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Purification, characterization and antiglycation activity of a novel polysaccharide from black currant

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ABSTRACT

A novel polysaccharide fraction (BCP-1) was extracted from the black currant fruit by ultrasound-assisted compound enzyme and purified by chromatography on macroporous resin D4006, anion-exchange Q-Sepharose FF and Sephadex G-100 columns. BCP-1 consisted of galacturonic acid, xylose, mannose, glucose and galactose in a ratio of 1.00:3.14:1.83:17.90:1.98 and its molecular weight was 14,050 Da. The preliminary structure features of BCP-1 were investigated by FT-IR and NMR. SEM and Congo red test showed that BCP-1 had honeycomb-like structure, but no triple helix structure. BCP-1 exhibited significant inhibitory abilities on protein glycation. Especially, BCP-1 showed obvious inhibitory effects on the formation of dicarbonyl compounds and AGEs (% inhibition of 66.95 ± 0.33% and 67.15 ± 0.40% respectively), but weaker inhibition Amadori compound production (max. 37.15 ± 0.36% inhibition). This suggested that the inhibitory action of BCP-1 on protein glycation was more effective on the later phases of dicarbonyl compounds and AGEs formation.

1. Introduction

Non-enzymatic glycation is a complicated reaction process between the aldehydic group in reducing sugars and amino groups in proteins react to produce advanced glycation end products (AGEs) (Li et al., 2014). AGEs is one of the major risk factors for the development of chronic complications of diabetes, such as diabetic atherosclerosis (Wu, Hsieh, Wang, & Chen, 2009), peripheral neuropathy (Wada & Yagihashi, 2005) and diabetic cataract (Lapolla, Traldi, & Fedele, 2005). Non-enzymatic glycation reaction of protein proceeds in 3 phases. In the early phase, the reducing sugar reacts with the free amino groups of proteins to form Schiff base, and then Amadori products are produced. In the intermediate phase, the Amadori products are converted to series of dicarbonyl compounds such as glycolaldehyde, glyoxal and methylglyoxal through oxidation and dehydration. In the last phase, the dicarbonyl compounds continue to react with amino groups to produce AGEs (Wang, Zhang, & Dong, 2012). Thus, inhibitors that can inhibit any process of the three phases can alleviate the formation of AGEs and be beneficial to treat chronic complications of diabetes. Some compounds or agents, including aminoguanidine, benfotiamine and pyridoxamine (Kim, Kim, Jung, & Kim, 2010; Peyroux & Sternberg, 2006), have been used to treat diabetic complications. However, these drugs were reported to have adverse effects (Singh, Barden, Mori, & Beilin, 2001). Therefore, searching for more

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effective and safer antiglycation drugs has received considerable attention.

Polysaccharides are natural polymers and widely distributed in plants and microorganisms. They are highly stable, safe, non-toxic, non-carcinogenic and possess a variety of bioactivities, such as antitumor, anticoagulant, antiviral, anti-inflammatory, anti-thrombotic, hypoglycemic and antiglycation activities (Song, Li, Hu, Ni, & Li, 2011). In recent years, some bioactive polysaccharides, such as the polysaccharides from *Dendrobium huoshanense* and *Polygonum multiflorum Thunb*, were found to have antiglycation activities (Li et al., 2014; Lv, Cheng, Zheng, Li, & Zhai, 2014; Qian et al., 2014). So polysaccharides may be a promising drug candidate for diabetic complications.

Black currant (*Ribes nigrum* L.), which originates from Northern Asia and Europe, is a shrubby tree with many health-beneficial substances, such as ascorbic acid, flavone, anthocyanin, polyphenol and polysaccharide (Khoo, Clausen, Pedersen, & Larsen, 2012; Liu, Kallio, & Yang, 2014; McDougall, Gordon, Brennan, & Stewart, 2005; Takata, Yamamoto, Yanai, Konno, & Okubo, 2005). Black currant fruits and leaves have been used in both Asian and European traditional medicine for treatment of a variety of diseases (Bishayee et al., 2011). Black currant berry has evoked tremendous interest due to its diverse health benefits such as the inhibition of hypertension, neurodegenerative disease, ocular disease and anti-carcinogenesis (Gopalan et al., 2012; Tabart, Kevers, Evers, & Dommes, 2011; Yamamoto et al., 2014). Recently, the polysaccharides from black currant were reported to be one of the main factors responsible for the health benefits, exhibiting antioxidant, antitumor, anti-inflammatory activities (Tabart et al., 2012; Takata et al., 2005). In our previous research, the crude polysaccharides isolated from black currant fruit were found to exhibit good antioxidation and antiglycation activities *in vitro* (Yu, Ren, Xu, & Li, 2012), but there exist no reports on the structure and antiglycation activity of the purified polysaccharides.

In this study, the polysaccharide (BCP-1) was obtained by purification using macroporous resins D4006, anion-exchange Q-Sepharose FF and Sephadex G-100. Then the structure of BCP-1 was characterized by Fourier-transform infrared (FT-IR) spectroscopy and nuclear magnetic resonance (NMR). Moreover, the antiglycation activity of BCP-1 was also evaluated using the non-enzymatic glycation of protein test *in vitro*.

