International Journal of Biological Macromolecules 84 (2016) 289–294

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Antioxidant and immunoregulatory activity of alkali-extractable polysaccharides from mung bean

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

[a] Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing 100081, PR China
[b] Animal Science Unit, Gembloux Agro-Bio Tech, University of Liège, Passage des Déportés 2, 5030 Gembloux, Belgium

ARTICLE INFO

Article history:
Received 7 October 2015
Received in revised form
30 November 2015
Accepted 14 December 2015
Available online 19 December 2015

Keywords:
Mung bean
Alkali-extractable polysaccharide
Antioxidant

ABSTRACT

Alkali-extractable polysaccharides from the seeds of mung beans and two polysaccharide sub-fractions (MAP-1 and MAP-2) were isolated and purified by anion-exchange and gel filtration chromatography. The average molecular weights (Mws) of MAP-1 and MAP-2 were 94.2 kDa and 60.4 kDa, respectively. Monosaccharide component analysis indicated that MAP-1 was composed of Rha, Ara, Glu, Gal, and GalA in a molar ratio of 1.1:0.4:0.7:0.5:0.3. MAP-2 consisted of Xyl, Rha, Gal, Glu and GalA with a relative molar ratio of 0.4:1.4:1.6:0.5:0.2. Antioxidant assays indicated that both MAP-1 and MAP-2 exhibit significant antioxidant activity in a dose-dependent manner. An *in vitro* study further showed that MAP-1 and MAP-2 were both able to stimulate the production of secretory molecules (NO, TNF-α and IL-6) by RAW 264.7 murine macrophages in a concentration-dependent manner. These findings suggest that the polysaccharides isolated in our study have immunoregulatory effects on macrophages and can be used as a beneficial health food.

1. Introduction

Interest in polysaccharides from natural sources has been growing in recent years due to their potential biological functions, especially antioxidant and immunomodulatory activities, such as inhibiting lipid oxidation, scavenging free radicals, promoting natural killer cell (NK) cytotoxicity, activating macrophages and potentiating interleukins [1–3]. The bioactivity of polysaccharides mainly depends on several structural parameters, including molecular weight, sugar composition, type of glycosidic bond, and degree of sulfation [4]. In view of their potential application in functional foods and medicine, more and more studies have been focused on the isolation and purification of polysaccharides from numerous plants, animals and microorganisms [5].

Many studies have proved that polysaccharides in mung beans show strong antioxidant activity [6,7]. However, most of the reported polysaccharides were prepared from water extracts of mung beans. In addition, after water extraction, the residue was usually abandoned. There are still some components in the residue that are of great use, such as alkali-extractable polysaccharides. As far as we know, there are no reports about the extraction and purification of alkali-extractable polysaccharides from mung beans. In this work, we isolated alkali-extractable polysaccharides from mung beans, identified their chemical characteristics, and determined their antioxidant and immunomodulatory effects.

2. Materials and methods

2.1. Materials and reagents

Mung bean seeds were purchased from Hebei (China). DEAE Sepharose Fast Flow and Sephacryl S-300 High Resolution were obtained from GE Healthcare Bio-Sciences Co. (Piscataway, NJ, USA). Lipopolysaccharide (LPS), DMEM, 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), Trolox, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, RPMI 1640 medium, Griess reagent, rhamnos (Rha), arabinose (Ara), xylose (Xyl), galactose (Gal), galacturonic acid (GalA) and glucose (Glu) monosaccharide standards were purchased from Sigma–Aldrich. Raw murine macrophages (RAW 264.7) were purchased from National Platform of Experimental Cell Resources for Sci-Tech (Beijing, China). Fetal bovine serum (FBS) was obtained from GE Healthcare Bio-Sciences (Piscataway, NJ, USA). All other chemicals and solvents used were of analytical grade unless otherwise specified.

* Corresponding author at: Institute of Crop Science, Chinese Academy of Agricultural Sciences, No. 80 South Xueyuan Road, Haidian District, Beijing 100081, PR China. Fax: +86 10 6215 6596.
E-mail address: renguixing@caas.cn (G. Ren).

