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Detection and homogeneity of cell wall pectic polysaccharides of *Lemna minor*

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Abstract

A method was developed for determining the size homogeneity of purified pectic polysaccharide based on analysis of polysaccharide in individual diethylaminoethyl (DEAE) column fractions by high performance size exclusion chromatography. When the method was used in combination with laser light scattering, absolute M_w and M_n values of pectic polysaccharides were obtained. The method was used to show that four pectic polysaccharides were present in the 22 °C ammonium oxalate-soluble fraction of cell walls of *Lemna minor*. The four polysaccharides, designated PS-I–PS-IV, had the following M_w ranges: PS-I, 50,200–75,400; PS-II, 18,800–99,700; PS-III, 24,200–150,000; and PS-IV, 6,170–163,000. PS-IIb and PS-IVb, the major portions of PS-II and PS-IV, respectively, were heterogeneous with respect to molecular size but were chemically homogeneous. The peak M_w range of PS-IIb was 28,100–99,700, while the range for PS-IVb was 53,300–163,000. PS-IIb was composed of: GalA (54.5 ± 3.2%) (std dev), Api (39.3 ± 1.4%), Xyl (2.5 ± 1.4%), Ara (1.4 ± 0.8%), and Rha (2.3 ± 0.3%), while PS-IVb was composed of: GalA (96.3 ± 0.8%), Api (2.1 ± 0.7%), and Xyl (1.6 ± 0.3%). Small amounts (< 0.5 mole %) of fucose, mannose, and glucose and rhamnose, fucose, arabinose, mannose, and glucose were detected in PS-IIb and PS-IVb, respectively. The degree of methyl esterification of both PS-IIb and PS-IVb was zero. The isolation of a pectic polysaccharide of high galacturonic acid content (96%) and low degree of methyl esterification (~ 0%) under mild isolation conditions has not been reported previously. © 1997 Elsevier Science Ltd.

Keywords: Lemnaceae; *Lemna minor*; Homogeneity; Pectic polysaccharides; Cell walls

1. Introduction

Pectic polysaccharides constitute one of the three major polysaccharide components of primary cell walls of plants [1]. Solubilization of pectic polysaccharide material from plant cell walls by various extractants has been reported in numerous studies. Commonly the solubilized polysaccharide material is fractionated by DEAE column chromatography, and the column fractions are combined, based on the uronic acid or total sugar peaks detected, and the peaks characterized. The homogeneity of the polysaccharide peaks eluted has not been established. Numerous studies have indicated that the cell wall

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pectic polysaccharide material solubilized by chelating agents at room temperature consists of more than one component [2–12]. Our goal was to determine the number of pectic polysaccharides that is present in this fraction from a higher plant. The 22 °C ammonium oxalate-soluble pectic polysaccharide fraction from cell walls of *Lemna minor* (duckweed) [13] was chromatographed on DEAE-Trisacryl Plus M. The homogeneity of the material eluted was determined by analyzing the pectic polysaccharide in individual DEAE column fractions for three properties — M_w, glycosyl residue composition, and degree of methyl esterification — and by comparing each property across column fractions.

2. Experimental

General methods.—L. minor was grown as described elsewhere [14]. Total carbohydrate at 490 nm and uronic acid at 520 nm were determined by the methods of Dubois et al. [15] and Blumenkratz and Asboe-Hansen [16], respectively. DEAE-Trisacryl Plus M and polygalacturonic acid were purchased from Sigma Chemical Co. Apple pectic acid and D-galacturonic acid · H_2O were purchased from Aldrich Chemical Co. and Pfanstiehl Laboratories, Inc., respectively. Information on the above two polysaccharides has been given previously [17].

Isolation of pectic polysaccharides.—Cell walls were isolated from fresh *L. minor* (596 g) as described by Kindel et al. [13]. The cell walls were extracted for 30 min at 22 °C with 1070 mL of 0.05 M ammonium oxalate (pH 5.5), and the suspension was filtered through 15 μm Nylon mesh (3–15/6) (Tetko, Inc., Briarcliff Manor, NY). The cell wall residue was resuspended in 0.05 M ammonium oxalate (pH 5.5), and the suspension was filtered. The combined filtrates were filtered successively through 5 and 1.2 μm membrane filters (MF-Millipore) (Millipore Corp., Bedford, MA). A 0.5-mL portion of the final filtrate was removed to determine recovery of uronic acid, and the remainder was applied to a DEAE-Trisacryl Plus M column (2.9 × 24 cm). The column was washed with 200 mL of 0.05 M ammonium oxalate (pH 5.5) and then developed with a linear gradient of 0–0.5 M NH_4Cl in 0.05 M ammonium oxalate (pH 5.5); total vol was 4800 mL. Salt concn was measured with an in-line conductivity meter. Fractions of 20 mL were collected at 1.06 mL/min, and a portion of each was assayed for uronic acid. The M_w, glycosyl residue composition, and degree of methyl esterification of the polysaccharide in selected column fractions were determined.

