The Japanese Society of Plant Physiologists

Plant Cell Physiol. 35(5): 719–727 (1994)
JSPP © 1994

Synthesis of Cell Wall Galactans from Flax (*Linum usitatissimum* L.) Suspension-Cultured Cells

Florence Goubet and Claudine Morvan

Equipe "Paroi et Polymères Pariétaux", SCUEOR URA 203 CNRS, Faculté des Sciences, Université de Rouen, 76821 Mont Saint Aignan Cedex, France

Cell walls of flax suspension-cultured cells contain both β 1 → 4 galactan (most of which can be extracted with boiling water) and β 1 → 3, β 1 → 6 galactan (most of which can be solubilized with alkali). The relative level of β 1 → 4 galactan in the cell walls is maximal during the lag phase, accounting for an average of 8% of the dry cell-wall mass, whereas the relative level of β 1 → 3, β 1 → 6 galactan increases throughout growth, reaching a maximum (up to 20%) during the exponential phase.

Two peaks of galactan synthase activity can be detected that catalyze the synthesis of β 1 → 4 galactan at pH 8 during the lag phase and the synthesis of β 1 → 3, β 1 → 6 galactan at pH 5 during the growth phase. At the end of the growth phase both activities are negligible.

Key words: Galactan synthase — Polysaccharides.

The genus *Linum* is one of the oldest sources of cellulosic fibers. In order to improve the quality of fibers, the composition of flax cell walls has to be characterized and the key enzymes involved in the synthesis of cell walls have to be defined. We showed previously that the main neutral sugar in polysaccharides linked to the cellulose in flax is galactose (Morvan et al. 1989, Goubet et al. 1993). Moreover, the pectic fraction is particularly rich in β 1 → 4 galactan (Davis et al. 1990). We have also identified β 1 → 3, β 1 → 6 galactans in the secondary wall of flax fibers (unpublished data).

The importance of galactan cell walls of many plant cells has emphasized. There are many reports of the presence of different galactans, for example, β 1 → 4 galactan (Aspinall 1980, Selvendran 1985, Lau et al. 1987, Hervé du Penhoat et al. 1987, Davis et al. 1990) and β 1 → 3, β 1 → 6 galactan (O'Neill et al. 1990, Carpita and Gibeaut 1993). However, little information about plant galactan synthases is available with the exception of the reports of McNab et al. (1968) and Panayotatos and Villemez (1973) for *Phaseolus aureus*. Recently, two galactan synthases were recognized in suspension-cultured cells of flax: the first one catalyzed the formation of β 1 → 4 galactan and the second one catalyzed the synthesis of β 1 → 3, β 1 → 6 galactan (Goubet and Morvan 1993). These two activities differed in terms of their optimal pH and appeared to differ also in their localization, being found either in Golgi bodies or in low-density vesicles in cells. These galactan synthase activities were inhibited by treatment with monensin (Goubet et al. 1994) but this treatment did not modify the composition of cell walls.

The present study deals with the relationship between galactan synthase activities and the variations in galactan content of the cell walls of flax suspension-cultured cells during the culture. Suspension-cultured cells were used because this system has been well defined (Schaumann et al. 1993) and galactan synthases in this system have already been investigated (Goubet and Morvan 1993, Goubet et al. 1994).

Abbreviations: BSA, bovine serum albumin; DMSO, dimethylsulfoxide; DTE, dithioerythritol; EDTA, ethylenediaminetetraacetic acid-(disodium salt); EGTA, [ethylenebis(oxyethylenenitrilo)]-tetraacetic acid; MES, 4-morpholine ethane sulfonic acid; PVP, polyvinylpyrrolidone; Tris, 2-amino-2(hydroxymethyl)-1;3-propanediol.

Material and Methods

Flax suspension-cultured cells—Flax cells (*Linum usitatissimum* L.) were cultivated in Murashige and Skoog's (1962) medium that contained 0.75 mg liter^{-1} kinetin and

NII-Electronic Library Service

The Japanese Society of Plant Physiologists

0.25 mg liter^{-1} 2,4-D on a gyratory shaker at 25°C, as described previously (Schaumann et al. 1993).

Preparation of microsomes—Flax cells were filtered through 30-μm nylon mesh. Cells were plasmolysed for 15 min and ground in a Tenbroeck glass Potter-Elvehjem homogenizer in 50 mM Tris-HCl, pH 7.5, 12% (w/w) sucrose, 1 mM EGTA, 1 mM DTE, 0.1 mM $MgCl_2$, 0.5% (w/v) BSA and 1.5% (w/v) PVP. The homogenate was filtered through 30-μm nylon mesh. The retentate contained cell walls. The filtrate was centrifuged at 10,000 × *g* for 15 min and the resultant supernatant was centrifuged at 180,000 × *g* for 45 min. The final pellet was suspended in 4 ml of 50 mM Tris-HCl, pH 7.5, 12% sucrose, 1 mM DTE, 0.1 mM $MgCl_2$ and was used as the crude microsomal fraction (Baydoun and Northcote 1980). All procedures were performed at 4°C in a minimum of 1 h.

