Carbohydrate Polymers 92 (2013) 345–352

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

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

Pectic polysaccharide from immature onion stick (*Allium cepa*): Structural and immunological investigation

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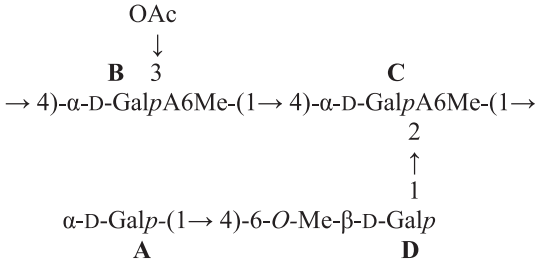
ARTICLE INFO

Article history:
Received 13 June 2012
Received in revised form 7 September 2012
Accepted 10 September 2012
Available online 17 September 2012

Keywords:
Immature onion stick (*Allium cepa*)
Pectic polysaccharide
Structure
NMR Spectroscopy
Immunological study

ABSTRACT

The structure of a water-soluble pectic polysaccharide (PS) isolated from immature onion stick (*Allium cepa*) was investigated using acid hydrolysis, methylation analysis, periodate oxidation study, and NMR studies (^{1}H, ^{13}C, DQF-COSY, TOCSY, NOESY, ROESY, HSQC, and HMBC). The results of the above experiments indicated that the PS contained D-galactose, 6-*O*-Me-D-galactose, 3-*O*-acetyl-D-methyl galacturonate and D-methyl galacturonate in a molar ratio of nearly 1:1:1:1 and possesses a backbone of [→4)-α-D-Gal*p*A6Me-(1 → 4)-α-D-Gal*p*A6Me-(1→] in which one methyl galacturonate was substituted at *O*-3 position by an acetyl group and the neighboring methyl galacturonate being substituted at *O*-2 with a side chain, α-D-Gal*p*-(1 → 4)-6-*O*-Me-β-D-Gal*p*-(1→. The probable structure of repeating unit of the pectic polysaccharide was established as:



The pectic polysaccharide showed in vitro splenocyte, thymocyte as well as macrophage activations.

1. Introduction

Pectic polysaccharides, a component of dietary fiber (Lunn & Buttriss, 2007) in fruits and vegetables, are excellent gelling and thickening agents for the production of jams and jellies in food industries and also display different pharmaceutical activities (Voragen, Pilnik, Thibault, Axelos, & Renard, 1995). They exhibit anti-inflammatory activity by oral administration (Ovodova et al., 2009; Popov, Popova, Ovodova & Ovodov, 2005; Popov et al., 2007) and prevent hyperlipidemia, as well as bowel cancer (Lim, Yamada, & Nonaka, 1998; Willats, McCartney, Mackie, & Knox, 2001). Ginseng pectin decreases blood glucose levels in normal and hyperglycemic mice (Konno, Sugiyama, Kano, Takahashi, & Hikino, 1984; Suzuki & Hiking, 1989), inhibit tumor growth and metastasis (Kim, Kang, & Kim, 1990; Shin et al., 2004), modulate the immune system (Du, Jiang, Wu, Won, & Choung, 2008; Han, Song, Yun, & Yi, 2005) and also protect animals from the lethal effects of ionizing radiation (Kim et al., 2007; Song et al., 2003). The branching and presence of acetyl groups on galacturonic acid are very important for the expression in bioactivities (Kravtchenko, Penci, Voragen, & Pilnik, 1993). A pectic polysaccharide isolated from the pods of green beans (*Phaseolus vulgaris* L) exhibited immunoenhancing activity as well as antioxidant property (Patra, Das, Behera, Maiti, & Islam, 2012). Immunoenhancing effects on T and B lymphocytes by a pectic polysaccharide, isolated from the stems of *Dendrobium nobile* was also reported (Wang, Luo, & Zha, 2010). People all over the India consume immature onion stick as a

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http://dx.doi.org/10.1016/j.carbpol.2012.09.013

delicious vegetable mainly at winter seasons. The presence of diallyl sulfide and flavonoid compounds in onion bulbs exhibit different pharmacological effects like inhibition of tumor and microbial cells, scavenging of free radicals and protection of cardiovascular diseases (Block, Calvey, Gillies, Gillies, & Uden, 1997; Elattar & Virji, 1999; Jang et al., 1997; Stavric, 1997). Onion bulbs promote bile production; reduce sugar and lipid levels (Augusti, 1990). It is reported that onion bulbs (Golovchenko, Khramova, Ovodova, Shashkov, & Ovodov, 2012) contained pectic polysaccharide in which the main constituents of the linear regions are joined by galacturonan and rhamnogalacturonan. Oxalate-citrate extracted fraction of onion cell walls was reported to compose of a range of pectic polysaccharides with varying proportions of neutral side-chains (Mankarios, Hall, Jarvis, Threlfall, & Friend, 1980). The present work is devoted to the elucidation of the detailed structural features and immunological activities of a pectic polysaccharide (PS) isolated from immature onion stick (*Allium cepa*) and reported herein.

2. Materials and methods

2.1. Isolation, fractionation, and purification of the crude pectic polysaccharide

The immature onion sticks (600 g) were collected from the local market, washed with water, cut into small pieces and boiled in 300 ml of distilled water for 8 h. The whole mixture was kept overnight at 4 °C and filtered through linen cloth. The filtrate was centrifuged at 8000 rpm (using a Heraeus Biofuge stratos centrifuge) for 1 h at 4 °C and supernatant was collected and precipitated in ethanol (1:5, v/v). It was kept overnight at 4 °C and again centrifuged as above. The precipitated material was washed five times with ethanol and then freezes dried followed by dialysis through cellulose membrane (Sigma–Aldrich, retaining > M_w 12,400) against distilled water for 36 h to remove low-molecular weight materials. The aqueous solution was then collected from the dialysis bag and freeze-dried, to yield crude polysaccharide (1.5 g).

