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Structural Characterization of an Arabinogalactan-Protein from the Fruits of *Lycium ruthenicum*

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ABSTRACT: A water-soluble arabinogalactan-protein (AGP), designated LRGP3, was isolated from the fruits of *Lycium ruthenicum* Murr. Its average molecular weight was 75.6 kDa. Monosaccharide composition analysis revealed that it was composed of rhamnose, arabinose, and galactose in a molar ratio of 1.0:14.9:10.4. Protein accounted for 1.7% of the AGP and was rich in hydroxyproline. On the basis of monosaccharide composition, partial acid hydrolysis, methylation analysis, ESI-MS, and NMR spectroscopy, LRGP3 was characterized as a highly branched polysaccharide with a backbone of (1→3)-linked β-D-galactopyranosyl residues, many of which were substituted at the O-6 position by galactosyl or arabinosyl groups. The branches were composed of (1→5)-linked arabinose, (1→2)-linked arabinose, (1→6)-linked galactose, (1→3)-linked galactose, and (1→2,4)-linked rhamnose, and the major nonreducing termini were α-L-arabinofuranosyl residues.

KEYWORDS: *Lycium ruthenicum, polysaccharide, structure, glycoconjugate, arabinogalactan-proteins*

INTRODUCTION

Lycium ruthenicum Murr. belongs to the Solanaceae family and is widely distributed in the salinized desert of northwestern China. *L. ruthenicum* is a nutraceutical food that has been used for the treatment of heart disease, hypertension, and menopause. *L. ruthenicum* contains many functional components such as pigments, essential oils, and polysaccharides. The structure and antioxidant activity of pigments have been demonstrated,[1] and the composition of the essential oils in *L. ruthenicum* has been well documented.[2] Up to now, no comprehensive studies have been conducted to explore *L. ruthenicum* polysaccharides. Arabinogalactan-proteins (AGPs) are widely distributed in higher plants, and they are localized in cytoplasmic organelles, plasma membranes and cell walls.[3] Both the intact AGPs and purfied arabinogalactan of many plants are highly water-soluble and possess immunomodulatory, hypoglycemic, and other biological activities, which are hot spots of research on functional factors of protective foods.[4−6]

In our previous studies, crude *L. ruthenicum* polysaccharide (CLRP) was isolated from the fruits of *L. ruthenicum*. Chemical analysis showed that CLRP was composed of 68.7% neutral sugar and 24.5% acid sugar; monosaccharide composition test indicated that CLRP was composed of arabinose (40.7%), galacturonic acid (26.4%), galactose (18.9%), xylose (5.1%), rhamnose (4.9%), glucose (2.7%), and mannose (1.3%). CLRP was further purified by anion-exchange chromatography and gel filtration, and five glycoconjugates, LRGP1−LRGP5, were isolated from *L. ruthenicum*.[7] In the present study, we report the structural characterization of the AGP LRGP3 based on monosaccharide composition, partial acid hydrolysis, methylation analysis, ESI-MS, and NMR, and our results provide a basis for future bioactivity studies.

MATERIALS AND METHODS

Materials. The dried mature fruit of *L. ruthenicum* was purchased from Jiahe Biological Engineering Co. (Qinghai, China). It was dried at 50 °C and crushed into powder.

Chemicals. Standard monosaccharides and T-dextran series of different standard molecular weights were purchased from Sigma Chemical Co. (St. Louis, MO, USA); DEAE-Cellulose-52 was purchased from HengXin Chemical Reagent Co. (Shanghai, China); Sephadex G-100 was purchased from Pharmacia Co. (Uppsala, Sweden); Dowex 50 WX8-400 cation exchange resin was from Sigma-Aldrich (St. Louis, MO, USA); other reagents used were of analytical grade.

General Methods. The carbohydrate content was determined by the H_2SO_4−phenol method using glucose as a standard.[8] The protein content was estimated according to the method of Bradford using ovalbumin as a standard.[9] All of the measurements were repeated three times. A Lambda 25 UV−vis spectrophotometer (Perkin-Elmer, Boston, MA, USA) was used to detect the absorbance. A UV scan in the region of 200−400 nm was performed on the spectrophotometer. The IR spectrum was determined using an EQUINOX 55 Fourier transform infrared spectrophotometer (Bruker, Germany).

