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CELL WALL POLYSACCHARIDES FROM ONIONS

ANWAR T. MANKARIOS,† MICHAEL A. HALL,* MICHAEL C. JARVIS,‡ DAVID R. THRELFALL and JOHN FRIEND

Department of Plant Biology, Hull University, Hull, HU6 7RX, U.K.;
*Department of Botany, University College of Wales, Aberystwyth, SY23 2AX, U.K.

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Abstract—Onion (*Allium cepa*) cell walls were fractionated by successive extraction with oxalate–citrate buffer and with alkali. The substantial oxalate–citrate extracted fraction comprised a range of pectic polysaccharides with varying proportions of neutral side-chains. Methylation analysis of the alkali extract indicated that (1,4′)-linked galactans and a substituted xyloglucan were probably major components. Onions thus resemble dicotyledonous plants more than the Gramineae in their cell wall composition.

INTRODUCTION

To provide the background for an investigation on onion pathogens and their polysaccharide-degrading enzymes [1, 2], we required some basic information on the structure of the onion cell wall. The cell walls of onions have long been known to contain pectin [3, 4], with β(1,4)-D-galactan side-chains [5], but otherwise their composition is unknown. We have therefore made cell wall preparations from onion bulbs, fractionated them by simple chemical means, and examined the structure of their constituent polysaccharides.

RESULTS

The preparation of cell walls from onion bulbs presented few problems, as the cells were readily broken during homogenization and did not contain appreciable amounts of starch. The cell walls were fractionated by extraction first with oxalate–citrate buffer, pH 4 and then with 3.5 M KOH. The amounts of polysaccharide extracted, and the monosaccharides determined by hydrolysis and GLC, are shown in Table 1. The total uronic acid, determined colorimetrically, was assumed to contain a predominance of galacturonic acid units since onion cell walls can be degraded to a considerable extent by polygalacturonases produced by *Sclerotium cepivorum* [1, 2].

Both extracts were examined by electrophoresis. The oxalate–citrate extract contained a range of acidic polysaccharides varying in mobility, apparently continuously, from 0.20 to 0.41 mm/min at pH 6.5 and with a potential gradient of 10 V/cm. Under these conditions the mobility of an unsubstituted galacturonan was 0.53 mm/min. The polysaccharides in the KOH extract did not move appreciably from the origin.

Table 1. Quantities and composition of onion cell wall fractions

	Pectins	Hemicelluloses	Cellulose
% of cell wall	42.4	36.6	21.0% (measured gravimetrically)
% Monosaccharide composition:			
Uronic acid	66.4	0	
Galactose	28.4	67.6	
Arabinose	2.4	3.5	
Rhamnose	0.6	0.2	
Glucose	1.4	14.1	
Xylose	0.3	12.1	
Mannose	0.2	0.8	
Fucose	0.2	1.8	

The KOH-extracted polysaccharides were methylated, and the chloroform–methanol soluble products hydrolysed and converted to the partially methylated alditol acetates listed in Table 2. There was some variation in the proportions of the products, particularly 2,3,6-tri-*O*-methyl galactose, amongst 3 analyses of the same polysaccharide preparation.

DISCUSSION

The chemical fractionation of the onion cell walls showed that pectic polysaccharides were the most abundant group of constituents. The pectic complex

†‡Present addresses: †Department of Botany, Mansoura University, Mansoura, Egypt; ‡Department of Agricultural Chemistry, Institute of Chemistry, Glasgow University, Glasgow, G12 8QQ, U.K.

comprised a range of polymers differing in acidity, and thus having varied proportions of neutral, largely (1,4′)-β-D-galactan [5], side-chains. In quantity and structure, therefore, onion pectin resembles the pectins of dicotyledons.

In our parallel studies on the potato cell wall ([6]; Jarvis, M. C., Hall, M. A., Threlfall, D. R. and Friend, J., unpublished), we noted that substantial quantities of pectic galacturonans, rich in galactan and arabinan side-chains, resisted extraction with hot oxalate–citrate and similar buffers but could be extracted subsequently under mildly alkaline conditions. However, the alkali extract from onion cell walls gave a negative result for uronic acids with the carbazole reagent and did not contain any acidic polysaccharides that were readily detectable by electrophoresis. (Neither of these methods of detecting the presence of uronic acid units is particularly sensitive in the presence of large amounts of neutral sugar.) Nevertheless the methylation analysis suggested that the KOH extract did contain a considerable quantity of galactan; most of the arabinose is probably (1,5′)-linked arabinose units (Table 2). Probably little of the arabinose is attached to galactan since less arabinose than galactose is released from onion cell walls incubated with wall-degrading enzymes of *Botrytis allii* or *Sclerotium cepivorum* [7]. If the chains of galactose and arabinose units were attached to a rhamnogalacturonan core, the latter must have been vestigial and very heavily substituted. There may alternatively be free galactans in the onion cell wall, as suggested by Sen *et al.* [5].

