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Structure of arabinogalactan from *Larix laricina* and its reactivity with antibodies directed against type-II-arabinogalactans

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ABSTRACT

An arabinogalactan from wood of the larch tree (*Larix laricina*), which is a source of dietary fiber, is composed of D-galactose and L-arabinose in a 6:1 molar ratio accompanied by small amounts of D-glucuronic acid. The molecular mass has been determined to be 38 kDa. Linkage analysis in combination with partial hydrolysis and spectroscopic investigations revealed a 1,3-linked Gal*p* backbone, branched at C6 to 1,6-linked Gal*p* side residues terminated by Gal*p*, Ara*p*, Ara*f* or Glc*p*A. Monoclonal antibodies directed against a type-II-arabinogalactan from *Echinacea purpurea* showed cross reactivity with the larch arabinogalactan and have been used to develop an ELISA for quantification of this polysaccharide.

1. Introduction

The genus *Larix* (larch) consists of tree species found in cool, temperate regions of the northern hemisphere. A characteristic feature of Larix trees is the occurrence of arabinogalactan (AG), a water soluble polysaccharide, which can be extracted from the wood of these trees in high yield (Stephen, 1983). The AG from Western larch (*Larix occidentalis*) especially has been studied extensively since the middle of the last century (Bouveng & Lindberg, 1956, 1958; Churms, Merrifield, & Stephen, 1978; Eremeeva & Bykova, 1992; Ponder, 1998; Ponder & Richards, 1997a, 1997b, 1997c; Prescott, Groman, & Gulyas, 1997; White, 1941) and found to consist of a (1 → 3)-β-D-galactopyranosidic backbone, branched at position 6 to mono- or oligosaccharide side chains consisting of Gal*p*, Ara*f*, Ara*p* and Glc*p*A. A first molecular modeling study proposes a triple helical structure for AG from Western larch (Chandrasekaran & Janaswamy, 2002). There is consensus on a molar galactose/arabinose (Gal/Ara) ratio of about 6:1, but the glucuronic acid (GlcA) content in larch AGs is sometimes not properly studied or even ignored, although it has been shown that even low levels of uronic acids have strong effects on size exclusion experiments and might even be important for biological activities of these molecules such as interaction with other charged substances (Ponder & Richards, 1997a). Furthermore, substantial discrepancies exist in molecular weight (MW) determined for larch AGs, which range from 3 to 100 kDa. Whereas some authors find a homogenous MW for larch AG (Adams, 1960; Eremeeva & Bykova, 1992), other AG samples from larch consist of two polysaccharides with different MW (Bouveng & Lindberg, 1958; Churms et al., 1978; Prescott, Enriquez, Jung, Menz, & Groman, 1995; Swenson, Kaustinen, Bachhuber, & Carlson, 1968).

Oral administration of larch AG has been shown to result in some beneficial effects on faecal microbial populations in humans (Robinson, Feirtag, & Slavin, 2001) and dogs (Grieshop, Flickinger, & Fahey, 2002). Besides the prebiotic effect, larch AG might also have potential benefits as an immune stimulating agent (Currier, Lejtenyi, & Miller, 2003), at least in combination with extracts of *Echinacea* (Classen, Thude, Blaschek, Wack, & Bodinet, 2006; Kim, Waters, & Burkholder, 2002). AG from larch might also be used as a carrier for delivering diagnostic or therapeutic agents to hepatocytes via the asialoglycoprotein receptor (Groman, Enriquez, Jung, & Josephson, 1994). Interestingly, blocking of hepatocyte lectins by larch AG inhibits liver tumor cell metastasis in a mice model (Beuth, Ko, Schirrmacher, Uhlenbruck, & Pulverer, 1988).

In contrast to AG from *L. occidentalis*, literature on AG from *Larix laricina* is very limited (Adams, 1960; Haq & Adams, 1961; Urbas, Bishop, & Adams, 1963), even though this AG has GRAS (generally

Abbreviations: AG, arabinogalactan; AGP, arabinogalactan-protein; Ara, arabinose; ELISA, enzyme-linked immunosorbent assay; Gal, galactose; GLC, gas liquid chromatography; GlcA, glucuronic acid; LAG, high molecular weight component of the aqueous extract of *Larix laricina*; MW, molecular weight; MWCO, molecular weight cut off; RI, refractive index; SEC, size exclusion chromatography.

