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Structural features of pectic polysaccharide from *Angelica sinensis* (Oliv.) Diels

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ABSTRACT

The structure of the pectic polysaccharide (ASP3) isolated from roots of *Angelica sinensis* (Oliv.) Diels was investigated using partial acid hydrolysis, enzymic digestion combined with methylation analysis, and further supported by ^{1}H and ^{13}C NMR spectroscopy techniques. The results indicated that ASP3 contained a backbone of linear homogalacturonan fragments as "smooth regions" and rhamnogalacturonan fragments as "hairy regions" with repeating unit of [→4)-α-D-Gal*p*A-(1 → 2)-α-L-Rha*p*-(1 →]. A total of 58.8% rhamnopyranose residues in the backbone were substituted at *O*-4 position by the side chains. The side chains contained mainly β-1,6- and β-1,4-galactopyranan bearing 3,6- and 4,6-substituted β-D-galactopyranose residues as branched points and short α-1,5-arabinofuranan possessing 3,5-substituted α-L-arabinofuranose residues as branching points. In addition, β-1,6-galactopyranan side chains were highly branched with α-1,5-arabinofuranan carrying 3-*O*-substituents (1,3,6-Gal) and terminated by the α-arabinofuranose residues which form arabinogalactan.

1. Introduction

Pectins are a family of complex heterogeneous polysaccharides that constitute a large proportion of the cell wall of many higher plants where greatly influence growth, development and senescence (O'Neill, 1990; Ridley, O'Neil, & Mohnen, 2001). Pectins are also traditional gelling and thickening agents for the production of jams and jellies, and the area of the use extends to the production of fruit, dairy, and dessert products and pharmaceuticals (Thakur, Singh, & Handa, 1997; Voragen, Pilnik, Thibault, Axelos, & Renard, 1995).

In recent years, pectin is increasingly recognized as an important precursor of substrates improving gastrointestinal functions. It plays an important role in the regulation of some physiological processes and therefore in the prevention of hyperlipidemia, as well as bowel cancer (Lim, Yamada, & Nonaka, 1998; Willats, McCartney, Mackie, & Knox, 2001). Earlier, we isolated a pectic polysaccharide named ASP3 from roots of *Angelica sinensis* (Sun, Tang, Gu, & Li, 2005) which is a well-known oriental herb (Zhang & Cheng, 1989). The sugar chain of ASP3 was found to contain residues of galacturonic acid, arabinose, galactose, and rhamnose as the main constituents. Our previous study has shown that ASP3 can protect leucocytes and lymphocytes of mice against radiation-induced damage, which has potential radioprotective effect on acute radiation injured mice.

According to reports, many of the bioactivities of pectins from various sources have been shown to have a relationship with complex branched structures (Wang, Dong, Zuo, & Fang, 2003; Yamada, 1994; Yu, Kiyohara, & Matsumoto, 2001), so elucidation of molecular fine structural features of the pectic substances is necessary for understanding the mechanism of physiological activity and clarifying the structure–activity relationships.

The primary structure of pectin obtained from various sources has been studied extensively by methods of partial chemical or enzymatic degradation (Bushneva, Ovodova, Shashkov, Chizhov, & Ovodov, 2003; Dong & Fang, 2001; Habibi, Mahrouz, & Vignon, 2005; Polle, Ovodova, Chizhov, Shashkov, & Ovodov, 2002a; Polle, Ovodova, Shashkov, & Ovodov, 2002b; Singthong & Cui, 2004). However, the precise chemical structure of pectin remains under debate, although the structural elements of pectin are rather well described (Coenen, Bakx, Verhoef, Schols, & Voragen, 2007). The present work is devoted to further elucidation of the detailed structural features of the pectic polysaccharide ASP3 from *A. sinensis* using mild acid hydrolysis and enzymic digestion followed by NMR spectroscopy and methylation analysis of fragments obtained.

2. Experimental

2.1. Plant materials and preparation of ASP3

The roots of *A. sinensis* (Oliv.) Diels, cultivated in Minxian County, Gansu Province, China, were provided by Shanhe

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Pharmaceutical Co. Ltd. (Wuxi, China). Isolation followed by purification of the pectic polysaccharide ASP3 from roots of *A. sinensis* was performed as described earlier (Sun et al., 2005).

2.2. General methods

Uronic acid content was determined by photometry with *m*-hydroxybiphenyl at 520 nm (Blumenkrantz & Asboe-Hansen, 1973), using D-galacturonic acid as standard. Total neutral sugar content was determined by the reaction with phenol in the presence of sulfuric acid using galactose and arabinose as standards. A correction was made for the response of galacturonic acid in the neutral sugar test. Neutral sugar composition was analyzed by GC after conversion of the hydrolysate into alditol acetates, as described earlier (Sun et al., 2005). The percentage of monosaccharides in the sample was calculated from the peak areas using response factors. The specific optical rotation was determined in H_2O at 25 °C using a WZZ-2A polarimeter.

2.3. Partial acid hydrolysis

Fragment ASP3 (150 mg) was hydrolyzed with 0.2 M trifluoroacetic acid (TFA) for 1 h at 121 °C. After cooling, TFA was evaporated under a stream of N_2. The hydrolysate was dissolved in distilled water and dialyzed against distilled water (M_w cut-off 3500 Da). The retentate and dialysate were concentrated and purified respectively on size exclusion chromatography (SEC) of Sepharose CL-6B column (*D*1.0 × 120 cm, Amersham Bioscience) at room temperature and eluted with degassed distilled water (12 mL/h). A purified high-molecular weight fraction (ASP3-PH) and a low-molecular weight fraction (ASP3-PL) were collected, concentrated and then lyophilized.