Preparation of cell walls—The wall-containing retentate was heated for 10 min in 90% ethanol at 100°C to inactivate enzymes and then it was washed successively with ethanol, a mixture of chloroform and methanol (1 : 1, v/v), acetone and DMSO (Goubet et al. 1993). The cell walls were then dried overnight at 45°C and for 1 h at 80°C, and then they were weighed. The yield of cell walls was defined as the weight (g) of dry cell walls per 100 g of fresh cells.

Extraction of polymers—Polymers were successively solubilized from cell walls by treatments with (i) water (1 h at 4°C; to give the fraction designated P), (ii) boiling water (2 h at 100°C; to give Pb), (iii) 0.5% EDTA (100°C, 1 h; to give Pe) and (iv) NaOH (60 g liter^{-1}, 100°C, under nitrogen, 4 times for 1 h each; to give HC). Pe and HC were freed of salts by ultrafiltration through a Pellicon membrane (10-kDa cut-off; Millipore, France).

Sugar composition—Polysaccharides were digested with methanol-HCl (80°C, 24 h) and derivatized with 1% trimethyl chlorosilane in *N,O* bis(trimethyl silyl fluoroacetamide) (Quemener and Thibault 1990). The trimethyl silylated derivatives were dried on a Ross injector and fractionated on a DB225 capillary column (J.W. Instruments, France). The temperature was held for 1 minute at 90°C, it was raised to 180°C at 2° min^{-1}, it was held at 180°C for 5 min, then it was raised from 180°C to 230°C at 20° min^{-1} and finally it was held at 230°C for 10 min.

Methylation analysis—Samples (0.3–1 mg) were solubilized in 1 ml of DMSO, frozen and methylated by incubation with 200 mg of NaOH and 1 ml of methyl iodide for 45 min at 4°C. The reaction was terminated by addition of 2 ml of $Na_2S_2O_3$ (Harris et al. 1984). Methylated polymers were extracted four times with chloroform (0.5 ml). The organic phase was washed ten times with water, dried and then solubilized in methanol and chloroform (1 : 1, v/v). Contaminating ions (Na^+, $S_2O_3^{2-}$) were eliminated by size-exclusion chromatography (LH-20 Sephadex; Pharmacia, France). Methylated polymers were hydrolysed at 100°C in 2 M trifluoroacetic acid for 2 h and acetylated by the method of Albersheim et al. (1967). The partially methylated alditol acetates were solubilized in dichloromethane and placed on a Ross injector. The material was fractionated on a DB225 capillary column. The temperature was raised from 120°C to 230°C at 2° min^{-1} and then it was held at 230°C for 15 min. Methylation was performed with duplicate samples when possible, and the derivatives were identified by reference to the work of Jansson et al. (1976).

Quantitation of protein—Protein was quantified by Bradford's microassay (Bradford 1976) with a kit from Bio-Rad (France) and BSA as the standard.

Assay of galactan synthase—Standard assay conditions were similar to those defined previously (Goubet and Morvan 1993). Reaction mixtures contained 620 nM UDP-D-galactose, 60 nM UDP-D-[U-^{14}C]galactose, 105 mM Tris-HCl, pH 8, or Tris-MES, pH 5, 5 mM $MgCl_2$, 0.5 mM DTE, 6% sucrose and 0.1–0.7 mg of microsomal protein. Incubation for 1 h was started by mixing an aliquot of the microsomal fraction with the substrate buffer at 25°C. The reaction was stopped by heating in a boiling water bath for 5 min. Radioactive glycans were incubated with 3 ml of 70% ethanol, 10 mg of cellulose powder and a crude mitochondrial fraction (4°C overnight). The precipitated polymers (which had been adsorbed by cellulose and mitochondrial proteins) were collected on glass-fiber filters (GF/C or GF/F; Whatman, France). Filters were washed three times with 70% ethanol to remove the remaining UDP-D-[U-^{14}C]galactose, dried and immersed in scintillation fluid (Lipoluma; Lumac The Netherlands).

Analysis of products generated in vitro—The preparative assay was performed as follows: 12 μM UDP-D-galactose, 60 nM UDP-D-[U-^{14}C]galactose, 105 mM Tris-HCl, pH 8, or Tris-MES, pH 5, 5 mM $MgCl_2$, 0.5 mM DTE, 6% sucrose and microsomal proteins (2–4 mg) were incubated in a volume of 2 ml. Incubation was started by mixing an aliquot of the microsomal fraction with the substrate mixture and was continued for 3 h at 25°C. The reaction was stopped by heating in a boiling water bath for 15 min. Radioactive glycans were incubated with 3 ml of 70% ethanol. The glycans were centrifuged (3,000 × *g* for 10 min at 25°C) and washed successively with 70% ethanol (once), methanol and chloroform (2 : 3, v/v; three times), boiling water (twice) and NaOH (60 g liter^{-1}; twice). Radioactivity was measured in an aliquot of each fraction.

Measurements of radioactivity—The filters with bound radioactive glycans were immersed in 4 ml of scintillation liquid (Lipoluma). Solutions containing radioactive soluble glycans (5 ml) were mixed with 10 ml of scintillation fluid (Lumagel SB; Lumac). Radioactivity was measured in a 2250 CA spectrometer (Packard; France).

Chemicals—UDP-D-[U-^{14}C]galactose (specific radioactivity, 12 GBq mmol^{-1}) was obtained from Amersham (France). Non-radioactive UDP-D-galactose was obtained

NII-Electronic Library Service

The Japanese Society of Plant Physiologists

from Boehringer-Mannheim (France).

