Carbohydrate Polymers 126 (2015) 179–184

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Structural characterization of a broccoli polysaccharide and evaluation of anti-cancer cell proliferation effects

Lishan Xu*, Jingjing Cao, Wenrong Chen

College of Chemistry and Life Science, Zhejiang Normal University, Jinhua 210043, China

ARTICLE INFO

Article history:
Received 15 December 2014
Received in revised form 6 February 2015
Accepted 3 March 2015
Available online 17 March 2015

Keywords:
Broccoli
Polysaccharide
Structure
NMR
Anti-cancer cell proliferation activity

ABSTRACT

Broccoli is a widely consumed vegetable with abundant amount of nutrients, which bring numerous beneficial effects on human health. The structural information of water-soluble polysaccharides in broccoli was eludicated for the first time in this work. A purified polysaccharide fraction (BPCa) was obtained by column chromatography. It comprised of arabinose (Ara), galactose (Gal), rhamnose (Rha) with a molar ratio of 5.3:0.8:1.0. Nuclear magnetic resonnance spectra data revealed that α-L-1,5-Ara*f* and α-L-1,3,5-Ara*f* are present in the backbone, while α-L-Ara*f* terminal was attended in side chain. α-L-1,2-Rha*p* was found to be linked to α-L-1,5-Ara*f* in heteronuclear multiple bond correlation spectra. The presences of β-D-1,4-Gal*p* and α-D-1,4-Gal*p*A were also detected. Furthermore, BPCa showed significant anti-cancer cell proliferation activities against HepG2, Siha and MDA-MB-231 carcinoma cell lines. The results indicated that BPCa had a good potential to be applied as functional food additives.

1. Introduction

Plant cell wall is mainly consisted of polysaccharides, including hemicelluloses, pectins and cellulose (Mohanty, Misra, & Drzal, 2001). These polysaccharides play an important role in the physiological behaviours of plant cell and defence against biotic and abiotic stress. Pectins usually have a backbone of (1 → 4)-α-D-galacturonan with varying degrees of carboxyl groups methylesterified or of rhamnogalacturonan (Thakur, Singh, & Handa, 1997). Hemicelluloses are the second most common polysaccharides in nature (Saha, 2003), including xylans, arabinogalactans and many other structural types. In addition to protective effects on plants, polysaccharides also exhibit healthcare benefits on human body. Immunomodulation activity, growth improvement of microorganisms in gastrointestinal tract, prevention of cardiovascular diseases, and inhibition of cancer are the main documented biological functionalities (Ma, Qiao, & Xiang, 2011). For example, a water-soluble polysaccharide (branched glucan) from *Ganoderma lucidum* can effectively induce MAPKs- and Syk-dependent TNF-α and IL-6 secretion in murine resident peritoneal macrophages. It can also potentiate the ConA-induced proliferative response of splenocytes and induce the anticancer behaviour (Guo et al., 2009).

As a widely consumed vegetable, broccoli has been reported to be capable of prevention of degenerative diseases and cancer. It can also improve the cardiovascular health (Velasco et al., 2011). In addition to the contribution of bioactive small molecules, like phenolics (Lee, Boyce, & Breadmore, 2011; Zhao et al., 2007), polysaccharides therein might be responsible for the observed functionality, as the level of polysaccharide component is high. However, little structural information was available in this regard. Therefore, in this work, the water-soluble polysaccharides were extracted from broccoli stem and structural components were analysed. The crude polysaccharides were purified by anion exchange chromatography and gel filtration chromatography. The molecular weight was determined by gel permeation chromatography. The chemical structure was identified. Furthermore, the anti-cancer cell proliferation activity of the purified polysaccharide was investigated.

2. Materials and methods

2.1. Plant materials

Fresh broccoli (*Brassica oleracea*) were purchased from a local grocery store. The raw materials were cleaned carefully by distilled

* Corresponding author. Tel.: +86 579 82282269.
E-mail address: xls@zjnu.edu.cn (L. Xu).

http://dx.doi.org/10.1016/j.carbpol.2015.03.011
0144-8617/

water, and the stems were subjected to polysaccharide extraction immediately.

2.2. Chemicals

Monosaccharide standards (glucose, Glc; galactose, Gal; fructose, Fru; mannose, Man; xylose, Xyl; arabinose, Ara; rhamnose, Rha) were purchased from Biomart (Hangzhou, China). Galacturonic acid (GalA), glucuronic acid (GlcA) and dextran standards were purchased from Sigma Chemical Co. (St. Louis, MO). Hydroxylamine hydrochloride, acetic anhydride, methyl iodine and sodium borohydride were purchased from Aladdin Reagent Inc. (Shanghai, China). All the other chemicals used were of analytical grade.

2.3. Extraction of water-soluble broccoli polysaccharides

The water-soluble polysaccharides were extracted by hot-water extraction technique (Zhu et al., 2013). Homogenization was conducted when five hundred grams of broccoli were added into 5 l of distilled water. The slurry was transferred to a water bath shaker. Extraction was manipulated at 80 °C for 2 h. The extract was then filtered and concentrated by using a vacuum rotary evaporator at 65 °C. Anhydrate ethanol was added to a final concentration of 40% (v/v), and maintained overnight at 4 °C. After centrifugation at 8000 *g* for 20 min, the supernatant was added with ethanol to a final concentration of 60% (v/v). They were incubated at 4 °C for 12 h and then centrifuged. The polysaccharide pellets were collected and lyophilized.

