An Acidic Polysaccharide Isolated from the Midrib of Leaves of *Nicotiana tabacum*

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An acidic polysaccharide (APS-H) purified from the hemicellulosic fraction of the midrib of *Nicotiana tabacum* was composed of D-galacturonic acid, L-rhamnose, L-arabinose and D-galactose in a molar ratio of 31.8:15.4:9.9:42.9. Its molecular weight was estimated to be 90,000 by gel filtration chromatography. APS-H had a pectin-like structure in which the rhamnogalacturonan backbone was composed of (1->2)-linked L-rhamnopyranosyl and (1->4)-linked D-galacturonic acid residues in a ratio of approximately 1:2.1. It also contained (1->4)-linked D-galactan and (1->5)-linked L-arabinofuranosyl moieties as the side chains. Branch points occurred mainly at C-4 of (1->2)-linked L-rhamnosyl residues in the backbone and at C-6 of (1->4)-linked D-galactosyl residues in the side chains.

Plant cell wall polysaccharides are traditionally classified into three groups, namely, pectic substances, hemicellulose and α-cellulose. For many years pectic and hemicellulosic polysaccharides were distinguished by their extractability with chelating solvents and alkali solutions. Pectic polysaccharides are essentially composed of rhamnogalacturonan and associated neutral polysaccharides such as galactan and arabinan.1,2 However, Jarvis *et al.* reported that there were two rhamnogalacturonan-based fractions in the potato cell wall.3,4 The first fraction, extractable with a hot chelating solvent, was probably associated with other polysaccharides only through calcium-complexing and the steric effect of its own molecular size. The second fraction, extracted subsequently with mild alkali, was retained by covalent bonding. These two fractions showed a close similarity in the mode of linkages of sugar residues, although the latter was rich in galactan and arabinan side chains.4

In the previous paper, we reported the structure of pectin isolated from tobacco midrib by extraction with EDTA.5 The pectin had a rhamnogalacturonan backbone to which side chains of galactan and arabinan were linked probably to C-4 of the L-rhamnosyl residues.

In the present work, we have examined a polysaccharide containing a rhamnogalacturonan backbone purified from the hemicellulosic fraction of the same origin as above by means of methylation analysis, mild acid hydrolysis and periodate oxidation.

**MATERIALS AND METHODS**

*Materials.* The water-soluble hemicellulosic fraction was isolated from the midrib of leaves of *Nicotiana tabacum* cv. BY as described previously.6

*General methods.* All evaporations were performed under reduced pressure at a bath temperature not exceeding 45°C. Optical rotations were measured with a JASCO DIP-181 polarimeter using a 100 mm micro cell at 25°C. PPC was performed on Whatman No. 1 paper using the

Abbreviations: EDTA, disodium ethylenediaminetetraacetic acid; PPC, paper-partition chromatography; GLC, gas-liquid chromatography; MS, mass spectrometry.
following solvent systems; (A) ethyl acetate–pyridine–water (8:2:1), (B) ethyl acetate–acetic acid–formic acid–water (18:3:1:4) and (C) ethyl acetate–acetic acid–formic acid–water (18:8:3:9). Sugars on a paper chromatogram were detected with aniline hydrogen phthalate in 1-butanol–saturated water. The concentration of total sugar was determined by the phenol–sulfuric acid method. The content of uronic acid was determined by the $m$-hydroxy-diphenyl method of Blumenkrantz and Asboe-Hansen. Zone electrophoresis was performed on Whatman GF/A glass micro fibre paper with 0.05 m sodium borate (pH 9.2) at 2 kV for 40 min. After electrophoresis, the paper was cut into 1 cm strips, and the sugar on each strip was eluted with distilled water. The contents of total sugar and uronic acid of each eluate were measured colorimetrically. GLC was performed with a Shimadzu GC-7A or GC-4CM instrument fitted with a flame ionization detector on (A) a glass column $0.3 \times 200\text{cm})$ containing Gas Chrom P (100/120 mesh) coated with a mixture of 0.2% PEGA, 0.2% PEGS and 0.4% silicone XF-1150 and (B) a glass capillary column (0.28 mm $\times 50\text{m})$ coated with silicone OV-101. Peak areas were measured with a Hewlett-Packard 3380A digital integrator. GLC-MS was performed with a Hitachi M-80 mass spectrometer (70 eV) using column (B).