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recognized as safe) in the United States and is commercially used as a dietary supplement. To enhance knowledge on this beneficial constituent of *L. laricina*, the structural characterization of the high MW AG from this species is the aim of this work. Furthermore, monoclonal antibodies directed against a type-II-AG have been used to develop an ELISA for detection of larch AG, which might be useful for quantification in preparations.

2. Experimental

2.1. Material

The crude aqueous AG-rich solutions, prepared by aqueous extraction of wood chips of *L. laricina* (DuRoi) K. Koch were provided by Lonza Inc. (Cohasset, Minnesota). The solutions were freeze-dried and then directly used for the initial investigations.

In order to gain the high MW component of the aqueous extract of *L. laricina* (LAG), the freeze-dried aqueous extract was resolved in water to a final concentration of 6% (m/V) and added to six volumes of ethanol 99% at 4 °C. The high MW precipitate was freeze-dried for further analytical investigations.

2.2. Elementary analysis

Elementary analysis for carbon, hydrogen, nitrogen and sulfur was carried out with a HEKAtech CHNS Analysator (Co. HEKATECH, Wegberg, Germany). 2.0 mg of the sample was weighed into a tin capsule. A blank value of an empty tin capsule was analyzed under the same conditions. Aminobenzenesulphonamide was used for the calibration of the system. The content of oxygen was calculated by difference.

2.3. Size exclusion chromatography (SEC)

The absolute molecular mass of the LAG polymer and its degradation product after acidic hydrolysis was determined by SEC on three PL aquagel–OH columns in series (temperature 35 °C, Co. Polymer Laboratories, Darmstadt, Germany). The samples were eluted with $NaNO_3$ (0.1 M) at a flow rate of 0.7 mL/min. The detection system consisted of a multi-angle laser light scattering instrument (mini DAWN, Co. Wyatt Technology, Santa Barbara, CA, USA), directly followed by a refractive index (RI) detector (Co. Polymer Laboratories) with a RI increment of dn/dc = 0.146 mL/g (Zhang, Zhang, & Whistler, 2003). The hydrodynamic volume was calculated by the retention time in comparison to different pullulans, stachyose and glucose with molecular masses of 112,000, 47,300, 22,800, 11,800, 5900, 667 and 180 Da (PL Polysaccharide Standard Kit, Co. Polymer Laboratories).

2.4. Partial acid hydrolysis

The mild acid hydrolysis of the LAG with oxalic acid was carried out according to Göllner, Blaschek, and Classen (2010) with 12.5 mM oxalic acid at 100 °C for 5 h.

2.5. Monosaccharide composition

To determine the monosaccharide composition, the sample was hydrolyzed with trifluoroacetic acid (2.0 mol/L) at 121 °C for 1 h. After evaporation of trifluoroacetic acid, monosaccharides were converted to their corresponding alditol acetates by reduction and acetylation as described by Blakeney, Harris, Henry, and Stone (1983) and analyzed by gas liquid chromatography (GLC) as described by Göllner, Ichinose, Kaneko, Blaschek, and Classen (2011).

2.6. Reduction and labeling of uronic acids with $NaBD_4$

Uronic acids in the LAG polymer were converted by reaction with water-soluble carbodiimides to esters which were then reduced with sodium borodeuteride to their corresponding alcohols as described by Taylor and Conrad (1972): 20.0 mg LAG were dissolved in 30 mL demineralized water. 216 mg N-cyclohexyl-N′-(2-morpholinoethyl)carbodiimide p-toluenesulfonate were added carefully with stirring. The reaction mixture was maintained at pH 4.75 by automatic titration (719 S Titrino, Co. Metrohm, Filderstadt, Germany) with 0.01 M hydrogen chloride for 4 h. The activated carboxylic ester was then reduced by dropwise addition of sodium borodeuteride (4.0 mL of 1.0 M, 5.0 mL of 2.0 M and 5.0 mL of 4.0 M) with maintenance of the pH at 7.0 again by automatic titration using 2.0 M hydrogen chloride. When the pH value remained stable, acetic acid was added to pH 6.5. The entire mixture was dialyzed against water for three days at 4 °C (Spectra/Por® membrane, molecular weight cut off (MWCO): 6–8000, Co. Spectrum Laboratories, Rancho Dominguez, CA, USA) and freeze dried. Due to this reaction uronic acids could be identified and quantified as their corresponding deuterium labeled, permethylated alditol acetates in linkage analysis.

