Characterization of arabinogalactans from *Larix principis-rupprechtii* and their effects on NO production by macrophages

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**A R T I C L E  I N F O**

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*Larix principis-rupprechtii*

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NMR

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**A B S T R A C T**

Arabinogalactans are a source of dietary fiber with health benefits. In this work, two arabinogalactans assigned as AGW and AGS were isolated from *Larix principis-rupprechtii*, and characterized by gel permeation chromatography (GPC), monosaccharide analysis, methylation analysis and NMR spectroscopy analysis. The average molecular weights of AGW and AGS were 1.53 × 10\(^4\) and 1.84 × 10\(^4\) Da, respectively. Methylation analysis and NMR spectra suggested that AGW and AGS have a 1,3-linked Gal backbone, branched at C6 with 1,6-linked Galp side residues. The Ara residues were substituted at C6 of 1,6-linked Galp backbone, branched at C6 hydroxyl position to either single D-Galp units or to extended oligosaccharide-like structures consisting of Galp, Arap, Araf, Rhap and GlcA (Churms, Merrifield, & Stephen, 1978; Goellner, Utermoehlen, Kramer, & Classen, 2011; Ponder & Richards, 1997a; Ponder, 1998, 1997b, 1997c; Prescott, Groman, & Gulyas, 1997). It is generally believed that the corresponding molar ratios of Gal: Ara in larch AG is about 6:1, and both of comprise more than 98% of the total carbohydrate content of AG. (Côté, Day, Simson, & Timell, 1966; Goellner et al., 2011; Willför & Holmbom, 2004). A trace amount of glucuronic acid moieties have also been identified in the AG, but further understanding of how it is connected is not yet known, due to a lack of elucidative analytical techniques (Odonmaiig, Ebringerova, Machova, & Alfijjadi, 1994; Ponder & Richards, 1997a). The significance of uronic acid groups in AG lie in the dramatic effect which a few uronic acid groups have on chromatographic behavior of isolated AG, where differences in separation were observed around system pH and uronic acid pKa (∼3) (Ponder & Richards, 1997a). The molecular weight distribution of larch

1. Introduction

Macromolecular and biological polysaccharides have begun to receive wide attention as benefactors to human health, due to their ability to function across several biological activities. Examples of such activities include polysaccharides serving as antioxidants, anti-virulents, antineoplastics and even immune system enhancers (Chen et al., 2017; Holmbom, 2004). A trace amount of glucuronic acid moieties have also been identified in the AG, but further understanding of how it is connected is not yet known, due to a lack of elucidative analytical techniques (Odonmaiig, Ebringerova, Machova, & Alfijjadi, 1994; Ponder & Richards, 1997a). The significance of uronic acid groups in AG lie in the dramatic effect which a few uronic acid groups have on chromatographic behavior of isolated AG, where differences in separation were observed around system pH and uronic acid pKa (∼3) (Ponder & Richards, 1997a). The molecular weight distribution of larch

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arabinogalactans is reported to be very broad, with molecular weight from 3 to 100 kDa having been reported with high polydispersities. (Churms et al., 1978; Eremeeva & Bykova, 1992; Prescott, Enriquez, Jung, Menz, & Groman, 1995, 1997; Teratani, Kato, Kai, & Yamashita, 1987).

AG extracted from different larch species have been shown to possess diverse biological properties, including immunological activity (Currier, Lejtenyi, & Miller, 2003; Groman & Gou, 1997; Kelly, 1999; Kim, Waters, & Burkholder, 2002), antitumor (Beuth, Ko, Schirmacher, Uhlenbruck, & Pulverer, 1988), fecal microbial population regulators (Grieshop, Flickinger, & Jr, 2002; Robinson, Feirtag, & Slavin, 2001), antiviral effects (Enriquez, Chu, Josephson, & Tennant, 1995), and ocular benefits (Burgalassi et al., 2007). AG from *Larix laricina* has even been demonstrated to play a unique role in reducing the incidence of the common cold (Riede, Grube, & Gruenwald, 2013). Finally, AG from larch has properties that make it suitable as a carrier for delivering diagnostic or therapeutic agents to hepatocytes via the asialoglycoprotein receptor (Groman, Enriquez, Jung, & Josephson, 1994), and as protecting agent for maintaining precious metal nanoparticles in colloidal suspension (Mucalo, Bullen, Manley-Harris, & McIntire, 2002).