Sugar composition analysis. The polysaccharide samples were hydrolyzed with 3% sulfuric acid at 110°C for 1 hr according to the method of Saeman et al. The neutral sugar composition was determined as the alditol acetates by GLC using column (A) according to the method of Albersheim et al. For quantitative analysis, methyl-β-D-glucopyranoside was added to the hydrolyzate as an internal standard before neutralization with barium carbonate. Identification of uronic acids was conducted by reduction with sodium borohydride followed by esterification of the aldonic acids with methanol containing dried Dowex 50W (H form) at 100°C for 1 hr, followed by reduction of methyl esters of aldonic acids with sodium borohydride and acetylation, and GLC of the resulting alditol acetates. The identity of the component sugars was confirmed by their optical rotations and by PPC with solvent systems (A), (B), and (C).

Gel filtration chromatography. Gel filtration was performed on a column ($1.6 \times 90\text{cm}$) of Sepharose CL-6B which had been equilibrated with 0.5 m sodium chloride containing 5 mM EDTA. The void and included volumes were determined with blue dextran (Pharmacia Co.) and d-glucose, respectively. Carbohydrate markers for molecular weight determination were standard dextrans, T-10 ($M_w$ 9,400), T-20 ($M_w$ 18,600), T-40 ($M_w$ 39,200), T-70 ($M_w$ 71,400) and T-150 ($M_w$ 146,000) (Pharmacia Co.).

Methylation analysis. The polysaccharide samples were methylated by the method of Hakomori, modified by Sandford and Conrad. The methylated product was extracted with chloroform and purified on a column (1.0 $\times$ 90 cm) of Sephadex LH-20 eluted with chloroform–methanol (1:1, v/v). A sample of the fully methylated derivative was reduced with lithium aluminum hydride in dry tetrahydrofuran according to the method of Aspinall and McNab. The methylated derivatives were hydrolyzed by heating with 88% formic acid at 100°C for 5 hr followed by heating with 2 N trifluoroacetic acid at 120°C for 1 hr, and converted to partially methylated alditol acetates in the usual manner. Each of the alditol acetates was identified from its retention time on GLC using column (B) and the mass spectrometric data given by Lindberg et al.

Mild acid hydrolysis. A sample of the polysaccharide (40 mg) was heated with 0.05 M trifluoroacetic acid for 1 hr in a boiling water bath under reflux. After cooling, the hydrolyzate was poured into four volumes of ethanol and centrifuged. The supernatant solution was concentrated and then freeze-dried (8 mg). It contained D-galacturonic acid, L-rhamnose, L-arabinose and D-galactose in a molar ratio of 8.3 : 4.3 : 67.8 : 19.6. The precipitates were suspended in water and freeze-dried to give a degraded polysaccharide (DPS, 32 mg). DPS gave on acid hydrolysis D-galacturonic acid, L-rhamnose and D-galactose in a molar ratio of 35.6 : 21.1 : 43.3.

Periodate oxidation. A sample of the polysaccharide (40 mg) was oxidized with 0.01 M sodium metaperiodate in 0.05 M sodium acetate buffer (pH 4.0) in the dark at 5°C for 7 days. The consumption of periodate was monitored by the spectrophotometric method and checked by the use of buffered sodium arsenite and iodine titration. After the excess periodate was destroyed with ethylene glycol, the reaction mixture was dialyzed against distilled water. The oxidized polysaccharide was reduced with sodium borohydride and the excess hydride was decomposed by addition of aqueous acetic acid. The resulting polyalcohol (32 mg) was freeze-dried after dialysis against distilled water. A sample of the polyalcohol was hydrolyzed with 2 N trifluoroacetic acid at 120°C for 1 hr. After the acid was removed by evaporation, the hydrolyzate was analyzed by GLC of the alditol acetates (column A) and dithioacetal trimethylsilyl derivatives (column B).

