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Structural study on arabinogalactan–proteins from *Picea abies* L. Karst

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Abstract

Two homogeneous arabinogalactan–proteins (AGPs) have been purified from spruce (*Picea abies* L. Karst) callus cells by ion-exchange and gel-permeation chromatography, followed by enzymic treatment. The apparent molecular masses of these highly glycosylated proteoglycans AGP-1 and AGP-2 were 43 and 19 kDa, respectively. Both macromolecules contained predominantly terminal α-L-arabinofuranosyl residues, terminal, 3-, 6-, and 3,6-linked β-D-galactopyranosyl residues, and terminal β-D-glucopyranosyluronic acid residues. The presence of a pyruvate substituent on O-4,6 of some of the 3-linked β-D-galactopyranosyl residues was proved for both AGPs. The protein moiety was rich in hydroxyproline, serine, threonine, and alanine. Reductive alkaline degradation of the AGPs indicated that serine and threonine were not involved in the carbohydrate–protein linkage. While sharing general structural similarity, these endoplasmic AGPs were distinguishable by composition, size, and shape of the macromolecules.

Keywords: Callus cultures; *Picea abies* L. Karst; Arabinogalactan–protein

1. Introduction

Arabinogalactan–proteins (AGPs), a heterogeneous group of proteoglycans, are common components of most higher plants and their exudates. In general, the carbohydrate moiety of these macromolecules comprises arabino-3,6-galactan (type II) and the protein moiety is characteristically rich in threonine, serine, alanine, and hydroxyproline [1]. At the subcellular level, AGPs are localized in cytoplasmic organelles [2,3], plasma membranes [4,5], cell walls [6], and extracellularly in the medium of suspension-cultured cells [5,7]. This multi-site localization of AGPs may reflect their structural heterogeneity and functional diversity. Recently it has been shown that different plant organs contain characteristic sets of AGPs or AGP epitopes that are dependent on the developmental stage of the tissue [8].

To date the biological function of AGPs is uncertain and may be diverse. AGPs, as components of the extracellular matrix and the plasma membrane, play a role as messengers in cell–cell interactions during differentiation [9,10]; specific AGPs are essential in somatic embryogenesis and are able to direct development in cells [11,12]. AGPs have been implicated in plant growth where

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they play the role of a sensor for the elongation growth of the cell wall [13]. The accumulation of AGPs in response to wounding has also been evidenced, suggesting a possible role for these molecules in plant defence systems [14].

The effect of soluble AGPs and their fragments isolated from spruce *P. abies* callus cells in auxin-stimulated elongation growth of pea *Pisum sativum* segments has been ascertained in our experiments (unpublished results). In the present paper we report on the isolation of spruce AGPs and the characterization of their carbohydrate moieties.

2. Experimental

Plant material.—Spruce callus *P. abies* L. Karst was cultured for several years on a modified Brown and Lawrence [15] medium supplemented with α-naphthaleneacetic acid (1 mg/L), kinetin (1 mg/L), casein hydrolyzate (500 mg/L), vitamins (three times strengthened), and 0.8% agar at 23 ± 1 °C in a 16 h photoperiod (45–60 $\mu Em^{-2}s^{-1}$ light intensity) and 68% relative humidity, and subcultured in 40 day intervals.

General.—Spectral data were recorded with a Perkin-Elmer G 9836 (IR) and a Specord UV-visible spectrometer. Optical rotations (1 mL cell) were measured with a Perkin-Elmer Model 141 polarimeter at 20 ± 1 °C. Free-boundary electrophoresis of polysaccharide solutions (10 mg/mL) was performed in 50 mM sodium tetraborate buffer (pH 9.2) using a Zeiss 35 apparatus for 30 min at 150 V and 8 mA. Polyacrylamide gel electrophoresis (PAGE) was carried out in a disc electrophoretic apparatus at pH 8.9 according to Ornstein and Davis [16]. Carbohydrate and proteins were stained by the periodate-Schiff reagent and a Coomassie Brilliant Blue R-250 (Serva) assay, respectively. GC–MS was performed with a Finnigan MAT SSQ 710 mass spectrometer coupled with a Varian 3400 gas chromatograph. A fused silica capillary column (SP-2330, 30 m × 0.25 mm i.d., 0.2 μm film thickness) was used: temperature range, 80–240 °C at 8 °C/min; helium (25 mL/min) as carrier gas. Column effluents were analyzed by CI–MS with pyridine as the reagent gas and by EI–MS. NMR spectra of the samples (3% in D_2O) were recorded with a Bruker AM-300 FT-spectrometer at 25 °C using $(CH_3)_2CO$ (2.2 ppm) or MeOH (50.15 ppm) as internal references for ^1H and ^{13}C, respectively.

Polysaccharides were hydrolyzed with aq 90% HCOOH at 100 °C for 7 h. Neutral sugars were analyzed as their alditol trifluoroacetates by GC on a PAS-1701 column (25 m × 0.32 mm) maintained at 110 °C for 2 min, then the temperature was raised to 165 °C at 20 °C/min, with H_2 (7 mL/min) as carrier gas. Myo-inositol hexaacetate was used as the internal standard. Amino acid compositions were determined after hydrolysis with 6 M HCl (100 °C, 20 h) on an automatic amino acid analyzer T-339 (Mikrotechna, Prague). Hydroxyproline was determined according to Drozdz et al. [17]. The pyruvate content was determined by the lactate dehydrogenase method of Duckworth and Yaphe [18] following acid hydrolysis in 40 mM oxalic acid (100 °C, 4 h). Reducing sugars, protein, and uronic acid contents were determined by the Somogyi [19], Lowry [20], and *m*-phenylphenol [21] methods. The absolute configuration (D or L) of the glycosyl residues was determined as described by Gerwig et al. [22].

High-performance gel-permeation chromatography (HPGPC).—HPGPC was performed using a commercial instrument (Laboratorní přístroje, Prague) equipped with two Tessek Separon HEMA BIO-100 exclusion columns (8 × 200 mm) and 0.1 M aq $NaNO_3$ as eluant. The eluate was monitored by RI and UV detectors (254 nm). The column was calibrated with pullulan standards P-5, P-10, P-20, and P-50 (Macherey-Nagel).

β-*Lectin assay*.—The β-lectin assay was carried out according to Jermyn and Yeow [23]. The substrate, i.e., 1,3,5-tris(4-β-D-galactopyranosyloxyphenylazo)-2,4,6-trihydroxybenzene [24], was synthesized by coupling diazophenyl β-D-galactopyranoside to phloroglucinol.

AGP.—(a) Isolation and purification. Approximately 1290 g fresh weight of callus tissue cells, at the late linear phase of growth (30 days after inoculation), were dispersed on a rotatory shaker in a mineral salts solution [15] supplemented with 0.4 M sucrose. The suspension obtained was filtered through a nylon net (100 μm openings) and washed exhaustively with cold (4 °C) distilled water. The washed cells (1204 g) were homogenized with an X-Press Cell Disintegrator (LKB-Biotec, Sweden) at −35 °C and 200 MPa. The homogenate was filtered through Miracloth and the cell walls were prepared according to Kivilaan et al. [25]. Cell wall material (CWM) from mechanically disrupted cells was exhaustively extracted with 2:1 CH_3Cl–MeOH, and the lipid-free material was

Table 1
Polysaccharides from *P. abies* L. Karst callus tissue cells

Polysaccharide [a]	EP	CWM
$[\alpha]_D^\circ$	−35	
Protein (%) [c]	10.0	12.6
Uronic acids (%) [c]	13.4	22.1
Pyruvic acid (%) [c]	1.9	
Methoxyl		0.6
Acetyl	0.6	1.5
Carbohydrate composition (mol %) [b]		
Fuc	tr [d]	2.1
Rha	1.4	2.4
Ara	16.1	23.5
Xyl	12.7	9.6
Man	0.6	2.4
Gal	63.5	8.9
Glc	5.7	51.1

[a] Polysaccharide after elimination of non-covalently bound protein and lipids.
[b] Determined as alditol trifluoroacetates by GC (see Experimental).
[c] On dry-weight bases.
[d] tr, trace.

