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Structural and conformational characterization of arabinoxylans from flaxseed mucilage

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ABSTRACT

The structure of neutral fraction gum from flaxseed mucilage (FM-NFG) was studied for better understanding of the relationship between primary structure and conformation. Based on methylation/GC–MS, nuclear magnetic resonance (NMR) spectroscopy, the structure of FM-NFG was proposed as arabinoxylans. Flaxseed mucilage arabinoxylans contained β-1,4-linked xylose backbones, which were mainly substituted at O-2 and/or O-3 positions by 1–3 sugar residues. The possible branches included → 5)-α-L-Ara*f*-(1 → (17.3 mol%), → 3)-α-L-Ara*f*-(1 → (4.9 mol%), and → 4)-α-D-Glc*p*-(1 → (3.5 mol%), which were ended with three terminal sugar units: T-β-D-Xyl*p*-(1 → (15.5 mol%), T-α-D-Gla*p*-(1 → (4.5 mol%), and T-α-L-Ara*f*-(1 → (2.6 mol%). The weight average molecular weight (*Mw*) of flaxseed mucilage arabinoxylans was calculated to be 1747 kDa by static light scattering (SLS), and it exhibited random coil conformation. The proposed structure and conformational models confirmed that different backbone sugar units especially substitution positions directly contributed to the conformational diversity and rigidity of polysaccharide molecules.

1. Introduction

Flaxseed (*Linum usitatissiumum* L.) is rich in dietary fibers (22–28%, w/w), alpha-linolenic acid (~23%, w/w), and lignan (0.6–1.3%, w/w) (Morris, 2007; Cui & Mazza, 1996; Johnsson, Kamal-Eldin, Lundgren, & Aman, 2000). Flaxseed consists of two cotyledons (~55%, w/w), an embryo (~4%, w/w), hull (36–40%, w/w), as well as mucilage (5–8%, w/w) from the outer layer of the hull (Vaughan, 1970; Cui, Kenaschuk, & Mazza, 1996; Naran, Chen, & Carpita, 2008; Singh, Mridula, Rehal, & Barnwal, 2011).

Our previous study revealed that flaxseed contained about 12% (w/w) of dietary fibers in kernel, and 2–5% (w/w) of soluble dietary fibers in mucilage. Flaxseed kernel dietary fibers (FKDF) were sequentially extracted from flaxseed kernel (i.e. cotyledons and embryo) (Ding et al., 2014), and two major dietary fiber fractions were characterized as arabinan-rich rhamnogalacturonan-I (RG-I) and xyloglucans (Ding et al., 2015a; Ding, Cui, Goff, Guo, & Wang, 2016). Soluble flaxseed mucilage dietary fibers were separated into an acidic and a neutral fraction using anion-exchange chromatography, and the acidic fraction was proposed as RG-I ((Qian, Cui, Nikiforuk, & Goff, 2012a). The neutral fraction gum from flaxseed mucilage (FM-NFG) was referred to as arabinoxylans (Cui, Mazza, & Biliaderis, 1994; Qian, Cui, Wu, & Goff, 2012b). The short-chain fatty acid profiles of four major flaxseed dietary fiber fractions after *in vitro* fermentation were evaluated (Ding, Cui, Goff, & Gong, 2015b) and compared with psyllium fibers (i.e. arabinoxylans), which have been commercialized and commonly accepted as prebiotics. Clinical trials were also conducted to compare flaxseed mucilage with yellow mustard mucilage and fenugreek gum on the attenuation of postprandial glycemic and insulinemic responses (Repin et al., 2017).

Flaxseed mucilage has been well characterized by many research groups. Earlier results of methylation analysis and/or NMR spectra showed the main linkages of the FM-NFG (i.e. arabinoxylan) included T-α-D-Xyl*p*, 1,4-linked α-D-Xyl*p*, and 1,3-linked or 1,5-linked α-L-Ara*f* (Hunt & Jones, 1962; Muralikrishna, Salimath, & Tharanathan, 1987; Cui et al., 1994; Naran et al., 2008). Previous publications on structural characterization of flaxseed dietary fibers are summarized in Table S1. The structural diversity and complexity are related to the source of material, the genotype and cellular origin, biological functionality, as well as the growth stage of the plants (Izydorczyk, 2009). Knowledge of the relationship between primary structure and conformation of flaxseed mucilage arabinoxylans is still limited from previously published papers.