Purification of LRGP3. LRP3 was isolated from the fruits of *L. ruthenicum* as described previously.[7] LRP3 (80 mg) was further purified by gel permeation chromatography on a Sephadex G-100 column (1.5 cm × 100 cm) using a 0.1 M NaCl solution as an eluent. The fractions were monitored by UV absorption at 280 nm for protein content and for total carbohydrate by the H_2SO_4−phenol method using glucose as a standard. The fractions corresponding to the main carbohydrate-containing peak of the chromatography elution profile were pooled. After dialysis and lyophilization, a white fluffy material

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(LRGP3) was obtained. This process was repeated until the necessary quantities of LRGP3 for future experiments were obtained.

Homogeneity and Molecular Weight Determination. The homogeneity and molecular weight of samples were determined by high-performance gel permeation chromatography on a TSK-Gel G3000SW column (Tosoh, Japan). A Waters 2695 high-performance liquid chromatography system coupled with a Waters Alliance 2414 refractive index detector (Waters, Milford, MA, USA) was used. The temperature of the column and the detector was kept at 30 °C. The sample concentration was 0.2% (w/v), and the injection volume was 20 μL. The eluent was the mixture of 0.1 M phosphate buffer (pH 6.0) and 0.1 M Na_2SO_4 with a volume ratio of 1:1, which was passed through Millipore filters (0.45 μm). The flow rate was 0.5 mL/min. The linear regression was calibrated with dextrans 5,000, 12,000, 25,000, 80,000, and 150,000. V_t and V_0 were calibrated with glucose and dextran blue (2,000,000), respectively.

Monosaccharide Composition Analysis. The sample (2 mg) was dissolved in 2 mL of 2 M TFA in a screw-capped vial filled with N_2 and hydrolyzed at 120 °C for 2 h. The hydrolysate was evaporated with a rotary evaporator. Neutral sugars and uronic acids were simultaneously detected by GC using the method described previously.[10] GC was performed by a Shimadzu GC2010 equipped (Shimadzu, Japan) with a capillary column of rtx-5 ms (30.0 m × 0.25 mm × 0.25 μm). The temperature program was as follows: 180 °C for 2 min, raised to 210 °C at 6 °C/min, then to 215 °C at 0.3 °C/min, then to 240 °C at 6 °C/min for 45 min. N_2 was used as the carrier gas at 0.6 mL/min.

Yariv Assay for AGPs. LRGP3 (4 mg) was dissolved in 1 mL of 0.15 M NaCl containing 0.02% NaN_3. Agarose gels (1%) containing 0.002% β-glucosyl-Yariv reagent (Biosupplies, Australia), 0.15 M NaCl, and 0.02% NaN_3 were poured into Petri dishes. Wells were made (4 mm in width) in the gel, and 15 μL of a polysaccharide solution was pipetted into the wells. The reagents without polysaccharide were used as blanks, and gum arabic and larch arabinogalactan were used as test polysaccharides. The Petri dishes were sealed with Parafilm and left in a dark cupboard at ambient temperature for 2 days to allow the colored halo to develop.[11]

Amino Acid Composition Analysis of the Protein Part. Amino acid composition analysis was performed by the Research Center for Proteome Analysis (RCPA), Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences.

Carbohydrate−Peptide Linkage Analysis. The carbohydrate−peptide linkage of LRGP3 was analyzed by the β-elimination reaction.[12] LRGP3 (10 mg/mL) was incubated in 0.1 M NaOH containing 1.0 M $NaBH_4$ at 45 °C for 24 h, and then the sample was scanned from 200 to 400 nm by UV spectrophotometer. The obtained data were compared with that of the sample without alkali treatment.

Releasing the Glycan from LRGP3. LRGP3 (80 mg) was dissolved in 3.5 mL of 0.1 M Tris-HCl buffer (pH 8.0) containing 0.1% $CaCl_2$. Then, Pronase E (1% of the weight of LRGP3) was added to the solution, which was incubated at 37 °C for 24 h, followed by another 48 h digestion with a supplement of Pronase E (0.5% of the weight of LRGP3). Finally, the glycan LRGP3-OL released from LRGP3 was purified by a Sephadex G-100 column and then lyophilized.

