Carbohydrate Polymers 163 (2017) 330–336

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Isolation of a lectin binding rhamnogalacturonan-I containing pectic polysaccharide from pumpkin

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ARTICLE INFO

Article history:
Received 3 December 2016
Received in revised form 2 January 2017
Accepted 18 January 2017
Available online 21 January 2017

Keywords:
Pectin
Rhamnogalacturonan-I
Pumpkin residues
Lectin
NMR

ABSTRACT

A rhamnogalacturonan-I (RG-I) containing pectic polysaccharide (PPc) was isolated from pumpkin following a low-temperature alkali treatment and a combination of gradual alcohol precipitation and ion-exchange. Monosaccharide compositional analysis of PPc revealed the presence of rhamnose, galacturonic acid, galactose, and arabinose in a molar ratio of 7.4: 25: 28: 2.6. Structural and linkage analysis by 1D NMR (^{1}H NMR and ^{13}C NMR), and 2D NMR (COSY, TOCSY, HSQC, and elevated temperature HMBC) suggested that PPc was a RG-I-like pectic polysaccharide, branched at the C-4 of some of the (about 29% of) rhamnosyl units, with relatively long β-1,4-D-galactan side chains to which were attached, through the C-3 of β-D-Gal, terminal non reducing α-Ara*f* units. The results of surface plasmon resonance (SPR) show that PPc binds to two types of lectin, *Ricinus communis* agglutinin 120 (RCA_{120}) and Galectin-3 (Gal-3). These binding studies show quick association and slow dissociation with a moderate binding affinity between PPc and Gal-3 of 1.26 μM. The interaction between PPc and Gal-3 suggest the potential use of pumpkin pectic polysaccharide as a Gal-3 inhibitor in functional food or drug development applications.

1. Introduction

Pectins are complex galacturonic acid-rich polysaccharides that are widely distributed in plants and have recently received considerable attention due to their potential bioactivities (Maxwell, Belshaw, Waldron, & Morris, 2012). Pectin is composed of distinct structural elements, which differ between plants and vary within individual tissues of the same plant. The most common elements include homogalacturonan (HG), rhamnogalacturonan I (RG-I), and substituted galacturonans, such as rhamnogalacturonan II (RG-II) and xylogalacturonan (XGA) (Mohnen, 2008). The linear HG portions tend to be partly methylesterified and/or acetylated forming the 'smooth' domains of pectin. The HG sections can be interspersed with RG elements, in which RG-I often bears galactosyl, arabinosyl and arabinogalactosyl side chains of different length, while RG-II contains some rare sugars and complex oligosaccharide chains (Caffall & Mohnen, 2009; Maxwell et al., 2012).

Pectin has been extracted from pumpkin fruit (Košťálová, Hromádková, & Ebringerová, 2013), pumpkin peel (Jun, Lee, Song, & Kim, 2006), and also from unutilized pumpkin biomass (Košťálová, Aguedo, & Hromádková, 2016). An acidic polysaccharide isolated from pumpkin by Yang, Zhao, & Lv (2007), suggested to exhibit potential cytoprotective and antioxidative activity, was composed of glucose (21.7%), galacturonic acid (18.9%), galactose (11.5%), arabinose (9.8%), xylose (4.4%), and rhamnose (2.8%). Košťálová, Hromádková, & Ebringerová (2010) isolated different pectin products from the seeded fruit of oil pumpkin and these polysaccharides

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http://dx.doi.org/10.1016/j.carbpol.2017.01.067
0144-8617/

mainly contain galactose, glucose, mannose, xylose, fucose and rhamnose as neutral sugars. It appears that pectins derived from different varieties of pumpkin share most of these neutral sugars. The presence of galactose, arabinose and rhamnose suggest that pumpkin pectin might contain an RG-I domain.

