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Chemical characterization of the immunomodulating polysaccharide of *Aloe vera* L.☆

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Abstract—The polysaccharide isolated by alcohol precipitation of *Aloe vera* mucilaginous gel was found to have a Man:Glc:Gal:GalA:Fuc:Ara:Xyl ratio of 120:9:6:3:2:2:1 with traces of Rha and GlcA. Linkage analysis of the *endo*-(1→4)-β-D-mannanase-treated sample yielded Man*p*-(1→ (∼26%), 4-Man*p* (∼53%), 2,4-Man*p* (∼3%), 3,4-Man*p* (∼1%), 4,6-Man*p* (∼1%), 4-Glc*p* (∼5%), 4-Xyl*p* (∼1%), Xyl*p*-(1→ (∼2%), Gal*p*-(1→ (∼5%), and traces of 4,6-Gal*p* and 3,6-Gal*p*. Hydrolysis with strong acids produced a mixture of short oligosaccharides and an acid-resistant fraction containing greater relative fractions of Man*p*-(1→, Ara*f*-(1→, Xyl*p*-(1→, and 4-Xyl*p* than the bulk polysaccharide. NMR analysis of oligosaccharides generated by *endo*-(1→4)-β-D-mannanase and acid hydrolysis showed the presence of di-, tri-, and tetrasaccharides of 4-β-Man*p*, β-Glc*p*-(1→4)-Man, β-Glc*p*-(1→4)-β-Man*p*-(1→4)-Man, and β-Man*p*-(1→4)-[α-Gal*p*-(1→6)]-Man, consistent with a backbone containing alternating →4)-β-Man*p*-(1→ and →4)-β-Glc*p*-(1→ residues in a ∼15:1 ratio. Analysis of the sample treated sequentially with *endo*-(1→4)-β-D-mannanase and α-D-galactosidase showed that the majority of α-Gal*p*-(1→ residues were linked to O-2, O-3, or O-6 of →4)-β-Man*p*-(1→ residues, with ∼16 →4)-β-Man*p*-(1→ residues between side chains. Our data provide direct evidence of a previously proposed glucomannan backbone, but draw into question previously proposed side-chain structures.

Keywords: ^{13}C NMR; ^{1}H NMR; Acemannan; Aloe vera; Polysaccharide; Immunostimulant; Immunomodulator

1. Introduction

Aloe vera L. is a tropical or sub-tropical plant with turgid lace-shaped green leaves with jagged edges and sharp points. The plant is a member of the lily family (Liliaceae), not the cactus family as many would believe from the rosette-like arrangement of the long spiked leaves on the central stem.[1] There are over 300 species of *Aloe* known, but *Aloe vera* L. is recognized as the 'true *Aloe vera*' for its widespread use and purported healing powers. The plant contains two separate juice materials, a yellow latex (exudate), extracted from the vascular bundles at the junction between the rind and the fillets, and a transparent mucilaginous gel, extruded from the inner pulp. While the dried exudate has been used as a cathartic, the gel has been widely accepted since the 4th century B.C. as a traditional medicine for alleviating pain and treating a variety of ailments.[1–9] Acemannan, an acetylated glucomannan, which makes up the majority of the mucilaginous *Aloe vera* gel,[10–17] has been incorporated in commercial wound care products and has been reported to effect wound closure in chronic wounds,[2–9,18] aphthous ulcers,[4,19,20] and reduction of dry socket associated with third-molar extraction sites.[21] Furthermore, the refined polysaccharide has been shown to act as an immunostimulant, displaying adjuvant activity on specific antibody production[15] and enhancing the release of interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and interferon-γ (INF-γ).[22] Release of these cytokines stimulates an increase of up to 300% in the replication of fibroblasts in tissue culture and enhances macrophage phagocytosis.[12,23] Proliferation of fibroblasts is known to be responsible for healing burns, ulcers, and other wounds of the skin and gastrointestinal lining. In addition, acemannan

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has been shown to inhibit AIDS virus replication in vitro, and an injectable form has been found to be of significant benefit in FIV-infected cats.[24,25]

Acemannan is not unique in its ability to elicit pharmacological activity. A variety of glycans have been reported to have antiviral and antitumor activity, believed to be a result of their ability to activate macrophages and T cells[17,23,26,27] or to stimulate complement.[15] In some cases, minor changes in structure, molecular weight or conformation of the polysaccharide may have dramatic effects on potency. For example, different antiviral activities of the xylo-mannans from *Nothogenia fastigiata* were explained on the basis of a flexible backbone, molecular size, composition, and distribution of sulfate groups.[27] Mouse macrophages were stimulated by an immobilized glycan but not by soluble glycan of the same structure, leading to the postulate that the conformation modulated its activity.[28]

There is considerable discrepancy in the literature as to the structure of the polysaccharide isolated from *Aloe vera* mucilaginous gel. Gowda et al.[13] were first to find that the polysaccharide isolated from gel by alcohol precipitation contained Man and Glc in a stoichiometric ratio of ~19:1. On the basis of linkage analysis and optical rotation they suggested the polysaccharide contained a backbone of *O*-acetylated →4)-β-Man*p*-(1→ residues, with randomly substituted →4)-β-Glc*p*-(1→ residues. Since their initial work, others have proposed structures containing (1) side chains of →4)-β-Gal*p*-(1→ residues attached from the O-2 of side-chain 2,4-β-Gal*p* residues to the O-6 of backbone →4)-β-D-Man*p*-(1→ residues, (2) side chains of →4)-β-Man*p*-(1→ residues attached to O-6 of backbone →4)-β-Man*p*-(1→ residues, or (3) neutral sugars found in minor abundance (Rha, Fuc, Ara, Xyl) integrated into the glucomannan backbone.[11,12,14–16] However, no definitive evidence of these structures exists.

An approach which has proven quite successful in the past for determining the structures of galactomannan, glucomannan, and galactoglucomannans is one in which oligosaccharides produced by enzymatic or acid hydrolysis are identified using a variety of chemical and spectroscopic methods.[29–40] In the present report, the structure of the *Aloe vera* polysaccharide is studied using a similar combined approach. Our results suggest that the polysaccharide has a β-glucomannan backbone with a Man:Glc ratio of ~15:1 and that branching occurs from the O-2, O-3, and O-6 of →4)-β-Man*p*-(1→ residues to single α-Gal*p*-(1→ side chains.

2. Experimental

2.1. Materials

2.1.1. BSW polysaccharides. Acemannan Hydrogel™ was obtained from Carrington Laboratories (Irving, TX) and was isolated by clarification and ethanol precipitation of the inner leaf gel of *Aloe vera* L. Acemannan Hydrogel was dissolved in distilled water by gentle shaking overnight and vacuum filtered through a 0.45 μm nitrocellulose membrane to yield bulk water-soluble polysaccharide (BSW). BSW was found to contain 90% soluble carbohydrate, 1–2% protein, less than 1% insoluble materials, and the remainder organic salts (oxalate, malate). The polysaccharide had an average molecular weight of 1.1×10^6, as determined by size exclusion chromatography.

2.1.2. Enzymes and other reagents. *endo*-(1→4)-β-D-Mannanase (*Aspergillus niger*, 500 U, E.C. 3.2.1.25) and α-D-galactosidase from *Cyanopsis tetragonobolus* (guar; 270 U, E.C. 3.2.1.22) were obtained from Megazyme, Ltd. (County Wicklow, Ireland). *endo*-(1→4)-β-D-Glucanase (*Trichoderma longibrachiatum*, 2250 BGLU/mL, E.C. 3.2.1.4) was obtained from Genencor, Inc. (Rochester, NY). The *endo*-(1→4)-β-D-mannanase and the α-D-galactosidase were used without further purification. A total of 100 units (50 μL) of *endo*-(1→4)-β-D-glucanase were dissolved in 0.2 mL of 10 mM sodium acetate, pH 4.5, and this solution was dialyzed twice against 1 L of buffer. All other chemical reagents were purchased from Sigma–Aldrich Company Co. (St. Louis, MO) and were used without further purification.