2. Materials and methods

2.1. Materials

The fruits of black currant (Heifeng) were provided by Mudanjiang Institute of Agricultural Science in Heilongjiang Province (Mudanjiang, China). The fruits at the fully mature stage were washed and stored at −20 °C until use. Before extraction process, the fruits were gently defrosted and then homogenized using a JJ-2 homogenizer (Changzhou Guohua Electric Appliance Co., Ltd., Jiangsu Province, China).

Papain (23.74 U/mg) was purchased from Beijing Obo Star Biotechnology Co., Ltd (Beijing, China). Pectinase (28.48 U/mg) was obtained from Shanghai Lanji Biotechnology Co., Ltd (Shanghai, China). D4006 Macroporous Resin was purchased from NanKai University Chemical Plant (Tianjin, China). Anion-exchange Q-Sepharose FF was obtained from Shanghai Yuanye Biotechnology Co., Ltd (Shanghai, China). Sephadex G-100 and standard monosaccharides (D-galacturonic acid, D-glucuronic acid, D-glucose, D-galactose, D-rhamnose, D-mannose, D-arabinose, D-fucose and D-fructose) were purchased from Sigma–Aldrich Co. (St, Louis, Mo, USA). Dextrans of different molecular weights (T-10, T-40, T-70, T-110) were provided from Baierdi Biotechnology Co. (Beijing, China). All other chemicals were of analytical grade.

2.2. Extraction and purification

The black currant fruit homogenate (20.0 g) with 2% enzyme mix (papain: pectinase = 2:1) were put into a 500 mL beaker, then a buffer solution (pH = 5.3) was added at 20:1 mL/g. The polysaccharides were extracted at 40 °C in the ultrasonic cell disintegrator (JY92-2D, Xinzhi Bio-Sciences Co. Ltd, Ningbo, China) for 45 min, and the ultrasonic power was 600 W. The extract was centrifuged at 3500 rpm for 20 min and the supernatant was filtrated, concentrated and precipitated with ethanol to a final concentration of 60% (*v*/*v*) at 4 °C overnight. The formed precipitate was gathered by membrane filtration (0.45 μm, Millipore, USA) under vacuum and lyophilized. Then the crude polysaccharides (BCP) were obtained.

The polysaccharide solution (4.00 mg/mL) was loaded on a D4006 macroporous resin column (2.0 cm × 30 cm) and eluted with deionized water at a flow rate of 1.00 mL/min. The obtained elute (1 mL/tube) was collected automatically and monitored by phenol–sulfuric acid method at 490 nm using D-glucose as a standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The collected fraction was lyophilized and dissolved in deionized water to a concentration of 20 mg/mL. Then, the sample was fractionated by anion-exchange chromatography of Q-Sepharose FF (2.0 cm × 30 cm) and eluted with deionized water at a flow rate of 2.00 mL/min. The obtained elute was pooled, concentrated and lyophilized. The eluted fraction (15 mg/mL) was further loaded on a Sephadex G-100 gel-permeation chromatography column (1.8 cm × 40 cm) and eluted with deionized water at a flow rate of 0.60 mL/min. Fractions were collected and enriched using the method mentioned above. The main fraction BCP-1 was obtained and lyophilized for further structure characterization and bioactivity assays.

2.3. Physicochemical properties and structural analysis of BCP-1

2.3.1. Physicochemical properties of BCP-1

BCP-1 was evaluated for solubility in water, ethanol, diethyl ether, ethyl acetate, acetone and chloroform in accordance with the British pharmacopoeia (BP) specification (Kannan, Manivannan, Balasubramaniam, & Kumar, 2010). The nature of BCP-1 was confirmed by Ninhydrin test, Iodine test, Felhing's test and Ferric chloride reaction. Uronic acid content was determined by the carbazole-sulfuric acid method using D-galacturonic acid as standard (Kintner & Buren, 1982).

2.3.2. Determination of molecular weight

The molecular weight of BCP-1 was determined by high performance liquid chromatography (HPLC, LC-10AVP, Shimadzu Corporation, Japan), which was performed on Waters Ultrahydrogel 2000 column (7.8 mm × 300 mm) and detected by differential refraction index detector (RID-10A) at 35 °C. The pressure was constantly kept at 1.4 MPa. BCP-1 was dissolved in deionized water and passed through 0.45 μm filter, applied to gel-filtration chromatographic column. Various standard dextrans of known molecular weight (T-10, T-40, T-70, T-110) were passed through the column. A standard curve was plotted according to the retention time and the logarithm of their respective molecular weights. The molecular weight of BCP-1 was estimated by reference to the calibration curve made above.

2.3.3. Analysis of monosaccharide composition

Gas chromatography (GC, GC-2010, Shimadzu Corporation, Japan) was used for identification and quantification of the monosaccharide compositions of BCP-1. Briefly, the polysaccharide