2.7. Smith degradation

The Smith degradation, following the method of Goldstein, Hay, Lewis, and Smith (1965), aims to provide more structural information by selectively degrading polysaccharides to smaller polysaccharides (high MW fraction) and detached sugar units.

20.0 mg of a previously dialyzed sample (MWCO: 12–14 kDa) was dissolved in 0.1 M aqueous sodium periodate solution and stored at room temperature for two days in the dark. Ethylene glycol (19.5 μL in periodate solution) was added, followed 3 h later by sodium borohydride (100 mg). After 20 h, the solution was neutralized with glacial acetic acid, dialyzed (MWCO: 3–6 kDa) and freeze dried. The polysaccharide was hydrolyzed under mild conditions with aqueous trifluoroacetic acid (0.5 M, 15 h at room temperature) and freeze dried again. This degraded sample was precipitated with ethanol to separate the high from the low MW components.

2.8. Linkage analyses

The linkage analysis was carried out as described by Harris, Henry, Blakeney, and Stone (1984). To form polyalkoxide ions the sample was treated with potassium hydride in dimethylsulfoxide (80 g/L), and afterwards methylated by addition of 340 μL methyl iodide. The methylated sample was washed with a chloroform/methanol/water mixture and then was hydrolyzed with 2.0 M trifluoroacetic acid at 121 °C for 1 h. Afterwards the permethylated monosaccharides were reduced with 0.5 M sodiumborodeuteride in 2 M ammonia at 60 °C for 1 h and the reaction was stopped by addition of acetone. Acetylation of the sample was performed by addition of 200 μL of glacial acid, 1.0 mL ethyl acetate, 3.0 mL acetic anhydride and 100 μL perchloric acid. By addition of demineralized water and 100 μL methylimidazole the reaction was stopped. After extraction with methylene chloride the permethylated alditol acetates were separated and detected by GLC-mass spectroscopy as described by Göllner et al. (2011).

2.9. ^{13}C NMR

For ^{13}C NMR analysis, the sample was solubilized in D_2O (10 mg/mL) and analyzed at 75.47 MHz on a Bruker ARX 300 spectrometer incorporating Fourier transform (Co. Bruker, Bremen, Germany) at 323 K for 6–9.5 h (8000–12,500 scans). Chemical shifts are expressed in ppm (δ), based on the resonance of the internal

Table 1
Neutral monosaccharide composition of aqueous extract of larch wood, LAG after ethanol precipitation and LAG after partial acid hydrolysis.

Monosaccharide	Aqueous extract of larch wood (%w/w)	LAG after ethanol precipitation (%w/w)	LAG after partial acid hydrolysis (%w/w)
Gal	80.5 ± 3.0	85.0 ± 1.5	88.0 ± 1.9
Ara	15.2 ± 1.4	12.7 ± 0.8	9.4 ± 1.6
Man	3.1 ± 1.7	1.4 ± 0.4	2.2 ± 0.9
Glc	0.7 ± 0.4	0.6 ± 0.1	0.4 ± 0.1
Xyl	0.5 ± 0.3	0.3 ± 0.2	–
Total carbohydrate content (%w/w)	90.3 ± 8.3	93.4 ± 12.9	95.9 ± 2.9
Ara:Gal	1:5.3	1:6.7	1:9.4
Sample size	$n = 6$	$n = 3$	$n = 5$

standard acetone at δ 31.5 (2 μL/mL D_2O). The data interpretation was accomplished in comparison to the chemical shifts that Ponder and Richards (1997b) and Willför, Sjöholm, Laine, and Holmbom (2002) measured for larch AG.