2.2. Hydrolysis of BSW

2.2.1. Acid hydrolysis of BSW and purification of BSW oligosaccharides. BSW (~5 mg/mL) was hydrolyzed with 2 M TFA (22 h, 85 °C or 3 h, 120 °C) or with 0.5 M H_2SO_4 (2 h, 90 °C). In the latter case the solution was neutralized with $BaCO_3$, and excess salts were removed by centrifugation. All hydrolysis reaction mixtures were dried under a stream of N_2, dissolved in water and lyophilized. Acid-hydrolyzed BSW was dissolved in distilled water, filtered through a 0.45 μm nitrocellulose membrane and injected into a Dionex liquid chromatograph equipped with a Phenomenex Rezec column (200 × 10 mm, 85 °C), pre-calibrated with dextran standard (MWCO 1000, Polymer Service—USA) to yield a correlation between retention time (t_R) and DP. Oligosaccharide fractions were eluted at 0.4 mL/min with water, detected with a Bio-Rad refractive index detector (Model 1770) connected to a Hitachi D-2500 integrator, and collected with a Bio-Rad fraction collector (Model 2110). Shorter oligosaccharides (DP <5) were further purified on a Dionex liquid chromatograph equipped with a Bio-Rad Aminex HPX-87C column (300 × 7.8 mm, 85 °C) or on a Rainin Microsorb-MV (C_{18}) column (250 × 5 mm) eluted with water at 0.2 mL/min.

2.2.2. Isolation of acid-resistant oligosaccharides. Saccharides (120 mg) obtained from TFA-hydrolyzed BSW (3 h, 120 °C) were dissolved in 25 mL of distilled water and put in 1000 Da nominal molecular weight dialysis tubing (Spectra/Por® CE, Cellulose Ester). The solution was dialyzed for 30 h against 2 L of distilled water. The aqueous sample (Fraction 1B) was removed from the dialysis tubing, lyophilized, and purified using a Bio-Rad Aminex HPX-87C column, as previously described.

2.2.3. Enzymatic hydrolysis of BSW. Prior to enzymatic hydrolysis, BSW was deacetylated by preparing an aqueous solution of the polysaccharide (3 mg/mL) in 0.06 M NaOH and allowing the deacetylation reaction to proceed for 1 h at room temperature. The solution was then adjusted to pH 4.5 with 5 M HOAc, and 1 U (4 μL) of *endo*-(1→4)-β-D-mannanase in 2 M NH_4SO_4 was added. Following incubation for 1 day at 45 °C, the reaction was stopped by heating to 100 °C for 5 min. The mixture was filtered through a 0.45 μm filter and lyophilized to dryness. Further hydrolysis was carried out by treating a solution of the *endo*-(1→4)-β-D-mannanase-treated polysaccharide (3 mg/mL, pH 4.5) with 2 U of α-D-galactosidase for 1 day at 45 °C. Following quenching of the reaction (5 min, 100 °C), an aliquot was removed for analysis, filtered and lyophilized. The remaining oligosaccharide mixture was treated with 34 U of freshly dialyzed *endo*-(1→4)-β-D-glucanase for 1 day at 45 °C. The reaction was terminated by heating at 100 °C for 5 min, and the quenched mixture was filtered through a 0.45-μm filter and lyophilized to dryness.

2.3. Monosaccharide composition by GC–MS of their per-*O*-trimethylsilyl (TMS) methyl glycosides

Methyl glycosides were prepared by heating 20–50 μg of polysaccharide and 50 μg of *myo*-inositol in 500 μL of dry 1 M HCl–MeOH for 16 h at 85 °C. Following cooling and evaporation of the HCl–MeOH under a stream of N_2, 200 μL of Sil-A reagent (Sigma Chemical Co.) was added, and the reaction mixture was heated to 80 °C for 20 min in a sealed vial with stirring. The sample was cooled, and the Sil-A reagent was evaporated under a stream of N_2. The dried sample was extracted with 500 μL of hexanes, filtered and was ready for GC–MS analysis.

The methylper-*O*-TMS-glycosides were analyzed by GC–MS on a Hewlett–Packard 5970 MSD instrument using a DB-1 column (J&W Scientific). Glycosides were eluted by first holding the temperature constant for 2 min at 80 °C, then increasing the temperature to 170 °C at 30 °C/min, increasing the temperature to 240 °C at 4 °C/min, and finally, holding the temperature at 240 °C for 15 min. Identification of the methylper-*O*-TMS-glycosides was made by comparing t_Rs of peaks occurring in the MS total ion chromatograph (TIC) to t_Rs of known standards. Concentrations with respect to *myo*-inositol (added as an internal standard) were determined by comparing the integrated TIC peak area to that for the per-*O*-TMS-inositol derivative, taking into account appropriate response factors.

2.4. Linkage analysis by GC–MS of partially methylated alditol acetates (PMAAs)

Methylation under basic conditions was performed using the NaOH method.[41,42] Samples (1–2 mg) were dissolved in 0.3–0.5 mL of Me_2SO, methylated, extracted into $CHCl_3$, and dried with Na_2SO_4. The permethylated material was dissolved in CH_3CN and passed through a Sep–Pak C_{18} reversed-phase cartridge, preconditioned with CH_3CN. The eluant (pale-yellow solution) was evaporated under a stream of N_2 at room temperature.

To the dried permethylated carbohydrate and 50 μg of *myo*-inositol was added 500 μL of 2 M TFA. The mixture was hydrolyzed for 2 h at 120 °C, and the acid was removed under a stream of N_2. Partially methylated monosaccharides were reduced with aqueous 1 M $NaBD_4$ (3 h, room temperature), neutralized by the dropwise addition of glacial HOAc, and the solvent removed under a stream of N_2. Partially methylated alditols were acetylated with 200 μL of Ac_2O and 20 μL of 1-methylimidazole (12 h, room temperature). The final acetylation reaction was quenched with water, and the PMAAs were extracted into CH_2Cl_2 and dried over Na_2SO_4. The PMAAs were analyzed by GC–MS[43,44] using a SP-2330 column (SUPELCO). A temperature gradient program identical to that described for elution of methyl glycosides was used for the chromatography of PMAAs.

2.5. NMR of BSW oligosaccharides

Samples were lyophilized three times from D_2O and dissolved in 0.5 mL of D_2O containing 0.75% TSP as an internal chemical shift standard. ^1H NMR spectra were recorded on a 500 MHz Varian spectrometer, with a single 90° pulse at 45 °C, using a sweep width of 8000 Hz. The residual HOD resonance was presaturated during the delay period. ^{13}C NMR spectra were recorded at 125 MHz at 45 °C and a sweep width of 28,996 Hz. Dioxane was used as an external standard (67.5 ppm).

COSY spectra were acquired using a sweep width of 1607 Hz and a matrix of 1024 × 1024 data points. When Fourier transformed, the absolute-value data was processed using sinebell weighting to diminish peak tailing effects. TOCSY spectra were acquired in the phase-sensitive mode using a sweep width of 1500 Hz and a mixing time of 70 ms. Data were processed (2048 × 2048 data matrix) with Gaussian weighting.

