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Characterization of galactomannans isolated from legume endosperms of Caesalpinioideae and Faboideae subfamilies by multidetection aqueous SEC

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ABSTRACT

Galactomannans isolated from legume seed endosperms, including those of commercial interest, have been characterized by multidetection aqueous SEC. Galactomannans derived from seeds of the Faboideae subfamily had substantially higher M_w than those from Caesalpinioideae seeds ($M_{w,Fab}$ = 2.4–3.1 × 10^6 g/mol, $M_{w,Caes.}$ = 0.86–2.1 × 10^6 g/mol) and within the latter botanical subfamily, an apparent correlation between M_w and the degree of galactose substitution DG was found. The molar mass distributions were unimodal and differed primarily by a scale factor, with distributional widths narrower than a true Flory 'most-probable distribution'; good fits to Schulz–Zimm model were obtained. Across subfamilies no differences were found in the exponents of $[\eta]$–M and R_v–M relationships (0.61 ± 0.02, 0.54 ± 0.01, respectively), the Flory chain stiffness ratio (C_∞ = 20 ± 1 (BSF analysis)), or the persistence length (L_p = 5.5 ± 0.2 nm) obtained from SEC fraction data. However, it was found that prefactors in the $[\eta]$–M and R_v–M relationships as well as the unperturbed parameter K_Θ decrease in proportion to DG and therefore chain density. Generalized relationships incorporating galactose-dependent prefactors were therefore developed to model SEC fraction data of native galactomannans ($[\eta]_{GM} = (1800 \pm 200) \times M_o^{-1.61} \times M^{0.61\pm0.02}$, $R_{v,GM} = 0.63 \pm 0.05 \times M_o^{-0.54} \times M^{0.54\pm0.01}$) as well as lower-$M$ fractions obtained by ultrasonication ($[\eta]_{GM} = (730 \pm 100) \times M_o^{-1.71} \times M_w^{0.71\pm0.02}$, $R_{v,GM} = 0.49 \pm 0.05 \times M_o^{-0.57} \times M_w^{0.57\pm0.01}$, $M \approx 1 \times 10^5$-native). As a consequence of this dependence and the observed patterns in molar mass variation, $[\eta]$ varies within a narrow range for galactomannans as a whole despite substantial M_w differences.

1. Introduction

Galactomannans are high molar mass, water-soluble nonionic polysaccharides extracted in high yield from the endosperm tissues of many legume seeds, where they possess energy-reserve and hydration functions. Milled endosperm powders of guar (*Cyamopsis tetragonoloba*), carob (or locust bean) (*Ceratonia siliqua*), and fenugreek (*Trigonella foenum-graecum*) seeds are currently exploited on an industrial scale, and within the food industry are used as mass-efficient aqueous thickeners, co-gellants, and nutritional supplements (Gidley & Reid, 1995).

The 'classical' structure of legume galactomannans refers to a linear mannan backbone, comprising β-D-mannose units linked at carbons 1 and 4 through a flexible glycosidic bond, variably substituted at carbon 6 with α-D-galactose units. In aqueous solution, galactomannans adopt random-coil conformations with very large molecular dimensions and consequently are mass-efficient aqueous viscosifiers. The average degree of galactose substitution DG, as well as its substitution pattern varies according to the specific plant source, and applications have evolved to exploit these differences. In aqueous solution low-DG galactomannans such as carob interact strongly through galactose-depleted chain segments with compatible helices (e.g., xanthan, k-carrageenan) or by self-interaction, producing network-type gels that are useful texturants in many food products. High DG galactomannans on the other hand do not interact strongly with other polysaccharides and are used primarily as mass-efficient thickeners. Average DG has been found in fact to vary within a limited range depending on the botanical origin, with low-DG galactomannans confined primarily to Caesalpinioideae legume subfamily, and high-DG galactomannans to the Faboideae subfamily, based on a frequency analysis (Buckeridge, Panegassi, Rocha, & Dietrich, 1995). Further information on the details of the repeat unit structure, composition, and structure–property relations of galactomannans are documented in several reviews (Gidley & Reid, 1995; Shcherbukhin & Anulov, 1999; Srivastava & Kapoor, 2005).

Despite the appeal of these endosperm powders as robust thickeners and gellants, it has not been trivial to achieve reliable measure of the 'native' molecular weight of galactomannans, as

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found within the viable seed's endosperm. A major problem is the reliance on commercially available products such guar gum or locust bean gum, which are manufactured through a series of potentially harsh thermal and mechanical treatments (e.g., acid or flame peeling to extract endosperms, attrition grinding, etc.), and there is no guarantee that the molecular weight in final products will be within a given range, based on current regulations. Beyond this are the difficulties to obtain stable, homogeneous solutions, even after standard solubilization and purification methods have been used. It still appears to be an open question to what extent the molecular weight of galactomannans depends on the botanical source, as opposed to other possible sources of variation. There is some evidence based on recent SEC measurements that the molecular weights of different galactomannan types are not the same, within typical experimental errors (Brummer, Cui, & Wang, 2003; Daas, Grolle, Vliet, Schols, & de Jongh, 2002; Picout, Ross-Murphy, Errington, & Harding, 2001; Picout, Ross-Murphy, Jumel, & Harding, 2002; Richardson, Willmer, & Foster, 1998), although these studies do not address this question in detail. Since average DG and the associated patterning are considered to be genetically controlled, based on the enzymes catalyzing polymerization, it seems likely that M_w and the associated distribution will also be controlled at a similar level, and then we may speak meaningfully of a species-characteristic value of M_w and molar mass distribution of galactomannans, as well as DG.