2.10. Enzyme-linked immunosorbent assay (ELISA)

For the direct ELISA, microtiter plates (Co. Nunc, Roskilde, Denmark) were coated with LAG in different concentrations (10–1000 μg/mL PBS-buffer for LAG, 100 μL/well) at 36 °C overnight. Coated plates were washed three times (PBS with 0.05% Tween 20) in a Tecan Columbus Plus automatic plate washer (Co. Tecan, Crailsheim, Germany). Unbound sites were blocked by addition of a solution of 0.1% bovine serum albumin (BSA) in PBS (200 μL/well, 1 h at 36 °C) and washing was repeated. The dilution of monoclonal antibodies (1:500 in PBS, 100 μL/well), raised against an arabinogalactan-protein (AGP) from pressed juice of *Echinacea purpurea*, was added to the plate (Classen, Csávás, Borbás, Dingermann, & Zündorf, 2004). After 1 h of incubation at 36 °C, the plates were washed and treated with 100 μL/well of alkaline-phosphatase-conjugated antibody (1:1000 in PBS) for 1 h at 36 °C. After washing, the dye was developed by addition of 100 μL/well of substrate solution containing 0.1 mg/mL para-nitrophenylphosphate in 0.2 M Tris-buffer. After 15 min the absorptions were measured at 405 nm in an ELISA reader (Tecan Spectra Thermo, Co. Tecan). On one plate, samples were tested in sextuplicate; the results shown are based on the data from 8 plates.

3. Results and discussion

3.1. Composition of LAG

Elementary analysis revealed a content of carbon of about 40.0% and a content of hydrogen of 6.6% of LAG. Oxygen was then calculated to be 53.4% of the entire LAG mass. Converted into molar percentages, carbon, hydrogen and oxygen are present in approximately a 1:2:1 ratio. The elements nitrogen and sulfur were not found in LAG samples from *L. laricina*. The complete absence of nitrogen demonstrates that there is no protein associated with LAG extract, although the occurrence of AGPs has sometimes been reported for other members of the Pinaceae (Altaner, Tokareva, Jarvi, & Harris, 2010; Putoczki et al., 2007).

The neutral monosaccharide analysis showed that the crude extracts consisted of galactose (80.5 ± 3.0%), arabinose (15.2 ± 1.4%) and smaller amounts of accompanying mannose, glucose and xylose residues (Table 1). Ethanol precipitation of the aqueous extracts was used to separate the LAG macromolecule from accompanying mono-, di- and oligosaccharides. The yield of the ethanol precipitated macromolecular LAG from the crude aqueous extract was about 88 ± 3% (of dry mass). The Ara content of the LAG was slightly lower, indicating that some Ara residues in the crude extracts were present as mono- or oligosaccharides. The ratio of Ara to Gal for the LAG macromolecule was found to be 1:6.7, which is in good correspondence with data for AG of *L. occidentalis* (Ponder & Richards, 1997a), but different to published data for AG of *L. laricina*: Adams (1960) isolated an AG from *L. laricina* with a 1:3.8 molar ratio of Ara to Gal. The different Ara:Gal ratios could be due to the extraction processes. Our extraction process for *L. laricina* is comparable with the aqueous extraction process of Ponder and Richards (1997a). The extraction method for the AG examined by Adams (1960) differed, especially by the pre-treatment of the wood material with benzene and ethanol. Furthermore the Ara:Gal ratio was determined by different analytical methods: In contrast to our gas chromatographic analysis, Adams (1960) performed a quantitative estimation separating Ara and Gal on a column followed by gravimetric determination of monosaccharide weights. Acidic hydrolysis of the macromolecule under mild conditions cleaves furanosidic Ara residues preferentially, whereas pyranosidic Ara and Gal bonds are more acid stable. This treatment led to a loss of about 26% of Ara residues at the periphery of the LAG.

Table 2
Linkage type analysis of aqueous extract of larch wood, LAG after partial acid hydrolysis and LAG after Smith degradation.