Results

Cell wall galactans

Cell wall polysaccharides—The time course of growth of flax cells was typical, having a two-day lag phase, a growth phase between the third and the eighth day and a maturation phase (Schaumann et al. 1993). During this time, the yield of cell walls decreased rapidly to 1–1.3% and then increased to a mean value of 2%.

The mean level of water-soluble components (P+Pb) accounted for 18% of the dry cell walls (Table 1) but this value increased during the two first days (to 26–31%). Showing an initial decrease, the NaOH-soluble fraction (HC) had a minimum relative level of 42% on day 1 which increased again from day 3 to 7 (to 61–73%). The mean percentages for the EDTA-soluble fraction (Pe) and the NaOH insoluble fraction (residue) were 3% and 18%, respectively, and they remained basically unchanged during the cell culture.

Sugar composition—The P fraction contained mainly galactose, and its relative level continued to increase after the sixth day of cell growth (Table 2A). The mean ratio of galactose to arabinose (gal/ara) was about 4. The mean relative level of galacturonic acid was moderate (24%) but this compound became prominent between days 4 to 7, the mean value of the ratio of galacturonic acid to rhamnose (galU/rham) being always larger than 10.

The Pb fraction contained mainly galacturonic acid and galU/rham was larger than 10 (Table 2B). Galactose, arabinose and glucose were the main neutral sugars. Two peaks in the relative level of galactose appeared, on the second and seventh day, respectively. The galactose/arabinose ratio was about 4 during the first increase in the relative level of galactose, whereas this ratio was close to 2 during the second increase.

The Pe fraction contained a high percentage of galacturonic acid (the mean relative level was 73.7%; Table 2C). The gal/ara ratio was close to 1 and, on average, galactose accounted for 30% of the neutral sugars.

Galactose was also abundant in the HC fraction (the mean relative level was 25%; Table 3). We observed two increases in this value, the first one on days 3–4 and the second on days 7–9. The gal/ara ratios were generally be low 1 except during the first increase in the relative level of galactose.

Sugar linkages—The linkages of sugars were studied in the polysaccharides that contained relatively high percentages of galactose and were abundant in cell walls, namely HC and Pb fractions. The relative levels of 2,3-di-*O*-methyl, 2,3,6- and 2,3,5-tri-*O*-methyl and 2,3,5,6-tetra-*O*-methyl derivatives of galactose in the Pb fraction indicated that galactose sugars were mainly substituted at position 4, but also at positions 3 and 6 (Table 4). The *O*-6 substitution was the most important on day 7. The pattern of glycosidic linkages of arabinose was consistent with some (→ 5)-*α*-ara*f*-(1 →)chains whereas short chains of glucose might be inferred from the 2,4,6-tri-*O*-methyl and 2,3,4,6-tetra-*O*-methyl derivatives of glucose.

The pattern of glycosidic linkages of galactose in the HC fractions was mainly characterized by the presence of 2,4-di-*O*-methyl and 2,4,6-tri-*O*-methyl derivatives of galactose, suggesting galactosyl residues substituted at positions 3 and 6 (Table 5). The high level (up to 36%) of 2,3,4,6-tetra-*O*-methyl derivatives of galactose indicated the presence of short chains of galactan. Some 4-linked galactose was also detected (mainly from day 4 to 9).

Comparison of the time courses of changes in levels of two types of galactan—The relative level of *β* 1 → 4 galactan was estimated from that of pectic components (P+Pb) in Table 1 and their galactose composition (Tables 2A, B), and it was compared with the relative level of *β* 1 → 3, *β* 1 → 6 galactans in the cell walls, which was calculated from the relative level of HC (Table 1) and its galactose composition (Table 3). As shown in Figure 1, a maximum level of *β* 1 → 4 galactan, accounting for about 4% of the dry cell-wall mass, was observed during the lag phase of cell growth, whereas the average relative level of *β* 1 → 3, *β* 1 → 6 galactans was about 13%, with a slight increase (up to

Table 1 Relative level (%) of polymers in cell walls during culture of flax cells

Day	0	1	2	3	4	5	7	9	11
P+Pb	14	31	26	9	14	17	12	22	18
Pe	3	6	2	3	5	2	3	1	4
HC	58	42	55	73	61	67	67	55	54
Residue	24	21	17	15	20	14	18	22	24

The mean results of two representative experiments are shown. As described in Material and Methods, P and Pb were solubilized with cold and boiling water, respectively; Pe was extracted with EDTA (0.5% at 100°C); HC was extracted with NaOH; and the residue was the NaOH-insoluble fraction of cell walls.

