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Structure and antioxidative property of a polysaccharide from an ammonium oxalate extract of *Phellinus linteus*

Jing-Kun Yan [a,*], Yao-Yao Wang [a], Zhen-Bin Wang [a], Hai-Le Ma [a], Juan-Juan Pei [a], Jian-Yong Wu [b,*]

[a] School of Food & Biological Engineering, Jiangsu University, Zhenjiang, 212013, China
[b] Department of Applied Biology & Chemical Technology, State Key Laboratory of Chinese Medicine and Molecular Pharmacology in Shenzhen, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong

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ABSTRACT

In this paper, the novel polysaccharide PL-A11 was purified from an ammonium oxalate extract of *Phellinus linteus* mycelia. Its physicochemical properties, structural characteristics, and antioxidant activities were investigated. Results showed that PL-A11 had a weight-average molecular weight (M_w) of 13.8 kDa and was mainly composed of arabinose, xylose, mannose, and glucose in a molar ratio of 1.1:1.3:1.0:6.6. The backbone of PL-A11 was composed of (1 → 4)-α-D-glucopyranosyl, (1 → 2)-α-D-xylopyranosyl, and (1 → 3)-α-D-arabinofuranosyl residues, whereas the (1 → 6)-α-D-mannopyranosyl residues formed branches at the O-2 position with 1-linked-α-D-glucopyranosyl terminal residues. From the antioxidative activity tests *in vivo*, the administration of PL-A11 obviously enhanced the activity of antioxidant enzymes and significantly reduced the level of malondiadehyde (MDA) in the serum and liver of D-galactose-treated aging mice in a dose-dependent manner, as well as effectively stimulated the immune system of aging mice. These findings implied that PL-A11 could be developed as a potential antioxidant for applications in the functional food, pharmaceutical, cosmetic or nutraceutical industries.

1. Introduction

Free radicals are intermediate products of biochemical reactions that occur in human the body during the life process; these compounds include reactive oxygen species (ROS), such as hydrogen peroxide, superoxide radicals, hydroxyl radicals, and singlet oxygen [1]. Under normal conditions, the generation and scavenging of free radicals are in a dynamic balance. However, excessive free radicals can induce irreversible oxidative damage to the body when the amount of free radicals produced by oxidative stress is beyond the capacity of the scavenging system. Free radicals further accelerate aging and may cause a wide variety of chronic diseases, such as cardiovascular disease, cancer, and diabetes [2,3]. Consequently, scavenging free radicals to protect cells and tissues from oxidative damage has been given considerable attention. The body's antioxidant levels can be improved effectively by exogenous administration of antioxidants to maintain the redox equilibrium of organisms, resist damage by free radicals, and increase protection against diseases.

Over the past few years, synthetic antioxidants including butylated hydroxyl anisole (BHA), butylated hydroxyl-toluene (BHT), tertiary butylhydroquinone (TBHQ), and propyl gallate (PG) have been used for industrial purposes [4]. However, most of these compounds require complicated synthesis with high production cost and high toxicity. In particular, these antioxidants show potential adverse effects, such as liver injury and carcinogenesis [5], thereby making people hesitant to their use. In addition, their clinical application has significant limitations. Natural antioxidants that are safe and have low toxicity can meet people's health demands. Thus, increasing attention has been directed to the exploration and development of effective and safe natural antioxidants to scavenge free radicals and prevent oxidative damage.

Natural polysaccharides derived from fungi (particularly mushrooms) have been reported to possess a broad spectrum of biological activities, such as immunological, antitumor, antioxidative, anti-inflammatory, and hypoglycemic activities [6]. These compounds have attracted increasing attention for their health benefits and use as therapeutic agents with low toxicity and minimal side effects. Interestingly, polysaccharides have an important role as dietary free radical scavengers in preventing oxidative

* Corresponding authors.
E-mail addresses: jkyan_27@163.com, jkyan27@ujs.edu.cn (J.-K. Yan), jian-yong.wu@polyu.edu.hk (J.-Y. Wu).

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damage in living organisms [7–9]. Up to now, there are lots of heteropolysaccharides from fungi or mushrooms have been demonstrated to have significant free radical scavenging capacity and antioxidant activity, such as IOPS from mushroom *Inonotus obliquus* [10], PPE and PPM from mycelium of *Phellinus pini* and culture medium [11], CMP-1 from the cultured *Cordyceps militaris* [12], HEP from *Hericium erinaceus* [13], and W-PTR and A-PTR from *Pleurotus tuber-regium* (Fr.) Sing [14], etc.

Phellinus linteus (Berkeley & M. A. Curtis) Teng, generally called Sanghuang, is a species of mushrooms belonging to the *Hymenochaetaceae* family of *Basidiomycetes* that is valuable in traditional Chinese medicine and is widely used in East Asia, particularly China, Japan, and Korea. Polysaccharides isolated from *P. linteus* are major active constituents with important biological activities and pharmacological functions, such as antitumor, antioxidative, and immunoregulatory properties [15,16]. Recently, our group isolated three water-soluble polysaccharides from cultured *P. linteus* mycelia by extraction with hot water, 1% $(NH_4)_2C_2O_4$, and 1.25 M NaOH/0.05% $NaBH_4$ [17]. Results revealed that water-soluble polysaccharides (PL-A) extracted by 1% $(NH_4)_2C_2O_4$ showed significant free radical scavenging capacity and antioxidative activity *in vitro*. However, to the best of our knowledge, purification, structural characterization, and *in vivo* antioxidative activity assay of PL-A have not been reported.