Macrophages are an important class of immune cells. It has demonstrated that some polysaccharides have unique patterns of influence upon the immune system (Wang et al., 2017; Xu, Chen, Zhang, & Ashida, 2012). Nitrous oxide (NO) is an important cytotoxic mediator contributing to both the antitumor and antimicrobial activity of macrophages (Bogdan, 2001). Among signaling qualities, NO affects cellular decisions of life and death either by turning on apoptotic pathways or by shutting them off (Brune, 2003). It has been reported that macrophage-derived NO can kill or reduce replication of infectious agents and cause cytostasis or kill tumor cells (Degroote & Fang, 1999; Pervin, Singh, & Chaudhuri, 2001; Xie, Dong, & Fidler, 1996). However, there is less research on larch arabinogalactans.

In this study, we isolated two different fractions of AG from a bulk source of AGs extracted from *L. principis-rupprechtii*. Molecular structures within each AG fraction were investigated using a variety of analytical techniques, such as high performance anion-exchange chromatography (HPAEC), methylation analysis, and a range of NMR analyses (1D, 1H, 13C, 2D COSY, TOCSY, HSQC, HMBC and NOESY). The analyzed AGs have been characterized in terms of physicochemical properties, monosaccharide composition, glycosidic bond types and macromolecular conformations. To relate this structural data to biological function, the effect of characterized AG upon NO production from macrophage RAW 264.7 cells was studied. The cumulative goal of this research is to contribute to the scientific discourse regarding the relationship between structure and function of AGs with respect to human health.

2. Materials and methods

2.1. Materials and reagents

Wood chips from *L. principis-rupprechtii* certified by plant taxonomist were supplied by Beijing Forest Bureau, Beijing, China. Prior to experimentation, the chips were crushed to 20–80 mesh wood powder. DEAE-Sepharose Fast Flow and Superdex 75 pre grade gel filtration media were purchased from GE Healthcare Bio-Sciences AB (Uppsala, Sweden). Deuterium oxide (D₂O), dextrans of different molecular weights and monosaccharide standards were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Fetal bovine serum (FBS) and RPMI 1640 medium were purchased from Gibco. Nitric Oxide (NO) assay kits were obtained from Beyotime (Shanghai, China). All other employed reagents were of analytical grade.

2.2. Extraction and purification of polysaccharides

Wood powder from *L. principis-rupprechtii* (300 g) was extracted twice with 3 L of distilled water for 2 h at 60 °C to isolate AG. After extraction, both solid and liquid were combined and centrifuged to obtain the AG-containing supernatant. To recover AG from the liquid phase, precipitation was induced by adding three volumes of 95% ethanol by holding at 4 °C overnight. After time, the precipitate was separated by centrifuging at 9600 g for 10 min and then collected. The recovered precipitate was then re-dissolved in distilled water, and the precipitation protocol was repeated twice more. After the conclusion of third cycle, the precipitate was freeze-dried to obtain crude AG.

Crude AG was redissolved in deionized water and purified with a DEAE-Sepharose Fast Flow column (XK1.6 × 15 cm, GE-healthcare). The column was eluted with deionized water, 0.05, 0.1, 0.2, 0.3 and 0.5 M NaCl. Resultant eluents were monitored by the phenol-sulfuric acid colorimetric assay (Dubois, Gilles, Hamilton, Rebers, & Smith, 1955). The eluted samples was collected and further purified on a Superdex 75 pre grade column (Tricorn 1 × 60 cm) using deionized water as eluent, collecting main fraction, respectively. Finally, two purified fractions named AGW (Water elution fraction) and AGS (NaCl elution fraction) were obtained.

