Note

Structural Characterization of a Heterogalactan from Antler-shaped *Ganoderma lucidum*

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Abstract: A heterogalactan fraction, accounting for approximately 0.5% of the dry weight of the fruit body, was derived from a water-extracted polysaccharide fraction from antler-shaped *Ganoderma lucidum*. The heterogalactan fraction was characterized by sugar composition and sugar linkage composition analyses. The results suggest that the heterogalactan fraction consists of a fucomannogalactan (a linear (1→6)-D-galactan substituted at the O-2 position with non-reducing fucosyl and mannosyl residues), or a mixture of fucogalactan (a linear (1→6)-D-galactan substituted at the O-2 position with non-reducing fucosyl residues) and mannogalactan (a linear (1→6)-D-galactan substituted at the O-2 position with non-reducing mannosyl residues). The molecular weight of the heterogalactan fraction was estimated to be 1.0×10^4 by gel filtration on Sepharose CL-6B.

Key words: polysaccharide, heterogalactan, antler-shaped *Ganoderma lucidum* fruit body, Reishi

It is well known that many medicinal and therapeutic properties are attributed to polysaccharides derived from various kinds of mushrooms.^{1–3)} Although the chemical structure of some polysaccharides in the fruit body of *Ganoderma lucidum* (Reishi) have been investigated, little is known regarding antler-shaped *G. lucidum* (AS *G. lucidum*) polysaccharides. To elucidate the constituents of the polysaccharides in the fruit body of AS *G. lucidum*, we investigated the influence of a culture medium on the composition of water-soluble polysaccharides (WSPs) in the fruit body of AS *G. lucidum*, using a medium of pruned persimmon, mulberry, Japanese cherry, Japanese plum, apple, Japanese oak, or grape branches.⁴⁾ Based on the neutral sugar composition and sugar linkage composition analyses, it was found that the kind and composition ratio of water-soluble polysaccharides in the fruit body of AS *G. lucidum* was dependent on the culture medium (*i.e.*, the type of branches used). Although there was a difference in the quantity, heterogalactan-like polysaccharides and β -glucan-like polysaccharides were found in all WSPs. Interestingly, the WSPs of AS *G. lucidum* from the persimmon and mulberry branches were rich in Ara- and Xyl-containing polysaccharides.⁴⁾ Many previous studies have been conducted on the biological functions of β -glucans,¹⁾ however, the biological function of heterogalactan remains unknown. Recently, studies on the anti-inflammatory and antinociceptive effects of fucogalactans from *Agaricus brasiliensis*⁵⁾ and *Agaricus bisporus* var. *hortensis*,⁵⁾ fucomannogalactans from *Lentinus edodes*,⁶⁾ and

mannogalactans from *Pleurotus pulmonarius*⁷⁾ have been carried out. We thought it would be of interest to determine whether or not the WSPs in the fruit body of AS *G. lucidum* contain fucogalactan and/or fucomannogalactan. This report deals with the structural characterization of a heterogalactan isolated from the WSPs of AS *G. lucidum* grown on apple branches (apple-WSPs).

The apple-WSPs (yield: 52.1 mg/10 g dry weight of fruit body. Fuc:Ara:Rha:Gal:Glc:Xyl:Man = 4.1:4.3:2.9:36.6:20.8:4.2:27.1) used in this study are described in a previous paper.⁴⁾ Unless otherwise stated, all materials and experimental procedures used in this study were as described in a previous paper.⁴⁾

To separate neutral polysaccharides from acidic polysaccharides, a portion of the apple-WSPs was subjected to DEAE-Sephadex A-25 chromatography (acetate form). WSP was resolved into five fractions, I–V (Fig. 1). Table 1 shows the yield and neutral sugar composition of fractions I–V obtained from apple-WSPs. Fractions I and II (neutral polysaccharide fractions), which were eluted with a sodium acetate buffer from the DEAE-Sephadex column, and which accounted for about 28.5 and 29.5% of the recovered carbohydrates, mainly consisted of Fuc, Man, and Gal residues. Fractions III, IV, and V (acidic polysaccharide fractions), which were eluted with NaCl in a sodium acetate buffer, mainly consisted of Gal, Man, and Glc residues. Small amounts of Ara, Xyl, and Glc residues discovered in a major neutral polysaccharide fraction, WSP-I, were considered to be derived from arabinoxylan-like and β -glucan-like polysaccharides, which were presumably admixed in the heterogalactan. Thus, the isolated WSP-I accounted for approximately 23% of the total WSPs and for about 0.5% of

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Abbreviations: Rha, rhamnose; Fuc, fucose; Ara, arabinose; Xyl, xylose; Man, mannose; Glc, glucose; Gal, galactose; AS, antler-shaped.

