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Structural identification of ginseng polysaccharides and testing of their antioxidant activities

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Abstract

A large number of polysaccharides were presented in boiling-water extraction of ginseng. A DEAE-Sepharose CL-6B column chromatography was used to isolate the major polysaccharides from ginseng. Two fractions were obtained, named GPII and GPIII. Both GPII and GPIII were a neutral polysaccharide and a single peak in HPLC with Sugar KS-804 column, with a molecular weight of 3×10^5 and 4×10^5, respectively, comprised mainly of glucose. Analysis by Periodate oxidation–Smith degradation indicated that GP was composed of 60.06% (1→)- or (1 → 6)-glycosidic linkages and 39.94% (1 → 3)-glycosidic linkages, and GPIII 16.23% (1→)- or (1 → 6)-glycosidic linkages, 25.98% (1 → 2)-glycosidic linkages, and 57.79% (1 → 3)-glycosidic linkages. On the basis of superoxide radical assay, hydroxyl radical assay and self-oxidation of 1,2,3-phentriol assay, their antioxidant activities were investigated. GPII exhibited equivalent inhibiting power for self-oxidation of 1,2,3-phentriol to Vc, a little higher scavenging activity of superoxide radical and hydroxyl radical than Vc, and should be explored as a novel potential antioxidant.

Keywords: Ginseng; Purification; Periodate oxidation–Smith degradation; Antioxidant activity

1. Introduction

Oxidation is essential to many organisms for the production of energy to fuel biological processes. However, the uncontrolled production of oxygen derived free radicals is involved in onset of many diseases such as cancer, rheumatoid arthritis, and atherosclerosis as well as in degenerative processes associated with aging (Mau, Lin, & Song, 2002). In order to reduce damage to the human body, synthetic antioxidants are used for industrial processing at the present time. However, the most commonly have been suspected of being responsible for liver damage and carcinogenesis (Grice, 1988; Qi et al., 2005). Thus, it is essential to develop and utilize effective and natural antioxidants so that they can protect the human body from free radicals and retard the progress of many chronic diseases (Kinsella, Frankel, German, & Kanner, 1993; Nandita & Rajini, 2004). Published data indicate that plant polysaccharides in general have strong antioxidant activities and can be explored as novel potential antioxidants (Hu, Xu, & Hu, 2003; Jiang, Jiang, Wang, & Hu, 2005; Ramarahnam, Osawa, Ochi, & Kawaishi, 1995).

Ginseng (*Panax ginseng* C.A. Meyer, ginseng) has been the most precious and renowned tonic drug in traditional Chinese medicine. To date, no investigation has been carried out on polysaccharides that may account for antioxidant activities of ginseng. Identification of the polysaccharides is necessary to better effectively exploit the structure and functional properties of these substances. In this study, we report on the extraction and purification of the major polysaccharides of ginseng using a DEAE-Sepharose CL-6B column chromatography. In addition, the structure properties and antioxidant activities of these major polysaccharides are also identified.

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2. Materials and methods

2.1. Materials and chemicals

Dried underground part of ginseng was purchased from a local drugstore (Quanzhou, Fujian Province, China). Nitro blue tetrazolium (NBT), phenazine methosulfate (PMS), dihydronicotineamidadenine dinucleotide (NADH), thiobarbituric acid (TBA), deoxyribose, L-rhamnose, D-glucose, D-arabinose, D-xylose, D-fructose, D-galactose and D-mannose were purchased from Sigma Chemical Co. (St. Louis, MO, USA), while DEAE-Sepharose CL-6B was from the Pharmacia Co. (Sweden). All other reagents used were of analytical grade.

