LWT - Food Science and Technology 70 (2016) 55–62

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Structural characterization and hypoglycemic activity of *Trichosanthes* peel polysaccharide

Tianle Chen [1], Min Zhang [1], Jinglei Li, Maheen Mahwish Surhio, Beibei Li, Ming Ye*

College of Biotechnology and Food Engineering, Hefei University of Technology, Hefei 230009, China

ARTICLE INFO

Article history:
Received 4 August 2015
Received in revised form
29 January 2016
Accepted 8 February 2016
Available online 17 February 2016

Keywords:
Trichosanthes peel
Polysaccharide
Purification
Structure
Hypoglycemic activity

ABSTRACT

Trichosanthes peel polysaccharide (TPP) was obtained from the aqueous extract of *Trichosanthes* peel by alcohol precipitation, deproteinization and decoloration. TPP was then separated and purified by Sephadex G-100 column to obtain the homogeneous component TPP-1 (1.2×10^5 Da). The compositions in monosaccharides were D-arabinose (Ara), D-mannose (Man), D-glucose (Glc) and D-galactose (Gal) with a molar ratio of 1.00:3.27:4.26:6.01. Its backbone was composed of 1,4,6-Gal*p*, 1,4-Gal*p*, 1,3,6-Man*p* and 1,4-Man*p*, while the branches comprised of 1,3-Ara*f*, 1-Ara*f* and 1-Glc*p*. Diabetic mice experiments showed that the blood glucose levels in hyperglycemia mice reduced by 22.47%, 15.38% and 12.72% after administration of high, medium and low doses of TPP-1, respectively. Compared with the negative control group, the contents of insulin and total superoxide dismutase of the hyperglycemia mice in groups treated with different doses of TPP-1 were increased significantly, while the contents of biochemical indexes including malondialdehyde, creatinine, triglyceride, total cholesterol low density lipoprotein cholesterin and blood urea nitrogen were decreased in different degrees. These results suggested that TPP-1 possessed strong hypoglycemic activity on streptozotocin-induced model mice.

1. Introduction

Diabetes mellitus (DM), a common endocrine disease, usually manifests as the disorder of blood glucose, fat and protein levels of the patients, and induces some complications including vascular lesions and diabetic neuropathy (Clements, 1979). The prevalence of DM has increased substantially over the past years being the third most prevalent disease, which is following by cardiovascular system diseases and cancer. It may pose great threat to human health and life in the developed countries (Wang et al., 2013). DM is mainly caused by the metabolic disorder, and presents as hyperglycemia, dyslipidemia and oxygen free radical metabolism enzyme defects (Kesavulu, Giri, Kameswara, & Apparao, 2000; Nawata, Sohmiya, Kawaguchi, Nishiki, & Kato, 2004; Scoppola, Montecchi, Menzinger, & Lala, 2001). Sulfonylureas, biguanides and thiazolidinediones applied as oral anti-diabetic medicines have the ability to increase the reduction rate of blood sugar level, but behave poorly in regulating the glucose tolerance and dyslipidemia (Tahrani, Piya, Kennedy, & Barnett, 2010). Moreover, these drugs may also produce toxicity and side effects if taken for a long time (Hu, Liu, Ni, & Lu, 2014). Therefore, it is necessary to explore and develop other natural and effective hypoglycemic drugs for the prevention and treatment of DM.

Plant polysaccharides contain many monosaccaride compositions with various structures and biological activities (Ding, Zhu, & Gao, 2012). Polysaccharides isolated from *Ophiopogon japonicas* and *Porphyra yezoensis* have been proven to exert hypoglycemic and hypolipidemic effects, respectively (Chen et al., 2011; Qian, Zhou, & Ma, 2014). Therefore, plant polysaccharides can be explored as a kind of natural medicines and new functional food.

