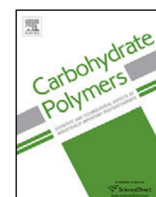
Carbohydrate Polymers 139 (2016) 61–66

Contents lists available at ScienceDirect

Carbohydrate Polymers

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



Immunoregulatory activities of polysaccharides from mung bean

Yang Yao[a], Yingying Zhu[b], Guixing Ren[a,*]

[a] Institute of Crop Science, Chinese Academy of Agricultural Sciences, No. 80 South Xueyuan Road, Haidian District, Beijing 100081, People's Republic of China
[b] Animal Science Unit, Gembloux Agro-Bio Tech, University of Liège, Passage des Déportés 2, B-5030 Gembloux, Belgium

ARTICLE INFO

Article history:
Received 1 July 2015
Received in revised form 22 October 2015
Accepted 1 December 2015
Available online 11 December 2015

Keywords:
Mung bean
Water-extractable polysaccharide
Ultrasonic

ABSTRACT

Ultrasonic treatment was performed on water-extractable polysaccharides from the seed of mung beans. Purified by anion-exchange and gel filtration chromatography, MWP-1′ and MWP-2′ were obtained. Average molecular weights (M_ws) of MWP-1′ and MWP-2′ were 68.4 kDa, and 52.4 kDa, respectively. Monosaccharides components analysis indicated that MWP-1′ was composed of Rha, Ara, Man and Gal in a molar percent of 0.4:2.6:5.3:0.7. MWP-2′ was composed of Ara, Man, Gal and Glc in a molar percent of 0.5:1.4:2.1:0.4. *In vitro* study showed that both polysaccharides samples were able to stimulate the production of secretory molecules (NO, TNF-α and IL-6) of RAW264.7 murine macrophages in a dosage dependent manner. MWP-2′ seemed to be the most potent and induced significantly higher the NO production. These findings suggest that the ultrasonic treatment polysaccharides isolated in our study have immune potentiation effects on macrophages.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Mung bean (*Vigna radiate* L.) is native to the northeastern India–Burma (Myanmar) region of Asia. There is now much interest in it for its physiological functionalities, such as antioxidant (Song, Zhang, Zhang, & Wang, 2010), antidiabetic (Wang et al., 2004), antitumor (Yang, Zhao, & Lv, 2008), antiangiotensin I-converting enzyme (ACE) (Zhu & Lin, 2006), and antimelanocytes (Li, Huang, Lu, & Hou, 2011). Polysaccharides from natural sources have attracted increasing attention due to their potential biological functions, especially for their antioxidant and immunomodulation activities such as scavenging free radical, inhibiting lipid oxidation, promoting natural killer cells (NK) cytotoxicity, activating macrophages and potentiation interleukins (Wang, Chen, Jia, Tang, & Ma, 2012; Yao, Cheng, & Ren, 2014; Yao, Cheng, Wang, Wang, & Ren, 2011a). The bioactivity of polysaccharides mainly depends on several structural parameters, including the sugar composition, molecular weight, type of glycosidic bond, and degree of sulfation (Mukai & Sato, 2011). In view of their potential applications in functional food and medicine, increasing numbers of studies have been focused on the isolation and purification of polysaccharide from a varieties of plants, animals and microorganisms (Sato et al., 2008).

Appropriate structural transformation can improve the biological activity of polysaccharides (Zhang, Zhang, Yang, & Liang, 2010). Modification methods of polysaccharides include chemical, physical and biological approach (Dubious, Gilles, & Hamilton, 1956). Ultrasonic treatment could enhance the mass transfer and the solvent's ease of access to the cell material of the fiber. The effects of cavitation, including macro-turbulence generated by the implosion of cavitation bubbles, and micro-jets caused by cavitation on the product surface to make the osmotic pressure between the inside and the outside of the cell different, so that the extraction of ultrasonic extraction quickly arrives at extraction equilibrium (Chen et al., 2012). Ultrasonic degradation, unlike chemical or biological decomposition, is a non-random process, with cleavage being taken roughly at the center of the molecule and with larger molecules degrading the fastest (Yu, Yang, Cui, Wang, & Ren, 2014). It is reported that polysaccharides extracted from mung beans using ultrasonic treatment to enhance its antioxidant activity (Yao & Ren, 2011).

