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Note

New molecular weight forms of arabinogalactan from *Larix occidentalis*

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Abstract

Arabinogalactan fractions with narrow molecular weight distributions were obtained from a crude extract of *Larix occidentalis* by gel-filtration chromatography. Molecular weight distributions of the fractions were determined by a combination of gel filtration chromatography (GFC) with intensity (Rayleigh) light scattering. Two distinct distributions were observed, with the fractions in the lower distribution as small as 3 kDa. A sensitive immunoassay confirmed that the polysaccharide fractions were arabinogalactans. Compositional analysis revealed that the sub-9 kDa arabinogalactan fractions obtained by gel filtration of the crude extract have significantly lower galactose to arabinose ratios than the previously isolated sub-9 kDa arabinogalactans obtained from arabinogalactan (37 kDa) by chemical methods [J.H. Prescott et al., *Carbohydr. Res.*, 278 (1995) 113–128]. © 1997 Elsevier Science Ltd.

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1. Introduction

Substantial discrepancies exist in molecular weights determined for larch arabinogalactans. Larch arabinogalactan has been reported to consist of two macromolecular components, one of high molecular weight distribution with values reported in the range of 37–100 kDa (70–95%) and a second distribution of lower molecular weight with values in the range 7.5–18 kDa (5–30%) [1–5]. The variation within each range may be attributed to analytical methodology and isolation procedure [6,7]. Ettling and Adams isolated arabinogalactans from larch using gel filtration chromatography (GFC) and identified two arabinogalactan distributions, and a third component corresponding to low molecular weight phenols and monosaccharides [8]. We have re-examined the molecular weight of arabinogalactan following the methodology of Ettling and Adams. Molecular weights were determined by a combination of GFC with intensity (Rayleigh) light scattering, an absolute method for the determination of molecular weight. Fractions were identified as arabinogalactans on the basis of their composition and immunoreactivity to an anti-arabinogalactan antibody. The arabinogalactan fractions range in molecular weight from 3 to 93 kDa. This is the first report of larch arabinogalactan with a molecular weight as low as 3 kDa.

We have previously reported isolation of a sub-9 kDa fragment from a purified, 37 kDa larch arabinogalactan using alkaline hydrolysis [1]. In view of

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previous reports of a low and high molecular weight arabinogalactan in crude extracts, together with our own finding of a sub-9 kDa arabinogalactan in crude extracts of *Larix occidentalis*, we asked if these two sub-9 kDa arabinogalactans would have identical compositions (molar ratio of galactose to arabinose) and thus similar chemical genesis. Compositional analysis revealed that the sub-9 kDa arabinogalactan fractions obtained by gel filtration of the crude extract have significantly lower galactose to arabinose ratios than the previously isolated sub-9 kDa arabinogalactans obtained from arabinogalactan (37 kDa) by chemical methods.

2. Experimental

Materials and procedures.—Fractions of arabinogalactan were obtained from crude arabinogalactan from *Larix occidentalis* (Stractan 2, Champion Corp., Tacoma, WA) by gel-filtration chromatography with a Sephadex G-75 column. Approximately 4 g of Sephadex was used to pack the column, which had dimensions of 4.6 mm by 25 cm. The mobile phase was 0.2 μm-filtered water, flowing at 1 mL/min. The crude arabinogalactan was fractionated by placing 1.5 mL of a 100 mg/mL solution on the column and eluting with distilled water. A total of 40 fractions were collected in ~3 mL increments. The molecular weight distributions of the fractions were determined by GFC with intensity (Rayleigh) light scattering as described previously [1]. The carbohydrate contents of the fractions were determined with the anthrone assay [9]. The monosaccharide compositions of selected fractions were determined by acid hydrolysis, column separation, and electrochemical detection as described by Lee and Manzi [10,11]. The arabinogalactan contents of the fractions were determined by a sensitive radioimmunoassay which we have developed using a rabbit anti-larch arabinogalactan antibody [12].

3. Results

The molecular weight values for the arabinogalactan fractions from Stractan 2 collected from the Sephadex G-75 column are listed in Table 1. No smoothing was applied to the collected data or to the molecular weight distributions. In general the fractions were nearly monodisperse, with detected molecular weights ranging from 3 to 93 kDa. Fractions 1 through 7 contained no carbohydrate, and neither a refractive index (RI) signal nor a light scattering signal was observed from these fractions. The light scattering and RI signals from fractions 8 through 25 were sufficient for performing molecular weight determinations. The peak average molecular weights for fractions 8 through 25 are plotted versus their corresponding carbohydrate concentrations determined by anthrone assay in Fig. 1. Also plotted in Fig. 1 is the molecular weight distribution of the crude arabinogalactan. A close correspondence of the relative amount of polysaccharide in each fraction as determined by carbohydrate (anthrone) assay and by differential refractive index detection is observed. The light scattering signals from fractions 26 through 40 were too weak to make molecular weight determinations.