Lectins are specific carbohydrate-binding proteins that can bind to cell membranes and, thus, influence cell–cell interactions (Gabor, Bogner, Weissenboeck, & Wirth, 2004). Lectins have been explored as cell-surface biosensors and as therapeutic targets in a number of diseases. *Ricinus communis* agglutinin 120 (RCA_{120}) is a lectin that recognizes a non-reducing terminal β-D-galactose and is used as probe to detect and target specific oligosaccharide sequences (Spain & Cameron, 2011). Galectin-3 (Gal-3) is a β-galactoside-binding lectin associated with cancer, cardiovascular diseases and fibrosis, which has been reported to interact with RG-I domain of pectin (Boer, Voors, Muntendam, Gilst, & Veldhuisen, 2009; Li, Li, & Gao, 2014). Gal-3 is characterized by a carbohydrate recognition domain (CRD) that naturally binds to specific carbohydrate molecular patterns of molecular receptors, inducing cell adhesion, migration, transformation, and apoptosis (Vladoiu, Labrie, & St-Pierre, 2014). The galactan side chain of RG-I pectin can occupy the binding site of Gal-3 and, thus, inhibits the activity of Gal-3 (Gao et al., 2013). There have been studies showing that pectic polysaccharides with higher arabinose and galactose content significantly inhibit hemagglutination (Sathisha, Jayaram, Nayaka, & Dharmesh, 2007). Modified citrus pectin (Kolatsi-Joannou, Price, Winyard, & Long, 2011), ginseng pectin (Gao et al., 2013), and potato pectin (Maxwell et al., 2015) have been identified to have Gal-3 inhibitory effects. Gal-3 lectin binding activity has not been reported for pectic polysaccharides from pumpkin.

In this study, a pectic polysaccharide isolated from pumpkin residues was found to contain an RG-I domain. The pumpkin residues were insoluble after hot water extraction, the standard approach used in producing pumpkin polysaccharides. The insoluble residues of pumpkin were rich in pectic polysaccharide as indicated by the monosaccharide compositional analysis. Thus, a low-temperature alkali treatment was used to recover additional pectic polysaccharide from the insoluble fraction. Size-exclusion chromatography (SEC), monosaccharide compositional analysis, Fourier-transform infrared (FT-IR) and nuclear magnetic resonance (NMR) spectroscopy were utilized for the characterization of the alkali-extracted pectic polysaccharide. The lectin binding behavior of RG-I containing pumpkin pectic polysaccharide was studied by surface plasmon resonance (SPR). The results of this study suggests that the pectic polysaccharide from pumpkin residue may represent a potentially safe, non-toxic Gal-3 inhibitor and used in preventing or reducing cancer, carcinogenesis and fibrosis.

2. Experimental

2.1. Pumpkin materials and chemicals

The fresh mature fruit of pumpkin (*Cucurbita moschata*) was cleaned with tap water and the skin and seeds were discarded. Fruit pulp was then cut into small pieces and freeze-dried before use. The pumpkin residues were obtained after hot water extraction of pumpkin polysaccharides as previously described (Song, Zhao, Ni, & Li, 2015). The remaining insoluble pumpkin residue was lyophilized and blended into powder for subsequent extraction. Ammonium acetate and sodium azide were purchased from Fisher Scientific (Springfield, NJ, USA). Monosaccharides standards, 1-phenyl-3-methyl-5-pyrazolone (PMP), and D_2O were all purchased from Sigma (St. Louis, MO, USA). All other used chemicals were of analytical grade.

2.2. Extraction of pectic polysaccharide from pumpkin residues

Isolation of pectic polysaccharide from insoluble pumpkin residue was performed by a low-temperature alkali treatment procedure. Pumpkin residue (50 g) was suspended in 1 M NaOH solution as extracting agent with a solid-liquid ratio of 1/30 (w/v). The mixture was warmed at 50 °C for 4 h with stirring. The supernatant was obtained by centrifugation at 8000 × *g* for 15 min, neutralized, and then concentrated to 200–300 mL by evaporation. The proteins in the extract were removed by Sevag reagent (Navarini et al., 1999). Ethanol was then added to the solution to obtain a final concentration of 80 vol% and the polysaccharide was precipitated at 4 °C for 12 h. The polysaccharide precipitate was recovered by centrifugation at 8000 × *g* for 25 min, dialyzed (membrane cut-off of 1000 Da) and lyophilized. The precipitated polysaccharide was applied to a DEAE Sepharose Fast Flow gel column (2.5 × 8 cm) and eluted by three column volumes of 0, 0.1, 0.2, 0.3, 0.5 M NaCl. Each fraction was collected and precipitated with ethanol, dialyzed (membrane cut-off of 1000 Da) and then lyophilized.