Monosaccharide	Deduced linkage	Relative amount (mol%)		
		Aqueous extract of larch wood	LAG after partial acid hydrolysis	LAG after Smith degradation
Gal	t*p*	19.5	25.7	7.2
	3*p*	3.0	4.9	69.3
	6*p*	26.2	25.8	4.4
	3,4*p*	2.1	1.0	0.0
	3,6*p*	33.0	28.9	11.1
Ara	t*f*	5.0	4.5	6.5
	t*p*	4.5	4.8	0.0
	3*f*	5.6	4.4	1.5
GlcA	t*p*	1.1	–[a]	–[a]

[a] GlcA was not detectable, because uronic acids were not reduced prior to methylation analysis.

3.2. Linkage analysis of LAG

Determination of linkage type by methylation analysis was carried out after reduction of uronic acids to the deuterium labeled corresponding neutral alditol acetates. The data revealed the presence of linkages characteristic for type-II-AGs (Table 2).

Gal, the main monosaccharide detected, was present only in the pyranosidic form. The dominant linkage types were 1,3,6-Gal*p*, 1,6-Gal*p* and terminal Gal*p*, accompanied by small amounts of 1,3-Gal*p* and 1,3,4-Gal*p*. The ratio of branched Gal residues to 1,3-linked Gal in the native LAG is very high (11.7:1), thus indicating a highly branched polymer. The low content of 1,3-linked Gal residue is in good correspondence with findings for AG from *L. occidentalis* (Ponder & Richards, 1997b) and *Larix sibirica* (Willför et al., 2002).

Ara was found as a terminal residue in its pyranosidic and furanosidic form as well as in 1,3-linkage in its furanosidic form. The occurrence of Ara in its pyranosidic form seems to be a typical feature of AGs from wood of different larch species such as *L. occidentalis* (Ponder & Richards, 1997b) and *Larix dahurica* (Odonmažig, Ebringerová, Machová, & Alföldi, 1994) and other gymnosperms such as spruce and pine (Willför et al., 2002), but is rather untypical for AGPs, in which the type-II-AGs are covalently linked to a protein moiety (Seifert & Roberts, 2007). GlcA was detected in its pyranosidic form as a terminal unit. The presence of GlcA in low amounts as a component of AG from *L. laricina* was first identified

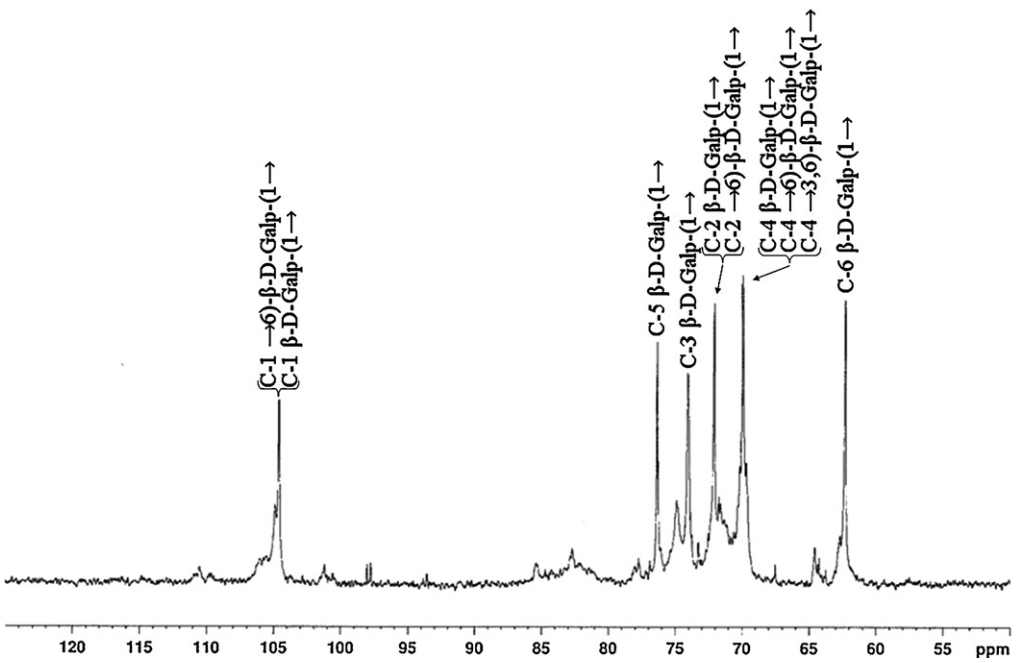

Fig. 1. ^{13}C NMR spectrum of LAG from *Larix laricina*.