Table 1
Molecular weights of fractionated crude arabinogalactan obtained by passage across Sephadex G-75

Fraction	M_p (Da)	M_w / M_n	Molar ratio, Gal/Ara
8	93325	1.12	
9	78524	1.08	6.99
10	63826	1.08	
11	51286	1.05	
12	45709	1.03	
13	42658	1.02	
14	38905	1.01	
15	35892	1.01	
16	33113	1.01	5.96
17	30200	1.11	
18	31261	1.13	
19	9120	1.16	
20	7499	1.06	3.69
21	6383	1.06	
22	5559	1.02	
23	4416	1.04	
24	3673	1.05	
25	2951	1.08	2.33
AG (37 kDa) from Stractan 2 [5] [b]	35842	1.17	5.45 [5]
AG (9 kDa) isolated from AG (37 kDa) [5] [b]	8414	1.15	5.71 [5]

[a] M_p is the weight average molecular weight for the maximum of the molecular weight distribution. The ratio of the weight averaged to number averaged molecular weights (M_w / M_n) is an indicator of molecular weight homogeneity for the sample. A homogenous material will have a ratio of 1.0. Commercial dextrans typically have M_w / M_n ratios on the order of 1.2 to 1.5.
[b] See Table 3 of Prescott et al. [5].

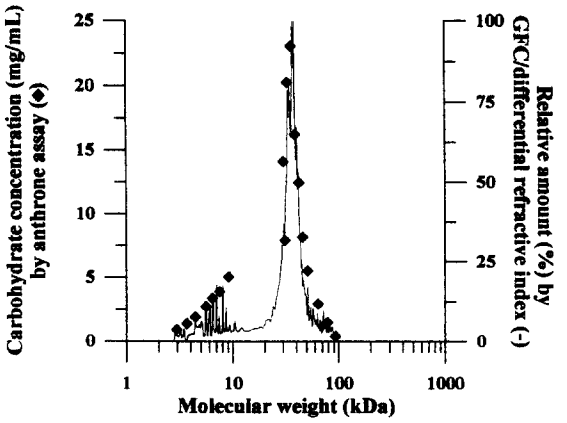

Fig. 1. Comparison of peak average molecular weights, M_p, for the fractions of crude larch arabinogalactan obtained by GFC as a function of their carbohydrate content (◆) with the molecular weight distribution of the crude arabinogalactan (Stractan 2) as determined by GFC/laser light scattering (—).

The carbohydrate-containing fractions were identified as arabinogalactans by testing for their immunoreactivity and composition. The immunoreactivity of each fraction was measured using a rabbit anti-larch arabinogalactan antibody [12]. A comparison of the amount of arabinogalactan in each fraction as determined by immunoassay and carbohydrate (anthrone) assay is presented in Fig. 2. The arabinogalactan immunoassay is very specific for measuring arabinogalactan from the larch, exhibiting little or no reactivity with a variety of polysaccharides, asialoglycoproteins, and neoglycoproteins [12]. The assay results indicate that fractions 8 through 25, with molecular weights from 3 to 93 kDa, are immunoresponsive to approximately the same extent on a weight basis. The high selectivity of the anti-larch arabinogalactan antibody strongly supports our belief that the 93 kDa component, the 3 kDa fraction, and all of the fractions in between are arabinogalactans.

The galactose to arabinose ratios of selected arabinogalactan fractions isolated from Stractan 2 by GFC are presented in Table 1. Also listed in Table 1 are the galactose to arabinose ratios of a purified arabinogalactan with a molecular weight of 37 kDa, denoted AG(37 kDa), and a chemically derived arabinogalactan with a molecular weight of 8.4 kDa, denoted AG(9 kDa) [1]. The monosaccharide composition of selected immunoresponsive fractions over the whole molecular weight range of fractionated Stractan 2 contained only galactose and arabinose. Thus, the material contained in fractions 8 through 25 is identified as arabinogalactan by three criteria: molecular weight (> 1 kDa), immunoreactivity, and composition.