2.3. Homogeneity and molecular weight

The homogeneity and average molecular weight (Mw) of pumpkin pectic polysaccharide were evaluated by SEC using TSK-GEL $G4000SW_{XL}$ (30 cm × 7.8 mm) and $G3000SW_{XL}$ (30 cm × 7.8 mm) in series and a refractive index (RI) detector. The mobile phase was 7.71 g ammonia acetate and 0.2 g sodium azide in 1 L water and a flow rate of 0.6 mL/min (40 °C) was used in SEC. Pumpkin pectic polysaccharide was dissolved in mobile phase (5 mg/mL) and filtered through a 0.45 μm membrane filter. A series of molecular weight standards (1, 5, 10, 25, and 50 kDa) were used. Sample (20 μL) was injected and the data obtained were analyzed by LCsolution Software (Shimadzu Scientific Instruments, MA, USA).

2.4. Monosaccharide composition and degree of esterification (DE)

The monosaccharide composition of pumpkin pectic polysaccharide was analyzed by reversed-phase high performance liquid chromatography (RP-HPLC) equipped with an Agilent XDB-C18 column (4.6 mm × 250 mm) and UV detector after pre-column derivatization as described by Chen et al. (2012) with some modification. Briefly, pumpkin pectic polysaccharide was hydrolyzed with 2 mol/L trifluoroacetic acid (TFA) at 105 °C for 6 h in a sealed tube. These stringent hydrolysis conditions were selected to ensure hydrolysis of resistant glycosidic linkages involving uronic acid residues. Excess acid was removed by co-distillation with methanol (four times) after the hydrolysis was completed. Dry hydrolysate (1 mg) was dissolved in 100 μL of 0.3 mol/L NaOH, and then added to 120 μL of 0.5 mol/L methanol solution of PMP at 70 °C for 1 h. Finally, the mixture was added 100 μL of 0.3 mol/L HC1 solution and vigorously shaken and centrifuged at 5000 × *g* for 5 min. The supernatant, containing the labeled carbohydrates, was filtered through 0.22 μm nylon membranes (Westborough, MA, USA) and 10 μL of the resulting solution was injected for analysis. The mobile phase was a mixture of 0.1 mol/L KH_2PO_4 and acetonitrile (83:17). The flow rate was 1.0 mL/min and column temperature was 35 °C. The absorbance of samples was detected at 245 nm. D-glucose, L-rhamnose, D-xylose, L-arabinose, D-mannose, L-fucose, D-galactose, and D-galacturonic acid were derivatized and used as standards. The degree of esterification (DE) of pumpkin pectic polysaccharide was determined by the titrimetric method (USP 26 NF 21, 2003) with some modifications.

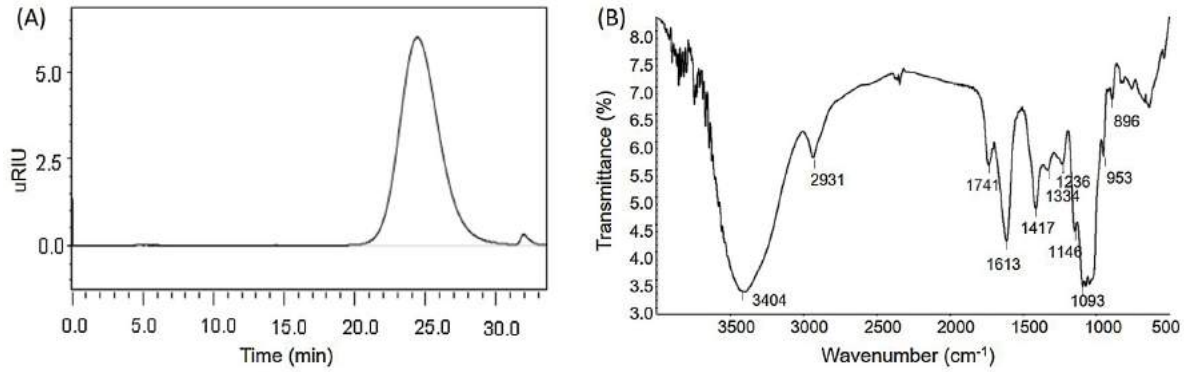

Fig. 1. Homogeneity and FT-IR spectrum of PPc. (A) SEC elution of PPc. (B) FT-IR spectrum of PPc.