High performance size exclusion chromatography (HPSEC).—The polysaccharides in the DEAE column fractions (Fig. 1) were chromatographed on a HPSEC column ($G4000PW_{XL}$, 7.8 mm × 30 cm, particle size = 10 μm) (TosoHaas, Montgomeryville, PA) and detected with an in-line differential refractometer (Knauer, λ_{max} from a light-emitting diode = 950 ± 30 nm). Centrifuged portions (100 μL) of fractions were analyzed shortly after being collected and before being frozen. The column was developed with 0.35 M NH_4Cl at a rate of 0.6 mL/min. HPSEC solvents were filtered through a 0.22-μm filter (Durapore-hydrophilic) (Millipore Corp.). The K_{av} of the polysaccharide in each column fraction analyzed was calculated by using the expression $K_{av} = V_e - V_o/V_t - V_o$, where V_e, V_o, and V_t are the elution times of pectic polysaccharide sample, Dextran T-2000, and D-xylose, respectively. The K_{av} was used to obtain the peak M_w of each polysaccharide sample graphically from the relationship K_{av} versus peak M_w. These peak M_w values of the samples are plotted in Fig. 1. The peak M_w values for the relationship K_{av} versus peak M_w were obtained from laser light scattering (LLS) as described in the next section.

Determination of M_w *and peak* M_w.—The M_w, M_n, peak M_w, peak M_n, and polydispersity of the polysaccharide(s) in column fractions 189, 207, 214, 217, 220, 223, 227, 270, 278, 282, 284, 286, 288, 290, 294, 296, and 298 (Fig. 1) were determined with a miniDawn triple-angle LLS detector (light scattering detectors are at 45°, 90°, and 135°) (Wyatt Technology Corp., Santa Barbara, CA) used in combination with the in-line differential refractometer and $G4000PW_{XL}$ HPSEC column described above. ASTRA 4.0 and EASI software (Wyatt Technology Corp.) were used to calculate M_w, M_n, peak M_w, peak M_n, and polydispersity. The K_{av} values for the above samples were calculated and graphs of K_{av} versus peak M_w were prepared. These graphs were used to obtain peak M_w values of polysaccharides from K_{av} values as described above. Peak M_w is defined as the M_w, determined either directly from light scattering data or graphically from the relationship K_{av} versus peak M_w (from LLS), of a narrow polysaccharide band at the peak of the polysaccharide band eluted from the HPSEC column and detected by differential refractometry (DR).

Refractive index increment (dn/dc) of polysaccharide samples.—The differential refractometer

was calibrated with NaCl. The Cauchy dispersion formula (terms of higher order than B/λ^2 were not used) was used to extrapolate the refractive index data of Kruis [18] at nine concns of NaCl (0.09412–6.9092 g/100 g H_2O) to 950 nm (the wavelength of light produced by the refractometer used). A plot of $(n - n_o)_{950\,nm}$ (from extrapolation) versus concn of NaCl was prepared, and the following polynomial in concn was obtained:

$$(n - n_o)_{950\,nm} \times 10^6 = 0.65345c^3 - 21.987c^2 + 1680.6c + 2.6472 \quad (1)$$

where $(n - n_o)_{950\,nm}$ is the difference in refractive index between soln (n) and solvent (n_o) at 950 nm and c is the concn of NaCl in g/100 g H_2O.

The relative refractive index (RRI) readings of five concns of NaCl (0.0056, 0.0190, 0.0571, 0.1942, and 0.5009 g/100 g H_2O), and the $(n - n_o)_{950\,nm}$ of these concns, calculated from Eq. (1), were plotted separately against concn of NaCl. The change in slope of the lines was small over the concn range of NaCl used, therefore linear regression analysis of the data was performed. The slope of the former plot, $(dRRI/dc)_{NaCl} = 2026.8$ RRI units (g NaCl/100 g $H_2O)^{-1}$, and the slope of the latter plot, $(dn/dc)_{NaCl} = 1669.9 \times 10^{-6}$ (g NaCl/100 g $H_2O)^{-1}$, were used in Eq. (2) to determine the refractive index increment of pectic polysaccharide samples.