Trichosanthes kirilowii Maxin belongs to *Trichosanthes* genus of cucurbitaceous, a perennial vine. *Trichosanthes* peel is the ripe pericarp of *T. kirilowii* Maxin and rich in oils, organic acid, polysaccharide, flavones and protein (Qian, Dan Liu, & Peng, 2010). It was reported that *Trichosanthes* peel was widely used in traditional Chinese medicine to treat diseases of cerebrovascular, cardiovascular, and respiration systems due to the abilities to clear heat and dissipate phlegm, regulate the flow of vital energy and relieve chest stuffiness (Chen, Huang, & Wang, 2006). However, there is no report with regard to the structure and hypoglycemic activity of *Trichosanthes* peel polysaccharide.

The aims of this study were to analysis the structure of the polysaccharide from *Trichosanthes* peel (TPP-1) and to evaluate its hypoglycemic activity.

* Corresponding author. Tel.: +86 551 62919368.
E-mail address: yeming123@sina.com (M. Ye).
[1] The authors contributed equally to this work.

http://dx.doi.org/10.1016/j.lwt.2016.02.024
0023-6438/

2. Materials and methods

2.1. Materials and reagents

Trichosanthes peel (*T. kirilowii* Maxin) was provided by Lushi Ecological Agricultural Technology Co., Ltd (Anhui).

Sephadex G-100 column was purchased from Sigma Chemical Co. (Zhengzhou, China). ELISA kits for the analysis of insulin (INS), triglyceride (TG), total cholesterol (TCH), low density lipoprotein cholesterin (LDLC), creatinine (Cr) and blood urea nitrogen (BUN) were purchased from Yansheng Biological Technology Co., Ltd. (Shanghai, China). Total superoxide dismutase (T-SOD) kit and malondialdehyde (MDA) kit were purchased from Nanjing Jiancheng Technology Co., Ltd. (Nanjing, China). Sinocare glucometer and test paper were purchased from Changsha Sinocare, Inc. (Changsha, China). The reagents were of analytical grade unless otherwise specified.

2.2. Experimental animals

Sixty Kunming mice with the body weight of 20 ± 2 g were purchased from Experimental Animal Center of Anhui Medical University, Hefei, China. All animals' treatments were strictly in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The animals were kept at 23 ± 2 °C with the humidity of 55 ± 5%, and cultured in a 14 h:10 h light–dark cycle.

2.3. Preparation of TPP-1

Trichosanthes peel was dried to a constant weight at the temperature of 40–50 °C in an oven, and grounded to 80–100 μm particles. The polysaccharide was extracted according to the method of Khodaei and Karboune (2013). *Trichosanthes* peel was suspended in NaOH alkaline solution, containing 0.02 M $NaBH_4$, and the mixture were incubated at 60 °C for 24 h. The supernatants were recovered after centrifugation (4,000g, 10 min) followed by filtration. The recovered polysaccharide solution was neutralized to pH 7.0 with 0.1 M acetic acid, and mixed with three volumes of 95% ethanol to obtain precipitate. Then, the precipitate was dialyzed in 14,000 Da dialysis bag against tap water and distilled water for 48 h successively. Crude polysaccharides (TPP) were obtained after freeze-drying for 24 h. The crude polysaccharide was fractionated by Sephadex G-100 chromatographic column. The major polysaccharide peak eluted by distilled water was collected and freeze-dried, and used for further structure analysis and animal experiments.

The chromatography conditions of Sephadex G-100 (1.6 cm × 60 cm) were as follows: the amount of loading sample was 20 mg; eluent was double distilled water at a flow rate of 0.4 mL min^{-1}, and 4 mL of the solution was collected in each tube.

