radioactivity of the membrane compartments had reached saturation value.

Sugar	Amount of material (pmol)		Rate of increase (pmol/min) into exportable polysaccharide
	Rough endoplasmic reticulum	Golgi apparatus	
Galactose	44.4	2.1	2.0
Glucose	25.2	0.9	1.7
Mannose	4.4	0.1	0.2
Arabinose	62.9	3.2	1.4
Xylose	118.3	6.8	2.3
Fucose	8.7	0.5	1.3
Total	263.9	16.7	8.9

Table 7. *Comparison of the amounts of root-slime polysaccharide components in the dictyosome and rough-endoplasmic-reticulum fractions, and the rate of increase (pmol/min) observed in root slime of maize roots*

The amount of root-slime polysaccharide components in the membrane fractions, and the rate of increase (pmol/min) of exported polysaccharide into root slime were calculated from a knowledge of the known composition of maize root slime (galactose 21%; glucose 22%; mannose 6%; arabinose 15%; xylose 4%; fucose 32%) and the values shown in Table 6.

Sugar	Amount of material (pmol)		Rate of increase (pmol/min) into slime polysaccharide
	Rough endoplasmic reticulum	Golgi apparatus	
Galactose	5.7	0.3	0.87
Glucose	6.0	0.3	0.91
Mannose	1.6	0.09	0.25
Arabinose	4.0	0.2	0.62
Xylose	1.1	0.06	0.17
Fucose	8.7	0.5	1.33
Total	27.1	1.5	4.15

1970; Bowles & Northcote, 1972; Wright & Northcote, 1974). These polysaccharides make up the root slime, which is characterized by the presence of fucose. It is possible, since fucose is not formed in appreciable amounts by any other cells of the root, to calculate the amounts of the slime polysaccharide

Table 8. *Comparison of the amounts of cell-wall polysaccharide components in the dictyosome and rough-endoplasmic-reticulum fractions, and the rate of increase (pmol/min) observed in cell-wall polysaccharides of maize roots*

The amount of cell-wall polysaccharide components in the membrane fractions, and the rate of increase (pmol/min) of exported polysaccharide into cell-wall material, were calculated by using the values shown in Table 6, corrected for root slime.

Sugar	Amount of material (pmol)		Rate of increase (pmol/min) into cell-wall polysaccharide
	Rough endoplasmic reticulum	Golgi apparatus	
Galactose	38.7	1.8	1.1
Glucose	19.2	0.56	0.8
Mannose	2.8	0.01	0.0
Arabinose	58.9	3.0	0.8
Xylose	117.2	6.7	2.1
Fucose	0	0	0
Total	236.8	12.1	4.8

within the root cap, and thus the amounts of polysaccharides other than the slime, i.e. those of the cell-wall material, can be obtained. Table 7 shows the amounts of slime components in the membrane fractions, and the rate of increase (pmol/min) of different sugars into exported slime. The amounts of non-slime components in the membrane fractions, and the rate of increase (pmol/min) of different sugars into cell-wall material are shown in Table 8.

If the only access of the slime polysaccharide to the cell surface is via dictyosome-derived vesicles, the amount of slime components in the Golgi apparatus would have to be displaced every 20s in order to maintain the observed rate of increase of approx. 4.1 pmol of the component sugars/min into exported slime. A turnover rate of one cisternal stack every 20s is very fast when compared with values given for turnover in animal systems: 20min/stack, and individual cisternae released every 1–4min (Morré *et al.*, 1971). However, recent work (Brown *et al.*, 1973) indicates that the highly complex surface scale of *Pleurochrysis* can be fully synthesized and discharged from the Golgi apparatus in under 2min. The observed rate of displacement of the slime does not necessarily indicate an equivalent rate of displacement of the whole membrane constituting the Golgi apparatus. Slime production and transport may occur in part of the cisternal stack, and only certain vesicles may be used for the export of slime polymers to the cell surface. There is some evidence to suggest that in certain instances different vesicles of one cisternal stack may be carrying different secretory products (Manton & Ettl, 1965; Schnepf, 1966; Schwab *et al.*, 1969; Fraser & Gunning, 1973;

Dauwalder & Whaley, 1973), or that different dictyosomes in the same cell may be carrying out different functions. If the amount of material in the Golgi apparatus is only maintained by inflow of polymeric material from the rough endoplasmic reticulum, then the total amount of polysaccharide within the rough endoplasmic reticulum would have to be displaced about every 7 min to maintain a constant pool size in the Golgi apparatus. No evidence was found for an involvement of endoplasmic reticulum in slime synthesis in wheat roots by using radioautographic techniques (Northcote & Pickett-Heaps, 1966). If the root tissue of wheat is comparable with that of maize, the result suggests that slime components in the endoplasmic reticulum are not in a form that would allow their detection by radioautography. A possible explanation could be the molecular-weight characteristics of the slime components in the endoplasmic reticulum. Low-molecular-weight polymers would be lost during the washing and fixation procedures for radioautography. Such an explanation is in accordance with evidence from periodic acid-silver hexamine staining of plant tissue (Pickett-Heaps, 1967c; Van der Woude *et al.*, 1971) which indicates that polymerization of low-molecular-weight polysaccharide precursors occurs within the cisternae and secretory vesicles of the Golgi apparatus.

The amounts of polymeric material in the Golgi apparatus and rough endoplasmic reticulum in non-slime-producing regions are greater than in the slime-synthesizing regions. This is to be expected, since the fractions are representative of membrane from the vast bulk of root tissue. However, the rate of increase of approx. 4.8 pmol of component sugars/min into cell-wall polysaccharide is not correspondingly greater. Therefore the displacement times necessary to maintain cell-wall formation alone are relatively slower, with a turnover rate of the amounts of polysaccharide material within the Golgi apparatus of approx. 2.5 min.

The amounts of cell-wall components in the rough endoplasmic reticulum are much larger than those in the Golgi apparatus. If the endoplasmic reticulum did contribute directly to the cell surface, the displacement times necessary to maintain the observed rate of polymer production would be very slow (approx. 55 min for xylose).

The rate of turnover of the intracellular membranes for the production of the root slime is eight times as great as for the production of wall material. It has previously been shown that the response of the membrane system to differentiation of maize root cells would be detected in a changed pattern of polysaccharides synthesized (Bowles & Northcote, 1972). The present results indicate that

another response to differentiation of root-cap and non-cap cells is a changed rate of synthesis and transport of the exportable polysaccharide and the turnover of membrane components.

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