This study focused on the structure of FM-NFG from flaxseed mucilage using methylation-GC/MS analysis and 1D/2D NMR spectroscopy. The conformation of FM-NFG was also characterized using static

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and dynamic light scattering, and further compared with the major hemicellulose fraction from flaxseed kernel (i.e. xyloglucans). A combination of knowledge regarding the detailed primary structure and conformation of flaxseed mucilage arabinoxylans may assist in further establishing the relationship between these parameters and functionality of flaxseed dietary fibers.

2. Material and methods

2.1. Materials

Soluble flaxseed mucilage gum was isolated by aqueous extraction from flaxseed hulls supplied by Natunola Health Inc. (Ontario, Canada). The cultivar of flaxseed is Bethune. FM-NFG was separated from soluble flaxseed gum after ion exchange chromatography (Qian et al., 2012b). All chemicals were of reagent grade.

2.2. Methylation and GC/MS analysis

The procedure of methylation followed that of Taylor & Conrad (1972), and Ciucanu & Kerek (1984). The resultant partially methylated alditol acetates (PMAAs) were analyzed by GC–MS (ThermoQuest Finnigan, San Diego, CA) with an SP-2330 (Supelco, US) column (30 m × 0.25 mm × 0.2 μm, 160 °C–210 °C at 2 °C/min, and then 210 °C–240 °C at 5 °C/min) equipped with an ion trap MS detector.

2.3. Nuclear magnetic resonance (NMR) spectroscopy analysis

FM-NFG was dissolved in deuterium oxide (3%, w/w) at 80 °C for 1 h. It was freeze-dried (repeated three freeze-thaw cycles), and redissolved in D_2O (2%, w/w) for NMR analysis. The ^1H and ^{13}C NMR spectra were recorded at 500.13 MHz and 125.78 MHz, respectively, on a Bruker ARX 500 NMR spectrometer (Bruker, Germany). The spectra of $^1H/^1H$ correlation spectroscopy (COSY), total correlation spectroscopy (TOCSY), $^1H/^{13}C$ heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple bond correlation spectroscopy (HMBC), and nuclear overhauser effect spectroscopy (NOESY) were collected at 70 °C. Trimethylsilyl propionate (TSP) and 1,4-dioxane in D_2O were used as ^1H and ^{13}C chemical shift standards, respectively.

2.4. Light scattering measurements

FM-NFG (1 mg/mL) was dissolved in Milli-Q water, 0.1 M NaCl, and 0.5 M NaOH, respectively. The solutions were filtered through a 0.45 μm nylon filter 4 times to remove dust, and diluted into various concentrations (0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL). Each sample solution was filtered directly into a cylindrical quartz cell (25 mm in diameter), and was analyzed within 24 h. Samples were fully recovered after filtration as there was no detected reduction of total sugar content in FM-NFG solutions.

Light scattering measurements followed that of previous work (Guo, Wang, Cui, Kan, & Hu, 2013; Ding et al., 2016). Briefly, static light scattering (SLS) and dynamic light scattering (DLS) measurements were conducted at 637 nm using Brookhaven BI-200SM laser light scattering instruments including a goniometer, a photomultiplier and a 128-channel BI-9000AT digital autocorrelator (Brookhaven Instruments, US). SLS measurements were carried out in the angular range of 40–150° at 25 °C, and toluene was used as a reference with the Rayleigh ratio of $1.40 \times 10^{-5}\,cm^{-1}$. Data were analyzed under Zimm plot method (Zimm, 1948). The results of dynamic light scattering measurements or the particle size distributions were calculated by either the constrained regularization (CONTIN) method or Non-Negatively Constrained Least Squares (NNLS) method using Brookhaven dynamic light scattering software.

Table 1
Linkage types of FM-NFG.