2.3. Elementary analysis

The elemental composition of AG was determined by measuring the atomic contents of carbon, hydrogen and nitrogen in AGW and AGS with Thermo Scientific Flash 2000. For analysis, ~1.5 mg of AG was weighed into a tin capsule. A blank value of an empty tin capsule was recorded under the same conditions to establish a baseline value.

2.4. Molecular weight determination

The average molecular weights and polydispersity of both AGW and AGS were determined by GPC on Agilent 1260 system (Agilent, USA) equipped with Ultrahydrogel 250 and Ultrahydrogel 2000 columns in series (7.8 × 300 mm, Waters Corp, USA). Column temperature was kept at 50 °C. The sample solution (2 g/L) was eluted with 0.05 M NaNO₃, flowing at the rate of 0.6 mL/min. The average molecular weights were calculated based upon retention time results of different dextran standards.

2.5. Monosaccharide composition analysis

To assay the monosaccharide constitution of various AG preparations, ~10 mg of the polysaccharide was hydrolyzed with 2 M trifluooroacetic acid (TFA) at 121 °C for 1 h. Excess TFA was removed by mixing in methanol and forcing co-evaporation under vacuum. Resultant monosaccharides from hydrolysate were analyzed by a high performance anion-exchange chromatography system (Dionex ICS-5000, USA) equipped with a CarboPac™ PA10 column (2 × 250 mm) and a pulse amperometric detector. The elution program consisted of an initial isocratic elution in 37 mM NaOH from 0 to 20 min, followed 200 mM CH₃COONa from 20 to 35 min, and finally equilibrated in 37 mM NaOH from 35 to 50 min.

2.6. Methylation analysis

Methylation of AGW and AGS was conducted according to the method of previous reports (Hakomori, 1964). Methylated polysaccharides were dialyzed with distilled water for 24 h and next extracted by chloroform. The chloroform layer was concentrated and dried. The methylated products were hydrolyzed with 2 mol/L TFA at 105 °C for 6 h. The hydrolyzate was reduced with sodium borodeuteride (10 mg) for 4 h at room temperature, following by an acetylation step utilizing acetic anhydride (0.5 mL) and pyridine (0.5 mL) for 2 h at 100 °C. Methylated alditol acetates were finally analyzed by a Thermo Trace IQS GC–MS system (Thermo Fisher Scientific, USA). The initial column temperature was set at 80 °C (held for 2 min), and programmed
to 280 °C (held for 10 min) at 10 °C/min. The partially methylated alditol acetates were identified by their mass spectra and relative retention time.

2.7. NMR analysis

The NMR spectra of AGW and AGS were acquired on a Bruker AVANCE 600 MHz spectrometer (Bremen, Germany). 30 mg of the dried sample was dissolved in deuterium oxide (D₂O, 0.5 mL) at room temperature. DSS (4,4-dimethyl-4-silapentane-1-sulfonic acid) was used as standard. Both ¹H (600 MHz), ¹³C (150 MHz), 2D NMR spectra including ¹H/¹H Correlating Spectroscopy (COSY), Total Correlation Spectroscopy (TOCSY), Nuclear Overhauser Enhancement Spectroscopy (NOESY), Heteronuclear Singular Quantum Correlation (HSQC) and Heteronuclear Multiple Bond Correlation (HMBC) were run using standard Bruker pulse sequences at 25 °C.

2.8. Cell culture

RAW 264.7 cells were cultured in RPMI 1640 medium supplemented with penicillin (100 units/mL), streptomycin (100 units/mL) and 10% (v/v) fetal bovine serum (FBS) at 37 °C in a humidified atmosphere with 5% CO₂.