RESULTS

Isolation of hemicellulosic polysaccharide

The water-soluble hemicellulosic polysaccharides were extracted with hot water from an alkali extract of the chlorite holocellulose of tobacco midrib as described previously. They were fractionated on a DEAE-cellulose (ace-
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In a molar ratio of 18.8 : 8.8 : 26.5 : 39.0 : 6.9.

**Anion exchange chromatography of F-3**

F-3 (1.12 g) was dissolved in 100 ml of 50 mM potassium phosphate buffer (pH 7.0) and applied to a column (3 x 35 cm) of Sephadex A-50 which had been equilibrated with the same buffer. After application of the sample, the column was washed with the buffer. The absorbed polysaccharides were eluted with a linear sodium chloride gradient, by using 400 ml of buffer and 400 ml of buffer containing m sodium chloride (Fig. 1). The contents of total sugar and uronic acid of each fraction

**Fig. 1. DEAE-Sephadex A-50 Column Chromatography of F-3.**

The eluate was collected in 10 ml fractions and analyzed for both total sugar and uronic acid. O-O, total sugar; 0-0, uronic acid.

**Table I. Sugar Compositions of Fractions from a DEAE-Sephadex A-50 Column**

<table>
<thead>
<tr>
<th>Fraction*</th>
<th>Weight (mg)</th>
<th>Sugar composition (%, wt/wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-3</td>
<td>1120</td>
<td>22.8</td>
</tr>
<tr>
<td>F-3-1</td>
<td>15</td>
<td>10.1</td>
</tr>
<tr>
<td>F-3-2</td>
<td>38</td>
<td>17.8</td>
</tr>
<tr>
<td>F-3-3</td>
<td>86</td>
<td>18.2</td>
</tr>
<tr>
<td>F-3-4</td>
<td>268</td>
<td>33.7</td>
</tr>
<tr>
<td>F-3-5</td>
<td>140</td>
<td>50.3</td>
</tr>
<tr>
<td>F-3-6</td>
<td>90</td>
<td>49.7</td>
</tr>
</tbody>
</table>

* F-3-1 was not absorbed on the column. The fractionation profile of F-3 is shown in Fig. 1.

**Table I. Sugar Compositions of Fractions from a DEAE-Sephadex A-50 Column**

- UA, uronic acid which was determined in terms of D-galacturonic acid.
Fig. 2. Gel Filtration of APS-H and APS-L on a Sepharose CL-6B Column.
Each 1 ml of 0.1% samples was applied on the column. The eluate was collected in 3.5 ml fractions and analyzed both for total sugar and uronic acid. O—O, total sugar; ●—●, uronic acid.

were measured colorimetrically. The individual fractions were collected separately, dialyzed and freeze-dried. Their yields and sugar compositions are listed in Table I.

Ultrafiltration of F-3-4
The major polysaccharide fraction (F-3-4, 250 mg), obtained by the above column chromatography, was rich in L-rhamnose and D-galactose residues. The polysaccharide was dissolved in 0.5 M sodium chloride containing 5 mM EDTA and fractionated by ultrafiltration using an Amicon PM-30 membrane under nitrogen pressure (1.0 kg/cm²). The fraction (APS-H, 160 mg), which remained on the membrane, and the filtrate (APS-L, 90 mg) were dialyzed against distilled water and freeze-dried, respectively. As shown in Fig. 2, each of them showed a single peak on Sepharose CL-6B gel filtration chromatography. The molecular weights were estimated to be 90,000 for APS-H and 16,000 for APS-L by gel filtration chromatography using standard dextrans of known molecular weights as markers. However, no significant differences in sugar compositions between these two fractions were observed.

Homogeneity of APS-H
When APS-H was re-chromatographed on a DEAE-Sephadex A-50 column in the same manner as described above, it gave a single and symmetric peak at approximately 0.33 M sodium chloride concentration. APS-H gave a single spot on zone electrophoresis in alkaline conditions.
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borate buffer (Fig. 3), and also gave a single, symmetric peak on gel filtration chromatography. APS-H showed $[\alpha]_D = +154^\circ$ (c = 0.1, 0.5 M NaCl containing 5 mM EDTA) and contained 34.2% of d-galacturonic acid residues. On acid hydrolysis it gave l-rhamnose, l-arabinose and D-galactose in relative proportions of 14.0 : 8.3 : 43.1. These results suggested that APS-H was a homogeneous acidic polysaccharide which was rich in neutral sugar residues, particularly l-rhamnose residues.