treated with amylase in 50 mM phosphate buffer (pH 7.0) to remove starch contamination. Cell walls were then washed with deionized water (300 mL) and dried with acetone. The filtrate was first centrifuged at 6000 *g* for 20 min to remove nuclei and cell debris, and then at 165,000 *g* for 90 min to sediment the membraneous organelles. The 165,000 *g* pellet was resuspended in water and resedimented in the same way. Both supernatants were combined, concentrated (50 mL), and then extracted (30 min with stirring) with an equal volume of 2:1:1 phenol–HOAc–water at 65 °C to remove non-covalently bound protein [26]. After centrifugation, the aqueous layer was removed, dialyzed against water (2 days), lyophilized, dissolved in 50 mM phosphate buffer (pH 7.0), and treated with amylase (type II A, Sigma, 100 μg/mL) at 37 °C for 2 days in the presence of toluene. The digest was concentrated, dialyzed, and the starch-free endopolysaccharide (EP), $[\alpha]_D$ −35° (c. 1, water) (86 mg/100 g of the tissue fresh weight) was recovered by freeze-drying. Table 1 contains the analytical characteristics of EP and CWM preparations.

EP (150 mg) in 2 mM KOAc (pH 6.4, 2 mL) was applied to a column (2.8×30 cm) of QAE-Sephadex Q-50 (AcO^-) and eluted with 2 mM KOAc (300 mL), and then with a linear concentration gradient of 2 mM→0.65 M KOAc (700 mL), followed by 0.3 M NaOH (300 mL). For preparative purposes the chromatographic runs were repeated four times and the eluate was separated into four parts by collecting fraction Nos 0–40 (**1**), 60–74 (**2**), 76–124 (**3**), and 160–174 (**4**) (Fig. 1). Rechromatography of subfractions **2** and **3** on the same column upon elution with a linear concentration gradient of 0→0.15 M and 0.15→0.6 M KOAc, respectively,

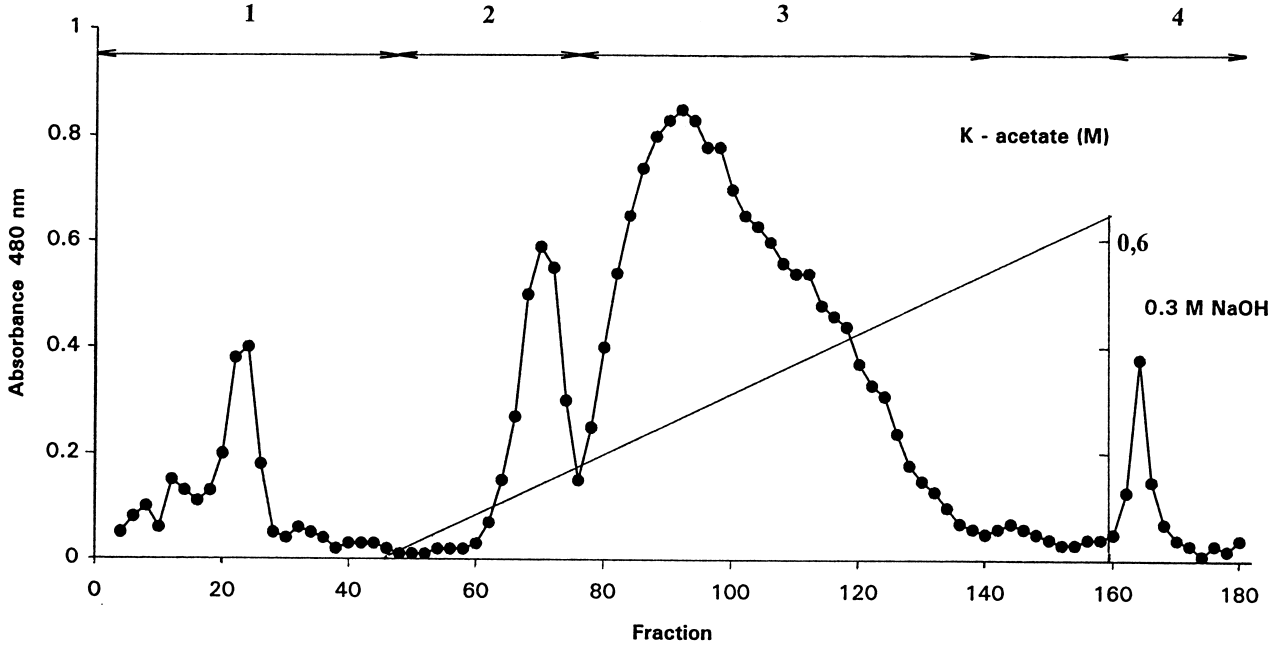

Fig. 1. Fractionation of EP by anion-exchange chromatography on QAE-Sephadex Q-50. **1–4**, collected fractions. Carbohydrate was assayed by the phenol–sulphuric acid method.

