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Structure characterisation of polysaccharides in vegetable "okra" and evaluation of hypoglycemic activity

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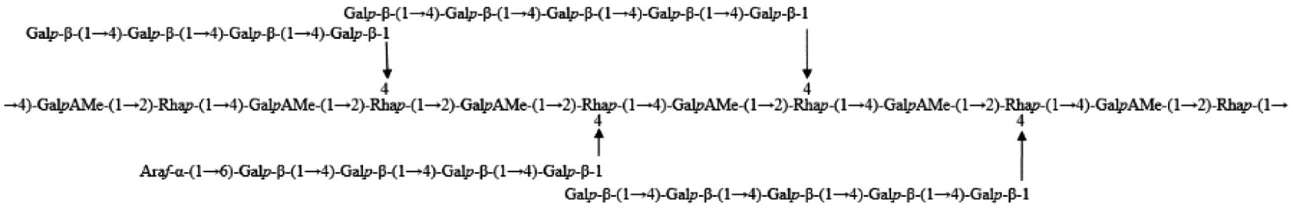
ABSTRACT

Okra is a widely accepted vegetable in subtropical and tropical regions due to the good palatability. However, the polysaccharide compositions remain unclear. In this work, the water extract of okra pod was prepared and the leading polysaccharide fraction was purified. The precise structural characteristics were identified. It was a polysaccharide "rhamnogalacturonan" as shown below, and the structure was different to previously reported rhamnogalacturonans. The hypoglycemic effect of rhamnogalacturonan was determined *in vivo*. By comparing with streptozotocin-induced diabetic mice group, the high-dose group showed decreased blood glucose level and glucose tolerance. The body weight of all groups were not significantly different. The results indicated that the rhamnogalacturonan was responsible for the hypoglycemic effect of okra.



1. Introduction

Okra (*Abelmoschus esculentus* L. Moench), also known as lady's finger or gumbo, is a vegetable mainly planted in tropical and subtropical regions. Besides supplying common nutrients like minerals and vitamins, it is also a good source of dietary fibres and bioactive chemicals. It has been reported that the peel and seed of okra have antidiabetic and antihyperlipidemic effects in streptozotocin-induced diabetic rats (Sabitha, Ramachandran, Naveen, & Panneerselvam, 2011). Some reports have revealed that okra can lower blood glucose level and lipid level in high-fat diet-induced obese C57BL/6 mice. These evidences indicate that okra may play an important role in the regulation of glucose and lipid metabolisms.

Okra is rich in bioactive polysaccharides, which have various biological activities (Deters, Lengsfeld, & Hensel, 2005; Lengsfeld, Titgemeyer, Faller, & Hensel, 2004; Panagiotis, 2008; Wittschier et al., 2007), and is used as a dietary therapy for blindness, cataract and glaucoma development in type 2 diabetic patients (Moise, Benjamin, Doris, Dalida, & Augustin, 2012). Recently, some reports have mentioned that okra polysaccharides can lower the body weight and glucose levels, improving glucose tolerance, and decreasing total serum cholesterol levels in high-fat diet-fed C57BL/6 mice (Fan et al., 2013). In addition, purified okra polysaccharides increase the spleen index, splenocyte proliferation, and cytokine secretion *in vivo*. These information indicates that okra polysaccharides may potentially serve as a novel immunomodulators (Chen et al., 2016). Furthermore, the

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okra water extract also has the potential to serve as an adjuvant for diabetic nephropathy (Peng et al., 2016). However, the precise structures of the major polysaccharides in okra remain unknown. To understand which chemical plays an important role in the antidiabetic activity of okra, it is required to identify the precise structure of polysaccharide and to evaluate the hypoglycemic activity. Therefore, in the present study, the water-soluble polysaccharides were extracted from okra pod and the leading polysaccharide was purified. NMR was used to identify the precise structure.

2. Materials and methods

2.1. Okra selection

Okra (*Abelmoschus esculentus*) pods were collected from a local market in October 2015. The fruits were selected for uniformity of size and color, and they were free from visible wounds and rottenness.

2.2. Extraction and purification of okra polysaccharide

Polysaccharides in okra were extracted by hot water as described below. Okra pods (7.5 kg) were cut into small pieces, and were added with 20 L of absolute ethanol. After incubation for 3 days at room temperature, the supernatants were removed. The residues were extracted by deionized water with a ratio of 1:10 (w/w) at 75 °C for 2 h, then centrifuged at 10,000*g* for 20 min to collect the supernatants. After concentrating at 50 °C by using a vacuum rotary evaporator (Eyela N-1100V-W, Tokyo Rikakikai Co. Ltd, Tokyo, Japan), ethanol was added to a final concentration of 60% (v/v), and maintained for 12 h at 4 °C to precipitate the crude polysaccharide. The pellets were collected by centrifugation at 7000*g* for 15 min and lyophilized.

The okra polysaccharides were dissolved in deionized water. A middle-pressure liquid chromatography (LC3000, Beijing ChunagXin TongHeng Science & Technology Co., Ltd., Beijing, China) equipped with a DEAE Sepharose Fast Flow column (15 × 460 mm) was used for purification. Solvent A (phosphate buffer) and solvent B (1 M NaCl in phosphate buffer) were used as elution buffers. A gradient elution was conducted as 100% solvent A, 0–50 min; 80% solvent A, 50–100 min; 20%A, 100–150 min. The flow rate was set at 4 mL/min. The dominant fractions at 0–50 min was collected, concentrated and freeze-dried. An ultrafiltration membrane with molecular weight cut-off of 100 kDa was used to remove small carbohydrate molecules. The retentates were lyophilized for further analysis.