Abbreviation	Deduced Linkage	RT[a]	Mol%[b]	PMAA
X^4	→4)-β-D-Xyl*p*-(→	1.255	25.0	2,3-Me_2-Xyl*p*
X^{234}	→2,3,4)-β-D-Xyl*p*-(1→	2.066	19.6	Xyl*p*-$(OAc)_5$
X^T	T-β-D-Xyl*p*-(1→	0.790	15.5	2,3,4-Me_3-Xyl*p*
$X^{24/34}$	→2/3,4)-β-D-Xyl*p*-(1→	1.648	5.1	3/2-Me-Xyl*p*
	Xylose		*65.1*	
A^5	→5)-α-Ara*f*-(1→	1.158	17.3	2,3-Me_2-Ara*f*
A^3	→3)-α-Ara*f*-(1→	1.045	4.9	2,5-Me_2-Ara*f*
A^T	T-α-Ara*f*-(1→	0.646	2.6	2,3,5-Me_3-Ara*f*
	Arabinose		*24.8*	
	Total Pentoses		***89.9***	
G^T	T-α-D-Gal*p*-(1→	1.114	4.5	2,3,4,6-Me_4-Gal*p*
C^4	→4)-α-D-Glc*p*-(1→	1.576	3.5	2,3,6-Me_3-Glc*p*
C^6	→6)-α-D-Glc*p*-(1→	1.517	1.4	2,3,4-Me_3-Glc*p*
	Total Hextoses		***9.4***	

[a] Retention time is relative to 2,3,4,6-Me_4-Glucose (14.591 min).
[b] Molar ratio of each sugar residue is based on the percentage of its peak area; sugar residues less than 0.3 mol% are not shown.

3. Results and discussion

3.1. Methylation and GC/MS analysis of FM-NFG

FM-NFG was composed of 65.1 mol% of Xyl*p* and 24.8 mol% of Ara*f* based on methylation-GC/MS analysis (Table 1). The results were also confirmed by the monosaccharide compositions determined by high performance anion exchange chromatography (Qian et al., 2012b). Four major linkage patterns constituted 77.4 mol% of total sugar residues, and they are: →4)-D-Xyl*p*-(1 → (25.0 mol%), → 2,3,4)-D-Xyl*p*-(1 → (19.6 mol%), *T*-D-Xyl*p*-(1 → (15.5 mol%), and → 1)-L-Ara*f*-(5 → (17.3 mol%).

Molar ratio of total non-reducing terminal residues and total branching points were 22.6 mol% and 44.3 mol%, respectively. The molar ratio of *T*-Ara*f*-(1→ and *T*-Xyl*p*-(1→ from GC–MS could be underestimated, because terminal sugar residues were relatively easily destroyed by acid during methylation analysis. Instead of terminal sugar residues, phenolic compounds could also be ester-linked to the branches (Ding et al., 2015a).

The degree of branching (DB) of FM-NFG was calculated to be 0.48 according to the equation:

$$DB = (N_T + N_B)/(N_T + N_B + N_L) \quad (1)$$

where N_T, N_B and N_L are the molar percentage of the terminal, branched, and linear residues, respectively (Hawker, Lee, & Fréchet, 1991). The DB value of FM-NFG (0.48) indicated it had a branched structure, but it was less branched than the acidic fraction gum from flaxseed mucilage (FM-AFG), of which DB value was 0.55 (Qian et al., 2012a).

3.2. NMR analysis and primary structure of FM-NFG

As shown in the ^1H NMR spectrum (Fig. 1a), eleven peaks in the anomeric region (δ 4.3–5.3 ppm) can be divided into three groups of sugar residues: three peaks from α-Ara*f* (δ 5.17–5.26 ppm), three peaks from α-Glc*p* and α-Gal*p* (δ 4.82–4.97 ppm), and five peaks from β-Xyl*p* (δ 4.29–4.55 ppm). Major sugar residues from FM-NFG were assigned in both ^1H and ^{13}C NMR spectra (Fig. 1a, b).

Xylose (65.1 mol%) is the most abundant component in FM-NFG including five linkage types: linear → 4)-β-D-Xyl*p*-(1 → (X, 25.0 mol%), substituted at O-2 or O-3 position ($X^{24/34}$, 5.1 mol%), and both of O-2 and O-3 position (X^{234}, 19.6 mol%), as well as T-β-D-Xyl*p*-(1 → (X^T, 15.5 mol%). The second abundant component in FM-NFG, arabinose (24.8 mol%), existed in three linkage patterns: →1)-L-Ara*f*-(5 → (A^5, 17.3 mol%), → 1)-L-Ara*f*-(3 → (A^3, 4.9 mol%), and *T*-L-Ara*f*-(1 → (A^T, 2.6 mol%). A minor portion of galactose and glucose residues in FM-