NII-Electronic Library Service

The Japanese Society of Plant Physiologists

Table 2 Variations in relative level (%) of sugars in pectins (P, Pb and Pe) during cell culture

A: Polysaccharides solubilized with cold water (P)

Day	0	1	2	3	4	5	6	7	9	11
Ara	4.8	14.9	7.8	4.5	10.8	16.7	11.2	15.1	15.9	6.2
Rham	1.0	3.2	1.7	1.7	3.8	6.3	2.9	2.6	2.9	0
Xyl	2.4	3.7	3.8	4.3	6.0	10.4	5.3	5.4	4.4	0
Man	1.6	2.5	5.9	3.4	6.2	3.3	5.3	4.3	4.3	0
Gal	42.0	49.7	48.3	46.1	46.2	48.9	47.7	58.2	59.7	80.2
Glc	48.2	26.0	32.5	40.0	27.0	14.4	27.6	14.4	12.8	13.6
% galU	8.0	20.7	20.6	17.1	30.6	44.8	29.6	27.1	22.9	14.9

B: Polysaccharides solubilized with boiling water (Pb)

Day	0	1	2	3	4	5	6	7	9
Ara	21.4	20.8	12.2	11.0	19.1	16.9	22.0	22.1	26.6
Rham	11.5	9.0	9.2	11.4	13.4	12.4	5.0	11.7	14.9
Xyl	9.3	6.7	3.8	5.6	8.9	6.4	6.1	3.4	5.6
Man	5.2	3.8	3.2	5.0	4.0	3.0	3.9	2.7	3.5
Gal	37.9	31.0	48.1	32.9	37.1	38.8	42.1	49.3	37.9
Glc	14.7	28.7	23.5	34.1	17.5	22.5	20.9	10.8	11.5
% galU	57.8	51.6	54.1	67.0	58.1	65.9	46.0	72.1	66.4

C: Polysaccharides extracted with EDTA (Pe)

Day	0	1	2	3	4	5	6	7	9	11
Ara	25.8	24.3	31.5	25.7	25.7	33.4	27.9	35.5	48.3	40.4
Rham	36.7	28.4	50.1	23.3	31.1	23.2	11.7	16.0	33.0	29.2
Xyl	0	0	0	0	0	6.9	2.4	5.0	1.8	3.1
Man	0	0	0	0	0	1.4	1.0	0	0	0
Gal	16.5	35.7	15.2	53.0	35.2	22.1	46.2	37.4	16.3	24.2
Glc	21.0	11.6	3.2	8.0	8.0	13.0	10.8	5.9	0.6	3.1
% galU	93.8	81.5	70.4	81.7	76.4	69.8	69.3	70.5	56.4	67.4

The mean results of two representative experiments are shown. The percentages of ara (arabinose), rham (rhamnose), xyl (xylose), man (mannose), gal (galactose) and glc (glucose) were calculated relative to the total amount of neutral sugars. The percentage of galU (galacturonic acid) is given relative to the total amount of sugars (neutral and acid). No glucuronic acid was detected.

20%) during the growth phase.

Galactan synthase activities

As previously described (Goubet and Morvan 1993), when incorporation of UDP-^{14}C-galactose was examined, the only labelled sugar in the polymers was galactose. Moreover, the results of methylation analysis showed that the major water-soluble fraction from the microsomes had a 4-β-gal-(1 $\rightarrow$) chain whereas short chains of 3,6 linked galactose were present in the products of the NaOH extract. Hence, in this study, the products of enzymatic reactions were not characterized again. We did, however, study the changes in galactan synthase activities at pH 5 and pH 8 during the culture of flax cells, using 2 methods: (1) facilitated precipitation (with cellulose and a mitochondrial fraction) and then collection on a filter, to give the total activity; and (2) a three-step treatment of the microsomal preparation with boiling water, EDTA and NaOH, to give the different products of the reaction.

Total galactan synthase activity—At both pH 5 and pH 8, two peaks of galactan synthase activity were observed during the culture (Figure 2A): the first one on days

NII-Electronic Library Service

The Japanese Society of Plant Physiologists

Table 3 Variations in relative level (%) of sugars in polysaccharides extracted with NaOH during cell culture

Day	0	1	2	3	4	5	6	7	9	11
Ara	22.1	33.9	34.9	21.8	25.2	31.6	39.3	26.4	34.6	44.0
Rham	19.1	18.5	16.0	10.6	11.9	15.0	9.6	11.9	9.2	13.0
Xyl	25.4	23.0	19.7	19.4	18.3	17.8	16.2	16.8	17.6	16.0
Man	2.5	2.2	2.6	1.6	2.3	3.2	2.2	3.4	2.9	2.1
Gal	20.5	15.7	20.7	33.0	30.5	24.0	25.9	28.3	29.8	21.0
Glc	10.4	6.7	6.1	13.6	11.8	8.4	6.8	13.2	5.9	3.9
% acid	30.9[b]	19.0[a]	15.9[a]	17.1[a]	17.0[a]	5.1[a]	3.4[a]	33.6[b]	16.4[a]	9.3[a]

The mean results of two representative experiments are shown. The percentages of ara (arabinose), rham (rhamnose), xyl (xylose), man (mannose), gal (galactose) and glc (glucose) were calculated relative to the total amount of neutral sugars. The percentage of acid sugars (% acid) is given relative to the total amount of sugars (neutral and acid).

[a] % acid corresponds to galacturonic acid, only.

[b] 5–10% of the acid sugars is glucuronic acid and the rest of acid sugars is galacturonic acid.

