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A hyperbranched β-D-glucan with compact coil conformation from *Lignosus rhinocerotis* sclerotia

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ABSTRACT

An alkali-soluble polysaccharide was extracted from *Lignosus rhinocerotis* sclerotia (LRP). Its structural characteristics were determined by GC–MS, FT-IR, GC, 1D and 2D NMR combined with Smith degradation and methylation analysis. The LRP had a (1 → 3)-β-D-Glcp backbone with every three residues bearing a (1 → 6)-linked and hyperbranched side chain that contained three (1 → 6)-β-D-Glcp residues as secondary main chain and two terminal β-D-Glcp residues linked at O3. The degree of branching was 0.76 from GC–MS analysis, implying a highly branched structure for LRP. The M_w, $\langle S^2\rangle_z^{1/2}$, R_h and $[\eta]$ values of LRP in 0.25 M LiCl/DMSO were measured by SEC-MALLS-Vis-RI combination technology to be 2.88×10^5 g/mol, 30.36 nm, 22.34 nm and 131.50 ml/g, respectively. Furthermore, the exponent α of $[\eta]$–M_w, β of $\langle S^2\rangle_z^{1/2}$–$M_w$, the fractal dimension d_f and molecular parameter ρ were determined to be 0.20, 0.33, 2.50 and 1.36, demonstrating that the LRP was a hyperbranched polysaccharide and adopted a compact coil conformation in LiCl/DMSO.

1. Introduction

Currently, the commercial value of mushroom or fungus polysaccharides has attracted much attention in functional foods, due to a wide range of nutritional and healthy benefits (Palacios, García-Lafuente, Guillamón, & Villares, 2012). *Lignosus rhinocerotis* (*L. rhinocerotis*), a popular edible mushroom, contains large amounts of beneficial components such as polysaccharides, proteins/peptides, polysaccharide-protein complexes, etc. It has been extensively used in South China, Malaysia and other Southeast Asian countries as a general tonic food to improve overall wellness for the nutritional characteristics (Lau, Abdullah, Aminudin, Lee, & Tan, 2015). The sclerotium of *L. rhinocerotis* is the important part with nutritional value and is rich in β-glucan, which is a non-digestible polysaccharide well known as dietary fiber (DF) (Wong

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& Cheung, 2005). The DF not only has desirable technological properties such as texture improvement, water retention, gel formation and thickening in food systems, but also is increasingly incorporated into health foods on account of its prebiotic effect (Ben Jeddou et al., 2016; Wong & Cheung, 2005). Moreover, β-glucan from *L. rhinocerotis* has been previously demonstrated to manifest prebiotic benefit and immunomodulatory activity (Cheung, 2013; Lau et al., 2015; Wong, Lai, & Cheung, 2009; Hu, Huang, Wong, & Yang, 2017).

Generally, (1 → 3)-β-D-glucans is known to be important for their prebiotic and immunomodulatory activities, and they have a strong nature to form a helical structure giving high viscosity and gelling properties (Synytsya et al., 2009; Xu, Xu, & Zhang, 2012). Lentinan, a typical (1 → 3)-β-D-glucan with (1 → 6)-linked glucose branches from *Lentinus edodes*, showed immunocompetence and high viscosity due to its triple helix and stiff conformation (Zhang, Li, Wang, Zhang, & Cheung, 2011). Besides, the degree of branches, even length of branches may also influence the physiological and technological benefits of polysaccharides. For instance, β-D-glucans with degree of branching (DB) between 0.20 and 0.33 seem to be the most active, while the highly branched (DB = 0.75) or much less branched (DB = 0.06) glucan is weakly active (Bohn & BeMiller, 1995). A comb-like (1 → 3)-β-D-glucan with short and intensive (1 → 6)-β-glucose branches from *Cordyceps sinensis* had high viscosity, whereas a hyperbranched β-D-glucan from *Pleurotus tuber-regium* showed low viscosity (Hu, Jiang, Huang, & Sun, 2016; Tao, Zhang, Yan, & Wu, 2007). On the basis of the literatures, the functional properties (nutritional, technological and physiological benefits) of polysaccharides are closely correlated to their monosaccharide composition, α/β-configuration, glycosidic linkages, degree of branches, length of branches, molecular weight and chain conformation (e.g., random coil, compact coil, helix chain, or rod chain) (Bohn & BeMiller, 1995; Huang, Jin, Zhang, Cheung, & Kennedy, 2007), so the elucidation of fine structure of polysaccharide is essential for successful interpretation of its functional properties and the structure-function relationships. However, the molecular structure and chain conformation of polysaccharide from the sclerotium of *L. rhinocerotis* (LRP) has not been fully clarified until now. Therefore, it is necessary to explore the structural characteristics and chain conformation of LRP.