Partial Acid Hydrolysis. LRGP3-OL (40 mg) was dissolved in 10 mL of 0.02 mol/L H_2SO_4 and heated at 80 °C for 12 h. After the solution was cooled to room temperature, the resulting solution was neutralized with NaOH and dialyzed against deionized water using a dialysis bag with a MWCO of 8 kDa, giving rise to dialyzable (outside the dialysis bag) sample and nondialyzable (inside the dialysis bag) sample. Both dialyzable and nondialyzable samples were lyophilized. The nondialyzable fraction was further purified on a Sephadex G-25 column, giving the partially hydrolyzed polysaccharide LRGP3-OL-I; the dialyzable oligomers were designated as LRGP3-OL-O. Sugar compositions of LRGP3-OL-I and LRGP3-OL-O were analyzed by GC. The structure of LRGP3-OL-I was further analyzed by methylation.

Methylation Analysis. The samples of both LRGP3-OL and LRGP3-OL-I were methylated using a modified Ciucanu method as described previously.[13] The methylation procedure was repeated three times. Complete methylation was confirmed by the disappearance of the O−H absorption band (3700−3100 cm^{-1}) in the FTIR spectrum. Next, the samples were hydrolyzed with 2 M TFA (121 °C, 2 h), reduced with $NaBH_4$, and acetylated to convert their monosaccharide units into partially methylated alditol acetates, which were analyzed by GC and GC-MS. Peaks of methylated sugars were identified by their mass spectra. Their relative molar ratios were estimated from the peak areas of GC and corresponding response factors. Response factors of partially methylated alditol acetates were calculated by the effective carbon response.[14] GC-MS was performed using a Shimadzu instrument GCMS-QP2010 equipped with an electron impact ion source (ionization energy = 70 eV). The capillary column used was rtx-5 ms (30 m × 0.25 mm × 0.25 μm); the temperature program was as follows: 140 °C for 2 min, raised to 250 °C at 2 °C/min in 30 min. Helium was used as a carrier gas, and the flow rate was 0.6 mL/min. The temperatures of the interface and the ion source were 200 and 250 °C, respectively.

ESI-MS Analysis of Oligosaccharides from the Hydrolysates of LRGP3-OL. One milligram of LRGP3-OL-O was dissolved in 0.5 mL of deionized water and loaded onto a Dowex 50 WX8-400 cation exchange column (1 mL) pre-equilibrated with 3 mL of deionized water. The oligosaccharides were eluted with 3 mL of deionized water, and the eluted fractions were collected and diluted 1000 times with MeOH for ESI-MS analysis.[15] The ESI-MS data were acquired on a Thermo Scientific LTQ XL ion trap mass spectrometer (Thermo-Fisher, USA) in positive ion mode. For the electrospray ion source, the spray voltage was set at 4 kV, with a sheath gas (N_2) flow rate of 30 arb, an auxiliary gas (N_2) flow rate of 5 arb, a capillary temperature of 275 °C, a capillary voltage of 350 V, and a tube lens voltage of 250 V. Samples were injected through a Rheodyne loop (Thermo-Fisher, USA), using a plastic capillary with a volume of 2 μL, and then diluted and taken into the electrospray ion source by a stream of 50% aqueous MeOH at a flow rate of 200 mL/min from the pump of the HPLC system to lessen contamination of the instrument. MS data were collected through Xcalibur software (Thermo-Fisher, USA).

NMR Spectroscopy. LRGP3 (70 mg) was kept in a vacuum oven (80 °C) for 6 h, and then the dried sample was dissolved in 5 mL of D_2O (4%, w/v) and freeze-dried. This procedure was repeated three times to completely replace H with D, and the sample was finally dissolved in D_2O at room temperature for 3 h before NMR analysis. Both ^1H and ^{13}C spectra were recorded on a Bruker AMX 500FT spectrometer (Bruker, Germany).

RESULTS AND DISCUSSION

Purification and Molecular Weight of LRGP3. LRP3 was isolated from *L. ruthenicum* as described previously.[7] After being further purified by gel permeation chromatography on a Sephadex G-100 column, a homogeneous glycoconjugate named LRGP3 was obtained with a yield of 0.008% of the fruit powder. The homogeneity and molecular weight of LRGP3 were determined by high-performance gel permeation chromatography. The elution peak was single, symmetric, and narrow, corresponding with the feature of homogeneous distribution. On the basis of a calibration with standard dextrans, the molecular weight of LRGP3 was estimated to be 7.56×10^4 Da.