Fig. 1. DEAE-Sephadex A-25 chromatography of apple-WSPs.

Apple-WSPs (50 mg in 10 mL of water) were applied to a column ($\phi 3.5 \times 20$ cm) pre-equilibrated with 50 mM sodium acetate buffer (pH 5.5). The column was washed with the same buffer (400 mL). Absorbed materials were eluted, stepwise, with 0.5 M NaCl (400 mL) and 1.0 M NaCl (400 mL) in 50 mM sodium acetate buffer: 10-mL fractions were collected and assayed for carbohydrates by the phenol-sulfuric acid method.⁸⁾ Tubes 3–9 (I), 10–17 (II), 46–51 (III), 52–56 (IV), and 86–93 (V) were separately combined, dialyzed and concentrated.

Table 1. Yields and neutral sugar composition of fractions obtained from apple-WSPs by DEAE-Sephadex A-25 chromatography (Fig. 1).

Fraction	Yield (Total sugar, mg)	%	Neutral sugar composition (mol%)						
			Fuc	Ara	Rha	Gal	Glc	Xyl	Man
I	11.5	28.5	12.4	0.1	0	81.3	0.4	0.2	5.7
II	11.9	29.5	5.6	1	0	56.6	13.8	5.3	17.8
III	10.4	25.8	2.2	6.9	5.3	14.8	32.5	5.2	33
IV	3.7	9.2	2	7.6	4.6	12.8	42.3	8.3	22.3
V	2.8	6.9	1.8	5.1	0	9	48.4	12.3	23.1

Fig. 2. Sepharose CL-6B chromatography of apple-WSP-I.

Apple-WSP-I (1.0 mg in 0.5 mL of 0.1 M NaOH) was applied to a column ($\phi 1.0$ cm $\times$ 45 cm) pre-equilibrated with 0.1 M NaOH, followed by filtration through the column with 0.1 M NaOH. Each tube was assayed for carbohydrates by the phenol-sulfuric acid method.⁸⁾ The arrows indicate the elution positions of the dextrans of mol wt 5.0×10^5 and 1.0×10^4 , respectively.

the total dry weight of the fruit body.

Apple-WSP-I (1 mg) was chromatographed in a calibrated Sepharose CL-6B column ($\phi 1.0$ cm $\times$ 45 cm) equilibrated with 0.1 M NaOH. It gave a single peak, and its average molecular weight was estimated to be approximately 1.0×10^4 (Fig. 2). Apple-WSP-I was methylated and hydrolyzed, and the sugar linkage composition was analyzed by gas-liquid

chromatography of the resulting alditol acetates. The non-reducing terminal of Fuc (10.8%), the non-reducing terminal of Man (4.5%), the 6-linked Gal (61.3%), and the 2,6-linked Gal (17.6%) residues were shown to be prominent structural units. In a previous sugar linkage analysis of apple-WSPs, 6-linked Gal, 3,4-linked Gal, 2,4-linked Gal, and 3,6-linked Gal residues were identified as the structural units of Gal

residues, and the 2,6-linked Gal residue was not identified⁴⁾ (Table 4 in the reference). The unidentified peak No. 22 in a previous study⁴⁾ (Table 4 in the reference) may correspond to the 2,6-linked Gal residue from the data on retention time.

Although some possible structures can be deduced from the present data, reference to previous works^{2,3,9-11)} would suggest that a heterogalactan fraction (WSP-I) of AS *G. lucidum* consists of a fucomannogalactan (a linear (1→6)-D-galactan substituted at the O-2 position with non-reducing fucosyl and mannosyl residues), or a mixture of fucogalactan (a linear (1→6)-D-galactan substituted at the O-2 position with non-reducing fucosyl residues) and mannogalactan (a linear (1→6)-D-galactan substituted at the O-2 position with non-reducing mannosyl residues).

Most heterogalactans have a main chain composed of (1→6)-linked α -D-galactopyranosyl units, with fucose and/or mannose as the main substituents. Heterogalactans, especially those that contain fucose, exhibit biological activities.⁵⁻⁷⁾ Usui *et al.*¹⁰⁾ and Mizuno *et al.*¹¹⁾ reported, respectively, that fucogalactan and mannofucogalactan were isolated from the hot-water extract of the fruit body of *Fomitopsis pinicola* and *G. lucidum* by a combination of fractionation procedures, including precipitation with ethanol and cetyltrimethylammonium bromide and chromatography on a column of DEAE-cellulose, Sephadex G-100 and concanavalin A-Sepharose CL 4B. A heterogalactan of AS *G. lucidum* may be fractionated into fucogalactan and mannofucogalactan. It is of interest to clarify whether or not the AS *G. lucidum* heterogalactan has some medicinal and therapeutic properties.

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