2.9. Measurement of NO

NO production in the conditioned media was determined based on the amount of nitrite present by the Griess reaction (Granger, Taintor, Boockvar, & Hibbs, 1996). After being cultured for 24 h in 96-well flat-bottom plate (2 × 10⁴ cells/well), cells were treated with various concentrations of AGW and AGS for 24 h. Then each supernatant (100 μL) was mixed with an equal volume of Griess reagent (50 μL of 0.1% N-1-naphthylethylenediamine dihydrochloride in distilled water) at room temperature in darkness place for 10 min. The absorbance was measured at 540 nm using microplate reader, which employed NaNO₂ as a standard in the range of 0–100 μM.

3. Results and discussion

3.1. Extraction, separation and molecular weight of polysaccharides

In effort to obtain pure polysaccharides from Larix principis-rupprechtii, a series of purification-oriented operations were applied. The process began with the initially-extracted crude AG polysaccharides, which were obtained at a yield of 11.3% (w/w). The obtained crude AG was then separated into two unique fractions by way of anion-exchange chromatography (Fig. 1). Then, the two fractions were further purified on column of Superdex 75 with distilled water. The main fraction was collected and lyophilized to obtain the pure polysaccharide (Fig. S1 and S2). Finally, the recovery yield of AGW and AGS were 3.0% and 6.6% (based on the dry weight of larch), respectively. GPC was used to determine the molecular weight distribution of each AG preparation. The GPC chromatogram of each isolated polysaccharide displayed a single symmetrical peak. From this it can be deduced that each preparation is relatively non-polydisperse (Fig. 2). Calibrating to standard dextrans, the Mₘ of AGW and AGS was 15.3 kDa and 18.4 kDa, respectively (Table 1). These results significantly vary from other reports across literature. For instance, AG from Larix occidentalis is reported to have molecular weight of 78 kDa and 18 kDa (Churms et al., 1978), 10 kDa from L. sibirica (Eremeeva & Bykova, 1992), 29 kDa from L. sibirica (Karácsényi, Kováč, Alföldi, & Kubášková, 1984), and 16 kDa from L. dahurica (Odonmaïg et al., 1994). This can be attributed mostly to differences in larch species and extraction processes. The short extraction and lower temperature can avoid breakage of polysaccharide glycosidic bonds.

3.2. Elemental and monosaccharide composition of AGW and AGS

Table 1

<table>
<thead>
<tr>
<th>Relative amount (mol %)</th>
<th>AGW</th>
<th>AGS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rha</td>
<td>0.03</td>
<td>0.21</td>
</tr>
<tr>
<td>Ara</td>
<td>9.15</td>
<td>8.16</td>
</tr>
<tr>
<td>Gal</td>
<td>90.79</td>
<td>90.89</td>
</tr>
<tr>
<td>GlcA</td>
<td>0.03</td>
<td>0.74</td>
</tr>
<tr>
<td>Ara/Gal</td>
<td>1:9.92</td>
<td>1:11.14</td>
</tr>
<tr>
<td>Mₘ (Da)</td>
<td>15300</td>
<td>18400</td>
</tr>
<tr>
<td>Mₘ (Da)</td>
<td>11800</td>
<td>13050</td>
</tr>
<tr>
<td>Mₘ/Mₘ</td>
<td>1.30</td>
<td>1.41</td>
</tr>
</tbody>
</table>