A portion (2 mL, 0.53–0.83 mg of pectic polysaccharide) of each of column fractions 222, 223, 284, and 290 from the separation shown in Fig. 1 (polysaccharides therein were designated F222, F223, F284, and F290, respectively) was dialyzed against 0.35 M NH_4Cl for 28 h. Dialysis tubing with a molecular weight cutoff of 6000–8000 was used. Three concns of each dialyzed polysaccharide soln were passed through the differential refractometer at a rate of 2 mL/min. The relative refractive index readings were obtained with 0.35 M NH_4Cl as the reference, and the concn of samples was obtained from total carbohydrate and uronic acid determinations. The dn/dc values of the polysaccharides were calculated with the equation:

$$\frac{(dRRI/dc)_{NaCl}}{(dn/dc)_{NaCl}} = \frac{(dRRI/dc)_{polysaccharide}}{(dn/dc)_{polysaccharide}} \quad (2)$$

where $(dRRI/dc)_{polysaccharide}$ is the slope from linear regression analysis of the data in the plot of relative refractometer reading versus concn of the pectic polysaccharide tested, and $(dn/dc)_{polysaccharide}$ is the refractive index increment of the polysaccharide. The dn/dc values of the four pectic polysaccharides and polygalacturonic acid are given in Table 1. Since the dn/dc value of the polysaccharide material in every column fraction examined by LLS was not determined, appropriate dn/dc values of Table 1 were averaged, and average values were used to calculate some of the values reported in Table 2.

Table 1
Refractive index increment of pectic polysaccharides

Sample	dn/dc
Polygalacturonic acid	0.1757
F222 [a]	0.1072
F223 [a]	0.1088
F284 [a]	0.1526
F290 [a]	0.1784

[a] Polysaccharide in corresponding DEAE column fraction of Fig. 1.

Glycosyl residue composition of pectic polysaccharides.—Pectic polysaccharide samples (approximately 0.14 mg dry weight) were dialyzed against water, freeze-dried, and polymerized (L. Cheng and P.K. Kindel, in preparation). Samples were treated with 2 M HCl in dry MeOH containing trimethylorthoformate at 80 °C for 8 h. The samples were dried with N_2 at 22 °C or less and were reduced with $NaBD_4$ in aq 75% (v/v) EtOH (2 mg/mL) at 22 °C for 10 h. Glacial acetic acid was added, borate was removed with MeOH, and the samples were dried with N_2 at 30 °C. The samples were taken through a second cycle of the above methanolysis/methyl esterification and reduction procedure, and then they were hydrolyzed with 2 M CF_3CO_2H at 110 °C for 1 h and dried with N_2 at 35 °C. Alditol peracetates were prepared as described elsewhere [19–21].

Gas chromatography and mass spectrometry.—Analysis of alditol acetates was as described previously [20,21], except data collection and processing was performed by computer (SRI Instruments, Las Vegas, NV).

Electron impact mass spectrometry of alditol acetates was performed with a JEOL JMS-HX110, double-focusing mass spectrometer (JEOL USA) interfaced to a gas chromatograph, Model 5890A (Hewlett–Packard Co.), which was equipped with a splitless injector and a DB-225 fused silica capillary column (30 m × 0.32 mm, i.d.; film thickness, 0.25 μm). Helium was the carrier gas and the flow rate was 4 mL/min. The proportion of galactose units to galacturonic acid units in samples was determined by selective ion monitoring. The ratio, 6,6-dideuterioga-

lactitol peracetate/6,6-dideuteriogalactitol peracetate + galactitol peracetate, was the mean of the ratios calculated from two ion clusters: (i) ions *m/z* 217, 218, and 219 and (ii) ions *m/z* 289, 290, and 291. Standard 6,6-dideuteriogalactitol acetate was used to calibrate the mass spectrometer and was prepared from D-galacturonic acid · H_2O.

Determination of degree of methyl esterification.—The procedure of Wood and Siddiqui [22] was used to determine the methyl ester content of the polysaccharides. The degree of methyl esterification is the molar ratio of methyl ester/uronosyl unit for each sample, expressed as a percent.

