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XYLOGLUCAN FROM XYLEM-DIFFERENTIATING ZONES OF *CRYPTOMERIA JAPONICA*

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Key Word Index—*Cryptomeria japonica*; Taxodiaceae; sugi; xylem-differentiating zone; cell walls; xyloglucan.

Abstract—Xyloglucan was isolated from xylem-differentiating zones of *Cryptomeria japonica*. Endo-1,4-β-glucanase digestion of the xyloglucan gave a series of oligosaccharides. These oligosaccharides were purified by gel permeation chromatography and normal-phase HPLC. Glycosyl-residue composition and glycosyl-linkage composition analyses, ^{1}H NMR spectroscopy and FAB-mass spectrometry of the oligosaccharides showed that the xyloglucan was composed of five kinds of oligosaccharide. These oligosaccharides are commonly found in xyloglucan from dicot plants and are characterized as XXXG, XXLG, XXFG, XLLG and XLFG. These results suggest that xyloglucan from gymnosperms has similar structure to that of dicots.

INTRODUCTION

Xyloglucan is the hemicellulosic polysaccharide, commonly found in the primary cell walls of higher plants [1]. Its structure, metabolism and physiological roles in cell walls have been studied [1]. There are many publications about the chemical structure of xyloglucan isolated from the storage polysaccharides of seeds [2], primary cell walls of intact herbaceous plants [3–6] and of cultured cells [7–9]. The basic unit of xyloglucan, according to the nomenclature for xyloglucan oligosaccharides established by Fry *et al.* [10], is a heptasaccharide (XXXG) composed of a 1,4-β-D-glucan backbone with three α-D-xylosyl residues attached to *O*-6 of separate glucosyl residues. In most dicots, the basic unit is more complex, consisting of octa- (XXLG), nona- (XXFG, XLLG) and decasaccharide (XLFG), in addition to XXXG, having a galactosyl residue, a 2-*O*-α-fucosyl galactosyl, two galactosyl or a galactosyl and a 2-*O*-α-fucosyl galactosyl residues, respectively, attached to the *O*-2 position of xylosyl unit of XXXG by a β-linkage [1, 4–8]. Recently, xyloglucans containing arabinosyl residues were isolated from tobacco and characterized [9]. In contrast, monocot xyloglucan is reported to contain fewer substituted xylosyl residues than that from dicots [1, 3, 11].

In dicots, it has been reported that xyloglucan plays an important role during auxin-induced elongation growth [12–14]. Xyloglucan is tightly bound to cellulose microfibrils by hydrogen bonds and forms a cellulose-xyloglucan network. Degradation and reconstruction of xyloglucan molecules, catalysed by enzymes, such as endo-1,4-β-glucanase [15], endo-xyloglucan transferase [16] and xyloglucan endo-transglycosylase [17], regulate the ability to loosen the network in the cell wall and the cells to expand and/or elongate during growth.

Structural studies of cell wall polysaccharides in woody plants have been focused extensively on lignified secondary cell walls [18–20]. Recently, the structure of cell wall polysaccharides from woody plants have been elucidated using suspension cultured cells [7, 21]. However, we have little knowledge about the chemical structures of polysaccharides in the primary cell walls of intact woody plants, especially gymnosperms. In previous work, we have reported the characterization of the main structural polysaccharides [22] and of pectic polysaccharides of sugi [23]. In this study, xyloglucan has been isolated from xylem-differentiating zones of Japanese red cedar (*Cryptomeria japonica*) and its structure has been determined.