Table 4 Methylation analysis of Pb fractions (solubilized from cell walls in boiling water) obtained on different days during cell culture

Day	1	2	4	5	6	7
Arabinose						
2,3,5	0	0	0	0	18.2	0
3,5	0	0	11.7	0	0	0
2,3	0	0	0	10.1	3.6	0
3	0	13.9	15.0	0	8.5	0
Total	0	13.9	26.7	10.1	30.3	0
Galactose						
2,3,5,6	0	0	0	15.5	0	5.3
2,3,4,6	32.7	3.9	18.4	15.6	0	0
2,4,6	0	5.9	0	0	0	0
2,3,6	26.2	6.7	0	0	15.7	0
2,3,4	0	0	0	5.1	4.1	29.3
2,3,5	29.4	8.2	0	6.8	2.9	29.6
2,3	0	19.2	8.9	18.7	14.9	6.4
Total	87.8	43.9	27.3	61.7	37.6	70.6
Mannose						
2,3,6	0	0	0	0	0	21.5
Total	0	0	0	0	0	21.5
Rhamnose						
2,3,4	0	22.6	32.3	28.2	23.1	5.7
3,4	0	0	9.4	0	0	0
3	0	13.7	0	0	0	0
Total	0	36.5	41.7	28.2	23.1	5.7
Glucose						
1,2,3,5,6	0	5.7	4.3	0	0	2.2
2,3,4,6	0	0	0	0	5.9	0
2,4,6	12.2	0	0	0	3.1	0
Total	12.2	5.7	4.3	0	9.0	2.2

The results for individual methylated sugars are given relative to the total amount of methylated sugars.

NII-Electronic Library Service

The Japanese Society of Plant Physiologists

Table 5 Methylation analysis of HC fractions (extracted from cell walls with NaOH) obtained on different days during cell culture

Day	1	2	3	4	6	7	9
Arabinose							
2,3,5	13.9	0	0	0	6.3	0	0
2,3,4	0	0	0	0	0	5.2	0
3,5	0	0	0	4.6	1.8	0	3.3
2,5	0	0	0	0	0	0	2.5
2,4	2.6	0	0	15.1	0	0	2.7
2,3	0	3.8	7.6	0	0	0	0
3	0	3.2	0	0	2.0	0	15.5
2	0	3.8	9.9	6.3	0	0	0
Total	16.5	10.8	17.5	26.0	10.1	5.2	24.1
Rhamnose							
3,4	0	0	0	0	0	0	7.9
2,3	6.7	0	0	3.9	4.6	0	8.3
2,4	6.8	0	8.4	0	6.2	4.4	0
3	0	16.5	0	0	1.4	2.5	0
Total	13.5	16.5	8.4	3.9	12.2	6.9	16.2
Xylose							
2,3,4	10.3	0	0	0	2.7	8.9	0
2,4	0	0	0	8.1	9.6	0	0
2,3	0	3.9	0	0	0	3.0	0
Total	10.3	3.9	0	8.1	12.3	11.9	0
Galactose							
2,3,4,6	34.6	11.2	36.0	7.4	18.5	19.0	19.8
2,4,6	0	5.1	5.0	12.3	8.7	15.2	11.5
2,3,6	0	0	0	7.0	4.2	6.3	5.0
2,3,4	0	0	0	0	0	3.7	0
2,6	2.0	5.5	0	4.3	0	3.4	0
3,4	0	4.8	0	0	0	5.4	0
2,4	0	9.2	10.5	6.2	1.4	5.4	10.7
2,3	0	5.0	0	0	11.6	0	0
3	2.7	0	0	3.5	1.6	2.8	0
Total	39.3	40.9	51.5	40.7	46.4	61.2	47.0
Glucose							
2,3,4,6	6.1	0	0	0	7.4	0	0
2,4,6	0	7.0	5.2	8.5	3.8	4.5	4.3
2,3,6	4.4	21.0	7.0	12.8	4.4	10.3	8.4
2,3,4	6.3	0	0	0	0	0	0
2,6	3.6	0	10.4	0	3.4	0	0
Total	20.4	28.0	22.6	21.3	19.0	14.8	12.7

The results for individual methylated sugars are given relative to the total amount of methylated sugars.

2–3 and the second one on days 6–7. The galactan synthase activity measured at pH 8 was highest on day 2, whereas that measured at pH 5 was highest on days 6–7.

Specific and cellular activities (at both pH values; Figures 2B, C) decreased slightly during growth, as a result of a large increase in levels of microsomal proteins (Schaumann et al. 1993) and in fresh-cell mass. After days 9–10, when the cells started to mature, both galactan synthase activities tended to disappear.

Incorporation of radioactivity into the in various frac-

NII-Electronic Library Service

The Japanese Society of Plant Physiologists

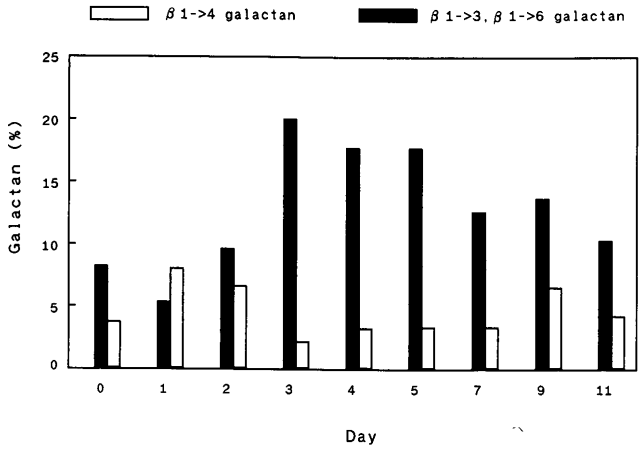

Fig. 1 Relative levels (%) of galactan during the culture of flax cells. Values were estimated from the results given in Tables 1, 3 and 5.