3. Results

3.1. BSW hydrolysis

Figure 1A shows the chromatographic profile observed for the separation of oligosaccharides arising from the TFA hydrolysis of BSW (2 M TFA, 22 h, 85 °C). Eight well-resolved fractions (*F*1, *F*3–*F*9) appear in the chromatogram and were assigned an apparent DP based on t_Rs of a calibration standard mixture of oligosaccharides. As estimated from peak area, *F*1 (DP >10) was found to comprise 37% of the bulk material. When the acid-hydrolyzed mixture of oligosaccharides was further hydrolyzed under more robust conditions (2 M TFA, 3 h, 120 °C; 0.5 M H_2SO_4, 2–6 h, 90 °C), *F*3 (DP 7)–*F*8 (DP 2) were degraded to monosaccharides, while *F*1 (DP >10) remained intact. *F*1 could, however, be further degraded to smaller oligosaccharides when treated with 1 M HCl–MeOH (22 h, 85 °C). Under these conditions, *F*1 made up no more than 3% of the total integrated peak intensity.

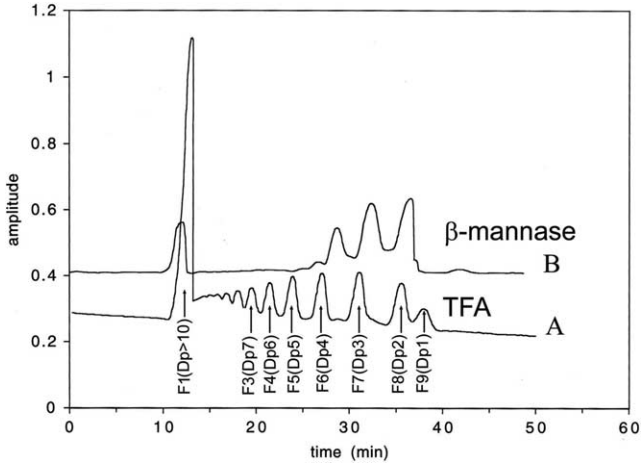


Figure 1. Chromatograph of *Aloe vera* BSW following (A) acid hydrolysis (2 M TFA at 85 °C for 22 h). t_Rs and percent of total integrated areas for each of the fractions were *F*1 (12.17 min, 36.6 ± 3.0%), *F*3 (19.73 min, 4.2 ± 1.0%), *F*4 (21.66 min, 6.4 ± 2.0%), *F*5 (24.07 min, 7.9 ± 1.0 %), *F*6 (27.14 min, 10.8 ± 1.0 %), *F*7 (31.13 min, 14.1 ± 3.0%), *F*8 (35.64 min, 14.4 ± 7.0%), and *F*9 (38.11 min, 5.2 ± 2.0%). *F*9–*F*3 corresponds to DP 1–7. *F*1 was found to have DP >10, (B) chromatograph following treatment of deacetylated BSW with *endo*-(1→4)-β-D-mannanase (1 U of enzyme at 45 °C for 24 h). Chromatography was carried out on a 200 × 10 mm Phenomenex Rezec column (85 °C) using water as eluant (0.4 mL/min). Peaks were detected using a refractive index detector.

In order to obtain a greater diversity of hydrolysis products that would complement those obtained by TFA hydrolysis, enzymatic methods of degrading BSW were explored.[45–48] Figure 1B shows the chromatographic profile of deacetylated BSW following treatment with *endo*-(1→4)-β-D-mannanase. By comparing the intensity of the peak co-eluting with *F*1 with a reaction blank, it was estimated that 90% of *F*1 was degraded to oligosaccharides having DP 2–4 (which eluted at 36.51, 32.32, and 28.64 min).

In summary, hydrolysis of BSW with strong acid at elevated temperatures yielded a mixture of oligosaccharides having DP 2–8 and an acid-resistant fraction with DP >10. The acid-resistant polysaccharide could be further degraded to monosaccharides with *endo*-(1→4)-β-D-mannanase or by HCl–MeOH.

3.2. Carbohydrate composition

The monosaccharide composition of BSW and of fractions chromatographically purified from the TFA hydrolyzate of BSW is summarized in Table 1. The ratio Man:Glc:Gal:GalA:Fuc:Ara:Xyl was 120:9:6:3:2:2:1 with traces of Rha and GlcA. The increased ratio of Ara, Fuc, Xyl, and Gal to Man in the monosaccharide fraction (F9) compared to BSW suggested that these saccharides were more labile to acid hydrolysis, while an increased abundance of Ara, Rha, Xyl, and uronic acid to Man in *F*1 suggested that these residues were an integral part of the oligosaccharides present in the acid-resistant fraction. Man, Glc, and Gal were the most abundant saccharides present in *F*3 through *F*8. On average, the relative abundance of Glc:Man or Gal:Man

Table 1. Monosaccharide composition (mol %) of BSW fractions[a]

Glycoside	BSW[b]	*F*1 DP >10	*F*3 DP 7	*F*4 DP 6	*F*5 DP 5	*F*6 DP 4	*F*7 DP 3	*F*8 DP 2	*F*9 DP 1
Ara	1.4 ± 0.2	8.00	0.26	ND	0.12	ND	ND	ND	17.15
Rha	0.1 ± 0.0	3.09	0.63	ND	0.09	0.11	ND	ND	0.33
Fuc	1.5 ± 0.1	ND	ND	ND	0.22	0.43	ND	0.33	2.67
Xyl	0.7 ± 0.1	8.15	3.85	1.24	1.24	1.21	1.42	1.1	2.58
Man	83.9 ± 3.8	43.86	58.85	68.37	63.48	75.16	80.07	82.8	59.65
Glc	6.2 ± 1.2	12.41	18.88	20.87	13.41	11.15	7.68	6.07	4.22
Gal	4.4 ± 1.2	21.06	13.4	4.81	21.44	11.95	10.83	9.69	13.4
GalA	2.4 ± 0.4	1.82	4.13	4.32	ND	ND	ND	ND	ND
GlcA	0.1 ± 0.0	1.61	ND	0.39	ND	ND	ND	ND	ND

[a] Fractions were obtained following hydrolysis in 2 M TFA at 85 °C for 22 h. Standard deviations are reported in cases where three determinations were carried out.

[b] Analysis was carried out on deacetylated BSW following treatment with *endo*-(1→4)-β-D-mannanase (1 U of enzyme 24 h at 45 °C).

was greater in longer oligosaccharides (i.e., *F*3, *F*5) than in shorter ones (i.e., *F*7, F8).

3.3. Linkage analysis of BSW oligosaccharides

We used GC–MS of PMAAs to determine the type of linkages present in *endo*-(1→4)-β-D-mannanase-treated BSW and the acid-resistant fraction of BSW (*F*1). Results are summarized in Table 2. In agreement with monosaccharide compositional analysis, the linkage analysis data suggested that Man was the most abundant residue present in BSW (~80%). This, along with the observed ~2:1 ratio of 4-Man*p*:Man*p*(1→ residues, suggested that the sample was composed predominantly of short Man-containing oligosaccharides. These structures were consistent with the specificity of *endo*-(1→4)-β-D-mannanase toward poly-(1→4)-mannans with residues in the β-D-configuration.[29–33] Glc in the enzyme-hydrolyzed BSW sample was found to be present only as 4-Glc*p* (4.9%), while Gal was present in greatest abundance as Gal*p*-(1→ in an amount (4.6%) that nearly matched the combined abundance of 3,4-Man*p*, 2,4-Man*p*, and 4,6-Man*p* (5.2%). Minor amounts of Ara*p*-(1→, Glc*p*A-(1→, Xyl*p*-(1→, 4-Xyl*p*, and branched Gal*p* were also found. While the *endo*-(1→4)-β-D-mannanase-treated acid-resistant fraction (F1) contained nearly the same fraction of total Man*p* residues as BSW (79.4% vs 84.8%), it contained significantly more Man*p*-(1→ (36.1% vs 26.7%), Ara*f*-(1→ (2.7% vs 0.3%), Xyl*p*-(1→ (6.1% vs 1.7%), 4-Xyl*p* (7.8% vs 1.2%) and branched Man*p* residues (7.1% vs 5.1%). The increase in the abundance of these residues was offset by the relative decrease in 4-Glc*p*, 4-Man*p*, and Gal*p*-(1→ residues. These results suggested that the *F*1 sample contained shorter Man-containing oligosaccharides having a greater abundance of Ara and Xyl residues than BSW.