Many of the remaining partially methylated derivatives in Table 2 may be attributed to the presence of a xyloglucan, although other arrangements of the residues are possible. The small amount of terminal xylose (0.7%) would indicate that even more of the xylose units must be substituted than in the xyloglucan from pea epicotyls [8]. A glucomannan may be a minor component, but there is no indication that substantial amounts of (1,4′)-linked xylan chains are present.

The onion belongs to the Monocotyledones. One might therefore expect that its primary cell walls would show the features that have been found in grasses and cereals: a relatively low pectin content, branched arabinoxylan hemicelluloses in considerable quantity, and no xyloglucans ([9–11]; Gilkes, N. R. and Hall, M. A., unpublished). Our results do not support this conclusion, and we suggest that the distinctive cell walls of the Gramineae may not be typical of other monocotyledonous plants.

EXPERIMENTAL

Preparation of onion cell walls. Onion cell walls were prepared from Giant Stuttgarten bulbs. Large healthy onions (1 kg) without dry scales or roots were stored in crushed ice. The bulbs were homogenized in a Waring blender in 3 ml/g chilled 1% Na deoxycholate with the addition of 1–2% *n*-octanol as antifoaming agent. The homogenate was transferred to a 120 mesh brass sieve and washed with 5 ml/g of ice-cold H_2O. The crude cell walls were transferred to a No.

Table 2. Products from methylation analysis of onion hemicelluloses; yields expressed as per cent of total partially methylated alditol acetates identified

Parent monosaccharide	*O*-Methyl ether	Mode of linkage	Retention time (min) on 3% OV-275*	Relative yield (%)
Galactose	2,3,4,6-tetra	terminal	36.0	6.4
	2,3,6-tri	4-linked	42.5	41.8
	2,4-di	3,6-linked	57.2	1.0
Arabinose	2,3,5-tri	terminal (furanose)	19.7	2.5
	2,3,4-tri	terminal (pyranose) phthalate§	24.9	[7.9]
	2,5-di	3-linked†	33.0	[0.7]
	2,3-di	5-linked	37.0	4.0
Glucose	2,4,6-tri	3-linked	42.3	0.4
	2,3,6-tri	4-linked	48.3	9.7
	2,3-di	4,6-linked	61.2	14.5
Xylose	2,3,4-tri	terminal	29.8	0.7
	3,4-di	2-linked‡	40.2	8.6
	4-	2,3-linked‡	51.8	1.2
Mannose	2,3,4,6-tetra	terminal†*	33.0	[0.7]
	2,3,6-tri	4-linked	45.1	0.6

* Carrier gas flow rate 50 ml/min. Oven temp. 120° held for 5 min then increased by 1°/min to 180° and held at that temp.

† Derivatives of 2,5-di-*O*-methyl arabinose and 2,3,4,6-tetra-*O*-methyl mannose not resolved, total 0.7%.

‡ MS of deuterated derivatives showed that these were the predominant linkages, but a trace of 4-linked xylose was also present.

§ From polypropylene extraction vessel.

2 sintered glass funnel and washed again with 6 ml/g $CHCl_3$–MeOH (1:1) to remove lipids and with 2 ml/g cold Me_2CO and Et_2O to remove the remaining H_2O. The cell walls were dried at 30° for 24 hr and stored in screw-top bottles, at −20° for future use.

Extraction of pectic fraction. Onion cell walls (0.5 g) were suspended in 0.2 M NH_4 oxalate–0.067 M citrate buffer, pH 4, at 100° and stirred rapidly at this temp. for 30 min. The non-pectic residue was filtered off on a coarse sinter and washed with 2×25 ml hot oxalate–citrate buffer and 25 ml hot dist. H_2O. The combined pectic extracts and washings were dialysed against 1 ml EDTA and recovered by freeze-drying from the dialysate. These extraction conditions have been shown to depolymerise esterified rhamnogalacturonan chains to a slight extent, but do not remove the neutral side-chains (Jarvis *et al.*, unpublished).

