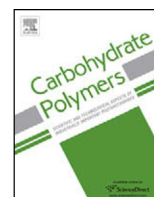
Carbohydrate Polymers 102 (2014) 481–488

Contents lists available at ScienceDirect

Carbohydrate Polymers

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



Analysis of compositional monosaccharides in fungus polysaccharides by capillary zone electrophoresis

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ARTICLE INFO

Article history:
Received 26 September 2013
Accepted 27 November 2013
Available online 6 December 2013

Keywords:
Termitomyces albuminosus
Panus giganteus
Polysaccharides
Monosaccharides
Capillary zone electrophoresis

ABSTRACT

A rapid analytical method of capillary zone electrophoresis (CZE) was established for the simultaneous separation and determination of 10 monosaccharides (aldoses and uronic acids). The monosaccharides were labeled with 1-phenyl-3-methyl-5-pyrazolone (PMP), and subsequently separated using an uncoated capillary (50 μm i.d. × 58.5 cm) and detected by UV at 245 nm with pH 11.0, 175 mM borate buffer at voltage 20 kV and capillary temperature 25 °C by CZE. The 10 PMP-labeled monosaccharides were rapidly baseline separated within 20 min. The optimized CZE method was successfully applied to the simultaneous separation and identification of the monosaccharide composition in *Termitomyces albuminosus* polysaccharides (TAPs) and *Panus giganteus* polysaccharides (PGPs). The quantitative recovery of the component monosaccharides in the fungus polysaccharides was in the range of 92.0–101.0% and the CV value was lower than 3.5%. The results demonstrate that the proposed CZE method is precise and practical for the monosaccharide analysis of fungus polysaccharides.

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1. Introduction

The nutritional properties and health benefits of edible or medicinal mushrooms have been recognized in China for over 2000 years (Dong, Cai, & Li, 2010; Mau, Chang, Huang, & Chen, 2004; Sadler, 2003). Interestingly, the active constituents that are involved in these effects of edible fungi mushrooms are considered to be polysaccharides, which possess numerous pharmacological effects on human physiology, such as satiety, gastric emptying, the regulation of blood glucose, insulin and cholesterol, as well as the balance of intestinal microflora (Adebayo-Tayo, Adegoke, Okoh, & Ajibesin, 2010; Lindequist, Niedermeyer, & Jülich, 2005; Mavundza et al., 2010). In recent years, rare edible fungi *Termitomyces albuminosus* and *Panus giganteus* used in popular medicines have been reported to contain water-soluble polysaccharides with a great variety of biological activities, including obvious antiviral and anticancer effects (Jong & Donovick, 1989; Lu et al., 2008; Wong et al., 2012). For these reasons, there has been great interest in reliable analytical methods for fungi polysaccharides, which can be used for routine quality control of commercial fungi mushrooms and their related polysaccharide products.

Monosaccharides are the most basic units of biologically important macromolecular polysaccharides. An understanding of the conjunct monosaccharides that frequently occur in natural polysaccharides can serve as the identification of basal chemical characteristic of commercial polysaccharides (Wang et al., 2012). However, the lack of chromophores or fluorophores in the structure of monosaccharides limits the modes of detection. High performance liquid chromatography (HPLC) with refractive index detection, and other related chromatographic methods do not often meet the demands of modern trace level analysis with regard to sensitivity and/or selectivity (Fu, Huang, Zhai, Li, & Liu, 2007; Wang & Fang, 2004). Therefore, the derivatisation of monosaccharides is indispensable to obtain highly sensitive detection (Honda, Akao, et al., 1989; Lv et al., 2009). The reagent 1-phenyl-3-methyl-5-pyrazolone (PMP) is one of the popular labels that may react with reducing carbohydrates under mild conditions, requiring no acid catalysts and causing no desialylation and isomerization (Zhang, Xu, Zhang, Zhang, & Zhang, 2003). Recently, we have described a capillary zone electrophoresis (CZE) method for separation of 8 monosaccharides, where the introduction of PMP into the reducing monosaccharide molecules can be easily achieved by chelation of monosaccharides with a suitable ion of borate (Hoffstetter-Kuhn, Paulus, Gassmann, & Widmer, 1991; Honda, Iwase, Makino, & Fujiwara, 1989; Nguyen, Lerch, Zemann, & Bonn, 1997; Stefansson & Novotny, 1993). The CZE is shown to be a powerful separation technique which provides high-resolution, high efficiency, good selectivity, and short analysis time, and is becoming a standard tool for the analysis of many compounds (Cortacero-Ramírez, Segura-Carretero, Cruces-Blanco, & Fernández-Gutiérrez, 2003; El Rassi,

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http://dx.doi.org/10.1016/j.carbpol.2013.11.054

1999; Morales, 2002; You et al., 2008). Although methods for the separation of the sugars in samples have been examined by numerous researchers, only 7–8 monosaccharides were usually baseline separated, which may significantly affect the analytical accuracy (Gao, Araiab, Lecka, & Emmerb, 2010; Rovio, Simolin, Koljonen, & Siren, 2008; Santos, Duarte, & Esteves, 2007). Therefore, potential CZE methods need to be further examined for the accurate identification of the 10 reductive monosaccharides possibly present in plant polysaccharides.

The aim of this study was therefore to develop a rapid and simple CZE analytical method for the simultaneous, efficient separation and determination of ten reducing monosaccharides (aldoses and uronic acids) possibly found in natural polysaccharides. This CZE method was successfully applied to the qualitative and quantitative analysis of the compositional monosaccharides of the polysaccharides derived from rare edible fungi *T. albuminosus* and *P. giganteus*.