In order to get polysaccharides of higher immunoregulatory activity, the water-soluble polysaccharides extracted from mung bean was treated by ultrasonic treatment. Purified by DEAE Sepharose Fast Flow and Sephacryl S-300 High Resolution column chromatography, the composition of polysaccharides was analyzed. The immunoregulatory of purified polysaccharide fractions was also investigated.

* Corresponding author. Tel.: +86 10 6211 5596; fax: +86 10 6215 6596.
E-mail address: renguixing@caas.cn (G. Ren).

http://dx.doi.org/10.1016/j.carbpol.2015.12.001
0144-8617/© 2015 Elsevier Ltd. All rights reserved.

2. Materials and methods

2.1. Materials and reagents

Mung beans were provided by the Chinese National Genebank (Beijing, China). DEAE Sepharose Fast Flow and Sephacryl S-300 High Resolution were purchased from GE Healthcare Bio-Sciences Co. (Piscataway, NJ, USA). DMEM, RPMI 1640 medium, lipopolysaccharide (LPS), Griess reagent, and monosaccharide standards rhamnose (Rha), arabinose (Ara), mannose (Man), galactose (Gal), Glucose (Glc), Trolox, fluoreseein fluorescein sodium salt, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), and 2,2-Azobis (2-amidinopropane) dihydrochloride (AAPH) were obtained from Sigma-Aldrich. Fetal bovine serum (FBS) was purchased from GE Healthcare Bio-Sciences (Piscataway, NJ, USA). Raw murine macrophages (RAW 264.7) were obtained from National Platform of Experimental Cell Resources for Sci-Tech (Beijing, China). All other chemicals and solvents used were of analytical grade unless otherwise specified.

2.2. Extraction of the polysaccharides

The seeds of the mung bean were ground into powder. The powder passing through a 60 mesh sieves was extracted with 95% ethanol for 3d and then centrifuged at 3000 rpm for 10 min. The precipitation was collected and extracted with distilled water at 90 °C for 4 h twice. After centrifuging (3500 rpm, 10 min), supernatant was collected and concentrated. The concentrated solution was dialyzed and deproteinated by the Sevag method (Wang, Yang, Yu, Yao, & Ren, 2013). Then, the water-soluble polysaccharide (MWP) was obtained.

2.3. Ultrasonic pretreatment

Ultrasonic treatment of 20 kHz, 700 W and pulsed at 10 s/3 s (on/off) was carried outusing a DEPU92-II ultrasonic reactor (Wuxi DePu Instrument Manufacturing Co., LTD). MWP solution was placed into a reaction vessel and kept at a stable temperature at 0 °C, and the ultrasonic probe dipped into the solution to about 20 mm. Thus, the solution of ultrasonic degraded MWP′ was obtained.

2.4. Separation, and purification of the polysaccharides

MWP′ was purified using ÄKTA explore 100 purification systems. Briefly, the sample was dissolved in distilled water and then centrifuged (13,000 rpm, 20 min), subsequently, the supernatant was loaded on a DEAE Sepharose Fast Flow column (2.6 × 100 cm) equilibrated with ultra-pure water. The column was first eluted with distilled water, then with a linear gradient from 0 to 2.0 M NaCl at a flow rate of 4 mL/min. Different fractions (8 mL/tube) were collected using an automatic fraction collector, then dialyzed and lyophilized. The fractions were purified further on a Sephacryl S-300 High Resolution column (1.6 × 100 cm) eluted with 0.15 M NaCl at a flow rate of 0.5 mL/min to yield two main final fractions, named MWP′-1 and MWP′-2. The fractions obtained were combined according to the total carbohydrate content quantified by the phenol-sulfuric acid method under 206 nm UV detection (Dini, Tenore, & Dini, 2009).

