Carbohydrate Research, 181 (1988) 273–277
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

Xyloglucan in the cell walls of cotton fiber

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(Received September 9th, 1987; accepted for publication, November 2nd, 1987)

Xyloglucans occur widely in the primary walls of higher plant cells where they are bound in close association with cellulose microfibrils. Evidence was recently presented that the polysaccharide functions in the organization of cellulose microfibril assembly by preventing the fasciation of the fibrils and could aid in spreading the fiber network over the cell surface[1]. In pea stems, auxin treatment induces the development of endo-(1→4)-β-D-glucanase activities and these are responsible for xyloglucan turnover[2,3]. The integrity of xyloglucan could then control the ability of microfibrils to loosen, and of cells to elongate and expand during growth.

Cotton fibers are highly elongated cells similar in shape to pollen tubes and root hairs. However, unlike these latter cell types, cotton fibers elongate not by tip growth, but by deposition of wall components and elongation throughout the length of the fiber[4]. During elongation, the fibers deposit a primary wall similar in composition to that of many other dicot species[5] that includes xyloglucan as one component[6,7]. We recently showed that xyloglucan deposition is restricted to the elongation phase of fiber development[7]. The mechanism by which cell shape is determined in cotton fibers is not understood, but the interaction of xyloglucan with cellulose microfibrils and the orientation of such fibrils might be expected to play some role. In this report, we have examined the fine structure of cotton fiber xyloglucan and compared its structure to that found in cell walls of other dicotyledonous plants.

Cotton fiber cell wall polysaccharides were sequentially fractionated into hot water-soluble and 24% potassium hydroxide, and an insoluble fraction. Each fraction was analyzed by methylation analysis[8] and the iodine–sodium sulfate method[9] in order to determine the xyloglucan content. The results indicated that 95% of total xyloglucan was concentrated in the 24% potassium hydroxide extract.

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0008-6215/88/$ 03.50

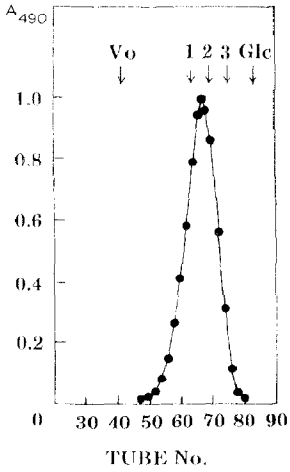


Fig. 1. Gel filtration on a Sepharose CL-6B column (1 × 100 cm) of xyloglucan isolated from the cell walls of cotton fibers. The polysaccharide markers for mol.wt. determination are: (V_0) Blue Dextran (2 000 000), (1) pea xyloglucan (230 000), (2) Dextran T-40 (40 000), and (3) Dextran T-9 (9000). The carbohydrate was detected by the phenol–sulfuric acid method (A_{490} and 0.5-mL fraction).

A purified cotton xyloglucan was observed to be homogeneous by gel filtration on Sepharose CL-6B, with an average mol.wt. of 80 000 (Fig. 1). The $[\alpha]_D$ value was +55.5°. On acid hydrolysis, the polysaccharide yielded glucose, xylose, galactose, and fucose in the ratio of 50:29:12:7. The g.l.c. of the alditols from fully methylated polysaccharide showed seven peaks corresponding to tri-*O*-methylxylitol, tri-*O*-methylfucitol, tetra-*O*-methylgalacitol, 3,4-di-*O*-methylxylitol, 3,4,6-tri-*O*-methylgalacitol, 2,3,6-tri-*O*-methylglucitol, and 2,3-di-*O*-methylglucitol in the

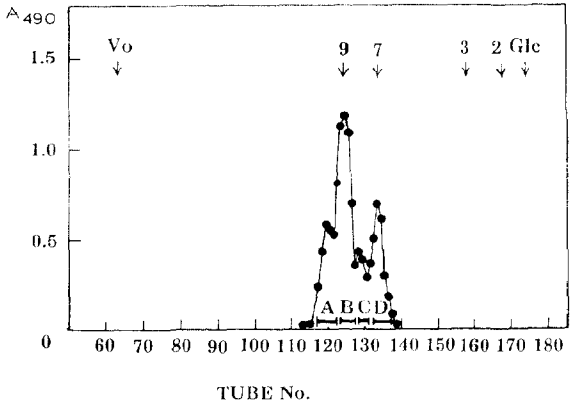


Fig. 2. Gel filtration on a Bio-Gel P-4 column (1 × 120 cm) of *endo*-β-D-glucanase-treated xyloglucan. The oligosaccharide markers for mol.wt. determination are: (9) pea xyloglucan nonasaccharide (1388), (7) heptasaccharide (1062), (3) maltotriose (504), and (2) maltose (342). The carbohydrate was detected by the phenol–sulfuric acid method (A_{490} and 1-mL fractions).