Therefore, the present study aimed to elucidate the structural characterization of a novel polysaccharide named PL-A11, which was isolated from an ammonium oxalate extract of *P. linteus* mycelia, by a combination of instrumental and chemical analyses. The *in vivo* antioxidative activity of PL-A11 was investigated using the D-galactose (D-Gal)-treated aging mice model.

2. Materials and methods

2.1. Materials and chemicals

PL-A was obtained from the ammonium oxalate extract of cultured *P. linteus* KCTC 6190 mycelia based on our previous study [17]. DEAE-Sepharose Fast Flow and Sephacryl S-400 HR were obtained from GE Healthcare Bio-Sciences AB (Uppsala, Sweden). Assay kits for malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). All other chemicals and solvents were of laboratory grade and used without further purification.

2.2. Isolation and purification of PL-A11

The polysaccharide PL-A was dissolved in deionized water and centrifuged before the supernatant was applied to a pre-equilibrated DEAE-Sepharose FF chromatography column (2.6 cm × 40 cm) and eluted with a gradient of 0–0.2 M NaCl solution at a flow rate of 1.0 mL/min. Each 10 mL fraction of the eluate was collected and analyzed by the phenol-sulfuric acid method. The main polysaccharide fraction was combined, concentrated, dialyzed, and lyophilized to yield two polysaccharide fractions, named PL-A1 and PL-A2 (Fig. 1A). PL-A1 was further purified by a gel-filtration chromatography on a Sephacryl S-400 HR column (1.5 cm × 60 cm) and eluted with deionized water at a flow rate of 1.0 mL/min to obtain the purified polysaccharide named PL-A11 (Fig. 1B), which was subjected to further structural elucidation and bioactivity evaluation.

2.3. Chemical properties analysis

The total carbohydrate content, protein content, and uronic acid content of PL-A11 were determined according to the phenol-sulfuric acid method with d-glucose as a standard, the Bradford method used bovine serum albumin (BSA) as a standard, and the sulfuric acid-carbazole method used glucuronic acid as a standard, respectively [18–20]. Optical rotation was measured for the PL-A11 sample dissolved in de-ionized water at 20 °C on a Perkin-Elmer 341 digital polarimeter at 589 nm. The UV–vis spectrum of PL-A11 (1.0 mg/mL) was recorded in a Varian Cary 100 spectrophotometer (Varian Co., USA) from 190 to 400 nm at room temperature.

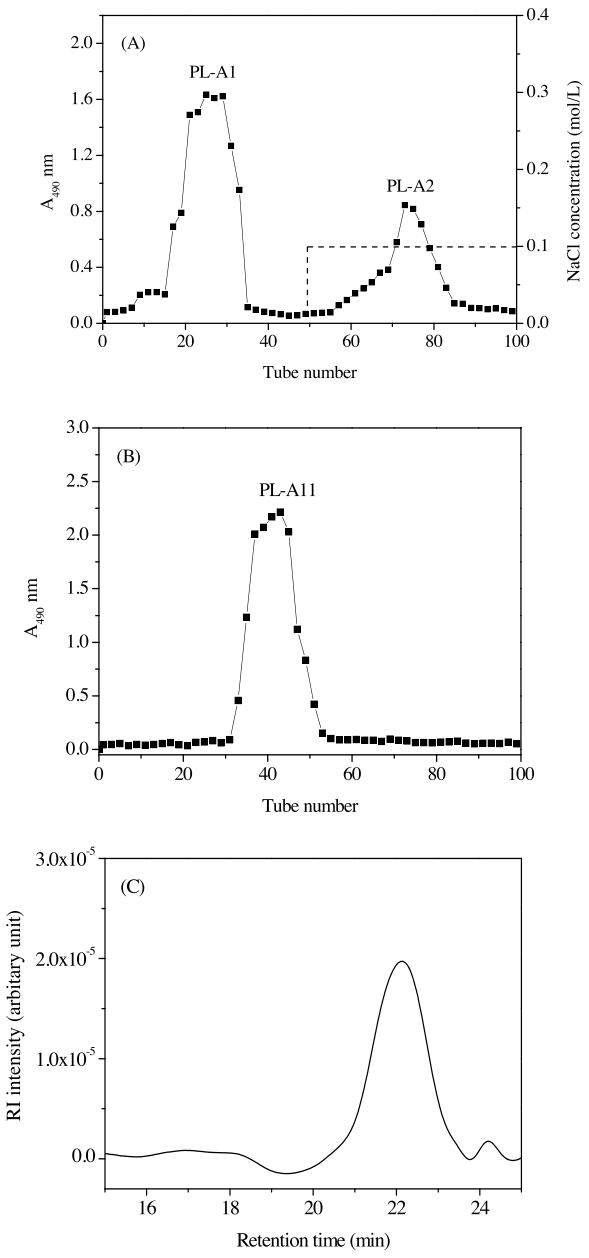

Fig. 1. (A) DEAE-Sepharose FF chromatographic profile for PL-A eluted with deionized water and 0.1 M NaCl solution; (B) sephacryl S-400 HR chromatographic profile for PL-A1 eluted with deionized water; (C) SEC elution pattern of PL-A11 in 0.1 M NaCl aqueous solution at 25 °C.

2.4. Homogeneity and molecular weight determination

The homogeneity and molecular weight (MW) of PL-A11 were investigated by size-exclusion chromatography with multi-angle laser-light scattering (SEC-MALLS, DAWN HELLOS II λ = 658 nm; Wyatt Technologies Corporation, USA) analysis. The SEC-MALLS was performed on an Agilent 1100 system equipped with two SEC