in 1963 and found to be linked to position 6 of a Gal residue (Urbas et al., 1963), but in recent publications, the occurrence of these acid groups is sometimes ignored (Chandrasekaran & Janaswamy, 2002).

Linkage analysis of LAG after mild oxalic acid hydrolysis revealed that only small amounts of Ara residues have been eliminated from the polysaccharide under these conditions (Table 2). The content of furanosidic Ara decreased from 10.6 to 8.9%, whereas pyranosidic Ara bonds remained stable. This indicates, that the pyranosidic Ara residues are linked to the galactan structure and remain attached to the macromolecule after oxalic treatment.

Smith degradation was carried out for selective hydrolysis of monosaccharides with two hydroxyl groups in vicinal positions. After this procedure, the majority of Gal residues were present in 1,3-linkage (Table 2). This linkage type increased from 3.0% in the aqueous larch extract to 69.3% in the degraded LAG and was accompanied by smaller amounts of terminal, 1,6- and 1,3,6-linked Gal residues. It can be concluded, that the majority of 1,3,6-linked Gal residues are part of the backbone of the native LAG and not part of the side chains. Ara residues are mainly furanosidic and terminally linked after periodate treatment, possibly originating from 1,3-linked Ara*f* residues.

3.3. ^{13}C NMR spectroscopy of LAG

The different linkage types found in methylation analysis were confirmed by ^{13}C NMR spectroscopy (Fig. 1 and Table 3). All Gal monosaccharides and pyranosidic Ara residues were present as β-conformers, whereas furanosidic Ara was detected in its α-conformation. The data obtained was in good correspondence with the data for larch AG from *L. sibirica* (Willför et al., 2002) and *L. occidentalis* (Ponder & Richards, 1997b). No signals from carboxyl carbons could be detected, probably due to the low amount of GlcA (Table 2).

3.4. Determination of molecular mass of LAG

For determination of the absolute molecular mass, a laser light scattering detector in combination with a RI detector was used.

Table 3
^{13}C chemical shifts of the most significant signals in LAG spectrum.

Linkage type	Chemical shift in ppm					
	C-1	C-2	C-3	C-4	C-5	C-6
β-D-Gal*p*-(1→	104.6	72.1	74.1	70.0	76.4	62.3
→6)-β-D-Gal*p*-(1→	104.6	72.1	73.3	70.0	74.9	70.2
→3,6)-β-D-Gal*p*-(1→	104.9	71.6	82.6	70.0	74.9	70.9
α-L-Ara*f*-(1→	110.5	81.4	77.7	85.4	62.7	
β-L-Ara*p*-(1→	101.2	69.7	69.9	70.2	64.6	
→3)-α-L-Ara*f*-(1→	109.5	nd	85.4	84.2	62.7	

nd: not detected.

Table 4
Absolute molecular mass and hydrodynamic volumes of aqueous extract of larch wood, LAG after reduction of uronic acids and LAG after partial acid hydrolysis.

