International Journal of Biological Macromolecules xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Structural elucidation and antiaging activity of polysaccharide from *Paris polyphylla* leaves

Shian Shen, Zhou Xu [1], Shiling Feng, Handong Wang, Jing Liu, Lijun Zhou, Ming Yuan, Yan Huang, Chunbang Ding *

College of Life Sciences, Sichuan Agricultural University, Yaan, Sichuan 625014, China

ARTICLE INFO

Article history:
Received 13 June 2017
Received in revised form 5 September 2017
Accepted 5 October 2017
Available online xxx

Keywords:
Paris polyphylla
Polysaccharide
NMR
Antioxidant

ABSTRACT

To optimize the use of *Paris polyphylla* resources, a homogenous polysaccharide (PPLP) was obtained from *P. polyphylla* leaves. Its average molecule weight was 2.95×10^4 Da, and the analysis of monosaccharide composition shown that PPLP consisted of L-arabinose and D-galactose with a molar ratio of 4.2:5.8. Methylation and nuclear magnetic resonance (NMR) spectroscopy data revealed that the backbone of PPLP was comprised of (1 → 6)-β-D-galactan, and the branched chains mainly consisted of arabinosyl residues which was linked to backbone *via* (1 → 3)-linkages. In addition, the antiaging effect of PPLP was investigated in a D-galactose induced mouse aging model. Compared with model group, the formations of malondialdehyde (MDA) were significantly prevented, and the levels of antioxidant enzymes and total antioxidant capacity (TAOC) were significantly improved in serum and liver in PPLP dose groups. These results demonstrated that PPLP possessed potent antiaging capacity.

1. Introduction

Paris polyphlla var. *yannanensis*, widely distributed in the southwest of China, has been used as a traditional Chinese herbal medicine for thousands of years. It usually plays an important role in detumescence, detoxifcation, acesodyne, and hematischesis [1]. With the development of extractive techniques, many bioactive constituents have been isolated and identified from *P. polyphylla*, such as saponins, ecdysones, phytosterols and flavonoid glycosides [2]. These effective components are widely used as antitumor, antimicrobial, hemostatic, and analgesic agents [3–6]. However, there are few studies on *P. polyphylla* leaves which are directly discarded by growers at present. Because *P. polyphylla* is a perennial herb and grows slowly, current supply of *P. polyphylla* rhizomes cannot meet the demand. At present the leaves are unutilized and directly discarded by growers. Thus, research on components of *P. polyphylla* leaves may provide a new approach for the utilization of *P. polyphylla* resources.

As a natural part of metabolism cell continuously generates reactive oxygen species (ROS) in metabolism. Although ROS can be balanced by natural antioxidative defense systems in normal cells, oxidative stress imposed by excessive ROS can damage the cellar macromolecules, such as DNA, proteins, lipids and carbohydrates, which can lead to many diseases [7,8]. According to the free radical theory of aging, free radicals react with cellular constituents, which result in organ senescence. The balance between antioxidant and prooxidant is tipped with aging, causing the antioxidant repair system of organism have insufficient ability to scavenge excessive free radical and maintain physiological function [9,10].

Plant polysaccharides, especially from medicinal herbs, have attracted considerable interest for their pharmacological effects and special structure. Many studies have documented that plant polysaccharides not only possess potent antioxidant, antihyperlipidemic, anticancer and immune activities [11–14], but also show low cytotoxicity and side effects [15]. In addition, to elucidate the mechanism of action of polysaccharides, many studies have been done to reveal the relationship between polysaccharide structures and their biological activities [16]. Our previous study has shown that the polysaccharide extracted from *P. polyphylla* leaves possesses strong antioxidant activities *in vitro*. However, there is scant information on *P. polyphylla* leaf polysaccharide structure and biological activities *in vivo*. In this study, the structure of *P. polyphylla* leaves polysaccharide (PPLP) was analyzed by methylation and nuclear magnetic resonance (NMR) spectroscopy. In addition, the *in vivo* antioxidant activity of PPLP was also investigated using a D-galactose induced mouse aging model.

* Corresponding author.
E-mail address: dcb@sicau.edu.cn (C. Ding).
[1] The same contributor with first author.

https://doi.org/10.1016/j.ijbiomac.2017.10.026
0141-8130/

2. Materials and methods

2.1. Extraction and purification of polysaccharide

The polysaccharide was extracted from *P. polyphylla* leaves as previous report [17]. Brief, the leaves were decocted under the optimum extraction conditions (extraction temperature 90.8 °C, ratio of water to raw material 21.3:1, and extraction time 4.8 h). The supernatant was collected and concentrated after centrifugation. The protein in the concentrate was removed by Sevag method [18]. The solution of crude polysaccharides was purified firstly by DEAE-cellulose DE52 column, and then further purified through a column of Superdex 200. The eluent was detected by a refractive index detector. A symmetrical peak named PPLP was collected and lyophilized for further study. The total sugar content of PPLP was measured by phenol-sulfuric acid method [19] with D-glucose as the standard. Protein content was determined by coomassie brilliant blue method [20] with bovine serum albumin as the standard. The uronic acid content of PPLP was determined by modified carbazole method [21] with glucuronic acid as the standard.

2.2. Structure determination

2.2.1. Molecular weight and determination

The average molecular weight of PPLP was determined using a high-performance gel permeation chromatography (HPGPC) equipped with a TSKgel GMPWXL column. The eluent was eluted by 20 mM ammonium acetate at a flow rate of 0.8 mL/min, and monitored with an evaporative light-scattering detector. Standard curve was created by plotting the elution volume versus logarithm of corresponding molecular weight with a series of standard detrains (Supplemental data 1). The average molecular weight of PPLP was estimated according to the standard curve.