TABLE I

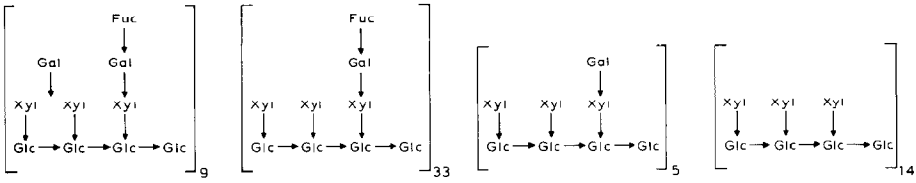
DEGREE OF POLYMERISATION AND SUGAR COMPOSITIONS OF OLIGOSACCHARIDE SUBUNITS[a]

Fraction	*Kd*[b]	*Mol.wt.*	*D.p.*	*Proportion*	*Molar ratio*			
					Glc	*Xyl*	*Gal*	*Fuc*
A	0.52	1550	10	2	4	3	2	1
B	0.55	1388	9	7	4	3	1	1
C	0.59	1224	8	1	4	3	1	
D	0.64	1062	7	3	4	3		

[a]Fractions were isolated from the Bio-Gel P-4 column shown in Fig. 2. [b]$Kd = V_e - V_o/V_{Glc} - V_o$.

ratio of 6:2.5:1:3.5:2.2:4.2:9.2, based on peak areas. The ^{13}C-n.m.r. spectrum (not shown) of the polysaccharide in di($^2H_3$)methyl sulfoxide solution was essentially identical to that from pea xyloglucan and to that reported for xyloglucan isolated[11] from walls of *Rosa glauca*.

The polysaccharide was hydrolyzed with *Streptomyces* endoglucanase and the digest was chromatographed on a Bio-Gel P-4 column. The gel filtration pattern of the digest indicated that cotton xyloglucan is composed of at least four different oligosaccharide subunits (Fig. 2). The molecular weight of each fragment was determined by calibration with oligosaccharide markers. The results indicated the presence of deca-, nona-, octa-, and hepta-saccharides in the ratio of 2:7:1:3, respectively (Fig. 2 and Table I). Based on sugar analysis by g.l.c., the composition of the decasaccharide is glucose, xylose, galactose, and fucose in the ratio of 4:3:2:1. The nonasaccharide was composed of glucose, xylose, galactose, and fucose in the ratio of 4:3:1:1; the octasaccharide of glucose, xylose, and galactose in the ratio of 4:3:1; and the heptasaccharide of glucose and xylose in the ratio of 4:3. Those results suggested structure **1** for the xyloglucan (Scheme 1).



Scheme 1. Proposed general structure of cotton fiber cell wall xyloglucan. The general structure is based upon a molecule of 80 000 mol.wt. The linear arrangement of the various oligosaccharides in such a molecule remains uncertain.

Soybean cells cultured in suspension contain three xyloglucans which are composed of nonasaccharide (glucose–xylose–galactose–fucose, 4:3:1:1) and heptasaccharide (glucose–xylose, 4:3) subunits in the molar ratio of 3:2 for the 180 000-mol.wt. polysaccharide, 2:1 for the 60 000-mol.wt. polysaccharide, and 1:1

for the 30 000-mol.wt. polysaccharide[10]. The arrangement of the two oligosaccharide units is irregular among soybean xyloglucan molecules. Multiple forms of xyloglucans have also been obtained by fractionation with alkaline and organic solvents of cell walls from suspension-cultured *Rosa glauca* cells[9]. Cotton xyloglucan, being composed of four different subunits, also contains such multiple forms of xyloglucan fragments. Thus, the multiplicity of xyloglucans found in a variety of primary cell walls is mainly due to the ratio of components and distribution of oligosaccharide subunits.

EXPERIMENTAL

General methods. — Polysaccharides or oligosaccharides were hydrolyzed in sealed tubes with 2M trifluoroacetic acid for 4 h at 100°. The molar ratio of neutral sugars was determined by converting them into alditol acetates, which were examined by g.l.c. (Hewlett–Packard) on a capillary column (0.25 mm × 15 m) of DB-225 (J & W Scientific) at 200°.

Cotton xyloglucan (5 mg) was digested with 0.5 mg of *Streptomyces* endoglucanase for 48 h at 40°. The hydrolyzate was fractionated on a Bio-Gel P-4 column (1 × 120 cm) eluted with water. Methylation analysis was done by the method of Hakomori[8]. Carbohydrate was determined by the phenol–H_2SO_4 method[12], and xyloglucan by the I_2–Na_2SO_4 method[9].

Materials. — Sepharose CL-6B and proteinase K (from *Tritirachium album*) were obtained from Sigma Chemical Co. Bio-Gel P-4 was from Bio-Rad Laboratories. Exo-(1→3)-β-D-glucanase (from *Basidiomycetes sp.* QM 806) was kindly provided by Dr. K. Matsuda (Tohoku University, Japan), and endo-(1→4)-β-D-glucanase (from *Streptomyces griseus*) by Dr. E. T. Reese (U.S. Army Laboratories, Natick, MA).

Isolation of cotton xyloglucan. — Cotton bolls were harvested at d 19 after flowering. The locules were removed and frozen immediately in liquid N_2, and the seeds were removed under N_2. The fibre cells were ground in a mortar and the resulting powder was homogenized with ice-chilled M $HClO_4$. The mixture was centrifuged at 0°, and the precipitate neutralized with M NaOH. It was homogenized and dialyzed against distilled water for 3 d. The cell wall preparation was extracted sequentially with hot water, 4% KOH–0.1% $NaBH_4$, and 24% KOH–0.1% $NaBH_4$ (ref. 10). Cotton xyloglucan was purified from the 24% KOH extract. The extracts were neutralized with acetic acid and dialyzed against distilled water for 2 d. The sample was treated with exo-(1→3)-β-D-glucanase for 24 h at 40° and with proteinase K (2 mg) for 24 h at 40°, and dialyzed against distilled water. After dialysis and freeze-drying, the sample was dissolved in M $CaCl_2$ solution and precipitated with 3% I_2 in 4% KI solution. After 2 h at 4°, the precipitate was collected by centrifugation and dissolved in hot water, and the mixture was treated with aqueous $Na_2S_2O_3$. The resulting solution was dialyzed against distilled water and freeze-dried (yield, 16 mg from 100 locules).

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