LAG sample	Molecular mass (Da)	Hydrodynamic volume (Da)
Aqueous extract of larch wood	37,936	17,052
LAG after reduction of uronic acids	37,320	13,511
LAG after partial acid hydrolysis	35,900	15,447

Furthermore, the hydrodynamic volume of LAG was determined in comparison to pullulan standards. Only one macromolecular component could be detected in all samples (Table 4). The absolute molecular mass of the high MW LAG was about 38 kDa, whereas the hydrodynamic volume was considerably smaller (about 17 kDa). This difference indicates a compact, highly branched structure of the LAG molecule. Furthermore, these values are in good correspondence with results of Groman et al. (1994) for *L. occidentalis*: AG from this species had a MW of 40 kDa and a hydrodynamic volume of 19 kDa. LAG samples after uronic acid reduction and labeling had a slightly smaller absolute MW of about 37 kDa as well as a smaller hydrodynamic volume of about 13.5 kDa. This was to be expected, since the carboxyl function is lost during this process and in consequence, charges and thereby hydration of LAG will clearly be reduced. LAG samples after oxalic acid treatment also showed a decrease of absolute molecular mass (36 kDa) and hydrodynamic volume (15.5 kDa) due to loss of labile Ara residues at the periph-

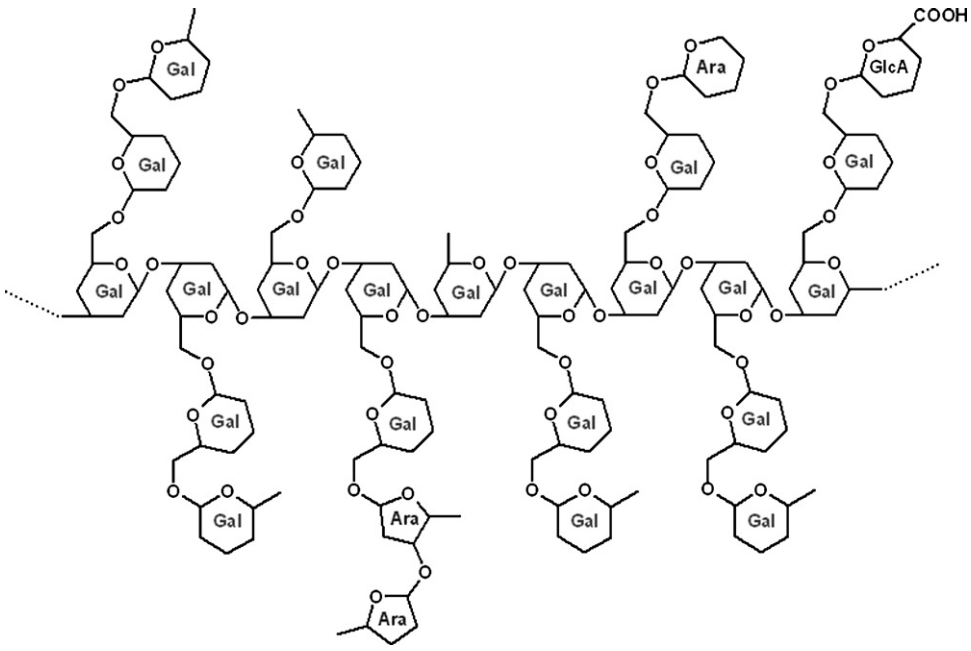

Fig. 2. Tentative structural model for LAG from *Larix laricina*.

ery of the molecule. This is nearly consistent with the loss of about 20% Ara that would lead to a resulting macromolecule of about 1 kDa less. The hydrodynamic volume of the mild hydrolyzed sample is clearly bigger compared to the hydrodynamic volume of the carboxyl-reduced sample. This indicates that GlcA is not liberated during the oxalic acid treatment which means that GlcA is linked to Gal and not to Ara.

In the literature, substantial discrepancies exist in MWs determined for larch AGs. Larch AG has been often reported to consist of two components of different MWs, one high MW polysaccharide with values recorded in the range of 37–100 kDa and a second component of lower MW in the range of 3–18 kDa (Bouveng & Lindberg, 1958; Clarke, Anderson, & Stone, 1979; Prescott et al., 1997; Swenson et al., 1968). Prescott et al. (1995) demonstrated that exposure of larch AG to alkaline solutions in the presence of sodium borohydride led to degradation resulting in 9 kDa AG fragments. Therefore it might be speculated that the low MW components found in different larch AG samples might not be present in native larch AGs but could be artefacts due to the isolation process.