2. Materials and methods

2.1. Materials and chemicals

The *T. albuminosus* and *P. giganteus* were purchased from local market of China, and identified according to the identification standard of Pharmacopeia of the People's Republic of China (2010 edition). Voucher specimens of the fungi mushroom materials were deposited at the School of Pharmacy, Fourth Military Medical University, China. The fungi mushrooms were thoroughly washed with water, air-dried and finely pulverized into a powder. D-Mannose, D-ribose, D-glucose, L-rhamnose, D-glucuronic acid, D-galacturonic acid, D-xylose, D-galactose, L-arabinose and D-fucose were obtained from Merck (Darmstadt, Germany). 1-Phenyl-3-methyl-5-pyrazolone (PMP) and trifluoroacetic acid (TFA) were the products of Beijing Reagent Plant (Beijing, China). HPLC grade methanol was purchased from Honeywell (USA). Water was purified on a Milli-Q system (Millipore, Bedford, MA). All of the other chemicals were of analytical-grade.

2.2. Extraction of the fungus polysaccharides

The fungi polysaccharides were isolated by hot-water extraction and ethanol precipitation as previously described (Tian, Zhao, Guo, & Yang, 2010). Briefly, the dried *T. albuminosus* and *P. giganteus* were defatted with 95% alcohol and then extracted three times with distilled water (w/v, 1:10) at 80 °C, 3 h each time. The combined extracts were pooled and concentrated under a reduced pressure and then centrifuged at 3000 × *g* for 15 min. The supernatant was collected and 95% alcohol was added slowly by stirring to precipitate the polysaccharide and adjusted to 75% alcohol, and then kept at 4 °C for 24 h. The polysaccharide pellets were obtained by centrifugation at 4000 × *g* for 15 min and repeatedly washed sequentially with ethanol, acetone and ether, respectively. The refined polysaccharide pellets were completely dissolved in appropriate volume of distilled water and intensively dialyzed for 3 days against distilled water (cut-off Mw 8000 Da), and then the retentate portion was deproteinized by a freeze-thaw process (FD-1, Henan Yuhua Instrument Co., China) repeated 10 times followed by filtration. Finally, the filtrate was lyophilized to yield brownish water-soluble polysaccharides.

2.3. Hydrolysis of the polysaccharides

Hydrolysis of the polysaccharides was performed according to our previous procedure (Lv et al., 2009). 18.6 mg of the analyte of *T. albuminosus* polysaccharides (TAPs) or 21.3 mg of *P. giganteus* polysaccharides (PGPs) was placed in 2 mL of 2 M TFA in an ampoule (10 mL). The ampoule was sealed under a nitrogen atmosphere and then kept in an oil bath at 110 °C for 8 h to hydrolyze the polysaccharide into component monosaccharides. The resultant mixture was cooled to room temperature, followed by a centrifugation at 1000 × *g* for 5 min. The supernatant was collected and dried to remove the excess TFA under a stream of nitrogen gas, and then 1.0 mL of distilled water was added for the further experiments.

2.4. PMP derivatization procedures for reducing monosaccharides

The PMP derivatization procedure was carried out as described previously (Yang, Zhao, Wang, Wang, & Mei, 2005). Briefly, 20 μL of standard monosaccharides (aqueous) or the monosaccharide hydrolysate of the polysaccharides was spiked with 400 μL of 0.3 M aqueous NaOH and 400 μL 0.5 M PMP-methanol solution. Galacturonic acid was added as an internal standard to each sample before the derivatization. After the solution was shaken for 10 s, the mixture was allowed to react for 30 min at 70 °C, and then cooled to room temperature and neutralized with 400 μL of 0.3 M HCl. The resulting solution was extracted with chloroform, and the organic phase was discarded, and the extraction process was repeated for 3 times. Finally, the aqueous layer was filtered through a 0.22 μm membrane for CZE analysis. The reagent solution was freshly prepared before derivatization.

2.5. Preparation of buffer and standard solution

The stock solution of 400 mM boric acid was prepared in water. The running buffers were prepared by diluting the stock solution to the appropriate concentrations and were adjusted to the desired pH values with sodium hydroxide (NaOH). The stock solution of standard monosaccharides (10 mM) were prepared and diluted with ultrapure water. Both of the buffer and sample solution were filtered through a 0.45 μm membrane filter and degassed by ultrasonic apparatus for 2 min before use. All the solutions prepared were stored in the dark at 4 °C until use.

2.6. Apparatus and electrophoretic procedures

Separation and analysis were performed on a P/ACE MDQ capillary electrophoresis instrument (Beckman-Coulter, Fullerton, CA, USA) equipped with a UV detector and an automatic injector. Data acquisition and processing were carried out with Beckman System Gold software. All fused-silica capillary tubes have an internal diameter of 50 μm with effective length of 48.5 cm in total length of 58.5 cm. The capillary (from Yongnian Optical Fiber Factory, Hebei) was conditioned prior to the first use by sequentially rinsing with 1 M NaOH, 0.1 M HCl, and ultrapure water for 20 min. At the start of each sequence, the capillary was washed with water for 5 min, 0.1 M HCl for 5 min, 0.1 M NaOH for 10 min and water for 5 min. Between analyses, the capillary was rinsed with 0.1 M NaOH for 3 min followed by water for 2 min and then equilibrated with running buffer at a pressure of 20 psi for 3 min. Samples were introduced into the capillary by pressure injection mode for 5 s at 0.5 psi. At the end of the working day, the capillary was washed with water for 5 min and the capillary end dipped in a vial containing water. Absorbance was detected with a UV detector set at 245 nm.

3. Results and discussion

3.1. Optimization of CZE separation of PMP-monosaccharides

3.1.1. Effects of buffer pH on separation

It is well known that the CZE separation of carbohydrates with vicinal hydroxyl groups is best performed in alkaline borate buffer, where the analytes can be transformed into negatively charged

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