Elemental analysis revealed great similarities in same carbon and hydrogen composition of AGW and AGS. Carbon and hydrogen elements accounted for 41.7% and 7.5% of AGW compared to 42.3% and 7.5% of AGS, respectively. No nitrogen and sulfur elements were found in either preparation, it indicated that there was no protein associated with the isolated AG preparations. The finding was also reported in a similar work (Goellner et al., 2011), although arabinogalactan-proteins have often been reported as been present when isolation from different (non-larch) biomass sources (Bartels et al., 2017; Tan et al., 2012). The total carbohydrate content of AGW and AGS was 92.6% and 95.9%, respectively, according to the phenol-sulfuric acid method using D-galactose as the standard. Monosaccharide analysis showed that AGW consisted of 9.15% Ara and 90.79% Gal. In slight difference, AGS...
was found to be comprised of 8.16% Ara and 90.89% Gal. In addition, it was found that small amounts of Rha and GlcA were present in AGW and AGS (Table 1). The molar ratio of Ara to Gal for AGW and AGS macromolecule was 1:11.4 and 1:11.1, respectively. These results closely matched what was found for AG of *Larix sibirica* L. (Karácsonyi et al., 1984), but differed from what is reported for AG of *Larix laricina* (1:6 M ratio of Ara to Gal) (Goellner et al., 2011) and *Larix occidentalis* (1:8 Ara:Gal) (Churms et al., 1978). The reason for the differences between the results obtained and what has been reported is again likely due to both differences in larch species as well as extraction protocol.

Interestingly, most published papers concerning larch AG have not discussed the occurrence of GlcA in the polysaccharide and how it influenced the structure and biological properties of AG. Despite the lack of significant difference in Gal and Ara constitution of AGW and AGS, the amount of GlcA in AGS was found to be 25 times than that of AGW (Table 1). This indicated that the presence of GlcA was the reason for separation of AGW and AGS by chromatography. To verify the effect of GlcA on the molecular configuration of AGW and AGS and subsequent separation behavior, different concentrations of NaNO₃ eluent were used to determine the solution properties of each AG preparation. As observed in Fig. 3, AGS retention time was more influenced by salt concentration than that of AGW. This phenomenon showed that we can change the spatial configuration of AGS by controlling salt concentration. The actual retention time is not only related to the absolute molecular weight of the sample, but also related to the spatial configuration. The difference in retention time of the same sample can be considered as a change in the three-dimensional structure. Ponder and Richards (1997a) also studied the effect which a few uronic acid groups have on the chromatographic behavior of AG from *L. occidentalis* implies possible differences in solution properties.

### 3.3. Linkage types of AGW and AGS

In order to characterize the glycosidic linkages in both AGW and AGS, methylation analysis was performed. Completion of methylation was determined by the absence of O-H band in the IR spectrum (Fig. S3). The abundant peaks derived from residues were shown in Fig. S4. The linkage patterns and corresponding molar ratios of AGW and AGS were shown in Table 2. The majority of the terminal units in AGW and AGS were T-Galp with the relative amounts of 30.1% and 32.5%, respectively. In addition, a small amount of T-Araf (6.9%, 4.7%) and T-Arap (3.7%, 2.6%) were identifiable, respectively. The sugar residue featuring the greatest extent of branching molecular structures was →3,6-Galp-(1→, accounting for 26.6% and 25.6% of total sugar residues, respectively. The results indicated that AGW and AGS are significantly branching polysaccharides. The main intra-chain of AGW and AGS was →6)-Galp-(1→ (23.7% and 28.2%, respectively), accompanied by a minor portion of →3)-Galp-(1→ and →3)-Araf-(1→. This findings suggested that almost all the backbone units featured attached residues at the O-6 position. In addition, it could be surmised that the side chain are also branching. Ara was found in its furanosidic and pyranosidic form as terminal residues (T-Arap and T-Araf), and as an intrachain residue →3)-Araf-(1→. The molar ratio of pyranosidic and furanosidic linkages in AGW and AGS were 1:3 and 1:2.9, respectively. The ratio is typical of the feature of larch arabinogalactan in good correspondence with findings for AG from *L. occidentalis* (Ponder & Richards, 1997c), *L. laricina* (Goellner et al., 2011) and *L. sibirica* L. (Karácsonyi et al., 1984). A small amounts of GlcA and Rha were detected during monosaccharide constitution analysis (Table 1), but it appeared that these structures were lost during methylation leading to their absence from our reported methylation results.

### 3.4. NMR analysis

Several 1D and 2D NMR methods were applied to characterize the main structural features of AGW and AGS. When comparing the spectra signals of AGW and AGS (Fig. 4A and B), no significant difference was observable. This was consistent with the results obtained from methylation analysis. Therefore, the $^{13}$C and 2D spectra of AGW were shown in the supplementary document (Fig. S5-9). From 1H spectrum (Fig. 4A and B), six anomic signals were observed at 4.45, 4.44, 4.70, 5.26,

![Fig. 3. AGW and AGS on Superdex 75 pre grade column with different eluent concentration (A) AGW; (B) AGS.](image-url)
4.99 and 5.32 ppm, respectively. The six signals were designated A, B, C, D, E and F in decreasing order of peak intensity. The signals indicated that these residues were β-glycosidically linked except residue D and residue F. All intense anomeric carbon signals were found at 106.05, 106.44, 111.94, 102.76 and 110.98 ppm, respectively (Fig. 5A and D). The chemical shift of H-2 can be assigned from the COSY spectrum based on the principle that H-2 correlates with H-1 (Fig. 5B). Other hydrogen signals (H-3 to H-6) were also assigned by the same analogy. All the proton chemical shifts can also be confirmed by TOCSY spectrum (Fig. 5C). In addition, all the 13C chemical shifts can also be identified from the cross peaks of HSQC spectrum (Fig. 5D). According to methylation analysis and literature data (Do Nascimento, Iacomini, & Cordeiro, 2017; Göllner et al., 2011; Kang et al., 2011; Liu, Wen, Kan, & Jin, 2015; Nie et al., 2013; Ponder & Richards, 1997b; Tan et al., 2010; Zhao, Zhang et al., 2017; Zhao, Li et al., 2017), these cross peaks were assigned to T-β-D-Galp (A), →6)-β-D-Galp(1→ (B), →3,6)-β-D-Galp(1→(C), T-α-L-Arapf (D), T-β-L-Arap (E) and →3)-α-L-Arapf(1→ (F), respectively. All the 13C NMR and 1H signals of sugar moieties were assigned completely, as showed in Table 3. However, the signals of Rha and GlcA residues were not clearly detected in the NMR spectra, which was probably due to the low contents.

To confirm the correlation between carbon and proton signals within the sugar residues, the HMBC and NOESY spectra are presented (Fig. 5E and F). The residue B correlated with two sugar residues at C-6 of residue B (B H-1, B C-6) and C-6 of residue C (B H-1, C C-6) at 4.44/71.88 ppm and 4.44/73.46 ppm. This indicates that C-1 of residue B was linked to O-6 of residue B and C-1 of residue C was linked to O-3 of residue C. Similar, the cross peaks at 4.45/71.88 and 5.26/86.50 ppm suggested that C-1 of residue A was linked to O-6 of residue B and C-1 of residue D was linked to O-3 of residue F. From the NOESY spectrum, we can also get the sequence of residue F. All intense anomeric carbon signals were found at 106.05, 106.44, 111.94, 102.76 and 110.98 ppm, respectively (Fig. 5A and D). The chemical shift of H-2 can be assigned from the COSY spectrum based on the principle that H-2 correlates with H-1 (Fig. 5B). Other hydrogen signals (H-3 to H-6) were also assigned by the same analogy. All the proton chemical shifts can also be confirmed by TOCSY spectrum (Fig. 5C). In addition, all the 13C chemical shifts can also be identified from the cross peaks of HSQC spectrum (Fig. 5D). According to methylation analysis and literature data (Do Nascimento, Iacomini, & Cordeiro, 2017; Göllner et al., 2011; Kang et al., 2011; Liu, Wen, Kan, & Jin, 2015; Nie et al., 2013; Ponder & Richards, 1997b; Tan et al., 2010; Zhao, Zhang et al., 2017; Zhao, Li et al., 2017), these cross peaks were assigned to T-β-D-Galp (A), →6)-β-D-Galp(1→ (B), →3,6)-β-D-Galp(1→(C), T-α-L-Arapf (D), T-β-L-Arap (E) and →3)-α-L-Arapf(1→ (F), respectively. All the 13C NMR and 1H signals of sugar moieties were assigned completely, as showed in Table 3. However, the signals of Rha and GlcA residues were not clearly detected in the NMR spectra, which was probably due to the low contents.

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3.5. Proposed structure of AGW and AGS

Based on the cumulative sum of the results provided above, possible molecular structural schemes for AGW and AGS are graphically proposed in Fig. 6A. Results demonstrated that the AG preparations, AGW and AGS, have a backbone consisting of 1,3-linked Gal residues which are branched at position O-6 to 1,6-linked Gal side chains. Araf-3 side chain is attached to the Galp-6 at C-6 position, T-Araf is linked to the Araf-3 residue, and T-Arap is attached directly to the Galp-6 at C-6 position. Based on our results and related research, GlcA is assumed to be at the end of the glycan structure (Göllner et al., 2011; Willför, Sjöholm, Laine, & Holmbom, 2002). The main difference between AGW and AGS is the content of GlcA, which leads to the existence of poly-electrolyte effect (Fig. 6B). This effect results in a conformation of AGS which is unique in aqueous and salt solutions.

3.6. Effect of AGW and AGS on NO production in RAW264.7 cells

NO has been identified as a consequential molecule produced by macrophages, serving as a pleiotropic regulator in different molecular and biological pathways to fights against infectious and inflammatory diseases (Brüne, 2003). This work sought to probe a relationship between the presence of AG (in the form of the preparations AGW and AGS) and macrophage NO generation. First as shown in Fig. 7, AGW and AGS were shown to be less influential on NO secretion at low AG concentrations. However, when the concentration of AGW and AGS was increased, observable stimulation of NO production by RAW264.7 cells could be observed. AGW demonstrated the strongest stimulation dose (400 μg/mL) resulting in 194% increase in NO output compared to the control group. This phenomenon with best effect concentration also existed in L2 from Lentinula edodes (Xu, Yan, & Zhang, 2012). It should be noted that the ability to stimulate NO secretion is closely related to the structure of polysaccharides. The polysaccharides from different sources have different ability to stimulate NO secretion in macrophages (Zhao, Zhang et al., 2017; Zhao, Li et al., 2017; Nie, Zhu, Ma, Wang, & Hu, 2018). For AGS, the increase of NO production mostly depended on the concentration. NO production does not increase linearly as the addition amount of AGW and AGS increased, so the possibility of contamination of LPS can be ruled out. The results indicated that AGW and AGS might play a significant role in promoting the secretion of NO. Even though there was no obvious difference between AGW and AGS in the neutral sugar, this difference could be reflected in their influences upon biological functions. The effect which a few uronic acid groups have on the chromatographic behavior of AG implies possible differences in solution properties and biological activities between the charged and uncharged species (Ponder & Richards, 1997a). To understand the role of uronic acid in bioactivity, more biological functions should be further investigated to tease out its exact mechanism of influence.
4. Conclusions

In this study, two different AG polysaccharides preparations (AGW and AGS) were purified from _Larix principis-rupprechtii_. AGW and AGS were shown to have almost the same primary structure, with a backbone comprised of →3)-β-D-Galp-(1→3)-β-D-Galp-(1→ residues and side chains of →6)-β-D-Galp-(1→ substituted in O-6 of →3,6)-β-D-Galp-(1→. Compared to AGW, AGS was revealed to have a higher uronic acid content, which established a polyelectrolyte effect. Both AGW and AGS were shown to stimulate macrophages secretion of NO, but each showed different extents of influence. This is the first study which has demonstrated that two structurally different arabinogalactans isolated

Fig. 5. 1D and 2D NMR Spectra of AGS in D_{2}O. (A) 13C NMR spectrum; (B) 1H-1H COSY spectrum; (C) 1H-1H TOCSY spectrum; (D) 1H-13C HSQC spectrum; (E) 1H-13C HMBC spectrum; (F) 1H-1H NOESY spectrum.
from larch have different ability to stimulate the NO production in macrophages. These findings should prove to be of great benefit towards developing a better understanding of the structure-function relationship between polysaccharides and their biological functions.