Table 2. Linkage analysis of BSW and *F*1[a]

Linkage[b]	BSW	*F*1[b]
Ara		
Ara*f*-(1→	0.3 ± 0.0	2.7 ± 1.4
GlcA		
Glc*p*A-(1→	1.4 ± 0.8	1.9 ± 0.3
Xyl		
Xyl*p*-(1→	1.7 ± 0.4	6.1 ± 4.1
4-Xyl*p*	1.2 ± 1.2	7.8 ± 2.5
Gal		
Gal*p*-(1→	4.6 ± 4.7	1.8 ± 0.5
4,6-Gal*p*	0.3 ± 0.4	ND
3,6-Galp	0.7 ± 0.2	1.1
Man		
Man*p*-(1→	26.7 ± 4.2	36.1 ± 0.9
4-Man*p*	53.0 ± 1.1	36.2 ± 3.8
3,4-Man*p*	1.2 ± 0.5	1.4
2,4-Man*p*	2.9 ± 1.8	5.7
4,6-Man*p*	1.0 ± 0.2	ND
Glc		
4-Glc*p*	4.9 ± 0.9	3.2 ± 0.6

[a] BSW and *F*1 were hydrolyzed with *endo*-(1→4)-β-D-mannanase prior to linkage analysis carried out using GC–MS of PMAAs. Standard deviations are reported in cases where two determinations were carried out.

[b] *F*1 (F1b) was prepared by dialysis of TFA hydrolyzed BSW (120 °C for 3 h).

3.4. ^{13}C NMR studies of enzymatically hydrolyzed BSW

The ^{13}C NMR spectrum of BSW treated with *endo*-(1→4)-β-D-mannanase is shown in Figure 2A. The most intense resonances in the spectrum likely arise from Man*p*-(1→ and 4-Man*p*, since these residues comprise ~27% and ~53% of the residues present. On the basis of their intensities and similarity of their chemical shifts with other oligosaccharides containing →4)-β-Man*p*-(1→ residues, peaks at 95.19 and 95.04 ppm were assigned to C-1 of reducing terminal Man*p* ($M_{R\alpha}$, $M_{R\beta}$), while the resonance at 101.56 ppm was assigned to C-1 of β-Man*p*-(1→ and →4)-β-Man*p*-(1→ residues (M_{NR} and M_I).[17] The near equal intensities of resonances arising from reducing terminal Man and β-Man*p*-(1→ or →4)-β-Man*p*-(1→ confirmed that the polymer had been hydrolyzed to short oligosaccharides. Other less intense peaks in the same region of the spectrum likely arise from residues less abundant than Man. Based on the similarity of chemical shift with other glucomannans, the resonance at 104.0 ppm could be assigned to the C-1 of →4)-β-Glc*p*-(1→ residues.[30,45,49,50] Nominal assignments for other resonances in the spectrum were made on the basis of intensity and assignments from other glucomannan and galactomannan oligosaccharides.

Figure 2B shows the ^{13}C NMR spectrum of BSW following treatment with α-D-galactosidase, an enzyme capable of hydrolyzing terminal α-D-Gal*p*-(1→ residues. Following enzyme treatment, the resonance at 77.94 ppm disappeared and the resonance at 78.15 ppm increased in intensity. A new resonance also appeared at 98.45 ppm, which could be assigned to C-1 of β-Gal*p*,[49] consistent with the generation of Gal monosaccharide as a product of enzyme hydrolysis. Previous NMR studies on galactomannans have shown that the C-4 resonance of →4)-β-Man*p*-(1→ residues branched through O-6 to Gal side chains has a chemical shift between 77.5 and 78.5 ppm and is shifted upfield relative to C-4 of →4)-β-Man*p*-(1→ residues.[30,34,37–39,51–53] Based on these studies, the resonance at 77.94 ppm was assigned to C-4 of →4)-β-Man*p*-(1→ residues branched from O-2, O-3, or O-6 to an α-Gal*p* containing side chain, and the resonance at 78.15 ppm was assigned to C-4 of unbranched →4)-β-Man*p*-(1→ residues.

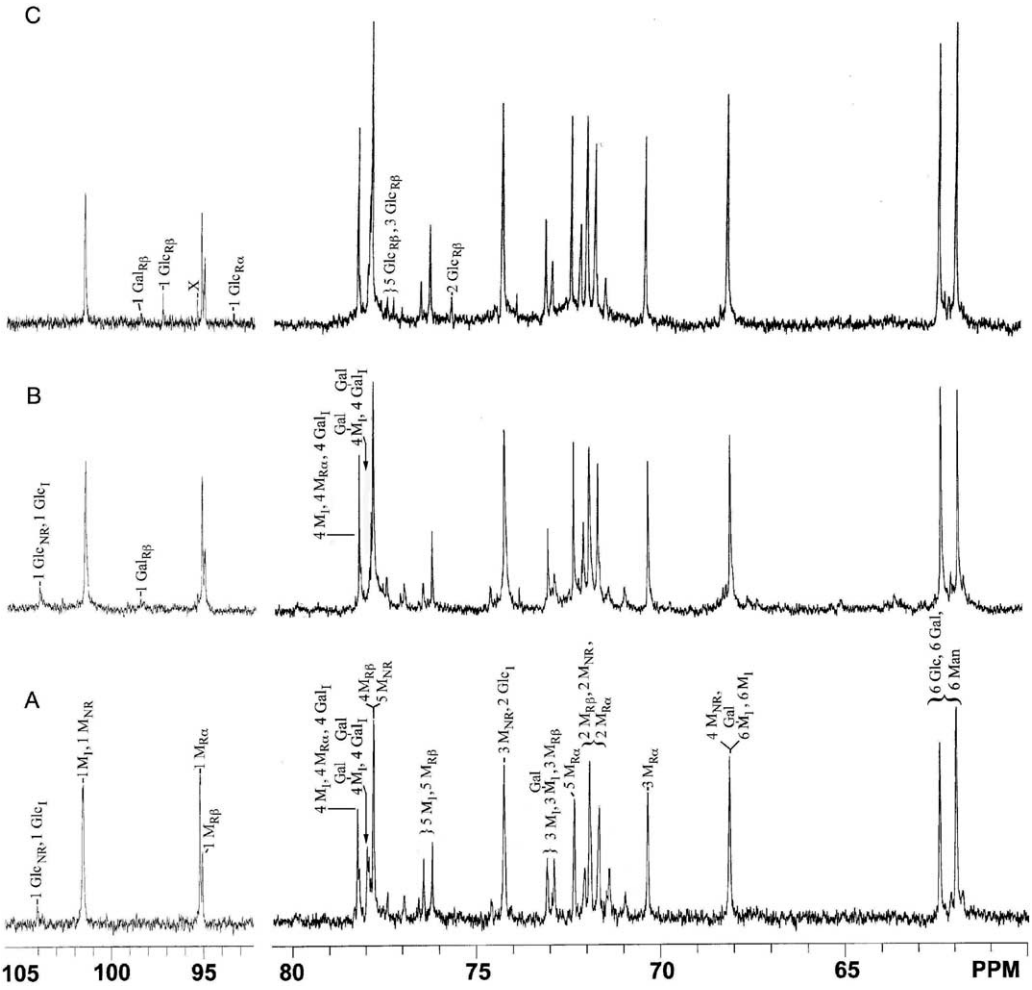


Figure 2. ^{13}C NMR spectra of deacetylated *Aloe vera* BSW (A) treated with *endo*-(1→4)-β-D-mannanase (1 U of enzyme at 45 °C for 24 h), (B) treated with *endo*-(1→4)-β-D-mannanase, followed by α-D-galactosidase (2 U of enzyme at 45 °C for 24 h) and (C) treated with *endo*-(1→4)-β-D-mannanase and α-D-galactosidase, followed by *endo*-(1→4)-β-D-glucanase (34 U of enzyme at 45 °C for 24 h). Peak 'X' is an unassigned resonance.