2.4. Purification of water-soluble broccoli polysaccharides

DEAE Sepharose Fast Flow column (15 × 450 mm) was used for purification. The polysaccharide pellets (500 mg) were dissolved in 10 ml of deionized water, filtered by 0.45-μm pore size membrane. Elution was programmed as follows: distilled water was used as elution solvent for 1 h, followed by elution with gradient NaCl solutions (0.1, 0.2, 0.3, 0.4 M NaCl, each gradient for 1 h). The flow rate was maintained at 5 ml/min. 4 ml each tube was collected. All tubes were quantified by phenol-sulphuric acid method. The chromatogram was drawn by Microsoft Excel 2003 (Microsoft, Seattle, WA). As the yields of polysaccharides eluted by 0.1 and 0.4 M NaCl were low, they were ignored for further analysis. Three polysaccharide peaks eluted by water, 0.2 M NaCl and 0.3 M NaCl were collected and freeze-dried. They were named BPA, BPB and BPC, respectively.

Gel filtration chromatography was used to purify BPC by difference in molecular weight. BPC was loaded onto a column (15 × 450 mm) filled with Sephacryl S-400HR. Deionized water was used as elution solvent at a flow rate of 0.5 ml/min. The main peak was collected and concentrated by rotary vacuum evaporator. Lyophilization was conducted to obtain BPCa.

2.5. Assays of monosaccharide composition and absolute configuration

10 mg of polysaccharides were hydrolysed by 2 M trifluoroacetic acid at 100 °C for 2 h. The hydrolysates were dried by rotary vacuum evaporator. Hydroxylamine hydrochloride and pyridine were added to the hydrolysates. They were incubated at 100 °C for 30 min. Then 2 ml of acetic anhydride were added. The reaction was kept at 100 °C for another 30 min. The acetylated derivatives were loaded onto gas chromatograph equipped with a HP-5 capillary column and a flame ionization detector. The following temperature program was adopted: Column temperature was programmed from 110 °C/min. It stayed at this temperature 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. Injection temperature: 230 °C; Detector temperature: 230 °C; Nitrogen was used as the carrier gas.

Hydrochloric acid (0.625 M) in (+)-2-butanol was added to the polysaccharide hydrolysates. They were incubated at 80 °C for 16 h. The reactants were dried by vacuum rotary evaporator, and per-*O*-trimethylsilyl derivatives were prepared by *N*,*O*-bis(trimethylsilyl) trifluoroacetamide. The products were analyzed by gas chromatography, and compared with those prepared from D- and L-enantiomers of the monosaccharide standards.

2.6. Methylation

Methylation was conducted by using the classical method (Yang et al., 2009) with minor revision. Dry polysaccharides were dissolved in dimethyl sulfoxide, then sodium hydroxide was added and treated with ultrasonic wave. After incubation for 20 min at room temperature, methyl iodide was added to initiate methylation. Distilled water was used to decompose the remained methyl iodide. The methylated polysaccharides were dialyzed to remove impurities. The retentates were concentrated by a rotary vacuum evaporator. After hydrolysis with 2 M trifluoacetic acid, the methylated polysaccharide hydrolysates were dissolved in 1 M NaOH. After reduction by $NaBH_4$, the sample was acetylated by adding acetic anhydride and pyridine. The reaction was kept at 100 °C for 1 h. Two milliliters of distilled water were used to decompose the remained acetic anhydride. The acetylated derivatives were extracted with 4 ml of methylene chloride. A gas chromatograph–mass spectrometer (GCMS-QP 2010, Shimadzu, Kyoto, Japan) was used to analyse the glycosidic linkage.

Reduction of uronic acids was conducted by L-cyclohexyl-3-(2-morpholinoethyl) carbodiimide metho-*p*-toluenesulfonate (Taylor & Conrad, 1972). Then methylation and acetylation were carried out as above. The linkage of GalA in BPCa was determined.

2.7. Nuclear magnetic resonance (NMR) spectroscopy analysis

BPCa was dissolved in 0.5 ml of D_2O. ^1H and ^{13}C NMR 1D and 2D spectra were recorded by a Bruker DRX-500 spectrometer (Bruker, Rheinstetten, Germany) at 20 °C. Chemical shift was expressed in ppm. Acetone (^1H 2.22 ppm, ^{13}C 30.89 ppm) was used to calibrate the chemical shift.

2.8. Determination of molecular weight

The molecular weight of polysaccharides were determined by using high-performance gel permeation chromatography (HPLC). A Shimadzu LC-20A HPLC instrument equipped with a refractive index detector was used. Tandem linked G6000PWXL and G3000PWXL columns (Tosoh Bioscience, Stuttgart, Germany) were used for polysaccharides separation. Dextran standards with molecular weights of 0.18–2457 kDa were applied for calibration.

2.9. Tumour cell proliferation assay

The assay was conducted following the protocol of Wen et al. (2014) with modifications. Three tumour cell lines, including HepG2 liver carcinoma cell, Siha cervica carcinoma cell and MDA-MB-231 breast carcinoma cell, were seeded at a density of 2.5×10^4 cells/well on a 96-well microplate. One hundred microlitres of the cell suspension were added. The 96-well microplate was incubated at 37 °C for 4 h. The medium was removed, and 100 μl of fresh medium containing BPCa (0.1–2.0 mg/ml) were added. Fresh medium without polysaccharides was used as control. The plates were incubated for 96 h at 37 °C. The medium was removed and the cells were incubated for 1 h at 37 °C after addition of 50 μl of methylene blue to each well. The staining solution was removed,