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Studies on polysaccharides from leaf skin of *Aloe barbadensis* Miller: Part II. Structural characteristics and molecular properties of two lower molecular weight fractions

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ABSTRACT

Aloe barbadensis Miller (*Aloe vera*) has long been used in food, cosmetic and pharmaceutical industries. The solid wastes such as the spikes and the outer green skins are usually separated from the inner gel and discarded during the process of *Aloe vera* leaves. Three kinds of polysaccharides (designated as ASP-4N, ASP-6N and ASP-8N) were successfully purified from the leaf skin of *Aloe vera* in our previous work. The present study aimed to analyze the structural and molecular features of ASP-6N and ASP-8N using methylation and GC-MS, NMR, high performance size-exclusion chromatography coupled with multi-angle laser light scattering (HPSEC-MALLS) and scanning electron microscopy (SEM) techniques. Results showed that ASP-6N and ASP-8N were β-(1→4)-glucomannans with acetyl groups, which may attach to *O*-2, *O*-3 or *O*-6 of mannopyranosyl residues in the backbone as mono-, di-, or tri-acetylated forms. HPSEC-MALLS analysis indicated that the polysaccharide fractions from *Aloe* leaf skin adopted various conformations in the aqueous solution depending on their molecular mass. The polysaccharides had fibrous, ribbon-like and spherical morphology by SEM observation.

1. Introduction

Aloe barbadensis Miller (*Aloe vera*) is a perennial plant belonging to the *Liliaceae* family. Its turgid green leaves are composed of mesophyll (gel) and thick epidermis (skin). It has been widely applied in folk medicine, functional food and cosmetics. By now, diverse bioactive substances such as carbohydrates, soluble sugars, organic acids, proteins, vitamins, minerals and amino acids extracted from the inner gel of *Aloe vera* have been widely studied (Boudreau & Beland, 2006; Eshun & He, 2004; Grindlay & Reynolds, 1986; Sánchez-Machado, López-Cervantes, Sendón, & Sanches-Silva, 2017). Among these functional chemicals, carbohydrates account for more than 60% of the dry material (McAnalley, 1993) and are found to be acemannan, glucomannan or pectic polysaccharides (Femenia, Sánchez, Simal, & Rosselló, 1999; Rodríguez-González et al., 2011).

During the process of *Aloe* gel products, a large amount of solid wastes generates without further use. These solid wastes (bagasses) include the spikes, bases and tips removed from the leaves and the skin resulting from the separation of the transparent gel. In early studies, Femenia et al. (1999) carried out a complete chemical composition analysis of the filets and skin from *Aloe vera* leaves. Results showed that there were comparable pectic polysaccharides, mannose-containing and xylose-containing polymers in the skin tissue. Later, a novel fraction (SAPS-1) was purified using DEAE-52 anion-exchange column chromatography and Sepharose-4B gel filtration from the skin of *Aloe barbadensis* Miller. It was consist of mannose, glucose and galactose in a ratio of 296:36:1 and identified to be β-(1→4)-D-galactoglucomannan with acetyl groups linked at C-6 by ^{13}C NMR spectroscopy (Liu, Wang, Xu, & Wang, 2007). Similarly, Chang, Chen, and Feng (2011) and Chang, Feng, and Wang (2011) used the column purification method (DEAE-Sephadex A-25 and Sepharose-4B columns) to isolate polysaccharides from the gel juice, skin juice and flowers of *Aloe* leaves. Both neutral and acidic polysaccharides existed in the skin of *Aloe* leaves. Further analysis revealed that the purified neutral fractions (SN1 & SN2) from the skin of *Aloe vera* were composed of mainly mannose and glucose, whereas the acidic fractions (SA1 and SA2) contained rhamnose, xylose, mannose, glucose, galactose, glucuronic acid and galacturonic acid (Chang, Chen, et al., 2011). In addition, a galacturonate polysaccharide was also isolated and

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identified from the rinds of *Aloe vera*. The molecular weight (M_w) was ranging from 200 to 523 kDa (McConaughy, Stroud, Boudreaux, Hester, & McCormick, 2008). Recent study by Flores-López et al. (2016) indicated that the ethanolic and aqueous extracts of the bagasse generated after *Aloe vera* processing leaves presented excellent antioxidant and antifungal activities.

In summary, great amounts of bioactive polysaccharides with different structures existed in the *Aloe vera* skin. Several monosaccharides, including rhamnose, glucose, mannose, galactose, xylose and uronic acids were found in these fractions. Detailed structural characteristics of polysaccharides such as glycosyl linkages and sequence of polymer chains were not well elucidated. In our previous investigation, three polysaccharide fractions (designated ASP-4N, ASP-6N and ASP-8N) from the leaf skin of *Aloe vera* were successfully obtained. Gradient ammonium sulfate precipitation rather than the complex and time-consuming column purification method was used to purify the polysaccharides. The three fractions had different molecular weights, degrees of acetylation and molar ratios of monosaccharide composition. One of the fraction, ASP-4N was deduced to be an *O*-acetyl- glucomannan after methylation and GC-MS, 1D/2D NMR analysis (Shi et al., 2017). In this part, fine structures of ASP-6N and ASP-8N were elucidated using methylation analysis and NMR spectroscopy. In addition, conformational analysis and solid surface properties of the three fractions are characterized by high performance size exclusion chromatograph coupled with multi-angle laser light scattering (HPSEC-MALLS) and scanning electron microscopy (SEM), respectively.