2.2.2. Monosaccharide composition

PPLP was first hydrolyzed with 2 M trifluoroacetatic acid at 100 °C for 3 h in ampoule bottle. The excessive acid was completely removed through continual addition of methanol in a vacuum rotary evaporator. Subsequently, the hydrolysate and the reference standards of monosaccharide were reduced by sodium borohydride, respectively. Finally, the resultant products were acetylated by acetic anhydride at 40 °C for 2 h. The resultant alditol acetates were determined using a DSQ II GC–MS system (Thermo Fisher Scientific) with HP-5 capillary column (30 m × 0.32 mm × 0.25 μm). The gradient temperature program was as follows: the initial temperature of column was 130 °C, increased to 200 °C at 2 °C/min, and then increased to 250 °C at 10 °C/min, holding for 5 min. The injection and detector temperatures were 250 °C, respectively. The carrier gas was helium with a flow rate of 1 mL/min.

2.2.3. Partial acid hydrolysis

20 mg PPLP was hydrolyzed with 5 mL 100 mM trifluoacetic acid (TFA) in a 10 mL sealed ampoule bottle at 105 °C for 1 h. After the residual TFA was removed, the resultant hydrolysate was redissolved in 5 mL distilled water and the solution was filtered with an ultrafiltration membrane with molecular weight cut-off of 3000 Da. The retentate and filtrate were collected, and then were determined by HPGPC–MS, respectively.

2.2.4. Methylation

Methylation was conducted as reported previously [22] with minor modification. PPLP (10 mg) and sodium hydroxide (10 mg) were added to 3 mL dimethylsulfoxide, and then the mixture was treated with ultrasound to complete dissolution. Methyl iodide was used to initiate the methylation reaction. Subsequently, the residual methyl iodide was consumed by distilled water. The methylated product was extracted with three volumes chloroform. The organic layer was collected, and then was dried by a vacuum rotary evaporator. The dried methylated polysaccharide was in turn hydrolyzed, reduced and acetylated. Finally, the acetylated derivative was measured using a DSQ II GC–MS system (Thermo Fisher Scientific) with HP-5 capillary column (30 m × 0.32 mm × 0.25 μm). The linkages were identified based on relative retention time and fragmentation patterns, and the molar ratios for each sugar residues were calculated from the peak areas and response factors of the flame ionization detector.

2.2.5. Fourier transform infrared (FT-IR) spectra

PPLP and methylated PPLP were mixed with KBr powder and pressed into pellets, respectively. The FT-IR spectra of samples were recorded with a Shimadzu IR spectrophotometer between 4000 and 450 cm^{-1}.

2.2.6. Nuclear magnetic resonance (NMR) spectroscopy

50 mg PPLP was dried for several days in a vacuum desiccator, and then was exchanged with deuterium by lyophilization from D_2O for three times. Finally, the product of deuterium exchange was dissolved in 0.5 mL D_2O. ^1H and ^{13}C NMR spectra were scanned at 25 °C by a Bruker DRX-500 spectrometer (Bruker, Rheinstetten, Germany). The distortionless enhancement by polarization transfer (DEPT 135°) spectrum was measured to analysis the hydrogenation of each carbon. Besides, ^1H–^{13}C heteronuclear multiple bond correlation spectroscopy (HMBC), ^1H–^1H correlated spectroscopy (COSY), and ^1H–^{13}C heteronuclear single quantum correlation spectroscopy (HSQC) were recorded using the pulse programs supplied with the apparatus. Acetone was used as internal standard to calibrate the chemical shift (^1H 2.12 ppm, and ^{13}C 29.6 ppm).

2.3. Animal treatment

2.3.1. Experimental design

Male Kunming mice were provided by Dashuo Experimental Animal Company (Chengdu, China). Each mouse was nearly 8 week old, and weighed 21 ± 2 g. The mice were housed at ambient temperature and 50–60% relative humidity with 12 h light per day, and allowed free access to food and water. After one week acclimatization period, the mice were randomly divided into six groups, and each group has 12 mice. All the mice except normal control group were administered 0.2 mL D-galactose (150 mg/Kg/d) by intraperitoneal injection, and the normal control group mice were injected the same volume of 0.9% saline solution once a day. After 15 days, the normal control group and the model control group were administered 0.4 mL 0.9% saline solution, and the positive control group was treated with 0.4 mL vitamin C (V_C 200 mg/Kg/d) by intragastric gavage for 45 consecutive days. Low dose group (100 mg/Kg/d), medium dose group (200 mg/Kg/d), and high dose group (400 mg/Kg/d) were received 0.4 mL PPLP solution by intragastric gavage for 45 consecutive days, respectively. After overnight fasting, all the mice were anesthetized with diethyl ether. Blood and organics were collected and reserved at −20 °C prior to use.

2.3.2. Biochemical assay

Serum samples were obtained by centrifuging the blood at 3000 × *g* at 4 °C holding 10 min. Organs were ground in liquid nitrogen, and supernatants were obtained from organ homogenates by centrifuging. The optimal concentrations of serum and supernatants were prepared with chilled 0.9% saline solution before use. The protein content of each supernatant was detected by Coomassie Brilliant Blue method. The content of malondialdehyde (MDA), the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and total antioxidant capacity

Please cite this article in press as: S. Shen, et al., Int. J. Biol. Macromol. (2017), https://doi.org/10.1016/j.ijbiomac.2017.10.026