Characterization of LRGP3. LRGP3 was a white fluffy protein−polysaccharide, and its solution had a neutral pH. As determined by the H_2SO_4−phenol method, the total carbohydrate content was 97.2%; the protein content was 1.7%, as determined by the Bradford method. Its monosaccharide composition is summarized in Table 1. LRGP3 mainly consisted of arabinose, galactose, and rhamnose in a molar ratio of 14.9:10.4:1.0, and uronic acid was not detected. The sugar composition of LRGP3 indicated that arabinose and galactose were the predominant sugars with only a trace

Table 1. Sugar Composition of LRPG3-OL, LRGP3-OL-I, and LRGP3-OL-O

	sugar composition[a] (%)		
polysaccharide fraction	rhamnose	arabinose	galactose
LRGP3-OL	3.8	56.6	39.6
LRGP3-OL-I	–	5.6	94.4
LRGP3-OL-O	5.7	82.9	11.4

[a]–, not detected.

amount of rhamnose (Table 1), implying the presence of arabinogalactan.

The UV scan spectrum of LRGP3 showed a strong absorbance band at about 200 nm and a weak absorbance band at about 280 nm, which further indicated that LRGP3 was a proteoglycan. By comparison of the untreated LRGP3 with the alkali-treated (0.2 M NaOH/0.1 M $NaBH_4$ at 45 °C for 24 h) LRGP3, the latter had a distinct absorbance band at 242 nm, suggesting that a β-elimination reaction had taken place. This revealed that the polysaccharide was conjugated with protein by the O-linkage.[16]

In the FT-IR spectrum, the characteristic bands in the regions of 3419, 2929, and 1641 cm^{-1} belonged to O−H bending, C−H bending, and associated water, respectively. The band at 1417 cm^{-1} was due to C−O bending; the absorption peaks between 1250 and 950 cm^{-1} indicated that galactose conformation of LRGP3 was of the pyranose type;[17] the occurrence of a band at 896.7 cm^{-1} indicated that pyranoses existed in the β-configuration.[18]

Identification of LRGP3 as AGPs. Monosaccharide compositional analysis of LRGP3 revealed that arabinose and galactose were the major sugars present. That LRGP3 as a protein−polysaccharide was further verified by Yariv plate assay, a test specific for AGPs; the β-glucosyl-Yariv reagent gave a strong positive reaction for LRGP3. Analyses of the amino acids of the pure LRGP3 show that the protein contained hydroxyproline, glycine, serine, proline, and glutamic acid as the main components (Table 2). The protein moiety of AGPs is

Table 2. Amino Acid Composition of the Protein Part of LRGP3

amino acid	mol %	amino acid	mol %
aspartic acid	7.5	alanine	7.5
glutamic acid	8.2	proline	8.9
hydroxyproline	21.6	tyrosine	1.5
serine	9.9	valine	2.8
glycine	10.9	isoleucine	3.1
histidine	0.9	leucine	3.8
arginine	3.2	phenylalanine	2.0
threonine	5.9	lysine	2.3

normally characterized by high amounts of hydroxyproline, serine, and alanine, and this pattern is reflected in the composition of *L. ruthenicum* AGP. Serine and hydroxyproline are known to be the main amino acids responsible for O-glycosidic linkages between protein and glycan moieties in AGPs. LRGP3 fulfilled the three criteria that generally identified AGPs, which are the presence of an arabinogalactan backbone, a positive test for the Yariv reagent, and a typical amino acid composition.

