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A XYLOGLUCAN FROM PERSIMMON FRUIT CELL WALLS

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Abstract—Persimmon (*Diospyros kaki* L.) fruit cell walls have been shown to contain a high proportion of xyloglucan. This hemicellulosic polysaccharide appears to play a crucial role in the softening process that accompanies fruit ripening. In the present work, xyloglucan from persimmon fruit cell walls was extracted, purified and chemically characterized from alkali-extracted hemicelluloses. Monosaccharide analysis of purified xyloglucan showed a molar ratio of Glc:Xyl:Gal:Fuc of 10.0:6.0:3.4:1.4. Linkage analysis showed a high proportion of terminal residues, indicating a low degree of polymerization of side-chains. © 1998 Elsevier Science Ltd. All rights reserved

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INTRODUCTION

The plant extracellular matrix, commonly referred to as the cell wall, is a heterogeneous macromolecular assembly consisting of cellulose microfibrils embedded in a matrix of complex polysaccharides and glycoproteins [1]. Xyloglucan is the major structural hemicellulosic polysaccharide in the primary cell wall of dicotyledonous plants [2], accounting for 20–25% of their dry mass. Its basic structure consists of a 1,4-β-glucan backbone with D-xylosyl side-chains α-linked to *O*-6 of some of the glucosyl residues [3]. About 30–50 % of the xylosyl residues have, attached to their position 2, a β-galactosyl residue or, more rarely, an L-arabinosyl residue [4–6]. In dicotyledons, many of the galactosyl residues have an α-L-fucosyl residue at *O*-2 [2, 4].

Xyloglucan binds tightly to the surface of individual cellulose microfibrils through hydrogen bonds, thereby cross-linking them into a complex polysaccharide network [1, 7]. This interaction between the semi-rigid cellulose microfibrils and the less rigid xyloglucan molecules plays a key role in the architecture of dicotyledons cell walls and is probably a major determinant of the mechanical properties of the cell wall [8]. This confers on xyloglucan a crucial role in some physiological events, such as the wall loosening process that initiates cell expansion during plant growth or tissue softening during fruit ripening. Wall-loosening has been explained as mediated by hydrolysis or transglycosylation of intermicrofibrillar xyloglucan polymers [9] or by the disruption of non-covalent bonds between xyloglucan and cellulose [10]. The degradation of xyloglucan has been described during the ripening of several fruits, such as tomato [11], muskmelon [12], kiwifruit [13] and persimmon [14].

In our previous work [14, 15], we found a large proportion of xyloglucan in persimmon fruit cell walls, particularly in the hemicelluloses closely associated with cellulose extracted from the cell wall with 4 M KOH. In addition, during fruit development, the xyloglucan average M_r showed a final decrease, concomitantly with the decrease in fruit firmness. In view of the important role that xyloglucan plays in persimmon fruit cell wall metabolism, we purified and characterized this hemicellulosic polysaccharide.

RESULT AND DISCUSSION

Cell walls (2.02 g) from 100 g of fresh persimmon fruit pericarp were firstly incubated with 200 ml of 1 M KOH during six periods of 24 h. The remaining residue was subjected to a second extraction with 200 ml of 4 M KOH (four periods of 24 h). The 4 M KOH-soluble polymers (50.6 mg) represented ca 2.5% of the cell wall dry wt and were mainly composed of neutral sugars (Table 1). The high proportion of Xyl and Glc suggested the presence of xyloglucan. This fraction was subjected to anion-exchange chromatography on a DEAE-Trisacryl column in order

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Table 1. Sugar composition of different fractions obtained during xyloglucan purification. Each value is the mean of three replicates and for any value the error was less than 5%

	mg		Molar (%)						
Fraction	TS	UA	Rha	Fuc	Ara	Xyl	Man	Gal	Glc
4 M KOH	50.6	8.9	2.0	5.4	8.2	34.2	5.8	15.3	28.9
4 M KOH acidic	10.2	7.6	10.0	N.D.	37.7	29.5	0.2	17.7	4.8
4 M KOH neutal	34.9	0.5	N.D.	8.0	1.2	30.4	7.9	16.6	35.9
$CaCl_2$-insoluble	0.9	N.D.	4.4	7.5	6.3	25.2	8.3	13.4	34.7
4 M KOH I_2-soluble	2.5	N.D.	0.2	6.6	8.0	30.1	7.1	14.2	33.7
4 M KOH I_2-insoluble	29.7	N.D.	0.2	6.2	1.3	26.3	7.6	14.9	43.5

TS, total sugars; UA, uronic acids; ND, not detected.

to eliminate the acidic polymers. Analysis of these polymers revealed a high proportion of uronic acids, arabinose and xylose (Table 1) and can be attributed to the presence of an arabinogalactan and a xylan. These acidic polymers have been described previously as major hemicellulosic polysaccharides of the primary cell wall together with xyloglucan [16]. The neutral fraction, which represented 69% of the 4 M KOH fraction, was composed mainly of residues of Glc, Xyl, Gal and Fuc, indicating the presence of xyloglucan as a major component. The low amount of acidic residues confirms the effectiveness of the previous anion-exchange chromatographic step. This fraction also contained Man residues. The presence of mannose during xylogucan purification has been described before [17, 18] and was explained as an impurity of the purification procedure [2].