2.4. Purity and molecular weight (M_W) determination

Ultraviolet (UV) absorption spectra of TPP-1 was recorded with a Agilent Cary5000 spectrophotometer (Agilent Technologies Co. USA). High performance liquid gel permeation chromatography (HPGPC) was further used to test the purity of TPP-1 and to calculate its molecular weight. The Waters-2414 HPLC host (Waters Co. USA) that was equipped with Waters-1515 parallax shading monitor was used. Double distilled water was used as eluent at the flow rate of 0.5 mL min^{-1} with the Ultrahydrogel™ 2000 analytical column temperature of 35 °C. The purity of the sample was determined according to the shape and distribution of the eluting peak. When the eluting peak was single, symmetric and narrow, with the feature of homogeneous distribution, it proved that TPP-1 was a homogeneous polysaccharide. To obtain the molecular weight of TPP-1, standard T-series dextrans were also eluted through the column, and a calibration curve was plotted. The molecular weight of TPP-1 was calculated according to the calibration curve established by standard T-series dextrans (M_W: 4400, 9900, 21,400, 43,500, 124,000, 196,000, 401,000 Da).

2.5. Analysis of monosaccharides

2 mL of 2 M Trifluoroacetic acid (TFA) was added into TPP-1 (5 mg) powder and the solution was hydrolyzed at 121 °C for 4 h. The subsequent steps were carried out according to the method of Ye et al. (2011). $NaBH_4$ (40 mg) was added and restored overnight under the room temperature. 4 mL of acetic anhydride and 3 mL of pyridine were added to the dried hydrolyzate and incubated at 110 °C for 1 h. The acetylated derivative was loaded into gas chromatography-mass spectrometry (GC–MS) (Shimadzu Co. Japan). Conditions of GC–MS: quartz capillary column HP-5 (30 m × 0.25 mm × 0.25 μm); temperature programming was selected for column temperature which increased from 50 °C to 250 °C at the rate of 10 °C min^{-1}; temperature of the injection port was 260 °C while the flow rate of He was 1 mL min^{-1}; ion source: EI, 70 eV; the molecular weight range: 35–650.

2.6. Periodate oxidation and smith degradation

The method of Linker, Evans, and Impallomeni (2001) was referred with slight amendments. TPP-1 (12.5 mg) was dissolved in 25 mL of 15 mM $NaIO_4$, and then the solution was placed in dark. An aliquot of this solution (1 mL) was taken out every 6 h and diluted 100 times with distilled water. The absorbance of the solution was detected at 223 nm by the UV spectrophotometer. When it was stable at 223 nm, 1 mL of reaction liquid was drawn out and titrated with 0.5 mM NaOH to measure the production of formic acid.

The purpose of Smith degradation is to reduce periodate oxidation products to polyol with boron hydrides, and evaluate the linkage types and order of the polysaccharide components. According to the method of Rout, Mondal, Chakraborty, and Islam (2008), 1.0 mL of ethylene glycol was added into the polysaccharide solution after periodate oxidation. The solution was shaken and then placed for 3 h, The mixture was dialyzed with distilled water for 48 h and concentrated by vacuum. $NaBH_4$ (80 mg) was added and later restored for 24 h under the room temperature. Then, 0.1 M acetic acid was added to the solution for neutralization and dialyzed for 48 h with distilled water, and evaporated to dryness under the decompression. 2 mL of 2.0 M TFA was added and sealed in a tube, and hydrolyzed at 120 °C for 2 h. Then 5 mL of methanol was added and evaporated to dryness again by decompression. The above procedures were repeated twice to eliminate TFA. The sample was acetylated according to the method described in the section 2.5 and then GC–MS analysis was carried out.

2.7. Methylation analysis

The method of Needs and Selvendran (1993) was referred. TPP-1 (28.0 mg) was taken and added into 0.1 mL of water. 5 mL of DMSO and 3A molecular sieve (5 mg) were added and filtrated with filter paper into the reaction flask. NaOH (80 mg) powder was added into the solution and N_2 was injected into the flask. Under the room temperature, it was treated by ultrasonication for 30 min to obtain the solution. Later, 1.0 mL of 98% CH_3I was added and N_2 was injected into the flask. Ultrasonic treatment was carried out in 20 °C for 1 h to expel the residual CH_3I. The procedures above were repeated for three times. The reaction was terminated by adding 2 mL of water and the solution was neutralized by 25% acetic acid. Then, it was