Figure 2C shows the spectrum of BSW sequentially treated with *endo*-(1→4)-β-D-mannanase, α-D-galactosidase, and *endo*-(1→4)-β-D-glucanase. The resonance at 104.0 ppm, previously assigned to the C-1 of →4)-β-Glc*p*-(1→ on the basis of its chemical shift, has disappeared and C-1 resonances of α-/β-Glc*p* appear at 93.5 and 97.0 ppm. New resonances also appear that can be assigned to C-2, C-3, and C-5 of β-Glc*p* monosaccharide.[49]

3.5. Structural characterization of purified BSW oligosaccharides

Fractions *F*4–*F*8, isolated from the acid hydrolysis of BSW, possess a greater number of monosaccharide components than their expected DP, suggesting that these fractions are impure mixtures of oligosaccharides. For example, *F*8 (DP 2) should contain two monosaccharides instead of three found. *F*7 and *F*8 (DP 3 and DP 2) and *endo*-(1→4)-β-D-mannanase-treated *F*1 were further purified by ion-exclusion chromatography, while *F*5 and *F*6 (DP 5 and DP 4) were purified using reversed-phase chromatography. Chromatograms for these purifications are shown in Fig. 3. Chromatograms of *F*7 and *F*8 showed a major peak (DP 3.2 and DP 2.2) with three smaller components (i.e., DP 2.1, DP 2.3, and DP 2.4 for chromatography of *F*8) while the chromatogram for the fractionation of *endo*-(1→4)-β-D-mannanase-treated *F*1 showed seven subfractions. The most abundant subfractions were collected and further characterized using a combination of carbohydrate compositional analysis and NMR spectroscopy.

Tables 3 and 4 summarize the compositions of DP 2.2, DP 2.3, DP 3.2, DP 4.2, DP 5.2, and AR.1 through AR.6. DP 2.2, and DP 2.3 contained greater than 80% Man with minor constituents of Gal and Glc, respectively, while DP 4.2 contained greater than 90% Man. In contrast, DP 3.2 and DP 5.2 contained 20–30% Glc and ~5% Gal, in addition to the more abundant Man. Of the *F*1 subfractions, AR.3 through AR.6 contained

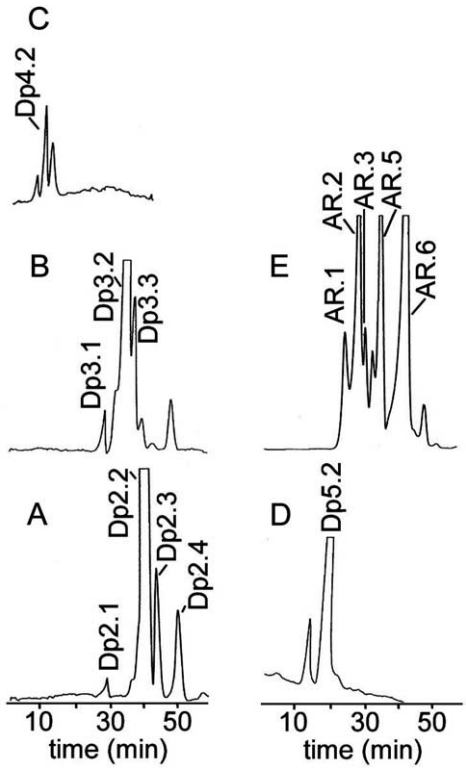


Figure 3. (A) Ion-exclusion chromatogram of *F*8 (DP 2), (B) ion-exclusion chromatogram of *F*7 (DP 3), (C) reversed-phase chromatogram of *F*6 (DP 4), (D) reversed-phase chromatogram of *F*5 (DP 5), (E) ion-exclusion chromatogram of *endo*-(1→4)-β-D-mannanase-treated acid-resistant fraction, *F*1.

predominantly Man with lesser amounts of Glc and Gal. AR.1 contained Ara, Xyl, Man, Glc, and GalA while AR.2 contained Gal, Glc, Man, Xyl, and GalA.

The ^{1}H NMR spectrum of DP 2.2 (Fig. 4A) showed resonances that could be assigned to the H-1 protons of the reducing terminal α-/β-Man at 5.27 and 5.00 ppm ($M_{R\alpha}$ and $M_{R\beta}$) and to the nonreducing terminal β-Man*p*-(1→ at 4.81 ppm ($M_{NR\beta}$). The intensity ratio of H-1 resonances assigned to β-Man*p*-(1→ and reducing terminal Man was ~1, as would be expected for a disaccharide. Additional assignments were made using COSY and TOCSY experiments (not shown).

Table 3. Composition (mol %) of soluble fractions obtained from TFA hydrolysis of BSW[a]

Glycoside	DP 2.2	DP 2.3	DP 3.2	DP 4.2	DP 5.2
Ara	0.1	0.3	ND	ND	ND
Rha	ND	0.1	ND	ND	ND
Fuc	ND	0.21	ND	0.1	ND
Xyl	0.5	0.5	1.2	0.1	1.3
Man	87.6	82.7	73.1	92.6	61.6
Glc	0.9	12.9	21.1	3.64	31.3
Gal	9.86	3.4	4.6	3.7	5.8
GalA	1.3	0.2	ND	ND	ND
GlcA	ND	ND	ND	ND	ND

[a] Determined by GC–MS of methylper-*O*-TMS-glycosides. ND = not detected.

These assignments, summarized in Table 5, were found to be in reasonable agreement with published assignments for β-Man*p*-(1→4)-Man disaccharide (β-Man_2),[33,34,38,39,50] and on this basis the structure of DP 2.2 was assigned to β-Man_2.

Resonances present in the ^{1}H NMR spectra of DP 3.2, DP 4.2, and DP 5.2 (Fig. 4B–D) could be assigned to H-1 of β-Man*p*-(1→ or →4)-β-Man*p*-(1→ residues (4.84 ppm) or to H-1 of reducing terminal α-/β-Man (5.27 ppm and 5.00 ppm) on the basis of similarity of chemical shifts with those of β-Man_2. The ratio of intensities of β-Man*p*-(1→ or -4)-β-Man*p*-(1→ to reducing terminal Man H-1 resonances in samples DP 4.2 and DP 5.2 are close to 2 and 3, suggesting that these fractions contained β-Man*p*-(1→4)-β-Man*p*-(1→4)-Man trisaccharide (β-Man_3) and β-Man*p*-(1→4)-β-Man*p*-(1→4)-β-Man*p*-(1→4)-Man tetrasaccharide (β-Man_4). The absence of most monosaccharides other than Man in DP 4.2 (Table 3) suggested that this sample was nearly pure trisaccharide. The complete assignment from the COSY and TOCSY spectra agree with previously published data for β-Man_3.[33,34,38,39] However, since DP 5.2 was shown by carbohydrate compositional analysis to contain ~ 30% Glc, other oligosaccharides may be present in this sample that were not observed in the ^{1}H NMR spectrum.

