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CELLULOSE AND CALLOSE OF THE POLLEN TUBE WALL OF *CAMELLIA JAPONICA*

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Key Word Index—*Camellia japonica*; Theaceae; pollen tube wall; cellulose; β-1,4-glucan; callose; β-1,3-glucan.

Abstract—By chemical analyses in addition to IR spectroscopy and staining reactions, cellulose and a β-1,3-glucan (callose) were demonstrated to occur in the α-cellulose and hemicellulose A fractions, respectively, of the pollen tube wall of *Camellia japonica*. The main component of the α-cellulose fraction was a β-1,3-glucan, probably from the callose plugs known to be present in this fraction. The cellulose content was estimated to be *ca* 20%, corresponding to only *ca* 4% of the original wall preparation. The β-1,3-glucan in the hemicellulose A fraction and in the DMSO-extractable hemicellulose fraction (reported in a previous publication) accounted for *ca* 40% of the pollen tube wall preparation.

INTRODUCTION

In addition to the morphological and cytochemical studies which have been reported so far, chemical studies of pollen tube walls are required to elucidate the mode of pollen tube growth. We reported that the pollen tube wall of *Camellia japonica* can be fractionated into DMSO-extractable hemicellulose, pectic substance, hemicellulose A, hemicellulose B and α-cellulose (plus callose plugs) fractions in yields of 46.6, 10.9, 8.2, 4.0 and 21.6%, respectively, and that *ca* 75% of the DMSO-extractable hemicellulose fraction consisted of a linear β-1,3-glucan of D.P. 21 [1]. Such a high content of hemicellulose β-1,3-glucan and a very low content of xylan compared with the cell walls of ordinary tissues [2] appear to be characteristic of at least *Camellia* pollen tube walls, and this may relate to the rapid apical growth of the pollen tube. Moreover, a major component of the pectic substance fraction was characterized as a partially esterified pectate [3].

This paper reports the chemical characterization of the α-cellulose and hemicellulose A fractions of the pollen tube wall of *C. japonica*.

RESULTS AND DISCUSSION

The main constituent sugar of the α-cellulose and hemicellulose A fractions from the pollen tube wall of *C. japonica* is glucose [1, 4]. However, the α-cellulose fraction contains microscopically detectable callose plugs [3].

To remove components other than cellulose from the α-cellulose fraction, it was either extracted with a nitric acid–acetic acid mixture or digested with a β-1,3-glucanase. The residues were microscopically freed from callose plugs and the yields (18.3 ± 4.0% in the acid treatment and 24.7 ± 9.7% in the enzyme treatment) were hardly changed on repeating the treatments. Absorbent cotton as a control was scarcely affected by either treatment.

Table 1 shows the sugar composition of the residue and extract thus obtained. The results indicate that the minor glycans and callose plugs in the α-cellulose fraction were removed by either the acid or the enzyme treatment, and that the remaining polysaccharide was probably cellulose.

In ordinary light microscopy, the purified α-cellulose fraction as well as absorbent cotton was negative in aniline blue and lacmoid stains, which are said to be specific for callose but have been questioned by some investigators for the specificity [5], and positive in zinc chloride–iodine and iodine–sulphuric acid stains, which are diagnostic of cellulose [6]. On the other hand, the tube wall β-1,3-glucan [1] as well as a bacterial β-1,3-glucan curdlan [7], was positive in the callose staining reactions but negative in the cellulose staining reactions. In fluorescence microscopy, all four samples were positive with either decolourized aniline blue or Calcofluor white, which has an affinity for both β-1,3- and β-1,4-glucans [8]. The original wall preparation and the unpurified α-cellulose fraction gave positive reactions with all the reagents.

The unpurified or purified α-cellulose fraction and absorbent cotton showed birefringence in polarized light microscopy. In addition, the IR spectra of the purified α-cellulose fraction and the acid-treated absorbent cotton were of the same pattern and showed a type 2b absorption at 890 cm^{-1} diagnostic of β-glucosidic linkages [9].