Table 2
Fractionation of polysaccharide EP[a] on QAE-Sephadex Q-50

Fraction	Yield[b]	$[\alpha]_D$[c]	Uronic acid	Protein	Molar ratios of monosaccharides						
	(%)	(°)	(%)	(%)	Gal	Glc	Man	Ara	Xyl	Rha	Fuc
1	2.8	−21.5	3.9	2.7	14.7	30.7	4.4	55.8	10.0		1.9
2	12.4	−42.4	11.6	7.3	11.0	0.7	0.3	5.8	10.0	0.2	0.3
3	55.7	−33.8	12.0	7.0	79.2	2.1		28.5	10.0	4.0	tr[d]
4	10.2	+61.7	5.2	0.9	23.6	5.3	2.6	13.3	10.0	2.2	3.6

[a] Cytoplasmic origin.
[b] Weight % of material applied to column.
[c] Water.
[d] tr, trace.

gave polysaccharide fractions **2** and **3**, the composition of which remained unchanged (Table 2).

Fractions **2** and **3** (2% w/v each) in 10 mM NaOAc buffer (pH 5.0, 50 mL) were separately incubated with a purified endo-(1→4)-β-D-xylanase (EC 3.2.1.8) from *Trametes hirsuta* (10 mg, 1.5 μ Kat) [27] at 40 °C for 2 days. The digest was heated for 10 min in a boiling water bath, centrifuged, dialyzed, and freeze-dried. The composition of polysaccharides AGP-1 and AGP-2 is detailed in Tables 3 and 4.

(b) Methylation analysis. Each polysaccharide (~10 mg) was methylated once by the Hakomori method [28], then repeatedly by the Purdie–Irvine procedure [29], and purified using a Sep-Pak C_{18} cartridge (Waters Assoc.) to give a product that had no IR absorption for hydroxyl groups. The methylated sample was carboxyl-reduced with lithium triethylborodeuteride (Super-Deuteride, Aldrich-Chemie) for 90 min at 20 °C. After addition of HOAc and dialysis, the recovered material was divided into two portions. One was remethylated [29] and the other was partially hydrolyzed with aq 40% HOAc (80 °C, 40 min), to cleave the pyruvate acetal residues, then trideuteriomethylated. Both samples were hydrolyzed with 2 M CF_3CO_2H (100 °C, 2 h), reduced with $NaBH_4$, acetylated, and analyzed by GC–MS [30,31]. The molar response of each methylated sugar was corrected according to Sweet et al. [32]. The results are given in Table 5.

(c) Periodate oxidation and Smith degradation [33]. Each polysaccharide (~40 mg) was stirred with 0.15 M $NaIO_4$ (10 mL) at 4 °C in the dark. After 96 h, ethylene glycol was added to reduce the excess of periodate, the oxidized polysaccharide was reduced with $NaBH_4$ for 16 h, HOAc was

Table 3
Data on purified AGPs

Polysaccharide	AGP-1	AGP-2
$[\alpha]_D$(water) (°)	−33.8	−15.7
Electrophoretic mobility ($cm^2V^{-1}s^{-1}$)	2.52×10^{-5}	n.d.[d]
Protein (%)	7.0	7.3
Uronic acids (%)	12.1	11.6
Pyruvic acid (%)	1.25	0.7
M_w[a]	43,000	19,000
M_n[b]	24,000	12,500
M_w/M_n	1.8	1.5
Carbohydrate composition (mol %)		
Fuc	tr[c]	1.2
Rha	3.2	1.1
Gal	68.7	77.3
Man		2.0
Ara	24.0	14.3
Xyl	4.1	4.1
Gal/Ara	2.9	5.4

[a] Data from HPGPC analysis.
[b] Data from osmometry.
[c] tr = trace.
[d] n.d. not determined.