Multidetection SEC measurements were originally begun to characterize galactomannans of commercial interest, in particular to determine their molecular weight and to further relate M_w to their intrinsic viscosity $[\eta]$. Even though the empirical $[\eta]$–M (i.e., MHS) relationships have been determined in numerous studies, only multidetection SEC can determine both molecular parameters simultaneously and independently, whether for separated near-monodisperse fractions or the global averages. For nonionic aqueous thickeners such as galactomannans, the intrinsic viscosity takes on particular importance in real applications, as solution viscosity depends primarily on this chain parameter (e.g., $\eta_o \sim ([\eta]c)^{1.4}$ for dilute solutions and $\sim c[\eta]^{3.3 \pm 0.3}$ for $c > c^*$ (Morris, Cutler, Ross-Murphy, & Rees, 1981) and thus only indirectly to molar mass via the coil-expansion rules for a random coil (e.g., $[\eta] \sim M^{0.72}$ for guar galactomannan (Beer, Wood, & Weisz, 1999)). It is well known that galactomannans exhibit some variations in their suspending and thickening properties even for highly pure samples, a fact often attributed to direct result of molar mass and/or chain length differences, yet $[\eta]$ could also result from coil expansion or contraction due to the influence of galactose groups.

In this publication, we describe the results of a comparative SEC study examining galactomannans extracted manually from seeds of two of the three legume subfamilies, Caesalpinioideae and Faboideae. We include galactomannans of commercial interest (these including *C. siliqua* 'carob' or 'LBG' and *Caesalpinia spinosa* 'tara' from the former subfamily, and *C. tetragonoloba* 'guar', and *T. foenum-graecum* 'fenugreek' from the latter) as well as non-traditional sources of galactomannans from seeds that are readily available. We have developed a gentle method for endosperm extraction and milling, and use a standard method for powder milling and further solubilizing, methods which provided the highest possible viscosity and M_w on dissolution according based on extensive optimization (Pollard et al., 2008). Checks on these methods have been repeated here in some instances, and this, in combination with measurements of comparable industrial samples, as well as purified samples, have led us to suggest that the galactomannans 'native' chain structure is preserved to a much better extent by this method. In addition to determination of M_w and $[\eta]$, the molecular weight distributions were determined based on a straightforward data analysis, and SEC fraction data was analyzed to obtain estimates of the unperturbed parameter K_Θ, and thereby the Flory chain stiffness C_∞ and chain persistence length L_p. We have also determined the $[\eta]$–M (i.e., MHS) and R_v–M relationships from SEC fractions as well as ultrasonicated fractions based on the multidetection approach, which will facilitate comparison with literature data.

2. Experimental

2.1. Seeds

Certified guar seeds (Lewis cultivar) were provided by Texas Foundation Seed, Vernon, TX, USA. Tara seeds and pre-processed guar endosperms for bulk measurements were provided by Unipektin AG, Eschenz, Switzerland. Honey locust seeds were collected locally in October 2006 in Oerlikon, Switzerland, and carob (locust bean) seeds were collected in April 2006 from an isolated tree in Mallorca, Spain. Fenugreek seeds were purchased in August 2006 from a local Indian food store. The remaining seeds were purchased from Sandeman Seeds, Lalongue, France. Industrial guar samples were Cargill Viscogum MP41230 (Blattmann Schweiz AG, Wädenswil, CH), Ecopol 1144 (Economy Polymers and Chemical, Houston, TX, USA), Fluka, Sigma G4129, Hercules Supercol U (Hercules, Inc., Wilmington, DE, USA), Provisco Provigel NAG903 (Provisco AG, Hauptwil, CH), Rhodia Meyprodor 400 (Danisco A/S, Copenhagen, DK), TIC VG506893 and G506858 (TIC Gums, Belcamp, MD, USA), Unipektin GH-200, A, and D (Unipektin AG, Eschenz, CH), Wolff 6382–200 (Sugro AG, Basel, CH). Commercial carob sample was Unipektin L-175, commercial tara gum sample was Unipektin SP-175, and commercial fenugreek was Adumim Fenupure (Adumim Food Ingredients, Israel).

2.2. Swelling tests, endosperm extraction, and powder preparation

The necessary conditions to extract endosperms from swollen seeds were determined by monitoring seed mass during soaking at room temperature over 3 days, or during soaking in boiling water over the course of several hours. Because seeds differed with respect to the efficiency and degree of swelling, three methods were used in practice: (1) swelling at room temperature; (2) holding at 95 °C for 5 min followed by room temperature swelling; (3) extended holding at 95 °C and removing swollen seeds at 1 h intervals (Table 1). Seeds having gummy or pasty endosperms or darkened germs after these treatments were discarded.

Powders were prepared by precutting the extracted endosperms to ca. 1 mm size, then hydrating the extracted endosperms in water, and milling the particles with a centrifugal rotor mill (Retsch ZM-200) to pass a 0.2 mm mesh. Powders were then dried under room temperature and humidity conditions for several days and then further dried in a desiccator. Selected galactomannans were further purified using a simple method. The powders were dissolved at ca. 0.5% (w/w) concentrations in deionized water at room temperature, hydrated for 1 h, held in a water bath at 85 °C for 10 min, then centrifuged to remove insoluble matter (40,000*g*, 15 min, 25 °C). The supernatant was precipitated into 2 volumes of ethanol, pressed and rewashed several times with ethanol, dried overnight in a hood with circulating airflow, then further dried in a desiccator. Per cent yields of purified galactomannan were 23–60% (Table 1).

2.3. Multidetection SEC

2.3.1. Instrument and methodology

The SEC instrument is a Viscotek TDA 302, with inline light-scattering (7° (LALS), 90° (RALS)), refractive index (RI), and differential bridge viscometer (DP) detectors. Other equipment includes: