Pergamon

0031-9422(95)00665-6

Phytochemistry, Vol. 41, No. 2, pp. 461–463, 1996
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0031-9422/96 $15.00 + 0.00

POLYSACCHARIDES IN PRIMARY CELL WALLS OF JAPANESE CYPRESS CELLS IN SUSPENSION CULTURE

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(*Received in revised form* 19 *July* 1995)

Key Word Index—*Chamaecyparis obtusa*; Cupressaceae; Hinoki; Japanese cypress; hemicellulose; primary cell wall; suspension culture.

Abstract—Extracellular polysaccharides in the medium of Hinoki cells in suspension culture were mainly composed of xyloglucan and 3,6-linked arabinogalactan. The non-cellulosic polysaccharides in the primary cell walls of the suspension-cultured cells were mainly composed of rhamnogalacturonan, xyloglucan, 4-linked galactan and 5-linked arabinan, which have been widely reported to be major non-cellulosic polysaccharides in dicotyledons.

INTRODUCTION

Growth and morphogenesis in plant cells are controlled by biochemical and structural changes in the primary cell walls and these changes are regulated by the expression of genetic information in each cell. The primary cell wall of higher plants appears to be composed of polysaccharides that are common to many plant species, although there are significant differences between monocotyledons and dicotyledons [1]. The polysaccharide constituents of secondary cell walls of wood have been reported by many authors because of their industrial usefulness, but there have been few reports on non-cellulosic matrix polysaccharides of primary cell walls of wood, especially of conifers.

Since intact plant tissues, such as seedlings, contain various types of cell, as well as a mixture of primary and secondary cell walls, suspension-cultured cells have been selected for some studies of the structure of plant cell walls [2–5] since cultured cells can be grown as a fairly homogeneous population with predominantly primary cell walls. In the present work, we have induced a suspension culture of Hinoki cells and report the polysaccharide constituents of the extracellular polysaccharides (ECP) and the cell walls. Hinoki (Japanese cypress) is an important conifer used for afforestation in Japan.

RESULTS AND DISCUSSION

Suspension-cultured cells of Hinoki were almost spherical and formed large clusters. During subculture, the fresh weight of cells increased logarithmically and was *ca* nine-fold after 18 days as compared with that at day 0. Table 1 shows the composition of two ECP fractions. In the neutral fraction that did not adsorb on DEAE cellulose (fr. ECP-A), the amount of uronic acid relative to the total amounts of carbohydrate was low. The main neutral sugars in this fraction were xylose (Xyl), galactose (Gal) and glucose (Glc); small amounts of fucose (Fuc), arabinose (Ara) and mannose (Man) were also detectable. Methylated ECP-A gave the following major sugars on hydrolysis: 2,3,4-tri-*O*-methyl-Xyl, 2,3- (or 3,4)-di-*O*-methyl-Xyl, 2,3,4-tri-*O*-methyl-Glc, 2,3,6-tri-*O*-methyl-Glc and 2,3-di-*O*-methyl-Glc. Meanwhile, the main neutral sugars in the acidic fraction that adsorbed on DEAE cellulose (fr. ECP-B) were Ara and Gal; the content of the other sugars was less than 5%. Methylated ECP-B gave the following major sugars on hydrolysis: 2,3,5-tri-*O*-methyl-Ara, 2,3,4,6-tetra-*O*-methyl-Gal, 2,4,6-tri-*O*-methyl-Gal and 2,4-di-*O*-methyl-Gal. Although some of the compounds present in small amounts have not yet been identified, it appears from these results that the ECP-A and ECP-B fractions are composed mainly of xyloglucan and 3,6-linked arabinogalactan, respectively. In the ECP-B fraction, the amount of uronic acid relative

Table 1. Composition of ECP in suspension culture of Hinoki cells

	Fraction	
	A	B
Uronic acid (% of total carbohydrate, w/w)	3.1	24.3
Neutral sugar composition (% of total neutral sugars, w/w)		
Rha	—*	1.3
Fuc	6.7	0.5
Ara	3.4	33.8
Xyl	22.4	1.7
Man	0.9	5.0
Gal	13.0	54.1
Glc	53.7	3.6

*Less than 0.5%.