2.3. Molecular weight determination

The molecular weight of polysaccharides was determined by a high-performance gel permeation chromatography (LC-20A, Shimadzu, Kyoto, Japan). A refractive index detector was used for monitor the chromatogram. G6000PWXL, G5000PWXL and G3000PWXL columns (Tosoh Bioscience, Stuttgart, Germany) were tandemly linked for analysis. Commercial dextran in the range of 5.22–2990 kDa were used to draw calibration curve. The molecular weight of analyte was calculated by the retention time (Liu, Jiang, Yang, & Yang, 2017).

2.4. Monosaccharide composition determination

Trifluoroacetic acid was used to hydrolyse the okra polysaccharides. The acid was removed by a vacuum rotary evaporator (Eyela N-1100V-W, Tokyo Rikakikai Co. Ltd, Tokyo, Japan). Hydroxylamine hydrochloride (10 mg) and pyridine (2 mL) were added to the hydrolysates. After incubation at 90 °C for 40 min, 2 mL of acetic anhydride were added and incubated for 30 min. 2 mL of water were added to terminate the reaction. The acetylated derivatives were extracted by 3 mL of chloroform for 3 times. The chloroform extracts were combined and re-extracted with 6 mL of water. The chloroform fraction was collected and concentrated at 40 °C. The monosaccharide derivatives were loaded into a gas chromatography equipped with a HP-5 capillary column and a flame ionization detector. The injection temperature and detector temperature were 230 °C. The column temperature was started from keeping at 110 °C for 1 min, increasing to 180 °C at 2 °C/min, holding for 3 min, then increasing to 220 °C at 10 °C/min, holding for 3 min, then increasing to 280 °C at 20 °C/min and finally holding for 10 min. Nitrogen was used as the carrier gas and maintained at 40 mL/min.l-Cyclohexyl-3-(2-morpholinoethyl) carbodiimide metho-*p*-toluenesulfonate was used to reduce the uronic acids in okra polysaccharides (Yuan et al., 2016). The reduced polysaccharides were subjected to above procedure for monosaccharide composition analysis.

2.5. NMR analysis

Okra polysaccharide was dissolved in 0.5 mL of D_2O and 1 μl of acetone was added (Yang, Prasad, & Jiang, 2016). 1D and 2D NMR spectra were recorded by a Bruker DRX-500 spectrometer (Bruker, Rheinstetten, Germany). The measured spectra included ^{1}H, ^{13}C, heteronuclear single quantum coherence spectroscopy (HSQC), heteronuclear multiple bond correlation spectroscopy (HMBC), heteronuclear single quantum coherence-total correlation spectroscopy (HSQC-TOCSY), selective ^{1}D total correlation spectroscopy (^{1}D TOCSY) and H/H correlation spectroscopy (COSY). The chemical shifts of acetone (2.22 and 30.89 ppm for methyl proton and carbon, respectively) were used for calibration.

2.6. Animals

Male C57BL/6 mice (16–18 g, age 6–7 weeks) were obtained from Guangdong Medical Animal Center. All mice were acclimated for 4 days. They were free access to tap water and standard laboratory diet under conditions of temperature (23 ± 3 °C) and a 12-h light-dark cycle. The experiment was approved by animal ethics guidelines of the Institutional Animal Ethics Committee.

2.6.1. Induction of diabetic model

The diabetic mice were forbidden from food for 16 h, and were induced by an intraperitoneal injection with 1% streptozotocin (45 mg/kg body weight, freshly prepared in 0.1 M sodium citrate buffer solution) once a day for five days. Streptozotocin-induced diabetes mice were allowed free access to food and water from the 2nd to 5th day. Three days later, all streptozotocin-injected mice were forbidden from food for 5 h and then were assessed by measuring glucose levels in tail vein. Glucose levels at 10–25 mM were considered as diabetic mice.

2.6.2. Experimental design

Five groups of mice were randomly divided, including model group, positive group, low-dose group of rhamnogalacturonan and high-dose group of rhamnogalacturonan. Group I (Normal control group, NG): normal mice as control group and injected with pure water (0.2 mL/10 g body weight, once a day for 10 days in total). Group II (Model control group, MG): streptozotocin-induced diabetic mice, the model group was intragastric administration with pure water (0.2 mL/10 g body weight, once a day for 10 days in total). Group III (Positive control, PG): streptozotocin-induced diabetic mice were intragastric administered with 2 mg/kg glimepiride and 200 mg/kg metformin hydrochloride. P50 group: streptozotocin-induced diabetic mice were given 50 mg/kg rhamnogalacturonan by intragastric administration. P200 group: streptozotocin-induced diabetic mice were intragastrically administered 200 mg/kg rhamnogalacturonan.

2.6.3. Determinations of blood glucose level and glucose tolerance

Blood glucose levels in tail vein at 0th, 5th and 10th day were determined by using a glucose analyser. At the 10th day, the blood glucose levels at 0.0, 0.5 and 2.0 h after intragastric administration of