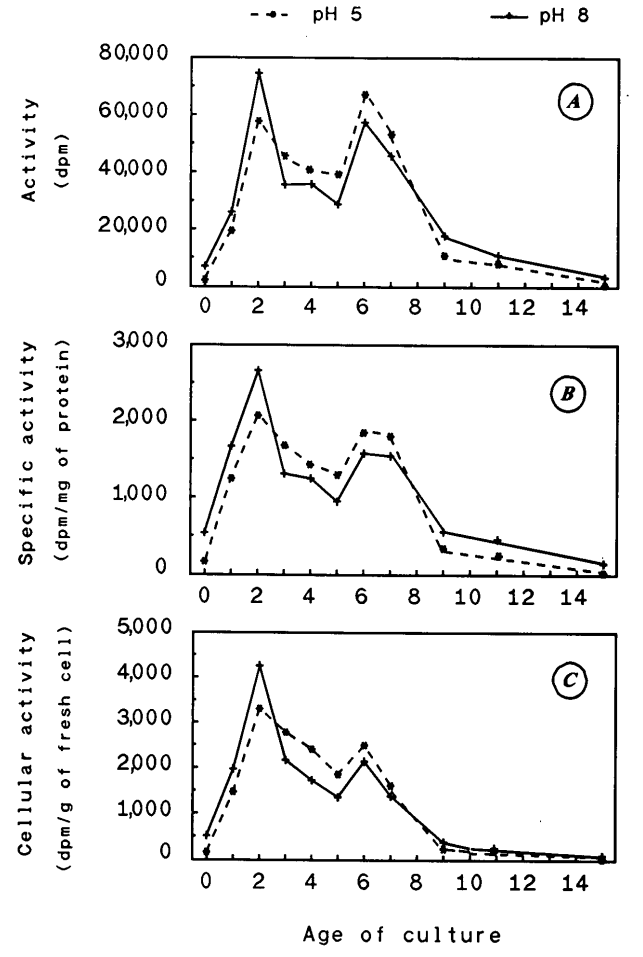

Fig. 2 Galactan synthase activities at pH 5 and pH 8 during the culture of flax cells. A. Total activity in dpm. B. Specific activity in dpm (mg protein)$^{-1}$. C. Cellular activity in dpm (g fresh cells)$^{-1}$. The experiment was repeated three times and the shape of the respective curves was identical in each case.

tions—Both at pH 5 (Figure 3A) and at pH 8 (Figure 3B), the amount of radioactivity incorporated into the water and the NaOH fractions decreased significantly throughout the duration of the culture. The extent of incorporation was generally larger in the NaOH fractions than in the water fractions, in particular at pH 5. When we were looked at each fraction separately, namely, the water (Figure 4A) or the NaOH (Figure 4B) fraction, it was clear that the relative amount of radioactivity incorporated into that fraction was similar, irrespective of the pH, during the lag phase. Then the relative amount of radioactivity incorporated into the water fraction increased at pH 8 and decreased at pH 5 (Figure 4A). Conversely, the relative amount of radioactivity incorporated into the NaOH fraction decreased when the galactan synthase was assayed at pH 8 and increased when it was assayed at pH 5 (Figure

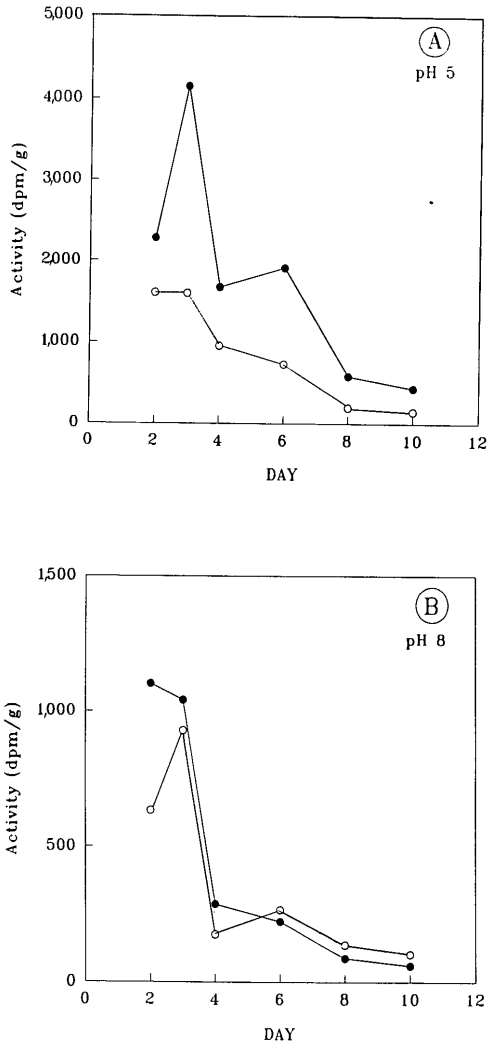

Fig. 3 Incorporation of radioactivity into water-soluble and NaOH-soluble fractions at pH 5 (A) and at pH 8 (B) during the culture of flax cells. Polymers extracted with boiling water —○—. Polymers extracted with NaOH —●—.