^1H NMR spectroscopy.—Pectic polysaccharides F289, F290, and F292 (Fig. 1) were dialyzed against water and adjusted to pH 6.0. F289 and F292 were exchanged four times with D_2O and then adjusted to pH 6.0 with NaOD in D_2O. F290 was adjusted to pH 6.0 with NaOH before evaporation and then exchanged with D_2O. The pH of F290 remained at 6.0. Polygalacturonic acid and apple pectic acid in water were adjusted to pH 5.0 with NaOH, lyophilized, and approximately 5 mg of each was dissolved in 0.8–1.0 mL of D_2O. F223 was treated the same, except no pH adjustment was made. The polysaccharides at 90 °C were analyzed at 500 MHz with a VXR-500 NMR spectrometer (Varian Instruments). Chemical shifts were expressed relative to the HDO (in D_2O) peak at 3.937 ppm. The HDO chemical shift (3.937 ppm) was obtained by reference to a *p*-dioxane peak which was locked at 3.530 ppm at 90 °C.

3. Results

Isolation of pectic polysaccharides.—The 22 °C ammonium oxalate-soluble pectic polysaccharides from cell walls of *L. minor* were chromatographed on a DEAE-Trisacryl Plus M column (Fig. 1). Recovery of uronic acid from the column was 99.4%. The total carbohydrate elution profile (data not shown) had the same general shape as the uronic acid profile. No carbohydrate was detected in column fractions 1–110.

HPSEC.—Four pectic polysaccharides, designated PS-I–PS-IV, were detected when the DEAE column fractions (Fig. 1) were analyzed by HPSEC-DR. For all four, the K_{av} (from the HPSEC refractometry

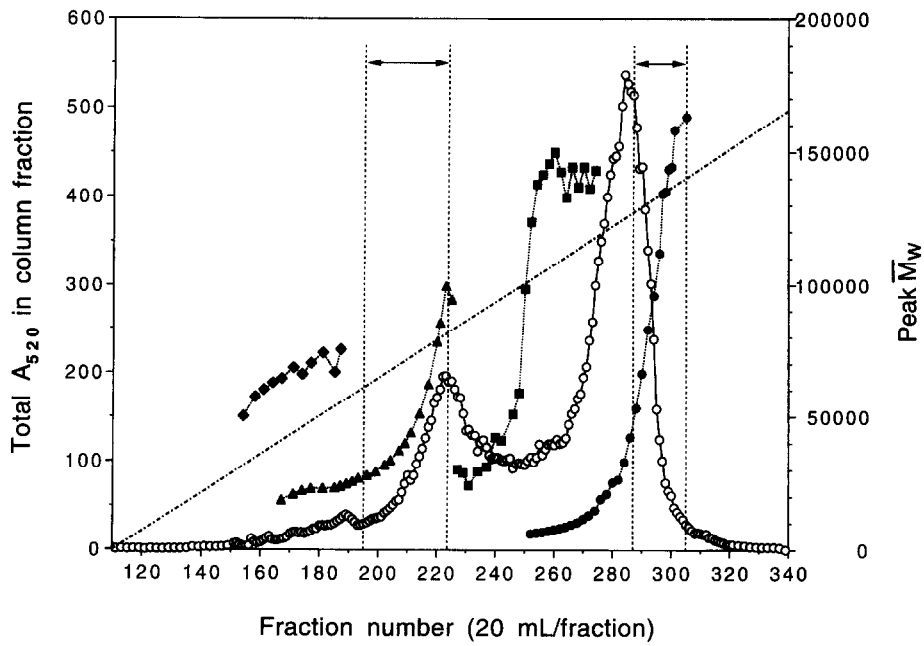

Fig. 1. Column chromatogram of the 22 °C ammonium oxalate-soluble fraction from purified cell walls of *L. minor*. The column was DEAE-Trisacryl Plus M. Column operation, determinations of uronic acid (○) and salt (– · –), and the concentration range of the salt gradient are described in the Experimental. PS-I, -II, -III, and -IV were present in column fractions designated as follows: PS-I (◆), PS-II (▲), PS-III (■), and PS-IV (●). The four polysaccharides were detected in the column fractions as described in the Experimental. The symbols showing the location of each polysaccharide also show the peak M_w values of the polysaccharides in the individual column fractions. Peak M_w values for the same polysaccharide are connected by a dotted line (· · ·). Peak M_w values were determined as described in the Experimental. The vertical dashed lines and double-headed arrows (↔) designate the column fractions constituting PS-IIb and PS-IVb.

profiles) generally decreased with increasing DEAE column fraction number until elution of polysaccharide could no longer be detected, indicating that the smaller molecules of each polysaccharide eluted first and the larger ones last. This result was confirmed by the M_w values obtained from light scattering.