The ratio of intensities of β-Man*p*-(1→ or -4)-β-Man*p*-(1→ to reducing terminal Man H-1 resonances

Table 4. Composition (mol %) of fractions purified from the enzymatic digestion of the acid-resistant fraction (*F*1) of BSW[a]

Residue	AR.1	AR.2	AR.3	AR.4	AR.5	AR.6
Ara	9.9 ± 0.5	3.8 ± 0.2	1.3 ± 0.2	0.2 ± 0.1	ND	0.6 ± 0.2
Rha	ND	ND	ND	ND	ND	ND
Fuc	2.6 ± 0.7	0.9 ± 0.01	ND	ND	ND	ND
Xyl	30.6 ± 3.5	11.6 ± 1.8	2.3 ± 0.1	0.4 ± 0.1	ND	1.0 ± 0.1
Man	27.2 ± 1.2	14.0 ± 0.4	60.6 ± 0.5	84.5 ± 2.1	56.5 ± 0.5	72.6 ± 0.3
Glc	9.0 ± 0.5	22.0 ± 1.1	26.1 ± 0.6	9.2 ± 0.7	23.8 ± 0.2	9.8 ± 0.1
Gal	1.7 ± 0.2	39.2 ± 1.1	6.2 ± 0.1	4.2 ± 0.9	18.2 ± 0.3	13.4 ± 0.1
GalA	19.3 ± 1.6	8.5 ± 1.6	2.9 ± 0.2	1.2 ± 0.2	ND	1.8 ± 0.1
GlcA	ND	0.3 ± 0.1	0.8 ± 0.2	0.3 ± 0.1	1.6 ± 0.1	1.0 ± 0.1

[a] *F*1 (F1b) was prepared by TFA hydrolysis of BSW at 120 °C for 3 h and was isolated after dialysis of the oligosaccharide mixture. Composition was determined by GC–MS of methylper-*O*-TMS-glycosides. Standard deviations are estimated from three separate sample preparations.

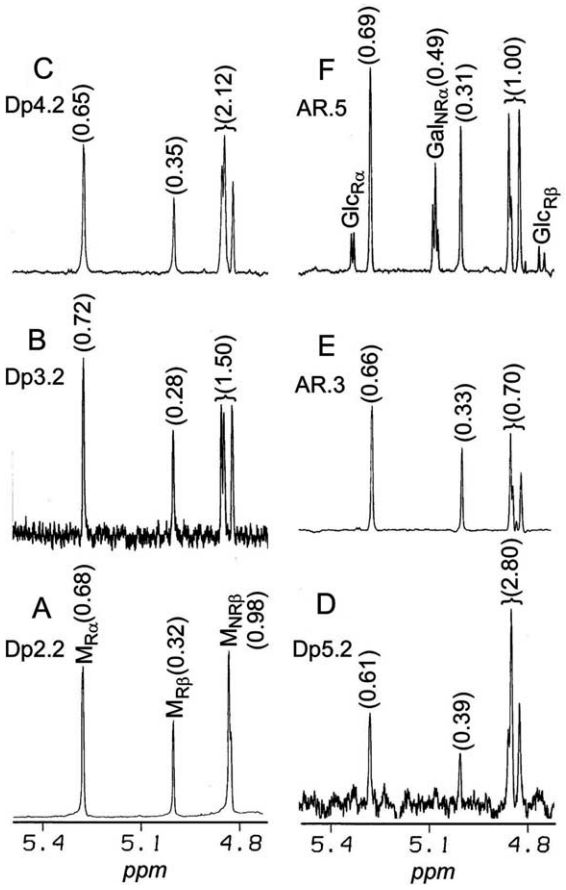


Figure 4. Partial ^{1}H NMR spectra showing the H-1 resonances of fractions (A) DP 2.2, (b) DP 3.2, (C) DP 4.2, (D) DP 5.2, (E) AR.3, and (F) AR.5. Numbers in parentheses are represent integrated peak intensities.

was ~1.5 for DP 3.2 (Fig. 4B). This result suggested that there was more reducing terminal Man present than would be expected for β-Man$_3$ but more β-Man*p*-(1→ -4)-β-Man*p*-(1→ present than would be expected from β-Man$_2$. Complete assignment from the COSY spectrum confirmed the presence of β-Glc*p*-(1→4)-β-Man*p*-(1→4)-Man.[39,54] Of the remaining subfractions obtained from the acid hydrolysis of BSW, which were characterized by NMR spectroscopy, DP 2.3 was found to be a mixture of β-Man$_2$ and Glc monosaccharide.

The ^{1}H NMR spectra of AR.3 and AR.5 are shown in Figure 4E and F. The ratio of intensities of nonreducing to reducing terminal Man H-1 resonances was ~0.7 for AR.3. The presence of β-Glc*p*-(1→ was evident from the anomeric carbon resonance at 104.0 ppm in the ^{13}C NMR spectrum of this fraction (not shown), and the complete proton assignments made from the COSY spectrum (Fig. 5 and Table 5) confirmed the presence of β-Glc*p*-(1→4)-Man.[50] From integration of the H-1 resonances in the ^{1}H NMR and the COSY spectrum, we estimated that AR.3 contained ~30% β-Glc*p*-(1→4)-Man and 70% β-Man$_2$. These results were consistent with the compositional analysis of AR.3 shown in Table 4. The ^{1}H NMR of AR.5 (Fig. 4F) showed the presence of a H-1 resonance that could be assigned to a α-Gal*p*-(1→ residue based on previously reported assignments in galactomannans.[34] The ratio of intensities of β-Man*p*-(1→ to reducing terminal Man H-1 resonances was exactly 1, which is consistent with the presence of β-Man$_2$ or a substructure containing β-Man$_2$. The apparent α-Gal*p* H-1 'triplet' likely arises from a partial overlap of doublets, each arising from a single anomer at the reducing terminal of β-Man*p*-(1→4)-[α-Gal*p*-(1→6)]-Man. This structure is consistent with the specificity of *endo*-(1→4)-β-D-mannanase for hydrolyzing mannans with Gal side chains substituted at the reducing Man residue.[30] However, since integration of the Gal H-1 is ~0.5 rather than 1, there must be more than a single oligosaccharide component present in the AR.5 subfraction. Assignment and integration of the COSY spectrum (Table 5) confirmed the presence of an equal mixture of β-Man*p*-(1→4)-[α-Gal*p*-(1→6)]-Man and β-Man$_2$. Carbohydrate compositional analysis of AR.5 (Table 4) suggested that this fraction also contained 23% Glc, consistent with resonances appearing in the ^{1}H NMR spectrum at 5.30 and 4.78 ppm.

The NMR spectra of AR.1 and AR.2 were not of sufficient quality to carry out structural determinations. A summary of the fractions analyzed and their structures is given in Table 6.

4. Discussion

The carbohydrate composition of the mucilaginous polysaccharide from *Aloe vera* gel has long been disputed.[9–17] Recent work in which the polysaccharide was either purified chromatographically or alcohol precipitated in a manner similar to the isolation method for BSW has shown that Man comprises greater than 75% of the carbohydrate fraction with traces of Xyl, Ara, Fuc, Rha, and uronic acids.[10,11,15] Except for the recent study by Femenia et al.,[10] most studies agree that Glc comprises 3–6% of the *Aloe vera* polysaccharide.[11–16] There appear to be more discrepancies in the relative fraction of Gal found. While some conclude that Gal comprises less than 1.5%,[10,13,16] others suggest that the composition with respect to Gal is roughly equal to that of Glc.[11,15] Our data agree with this latter finding, with Glc and Gal making up ~6% and ~4%, respectively. The ratio of Man:Glc:Gal:GalA:Fuc:Ara:Xyl was found to be about 120:9:6:3:2:2:1 with trace amounts of GlcA and Rha. It has been suggested that discrepancies in carbohydrate composition may be traced for differences in methods used to isolate the polysaccharide, time of harvest and growing conditions.[10,11]

From the chromatographic profile in Figure 1 we estimated that less than 60% of BSW was hydrolyzed under

Table 5. Summary of assigned ^{1}H NMR shifts[a]