Acknowledgment

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.carbpol.2018.08.027.

References


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Table 3

13C NMR and 1H NMR chemical shifts (ppm) for AGW and AGS (residues: A, B, C, D, E and F).

<table>
<thead>
<tr>
<th>Residues</th>
<th>C-1/H-1</th>
<th>C-2/H-2</th>
<th>C-3/H-3</th>
<th>C-4/H-4</th>
<th>C-5/H-5</th>
<th>C-6/H-6</th>
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<tr>
<td>T-β-D-Galp</td>
<td>106.05</td>
<td>73.45</td>
<td>75.40</td>
<td>71.69</td>
<td>77.83</td>
<td>63.75</td>
</tr>
<tr>
<td>(A)</td>
<td>4.45</td>
<td>3.56</td>
<td>3.65</td>
<td>3.92</td>
<td>3.71</td>
<td>3.76</td>
</tr>
<tr>
<td>→6)-β-D-Galp-(1→</td>
<td>106.05</td>
<td>73.45</td>
<td>74.52</td>
<td>71.33</td>
<td>76.30</td>
<td>71.92</td>
</tr>
<tr>
<td>(B)</td>
<td>4.44</td>
<td>3.54</td>
<td>3.62</td>
<td>3.96</td>
<td>3.92</td>
<td>3.92,4.05</td>
</tr>
<tr>
<td>→3,6)-β-D-Galp-(1→</td>
<td>106.44</td>
<td>72.97</td>
<td>84.06</td>
<td>71.33</td>
<td>76.30</td>
<td>72.72</td>
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<tr>
<td>(C)</td>
<td>4.70</td>
<td>3.78</td>
<td>3.90</td>
<td>4.22</td>
<td>3.92</td>
<td>3.92,4.04</td>
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<td>T-α-L-Araf/</td>
<td>111.94</td>
<td>83.43</td>
<td>79.31</td>
<td>86.11</td>
<td>63.75</td>
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<tr>
<td>(D)</td>
<td>5.26</td>
<td>4.12</td>
<td>3.96</td>
<td>4.13</td>
<td>3.83,3.76</td>
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<tr>
<td>T-β-L-Arap</td>
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<td>71.32</td>
<td>71.94</td>
<td>65.91</td>
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<tr>
<td>(E)</td>
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<td>4.22</td>
<td>4.22</td>
<td>4.30</td>
<td>3.79,3.66</td>
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<tr>
<td>→3)-α-L-Araf-(1→</td>
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<td>82.58</td>
<td>86.54</td>
<td>86.74</td>
<td>63.75</td>
<td></td>
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<tr>
<td>(F)</td>
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<td>4.22</td>
<td>3.96</td>
<td>4.28</td>
<td>3.83,3.76</td>
<td></td>
</tr>
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</table>

Fig. 6. (A) Proposed structure of AGW and AGS from Larix principis-rupprechti; (B) Representation of conformation of AGW and AGS.

Fig. 7. Effects of AGW and AGS on the production amount of NO in RAW 264.7 cells. Error bars in represent the S.D (N = 3 independent experiments). *p < 0.05, **p < 0.01 vs control group.

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from larch have different ability to stimulate the NO production in macrophages. These findings should prove to be of great benefit towards developing a better understanding of the structure-function relationship between polysaccharides and their biological functions.