2.4. Enzymic hydrolysis

Fragment ASP3 (100 mg) was dissolved in 24 mL of water, and 1 M NaOH (6 mL) was added. The solution was kept for 2 h at 25 °C. Excess alkali was neutralized with acetic acid to pH 5.5. Mould endo-α-(1,4)-polygalacturonase (EndoPG, EC 3.2.1.15, Fluka – 467 U/g) was added and the mixture was incubated at 30 °C for 72 h. The enzyme was inactivated by heating at 100 °C for 10 min and the denatured protein was removed by centrifugation. The digestion product obtained was concentrated and subjected to Sepharose CL-6B column (*D*1.0 × 120 cm, Amersham Bioscience) to give an enzyme-resistant fraction (ASP3-EH) and an enzyme-sensitive fraction (ASP3-EL). Fractions were collected, concentrated and then lyophilized.

2.5. Determination of the glycosidic linkage composition

The glycosidic linkage analysis was determined by methylation and gas chromatography–mass spectroscopy (GC–MS). Prior to methylation, the sample containing uronic acid was reduced to the corresponding neutral sugar by using 1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide methyl-*p*-toluenesulfonate (CMC, Fluka) and sodium borodeuteride ($NaBD_4$, Acpos), following a procedure described by Taylor and Conrad (1972) and York, Darvill, McNeil, Stevenson, and Albersheim (1986) with slight modification (Cui, 2005). After this reduction, methylation analysis was carried out according to the method of Ciucanu and Kerek (1984) with slight modification to give a fully methylated product (Cui, 2005). The methylated product was then converted into partially methylated alditol acetates (PMAA) by hydrolysis, reduction with $NaBH_4$, and acetylation followed by linkage analysis using GC–MS (OV1701 capillary column, 0.25 mm × 30 m, 0.25 mm film thickness coupled to a Trace Mass Spectrometer, Finnigan). The carrier gas was helium; 3 °C/min gradient from 150 to 250 °C. The temperature of the interface was 250 °C; Energy of ionizing electrons was 70 eV. Peak identification was based on retention times using partially methylated alditol acetates as standards. The percentage of the methylated sugars was estimated as ratios of the peak areas (total ion current).

2.6. ^1H and ^{13}C NMR spectroscopy

The polysaccharide samples were exchanged 3 times in D_2O (at concentrations of approximately 40 mg/mL), with intermediate freeze-drying. Finally, samples were dissolved in D_2O. ^1H and ^{13}C NMR spectroscopy were performed on a Brucker AMX500 NMR spectrometer (Germany) using standard pulse sequences with 5 and 10 mm tubes at 65 °C. Internal 1,4-dioxane was used as an internal chemical-shift reference for spectra. Two-dimensional spectra (COSY-45, TOCSY, and HMQC) were recorded using the standard Bruker procedures (Cui, Eskin, Biliaderis, & Marat, 1996).

3. Results and discussion

3.1. Preparation of ASP3

The pectic polysaccharide named ASP3 has been isolated and purified from roots of *A. sinensis* as described earlier (Sun et al., 2005). The sugar composition of ASP3 was listed in Table 1.

3.2. Partial acid hydrolysis of ASP3

Partial degradation of polysaccharides by acid hydrolysis is based on the fact that some glycosidic linkages are more labile to acid than others. For example, linkages between neutral sugars are the most susceptible to acid hydrolysis; hence controlled acid hydrolysis is frequently used to remove neutral sugars (Coenen et al., 2007). To study the linkages between backbone and side chains of polysaccharide, the native polysaccharide ASP3 was partially hydrolyzed with 0.2 M TFA. Partial acid hydrolysis resulted in two subfractions of ASP3: high-molecular (ASP3-PH) and low-molecular (ASP3-PL) ones. The sugar composition of fractions was given in Table 1. It showed that for the polymer fraction ASP3-PH, the amount of arabinose, galactose, mannose and glucose decreased considerably compared with ASP3, whereas the amount of rhamnose and galacturonic acid increased, suggesting that linkages between two GalA sugars are more stable then aldobiuronic linkages (GalA-Rha) or pseudo-aldobiuronic (Rha-GalA) sugars and linkages between neutral sugars are most susceptible to acid hydrolysis. ASP3-PH, yield 70% of the parent ASP3, composed of galacturonic acid (76.5%), rhamnose (2.7%), galactose (19.2%) and small amount of arabinose (0.2%), suggesting the presence of a typical homogalacturonan and rhamnogalacturonan substituted by the side chains of mainly galactosyl and arabinosyl residues.

For the oligomer fraction ASP3-PL, the sugar composition presented in Table 1 showed that the neutral sugars (arabinose, galactose, mannose and glucose) were the main constituent. These results confirmed that galacturonic acid and rhamnose present in the backbone which were not capable of mild hydrolysis, whereas the neutral sugars were attached to the side chains and easy to be hydrolyzed.

The subfraction ASP3-PH possessed higher positive rotation $[\alpha]_D^{25}$ + 194° than that of the raw fraction ASP3 (+131°) measured at the same conditions (*c* 0.1; H_2O; 25 °C) due to the prevalence of D-Gal*p*A, the main constituent of ASP3-PH.