RESULTS AND DISCUSSION

Purification of xyloglucan

Xyloglucan was extracted from de-pectinated cell walls isolated from xylem-differentiating zones with 1

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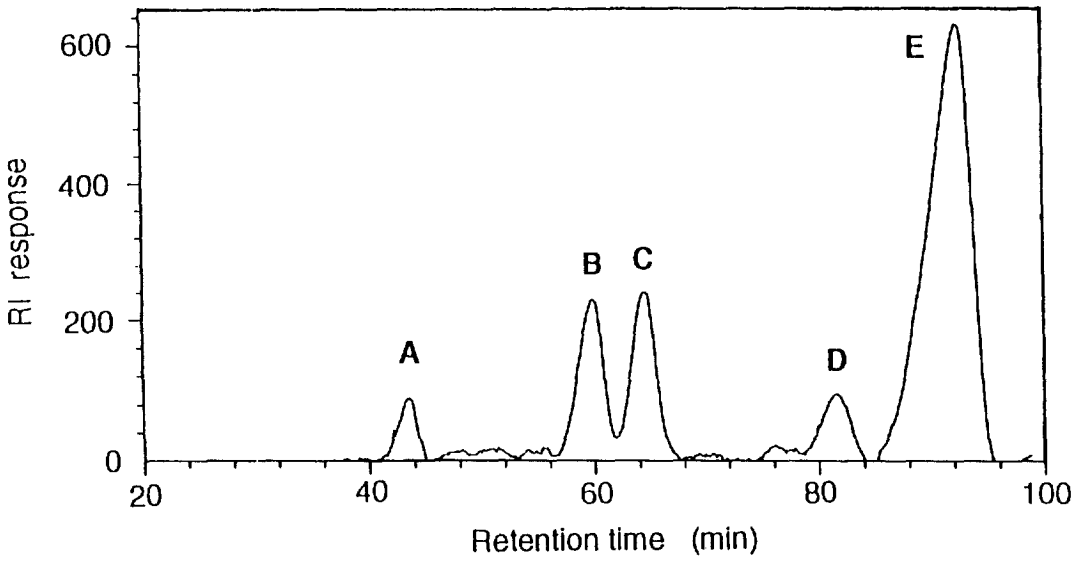

Fig. 1. Normal-phase HPLC on an Amide-80 column of the endo-1,4-β-glucanase digested xyloglucan from *Cryptomeria japonica*.

M and 4 M KOH solutions. Acidic polysaccharides which contaminated the extracts were removed by passage through a DEAE-Sephadex A-25 column. Acidic polysaccharides were absorbed onto the resin and the non-bound material was collected as neutral polysaccharide fractions. A sufficient amount of a pure xyloglucan was obtained from the 1 M KOH extract, while the 4 M KOH extract was a mixture of xyloglucan and glucomannan. Xyloglucans obtained from each extract had the same sugar compositions. The M_r of xyloglucan derived from the 1 M KOH extract was lower than that of 4 M KOH (data not shown). The 4 M KOH fraction was purified by the iodine precipitation method and β-mannanase digestion. The digested glucomannan was separated from the xyloglucan by GPC. Xyloglucan fractions purified by the above procedure were used for structural analysis.

The xyloglucan was partially hydrolyzed by β-1,4-glucanase and the digest separated into two oligosaccharide fractions by Bio-gel P-2 column chromatography. The fraction in the higher M_r region was further separated by normal phase HPLC to give five xyloglucan oligosaccharide fractions [Fig. 1(A–E)]. The lower M_r fraction contained only one oligosaccharide, which was identified as the same oligosaccharide present in fraction A by HPLC.

Characterization of xyloglucan oligosaccharide in fraction A

Sugar composition analysis showed that fraction A contained only xylosyl and glucosyl residues. From FAB-mass spectral data, its M_r was determined to be 1062 (Table 1). These results indicated that fraction A consists of four glucosyl and three xylosyl residues. Glycosyl linkage analysis showed that this fraction included 4-, 6-, and 4,6-linked glucosyl residues and terminal xylosyl residues, indicating the presence of a 1,4-linked glucan backbone and a single xylosyl side-chain attached to the *O*-6 of glucosyl residues. As chemical shifts of anomeric protons of glucosyl and xylosyl residues agreed with those of XXXG reported by Guillén *et al.* [6], glucosyl residues in the 1,4-linked glucan backbone were determined to be β-anomer and branched terminal xylosyl residues were α-anomer, respectively (Table 2). Fraction A had the same retention time on normal phase HPLC as authentic XXXG from tamarind (*Tamarindus indica*) seed xyloglucan (data not shown). From these results, the structure of fraction A was concluded to be XXXG [Fig. 2(A)].