All the above results suggested that the purified α-cellulose fraction was probably a true cellulose. However, there was another possibility, for alkali-resistant fibrils of crystalline β-1,3-glucan different from amorphous 'callose' have been reported to occur in lily pollen [10]. To confirm the presence of cellulose in the preparation, methylation analyses were performed (Table 2). The extract obtained from the α-cellulose fraction following treatment with the acid mixture gave 2,4,6-*O*-Me-glucose as the main component. This indicated that the callose plug glucan in the α-cellulose fraction might be selectively degraded and extracted by acid treatment. Methylation of the purified α-cellulose fraction was still incomplete, but the main component of the product was 2,3,6-*O*-Me-

Table 1. Sugar composition of the extract and the residue obtained from the α-cellulose fraction following treatment with either the acid mixture or β-1,3-glucanase

	Amount, mg (% total)		
	Acid mixture treatment		β-1,3-Glucanase treatment
Sugar	Extract	Residue	Residue
Rhamnose	0.029 (0.3)	n.d.*	n.d.
Arabinose	0.250 (2.6)	0.005 (0.4)	0.007 (0.2)
Xylose	n.d.	n.d.	tr*
Mannose	0.019 (0.2)	0.011 (0.9)	0.003 (0.2)
Galactose	0.042 (0.4)	n.d.	n.d.
Glucose	9.160 (96.4)	1.211 (98.7)	1.808 (99.4)
Total	9.500 (100)	1.227 (100)	1.818 (100)
Recovery (%)	91.3	38.8	70.6

*n.d., Not detected; tr, trace (< 0.1%).

Table 2. Methylation analysis of the hemicellulose A glucan and the extract and the residue obtained from the α-cellulose fraction following treatment with the acid mixture

	α-Cellulose fraction		
Methylated glucose*	Extract	Residue	Hemicellulose A glucan
2,3,4,6-Me_4	+ (1.0)†	+ (1.0)	+ (1.0)
2,4,6-Me_3	+ + (16.2)	+ (2.3)	+ + (35.7)
2,3,6-Me_3	−	+ + + (129.6)	+ (0.2)‡
2,6-Me_2	+	+	+
4,6-Me_2	−	+	−
3,6-Me_2	+	−	+
2,4-Me_2	−	+	−
2-Me	+	−	−

*Me_4- and Me_3-glucose were identified with the aid of authentic samples. The identities of the other minor peaks of Me-glucose were inferred from their RR_ts (Me_4-glucose = 1).

†Figures in parentheses indicate the molar ratio to 2,3,4,6-*O*-methyl-D-glucose (Me_4-glucose).

‡This may be 2,3,4-*O*-Me-D-glucose.

glucose, indicating that the glucan was cellulose. A small amount of 2,4,6-*O*-Me-glucose was also detected, but this may be derived from a β-1,3-glucan contaminant.

A glucan previously isolated from the hemicellulose A fraction of the tube wall preparation [1] was also examined by methylation analysis (Table 2). The main product was 2,4,6-*O*-Me-glucose. Thus the hemicellulose A glucan seemed to be 1,3-linked, possibly in β-configuration, with a degree of polymerization of *ca* 37. As for the molecular size, this glucan may differ from the tube wall callose of D.P. 21 [1] and the callose plug glucan of D.P. > 90 (unpublished results).

Microfibrils observed by electron microscopy in the pollen tube wall have been assumed to be cellulosic [11–13]. The present results furnish for the first time chemical evidence for the occurrence of cellulose in the pollen tube.

From the present results, the cellulose content of the *Camellia* pollen tube wall was calculated to be 4%. The cellulosic glucan fraction of lily pollen tube walls has been reported to be less than 7% [14]. The cellulose content of pollen tube walls seems to be low compared with the primary walls of ordinary tissue cells whose cellulose content is around 20–40% [15]. Cellulose may offer a minimum necessary plasticity for the thin wall at the apical growth region of the tube, where the callose staining reaction is negative.

By contrast, large amounts of β-1,3-glucan occur in the pollen tube wall. The overall yield of the tube wall callose is *ca* 35% [1]. The hemicellulose A glucan, suggested here to be β-1,3-glucan, corresponds to at least 4% of the tube wall material. In summary, the wall β-1,3-glucans and the callose plug β-1,3-glucan (unpublished) corresponding to about half of the α-cellulose fraction accounted for approximately 50% of the *Camellia* pollen tube wall preparation. Callose seems to be involved in (a) the lining and the reinforcing of the flexible primary wall formed at the growth zone and (b) callose plug formation.

EXPERIMENTAL

Fractionation of pollen tube wall polysaccharides. The pollen ube cell wall of *C. japonica* was prepared and fractionated into)MSO-extractable hemicellulose, pectic substance, hemicel-ulose A, hemicellulose B and α-cellulose fractions [1]. The α-ellulose fraction (0.5 g) was treated twice with 15 ml HNO_3–80% HOAc (1:10) [16] in a boiling water bath for 30 min nd centrifuged at 3000 rpm for 10 min. The supernatant was vapd to dryness *in vacuo*, and the residue suspended in H_2O and yophilized. The ppt. was washed several times with H_2O and yophilized. The α-cellulose fraction (1 g) was digested with 50 ml % β-1,3-glucanase soln in 0.1 M OAc buffer, pH 4.8, for 24 hr at 5°. The reaction mixture was centrifuged, and the ppt. was vashed and lyophilized as above.