In column fractions containing both PS-I and PS-II (Fig. 1), the two polysaccharides were separated completely by HPSEC and appeared as two peaks in the profile. In column fractions containing both PS-III and PS-IV (Fig. 1), the two polysaccharides were only partially separated and appeared as a double-headed peak or a peak with a shoulder. PS-II and PS-III were not detected together in any column fraction, although evidence presented in the following section suggested F227 may have contained both. In all other DEAE column fractions analyzed, the polysaccharide appeared as a single peak in the HPSEC profile. Major portions of PS-II and PS-IV were free of contaminating polysaccharides. These portions were called PS-IIb and PS-IVb, respectively. PS-IIb constitutes DEAE column fractions 195–224, while PS-IVb constitutes column fractions 287–305 (Fig. 1).

M_w and peak M_w.—The M_w, M_n, and polydispersity of the polysaccharide in the DEAE column fractions that were analyzed directly by LLS-HPSEC-DR are given in Table 2. The M_w, M_n, and polydispersity of the peak region of these samples are also given in Table 2. The two HPSEC chromatogram profiles, from light scattering and refractometry, for each of the samples listed in Table 2 were similar, except those for F227, F270, F278, F282, and F284. Each had a single peak, and the peak maxima were coincident, further indicating each sample was a single polysaccharide. In contrast, the profiles showed F270, F278, F282, F284, and possibly F227 consisted of at least two, partially resolved polysaccharides. These profiles, except those for F227, showed either two peaks or a peak and a shoulder. The profiles for F227 each showed a single peak, but the peak maxima were not coincident and the peaks were noticeably broader than those for the polysaccharide in the preceding fractions, suggesting F227 may have consisted of more than one polysaccharide.

All light scattering profiles had a peak at V_o due to polysaccharide aggregation. The aggregated material in each sample accounted for 1.9% or less of the total

Table 2
The M_w, M_n, and polydispersity of selected pectic polysaccharide samples from DEAE column chromatography of Fig. 1 [a]

Sample		dn/dc used	Entire sample			Peak (top) region of sample [b]	
			M_w	M_n	M_w/M_n	M_w	M_n
F189		0.108 [d]	42,240	26,000	1.625	25,740	23,800
F207		0.108 [d]	41,650	31,050	1.324	30,540	29,950
F214		0.108 [d]	56,790	50,020	1.135	48,890	48,430
F217		0.108 [d]	70,100	63,670	1.101	62,090	62,000
F220		0.108 [d]	80,910	72,940	1.109	74,610	74,530
F223		0.108 [d]	106,000	93,290	1.136	101,500	101,400
F227		0.108 [d]	56,020	34,370	1.630	39,270	37,500
F270	PS-III	0.130 [e]	192,200	106,300	1.808	143,400	142,400
	PS-IV	0.153	27,150	23,340	1.163	22,120	20,940
F278 [c]	PS-IV	0.153	26,860	25,180	1.067	22,210	22,100
F282 [c]	PS-IV	0.153	35,700	33,610	1.062	31,230	31,160
F284 [c]	PS-IV	0.153	41,450	38,860	1.067	35,770	35,700
F286		0.166 [f]	70,410	44,300	1.589	38,000	37,930
F288		0.166 [f]	72,520	47,840	1.516	44,910	44,880
F290		0.178	219,300	67,610	3.111	57,630	57,460
F294		0.178	191,500	109,700	1.745	95,850	90,360
F296		0.178	230,900	132,100	1.748	122,200	113,200
F298		0.178	250,000	127,800	1.916	119,500	117,200

[a] Values were obtained from LLS-HPSEC-DR as described in the Experimental.
[b] M_w/M_n (polydispersity) ranged from 1.001 to 1.082.
[c] In these samples PS-III was a shoulder.
[d] Average dn/dc of F222 and F223 of Table 1.
[e] Average dn/dc of F222, F223, and F284 of Table 1.
[f] Average dn/dc of F284 and F290 of Table 1.

material on a mass basis. The amount of aggregated material was too low to be detected by differential refractometry. There was no overlap of the sample and aggregation peaks for F189 through F288. With F290 there was a slight overlap which increased slowly for each subsequent sample to F298. Consequently the M_w and M_n values for F290 through F298 are somewhat higher than the true values. However, the similarity of M_w and peak M_w values (Table 2) indicates peak overlap is not a significant problem.