Characterization of xyloglucan oligosaccharides in other fractions

The structures of the four fractions [Fig. 1(B–E)] were determined in the same manner as described above. Fraction B was composed of glucosyl, xylosyl and galactosyl residues (Table 3). The FAB-mass spectrum showed that the M_r was 1224, indicating that fraction B had one more galactosyl residue, in addition to XXXG. Terminal galactosyl and 2-linked xylosyl residues were detected by methylation analysis (Table 4), showing that the galactosyl residue was attached to the *O*-2 position of one of the xylosyl residues. The chemical shift of anomeric proton of this terminal galactosyl residue (Table 2) showed that the galactosyl residue was attached to a xylosyl residue by a β-linkage [6]. These results showed that fraction B was XXLG [Fig. 2(B)].

Table 1. Positive-ion mode FAB-MS of xyloglucan oligosaccharide isolated from xylem-differentiating zones of *C. japonica*

Fraction	Quasi-molecular ion (*m/z*) $[M+H]^+$	$[M+NH_4]^+$	$[M+Na]^+$	$[M+K]^+$	M_r
A	1063	1080	1085	1101	1062
B	1225	1242	1247	n.d	1224
C	1371	1388	1393	n.d	1370
D	1387	1404	1409	n.d	1386
E	1533	1550	1555	n.d	1532

n.d: not detected.

Table 2. ^{1}H-chemical shifts and coupling constants of anomeric protons of xyloglucan oligosaccharides isolated from xylem-differentiating zones of *C. japonica*

Residue*	Chemical shift (δ) [coupling constant (Hz)] Fraction A	B	C	D	E
Glcol	n.a.	n.a.	n.a.	n.a.	n.a.
β-Glc^a	4.69 [8.1]	4.70 *br*	4.68 [1.5]	4.69 [1.5]	4.69 [2.9]
β-Glc^b	4.64 [8.1]	n.a.	4.58 *br*	4.62 *br*	4.57 [1.5]
β-Glc^c	4.60 [8.1]	4.64 *br*	4.60 *br*	4.60 *br*	4.59 [3.7]
α-Xyl^a	5.00 [2.9]	5.22 [2.9]	5.18 [2.9]	5.21 [5.9]	5.18 [3.7]
α-Xyl^b	5.01 [2.2]	n.a.	5.00 [3.7]	5.21 [2.2]	5.22 [3.7]
α-Xyl^c	4.99 [3.7]	n.a.	4.99 [3.7]	4.99 [2.9]	4.99 [3.7]
β-Gal^a	—	4.67 [2.2]	4.66 *br*	4.67 [2.9]	4.67 [2.2]
β-Gal^b	—	—	—	4.64 *br*	4.62 *br*
α-Fuc	—	—	5.31 [3.7]	—	5.31 [3.7]

* Indicates the position of residue in the oligosaccharide; see Fig. 2(E).
n.a. Could not be assigned.

The results of sugar composition analysis (Table 3) and positive-ion mode FAB-mass spectrometry (Table 1) indicated that fraction C includes one fucosyl residue more than fraction B. Because of the absence of a terminal galactosyl residue and the presence of terminal fucosyl and 2-linked galactosyl residues, the terminal fucosyl residue was shown to be attached at the *O*-2 position of the galactosyl residue. The chemical shift of the fucosyl residue (Table 2) indicated that the fucosyl residue was attached by an α-linkage [6]. From these results, fraction C was determined to be XLFG [Fig. 2(C)].

Fraction D was composed of glucosyl, xylosyl and galactosyl residues (Table 3). Comparison of the relative proportion of galactose with that in fraction B indicated that fraction D had two galactosyl residues. The positive-ion FAB-mass spectrum of fraction D also showed an additional hexosyl residue (Table 1). The presence of terminal galactosyl and 2-linked xylosyl residues suggested that both galactosyl residues were attached to xylosyl residues at the *O*-2 position (Table 4). The chemical shifts of the anomeric protons of the galactosyl residues indicated that both galactosyl residues were attached by a β-linkage (Table 2) [6]. From these results, the compound in fraction D was assigned as XLLG [Fig. 2(D)].