Partial Acid Hydrolysis. After Pronase E treatment, the glycan of LRGP3 was obtained and named LRGP3-OL. Its molecular weight was 7.41 × 10^4 Da. To study the linkages between backbone and side chains, LRGP3-OL (40 mg) was partially hydrolyzed with 0.02 M H_2SO_4. Dialysis resulted in two subfractions: nondialyzable fraction LRGP3-OL-I (14.3 mg) and dialyzable fraction LRGP3-OL-O (23.8 mg). Each fraction was subjected to GC analysis, and the sugar composition of these fractions is given in Table 1. LRGP3-OL-I contained galactose and trace arabinose, with a M_w of 2.81 × 10^4 Da, indicating almost all of the arabinose residues were released as monosaccharides or oligosaccharides upon mild hydrolysis. On the basis of their molecular weight and monosaccharide composition, the total molar ratio of LRGP3-OL was about 3 times as much as LRGP3-OL-I. As compared to LRGP3-OL, the amount of arabinose of LRGP3-OL-I decreased considerably, whereas the amount of galactose increased, suggesting that LRGP3-OL contains a galactan backbone with arabinose distributed in branches. LRGP3-OL-I had no rhamnose, indicating the presence of rhamnose in side chains of LRGP3-OL.

The sugar composition of LRGP3-OL-O presented in Table 1 showed that arabinose residues were the predominant monosaccharide in the side chains, confirming the presence of a galactan backbone in LRGP3-OL that was substituted with branches containing mainly arabinosyl residues. The mass spectrum of LRGP3-OL-O revealed numerous peaks of oligosaccharide fragments (Figure 1). Compositions of the

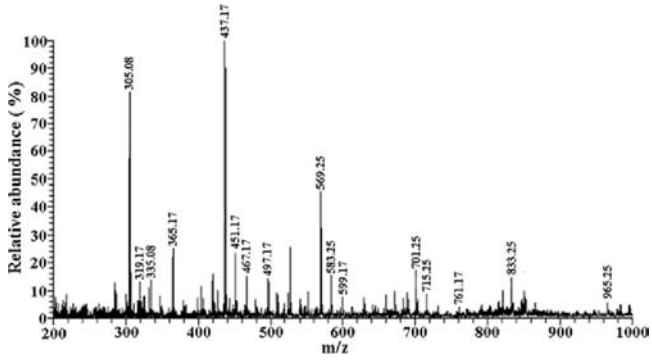

Figure 1. Positive ion ESI-MS spectra of oligosaccharides from LRGP3-OL-O. The molecular ion peaks were assigned to $[M + Na]^+$.

arabinogalactan-derived oligomers were determined from the possible sugars present and the molecular masses. Multiple sodium-cationized pseudomolecular ions at m/z 305.08, 437.17, 569.25, 701.25, 833.25, and 965.25 correspond to $[M + Na]^+$ adducts of pentose polymers with degrees of polymerization ranging from two to seven, respectively, suggesting that LRGP3-OL side chains contained abundant pentose residues of arabinose, consistent with the results by GC results (monosaccharide composition test). Part of these oligosaccharides, the ones observed at m/z values of 319.17, 451.17, 583.25, and 715.25, were assigned to RhaAra, $RhaAra_2$, $RhaAra_3$, and $RhaAra_4$. This further suggested the presence of rhamnose in the side chains. In addition, ions at m/z 335.08, 365.17, 467.17, 497.17, 599.17, and 761.17 can be ascribed to $[M + Na]^+$ adducts of GalAra, Gal_2, $GalAra_2$, Gal_2Ara, $GalAra_3$, and Gal_2Ara_3, respectively.

Glycosidic Linkage Analysis. LRGP3-OL and LRGP3-OL-I were methylated according to the modified Ciucanu method.[13] After hydrolysis and alditol acetate derivatization, partially methylated alditol acetates were analyzed by GC and

GC-MS. The results of methylation analysis are given in Table 3. The methylation analysis results indicated that the main

Table 3. Results of Methylation Analysis of LRGP3-OL and LRGP3-OL-I

		molar ratio[c]	
methylated sugar[a]	deduced linkage[b]	OL	OL-I
2,3,5-Me_3-Ara	Ara-(1−	8	1
3,5-Me_2-Ara	−2)-Ara-(1−	10	−
2,3-Me_2-Ara	−5)-Ara-(1−	12	−
2,3,4,6-Me_4-Gal	Gal-(1−	1	4
3-Me-Rha	−2,4)-Rha	2	−
2,4,6-Me_3-Gal	−3)-Gal-(1−	10	3
2,3,4-Me_3-Gal	−6)-Gal-(1−	3	5
2,4-Me_2-Gal	−3,6)-Gal-(1−	7	5