Table 4
Amino acid composition of spruce AGPs

Amino acid	mole %	
	AGP-1	AGP-2
Lys	1.5	1.4
His	1.6	tr[a]
Hyp	12.8	13.5
Asp	10.2	10.4
Thr	10.4	8.9
Ser	20.1	19.8
Glu	3.6	4.9
Pro	4.1	tr[a]
Gly	7.4	9.8
Ala	15.5	14.3
Val	4.2	4.5
Ile	1.6	2.0
Leu	3.5	8.2
Tyr	tr	tr
Phe	3.5	2.3

[a] tr = trace.

Table 5
Methylation analysis of spruce AGPs

Glycosyl residue	Position of		Linkage	Mole %						$[M+PyrH]^+$
	OCH_3	OCD_3		AGP-1[a]			AGP-2[a]			
				A	B	C	A	B	C	
Ara	2,3,5		T[c]	9.7	10.5	2.6	8.5	8.3		358
	2,5		3	1.5	1.0		1.5	1.2		386
	2,3		5	5.9	6.0	3.0	3.5	3.9		386
	3		2,5	1.5	1.7					414
	2		3,5	3.2	2.9					414
Xyl	2,3,4		T	tr[d]	tr		1.4	1.6		358
	2,3 (3,4)		4 (2)	4.2	4.4		1.8	2.6		386
Man	2,3,4,6		T				1.4	1.4		402
Gal	2,3,4,6		T	9.8	11.9	31.6	13.0	12.5	35.7	402
	2,4,6		3	21.3	20.9	27.9	22.6	21.9	23.5	430
	2	4,6	3(4,6-pyruvyl)		2.2			0.9		436
	2,3,6		4	3.2	2.1		5.8	6.2		430
	2,3,4		6	5.1	5.2	10.9	8.8	9.2	23.0	430
	2,6		3,4				2.4	1.9		458
	2,3		4,6				2.4	1.8		458
	2,4		3,6	16.2	15.4	16.1	10.1	9.5	8.3	458
	2		3,4,6	3.9	2.6	6.5	5.7	5.2	9.5	486
Rha	2,3,4		T	tr	tr		tr	tr		372
	3,4		2	1.1	1.1	1.3	1.0	0.8		400
	3		2,4	1.2	1.1		tr			428
Fuc	2,3,4		T				0.8	1.0		372
GlcA[b]	2,3,4,6		T	8.9	8.1		4.4	5.7		404
	2,3,6		4				0.7	1.0		432
GalA[b]	2,3,6		4	3.3	3.0		4.2	3.4		432

[a]A, AGP methylated, carboxyl-reduced ($LiB(C_2H_5)_3D$) followed by remethylation with CH_3I and hydrolysis; B, AGP methylated, carboxyl-reduced ($LiB(C_2H_5)_3D$), hydrolyzed with HOAc followed by remethylation with CD_3I and hydrolyzed; C, AGP after Smith degradation.
[b]Deuterium-labelled at C-6.
[c]T, a nonreducing terminal residue.
[d]Trace.

added, and the solution was dialyzed. After mild hydrolysis with 0.5 M CF_3CO_2H at ambient temperature for 70 h, the solution was dialyzed and the degraded polysaccharide was recovered by lyophilization. The degraded polymers were analyzed by HPGPC and for their glycosyl linkage composition.

(d) Treatment of AGPs with alkaline $NaBH_4$. Each polysaccharide (20 mg) was treated with 0.1 M NaOH (10 mL) containing 0.3 M $NaBH_4$ at 5 °C for 16 h [34]. The solution was adjusted to pH 6.0 with 1 M HOAc, concentrated, and desalted on Bio-Gel P-2. The product was analyzed by HPGPC and for its amino acid composition.

3. Results and discussion

A homogenate of spruce *P. abies* callus tissue cells harvested at the late linear phase of growth was separated into a soluble endopolysaccharide preparation EP and insoluble cell wall material CWM. The EP, after removal of starch and noncovalently bound protein [26], contained 75% neutral sugars represented by D-galactose and L-arabinose (molar ratio 3.9), and small proportions of D-xylose, D-glucose, L-rhamnose, L-fucose, and D-mannose, 13.4% uronic acid, 10% protein, and 1.9% pyruvic acid. As seen from Table 1, the chemical composition of this preparation was different from that of the insoluble CWM suggesting differences in the types and content of constituent polysaccharides.