To further purify the xyloglucan, the 4 M KOH neutral fraction was subjected to precipitation with $CaCl_2$, followed by a second precipitation of the supernatant with an I_2 solution. This last step in the xyloglucan purification procedure was designed in order to separate linear (insoluble) from branched (soluble) polysaccharides [19]. Xyloglucan was recovered as an I_2-insoluble complex and its sugar composition analysis showed the presence of Glc:Xyl:Gal:Fuc in a molar ratio of 10.0:6.0:3.4:1.4 (Table 1). This is in good agreement with data of xyloglucan extracted from *Phaseolus* (10:7:2.5:1) [20], *Pisum* (10:6:1.8:1) [21] or *Pinus* (10:6.8:2.9:0.5) [16].

The I_2 insoluble fraction was methylated and hydrolyzed, and the sugar linkage composition analyzed by gas-liquid chromatography. The results (Table 2) showed a good recovery of the methylated alditols (98%); 55.9% of the glucosyl residues were substituted at the *O*-6 position. However, the 7.6% of *t*-Man detected probably indicates the presence of a glucomannan. Thus, without further information, it is impossible to know what proportion of the glucose is in the xyloglucan and what proportion in the glucomannan. If we assume that the glucomannan has a backbone of 50% Glc and 50% Man, only 11.6% (19.2–7.6) of the 4-Glc is associated with xyloglucan. Thus, the ratio of 4,6-Glc:4-Glc would be 24.3:11.6 rather than the value 24.3:19.2 obtained and the degree of substitution of Glc at the *O*-6 position would be 67.7%. The *Diospyros* xyloglucan would then be concluded to have a rather typical degree of glucose substitution, comparable with many other dicotyledons, like Gobo (75%) [22] or Gymnosperms, like *Pinus* (80%) [16].

Table 2. Glycosyl-linkage composition of the 4 M KOH I_2 insoluble fraction

Glycosyl	Linkage	Molar (%)
Glucosyl	4-Linked	19.2
	4,6-Linked	24.3
Xylosyl	Terminal	14.6
	2-Linked	11.6
Galactosyl	Terminal	7.0
	2-Linked	7.9
Fucosyl	Terminal	6.2
Mannosyl	4-Linked	7.6

The high proportion of terminal residues (ca 27.8%) indicated that side-chain oligosaccharides had a low degree of polymerization, which is consistent with the structure of xyloglucans from other sources [2, 23].

We also used the 4 M KOH I_2-insoluble fraction to determine the response of persimmon fruit xyloglucan to the Kooiman's method [24]. We found that an A at 640 nm of 1.0 corresponds to a xyloglucan concentration of 325 μg ml^{-1}. This relationship can be used for further quantification of persimmon fruit xyloglucan.

EXPERIMENTAL

Plant material

Persimmon fruits (*D. kaki* L.) at developmental stage IV [15], i.e., fruits that had reached their maximum size and the change in colour had taken place, were obtained from horticultural unit in Torrent (Valencia, Spain), harvested and stored at −20° until used.

Cell wall isolation and fractionation

Fruits were thawed, peeled and the cell walls isolated as previously described [15]. Dry cell walls were first suspended (10 mg ml^{-1}) in 1 M KOH containing 20 mM $NaBH_4$ under a N_2 atmosphere, stirred for 24 h at 20°, centrifuged at 30,000 *g* for 10 min and the sediment re-extracted for another 5 periods. The remaining residue was then extracted × 4 for 24 h with 4 M KOH containing 20 mM $NaBH_4$ under a N_2 atmosphere. These last 4 extracts were combined, neutralized with HOAc, extensively dialyzed against H_2O, concd with a rotary evaporator and freeze-dried, and are referred to as the 4 M KOH fr.

Ion-exchange chromatography

Ion-exchange chromatography of the 4 M KOH fr. was carried out on a DEAE-Trisacryl column (15 × 1.6 cm) equilibrated with 10 mM imidazolium chloride. The sample (50.6 mg) was dissolved in 3 ml of 10 mM imidazolium chloride (pH 7) and eluted with 300 ml of the same buffer. Neutral carbohydrates were collected in 3 ml frs, assayed for total carbohydrates [25], freeze-dried and are referred to as the 4 M KOH neutral fr. After that, the retained carbohydrates were eluted with 300 ml of 10 mM imidazolium chloride, pH 7 containing 3 M NaCl, collected in 3 ml fractions, freeze-dried and referred as 4M KOH acidic fraction.