NII-Electronic Library Service

The Japanese Society of Plant Physiologists

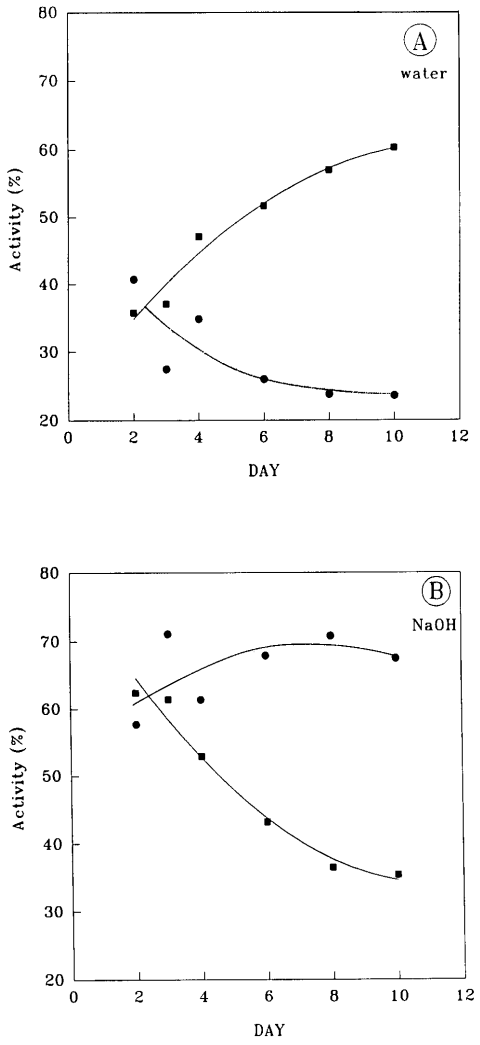

Fig. 4 Relative amounts (%) of radioactivity incorporated into the water-soluble (A) and NaOH-soluble (B) fractions during the culture of flax cells. The incorporation of radioactivity was measured at both pH 5 (-●-) and pH 8 (-■-). The extractions were performed with boiling water and NaOH as described in Material and Methods.

4B). At the end of the growth phase, the extent of incorporation of radioactivity at pH 8 was larger than at pH 5 in the water fraction (Figure 4A) whereas it was smaller at pH 8 than at pH 5 in the NaOH fraction (Figure 4B).

Discussion

In flax cell walls, as in other species (for example, in mung bean, Hervé du Penhoat et al. 1987), the pattern of glycosidic linkages of pectic polymers extracted with boiling water indicated a high level of β 1 → 4 galactan. As described by Carpita and Gibeaut (1993), some glycosyl units might be linked at the *O*-3 site of galactosyl units. For example, arabinosyl units at the *O*-3 site of galactosyl units were described by Bacic et al. (1988). Morever, at the end of cell growth, further substitution at the *O*-6 position of galactosyl moieties was observed.

The pattern of glycosidic linkages of galactan polymers extracted with alkali demonstrated that they were mainly composed of (1 → 3) and (1 → 6) β-D chains. The significant amount of arabinose, which was detected by methylation, may be present as branched chains on β 1 → 6 galactan, as described for type II arabinogalactan (Aspinall 1980, Selvendran 1985).

During cell growth, pectic β 1 → 4 galactan was synthetized mainly during the lag phase whereas the greatest amount of hemicellulosic β 1 → 3, β 1 → 6 galactan (HC fraction) was detected during the growth phase. During the same period, two peaks of galactan synthase activity were detected, on days 2–3 (maximum at pH 8) and days 6–7 (maximum at pH 5), respectively. Similar peaks of galactan synthase activity were observed during the growth of seedlings of *Phaseolus aureus* (McNab et al. 1968).

When the incorporation of UDP-galactose was examined at pH 5, the majority of the radioactivity was found in polymers that had been solubilized by NaOH (see Figure 3B). It was shown previously (Goubet and Morvan 1993) that these polymers were mainly β 1 → 3, β 1 → 6 galactan. Hence, we can associate the maximum activity at pH 5, detected from days 6–7, with a β 1 → 3, β 1 → 6 galactan synthase. From these results and those reported previously, which showed that the pH optimum for incorporation of UDP-^{14}C-galactose was in the region of pH 6.5 with some incorporation of radioactivity at pH 5 (Goubet and Morvan 1993), we can deduce that the optimal pH of β 1 → 3, β 1 → 6 galactan synthase is most probably close to pH 6.5 rather than pH 5. Working at pH 5 just allows us to reduce the extent of interference by the β 1 → 4 galactan synthase.

When the incorporation of UDP-galactose was examined at pH 8, a pH at which a shoulder of significant activity was observed previously (Goubet and Morvan 1993), the majority of the radioactivity was found in the products that were solubilized in water. It was shown previously that these polymers were mainly β 1 → 4 galactan (Goubet et Morvan 1993). Hence, we can associate the maximum activity at pH 8, detected during the lag phase, with a β 1 → 4 galactan synthase.