The crude EP, heterogeneous by free-boundary electrophoresis, was fractionated on a column of QAE-Sephadex Q-50 (Fig. 1) into four fractions. The most abundant fractions, **2** and **3**, after rechromatography (Table 2) and treatment with a purified *Trametes hirsuta* β-D-xylanase [27], gave two protein-containing acidic polysaccharides, AGP-1 and AGP-2, homogeneous by PAGE and HPGPC. Both polysaccharides were eluted as

nearly symmetrical peaks with apparent molecular masses of 43 and 19 kDa for AGP-1 and AGP-2, respectively. The identical elution profiles and electrophoretic mobilities of carbohydrates and proteins (Fig. 2) indicated that protein was an integral part of these proteoglycans.

Physico-chemical constants and chemical compositions of spruce AGPs are presented in Table 3. Both macromolecules, with a binding affinity for the β-galactosyl Yariv reagent [23], were dominated by galactose and arabinose associated with minor amounts of xylose, rhamnose, fucose, and mannose, but differed in the Gal/Ara molar ratio, which was 1.86 times higher in AGP-2 than in AGP-1. The content of uronic acids, represented mainly by glucuronic acid and a smaller amount of galacturonic acid, was similar in both polysaccharides. The presence of pyruvate groups in amounts of 1.3% for AGP-1 and 0.7% for AGP-2 (Table 3) is notable. This was shown to be a characteristic feature of spruce AGPs. As estimated by a colorimetric assay, the protein contents of AGP-1 and AGP-2 were 7.0 and 8.2%, respectively, or 5.5 and 6.3%, respectively, when calculated from an amino acid analysis. These data, with the marked exception of the pyruvate acetal substitution, are in general agreement with the compositions of previously studied AGPs [1].

Glycosyl linkage analysis of the methylated, carboxyl-reduced AGPs (Table 5) indicated similar structures with high degree of branching. The main features of the galactan moiety were terminal,

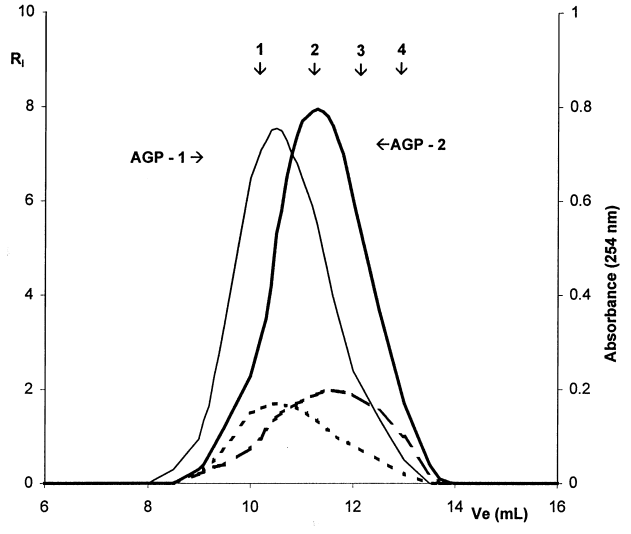

Fig. 2. HPGPC chromatogram of AGPs on Separon HEMA BIO-100; (—,—) total sugar, (—,···) protein. Arrows 1–4 indicate positions of molecular mass standards of 50, 20, 10, and 5 kDa, respectively.

3-, and 3,6-linked galactopyranose, though 6-, 3,4,6-, 4-, and 4,6-linked galactopyranose residues were also detected in minor proportions. Arabinofuranose was predominantly present as terminal and 5-linked residues, but 3-, 2,5-, and 3,5-linked residues were also detected. Rhamnose was shown to be 2- and 2,4-linked, whereas xylose was found mainly in the 4- or 2-linked form. The appearance of deuterated terminal and 4-linked glucose and 4-linked galactose indicated that glucuronic acid occupied terminal and 4-linked positions, while galacturonic acid was linked through O-4.