Iodine precipitation

The freeze-dried 4 M KOH neutral fr. was subjected to I_2 precipitation [20] in order to separate linear from branched polysaccharides. The fr. (34.9 mg) was first precipitated by the addition of 20 ml of 5.9 M $CaCl_2$ soln, followed by centrifugation at 30,000 *g* during 30 min. The supernatant was mixed with 2 ml of an I_2 soln (3% I_2 and 4% KI) and stirred at 4° during 2 h. The mixt. was then centrifuged at 30,000 *g* for 30 min, the supernatant collected, dialysed against H_2O, freeze-dried and referred to as the 4 M KOH I_2-sol. fr. The ppt. was redissolved in hot H_2O, neutralized with $Na_2S_2O_3$, dialyzed against H_2O, freeze-dried and referred to as the 4 M KOH I_2-insol fr.

Linkage analysis

The freeze-dried 4 M KOH I_2 insol. fr. was methylated [26] and then converted into partially methylated alditol acetates, which were separated by GC-MS on an SP-2330 capillary column (30 m), using the molar response factors of Ref. [27].

Sugar analysis

Total sugars and uronic acid contents were determined by the pHOH-H_2SO_4 [25] and *m*-hydroxydiphenyl method, [28], respectively. Neutral sugars were quantified following the method described by in ref. [29], *viz*, lyophilized powders (5 mg) were hydrolyzed with 2 M CF_3CO_2H at 121° for 1 h, the released neutral sugars derivatized to alditol acetates and then analyzed by GC.

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REFERENCES

1. Carpita, N. C. and Gibeaut, D. M., *The Plant Journal*, 1993, **3**, 1.
2. Fry, S. C., *Journal of Experimental Botany*, 1989, **40**, 1.
3. McNeill, M. Darvill, A. G., Fry, S. C. and Albersheim, P., *Annual Review of Biochemistry*, 1984, **53**, 625.
4. Bauer, W. D., Talmadge, K. W., Keegstra, K. and Albersheim, P., *Plant Physiology*, 1973, **51**, 174.
5. Akiyama, Y. and Kato, Y., *Phytochemistry*, 1982, **21**, 2112.
6. Ring, S. G. and Selvendran, R. R., *Phytochemistry*, 1981, **20**, 2511.
7. Nishitani, K. and Tominaga, R., *Journal of Biological Chemistry*, 1992, **267**, 1.
8. Levy, S., York, W. S., Stuike-Prill, R., Meyer, B. and Staehelin, A., *The Plant Journal*, 1991, **1**, 195.
9. Baydoun, E. A-H. and Fry, S. C., *Journal of Plant Physiology*, 1989, **134**, 453.
10. McQueen-Mason, S. J., *Journal of Experimental Botany*, 1995, **46**, 1639.
11. Tong, C. B. S. and Gross, K. C., *Physiologia Plantarum*, 1988, **74**, 365.
12. McCollum, T. G., Huber, D. J. and Cantliffe, D. J., *Physiologia Plantarum*, 1989, **76**, 303.
13. Redgwell, R. J., Melton, L. D. and Brasch, D. J., *Carbohydrate Research*, 1991, **209**, 191.
14. Cutillas-Iturralde, A., Zarra, I., Fry, S. C. and Lorences, E. P., *Physiologia Plantarum*, 1994, **91**, 169.
15. Cutillas-Iturralde, A., Zarra, I. and Lorences, E. P., *Physiologia Plantarum*, 1993, **89**, 369.
16. Acebes, J. L., Moral, R. and Zarra, I., *Phytochemistry*, 1993, **33**, 1346.
17. O'Neill, M. A. and Selvendran, R. R., *Carbohydrate Research*, 1983, **111**, 239.
18. Redgwell, R. J. and Selvendran, R. R., *Carbohydrate Research*, 1986, **157**, 183.

19. Gaillard, B. D. E., *Nature*, 1961, **191**, 1295.
20. Kato, Y. K. and Matsuda, K., *Plant and Cell Physiology*, 1976, **17**, 1185.
21. Hayashi, T. and Maclachlan, G., *Plant Physiology*, 1984, **75**, 596.
22. Kato, Y. and Watanabe, T., *Bioscience, Biotechnology and Biochemistry*, 1993, **57**, 1591.
23. Hayashi, T., *Annual Review of Plant Physiology and Plant Molecular Biology*, 1989, **40**, 139.
24. Kooiman, P. *Recueil des Travaux Chimiques des Pays-Bas et Belgique*, 1960, **79**, 675.
25. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A and Smith, F. *Analytical Chemistry*, 1956, **28**, 350.
26. Hakomori, S., *Journal of Biochemistry*, 1984, **55**, 205.
27. Sweet, D. P., Shapiro, H. and Albersheim, P., *Carbohydrate Research*, 1975, **40**, 217.
28. Blumenkrantz, N. and Asboe-Hansen, G., *Analytical Biochemistry*, 1973, **54**, 484.
29. Albersheim, P., Nevins, D. J. and English, P. D., *Carbohydrate Research*, 1967, **5**, 340.