The lyophilized preparation of β-1,3-glucanase was obtained rom a crude enzyme sample (A-12-C from *Trametes sanguinea*; upplied by Takeda Chemicals, Japan) according to the method)f ref. [17].

A hemicellulose A glucan was isolated from the hemicellulose A fraction of the *Camellia* pollen tube wall preparation in an)verall yield of 4%; it was composed of 84.4% glucose [1]. A β-,3-glucan (tube wall callose) isolated from DMSO-extractable iemicellulose fraction [1] was also used as a reference.

Other polysaccharides. Absorbent cotton was from Japanese Pharmacopoeia and curdlan was purchased from Wako Pure Chemical Ind.

Staining and microscopy. In light microscopy, the samples were tained with 0.04% aniline blue soln or 0.5% lacmoid soln (50% EtOH). $ZnCl_2$–I_2–KI and I_2–KI–H_2SO_4 reactions were per-ormed by the method of ref. [6]. In fluorescence microscopy, the amples were stained with 0.01% Calcofluor white RS soln or 0.01% decolourized aniline blue soln adjusted to pH 11 with NH_3.

Hydrolysis and sugar analysis. As in the previous paper [1], the neutral sugar composition of the wall polysaccharides was determined by GLC after hydrolysis, reduction and acetylation [18].

Methylation analysis. The polysaccharide (50 mg) dissolved in DMSO was methylated twice according to the method of ref. [19]. The α-cellulose fraction purified by acid extraction was dissolved in 4-methylmorpholine *N*-oxide at 120° [20], mixed with DMSO, and the mixture methylated using dimsyl potassium in place of dimsyl sodium [21]. The product was analysed in the same way as for the analysis of sugar composition.

IR spectra were determined in KBr discs.

REFERENCES

1. Nakamura, N., Yoshida, K. and Suzuki, H. (1980) *Plant Cell Physiol.* **21**, 1383.
2. Burke, D., Kaufman, P., McNeil, M. and Albersheim, P. (1974) *Plant Physiol.* **54**, 109.
3. Nakamura, N. and Yoshida, K. (1980) *Jpn. J. Palyn.* **25**, 11.
4. Nakamura, N. and Suzuki, H. (1981) *Phytochemistry* **20**, 981.
5. Reynolds, J. D. and Dashek, W. V. (1976) *Ann. Botany* **40**, 409.
6. Rollins, M. L. and Tripp, V. W. (1963) in *Methods in Carbohydrate Chemistry* III (Whistler, R. L., ed.), p. 335. Academic Press, New York.
7. Harada, T., Misaki, A. and Saito, H. (1968) *Arch. Biochem. Biophys.* **124**, 292.
8. Maeda, H. and Ishida, N. (1967) *J. Biochem.* **62**, 276.
9. Barcker, S. A., Bourne, E., Stacey, M. and Whiffen, D. H. (1954) *J. Chem. Soc.* 171.
10. Herth, W., Franke, W. W., Bittiger, H., Kuppel, A. and Keilich, G. (1974) *Cytobiologie* **9**, 344.
11. O'Kelley, J. C. and Carr, P. H. (1954) *Am. J. Botany* **41**, 261.
12. Mühlerthaler, K. and Linskens, H. F. (1956) *Experientia* **12**, 253.
13. Sessen, M. M. A. (1964) *Acta Bot. Neerl.* **13**, 175.
14. VanDerWoude, W. J., Morré, D. J. and Bracker, C. E. (1971) *J. Cell. Sci.* **8**, 331.
15. Setterfield, G. and Bayley, S. T. (1961) *Annu. Rev. Plant Physiol.* **12**, 35.
16. Updegraff, D. M. (1969) *Analyt. Biochem.* **32**, 420.
17. Nara, K., Fugono, K. and Yoshino, H. (1964) *Hakkokogaku Kaishi* **42**, 410.
18. Albersheim, P., Nevins, D. J., English, P. D. and Karr, A. (1967) *Carbohydr. Res.* **5**, 340.
19. Sandford, P. A. and Conrad, H. E. (1966) *Biochemistry* **5**, 1508.
20. Joseleau, J. P., Chambat, G. and Chumpitazi-Hermaza, B. (1981) *Carbohydr. Res.* **90**, 339.
21. Phillips, L. and Fraser, B. A. (1981) *Carbohydr. Res.* **90**, 149.