Glucose, galactose, xylose, and fucose were present as major sugar residues in fraction E (Table 3). From the FAB-mass spectral data, its M_r was determined to be 1532 (Table 1). These results indicated that one more galactosyl residue was present in comparison with fraction C. Methylation analysis and ^{1}H NMR spectroscopy showed that this galactosyl residue was attached to a xylosyl residue at the *O*-2 position as the terminal sugar and by β-linkage (Table 2 and 4) [6]. These results suggested that fraction E was XLFG [Fig. 2(E)].

Xyloglucan isolated from the cell walls of the xylem-differentiating zones of *Cryptomeria japonica* consisted of five types of oligosaccharides, namely, XXXG, XXLG, XXFG, XLLG and XLFG (Fig. 2). These xyloglucan oligosaccharides have been isolated and characterized in many dicot plants [1, 4–8]. In gymnosperms, the same oligosaccharides have been isolated from suspension-cultured Douglas fir cells [21]. Our results showed that the repeating units of xyloglucan from *Cryptomeria japonica* are similar to those of dicot xyloglucans, rather than those from

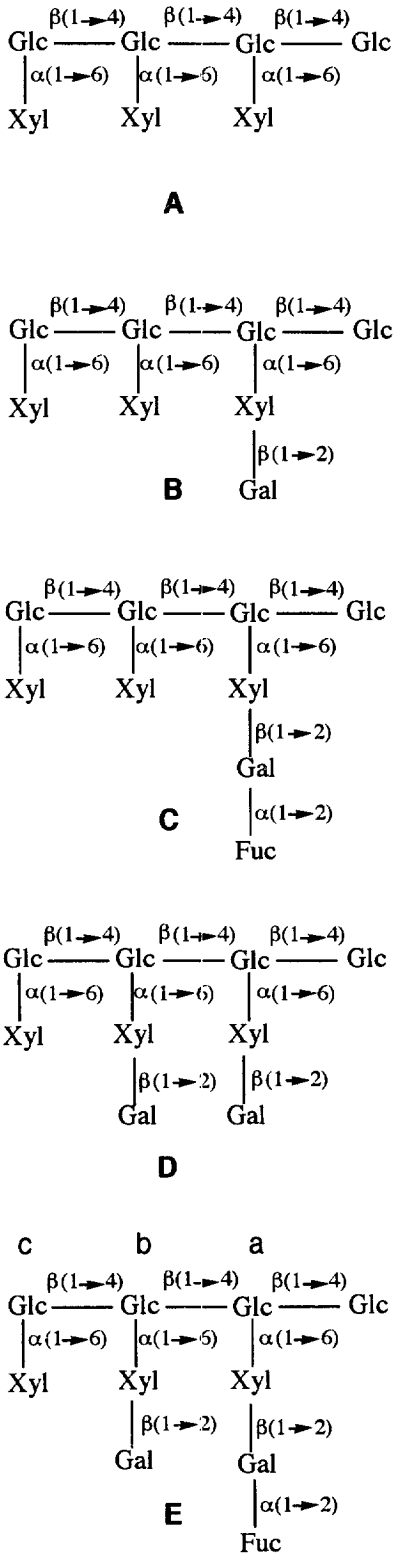

Fig. 2. Proposed structures of xyloglucan oligosaccharides isolated from the cells of xylem-differentiating zones of *Cryptomeria japonica*.