**Mild acid hydrolysis of APS-H**

Under mild conditions, partial acid hydrolysis of APS-H resulted in preferential cleavage of l-arabinose residues and afforded a degraded polysaccharide (DPS). When DPS was chromatographed on a DEAE-Sephadex A-50 column, it gave a single and symmetric peak at approximately 0.5 M sodium chloride concentration. DPS also gave a single and symmetric peak at 1.93 $V_0$ on gel filtration chromatography, indicating a molecular weight of approximately 40,000. It was composed of D-galacturonic acid, L-rhamnose and D-galactose in a molar ratio of 35.6 : 21.1 : 43.3. These results suggested that DPS was a homogeneous polysaccharide which was free from l-arabinose residues.

**Methylation analyses of APS-H and DPS**

The results of methylation analyses of APS with and without carboxyl-reduction of the fully methylated polysaccharide are shown in Table II, columns B and A. For these methylation analyses, the methylated sugars were converted to their alditol acetates and analyzed by GLC. Identification of the partially methylated alditol acetates was performed based on retention times on GLC and mass spectrometric data.17) As shown in Table II, column A, the following methylated sugars were identified as the main components of the methylated APS-H; 2,3-di-O-methyl-L-arabinose, 3,4-di- and 3-O-methyl-L-rhamnose, 2,3,4,6-tetra-, 2,3,6-tri- and 2,3-di-O-methyl-D-galactose. As shown in Table II, column B, carboxyl-reduction of the methylated APS-H gave an increased amount of 2,3-di-O-methyl-D-galactose, which should be derived from 2,3-di-O-methyl-D-galacturonic acid residues, together with the above methylated sugars.

The results of methylation analysis of DPS are shown in Table II, column C. The follow-

<table>
<thead>
<tr>
<th>Methylated sugars*</th>
<th>$t_R^b$</th>
<th>Mol%*</th>
<th>(A)</th>
<th>(B)</th>
<th>(C)</th>
<th>(D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,5-Me$_3$L-Ara.</td>
<td>0.65</td>
<td>3.1</td>
<td>3.0</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2,3-Me$_2$L-Ara.</td>
<td>0.88</td>
<td>10.2</td>
<td>8.2</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3,4-Me$_2$L-Rha.</td>
<td>0.89</td>
<td>11.1</td>
<td>10.3</td>
<td>15.0</td>
<td>3.3</td>
<td>—</td>
</tr>
<tr>
<td>3-Me$_3$L-Rha.</td>
<td>1.05</td>
<td>13.9</td>
<td>12.3</td>
<td>9.6</td>
<td>29.8</td>
<td>—</td>
</tr>
<tr>
<td>2,3,4,6-Me$_4$L-Gal.</td>
<td>1.00</td>
<td>5.6</td>
<td>5.6</td>
<td>18.7</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2,3,6-Me$_3$L-Gal.</td>
<td>1.14</td>
<td>39.5</td>
<td>35.6</td>
<td>38.6</td>
<td>3.3</td>
<td>—</td>
</tr>
<tr>
<td>2,4,6-Me$_3$L-Gal.</td>
<td>1.17</td>
<td>3.6</td>
<td>1.7</td>
<td>6.9</td>
<td>8.3</td>
<td>—</td>
</tr>
<tr>
<td>2,3,4-Me$_2$L-Gal.</td>
<td>1.25</td>
<td>2.6</td>
<td>1.7</td>
<td>6.1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2,3-Me$_2$L-Gal.</td>
<td>1.37</td>
<td>8.3</td>
<td>20.9</td>
<td>1.4</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2,4-Me$_2$L-Gal.</td>
<td>1.43</td>
<td>1.9</td>
<td>0.6</td>
<td>3.7</td>
<td>2.1</td>
<td>—</td>
</tr>
<tr>
<td>1,4-Me$_3$L-threitol</td>
<td>0.42</td>
<td>43.7</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1-Me$_3$L-threitol</td>
<td>0.58</td>
<td>9.5</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

* 2,3,5-Me$_3$L-Ara. = 2,3,5-tri-O-methyl-L-arabinose.