To identify the sugar residues bearing pyruvate acetal groups and to locate their positions the following strategy was followed. Treatment of the methylated and carboxyl-reduced polysaccharides with acid, followed by trideuteriomethylation, led to specific cleavage of the pyruvyl residues and trideuteriomethylation of the generated free hydroxyl groups. The products, converted conventionally into partially methylated alditol acetates, were analyzed by GC–MS. Under the conditions of CI (pyridine) MS, protonated pyridine $[PyrH]^+$ and partially methylated alditol acetates form stable $[M+PyrH]^+$ adduct ions, which enable the molecular mass and, consequently, the number of OCD_3 groups [31] to be determined, thereby complementing the EI–MS analysis. The appearance of 4,6-di-*O*-trideuteriomethyl-2-*O*-methylgalactose, in addition to the residues mentioned above, was evidence that in both polymers some of the 3-linked galactose residues contained pyruvate substituents at O-4,6 (Table 5, treatment B).

Smith degradation [33] of AGP-1 and AGP-2 resulted in a 53 and 60% loss of material in accord with the predicted amounts based on the presence of periodate-sensitive residues. HPGPC indicated single fragments with apparent molecular masses of 4.8 and 2.9 kDa that consisted of galactose and arabinose in the molar proportions of 93:7 for AGP-1 and entirely of galactose for AGP-2. The marked decrease in the molecular masses of the polysaccharides does not agree with the predicted value calculated from the loss of carbohydrates during Smith degradation. Thus, the presence of a sub-unit structure, as proposed for other AGPs [35,36], cannot be excluded. The glycosyl linkage compositions of the degraded polymers (Table 5, column C) revealed increased proportions of terminal and 6-linked galactose with a concominant decrease in proportions of terminal arabinose, 3-, and 3,6-linked galactose residues. As expected, the

uronic acid constituents of the AGPs were completely oxidized by periodate. The survival of arabinose residues in the AGPs may have been the result of the presence of a few internal periodate-resistant residues. These results suggested that most of the arabinose residues that attached as single terminal units and 2,5- or 3,5-linked residues to O-3 of the terminal or the interchain 6-linked galactose residues that constitute the side chains of the arabinogalactan framework.

The AGPs contained small proportions of 4-linked galacturonic acid together with 2- and 2,4-linked rhamnose. After Smith degradation only 2-linked rhamnose was detected in the degraded polymer, indicating that a fragment of the rhamnogalacturonan core and the arabinogalactan moiety of the AGPs might be interlinked through O-4 of rhamnose to form a pectin–arabinogalactan–protein complex. The xylose present as terminal groups or as 4- or 2-linked residues remaining after *T. hirsuta* β-D-xylanase treatment [27] led to the assumption that xylose residues may be linked to the AGPs framework; however, the possibility of contamination with a co-purified xylan cannot be excluded.

The essentially similar structural features of AGP-1 and AGP-2 were confirmed also by the ^{13}C and ^{1}H NMR spectra, which differed only in the relative intensities of the signals present (Fig. 3). The signals due to anomeric carbons at 110.5 and 108.7 ppm could be assigned to terminal and internal α-L-Araf residues, and the signals at 104.9, 103.9, 103.1, 102.1, 100.6, and 99.2 ppm to β-D-Gal*p*, β-D-Xyl*p*, β-D-Glc*p*A, α-L-Rha*p*, α-L-Fuc*p*, and α-D-Gal*p*A residues by comparison with literature data [37,38]. Diagnostic ^{1}H and ^{13}C chemical shift values were observed at 1.47 and 25.0 ppm, respectively, for the methyl groups of the