Table 3. Glycosyl composition of xyloglucan oligosaccharides isolated from xylem-differentiating zones of *C. japonica*

Fraction	Sugar composition (mol%) Rha	Fuc	Ara	Xyl	Man	Gal	Glc
A	0.2	0.6	0.2	26.7	0.2	3.2	69.0
B	0.2	2.2	0.2	25.0	1.0	17.7	53.8
C	0.2	8.3	0.5	21.1	0.5	14.9	54.6
D	0.2	3.1	0.2	18.1	1.0	26.2	51.2
E	0.2	7.7	0.4	17.9	0.5	23.9	49.4

Table 4. Sugar linkages of xyloglucan oligosaccharides isolated from xylem-differentiating zones of *C. japonica*

Glycosyl residue	Linkage	Sugar composition (mol%) Fraction A	B	C	D	E
Fucose	Terminal-	0.7	1.4	9.6	3.2	6.8
Xylose	Terminal-	27.4	14.9	11.6	5.4	4.5
	2-	1.7	10.0	9.1	22.4	13.6
Galactose	Terminal-	0.1	12.8	2.3	15.6	9.9
	2-	0.0	0.0	9.8	3.7	9.4
Glucose	Terminal-	0.0	0.6	0.5	2.8	0.3
	4-	17.9	13.7	14.8	11.9	13.1
	6-	16.5	15.1	13.5	8.2	10.6
	4,6-	35.6	31.6	28.9	26.8	31.8

monocots; the xyloglucan isolated from the xylem-differentiating zones was similar to Douglas fir cells. It is well known that xyloglucan plays a regulatory role in the elongation growth of dicot plants [12–17]. Therefore, it is possible that elongation growth of cells in xylem-differentiating zone of conifers is also controlled by xyloglucan in the same manner as dicot plants.

EXPERIMENTAL

Plant material. Cryptomeria japonica trees (*ca* 10 years old), grown in the experimental forest of the Forestry and Forest Products Research Institute (Ibaraki, Japan), were harvested in May and June from 1987 to 1992.

Preparation of cell walls. Cell walls were obtained from xylem differentiating zones of *Cryptomeria japonica* as described in ref. [22].

Isolation of xyloglucan and oligosaccharides. Cell walls were extracted sequentially with 0.05 M CDTA, 0.05 M Na_2CO_3, 1 M and 4 M KOH according to the method of ref. [24]. Xyloglucan was predominantly present in the 1 M and 4 M KOH extractions. The 4 M KOH extract was passed through a DEAE-Sephadex A-25 column (45 × 2.5 cm i.d., phosphate form), that had been equilibrated with 10 mM KPi buffer (pH 6), to remove acidic polysaccharides with

the same buffer. The 1 M KOH extract was purified using the same column and H_2O as eluant; this xyloglucan was of sufficient purity to use for structural analysis. The xyloglucan obtained from the 4 M KOH fr. was contaminated with glucomannan and was further purified by I_2 ppn [25] and treatment with β-mannanase in 0.1 M NaOAc buffer (pH 5). The digested glucomannan was sepd from xyloglucan by gel permeation chromatography on a Sephadex G-75 column (75 × 1.5 cm i.d.). Purified xyloglucan was partially hydrolysed by endo-1,4-β-glucanase from *Trichoderma* sp. (Megazyme) in 0.1 M NaOAc buffer (pH 5) at 40° for 48 hr. The reaction mixt. was applied to a Bio-Gel P-2 column (90 × 1.5 cm i.d.) and eluted with H_2O. The frs, including oligosaccharides, were collected and further purified by normal-phase HPLC on a column of Amide-80 (250 × 4.6 mm i.d. TOSOH) at 1 ml min^{-1} with MeCN-H_2O (7:3).

Glycosyl composition analysis. Glycosyl composition of xyloglucan oligosaccharides was determined according to the methods of ref. [26].

Glycosyl linkage analysis. Xyloglucan oligosaccharides were methylated by a modified Hakomori procedure [27] and then converted into partially methylated alditol acetates. Glycosyl-linkage composition was determined by GC and GC-MS as described in ref. [28].

FAB-MS. Xyloglucan oligosaccharides were dissolved in 20 μl of H_2O and glycerol–thioglycerol (1:1) used as matrix. Spectra were recorded at 10 kV and Xe as bombardment gas.

NMR. $NaBH_4$-reduced xyloglucan oligosaccharides were dissolved in D_2O (99.98%). ^1H NMR spectra were recorded at 25° and chemical shifts measured relative to HDO (δ 4.73).