$^{a}$ $t_R$, retention times of the corresponding alditol acetates on an OV-101 glass capillary column, relative to that of 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-D-galactitol. Separations were performed by temperature programming at 2°C/min from 150°C to 220°C with a helium flow rate of 1 ml/min.

* A, original APS-H; B, carboxyl-reduced methylated APS-H; C, mild acid degraded APS-H (DPS); D, polyalcohol.
ing methylated sugars were identified as the main components of the methylated DPS; 3,4-di- and 3-O-methyl-L-rhamnose, 2,3,4,6-tetra- and 2,3,6-tri-O-methyl-D-galactose.

**Periodate oxidation of APS-H**

On periodate oxidation, APS-H consumed 0.86 mol of periodate per mole of anhydrogalactose residues. After hydrolysis of the polyalcohol obtained by reduction of the oxidized polysaccharide, the hydrolyzate was analyzed by GLC both as alditol acetates and di-thioacetal trimethylsilylation derivatives. The polyalcohol gave ethylene glycol, propane-1,2-diol, glycerol, D-threitol, L-rhamnose and D-galactose in a molar ratio of 27.2 : 5.4 : 26.4 : 27.1 : 9.2 : 4.7 as neutral components, analyzed as their alditol acetates. As shown in Table III, the polyalcohol also gave D-threonic acid and threono-δ-lactone which were detected by the latter method together with the above neutral components. As shown in Table II, column D, methylation analysis of the polyalcohol showed mainly 3-O-methyl-L-rhamnose, 1,4-di- and 1-O-methyl-D-threitol with small amounts of other methylated sugars.

**DISCUSSION**

An acidic polysaccharide (APS-H) was purified from the hemicellulosic fraction of tobacco midrib by successive fractionation by DEAE-cellulose and DEAE-Sephadex A-50 column chromatography followed by ultrafiltration using an Amicon PM-30 membrane. APS-H was chromatographically and electrophoretically homogeneous. The molecular weight was estimated to be 90,000 by gel filtration chromatography. APS-H showed \([\alpha]_D = +154^\circ\) and was composed of D-galacturonic acid, L-rhamnose, L-arabinose and D-galactose in a molar ratio of 31.8 : 15.4 : 9.9 : 42.9. Structural analyses were conducted on APS-H by methylation analysis, mild acid hydrolysis and periodate oxidation.

As shown in Table II, methylation analysis indicated that APS-H was a highly branched polysaccharide, in which (1→2)- and (1→2,4)-linked L-rhamnopyranosyl residues, (1→4)-linked D-galactopyranosyl residues and (1→4)-linked D-galactopyranosyluronic acid residues were predominant. These results indicate that APS-H has a rhamnogalacturonan backbone which is composed of (1→2)-linked L-rhamnopyranosyl residues and (1→4)-linked D-galactopyranosyluronic acid residues in a ratio of approximately 1:2:1. APS-H also contained a (1→4)-linked D-galactan chain, a (1→5)-linked L-arabinofuranosyl moiety and small amounts of (1→3)- and/or (1→6)-linked D-galactan.

Mild acid hydrolysis of APS-H gave a degraded polysaccharide (DPS) which was free from L-arabinose residues. Methylation analysis of DPS suggested that galactan chains were directly linked to the rhamnogalacturonan backbone, probably through C-4 of (1→2)-linked L-rhamnopyranosyl residues. L-Arabinose residues joined by (1→5)-L-arabinofuranosidic linkages were present in APS-H as short side chains and were linked to the rhamnogalacturonan backbone directly or