[a]Analyzed by GC-MS, after per-O-methylation, total acid hydrolysis, reduction, and acetylation. 2,3,5-Me_3-Ara = 1,4-di-O-acetyl-2,3,5-tri-*O*-methylarabinose, etc. [b]Based on derived *O*-methylalditol acetates. [c]−, not detected.

sugar residues of LRGP3-OL were arabinose and galactose, which was consistent with the monosaccharide composition analysis determined by GC. The linkage analysis of LRGP3-OL showed high proportions of (1→3)-linked, (1→6)-linked, and (1→3,6)-linked galactose consistent with polymers comprising a highly branched galactan core, with side chains terminated variously by arabinose and galactose. The terminals consisted of arabinosyl residues and galactosyl residues. Branches consisted of galactosyl residues and rhamnosyl residues. The total percentage of terminal sugars matched with the branched portion. In addition, these molar ratios also agreed with the overall monosaccharide composition of LRGP3 described above. The arabinosyl residues were composed of terminal, (1→2)-linked, and (1→5)-linked arabinose, indicating that at least some of the arabinosyl residues were in the furanose ring form.

LRGP3-OL-I had five sugar residues, namely, terminal arabinose, terminal galactose, (1→3)-linked galactose, (1→6)-linked galactose, and (1→3,6)-linked galactose, in a molar ratio of 1:4:3:5:5 (Table 3). The branching unit of LRGP3-OL-I was (1→3,6)-linked galactose, which indicated that the backbone of LRGP3-OL-I was composed of either (1→3)-linked galactose or (1→6)-linked galactose. Linkage analysis of type II arabinogalactans found in many AGPs showed for the most part (1→3)-linked galactose residues, some of which are substituted at O-6 with arabinose and galactose residues.[19−22] The linkage analysis of LRGP3-OL was consistent with a main chain of (1→3)-linked galactosyl residues substituted at O-6.

LRGP3-OL-I contained no (1→2)-linked arabinosyl, (1→5)-linked arabinosyl, or (1→2,4)-linked rhamnosyl residues, indicating that they existed in branches. As compared to LRGP3-OL, the amount of (1→6)-linked galactosyl and terminal galactosyl residues of LRGP3-OL-I increased, whereas the amount of (1→3,6)-linked galactosyl and terminal arabinosyl residues decreased. As given in Table 3, the increase by two of (1→6)-linked galactosyl residues in LRGP3-OL-I was concomitant with the decrease by two of (1→3,6)-linked galactosyl residues. Partial acid hydrolysis (0.02 M H_2SO_4) led to a decrease of (1→3,6)-linked galactosyl residues and the conversion of (1→3,6)-linked galactosyl residues to (1→6)-linked galactosyl residues. All of the results agree with the sugar composition analysis. The results indicated that the backbone of LRGP3-OL was made up of (1→3)-linked galactose. In addition, the three new terminal galactose in LRGP3-OL-I were accompanied by the decrease of (1→3)-linked galactose, indicating that (1→3)-linked galactose were substituted by terminal arabinose. These sugar linkages were consistent with type II AGPs.[19,23]

NMR Analysis of LRGP3. The anomeric signals in the ^{1}H NMR spectrum of LRGP3 (Figure 2A) were assigned

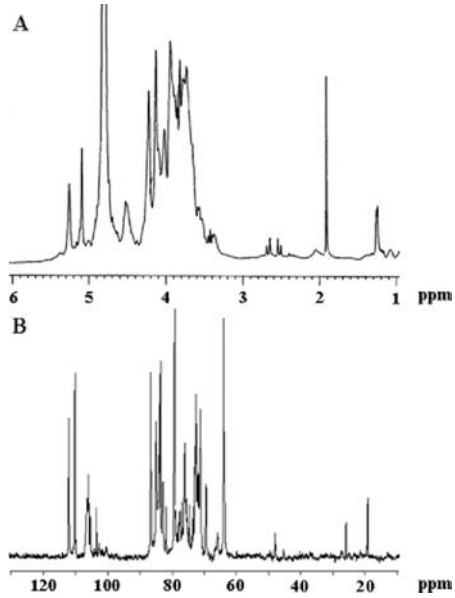

Figure 2. NMR spectrum of LRGP3: (A) ^{1}H NMR spectra of LRGP3 recorded on a 400 MHz spectrometer in D_2O at 80 °C; (B) ^{13}C NMR spectra of LRGP3 recorded on a 400 MHz spectrometer in D_2O at 80 °C.