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REFERENCES

1. Hayashi, T., *Annual Review of Plant Physiology and Plant Molecular Biology*, 1989, **40**, 139.
2. Kooiman, P., *Recuiel Travaux Chimica Pays-Bas*, 1960, **79**, 675.
3. Kato, Y., Ito, S., Iki, K. and Matsuda, K., *Plant Cell Physiology*, 1982, **23**, 351.
4. Ruperez, P., Selvendran, R. R. and Stevens, B. J. H., *Carbohydrate Research*, 1985, **142**, 107.
5. Hayashi, T. and Delmer, D. P., *Carbohydrate Research*, 1988, **181**, 273.
6. Guillén, R., York, W. S., Pauly, M., An, J., Impallomeni, G., Albersheim, P. and Darvill, A. G., *Carbohydrate Research*, 1995, **277**, 291.
7. Bauer, D., Talmadge, K. W., Keegstra, K. and Albersheim, P., *Plant Physiology*, 1973, **51**, 174.
8. Hayashi, T., Kato, Y. and Matsuda, K., *Plant Cell Physiology*, 1980, **21**, 1405.
9. York, W. S., Kolli, V. S. K., Orlando, R., Albersheim, P. and Darvill, A. G., *Carbohydrate Research*, 1996, **285**, 99.
10. Fry, S. C., York, W. S., Albersheim, P., Darvill, A., Hayashi, T., Joseleau, J., Kato, Y., Lorences, E. P., Maclachlan, G. A., McNeil, M., Mort, A. J., Reid, J. S. G., Seitz, H. U., Selvendran, R. R., Voragen, A. G. J. and White, A. R., *Physiology of Plants*, 1993, **89**, 1.
11. Kato, Y. and Matsuda, K., *Plant Cell Physiology*, 1985, **26**, 437.
12. Labavitch, J. M. and Ray, P. M., *Plant Physiology*, 1974, **54**, 499.
13. Nishitani, K. and Masuda, Y., *Plant Cell Physiology*, 1983, **24**, 345.
14. Hoson, T., Masuda, Y., Sone, R. and Misaki, A., *Plant Physiology*, 1991, **96**, 551.
15. Hayashi, T. and MacLachlan, G., *Plant Physiology*, 1984, **76**, 739.
16. Nishitani, K. and Tominaga, R., *Journal of Biological Chemistry*, 1992, **267**, 21058.
17. Fry, S. C., Smith, R. C., Renwick, K. F., Martin, D. J., Hodge, S. K. and Matthews, K. J., *Biochemistry Journal*, 1992, **282**, 821.
18. Aspinall, G. O., *Advances in Carbohydrate Chemistry*, 1959, **14**, 429.
19. Timell, T. E., *Advances in Carbohydrate Chemistry*, 1964, **19**, 247.
20. Timell, T. E., *Advances in Carbohydrate Chemistry*, 1964, **19**, 409.
21. Thomas, J. R., MacNeil, M., Darvill, A. G. and Albersheim, P., *Plant Physiology*, 1987, **83**, 659.
22. Edashige, Y., Ishii, T., Hiroi, T. and Fujii, T., *Holzforschung*, 1995, **49**, 197.
23. Edashige, Y. and Ishii, T., *Phytochemistry*, 1996, **42**, 611.
24. Selvendran, R. R. and O'Neill, M. A., *Methods in Biochemical Analysis*, 1987, **32**, 25.
25. Gaillard, B. D. E., Thompson, N. S. and Morak, A. J., *Carbohydrate Research*, 1969, **11**, 509.
26. Albersheim, P., Nevins, D. J., English, P. D. and Karr, A., *Carbohydrate Research*, 1967, **5**, 340.
27. Sandford, P. A. and Conrad, H. E., *Biochemistry*, 1966, **5**, 1508.
28. York, W. S., Darvill, A. G., McNeil, M. and Albersheim, P., *Methods in Enzymology*, 1986, **118**, 3.