Alkali extraction. The residue from pectin extraction was suspended in 50 ml of 20% KOH in a polypropylene vessel and stirred overnight at room temp. under N_2. The cellulosic residue was filtered off and washed with 2×25 ml of 20% KOH. The combined extract and washings were neutralized carefully with HOAc. The extracted polysaccharides were precipitated with 95% EtOH (200 ml), collected by centrifugation and dried *in vacuo*. The cellulose fraction was washed thoroughly with dist. H_2O and freeze-dried.

Characterisation of extracted polysaccharides. The methods used for hydrolysis, GLC and colorimetric sugar determination are described in ref. [12]. Identifications of monosaccharides were checked by PC in EtOAc–HOAc–Py–H_2O (25:6:9:5) [13]. Electrophoresis was carried out on trimethylsilylated glass-fibre paper strips [14], in 0.1 M acetate–0.02 M EDTA buffer, pH 6.5, with a potential gradient of 10 V/cm. Carbohydrates were located with α-naphthol–H_2SO_4. Dextran (Pharmacia) and a citrus galacturonan (Sunkist), containing <0.5% of neutral side-chains (Jarvis *et al.*, unpublished), were used as markers. Hemicelluloses were stirred overnight in dry DMSO at 40° and methylated by the Hakomori method, generally as described in ref. [15], but using the K salt of the DIME–sulphinyl anion (Bauer, W. D. and Bhuvaneswari, T.V., personal communication). Insoluble material was removed by centrifugation and the DMSO soluble methylated polysaccharides recovered by chromatography on Sephadex LH-20 in $CHCl_3$–MeOH (1:1) (Bauer and Bhuvaneswari, personal communication) or by diluting with H_2O, and freeze-drying. Hydrolysis with 2 M TFA and derivatization were as described for non-methylated polysaccharides, except that $NaBD_4$ was used for reduction to assist the MS analysis, and solvents were removed in a stream of filtered air at room temp. to prevent losses of the more volatile methylated sugars. Partially methylated alditol acetates were separated by GLC on 0.3% Reoplex-400–0.6% OV-275 [12] or on 3% OV-275, and subjected to GLC–MS under the conditions previously described [9].

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REFERENCES

1. Mankarios, A. T. and Friend, J. (1978) *Trans Biochem. Soc.* **6**, 441.
2. Beck, M. J. (1977) Ph.D Thesis, University of Hull.
3. Trehan, Y. N. and Ahmad, B. (1947) *J. Sci. Ind. Res. (India)* **6**, 16.
4. Sen, S. K. and Rao, C. V. N. (1966) *Indian J. Appl. Chem.* **29**, 127.
5. Sen, S. K., Chatterjee, B. P. and Rao, C. V. N. (1971) *J. Chem. Soc. C* 66.
6. Jarvis, M. C., Threlfall, D. R. and Friend, J. (1978) *Biochem. Soc. Trans.* **6**, 391.
7. Mankarios, A. T. and Friend, J. (1980) *Physiol. Plant Path.* **17**, (in press).
8. Gilkes, N. R. and Hall, M. A. (1977) *New Phytol.* **78**, 1.
9. Gordon, A. H., Hay, A. J., Dinsdale, D. and Bacon, J. S. D. (1977) *Carbohydr. Res.* **57**, 235.
10. Burke, D., Kaufman, P., McNeil, M. and Albersheim, P. (1974) *Plant Physiol. (Lancaster)* **54**, 109.
11. Mares, D. J. and Stone, B. A. (1973) *Aust. J. Biol. Sci.* **26**, 793.
12. Mankarios, A. T., Jones, C. F. G., Jarvis, M. C., Threlfall, D. R. and Friend, J. (1979) *Phytochemistry* **18**, 419.
13. Jarvis, M. C. and Duncan, H. J. (1974) *J. Chromatogr.* **92**, 454.
14. Jarvis, M. C., Threlfall, D. R. and Friend, J. (1977) *Phytochemistry* **16**, 849.
15. Lindberg, B. (1972) in *Methods in Enzymology* (Ginsburg, V., ed.) Vol. 28, Part B, p. 178. Academic Press, New York.