2. Materials and methods

2.1. Materials and chemicals

The whole *Aloe vera* leaves were purchased from Jiangxia *Aloe* Development Co., Ltd (Putian, Fujian province, China). The skin was separated, dried and milled from the clean leaves of *Aloe vera.* ASP-6N and ASP-8N were prepared using the gradient ammonium sulfate precipitation as described in part I of this series (Shi et al., 2017). Briefly, the water-extract (17.4%, w/w) was isolated by hot water extraction, ethanol precipitation and freeze-drying. Twenty grams of dried water-extract were dissolved in 200 mL of distilled water and then centrifuged to remove insoluble contaminants. Ammonium sulfate was added to the supernatant stepwisely to precipitate each fraction at saturations of 40%, 60% and 80% at room temperature, successively. Finally, three fractions designated as ASP-4N, ASP-6N and ASP-8N were obtained after dialysis and freeze-drying. Mannose and glucose were the dominant monosaccharides found in the three fractions.

Methyl iodide, deuterium oxide (99.9% D) and sodium borodeuteride (98 atom% D) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Trifluoroacetic acid (TFA) and methylene dichloride (CH_2Cl_2) of chromatographic grade were bought from Aladdin Chemical Co. (Shanghai, China). All the other chemicals were of analytical grade otherwise specified.

2.2. Methylation and GC-MS analysis

The methylation reaction was conducted according to the method derived from Ciucanu and Kerek (1984). Briefly, vacuum-dried polysaccharide (2−3 mg) was dissolved in 2 mL anhydrous dimethyl sulfoxide (DMSO). Dry powder of NaOH (30 mg) was added and the mixture was kept stirring for 2.5 h. After that, methyl iodide (0.8 mL) was added slowly to the system to start the methylation reaction. Methylene chloride (CH_2Cl_2) was used to extract the methylated polysaccharides in the mixture after 3 h and then the methylated polysaccharides were hydrolyzed, reduced and acetylated to get the partially methylated alditol acetates (PMAAs). The PMAAs were re-dissolved in CH_2Cl_2 and analyzed by the GC-MS system (Agilent Technology 7890A/5975C, USA), which was performed in the same manner as described before (Yin et al., 2012).

2.3. NMR spectroscopy

The deuterium-exchanged polysaccharide (30 mg) was dissolved in 0.6 mL D_2O and subjected to 1D and 2D NMR experiments. ^1H and ^{13}C NMR spectra of ASP-6N and ASP-8N were recorded at 600.58 and 151.01 MHz on a Bruker Avance 600 MHz NMR spectrometer (Brucker, Rheinstetten, Germany) at 295 K and 294 K, respectively. Two dimensional NMR experiments, including the total correlation spectroscopy (TOCSY), homonuclear 1H/1H correlation spectroscopy (COSY), heteronuclear single-quantum coherence (HSQC), heteronuclear multiple-bond correlation (HMBC) and nuclear overhauser effect spectroscopy (NOESY) using the standard Bruker pulse sequence, were also conducted for structure analysis.

2.4. Molecular properties determined by HPSEC-MALLS

The measurement of molecular properties was carried out on an HPSEC system with a Wyatt Model 1500 dual piston pump, a multi-angle laser light scattering detector (MALLS, DAWN HELEOS II), a differential pressure viscometer detector (ViscoStar II) and a refractive index detector (Optilab T-rEX) (Wyatt Technology Co., Santa Barbara, CA, USA). Three columns as described before (Yin et al., 2015) were kept in a model 200 column heater (CBL photoelectron technology, China) at 35.0 °C. The 0.1M $NaNO_3$ solution containing 0.02% (w/w) NaN_3 at a flow rate of 0.6 mL/min was used as eluent. Samples at concentrations of 1.0 mg/mL (ASP-4N) or 2.0 mg/mL (ASP-6N and ASP-8N) were fully dissolved in the eluent. Before injection, each sample was filtered through a 0.22 μm filter membrane for three times. The data was collected and analyzed using the ASTRA (Version 6.1.1.84) software. A recommended refractive index increment (dn/dc) of 0.150 mL/g was used in the calculation (Cheong, Wu, Zhao, & Li, 2015).

2.5. Scanning electron microscopy (SEM)

The polysaccharides dissolved in ultrapure water (1.0 mg/mL) were freeze-dried and observed using a JSM-6701F scanning electron microscopy (SEM) (JEOL Ltd., Japan). Samples were dispersed onto an aluminum stub and gold coated with an ion sputter coater. An acceleration voltage of 5 kV was applied for SEM observation.

3. Results

3.1. Linkage patterns by methylation and GC-MS analysis

To identify the linkage patterns, ASP-6N and ASP-8N were methylated, hydrolyzed and reduced to get the corresponding PMAAs before being analyzed by GC-MS system. The total ion chromatogram was provided in the supplementary material (Fig. S1). Four major peaks in the figure indicated that there were mainly four kinds of linkage patterns in ASP-6N and ASP-8N. Referring to the monosaccharide compositions reported in part I of this series (Shi et al., 2017) and mass spectra data from literature

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