3.5. Proposed structure of LAG

Our analytical data led to a structural model for LAG from *L. laricina* in which the molecule has a backbone of 1,3-linked Gal residues, branched in position 6 to 1,6-linked Gal side chains (Fig. 2) that is in agreement with the general structure of type-II-AGs. Smith degradation resulted in very high amount of 1,3-linked Gal residues, indicating that side chains have been degraded and 1,3,6-Gal residues are part of the backbone and not of the side chains. Based on an absolute molecular mass of 38 kDa, the LAG macromolecule should be composed of about 10 repetitions of the subunit shown in Fig. 2. The difference between absolute molecular mass and hydrodynamic volume is possibly caused by a very compact structure of the LAG macromolecule, thus leading to a high mass in a small volume. Structural investigations on larch AG from *L. laricina* have been carried out only in the middle of the last century and the authors proposed a backbone consisting of 1,3- and 1,6-linked Gal residues (Adams, 1960; Haq & Adams, 1961), which could not be confirmed by our investigations. A molecular modeling study by Chandrasekaran and Janaswamy (2002) suggest a helical structure for larch AG from *L. occidentalis*.

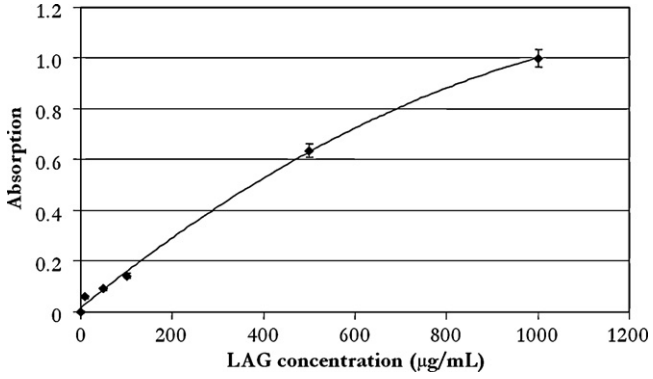

Fig. 3. Reactivity of monoclonal antibody raised against *Echinacea* AGP with LAG from *Larix laricina*.

3.6. Quantification of LAG by ELISA

Comparing the structure of LAG to the structure of AGP from *E. purpurea* (Classen, Witthohn, & Blaschek, 2000; Classen, Mau, & Bacic, 2005) showed that there were similarities. For this reason, monoclonal antibodies raised against the *Echinacea* AGP with a type-II-AG carbohydrate moiety (Classen et al., 2004) have been tested for cross reactivity with LAG from *L. laricina*. It could be shown that antibodies interact with this LAG in a concentration range between 10 and 1000 µg LAG/mL. Standard deviations are based on data of eight plates (Fig. 3). Reactivity of antibodies with LAG was around 200-fold weaker compared to AGP from *Echinacea* (data not shown). It has been evaluated before, that monoclonal antibodies against *Echinacea* AGP are not strongly specific and show cross reactivity towards AGPs from other species. It has been proposed that these antibodies interact with a galactan epitope present in most AGPs such as 1,3-, 1,6- and/or 1,3,6-linked Gal-oligosaccharides (Classen et al., 2004). As shown in Fig. 2, similar Gal-epitopes are present also in LAG from *L. laricina* and might be responsible for binding of the antibody. The weaker binding of

the antibodies to the LAG compared to the *Echinacea* AGP may be caused by the different structure of the Gal backbone. The LAG is highly branched and thereby has less 1,3-Gal units. Furthermore, in average the LAG has shorter side chains than the AGP from *Echinacea* which might also lead to reduced binding capability. In summary, antibodies might be a useful tool for quantification of LAG, e.g. for quality control of dietary products containing LAG. Production of antibodies specific for LAG will be an interesting task for the future.

4. Conclusion

Although LAG from *L. laricina* is commercially used as a dietary supplement, literature on the LAG from this source is very limited. Using GLC-MS, ^{13}C NMR, SEC and ELISA, we were able to assign the structure of this LAG to the type-II-AGs and propose a model for this polysaccharide. Antibodies have been used to establish an ELISA for detection of LAG, which might be used for quantification of LAG in dietary supplements.

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