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The characterization, selenylation and antidiabetic activity of mycelial polysaccharides from *Catathelasma ventricosum*

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ABSTRACT

The mycelial polysaccharide from *Catathelasma ventricosum* (mCVP-1S) was found to be a heteropolysaccharide with an average size of 230 kDa composed mainly of β-glucopyranosyl residues. The selenylation of mCVP-1S, performed using an HNO_3–Na_2SeO_3 method, produced a series of selenized mCVP-1Ss (SemCVP-1Ss). Varying the reaction time, temperature and Na_2SeO_3 dosage altered the yield and selenium content of the SemCVP-1Ss. NMR spectra showed substitution mostly at C-6, and Congo red tests indicated excessive selenylation might destroy the triple-helical structure of SemCVP-1Ss. The antidiabetic activities of SemCVP-1Ss with varying selenium contents (low, middle and high) were tested in streptozotocin-induced diabetic mice. In SemCVP-1Ss with triple-helical structure, increasing selenium content enhanced antidiabetic activity, but damage to the triple-helical structure weakened antidiabetic activity. The ability of SemCVP-1Ss to normalize key biochemical parameters in diabetic mice was greater than that of the polysaccharide from the fruiting body of *C. ventricosum*.

1. Introduction

Diabetes mellitus, a prevalent and serious metabolic disease characterized by chronic hyperglycemia and an absolute or relative lack of insulin (Zhang et al., 2016), ranks fourth in prevalence worldwide after cardiovascular diseases, cancers, and chronic respiratory diseases (Wang & Zhu, 2016). More than 371 million people have been diagnosed with diabetes mellitus (Zhang et al., 2015). The persistent hyperglycemia characteristic of uncontrolled diabetes mellitus results in oxidative damage in various tissues, especially the eyes, kidney, heart, blood vessel and nerves, and leads to tissue dysfunction. Recent discoveries have opened up an exciting opportunity to develop new types of therapeutics to control diabetes mellitus and its complications (De Silva, Rapior, Hyde, & Bahkali, 2012; Lo & Wasser, 2011).

Edible fungi, which possess a wide number of bioactive compounds, have traditionally been used in the prevention and treatment of several diseases (Zhang et al., 2015). The polysaccharides of edible fungi are important biological macromolecules that have been shown to have antioxidant (Li & Wang, 2016), antitumor (Ren, Jiao et al., 2015), antiinflammatory (Cheng, Chao, Chang, & Lu, 2016) and antidiabetic (Xiao et al., 2017) activities. Chemical modification of these polysaccharides is an effective way to enhance their useful features (Paramakrishnan, Jha, & Jayaram Kumar, 2016). Recent studies have shown that selenized polysaccharides, obtained by chemical selenylation, possess greater (two-fold or more) biological activity than either selenium or polysaccharide alone (Liu et al., 2015). Furthermore, the bodies of diabetic patients generally contain less selenium than the bodies of normal humans (Askari, Iraj, Salehi-Abargouei, Fallah, & Jafari, 2015). Selenium is an essential trace element that participates in many important physiological activities. For example, it can enhance antioxidant levels in animals via glutathione peroxidase (GSH-Px) (Qiu et al., 2014). Thus, rational selenium supplementation can help alleviate the related symptoms of diabetic patients and can effectively prevent diabetes. Researchers have been exploring chemical modification methods in order to obtain polysaccharides with higher selenium content (Liu et al., 2015). Yield and selenium content are important indexes commonly used to measure selenylation. Common selenylation methods give variable yields and selenium contents with different kinds of polysaccharides. Moreover, research has shown that the two indexes can be significantly affected by reaction

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time, temperature, and sodium selenite dosage (Chen et al., 2014). Despite these efforts, the rules concerning the influence of these parameters on yield and selenium content remain unclear and are worthy of further investigation.

Catathelasma ventricosum, an edible mushroom from southwest China, has been found to have diverse medicinal values and a pleasant taste. Previous studies have shown that the polysaccharide extracted from the fruiting body of *C. ventricosum* (CVP-1S) has antioxidant, hypolipidemic, and hypoglycemic activities and can also protect the liver, kidney, and pancreas from diabetes-induced injuries in streptozotocin (STZ)-induced diabetic mice (Liu, Chen et al., 2016). The quality of the polysaccharides extracted directly from the fruiting body is influenced by both the area and season in which the mushroom is harvested. In contrast, liquid fermentation can make the quality of mycelia more consistent. Adding selenium to the liquid medium can also enhance the bioactivity of the polysaccharides, but the selenium translation rate is usually lower than that of chemical selenylation. Thus, it is speculated that chemical selenylation can further improve the biological activity of selenium-enriched mycelial polysaccharides.

There have been no reports describing selenylation of the mycelial polysaccharides from *C. ventricosum* (mCVP-1Ss) and its effect on their antidiabetic activity. Therefore, the main purpose of this study was to explore the ability of different selenylation conditions (reaction time, reaction temperature and Na_2SeO_3 dosage) to enhance the antidiabetic activity of SemCVP-1S. The results of this study reveal the structure-function relationships of these polysaccharides, and provide a theoretical basis for the development of novel antidiabetic agents.