The ratio of galactose to arabinose in the fractions obtained from Stractan 2 are observed to increase with increasing molecular weight, in agreement with Swenson et al. [4] In contrast to arabinogalactan with a molecular weight less than 9 kDa isolated from Stractan 2 by gel filtration chromatography, the chemically derived, sub-9 kDa arabinogalactan has a composition which is nearly identical to the higher molecular weight AG(37 kDa) [1].

The peak average molecular weights (Mp) as determined by light scattering are plotted as a function of the fraction number in Fig. 3. Two distinct macromolecular weight distributions are seen, consistent with previous reports [2,13,14]. A dramatic shift in the molecular weight is observed between fractions 18 and 19. Components corresponding to arabinogalactan with a molecular weight of 30–95 and 3–9 kDa represent ~ 75 and 20% of the crude arabinogalactan by mass, respectively. While this bimodality in the molecular weight distribution of larch arabinogalactan has been seen previously, the polydispersity within each component was not been given much attention. Swensen et al. reported collecting narrow molecular weight fractions from larch arabinogalactan ranging from 6.4 to 61 kDa [4]. We have applied similar separation methods and an absolute method for the determination of molecular weight to expand this range to a low of 3 kDa and to a high of 93 kDa. The fractions in the sub-5 kDa range have not been reported previously.

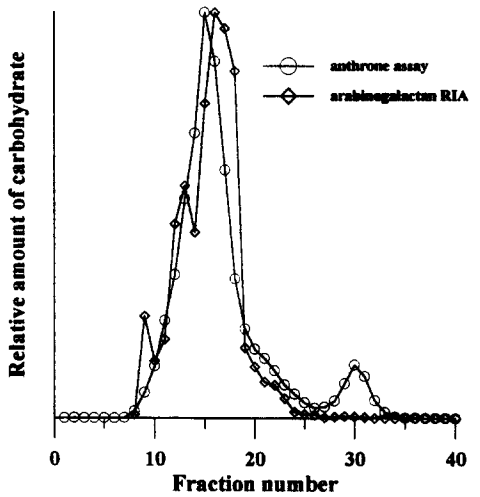

Fig. 2. Relative comparison between the carbohydrate content of the fractions from crude arabinogalactan (Stractan 2) as determined by the anthrone assay and by immunoassay.

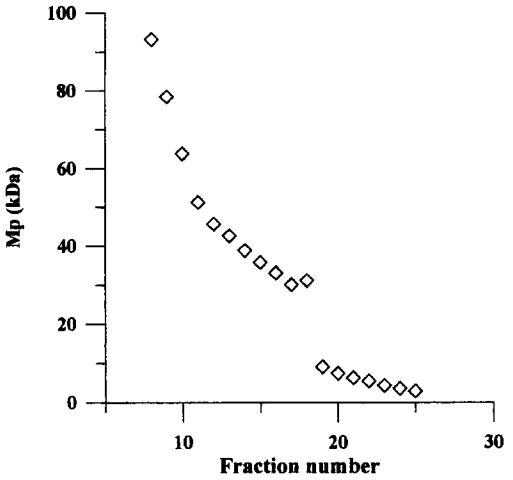

Fig. 3. Peak average molecular weights for the arabinogalactans obtained from crude arabinogalactan by fractionation with Sephadex G-75. Mp is the weight average molecular weight for the maximum of the molecular weight distribution of each fraction.

4. Discussion

Our finding of two molecular weight distributions in an arabinogalactan extract from *Larix occidentalis* is consistent with previous observations [1–5]. Reported molecular weights for arabinogalactans from *Larix occidentalis* have been determined by sedimentation equilibrium [2–4,13,14] and osmometry [4], and bimodal distributions were observed, with the high molecular weight form predominating. However, the molecular weights reported within each distribution have been variable, ranging from 37 to 100 kDa for the high molecular weight distribution and from 7.5 to 18 kDa for the low molecular weight distribution. Despite the variation in the reported values, the ratio of the mean of the high molecular weight distribution to that of the lower molecular weight distribution have consistently been on the order of 4:1. This suggests that the discrepancy in the reported molecular weights of arabinogalactans from larch is a consequence of the analytical methodology used.

