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Extraction, characterization and antioxidant activities of Se-enriched tea polysaccharides

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ABSTRACT

Se-polysaccharides from Se-enriched tea leaves were purified by DEAE–sepharose fast flow gel column (2.5 × 60 cm) and three polysaccharide fractions (Se-TPS1, Se-TPS2, and Se-TPS3) were isolated and purified with yields of 6.5, 37.14, and 8.57%, respectively. The average sizes of Se-TPS1 and Se-TPS2 were determined by HPGPC system, with molecular weights of 1.1×10^5 and 2.4×10^5 Da, respectively. Se-TPS3 was a polysaccharide polymer with two peaks with molecular weights of 9.2×10^5 and 2.5×10^5 Da. Monosaccharide components analysis by ion chromatography revealed Se-polysaccharides were acidic polysaccharoses and different from each other in monosaccharide kinds and molar ratio. Elements of Se, C, H, N, S, and 14 kinds of mineral elements were analyzed by AFS, EA, and ICP-AES, respectively. Spectral analysis (IR and UV) indicated Se-polysaccharides were typical glycoproteins. Morphological analyses of the samples were determined by SEM and AFM. In addition, the DPPH and superoxide radicals scavenging activities were also discussed to assess antioxidant activities of the samples, and Se-polysaccharides showed higher antioxidant activities compared to the ordinary polysaccharides.

1. Introduction

Se, an essential trace element for human health, has received considerable attention due to its special qualities, such as antioxidant function [1], insulin-mimetic activity [2], enhancement of body's immune [3,4], as well as inhibition of cancer and chromosome damage [5,6]. Se is also a necessary element for nutrition in the human biology. This element is a cofactor of a mass of selenium-dependent enzymes such as an antioxidant enzyme, which is involved in cellular protection from severe oxidation by free radicals [7]. The bioavailability of Se is affected by its chemical form (generally, organic compounds of Se are more bioavailable than the inorganic forms). For example, Se is a component of several selenoproteins, such as glutathione peroxidases (GSHPx), thioredoxin reductase (TRx), iodothyronine deiodinases, selenophosphate synthetase, selenoprotein P, and other selenoproteins [8,9].

The activity of polysaccharide is mainly related with sulfated group, glycosidic bond- and ion-complexing. However, Se-polysaccharide is different from the ordinary polysaccharide because of its unique bond of Se oxygen. Thus, Se-polysaccharide possesses more advantages [10–15]. He found that Ziyang Se-rich tea polysaccharide had an inhibitory effect on the human breast cancer cells MCF-7 [10]. What is more, Wang also reported that a Se-polysaccharide from Ziyang green tea had tumoricidal effects on human osteosarcoma U-2 OS cells [11]. Liu Y. T. has studied that mycelia Se-polysaccharide from *Catathelasma ventricosum* had antidiabetic activity in STZ-induced diabetic mice [12]. To the best of our knowledge, there is little work on purified Se-enriched tea polysaccharides. In this work, the crude Se-enriched tea polysaccharide was purified and isolated to Se-TPS1, Se-TPS2, and Se-TPS3. The molecular weights, chemical compositions, and morphological characteristics of the samples were investigated. Furthermore, the DPPH radical scavenging activity and scavenging effect on superoxide radicals of the samples were also discussed.

2. Materials and Methods

2.1. Chemicals and reagents

Se-enriched tea leaves were purchased from Enshi, Hubei province of China. Dialysis bags (3500D) and bovine serum albumin (BSA) were obtained from Solarbio Co. Ltd. (Shanghai, China). Galacturonic acid was bought from Sigma (MO, USA). DEAE–Sepharose FF was purchased from GE Co. (USA). Coomassie brilliant blue G-250 and glucose were obtained from Sinopharm Chemical Reagent Co. (Shanghai, China). Macroporous adsorption resin (ANP 16) was

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acquired from Shanghai East China University of Science and Technology. All other chemicals and solvents were of analytical grade and used without further purification, unless otherwise noted.

2.2. Preparation of the Se-enriched tea polysaccharides

The Se-enriched tea polysaccharides were obtained after twice extracting with distilled water (ratio of solid to liquid = 1:10, 1:8) and precipitating by 75% ethanol (concentrated solution: pure ethanol = 1:3). The precipitation was then dissolved with distilled water. Subsequently, the solution was concentrated in a rotary evaporator, followed by 2-d dialysis (with size of 3.5 kDa) to remove low molecular weight compounds. After lyophilization, crude Se-enriched tea polysaccharides were produced.

2.3. Isolation and purification of the Se-enriched tea polysaccharides

The crude Se-enriched tea polysaccharides were re-dissolved in distilled water, and then the solution was decolorized with dynamic adsorption of macroporous adsorption resin (ANP l6). Proteins in the extract were removed by using trichloroacetic acid. Then, the crude Se-enriched tea polysaccharide was dissolved in 0.02 M phosphate buffer solution (pH 6.0). After centrifugation (5000 rpm, 10 min), the solution was filtered through a filter paper (0.22 μm) and then was passed through a DEAE–sepharose fast flow gel column (2.5 × 60 cm). The Se-polysaccharides were eluted with phosphate buffer solution, followed by elution using a linear gradient (420 ml) from 0 to 0.3 M NaCl at a flow rate of 3 ml/min. After drawing curves based on ultraviolet absorption value and number of elution tubes, fractions of ultraviolet absorption value more than 0.3 were collected and monitored by the phenol–sulfuric acid method at 486 nm. Lastly, fractions containing Se-polysaccharides were pooled, dialyzed and lyophilized to give Se-NTPS, Se-TPS1, Se-TPS2, and Se-TPS3. The scheme of separation procedure is shown in Fig. 1.

