International Journal of Biological Macromolecules 111 (2018) 862–869

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: https://www.journals.elsevier.com/biomac

Structural characterization and antioxidant activity of polysaccharide from ginger

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ARTICLE INFO

Article history:
Received 5 December 2017
Received in revised form 4 January 2018
Accepted 13 January 2018
Available online xxxx

Keywords:
ginger polysaccharide
antioxidant activity
structural characterization

ABSTRACT

Two components ginger polysaccharide 1 (GP1) and ginger polysaccharide 2 (GP2) were extracted. The results showed that the molecular weights of GP1 and GP2 were 6128 Da and 12,619 Da, respectively. The composition and proportion of GP1 and GP2 were mannose, glucose and galactose in a molar ratio of 4.96: 92.24: 2.80 and arabinose, mannose, glucose and galactose in a molar ratio of 4.78: 16.70: 61.77: 16.75, respectively, illustrating that GP1 and GP2 were not a kind of homopolysaccharide. GP1 has a three-helix structure, and the structure is closely linked. GP2 contains sulfuric acid groups, and has a high oxidation resistance, its structure is more evacuated and messy.

1. Introduction

Ginger is a fresh rhizome of *Zingiber officinale Rosc*. As a perennial herb, a traditional medicinal and edible plant, it is also a natural raw material for ancient spices and cosmetic industries. It is rich in various active ingredients such as ginger polyphenol, curcumin, gingerol, ginger essential oil and ginger polysaccharide [1]. Pharmacological research shows that ginger has extensive pharmacological activities, such as antioxidant [2], antitumor [3], antibacterial [4], anti-inflammatory [5], lower cholesterol [6] and platelet aggregation [7]. The research covers in-vivo, in-vitro and epidemiological investigation and so on [8].

Several research teams have made some progress in extraction, purification and structural characterization of ginger polysaccharide. Zhang et al. [9] compared the antioxidant properties of ginger polysaccharide by three extractions, and determined the monosaccharide composition of mixed polysaccharide. Ma et al. [10] determined the monosaccharide composition of mixed polysaccharides by thin layer chromatography. Feng et al. [11] purified three polysaccharides from the ginger skin, and determined some structural characterization. However, to our knowledge, there is a lack of available information concerning the separation and purification, detailed structural elucidation, conformation and structural-activity correlation of polysaccharides from peeled ginger.

Therefore, the purpose of this work is to isolate and purify polysaccharides, explore the structural features of the purified polysaccharide fraction, and reveal the relationship between polysaccharide structures and their functions. In this study, two fragments (GP1 and GP2) of the ginger polysaccharide were purified by DEAE-52 cellulose chromatography and Sephadex G-200 chromatography after using Sevag method combined with microporous resin to remove the protein and pigment from ginger polysaccharide. The structures of GP1 and GP2 were determined using Fourier transform-infrared spectrometry (FT-IR), gas chromatography–mass spectrometry (GC–MS), nuclear magnetic resonance (NMR), gel permeation chromatography, colorimetric determination with Congo red, and transmission electron microscope (SEM) analysis. The -in-vitro antioxidant properties and the structural-activity correlation were also analyzed. This strategy provides a promising application for novel functional foods or drugs using polysaccharides as ingredient.

2. Materials and method

2.1. Materials and reagents

The fresh ginger was obtained from Anqiu City, China. DEAE-cellulose, Sephadex G-200, S-8 macroporous resin, bovine serum albumin (BSA), and dialysis bag (3500 Da) were purchased from Solarbio (Beijing, China), series of standard Dextrans of known MW (615, 1480,

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https://doi.org/10.1016/j.ijbiomac.2018.01.087
0141-8130/

3870, 8160, 16,100, 21,160 Da) and standard monosaccharides of D-glucose (Glc), D-galactose (Gal), D-arabinose (Ara), L-rhamnose (Rha), D-mannose (Man), D-xylose (Xyl) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals and reagents were of analytical grade.

2.2. Extraction and purification of GPS

The polysaccharides were extracted following the complex-enzyme-hydrolysis extraction method [12]. The crude polysaccharides (GPS) were dissolved in distilled water, and then the protein was removed using Sevag method [13] for 3 times. The supernatant was concentrated in a rotary evaporator under vacuum at 55 °C and filtered. According to the experimental results of the laboratory, the supernatant was injected to a column (1.6 × 50 cm) of S-8 macroporous resin, using a method similar to the extraction procedure of Lu et al. [14]. Eluate (5.0 mL/tube) was collected by automatic collector, and the carbohydrates and proteins were monitored by the anthrone colorimetry and coomassie brilliant blue method. The eluate was combined, concentrated, dialyzed, and then the supernatant was injected to a column (1.6 × 50 cm) of DEAE-52 cellulose equilibrated with distilled water. After loading the sample, the column was eluted with 0.1–1.0 mol/L NaCl solution for linear gradient elution, at a flow rate of 1 mL/min. Eluate (4.0 mL/tube) was collected by automatic collector and the carbohydrates were monitored by the anthrone colorimetric method [15].