The peak M_w ranges for the four pectic polysaccharides were: PS-I, 50,200–75,400; PS-II, 18,800–99,700; PS-III, 24,200–150,000; PS-IV, 6,170–163,000.

Glycosyl residue composition of polysaccharides. —The glycosyl residue composition of the polysaccharide in individual column fractions from DEAE chromatography (Fig. 1) was determined, and the results are shown in Fig. 2. Galacturonic acid is the major component of PS-II, PS-III, and PS-IV. The apiose content of PS-II is about 40%, and this drops to about 2% in PS-IV. The sugar composition of the polysaccharide in the column fractions containing PS-IIb and PS-IVb was reasonably constant (Fig. 2), indicating PS-IIb and PS-IVb are homogeneous with respect to sugar composition. The average sugar composition of PS-IIb was: GalA (54.5 ± 3.2%) (std dev), Api (39.3 ± 1.4%), Xyl (2.5 ± 1.4%), Ara (1.4 ± 0.8%), and Rha (2.3 ± 0.3%). The average sugar composition of PS-IVb was: GalA (96.3 ± 0.8%), Api (2.1 ± 0.7%), and Xyl (1.6 ± 0.3%). The inclusion of xylose, arabinose, and rhamnose and apiose and xylose as constituents of PS-IIb and PS-IVb, respectively, is tentative. Fucose, mannose, and glucose and rhamnose, fucose, arabinose, mannose, and glucose, each at less than 0.5 mole%, were also detected by gas chromatography in PS-IIb and PS-IVb, respectively. Sugar composition analysis of the polysaccharide in fractions 301–305 (Fig. 2) was not performed because the quantity of material was too low.

Galactose was not detected in polysaccharide from twenty-three column fractions between fractions 207 and 301 (Fig. 1). Selective ion monitoring analysis showed that 100.4 ± 0.8% (std dev) of the galactitol peracetate, on a mole basis, was derived from the galacturonic acid residues of the polysaccharide and none from galactose residues. For column fractions 189 and 199, 91.3% and 96.7%, respectively, of the

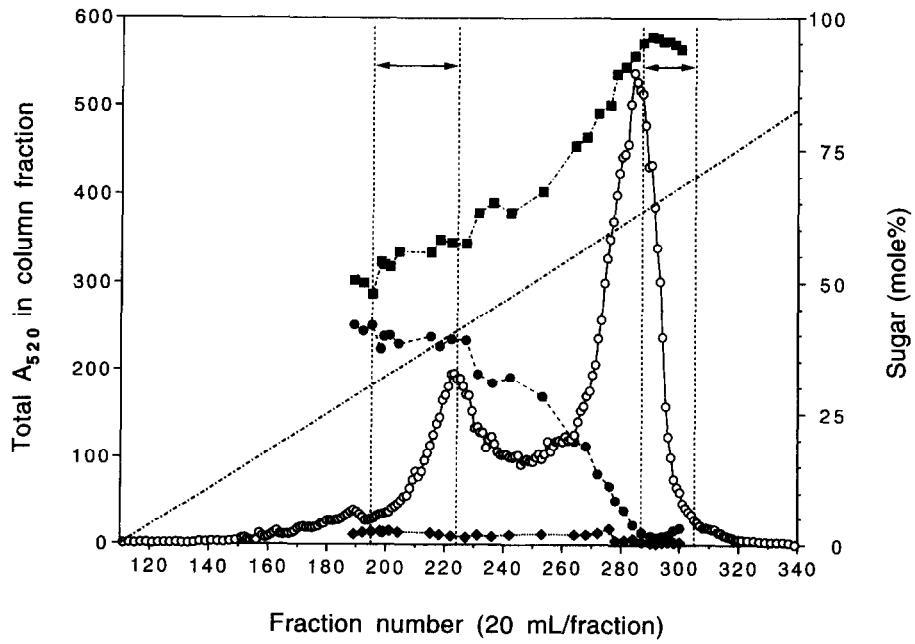

Fig. 2. Glycosyl residue composition of the pectic polysaccharide in individual column fractions from the DEAE-Trisacryl Plus M column chromatography reported in Fig. 1. For reference purposes the uronic acid (○) and salt concentration (– · –) profiles from Fig. 1 are reproduced here. Sugars are: galacturonic acid (■), apiose (●), and rhamnose (◆). Sugar composition was determined by gas chromatography as described in the Experimental. The vertical dashed lines and double-headed arrows have the same meaning as in Fig. 1.

galactitol peracetate was derived from galacturonic acid residues and 8.7% and 3.3%, respectively, from galactose residues.

