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The gel-forming polysaccharide of psyllium husk (*Plantago ovata* Forsk)

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Abstract—The physiologically active, gel-forming fraction of the alkali-extractable polysaccharides of *Plantago ovata* Forsk seed husk (psyllium seed) and some derived partial hydrolysis products were studied by compositional and methylation analysis and NMR spectroscopy. Resolving the conflicting claims of previous investigators, the material was found to be a *neutral* arabinoxylan (arabinose 22.6%, xylose 74.6%, molar basis; only traces of other sugars). With about 35% of nonreducing terminal residues, the polysaccharide is highly branched. The data are compatible with a structure consisting of a densely substituted main chain of β-(1 → 4)-linked D-xylopyranosyl residues, some carrying single xylopyranosyl side chains at position 2, others bearing, at position 3, trisaccharide branches having the sequence L-Ara*f*-α-(1 → 3)-D-Xyl*p*-β-(1 → 3)-L-Ara*f*. The presence of this sequence is supported by methylation and NMR data, and by the isolation of the disaccharide 3-*O*-β-D-xylopyranosyl-L-arabinose as a product of partial acid hydrolysis of the polysaccharide.

Keywords: *Plantago ovata* mucilage; Psyllium seed polysaccharide; Arabinoxylan; 3-*O*-β-D-Xylopyranosyl-L-arabinose

1. Introduction

The seed husk of *Plantago ovata* Forsk has a long history of use as a dietary fiber supplement to promote the regulation of large bowel function,[1] and more recently it has been shown to lower blood cholesterol levels.[2] In early chemical studies Laidlaw and Percival[3,4] analyzed the polysaccharide mucilage extracted from whole seeds by first cold, then hot water. They secured evidence for two components, which they characterized as a polyuronide and a neutral arabinoxylan. Later Kennedy and co-workers[5,6] studied the mucilage obtained from *Plantago* seed husk by extraction with alkali and concluded that the preparation, although polydisperse, represented a single species of polysaccharide, a highly branched, acidic arabinoxylan.

In our laboratory we developed an efficient, reproducible process for the alkaline extraction and fractionation of the polysaccharide from the husk in order to obtain material for biological studies and to address issues relating to the composition and structure of the active substance.[7] Through animal and human feeding experiments, we could show that a gel-forming fraction, amounting to some 55–60% of the husk, is responsible for both the laxative and cholesterol-lowering activities.[7,8] Other viscous, non-nutrient polysaccharides, such as β-glucans and pectins, lower blood cholesterol levels by the same mechanism as psyllium,[9] but these substances have negligible effects on bowel function. They are rapidly and completely fermented in the gut, whereas

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psyllium husk largely survives, increasing stool output and imparting a gel-like consistency to the excreta.[8,10] In the present paper we present the results of chemical and physical studies of the active fraction of psyllium mucilage.

2. Experimental

2.1. Materials

Anion-exchange resin AG3-X4, 100–200 mesh and cation-exchange resin AG50W-X8, 100–200 mesh were supplied by the BioRad Corp., Richmond, CA. α-L-Arabinofuranosidase (EC 3.2.1.55) from *A. niger* was obtained from Megazyme, Wicklow, Ireland. Psyllium seed husk, identical with the principal component of the preparation marketed as Metamucil®, was provided by the Procter & Gamble Company, Cincinnati, Ohio.

2.2. Initial preparation

The procedure for the isolation of the gel-forming polysaccharide (fraction B) and two additional fractions (A and C) from the husk is described elsewhere.[11] Fraction A amounted to 17%, B 57.5%, and C 12.9% of the weight of the husk.[7] Thus the three fractions account for nearly all of the carbohydrate and about 90% of the mass of the starting material. Neutral sugars in the husk and fractions were measured by GLC as alditol acetate derivatives using the method of Kraus et al.,[12] as modified.[13] Uronic acids were measured by a colorimetric assay employing 3-phenylphenol (3-hydroxybiphenyl).[14] The results of these analyses are recorded in our previous paper.[7]

Purified fraction B was used for all structural studies. It was prepared by subjecting isolated fraction B sequentially to two further rounds of alkali solubilization followed by acidification, centrifugation, and dehydration, as in the original isolation procedure. The monosaccharide composition of this purified material appears in Table 1.

2.3. Partial hydrolysis

2.3.1. With acid. Two dry samples (50 mg) of fraction B were triturated with successive 0.3-mL portions of 0.005 M sulfuric acid to hydrate them, after which acid was added to final volumes of 10 mL. The samples were placed in a heating block at 95 °C and vortexed periodically to facilitate solution of the gel. One sample was removed from the heat source after 2 h and the other after 6 h. When the cooled solutions were examined in a Ubbelohde capillary viscometer the observed efflux times were 161 s for the 2 h sample, 108 s for the 6 h sample, and 93 s for the acid solvent. After measurement, the solutions were poured into ethanol to a final alcohol concentration of 80%, giving precipitates that could be recovered by centrifugation. These were washed with 80% ethanol, 95% ethanol, and ether, then dried for 2 d at 40 °C. The yields of partially hydrolyzed products, B-H^+, were 41 and 33 mg, respectively. When 0.17% solutions of the products were dialyzed against water, using a membrane having a cutoff at MW 6000–8000, B-H^+ (2 h) was 100% retained, and B-H^+ (6 h) was 95% retained. On examination by paper chromatography the supernatants from the alcohol precipitations were found to contain both arabinose and xylose; no oligosaccharides were detected. From the compositions of B-H^+ (2 h) and (6 h), recorded in Table 1, and the weights of these fractions, it could be calculated that the amounts of monosaccharides released were: after 2 h heating, Ara 3.6 mg, Xyl 5.3 mg; after 6 h, Ara 8.4 mg, Xyl 9.7 mg.

2.3.2. Isolation and characterization of a disaccharide. Sixteen 50-mg samples of fraction B were triturated with 0.125 M sulfuric acid, acid was added to a final volume of 5 mL per sample, and all were heated at 100 °C for 45 min.[15] The samples were combined, and the solution was neutralized with barium carbonate, then filtered. The filtrate was concentrated in vacuo and passed through AG50W-X8 cation-exchange resin. The effluent was reduced in volume to 20 mL, and 95% ethanol was added to a final concentration of 79%. The resulting precipitate was recovered by centrifugation, and the supernatant was concentrated to a syrup. On analytical

Table 1. Monosaccharide composition of selected fractions and hydrolysis products

Monosaccharide	Compositions (mol%)						
	Purified B	B-H^+ (2 h)	B-H^+ (6 h)	B-E1	B-E2	C-1	C-2
Arabinose	22.6	19.4	11.4	13.7	7.2	16.0	3.9
Xylose	74.6	78.7	87.0	84.8	91.5	79.9	11.9
Galactose	1.5	1.5	1.3	1.3	1.2	2.3	1.8
Glucose	0.3	0.2	0.2	0.2	0.1	0.9	0.6
Rhamnose	0.4	0.1	0.1	0	0	0.3	40.5
Uronic acid	0.7	nd[a]	nd[a]	nd[a]	nd[a]	0.6	41.4

[a] nd = not determined.