http://dx.doi.org/10.1016/j.ijbiomac.2015.12.045
0141-8130/

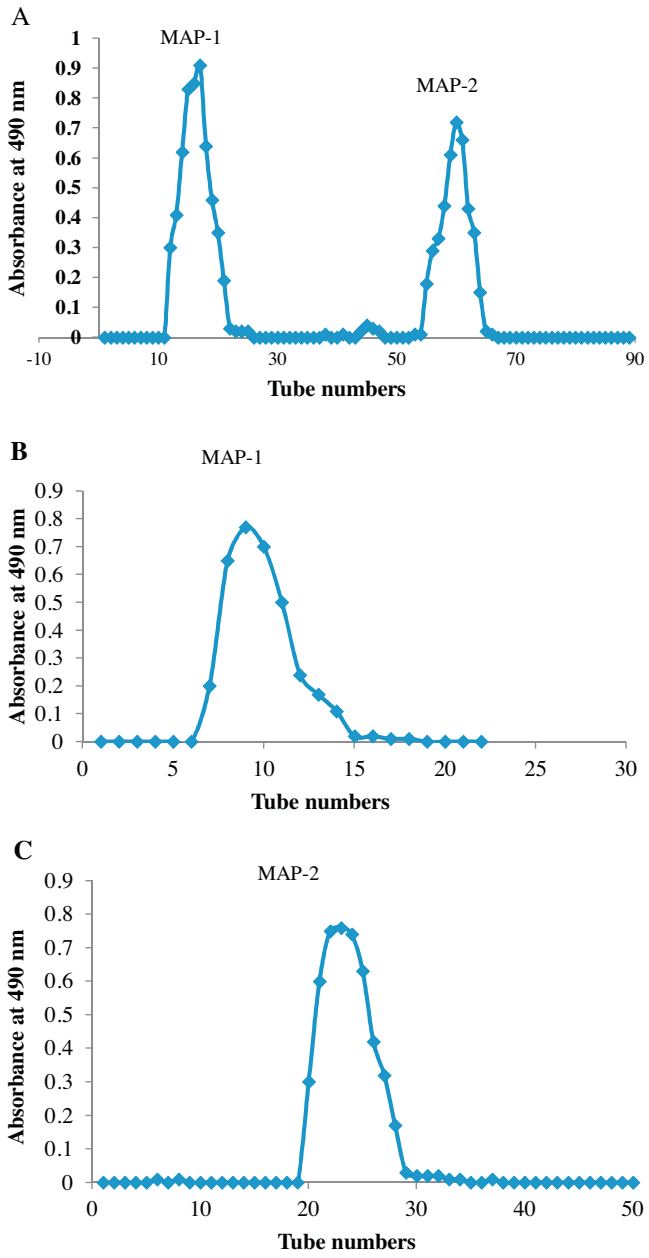

Fig. 1. (A) Elution curve of MAP on Sepharose Flast Flow column. Elution profiles of (B) MAP-1 and (C) MAP-2 on Sephacryl S-300 column, respectively.

2.2. Extraction, separation, and purification of polysaccharides

Seeds of mung beans were ground into powder. The powder was passed through a 60 mesh sieve and extracted with 95% ethanol for 3 days and distilled water at 90 °C for 4 h twice. Then, the water-unextractable solid was washed, dried and extracted with a 0.3 M NaOH solution that contained 0.3% (w/w) $NaBH_4$ at room temperature for 4 h. After centrifugation at 3500 × *g* for 15 min, the supernatant was collected and adjusted to neutrality with hydrochloric acid (5 M) and then concentrated, dialyzed, and deproteinated by the Sevag method [8]. The supernatant containing alkali-extractable polysaccharides was precipitated with ethanol at 4 °C for 24 h and then centrifuged and lyophilized to obtain MAP.

The MAP was dissolved in distilled water, centrifuged (13,000 rpm, 20 min), and then the supernatant was loaded on an ÄKTA explore purification system with a DEAE Sepharose Fast Flow column (2.6 × 30 cm) equilibrated with ultrapure 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 × 80 cm) and eluted with 0.15 M NaCl at a flow rate of 0.5 mL/min to yield two main final fractions named MAP-1 and MAP-2, respectively. The fractions obtained were combined according to the total carbohydrate content as quantified by the phenol–sulfuric acid method under 206 nm UV detection [9].

2.3. Chemical composition analysis

The carbohydrate content was measured by the phenol–sulfuric acid method using D-glucose as the standard [10]. The uronic acid content was assessed by the meta-hydroxydiphenyl method using glucuronic acid as the standard [11].

2.4. Infrared spectrum analysis

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

2.5. Analysis of molecular weight

The molecular weight of the samples was measured using a high performance size exclusion chromatography system 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-805HQ, 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). The samples were filtered through a 0.45 μm pore membrane before injection (200 μL) and eluted with 0.1 M NaCl (0.5 mL/min). The column temperature was kept at 40 °C.

2.6. Analysis of monosaccharide composition

Gas chromatography (GC) was used for identification and quantification of monosaccharide components. MAP-1 and MAP-2 were hydrolysed with trifluoroacetic acid (2 M) at 120 °C for 4 h. The released monosaccharides were converted into trimethylated derivatives, then analysed by 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 a flame ionization detector (FID). The column temperature and other parameters were set according to a previous method [12].

2.7. Evaluation of antioxidant activity

DPPH radical-scavenging activity was determined using the method reported by Yao and Ren [13]. MAP-1 and MAP-2 were dissolved in ethanol at a ratio of 1:3. The DPPH solution (1 mL) was mixed with 1 mL of the polysaccharide solution. The mixture was shaken and allowed to stand at room temperature in the dark for 10 min. The decrease in absorbance of the resulting solution was monitored at 517 nm after 10 min. The results were corrected for dilution and expressed in micromolar Trolox equivalents (TE) per 100 g of dw.

ORAC activity was determined as described previously with some slight modifications [14]. Fifty microlitres of the polysaccharide solution was mixed with 50 μL of fluorescein solution in a 96-well microplate, and then 150 μL of AAPH was added to each well rapidly. To build a blank decay curve and a Trolox standard