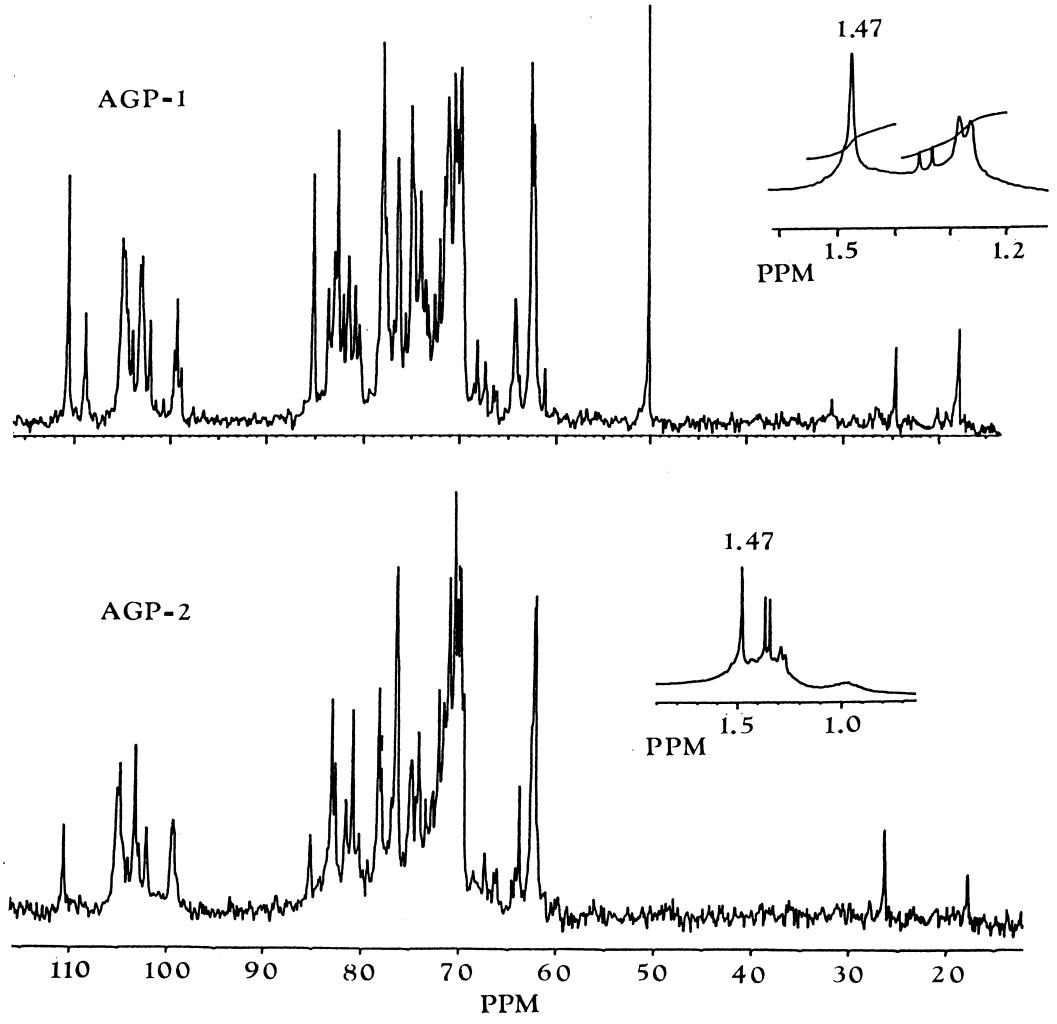

Fig. 3. ^{13}C and partial ^{1}H NMR spectra of AGP-1 and AGP-2 isolated from EP of spruce.

pyruvate acetal [39]. From these values, especially in the ^{13}C NMR spectra, it was possible to assign the *R*-configuration to the acetal carbon atom. Thus, the identified 4,6-*O*-[(*R*)-pyruvyl]-β-D-galactopyranosyl unit, a common component in extracellular bacterial polysaccharides [40], was found to be a characteristic feature of AGPs from spruce callus cell.

The protein moieties of the AGPs were similar and rich in serine, alanine, hydroxyproline, and threonine, corresponding to half of the amino acid content. The amino acid compositions of alkali-treated AGPs remained essentially unaltered, suggesting that O-glycosyl linkages through serine and/or threonine are unlikely in spruce proteoglycans. In view of these results, the most probable mode of linkage between arabinogalactan and protein is an alkali-resistant linkage through hydroxyproline. From the preceding results it may be concluded that the glycan portion of both spruce AGPs is a highly branched acidic arabino-3,6-galactan with different side branchings attached to O-6 of the 3-linked β-D-galactan core. The polysaccharide moieties differed from each other in sugar composition, linkage types, and physico-chemical properties.

Acidic AGPs from spruce callus cells have structural features essentially the same as those reported for proteoglycans from leaves of *Nicotiana tabacum* [41] and *Raphanus sativus* [42], cell cultures of *Arnica montana* [43], culture medium, and plasma membrane of Paul's Scarlet rose [5,44]. Some differences are apparent in chemical composition and fine structure related to plant origin. In addition, however, spruce AGPs possess pyruvate acetal groups linked to O-4,6 of some of the 3-linked galactosyl residues, which is a unique structural feature.

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