We have applied state of the art methods for determining the molecular weights of polymers in solution to fractions of a crude extract of *Larix occidentalis*. Weight-averaged molecular weight distributions were determined by combining gel filtration chromatography (GFC) with an in-line intensity light scattering photometer and a mass-sensitive detector. The molecular weight of polymers in solution can be accurately determined by Rayleigh light scattering. However, the exponential dependence of the scattered intensity, I_s, on the hydrodynamic diameter, d_h, of the scattering center has limited the practical usefulness of the technique to monodisperse systems. This limitation can be overcome by fractionating a polydisperse sample on a gel filtration bed prior to the scattering measurement, and extremely accurate, mass-weighted molecular weight distributions can be obtained. The gel filtration-light scattering technique is fast, easy to perform with commercially available instrumentation, not limited to monodisperse samples, and sample requirements for analysis are typically tens of micrograms or less. Unlike molecular weights calculated from a gel filtration elution volume relative to the elution of standards of known molecular weight, the technique does not use a standard curve and makes no assumptions regarding hydrodynamic shape or volume [15]. Unlike sedimentation equilibrium, the technique can accurately provide the distribution of molecular weights, and no assumptions need to be made regarding the shape of the molecules in solution if the radius of gyration (R_g) is less than approximately 60 nm [16]. Polysaccharides with molecular weights on the order of kilodaltons or greater are ill conditioned for molecular weight determinations by matrix assisted-laser desorption ionization-time of flight-mass spec (MALDI-TOF-MS) because of the tenacity with which they bind to the matrices, and their poor ionization. By applying the GFC-light scattering technique to fractions of larch arabinogalactan, two distinct molecular weight distributions were observed, and nearly monodisperse fractions of arabinogalactan within each distribution were isolated. Observed molecular weights were as low as 3 kDa. This represents the lowest molecular weight arabinogalactans isolated from larch to date.

Swenson et al. previously reported the presence of a high and low molecular weight arabinogalactan, designated polymer A and polymer B, respectively [4]. On the basis of viscosity measurements they proposed that polymer B was not a breakdown product of polymer A. In addition, they observed a relative increase in arabinose content with decreasing molecular weight of arabinogalactan. We have seen a similar increase in arabinose content in low molecular weight (≤ 9 kDa) arabinogalactan fractions from Stractan 2 relative to the high molecular weight fractions (≥ 30 kDa). In contrast to this monotonic increase in arabinose content with decreasing molecular weight, we have found that a chemically derived low molecular weight arabinogalactan [AG(9 kDa)], an in vitro chemical breakdown product, has nearly the same galactose to arabinose ratio as AG(37 kDa).

This is the first report of a measured difference in composition between a sub-9 kDa arabinogalactan isolated by physical methods from a crude arabinogalactan extract and a sub-9 kDa arabinogalactan obtained in quantitative yield from the 37 kDa arabinogalactan by chemical methods. The compositional differences between sub-9 kDa arabinogalactans obtained by either gel filtration or by chemical methods are both statistically significant and intriguing. Our observation is consistent with the conjecture of Swenson et al. that the sub-9 kDa arabinogalactan in the crude arabinogalactan extract is not a degradation product of the higher molecular weight fractions [1,4]. The low molecular weight material in the crude extract is possibly a biological precursor of the predominant, larger molecular weight form of arabinogalactan in the extract.

Immunoreactivity is dependent on structure and, to some extent on molecular size. It has been demonstrated that terminal galactose residues are the principal moiety responsible for binding to this anti-arabinogalactan antibody, and that arabinose does not bind to this antibody [12]. While the carbohydrate in the fractions from GFC was immunoresponsive to nearly the same extent on a gravimetric basis, the higher molecular weight fractions are slightly more immunoreactive. The lower gravimetric immunoreactivity of the low molecular weight fractions (≤ 9 kDa) compared to the higher molecular weight fractions, shown in Fig. 2, may be explained by their increasing arabinose content. A second factor contributing to decreased immunoreactivity is the molecular weight. A smaller antigen with a decreased number of terminal galactose residues per gram may not bind as effectively as a higher molecular weight antigen of similar structure, so that the immunoreactivity of the lower molecular weight forms of arabinogalactan may be diminished.

The anthrone assay shows a peak centered about fraction 30 which did not interact with the antibody. This latter component exhibited no immunoreactivity and a strong UV absorbance. This is consistent with the monomeric carbohydrate and phenolic components seen previously by Ettling and Adams [8].

Although arabinogalactan has a complex structure, its similar reactivity compared with monosaccharides and polysaccharides in assays that detect carbohydrate makes it difficult to determine arabinogalactan concentrations in plant extracts and plant cell culture. In this paper we have presented an example of how immunoassay can be used to detect trace amounts of arabinogalactan in larch extracts and suggest that this methodology presents opportunities for studying arabinogalactan synthesis and metabolism without isolation of trace fractions.

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