2.4. Determination of molecular weight-HPGPC

Molecular weights of Se-enriched tea polysaccharides were evaluated by high-performance gel permeation chromatography (HPGPC) with a refractive index detector (RI2000, Schambeck SFD GmbH, Germany), which was carried out at a flow rate of 0.5 ml/min under the column temperature of 30 °C and detector temperature of 35 °C, respectively. Samples (5 mg) were dissolved in 0.5 ml of 0.02 M phosphate buffer solution and centrifuged for 10 min at a speed of 10,000 r/min to obtain the supernatant, then 20 μl supernatant was injected for HPGPC analysis. The molecular weight was calculated by the calibration curve obtained by using various standard dextrans with different molecular weights (T3, T6, T10, T40, T100, T500, and T1000).

2.5. Analysis of monosaccharide composition

The monosaccharide composition of Se-enriched tea polysaccharides was identified and quantified by ion chromatography (IC), performed on a Dionex ICS2500 chromatographic system (CA, USA) with an efficient anion exchange column of Dionex Carbopac PA20 column (150 mm × 3 mm) and a Dionex pulsed amperometric detector equipped with an Au electrode. The detailed procedure was as follows: the sample (10 mg) was dissolved in 4 ml distilled water, and 0.9 ml trifluoroacetic acid (TFA) was added into the solution after it dissolved completely, following which the tube was sealed under nitrogen atmosphere. The mixture was hydrolyzed at 120 °C for 6 h and centrifugated at 5000 rpm for 10 min. The hydrolysates of Se-TPS were evaporated to dry under reduced pressure. Then, TFA was absolutely removed by washing with methanol. The dried hydrolysates were dissolved with 1 ml deionized water and measured by diluting 10-fold. The temperature was kept at 30 °C and the injection volume was 25 μl. NaOH solution (2 mM) were used as eluents at a flow rate of 0.45 ml/min. Fucose, rhamnose, arabinose, galactose, glucose, xylose, mannose, fructose, ribose, glucuronic acid, and galacturonic acid, were used as references.

2.6. Elemental analysis

Elemental analyses of C, H, N, and S, were performed by a Vario EL-III instrument (Elementar Analysensysteme GmbH). Generally, 3–5 mg samples were wrapped in foil bags and burned in catalytic oxidation tube. After separation of the components, C, H, N, and S elements in the sample were detected by a TCD detector.

The determination of selenium content was measured on a PF-6 atomic fluorescence spectrometry (Beijing's General Instrument Co., Ltd). First, the sample was predigested by nitric acid and hydrogen peroxide (volume ratio = 5:1) for 0.5 h in an ETFE digestion tube. Then, the tube was covered, sealed, and digested, in the microwave digestion workstation as a preset program. After cooling to 60 °C, the sample was transferred to a conical flask and heated until almost dry. Subsequently, 5 ml HCl was added and then heated until the solution became clear, releasing white smoke. Lastly, the digestion solution was transferred into a 25 ml volumetric flask and diluted with 20% HCl to volume for determination. All other elements were obtained by an IRIS Advantage ICP-AES (Thermo Electron, USA) and the processing method of the sample is similar to the determination of Se content.

2.7. IR spectroscopy and ultraviolet spectrum scan

The FT-IR spectra were recorded on a Nicolet 5700 IR spectrometer. The sample was ground with spectroscopic grade KBr powder and then pressed into 1 mm pellets for FT-IR measurement in the frequency range of 4000–400 cm^{-1}. Ultraviolet spectra of the samples were obtained on a T60 UV–Vis spectrophotometer. The scanning range was 200–700 nm at 2 nm intervals resulting in 250 points spectra for each sample.

2.8. Morphological analysis

Scanning electron micrographs (SEM, S-570, Hitachi) of the samples were conducted. Samples of Se-TPS, Se-TPS1, Se-TPS2, and Se-TPS3 were placed on a specimen holder with the help of double-sided adhesive tapes and coated with gold powder by using vacuum coating apparatus. Each sample was observed at an accelerating potential of 15 kV during micrography.

The topographies of samples were obtained using an atomic force microscope (AFM, Nanofirst 3600 A, Suzhou NZS-Nanosurf Nanotechnology Co., Ltd. China). Briefly, the sample in ultrapure water at 1 μg/ml was stirred for 10 min on a magnetic stirring apparatus. Subsequently, 5 μl of this solution was dropped onto freshly cleaved mica substrate and allowed to air-dry under ambient pressure, temperature and humidity. The atomic force microscope was operated in the tapping mode at room temperature. The scanning size was 5 × 5 μm.

2.9. Thermogravimetric analysis

The thermal decomposition process was studied on a DTG-60H thermal instrument which was carried out from room temperature to 500 °C at a heating rate of 10 °C min^{-1} under nitrogen atmosphere.