Table 2. Relative amounts of partially methylated alditol acetates in hemicellulose fractions of Hinoki cells. Each value is expressed as a percentage of total partially methylated alditol acetates; values in parentheses indicate total amount of partially methylated alditol acetates of the same sugar

Sugar/linkage	Methylated sugar	Fraction			
		HC-I-A	HC-I-B	HC-II-A	HC-II-B
T-Fuc	2,3,4-Me_3-Fuc	3.9	—*	1.8	—
2,4-Rha	3-Me-Rha	—	3.4	—	5.0
Ara		(—)	(34.9)	(—)	(31.8)
T-Ara	2,3,5-Me_3-Ara	—	6.8	—	4.1
5-Ara	2,3-Me_2-Ara	—	16.9	—	16.3
2,5-Ara	3-Me-Ara	—	5.3	—	5.5
2,3,5-Ara	Ara	—	5.8	—	5.8
Xyl		(22.6)	(11.1)	(18.2)	(8.3)
T-Xyl	2,3,4-Me_3-Xyl	15.2	—	10.7	—
3-Xyl	2,4-Me_2-Xyl	—	11.1	—	8.3
2 or 4-Xyl	2,3-(or 3,4)-Me_2-Xyl	7.5	—	7.5	—
Gal		(28.2)	(47.5)	(14.9)	(49.7)
T-Gal	2,3,4,6-Me_4-Gal	5.3	10.8	7.1	11.0
2-Gal	3,4,6-Me_3-Gal	5.8	—	7.8	—
3-Gal	2,4,6-Me_3-Gal	—	2.0	—	2.4
4-Gal	2,3,6-Me_3-Gal	17.1	30.5	—	32.5
4,6-Gal	2,3-Me_2-Gal	—	4.2	—	3.8
Glc		(45.3)	(3.2)	(65.1)	(5.2)
4-Glc	2,3,6-Me_3-Glc	11.8	—	17.3	—
4,6-Glc	2,3-Me_2-Glc	33.5	3.2	47.8	5.2

*Less than 1%.

to the total amounts of carbohydrate was *ca* 24%, suggesting that side-chains containing uronic acid might be attached to arabinogalactan.

The relative amount of the EDTA-soluble fraction (pectic substances) in the total cell-wall components was *ca* 30%; the content of uronic acid was 85% of the total carbohydrate in this fraction. The main neutral sugars in this fraction were Ara (*ca* 37% of total neutral sugars, w/w) and Gal (*ca* 36%), indicating that pectic substances in Hinoki cells in suspension are mainly composed of rhamnogalacturonan, which contain side-chains composed principally of Ara and Gal [6].

The main neutral sugars in the neutral fractions of hemicelluloses (HC-I-A and HC-II-A) were Xyl (23–25%) and Glc (54–59%). The major sugars derived from methylated polysaccharides were similar in HC-I-A and HC-II-A, with 2,3,4-tri-*O*-methyl-Xyl, 2,3,6-tri-*O*-methyl-Glc and 2,3-di-*O*-methyl-Glc being predominant (Table 2). It appears from these results that the neutral fraction of hemicellulose in Hinoki suspension culture is mainly composed of xyloglucan [7]. In the HC-I-A fraction, a considerable amount of 2,3,6-tri-*O*-methyl-Gal was present, but was not detectable in the HC-II-A fraction, suggesting that the presence of 4-linked galactan or Gal residues as side-chains could be the cause of the difference in extractability between these two fractions.