### Table III. Compositions of APS-H and Polyalcohol Determined by the Diethylthiobisacetate Trimethylsilylate Method

<table>
<thead>
<tr>
<th>Component</th>
<th>Peak area ratio&lt;sup&gt;a&lt;/sup&gt;</th>
<th>APS-H</th>
<th>Polyalcohol</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Rhamnose</td>
<td>1.07</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>L-Arabinose</td>
<td>0.96</td>
<td>8.0</td>
<td>—</td>
</tr>
<tr>
<td>D-Galactose</td>
<td>1.43</td>
<td>27.9</td>
<td>5.5</td>
</tr>
<tr>
<td>D-Galacturonic acid</td>
<td>1.17</td>
<td>7.2</td>
<td>—</td>
</tr>
<tr>
<td>D-Threitol</td>
<td>0.53</td>
<td>—</td>
<td>16.6</td>
</tr>
<tr>
<td>D-Threonic acid</td>
<td>0.57</td>
<td>—</td>
<td>8.5</td>
</tr>
<tr>
<td>Threono-δ-lactone</td>
<td>0.42</td>
<td>—</td>
<td>3.2</td>
</tr>
<tr>
<td>Glycerol</td>
<td>0.35</td>
<td>—</td>
<td>22.7</td>
</tr>
<tr>
<td>Propane-1,2-diol</td>
<td>0.22</td>
<td>—</td>
<td>5.3</td>
</tr>
</tbody>
</table>

<sup>a</sup> Retention times of the corresponding diethylthiobisacetate trimethylsilylates on a OV-101 glass capillary column, relative to that of inositol hexamethylsilylate. Separations were performed by temperature programming at 3°C/min from 100°C to 225°C with a helium flow rate of 1 ml/min.

<sup>b</sup> Expressed as peak area ratio relative to that of L-rhamnose (10.0).
I. Introduction

Acidic polysaccharides are isolated from tobacco midrib through galactan chains. If the arabinose residues are linked to the galactan chains, it is uncertain whether they are linked to (1→4)-linked or (1→3)- and/or (1→6)-linked galactan chains.

Methylation analysis showed that the proportion of non-reducing terminal D-galactopyranosyl residues to other sugar residues in DPS was higher than that in APS-H (Table II). This result suggested that, along with hydrolysis of L-arabinofuranosidic linkages, galactan chains were also partially hydrolyzed. Accordingly, it was assumed that the chain length of D-galactopyranosyl residues in DPS was shorter than that in APS-H.

APS-H was almost completely oxidized with periodate, although small amounts of (1→4)-linked D-galactopyranosyl residues and (1→2)-linked L-rhamnopyranosyl residues, which should be oxidized, were detected on methylation analysis, probably due to the somewhat incomplete oxidation (Table II, column D). The compounds in the acid hydrolyzate of the polyalcohol should be derived from the following sugar residues in APS-H; propane-1,2-diol from (1→2)-linked L-rhamnopyranosyl residues, D-threitol from (1→4)- and (1→4,6)-linked D-galactopyranosyluronic acid residues. On methylation analysis of the polyalcohol, the presence of 1-O-methyl-D-threitol and 3-O-methyl-L-rhamnose showed that the major branch points occurred through C-6 of (1→4)-linked D-galactopyranosyl residues and C-4 of (1→2)-linked L-rhamnopyranosyl residues. These results of periodate oxidation and methylation analysis of the polyalcohol are consistent with the structural features of APS-H assumed from the results of methylation analyses of APS-H and DPS.

We previously reported the structure of a pectin extracted with EDTA from the same source as in this study. The main structural features of APS-H seem to be similar to those of the pectin, although the latter had a somewhat long chain of (1→4)-linked D-galactopyranosyluronic acid residues. Recently, Albersheim et al. isolated and characterized a pectic polysaccharide, RG-I, from the endopolygalacturonase-solubilized material of suspension-cultured sycamore cell walls. Structural analysis indicated a close similarity between APS-H and RG-I in the mode of linkages of sugar residues, and also indicated a difference in the content of D-galactose.

APS-H could not be extracted under the conditions which are generally used for extraction of pectic polysaccharide from plant cell walls. It was extractable with an alkaline solution together with the hemicellulosic polysaccharides such as arabinoxylglucan and 4-O-methylglucuronoxylan. This finding suggests that there are two types of polysaccharides containing a rhamnogalacturonan backbone in the cell wall of tobacco midrib as reported for the potato cell wall by Jarvis et al. This also suggests that APS-H is linked to the hemicellulosic polysaccharide in the intact cell wall by a covalent or non-covalent bond. Further investigations are currently underway to clarify these points.

REFERENCES