^{1}H	β-Man$_2$[b]	β-Man$_3$[c]	β Glc*p*-(1→4)-Man*p*[d]	β-Man*p*-(1→4)-[α-Gal*p*-(1→6)]-Man*p*[e]
1α	5.27 (5.27)	5.27 (5.27)	5.27 (5.26)	5.27 (5.28)
1β	5.00 (5.00)	5.00 (5.00)	5.00 (5.00)	5.00 (5.01)
2α	4.08 (4.09)	4.08 (4.09)	4.08 (4.08)	4.08 (4.10)
2β	4.09 (4.09)	4.09 (4.09)	4.09 (4.08)	4.08 (4.10)
3α	4.05 (4.08)	4.05 (4.08)	4.05 (4.08)	ND (4.09)
3β	3.90 (3.89)	3.91 (3.89)	3.90 (3.89)	3.95 (3.90)
4α	3.98 (3.99)	3.98 (3.99)	3.98 (3.99)	ND
4β	3.92 (3.95)	3.92 (3.95)	3.92 (3.95)	ND
5α	3.96 (3.99)	3.96 (3.99)	3.96 (3.99)	ND
5β	3.71 (3.66)	3.71 (3.66)	3.71 (3.66)	ND
6aα	3.92 (3.94)	3.92 (3.94)	3.92 (3.94)	ND
6aβ	3.95 (3.99)	3.97 (3.99)	3.95 (3.99)	ND
6bα	3.82 (3.85)	3.82 (3.85)	3.82 (3.85)	ND
6bβ	3.83 (3.85)	3.83 (3.85)	3.83 (3.85)	ND
1′α	4.82 (4.83)	4.84 (4.85)	4.52 (4.60)	4.84
1′β		4.81 (4.85)		4.81
2′α	4.15 (4.16)	4.22 (4.22)	3.35 (3.41)	4.22
2′β		4.16		4.17
3′α	3.75 (3.75)	3.90 (3.89)	3.54 (3.61)	3.92
3′β		3.75		3.78
4′α	3.69 (3.74)	ND (3.94)	3.47 (3.51)	3.88
4′β		3.67		3.7
5′α	3.53 (3.52)	ND (3.65)	3.52 (3.59)	ND
5′β		3.57		ND
6′aα	3.84 (3.83)	ND (3.89)	ND (4.01)	ND
6′aβ		3.85		ND
6′bα	4.04 (3.04)	ND	ND (3.82)	
6′bβ		4.06 (4.01)		
1″α/β		4.84 (4.86)		5.06 (5.13)
2″α/β		4.22 (4.23)		3.92 (3.94)
3″α/β		3.91 (3.91)		3.86 (3.94)
4″α/β		3.95 (3.99)		ND (4.11)
5″α/β		3.83 (3.86)		ND (4.01)
6″aα/β		3.86 (3.89)		ND (3.86)
6″bα/β		4.04 (4.08)		ND

NMR shifts in parentheses were taken from Ref. 34.

[a] Determined from COSY and TOCSY ^{1}H NMR spectra. NMR shifts in parenthesis were taken from Refs. 34,38,39,50 and 53.

[b] β-Man$_2$ was found to be the only component of fractions DP 2.2 and AR.6, and was a major component of fraction DP 2.3, AR.3, AR.4, and AR.5.

[c] β-Man$_3$ was found to be the only component of fraction DP 4.2 and a major component of fraction DP 3.2.

[d] β-D-Glc*p*-(1→4)-D-Man*p* occurred as a component of fractions AR.3 and AR.4.

[e] β-Man*p*-(1→4)-[α-Gal*p*-(1→6)]-Man*p* was found in fraction AR.5.

conditions more robust (2 M TFA, 120 °C, 48 h) than are commonly used for compositional analysis.[10] Although acid resistance has been previously observed for pectins where the backbone arises from 4-α-Gal*p*A residues,[44] there is no evidence for such a structure in BSW, given the relatively low uronic acid composition. The fact that Xyl, Ara, Fuc, Rha, and GalA were concentrated in the acid-resistant fraction and in the monosaccharide fraction suggests that the BSW sample is heterogeneous with respect to hydrolysis by strong acids. The greater relative abundance of Ara and Xyl in the monosaccharide fraction may be explained by the finding that pentoses are more acid-labile than the corresponding hexoses by a factor of 10–1000.[43] Similarly, the fact that deoxyhexoses or methylhexoses hydrolyze five times faster than regular hexoses[45] may explain why Fuc was relatively more abundant in smaller oligosaccharide fractions. The increased relative abundance of Man in the smaller oligosaccharides (DP 2–5) is probably the result of the relative decrease of Gal and Glc in these fractions. Because Xyl, Ara, Fuc, Rha, and GalA were not found in oligosaccharides purified from the acid hydrolysis fractions and later characterized by NMR, it was not possible to determine if these minor components were integral components in the BSW structure or if they arose from contaminating pectins and hemicelluloses.

The most abundant internally linked residues in BSW were found to be 4-Man*p* (~53%), 4-Glc*p* (~5%), 2,4-Man*p* (~3%), 3,4-Man*p* (~1%), 4-Xyl*p* (~1%), and 4,6-Man*p* (~1%). Branched 4,6-Gal*p* and 3,6-Gal*p* were also found in trace abundance. The specificity of

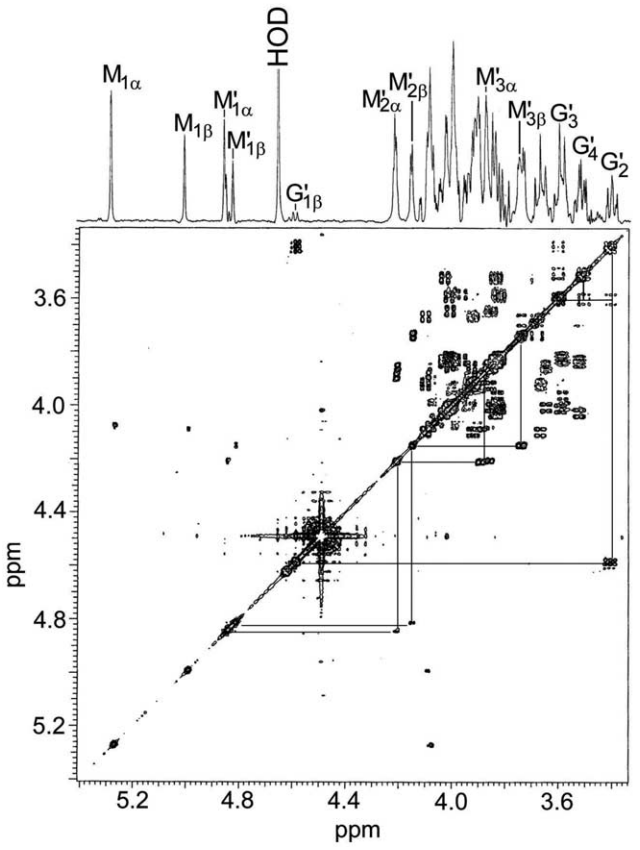


Figure 5. ^{1}H COSY spectrum of AR.3. As estimated from integration, this fraction is composed of approximately 70% β-Man$_2$ and 30% β-Glc*p*-(1→4)-Man. Coupling networks (H-2 through H-4) are shown for the β-Man-(1→ residue of β-Man$_2$ and β-Glc*p*-(1→ of β-Glc*p*-(1→4)-Man (nonreducing terminal is represented by the primed nomenclature; G = Glc, M = Man).