according to sugar composition and literature data (Table 4).[24] The signal at δ 5.26 was assigned to the nonreducing

Table 4. ^{1}H and ^{13}C NMR Chemical Shifts of LRGP3

	chemical shift (δ)	residue
^{1}H NMR	5.26	α-Ara*f*
	4.67	β-Gal*p*
	1.26	α-Rha*p*
^{13}C NMR	110.1	α-Ara*f*(1→
	105−107	→3)β-Gal*p*(1→
		→ 6)β-Gal*p*(1→
		→ 3,6)β-Gal*p*(1→
	86.6	→ 2)β-Ara*f*(1→
	65.9	→ 5)β-Ara*f*(1→
	19.2	→ 2,4)α-Rha*p*(1→

terminal arabinofuranosyl residues. The signals at δ 4.67 and 4.55 could be assigned to β-D-galactopyranose. The H-6 signal for rhamnose was observed at 1.26, indicating an α configuration. In the ^{13}C NMR spectrum (Figure 2B), the strong signal at δ 110.1 was correlated with the C-1 of terminal arabinofuranose. The peak at δ 106.2 belonged to β-D-galactopyranosyl residues, which corresponded to the characteristic absorption at 896.7 cm^{-1} in the IR spectrum, and a small peak observed at δ 19.2 was a typical signal of the C-6 of α-L-rhamnopyranose.[25] There were no signals attributable to α-

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glycosidic linkages of galactopyranose. Resonances corresponding to C-1 of (1→3)-linked, (1→6)-linked, and (1→3,6)-linked β-D-galactopyranose appeared as a cluster of signals from 105 to 107 ppm. The signals at δ 86.6 and 65.9 were attributable to (1→2)-linked and (1→5)-linked α-L-arabinofuranose, respectively.

In summary, we obtained a homogeneous AGP sample LRGP3, which is a neutral polysaccharide isolated from the furits of *L. ruthenicum*. Its molecular weight is 75.6 kDa. Monosaccharide composition analysis revealed that it is composed of rhamnose, arabinose, and galactose in a molar ratio of 1.0:14.9:10.4. The protein part of the AGP is rich in hydroxyproline. On the basis of the results of structural analyses, we conclude that LRGP3 is a highly branched arabinogalactan-protein with a backbone of (1→3)-linked β-D-galactopyranosyl residues. The backbone is partially substituted at O-6 of galactosyl residues by arabinosyl and galactosyl residues. Its branches are composed of (1→5)-linked β-D-arabinofuranosyl, (1→2)-linked β-D-arabinofuranosyl, (1→6)-linked β-D-galactopyranosyl, (1→3)-linked β-D-galactopyranosyl, and (1→2,4)-linked α-L-rhamnopyranosyl residues, terminated mostly with α-LL-arabinofuranosyl residues. On the basis of the above results, a proposed structure of an average repeating unit of the glycan of LRGP3 is given in Figure 3. The structure is similar to that of AGPs isolated from coffee bean and larch.[21,26] Further studies on the immunostimulating activity of the AGPs are in progress.

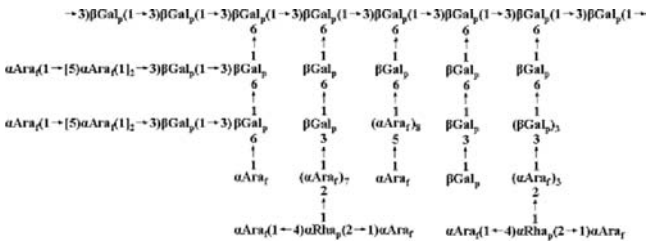

Figure 3. Hypothetical structure of the repeat unit of the glycan of LRGP3.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

LRGP3, *Lycium ruthenicum* glycoconjugate polysaccharide 3; LRGP3-OL, glycan of the LRGP3; HPGPC, high-performance gel permeation chromatography; M_w, molecular weight.

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