Degree of methyl esterification.—The degree of methyl esterification of polysaccharide in column fractions 208, 215, 218, 222, 287, 289, 290, 292, 294, and 297 of Fig. 1 ranged from 0% to 1.6% and averaged 0.25 ± 0.56% (std dev).

^{1}H NMR spectroscopy.—The low-field portion of the 500 MHz ^{1}H NMR spectra for F289, F290, F292, polygalacturonic acid, and apple pectic acid were similar (data not shown). The H-1 and H-5 signals showed no sign of splitting [23]. These results, the similarity of these spectra to those of polygalacturonic acid and apple pectic acid, and the absence of signals for other sugars are consistent with PS-IVb having a low degree of methyl esterification and a low neutral sugar content. The spectrum of F223, a polysaccharide of PS-II, was too complex to determine whether there was splitting in the H-1 and H-5 signals. The ^{1}H NMR spectrum of F223 indicated that one or more sugars, in addition to galacturonic acid, were present in PS-IIb.

4. Discussion

A method was developed for determining the homogeneity of pectic polysaccharide peaks eluted from ion-exchange columns. The method showed four distinct pectic polysaccharides were present in the 22 °C ammonium oxalate-soluble fraction prepared from cell walls of *L. minor*. The four were detected by analyzing the polysaccharide in individual column fractions from DEAE-Trisacryl Plus M column chromatography by HPSEC-DR. In contrast, colorimetric analysis showed only two major polysaccharide peaks were eluted from the DEAE column. DEAE column chromatography was only able to partially resolve the four polysaccharides; however, PS-IIb and PS-IVb, the major portions of PS-II and PS-IV, respectively (Fig. 1), were obtained completely resolved from the other polysaccharides. The results show that the analysis of DEAE column fractions with HPSEC-DR is an effective method for determining polysaccharide homogeneity. Moreover, when these two are used in combination with LSS, absolute M_w and M_n values of polysaccharides are obtained. The results also show there is not a continuum in the structure of the pectic polysaccharide material in the fraction.

The chemical homogeneity of PS-I through PS-IV could not be determined because none was obtained completely free of the others. However, PS-IIb and PS-IVb were obtained free of the other polysaccharides and both were homogeneous with respect to glycosyl residue composition and degree of methyl esterification. PS-IIb is an apiogalacturonan while PS-IVb is a linear homogalacturonan [24,25]. The isolation of a pectic polysaccharide (PS-IVb) of high galacturonic acid content (96%) and low degree of methyl esterification (~0%) under mild isolation conditions has not been reported previously. PS-IVb was discovered only after conditions were found that gave quantitative recovery of pectic polysaccharides from the DEAE-Trisacryl Plus M column [17]. This type of pectic polysaccharide may be present in the cell walls of other plants but has not been detected because it remained bound to anion-exchange columns. The chemical structures of PS-I and PS-III have not been determined yet.

PS-I–PS-IV are heterogeneous with respect to molecular size. PS-II, -III, and -IV have broad but different M_w ranges. The M_w range of PS-I is considerably narrower. PS-IIb and PS-IVb also are heterogeneous in molecular size. The peak M_w range of PS-IIb is 28,100–99,700 while that of PS-IVb is 53,300–163,000.

The procedure that was used for isolating pectic polysaccharides eliminated or minimized losses and degradation reactions of polysaccharides. All steps that involved concentration, dialysis, or freeze-drying of polysaccharide solutions and precipitation of polysaccharides from solution were eliminated. Highly purified cell walls were isolated from fresh plants at 0–4 °C and pH 5.5, the extraction time with ammonium oxalate was short (30 min [13]) and the extraction was performed at pH 5.5 and 22 °C. The recovery of pectic polysaccharides from the DEAE column was quantitative.

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