2. Materials and methods

2.1. Materials and reagents

Samples of *C. ventricosum* were purchased from the Mianyang Edible Fungi Research Institute (China) and maintained using synthetic potato dextrose agar (PDA) medium. Glibenclamide and STZ were purchased from Sigma-Aldrich Co. LLC. (USA). Sodium selenite was obtained from Shanghai Tiancifu Biological Engineering Co., Ltd. (China). Reagent kits for the determination of glucose level, total cholesterol (TC), triglyceride (TG), low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) were acquired from the Jianchen Bioengineering Institute (China). The other chemicals were purchased from Sinopharm Chemical Reagent Co., Ltd. (China). The solvents and other chemicals used in this work were of an analytically pure reagent grade.

2.2. Liquid fermentation

The *C. ventricosum* samples were initially grown at 22 °C for 4 d in liquid PDA medium (200 g potato, 20 g glucose, 2 g peptone, 2 g KH_2PO_4, 1 g $MgSO_4$ and 0.01 g vitamin B1 dissolved in 1 L ultrapure water). The resulting liquid culture (2.5 mL) was transferred to a 250-mL flask containing 47.5 mL of fermentation culture medium (4% maize powder, 2% glucose, 0.4% peanut meal, 0.2% KH_2PO_4, 0.5% K_2HPO_4, 0.1% $MgSO_4$ and 0.01% vitamin B1) and incubated with shaking (100 rpm) for 15 d at 22 °C. The mycelia obtained after centrifugation (625*g*, 10 min) were freeze-dried and ground to a powder (40 meshes).

2.3. Extraction of crude mycelial polysaccharides

Water-soluble mycelial polysaccharides were obtained by extracting the mycelial powder for 3 h with 4 L of distilled water at 85 °C. The extraction was performed three times and the combined extract was concentrated in a rotary evaporator. Proteins were removed using the Sevag method (Chai & Zhao, 2016) and low-molecular-weight compounds (≤5 kDa) were removed via 2-d dialysis. The resulting solution was precipitated with three volumes of ethanol and kept at 4 °C overnight. The crude mycelial polysaccharides produced after centrifugation (1738*g*, 10 min) and lyophilization will be referred to as mCVPs.

2.4. Purification of the polysaccharide

The mCVPs produced using the procedure in Section 2.3 were dissolved with distilled water to a final concentration of 100 mg/mL, and 3 mL of this solution was added to a DEAE-52 column (3.5 cm × 20 cm) equilibrated with ultrapure water. The column was washed with one column volume of ultrapure water, and then eluted using a linear gradient from 0 to 2 M NaCl at a flow rate of 1 mL/min. Fractions were collected (10 mL per tube) and the sugar profile was monitored using the phenol-sulfuric acid method (Masuko et al., 2005). Fractions with the highest antioxidant activity (data not shown) were combined, concentrated and lyophilized. This sample was further purified using a Sephadex G-100 column (2.6 cm × 80 cm). The column was eluted with ultrapure water at a flow rate of 0.3 mL/min. The eluent was collected and tested for the presence of polysaccharide using the phenol-sulfuric acid method. The purified polysaccharide mixture produced using this method is referred to as mCVP-1S.

2.5. Characterization of mCVP-1S

2.5.1. General analysis

Protein and nucleic acid contamination was investigated by scanning a mCVP-1S solution (1 mg/mL) from 200 to 400 nm using a UV spectrometer.

The molecular weight of mCVP-1S was determined using high-performance gas permeation chromatography (HPGPC) performed with a TSK gel G5000PWxL column (7.8 mm × 30 cm, TOSOH Co.) on an Agilent 1100HPLC system equipped with a Waters-2410 refractive index detector. The column was eluted at a flow rate of 0.8 mL/min with ultrapure water at 30 °C. A set of dextran standards was used to prepare the external calibration curves. The peak distribution was used to judge the purity of the mCVP-1S (Li, Fan, & Ding, 2011).

The monosaccharide compositions of mCVP-1S were assessed using gas chromatography (GC). A sample of mCVP-1S (10 mg) was hydrolyzed with trifluoroacetic acid (2 M, 2 mL) at 110 °C for 2 h. After removing excess trifluoroacetic acid, the resulting products were reduced using $NaBH_4$ (10 mg/mL, 3 mL) for 3 h and then vacuum dried. The reduced mixtures were acetylated with acetic anhydride and analyzed by GC. Components were identified by their typical retention times and peak areas, and then quantified using a calibration curve constructed using a mixed standard solution of monosaccharides (fucose, arabinose, xylopyranose, mannose, glucose and galactose). The results were given as weight percentages based upon the total sugar content.

Fourier transform infrared (FTIR) spectra (4000–400 cm^{-1}) were obtained using a Nicolet 5 DXC Nexus spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). Samples were prepared using the KBr pellet method.

2.5.2. Periodate oxidation and Smith degradation

A 30-mg sample of mCVP-1S was dissolved in 20 mL of distilled water and oxidized with 0.015 M $NaIO_4$ (60 mL) at 4 °C in the dark. The reaction was monitored by withdrawing 0.2 mL aliquots at 6 h intervals, diluting them to 50 mL with distilled water, and then determining their absorbance at 223 nm. When the absorbance