2.2. Isolation and purification of polysaccharide

The ginseng (250 g) was extracted with 80% ethanol at 50 °C for 6 h, dried, and then extracted with distilled water at 80 °C for 2 h twice. After each extraction, the soluble polymers were separated from residues by filtration, and extracts were combined, concentrated and dialyzed against running water for 48 h. The above extract was submitted to graded precipitation with four volumes of ethanol and the mixture was kept overnight at 4 °C to precipitate the polysaccharides. The precipitate was collected by centrifugation, washed successively with ethanol and a ether, and dried at reduced pressure, giving a crude polysaccharide (GP). Size-exclusion and anion-exchange chromatography were used for the fractionation of this preparation. GP (500 mg) was dissolved in 10 mL distilled water, centrifuged, and then the supernatant was injected to a column (4.6×30 cm) of DEAE-Sepharose CL-6B equilibrated with distilled water. After loading with sample, the column was eluted with distilled water for 1000 mL at 4 mL/6 min, followed stepwise by NaCl aqueous solution (0 and 1 M) for 400 mL respectively at 40 mL/h. The major polysaccharide fractions were collected with a fraction collector, dialyzed against tap water and distilled water for 48 h, respectively.

2.3. Monosaccharide composition and properties

Total carbohydrate and protein of these polysaccharides were determined by the phenol–sulfuric acid (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) and (Bradford, 1976), respectively. Paper chromatography (PC, Wang, Luo, & Liang, 2004) and gas chromatography (GC) were used for identification and quantification. GC was performed on a 6890N instrument (Angilent, USA) with a column HP-5 ($30\ m \times 0.32\ mm \times 0.25\ \mu m$). First, the polysaccharide (10 mg) was dissolved in 10 ml of a 2-M TFA and hydrolyzed at 120 °C for 6 h, and then hydrolyzed products were evicted TFA by ethanol and dried. Derivation was then carried out using the trimethylsilylation reagent according to the method of Guentas et al. (2001) with some modifications (Wang & Luo, 2007).

The IR spectrum of the polysaccharide was determined using a Fourier transform infrared spectrophotometer (FTIR, Bruker, Germany) equipped. The purified polysaccharide was ground with KBr powder and then pressed into pellets for FTIR measurement in the frequency range of 4000 to 500 cm^{-1} (Kumar, Joo, Choi, Koo, & Chang, 2004).

2.4. Determination of the molecular weight

The molecular weight of the fractions was determined by gel-permeation chromatography, in combination with a high-performance liquid chromatography instrument (Angilent1100, USA). Sample (2.0 mg) was dissolved in distilled water (2 mL) and passed through a 0.45-μm filter, applied to a gel-filtration chromatographic column of Shodex Sugar KS-804 (SHOWA DENKO K.K, Japan), maintained at a temperature of 50 °C, eluted with the distilled water at a flow rate of 1.0 mL/min and detected by a refractive index detector. Preliminary calibration of the column was conducted using dextrans of different molecular weight (Dextran Blue, Dextran T10, T40, T70, T500 and Glucose). The molecular weight was calculated by the calibration curve obtained by using various standard dextrans (Wang, Liang, & Zhang, 2001).

2.5. Periodate oxidation–Smith degradation

For analytical purpose, 50 mg of sample was dissolved in 25 ml of distilled water and 25 ml of 30 mmol/L $NaIO_4$ were added. The solution was kept in the dark at RT, 0.1 ml aliquots were withdrawn at 6 h intervals, diluted to 25 ml with distilled water and read in a spectrophotometer at 223 nm. Glycol (2 ml) was added, and then the experiment of periodate oxidation was over. The solution of periodate product (2 ml) was sampled to calculate the yield of formic acid by 0.005 M sodium hydroxide, and the rest was extensively dialyzed against tap water and distilled water for 24 h, respectively. The content inside was concentrated and reduced with sodium borohydride (160 mg), and the mixture was left for 24 h at room temperature, neutralized to pH 6.0 with 50% acetic acid, dialyzed as described above, and concentrated to a volume (10 ml). One-third of solution described above was freeze-dried and analyzed with GC. Others were added to the same volume of 1 M sulfuric acid, kept for 40 h at 25, neutralized to pH 6.0 with barium carbonate, and filtered. The filtrate was dialyzed as foresaid, and the content out of sack was desiccated for GC analysis; the content inside was diluted with ethanol, and after centrifugation, the supernatant and precipitate were also dried out for the GC analysis.

2.6. Assay for antioxidant activity

2.6.1. Hydroxyl radical assay

The hydroxyl radical assay was measured by the method of Ghiselli, Nardini, Baldi, and Scaccini (1998) with a