Table 6. Summary of structures for oligosaccharide fractions determined by NMR spectroscopy

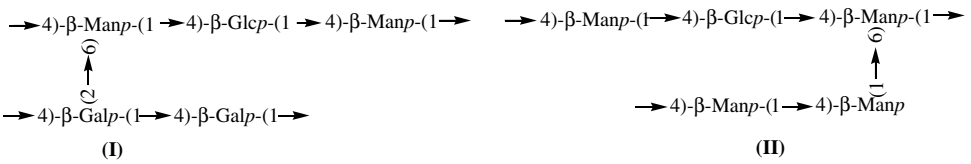
Fraction	Structures
DP 2.2	β-Man$_2$
DP 2.3	β-Man$_2$ + Glc*p*
DP 3.2	β-Man$_3$ +β-Glc*p*-(1→4)-β-Man*p*-(1→4)-Man
DP 4.2	β-Man$_3$
DP 5.2	β-Man$_4$ + other components
AR.3	70% β-Man$_2$ + 30% β-Glc*p*-(1→4)-Man
AR.4	85% β-Man$_2$ + 15% β-Glc*p*-(1→4)-β-Man*p*-(1→4)-Man
AR.5	50% β-Man$_2$ + 50% β-Man*p*-(1→4)-[α-Gal*p*-(1→6)]-Man

endo-(1→4)-β-D-mannanase from *A. niger* toward galactoglucomannans (GGMs) has been studied in detail, and it is known that the enzyme readily hydrolyzes the glucomannan backbone to yield predominantly di- and trisaccharides.[30–33] This specificity accounts for the ~2:1 ratio of Man*p*-(1→:4-Man*p* observed by linkage analysis and the near equal intensities of reducing terminal Man*p* and β-Man*p*-(1→ (or →4)-β-Man*p*-(1→) C-1 resonances observed in the ^{13}C NMR spectrum. Studies of the activity of *A. niger endo*-(1→4)-β-D-mannanase toward a variety of oligosaccharide substrates also suggests the enzyme binds a β-D-mannopentose moiety in the active site ($_{NR}$α-β-χ-δ-ε$_R$) and hydrolyzes the linkage between the second (δ) and third (χ) residues from the reducing end of the pentosyl unit. The enzyme is tolerant of →4)-β-Glc*p*-(1→ substitution of →4)-β-Man*p*-(1→ at the second (δ) and fourth (β) positions from the reducing end (ε) and side-chain branching from →4)-β-Man*p*-(1→ residues at the first (ε) and third (χ) positions. Hence, hydrolysis of GGMs by the enzyme can yield tetra-, tri-, and disaccharides having β-Glc*p* as the nonreducing terminal residue and branching from Man at the χ or ε positions.[30] Substitution of →4)-β-Glc*p*-(1→ for →4)-β-Man*p*-(1→ residues in the backbone of BSW is suggested by the presence of 4-Glc*p* determined by linkage analysis, by the disappearance of the resonance assigned to the C-1 of →4)-β-Glc*p*-(1→ in the ^{13}C NMR spectrum of BSW following treatment with *endo*-(1→4)-β-D-glucanase, and by the presence of β-Glc*p*-(1→4)-β-Man*p*-(1→4)-Man in oligosaccharides purified from BSW hydrolyzate. Although 4,6-Gal*p* and 3,6-Gal*p* were found by linkage analysis, they were not present in great enough abundance to be observed by NMR spectroscopy of the enzyme-hydrolyzed BSW or of purified oligosaccharides. Hence, our data offers no evidence as to whether or not these branched Gal residues are incorporated into the glucomannan backbone. Others have previously suggested a glucomannan structure for alcohol precipitable *Aloe vera* polysaccharide based on linkage analysis and optical rotation.[11,13,16] Our study provides more direct evidence for this structure based on NMR spectroscopy and the specificity of hydrolyzing enzymes.

The shift in the C-4 resonance in the ^{13}C NMR spectrum of BSW oligosaccharides following treatment with α-D-galactosidase indicates that branched residues have been converted to nonbranched residues as a result of hydrolysis of α-Gal containing side chains.[36–39] Since the three branched Man and two branched Gal residues found by linkage analysis would be expected to have their C-4 resonance in the same region of the spectrum,[52] the changes observed cannot unambiguously assigned to one type of branched residue. However, branched Man residues are more abundant than branched Gal residues (~5% vs ~1%), suggesting that the changes observed cannot be accounted for by hydrolysis of the side chains from branched Gal residues alone. Aside from the small fraction of branched Gal residues, only Gal*p*-(1→ residues were detected in BSW. Since α-D-galactosidase is an *exo*-glycosidase specific for hydrolyzing α-Gal*p* residues from the nonreducing end of an oligosaccharide, the changes observed in the ^{13}C NMR spectrum following enzyme treatment likely result from the hydrolysis of a single terminal α-

Gal*p*-(1→ residue from 2,4-β-Man*p*, 3,4-β-Man*p*, and 4,6-β-Man*p* residues in the backbone. The presence of β-Man*p*-(1→4)-[α-Gal*p*-(1→6)]-Man in AR.5 further confirms that single α-Gal*p*-(1→ side chains are attached to the O-6 of →4)-β-Man*p*-(1→ residues.

Previous workers have found Man residues in the *Aloe vera* polysaccharide to be acetylated at O-2, O-3, and O-6 in a ratio of ~1:1, with an overall degree of acetylation of 0.78/residue.[11–14] Because our BSW sample was deacetylated prior to linkage analysis, 2,4-Man*p*, 3,4-Man*p*, and 4,6-Man*p* must arise from branching to side chains. While we find direct structural evidence for single α-Gal*p*-(1→ side-chains attached to O-6 of →4)-β-Man*p*-(1→ residues, branching must also exist through O-2 or O-3, either to α-Gal*p*-(1→ or other side-chain residues. Side chains of (1) →4)-β-Gal*p*-(1→ residues attached from the O-2 of a 2,4-Gal*p* to the O-6 of backbone →4)-β-Man*p*-(1→ residues (**I**) or (2) →4)-β-Man*p*-(1→ residues attached from O-1 of a reducing terminal Man*p* to O-6 of backbone →4)-β-Man*p*-(1→residues (**II**) have been suggested by others.[11,16]



We found no evidence for 4-Gal*p* by linkage analysis or for the presence of →4)-β-Gal*p*-(1→ residues by NMR spectroscopy of BSW oligosaccharides, thus drawing into question the existence of structure **I**. The finding that α-D-galactosidase converts branched Man*p* residues in the backbone to unbranched ones also weighs against the existence of **II**, although its existence in minor abundance cannot be ruled out.

In summary, our results provide evidence that the polysaccharide from *Aloe vera* is a GGM having a backbone containing →4)-β-Man*p*-(1→ and →4)-β-Glc*p*-(1→ residues in ~15:1 ratio. Furthermore, our data suggests that single α-Gal*p*-(1→ residues are linked through the O-2, O-3, and O-6 of →4)-β-Man*p*-(1→ backbone residues, where, on average, side-chain substitutions occur every 16 β-Man*p* residues. Our data do not eliminate the possibility of other, less abundant types of side chains but draw into question previously proposed structures. Like BSW, other GGMs have a acetylated →4)-β-Man*p*-(1→ backbone with alternating →4)-β-Glc*p*-(1→ residues.[29–34] However, most GGMs have ratios of Man:Glc:Gal of approximately 1:1:1 and have side-chains more complex in structure than single α-Gal*p*-(1→ residues.[33]

Finally it should be emphasized that while the majority structure proposed for BSW is based on the most abundant fractions, those are the most readily available for analysis. The acid-resistant fraction makes up 37% of BSW and 60% of the carbohydrate contained in this fraction are non-Man residues. The fact that an acid-resistant fraction of BSW exists and comprises over 37% of the bulk polysaccharide is remarkable and emphasizes the heterogeneity present in the sample. It is possible that these unique substructures within BSW are responsible for its potent pharmacological activity.

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