The crude polysaccharide (30 mg) was purified by gel permeation chromatography on column (90 cm × 2.1 cm) of Sepharose 6B in water as eluant with a flow rate of 0.5 mL/min using Redifrac fraction collector. Forty five test tubes (2 mL each) were collected and monitored spectrophotometrically at 490 nm with phenol-sulfuric acid reagent (York, Darvill, McNeil, Stevenson, & Albersheim, 1985) using Shimadzu UV-VIS spectrophotometer, model-1601. A single fraction was collected and freeze-dried, to yield 12 mg pure polysaccharide (PS). The purification process was carried out in several lots and 95 mg PS was collected.

2.2. Monosaccharide analysis

2.2.1. Alditol acetate analysis

4.0 mg PS was hydrolyzed with 2 M CF_3COOH (2 mL) in a round-bottom flask at 100 °C for 18 h in a boiling water bath. The excess acid was completely removed by co-distillation with water. Then, the hydrolyzed product was divided into two parts. One part was examined by paper chromatography (Hoffman, Lindberg, & Svensson, 1972) in solvent systems *X* and *Y*. Another part was reduced with $NaBH_4$, followed by acidification with dilute CH_3COOH. It was then co-distilled with pure CH_3OH to remove excess boric acid. The reduced sugars were acetylated with 1:1 pyridine–acetic anhydride in a boiling water bath for 1 h to give the alditol acetates, which were analyzed by GLC and GLC–MS using Hewlett-Packard 5970A automatic GLC–MS system, fitted with an HP-5 capillary column (25 m × 25 mm). The program was isothermal at 150 °C; hold time 2 min, with a temperature gradient of 4 °C/min up to a final temperature of 200 °C. Quantification was carried out from the peak area, using response factors from standard monosaccharide's using inositol as standard.

2.2.2. Preparation of carboxyl reduced polysaccharide

PS (3.5 mg) was dissolved in 1 M imidazole–hydrochloric acid buffer, pH 7.0 (200 μL/mg) and cooled on ice. Sodium borohydride (40 mg) was then added and reaction mixture was maintained on ice for at least 1 h. The excess borohydride was decomposed by adding glacial acetic acid (100 μL/40 mg borohydride) slowly to the cooled sample. An equal volume of redistilled water was then added, and the reduced PS was precipitated by adding 3–4 volume of 95% (v/v) ethanol (2 mL). The sample was reprecipitated two more times with 95% ethanol, centrifuged and the sedimented material was freeze-dried. The carboxyl-reduced PS (Maness, Ryan, & Mort, 1990) was hydrolyzed with 2 M CF_3COOH for 18 h at 100 °C, and after usual treatment, the sugars were analyzed by GLC and GLC–MS on a Hewlett-Packard Model 5730A.

2.3. Methylation analysis

PS (6.0 mg) was methylated using the procedure described by Ciucanu and Kerek (1984) method. The methylated products were isolated by partition between $CHCl_3$ and H_2O (5:2, v/v). The organic layer containing products was washed with water for several times by taking 5 mL of water in each time and then dried. The methylated product was then hydrolyzed with 90% formic acid (1 mL) at 100 °C for 1 h, and excess HCOOH was evaporated by co-distillation with distilled water. The hydrolyzed product was then reduced with sodium borohydride, acetylated with (1:1) acetic anhydride-pyridine, and then the alditol acetates of the methylated sugars were analyzed by GLC (using columns A and B) and GLC–MS (using HP-5 fused silica capillary column).

Another portion of the methylated product (2.0 mg) was dissolved in dry THF (2 mL) and refluxed with lithium aluminium hydride (LAH) (Abdel-Akher & Smith, 1950) (40 mg) for 5 h, and kept overnight at room temperature. The excess of the reducing agent was decomposed by drop wise addition of ethyl acetate and aqueous THF. The inorganic materials were filtered off. The filtrate was evaporated to dryness giving the permethylated carboxyl-reduced product. The product was hydrolyzed with formic acid as before, and the alditol acetates of the methylated, carboxyl-reduced sugars were prepared, and analyzed by GLC and GLC–MS.

2.4. Periodate oxidation

The PS (6.0 mg) was oxidized by 2 mL 0.1 M sodium metaperiodate at room temperature in the dark for 48 h. The excess of $NaIO_4$ was destroyed by adding of ethylene glycol, and the solution was dialyzed against distilled water. The dialyzed material was reduced by $NaBH_4$ for 15 h and treated with acetic acid to make the solution slightly acidic. The resulting material was obtained by co-distillation with methanol. The periodate-oxidized material (Goldstein, Hay, Lewis, & Smith, 1965; Hay, Lewis, & Smith, 1965) was divided into three portions. One portion was hydrolyzed with 2 M CF_3COOH for 18 h, and alditol acetate was prepared as usual. Another portion was methylated by the method of Ciucanu and Kerek (1984), followed by preparation of alditol acetates which were analyzed by GLC and GLC–MS. Another portion was reduced by LAH and then kept with 0.5 M CF_3COOH for 48 h at room temperature. The acid was removed and the hydrolyzed material was analyzed by GLC (as alditol acetates).