2.3. Characterization of GP1 and GP2

2.3.1. UV–vis spectroscopic analysis

GP1 and GP2 polysaccharides were dissolved in distilled water, and then the UV–vis absorption spectra was recorded in the wavelength range of 190–700 nm using a UV-5600 Ultraviolet–visible (UV–vis) spectrometer (Shanghai Metash Instruments Co., Ltd., Shanghai, China).

2.3.2. FT-IR spectroscopic analysis

The FT-IR spectrum of the polysaccharides was determined using a Fourier transform infrared spectrophotometer (FT-IR) (Spectrum one FT-IR, PerkinElmer Co., USA). Polysaccharide samples were ground with KBr powder, and then pressed into pellets for FT-IR determination in the frequency range of 4000–400 cm^{-1}.

2.3.3. Monosaccharide composition analysis

Five milligrams of GP1 and GP2were hydrolyzed with 3 M H_2SO_4 at 110 °C for 8 h. After completely removing the excessive H_2SO_4, the resultant monosaccharides were converted into alditol acetates as described previously [16] and then analyzed by GC.

2.3.4. Determination of molecular weight

The molecular weight of GP1 and GP2 was determined by high performance gel permeation chromatography (HPGPC) (Malvern, TDA MAX270, UK). The HPGPC conditions were followed the method of Meng et al. [17].

2.3.5. NMR analysis

GP1 and GP2 were dried in a vacuum over P_2O_5 for 3 days, and then 20 mg of sample was dissolved in 2.0 mL of D_2O. ^1H NMR and ^{13}C NMR spectrum recorded by AVANCE III 400 MHz NMR spectrometer (Bruker Corporation, Switzerland). Chemical shifts were given in ppm.

2.3.6. Surface microscopic analysis

The molecular architecture of GP1 and GP2 in solution were observed on a field-emission scanning electron microscopy (Hitachi, SU1080, Japan) under a high vacuum condition. The dried sample powder was mounted on an aluminum stub with doublesided adhesive tapes and sputtered with a thin gold film.

2.3.7. Triple-helical structure determination with Congo red

One milliliter of 2 mg/mL sample solutions were mixed with 1 mL of NaOH solutions (0, 0.2, 0.4, 0.6, 0.8, 1 M), respectively. This step made the final concentration of NaOH solution to 0, 0.1, 0.2, 0.3, 0.4, 0.5 M. Then 2 mL of 80 μmol Congo red were added and shaken vigorously. The alkaline solution without NaOH was used as the reference. After being incubated at room temperature for 1 h, the visible absorption spectra of GP1 and GP2 mixture were performed on a UV-5600.

2.3.8. Assay of DPPH radical scavenging activity

DPPH radical scavenging activity was determined using the reported method [18], 2.0 mL of 0.1–1.0 mg/mL sample solutions and 3.0 mL of 0.08 M DPPH solutions (dissolved in ethanol) were mixed and shaken vigorously. After being incubated in the dark at room temperature for 30 min, the absorbance of reaction mixture at 517 nm was measured against a blank (distilled water). Vitamin C was used as a positive control. DPPH radical scavenging activity was calculated using the following equation:

$$\text{Scavenging activity}\ (\%) = [A_0 - (A_1 - A_2)]/A_0 \times 100\%$$

2.3.9. Statistical analysis

Values were expressed as means ± standard deviation. Differences in mean values between groups were analyzed by student's *t*-test using SPSS statistical software (version 16.0 for Windows, SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Isolation and purification of polysaccharide

The crude polysaccharide was purified on a S-8 macroporous resin column, which could improve the purity and quality of the polysaccharide. As demonstrated in Fig. 1, polysaccharide and protein were finally obtained. The carbohydrate content and proteins were monitored by the anthrone colorimetry and coomassie brilliant blue method. The UV–vis absorption spectra of crude polysaccharide and purification were recorded using the wavelength range of 190–700 nm as shown in Fig. 2, compared with the crude polysaccharides, purification procedure removed most of the impurities between 240 nm and 350 nm, such as proteins, pigment and nucleic acids, because free proteins in the crude polysaccharides had been removed by Sevag method and S-8 macroporous resin method [19, 20].

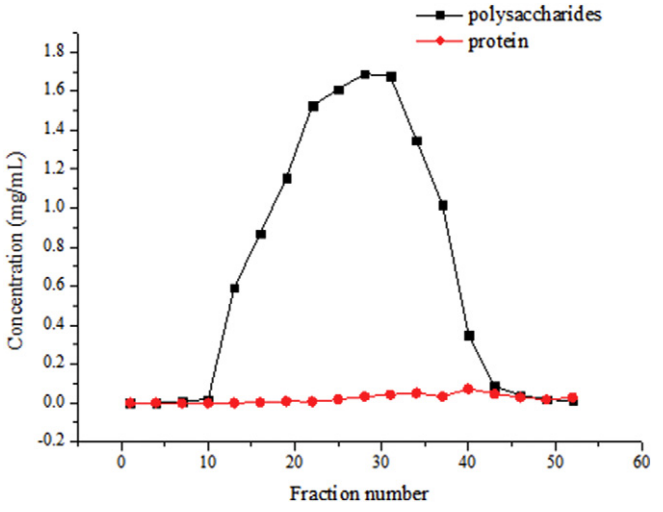

Fig. 1. Dynamic elution curve on of the ginger crude polysaccharides S-8 macroporous resin.