From these results and those reported previously (Goubet and Morvan 1993), flax suspension-cultured cells appear to be a good model system for studies of β 1 → 3, β 1 → 6 galactan synthase in plants since (1) the corresponding β 1 → 3, β 1 → 6 galactans account for an average of 13% of the mass of the cell wall; (2) the enzymatic activity is mainly expressed during the exponential phase; and (3) the activity is still detectable at pH 5 whereas that of β 1 → 4 galactan synthase is not. Moreover, β 1 → 4 galactan synthase activity can be also studied at pH 8, but at the early stages of cell development.

NII-Electronic Library Service

The Japanese Society of Plant Physiologists

References

Albersheim, P., Nevins, D.J., English, P.D. and Karr, A. (1967) A method for the analysis of sugars in plant cell-wall polysaccharides by gas-liquid chromatography. *Carbohydr. Res.* 5: 340–345.

Aspinall, G.O. (1980) Chemistry of cell wall polysaccharides. *In* The Biochemistry of Plants, a Comprehensive Treatise. Vol. 3. Edited by Preiss, J. pp. 473–499. Academic Press, New York.

Bacic, A., Harris, P.J. and Craig, J.W.T. (1988) Structure and function of plant cell walls. *In* The Biochemistry of Plants. Vol. 14. Edited by Preiss, J. pp. 297–371. Academic Press, New York.

Baydoun, E.A.H. and Northcote, D.H. (1980) Isolation and characterization of membranes from the cells of maize root tips. *J. Cell Sci.* 45: 147–167.

Bradford, M.M. (1976) A rapid and sensitive method of the quantitation of microgram quantities of proteins utilising the principle of protein-dye binding. *Anal. Biochem.* 72: 248–254.

Carpita, N.C. and Gibeaut, D.M. (1993) Structural models of primary cell walls in flowering plants: consistency of molecular struture with the physical properties of the walls during growth. *Plant J.* 3: 1–30.

Davis, E.A., Derouet, C., Hervé du Penhoat, C. and Morvan, C. (1990) Isolation and an n.m.r. study of pectins from flax (*Linum usitatissimum* L.). *Carbohydr. Res.* 197: 205–215.

Goubet, F., Chekkafi, A., Bruyant, M.P. and Morvan, C. (1994) Effects of monensin on cell-wall metabolism of flax suspension-cultured cells. *Plant Sci.* 97: 161–168.

Goubet, F., Martini, F., Thoiron, B. and Morvan, C. (1993) Incorporation of D-[U-^{14}C]-glucose and $^{14}CO_2$ into the cell-wall polymers of flax hypocotyl, in the course of the fibre differentiation. *Plant Cell Physiol.* 34: 841–848.

Goubet, F. and Morvan, C. (1993) Evidence for several galactan synthases in suspension-cultured flax (*Linum usitatissimum*) cells. *Plant Cell Physiol.* 34: 1297–1303.

Harris, P.J., Henry, R.J., Blackeney, A.B. and Stone, B.A. (1984) An improved procedure for the methylation analysis of oligosaccharides and polysaccharides. *Carbohydr. Res.* 127: 59–73.

Hervé du Penhoat, C., Michon, V. and Goldberg, R. (1987) Development of arabinans and galactans during the maturation of hypocotyl cells of mung bean (*Vigna radiata* Wilczek). *Carbohydr. Res.* 165: 31–42.

Jansson, P.E., Kenne, L., Liedgren, H., Lindberg, B. and Lonngren, J. (1976) A practical guide to the analysis of carbohydrate. *Chem. Commun. Univ. Stockholm* 8: 1–75.

Lau, J.M., McNeil, M., Darvill, A.G. and Albersheim, P. (1987) Treatment of rhamnogalacturonan I with lithium in ethylenediamine. *Carbohydr. Res.* 168: 245–274.

McNab, J., Villemez, C.L. and Albersheim, P. (1968) Biosynthesis of galactan by a particulate enzyme preparation from *Phaseolus aureus* seedlings. *Biochem. J.* 106: 355–360.

Morvan, C., Abdul-Hafez, A., Morvan, O., Jauneau, A. and Demarty, M. (1989) Etude physicochimique et biochimique de polysaccharides extraits de lin sous-roui. *Plant Physiol. Biochem.* 27: 451–459.

Murashige, T. and Skoog, F. (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant* 45: 473–497.

O'Neill, M., Albersheim, P. and Darvill, A. (1990) *In* Methods in Plant Biochemistry. Vol. 2. Edited by Dey, P.M. pp. 415–441. Academic Press, London.

Panayotatos, N. and Villemez, C.L. (1973) The formation of a β-(1 → 4)-D-galactan chain catalysed a *Phaseolus aureus* enzyme. *Biochem. J.* 133: 263–271.

Quemener, B. and Thibault, J.F. (1990) Assesment of methanolysis for the determination of sugars in pectins. *Carbohydr. Res.* 206: 277–287.

Schaumann, A., Bruyant, M.P., Goubet, F. and Morvan, C. (1993) Pectic metabolism of suspension of cultured cells of *Linum usitatissimum*. *Plant Cell Physiol.* 34: 891–897.

Selvendran, R.R. (1985) Developments in the chemistry and biochemistry of pectic and hemicellulosic polymers. *J. Cell Sci. Suppl.* 2: 51–88.

(Received December 6, 1993; Accepted April 8, 1994)

NII-Electronic Library Service