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Structure and conformation of α-glucan extracted from *Agaricus blazei* Murill by high-speed shearing homogenization

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ABSTRACT

Agaricus blazei Murill is an edible and medicinal mushroom favored in many countries, by virtue of both its delicious taste and its potential health benefits such as its purported anticancer activity. A neutral α-glucan (ABM40-1) with a carbohydrate content of 96% was purified from the high-speed shearing homogenization extracts of *A. Blazei* Murill by ethanol precipitation and column chromatography. Methylation analysis along with nuclear magnetic resonance spectroscopy revealed that ABM40-1 was an α-(1 → 4)-D-glucopyranan with *O*-6 position occasionally occupied with α-Glc*p*-(1 → or α-Glc*p*-(1 → 6)-β-Glc*p*-(1 → side chains. A weight-average molecular weight of 7.34×10^6 Da was determined for ABM40-1 and its chain in solution was revealed as a compact sphere by size exclusion chromatography (SEC) coupled with a laser light scattering. This spherical conformation was also further confirmed by Congo red test and using atom force microscopy. These results suggest it would be worthwhile to further study the potential bioactivities of ABM40-1.

1. Introduction

Agaricus blazei Murill, also known as *Himematsutake* in Japan and *Jisongrong* in China, is an edible and medicinal mushroom native to Brazil. It is widely cultivated and consumed in countries including Japan and China based on demands for it as a food and also for its potential health benefits. A wide range of biological functions has been reported for this mushroom, including anticancer, anti-mutagenicity, chemopreventive, antioxidant activities [1–4]. Among the components determining these functions of *A. Blazei* Murill are polysaccharides, which are believed to significantly contribute to the anticancer, antioxidation and immunomodulating bioactivities of *A. Blazei* Murill [5–7]. The structural characteristics of polysaccharides from *A. Blazei* Murill have also attracted increasing attention because of their vital roles in the activities, for example, the structure of β-D-glucans isolated from *A. Blazei* Murill have been reported [8,9].

Hot water extraction is the most common technique for the preparation of polysaccharides, but it is generally of low efficiency, time-consuming and energy-intensive. Some alternative extraction methods have been developed, such as ultrasonic-assisted and enzyme-assisted extraction [10,11]. High-speed shearing homogenization extraction, an emerging novel method to extract bioactive ingredients, has also been successfully used for the high efficiency extraction of polysaccharides like pectin [12].

The present study focuses on the extraction of a homogenous glucan isolated from *A. Blazei* Murill using high-speed shearing homogenization. The detailed structural features of this glucan were determined using chemical analysis and NMR spectroscopy. A determination of the solution conformation of this glucan was also performed since its conformation impacts its activities.

2. Materials and methods

2.1. Materials

The fruiting bodies of *A. blazei* Murill were provided by Hangzhou Baishanzu Biological Technology Co., Ltd., China and identified by Prof. Weiming Cai, Zhejiang Forestry Academy.

Diethylaminoethyl (DEAE) Sepharose fast flow and Sephacryl S-500 high-resolution were purchased from GE Healthcare. Monosaccharide standards (L-Rha, L-Fuc, L-Ara, D-Xyl, D-Man, D-Glc, D-Gal), dimethyl sulfoxide (DMSO), methyl iodide (CH_3I), sodium borohydride ($NaBH_4$), trifluoroacetic acid (TFA) and Congo red were obtained from Sigma.

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All other reagents and solvents were of analytical grade and made in China.

2.2. Isolation and purification of polysaccharide

The fruiting bodies of *A. blazei* Murill were ground and then soaked overnight with 95% ethanol to remove ethanol extractable molecules. After removing alcohol by filtration, the residues were extracted with distilled water through a high-speed shearing homogenization technique at room temperature. The aqueous extracts were pooled and concentrated under reduced pressure. Furthermore, removing protein using Sevag method was repeatedly performed six times for the concentrate, followed by ethanol precipitation by slowly adding ethanol to a final concentration of 40 vol%. The resulting crude polysaccharide fraction (sediments) was collected, lyophilized, and designated as ABM40.

ABM40 was dissolved in distilled water and then subjected to the DEAE Sepharose column (26 mm × 100 cm) chromatography with step-wise salt elution (0, 0.1, 0.2 M). Additional purification of the fraction eluting at salt concentration of 0.1 M was performed by chromatography on Sephacryl S-500 column (16 mm × 100 cm) eluted with water. Elution was monitored by determining sugar content in the eluent by phenol sulfuric acid method [13]. The main polysaccharide fractions were pooled, lyophilized, and designated as ABM40-1.

2.3. Physicochemical properties

The ultraviolet (UV) scan of ABM40-1 from 400 to 200 nm was conducted using a UV-2450 scanning ultraviolet/visible (UV/Vis) spectrophotometer (Shimadzu Co., Japan).

Fourier transform infrared (FT–IR) spectroscopy of ABM40-1 was recorded using KBr pellets on a Nicolet 6700 FT–IR spectrometer (Thermo, USA) in the range between 4000 and 500 cm^{-1}.