In the present work, the structural characteristics of LRP were determined by Fourier transform infrared spectroscopy (FT-IR), gas chromatography (GC), gas chromatography-mass spectroscopy (GC–MS), 1D and 2D NMR (^{1}H NMR, ^{13}C NMR, HMQC and NOESY) combined with monosaccharide composition analysis, periodate oxidation, Smith degradation (including controlled Smith degradation) and methylation analysis. To further investigate its molecular weight and chain conformation, size exclusion chromatography combined with multi-angle laser light scattering, viscometer and differential refractive index detector (SEC-MALLS-Vis-RI) instrument, together with the theory of polymer solution was applied. This work reveals the precise structure and chain conformation of LRP to supply a theoretical basis for interpretation of the LRP functional properties associated with its molecular structure, and facilitates the LRP development in food industry.

2. Materials and methods

2.1. Materials

Six monosaccharides (D-rhamnose, D-arabinose, D-xylose, D-mannose, D-glucose, D-galactose), erythritol and glycerol were purchased from Shanghai Yuanye Biological Technology CO., Ltd (Shanghai, China). Methyl iodide (CH_3I) was provided by Tianjin Fengchuan Chemical Reagent Technologies Co., Ltd (Tianjin, China). Trifluoroacetic acid (TFA), sodium borohydride ($NaBH_4$), hydroxylamine hydrochloride and sodium periodate ($NaIO_4$) were provided by Aladdin Industrial Corporation (Shanghai, China). Sodium hydride (NaH), acetic anhydride, phenol and other chemical reagents were provided by Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Dimethyl sulfoxide (DMSO) and DMSO-d_6 were purchased from Sigma-Aldrich. All chemical reagents were of analytical grade.

2.2. Extraction of LRP

L. rhinocerotis sclerotia were provided by Hong Kong Polytechnic University. After being milled and dried, the sclerotia power (30 g) was defatted sequentially by Soxhlet extraction with ethyl acetate and acetone for 6 h, respectively. The resultant residue was immersed in distilled water at 25, 95, and 100 °C stepwise and extracted at each temperature for five times. After being centrifuged, the final residue was extracted in 0.5 M NaOH aqueous solution at 4 °C for 4 h and repeated for three times, followed by centrifugation (10,000 rpm, 20 min). The supernatant was neutralized with hydrochloric acid, dialyzed using regenerated cellulose tube (M_w cut-off 8000–12,000) against tap water for 5 days and distilled water for 4 days, and then lyophilized (FD-1-50, Beijing Boyikang Laboratory Instruments Co., Ltd., China) to yield a polysaccharide (17.36 g) designated as LRP.

2.3. Basic chemical composition and monosaccharide composition analysis

Total sugar content of LRP was determined by the phenol-sulfuric acid method with glucose as a standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Protein content was measured according to the modified Lowry method (Bensadoun & Weinstein, 1976).

Monosaccharide composition of LRP was examined by aldononitrile acetate method and detected with GC as previous description (Hu et al., 2016). Briefly, LRP (5 mg) was hydrolyzed with 8 M TFA (5 ml) for 12 h at 100 °C. After TFA removed, the hydrolysate was reduced by hydroxylamine hydrochloride (5 mg) and acetylated with acetic anhydride (1 ml) for 30 min at 90 °C. The final aldononitrile acetate derivative was detected by Agilent GC 6890N system with a HP-5 column. The column temperature was kept at 180 °C for 2 min, raised to 300 °C at 2 °C/min. The injector and detector were at 250 and 300 °C. Six monosacharide standards also executed the same procedures as the LRP sample.

2.4. UV and FT-IR spectra

UV–visible spectrum of LRP was scanned on a UV–vis spectrophotometer (UV-1700, Shimadzu, Japan) from 190 nm to 700 nm. FT-IR spectrum of LRP was recorded on a FT-IR spectrometer (Nexus 470, Nicolet, UK) using KBr-pellets method in the range of 400–4000 cm^{-1}.

2.5. Periodate oxidation and Smith degradation

2.5.1. Periodate oxidation

The LRP (200 mg) was oxidized with 15 mM $NaIO_4$ (200 ml) at 4 °C and kept in dark. The absorption at 223 nm was measured by a UV–vis spectrophotometer (UV-1750, Shimadzu, Japan) to monitor the oxidation reaction and ethylene glycol was used to terminate when the absorption was stable. The consumption of $NaIO_4$ was determined by spectrophotometry, and the production of formic acid was measured by titration with 5 mM NaOH. The reaction solution was dialyzed against distilled water, reduced