2.5. Infrared of the spectrum analysis

Fourier transform infrared (FT-IR) spectra were obtained using a PerkinElmer FT-IR spectrometer (Massachusetts, USA) in the range of 4000–500 cm^{-1}.

2.6. Analysis of the molecular weight

The molecular weight of MWP-1′ and MWP-2′ were measured using a high performance size elusion chromatography coupled with a multi angle laser light scattering and refractive index (HPSEC–MALLS–RID) system, which consisted of a pump (LC-20AD, Shimadzu, Kyoto, Japan), a HPSEC column (SB-805 HQ, Shodex, Kyoto, Japan), a MALLS detector (DAWN HELEOS-II, Wyatt Technology, Santa Barbara, CA, USA), and a RI detector (Optilab Rex, Wyatt Technology, Santa Barbara, CA, USA). MWP-1′ and MWP-2′ were filtered on a 0.45 μm pore membranes before injection (200 μL) and eluted with 0.1 M NaCl (0.5 mL/min). The column temperature was kept at 40 °C.

2.7. Analysis of monosaccharide composition

Gas chromatography (GC) was used for identification and quantification of monosaccharide compositions. MWP-1′ and MWP-2′ were hydrolyzed by trifluoroacetic acid (2 M) at 120 °C for 4 h. The released monosaccharides were converted into the trimethylsilylated derivatives and then analyzed using a GC on an Agilent 6890 instrument (Agilent Technologies, USA) equipped with HP-5MS column (0.25 mm × 30 m × 0.25 μm) and determined by flame ionization detector (FID). The column temperatures and other parameters were set according to the previous method (Beretta, Granata, Ferrero, Orioli, & Maffei Facino, 2005).

2.8. Assay for immunomodulatory activity

Immunomodulatory activity was performed by a previously reported method (Methacanon, Madla, Kirtikara, & Prasitsil, 2005). Briefly, cells were incubated in medium alone (control group) or medium containing various concentrations of polysaccharides fractions (50, 100, and 200 μg/mL) or lipopolysaccharide (LPS, 1 μg/mL) as a positive control. The cells were incubated at 37 °C in 5% CO_2 for 24 h, and then the supernatants (50 μL) were pipetted from the medium and mixed with an equal volume of Griess reagent. After incubation for 15 min at room temperature, the absorbance was measured at 540 nm in a Spectrum microplate reader (Max plus 384, Molecular Devices, California, USA) (Wang et al., 2013). Nitrite concentrations were estimated from a sodium nitrite standard calibration curve (0–100 μM). The supernatants were also collected for the detection of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) production using a commercial ELISA kit (BD Biosciences Pharmingen, San Diego, USA) according to the instructions of these kits. The absorbance was measured at 450 nm and 570 nm using an ELISA reader. Cytokine quantities in the samples were calculated from standard curves of recombinant cytokines using a linear regression method.

2.9. Statistics

Data, which were expressed as the mean ± SD, included at least three replicates per sample. ANOVA and Tukey's test were performed using SPSS (Statistics for Social Science) version 17.0. All graphical representations were performed using Sigmaplot version 11.0 (SPSS, USA). Statistical significance was established at $p < 0.05$.

3. Results and discussion

3.1. Extraction and purification of the polysaccharide

MWP was isolated from seeds of mung bean with the yield of approximately 3.25%. After ultrasonic treatment, MWP′ was purified on a DEAE Sepharose Fast Flow column to obtain the water-eluted and salt-eluted fractions, accounting for 50.92% and 44.08%

Download English Version:

https://daneshyari.com/en/article/1374823

Download Persian Version:

https://daneshyari.com/article/1374823

Daneshyari.com