The main neutral sugars in the acidic fractions of hemicelluloses (HC-I-B and HC-II-B) were Ara (38–40%) and Gal (44–45%); the amounts of uronic acid relative to the total amounts of carbohydrate in HC-I-B and HC-II-B were *ca* 23 and 27%, respectively. The major sugars derived from the methylated polysaccharides in these fractions are shown in Table 2. The relative amounts of methylated sugars were similar in HC-I-B and HC-II-B, with 2,3,4,6-tetra-*O*-methyl-Gal, 2,3-di-*O*-methyl-Ara and 2,3,6-tri-*O*-methyl-Gal being predominant. It appears from these results that the acidic fraction of hemicellulose in Hinoki suspension culture is mainly composed of 1,4-galactan and arabinan [6]; uronic acid may be present as side-chains linked to these polysaccharides. However, it remains unclear whether they exist either as independent molecules or as a single molecule. To examine this aspect, these fractions were applied to a Sephacryl S-200 column (105 × 1.5 cm) equilibrated with 0.1 M Tris–HCl buffer (pH 8.0) containing 0.5 M NaCl. The amount of carbohydrate in each fraction was determined by the phenol–H_2SO_4 method [8] and high M_r fractions were subjected to GC analyses. Neutral sugar compositions and partially methylated alditol acetates in the high M_r fractions were similar to those of the original fractions (HC-I-B and HC-II-B), indicating that they exist as single molecules and that arabinan may be present as side-chains linked to galactan [6].

As a whole, the constituents of the primary cell walls of Hinoki cells in suspension culture are similar to those reported for dicotyledonous plants.

EXPERIMENTAL

Suspension culture. Seeds of Hinoki (*Chamaecyparis obtusa* Endle.) were a gift from Dr K. Ishii (Forestry and Forest Products Research Institute, Tsukuba, Japan). They were surface-sterilized for 10 min in Purelox (a soln of *ca* 6% NaOCl) and germinated on 0.8% (w/v) agar at 25° under continuous light. Hypocotyl segments, 5 mm long, were excised from the seedlings and transferred into the medium of ref. [9] containing 10^{-5} M NAA and 2% (w/v) sucrose. Cells were maintained and subcultured at 12 to 15-day intervals in 50 ml of the medium in 300-ml flasks, which were shaken at 84 strokes min^{-1} on a reciprocal shaker at 25° in the dark. The fr. wt of cells was determined after harvesting by filtration under red. pres.

Isolation and fractionation of cell-wall components. ECP and cell walls were isolated from cell-free filtrates of the medium and from cells, respectively, using the method described previously [4]. To remove starch from the prepn of cell walls, samples of cell walls were treated with pancreatic α-amylase [10]. Cell walls were extracted with 50 mM Na_2EDTA in 50 mM NaOAc buffer (pH 4.5) for 4 hr at 100°. After the removal of insoluble material by filtration, the extract was dialysed against distilled H_2O to give the EDTA-soluble fr. (pectic substances). The residue after extraction with Na_2EDTA was extracted with 5% KOH (w/w) soln and then 24% KOH soln for 24 hr at 27°. Each extract was neutralized with HOAc and centrifuged at 10000 *g* for 20 min to remove insoluble material. The supernatant was dialysed to give the hemicellulose I (HC I) and hemicellulose II (HC II) frs, respectively [4].

Fractionation of ECP and hemicellulose polysaccharides. ECP and the hemicellulose frs HC I and HC II were applied to a column (30 × 1.3 cm) of DEAE cellulose and the column eluted successively with H_2O and 1M NaOAc. The fr. eluted with H_2O was referred to as fr. A (ECP-A, HC-I-A and HC-II-A), and the fr. eluted with NaOAc was dialysed to give fr. B (ECP-B, HC-I-B and HC-II-B).

Analytical methods. Amount of total carbohydrate in each fr. was determined by the PhOH–H_2SO_4 method with glucose as standard [10]. The concn of uronic acid was determined by the modified carbazole method of ref. [11] with glucuronic acid as standard. After hydrolysis with 2 M TFA in sealed tubes at 120° for 1 hr, the neutral sugar composition of the polysaccharides was determined by GC as the corresponding alditol acetate derivatives with *myo*-inositol as int. standard [12]. Polysaccharide samples were methylated by the method of ref. [13], as modified in ref. [14]. Methylated polysaccharides were extracted with $CHCl_3$ and then hydrolysed with 2 M TFA at 120° for 1 hr. Methylated sugars were reduced with $NaBH_4$, acetylated and analysed by GC. Identification of partially methylated alditol acetates was made from their GC R_t and by GC–MS.

Acknowledgement—The authors wish to express their gratitude to Dr K. Ishii of the Forestry and Forest Products Research Institute for supplying the seeds of Hinoki.

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