2.4. Monosaccharide compositional analysis

Acetylation was performed before gas chromatography–mass spectrometry (GC–MS) analysis of monosaccharide compositions of ABM40-1 as described in our previous report [14]. Briefly, 2 mg of ABM40-1 was hydrolyzed with TFA (2 M, 4 mL) at 110 °C for 2 h, followed by rotary evaporation to remove excess TFA with methanol. The released monosaccharides were reduced through addition of $NaBH_4$ solution (10 mg/mL, 3 mL) for 3 h and then acetylated with acetic anhydride (4 mL) at 100 °C for 1 h. The resulting alditol acetates were determined by GC–MS equipped with an HP–5MS capillary column (30 m × 0.32 mm × 0.25 μm). The oven temperature program was set: 120 °C (holding for 1 min) to 165 °C (for 1 min) at 10 °C/min, to 169 °C (for 0.5 min) at 1 °C/min, to 196 °C (for 0.5 min) at 10 °C/min, to 200 °C (for 1 min) at 1 °C/min, to 240 °C (for 5 min) at 10 °C/min. The temperatures of both injector and detector were set at 250 °C. Helium was used as carrier gas. For qualitative analysis of monosaccharides in ABM40-1, monosaccharide standards were subjected to the same treatments of reduction and acetylation as above.

2.5. Methylation analysis

ABM40-1 was converted into partially methylated alditol acetates (PMAAs) as described by Anumula and Taylor [15]. Briefly, 3 mg of ABM40-1 was dissolved in DMSO (0.6 mL), followed by addition of a fine slurry of NaOH in DMSO (0.6 mL) and methyl iodide (0.6 mL). After incubating for 10 min, distilled water was added to terminate the methylation reaction and $CHCl_3$ was then added to extract the resulting products. For complete methylation this procedure was repeated again on the $CHCl_3$ extract after evaporation resulting in the disappearance of the hydroxyl absorption in IR spectrum. The permethylated polysaccharide was successively hydrolyzed with HCO_2H (88%, 3 mL) at 100 °C for 3 h and TFA (2 M, 4 mL) at 110 °C for 6 h. Reduction and acetylation of the hydrolysate were then performed as described in the monosaccharide analysis. The generated PMAAs were recovered by extraction with $CHCl_3$ and determined by GC–MS. The oven temperature was programmed as follows: initial temperature of 120 °C after holding 1 min were elevated to 240 °C at 10 °C/min and keeping constant for 6.5 min. The other parameters of GC–MS were set as described in the monosaccharide analysis.

2.6. NMR analysis

ABM40-1 (30 mg) was thrice deuterium exchanged by freeze-drying from D_2O before dissolution in D_2O (0.5 mL) for NMR analysis on a Bruker AVANCE III (500 MHz) NMR spectrometer. The NMR spectra were recorded including ^1H–(25 °C and 60 °C) and ^{13}C NMR, ^1H–^1H correlation spectroscopy (COSY), total correlation spectroscopy (TOCSY), nuclear Overhauser enhancement spectroscopy (NOESY), heteronuclear single-quantum correlation (HSQC), heteronuclear multiple bond correlation (HMBC). ^1H and ^{13}C chemical shifts were calibrated through δ 4.78 for HOD (25 °C) and δ 0.00 for 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS), respectively.

2.7. Congo red test

An aliquot of ABM40-1 aqueous solution (2 mL) was mixed with the same volume of Congo red (80 μM) aqueous solution. NaOH solution (1 M) and water were also added into the mixture to bring the final NaOH concentrations to 0, 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45, 0.50 M. Water was used in place of ABM40-1 as negative control. The maximum absorbance wavelength (λ_{max}) in the range from 400 to 600 nm was measured using a scanning ultraviolet/visible spectrometer.

2.8. SEC-MALLS-RI

Molecular weight and conformation parameters in solution of ABM40-1 were determined using size exclusion chromatography (SEC) equipped with a multi-angle light scatter detector and a refractive index detector (SEC-MALLS-RI). This determination was performed on TSK-Gel G5000 PW_{XL} and TSK-Gel G3000 PW_{XL} columns coupled in series. $NaNO_3$ solution (1.0 M) containing 0.05% (w/w) NaN_3 was used at a flow rate of 1.0 mL/min for elution. ABM40-1 solution (3.0 mg/mL) was prepared by completely dissolving sample in mobile phase and filtered through a 0.45-μm filter before injection on the column. The refractive index increment value (dn/dc) of 0.138 mL/g was adopted in this study. ASTRA software was used to acquire and analyze the experimental data.

2.9. Atomic force microscopy

ABM40-1 was dissolved in Milli-Q water and stirred for 4 h at 80 °C to yield a 1.0 mg/mL stock solution. The test sample solution was prepared by dilution of the stock solution into 5 μg/mL with Milli-Q water and stirred for 2 h at 80 °C before deposition on cleaved mica. After adsorption for 30 s at RT, the sample on mica was thoroughly rinsed with 1.0 mL Milli-Q water and dried in air for Atomic force microscopy (AFM) imaging. AFM was operated on a Park XE-70 Atomic Force Microscope (Park Scientific Instruments, Korea) and in tapping mode under ambient conditions using commercial silicon nitride cantilevers. While flattening was used to remove background curvature, no further image processing was carried out on the AFM images.

3. Results and discussion

3.1. Preparation and physicochemical properties of ABM40-1

Isolated of extracts of fruiting bodies of *A. blazei* using high-speed shearing homogenization and chromatography on a DEAE-Sepharose