2.5. FT-IR spectroscopy

The FT-IR spectrum of pumpkin pectic polysaccharide was measured on a Perkin Elmer Spectrum One FT-IR Spectrometer. One portion pectic polysaccharide was mixed with 9 parts KBr powder, ground and then pressed into a 3 mm pellet for FT-IR spectral measurement in the frequency range of 4000–500 cm^{-1}. Data obtained were processed by Omnic 32 software.

2.6. NMR spectroscopy

The NMR spectra of pumpkin pectic polysaccharides were obtained on a Bruker 800 MHz (18.8 T) standard-bore NMR spectrometer equipped with a $^{1}H/^{2}H/^{13}C/^{15}N$ cryoprobe with z-axis gradients. Polysaccharide (20 mg) was dissolved in 1 mL of 99.6% D_2O centrifuged at 5000 × *g* for 5 min and lyophilized. The process was repeated twice, and the final sample was dissolved in 0.5 mL of 99.98% D_2O. ^{1}H spectroscopy, ^{13}C spectroscopy, $^{1}H–^{1}H$ correlated spectroscopy (COSY), $^{1}H–^{1}H$ total correlation spectroscopy (TOCSY), and $^{1}H–^{13}C$ heteronuclear single quantum coherence spectroscopy (HSQC) experiments were all carried out at 25 °C.$^{1}H–^{13}C$ heteronuclear multiple bond correlation spectroscopy (HMBC) experiment was carried out at 45 °C to obtain a high-resolution coherence signal within three bonds using specific pulse programs.

2.7. RCA_{120} and Gal-3 binding studies

The binding behavior of pumpkin pectic polysaccharide with RCA_{120}/Gal-3 was measured using SPR on a BIAcore 3000 system. First, RCA_{120} (or Gal-3) was immobilized on a CM5 chip through its primary amino groups using EDC/NHS according to the standard amine coupling protocol. A 10 μL solution of 0.1 mg/mL RCA_{120} (or Gal-3) was injected over the flow cell at 5 μL/min. Successful immobilization of Gal-3 was confirmed by an ~10000 resonance unit (RU) increase in the sensor chip. The first flow cell (control) was prepared without injection of RCA_{120} (or Gal-3). After immobilization, the pectic polysaccharide sample was diluted in HBS-EP buffer (0.01 M HEPES, 0.15 M NaCl, 3 mM EDTA, and 0.005% surfactant P20, (pH 7.4)). Different dilutions of the pectic polysaccharide sample were injected at a flow rate of 30 μL/min. Following sample injection, HBS-EP buffer was passed over the sensor surface for dissociation. The sensor surface was regenerated by injection of 150 mM lactose after a 3 min dissociation time to fully regenerate the surface. The response was determined as a function of time (sensorgram) at 25 °C.

3. Results and discussion

3.1. Isolation and homogeneity of pumpkin pectic polysaccharide (PPc)

Following the treatment of pumpkin residue with alkali, and ethanol precipitation, the eluant in 0.2 M NaCl fraction from a DEAE column corresponded to a homogenous pectic polysaccharide (PPc). The yields of the crude polysaccharide and the 0.2 M NaCl fraction were 5 g and 1.25 g from 100 g of pumpkin residue. The purity was evidenced by the elution of a single symmetrical peak in SEC at ~25 min (Fig. 1A). The molecular weight of PPc was determined to be 22.6 kDa based on a linear regression curve plotted using dextran standards.

3.2. FT-IR analysis of PPc

The FT-IR spectrum (Fig. 1B) of PPc revealed the characteristic absorption bands of pectic polysaccharide. The strong and broad absorption area at 3404 cm^{-1} was attributed to the O—H stretching vibration due to intermolecular and intramolecular hydrogen bonds (Chylinska, Szymanska-Chargot, & Zdunek, 2016). The absorption at 2931 cm^{-1} was assigned to the C—H stretching vibration (Jeff et al., 2013). The absorption at 1741 cm^{-1} was assigned to methyl ester group (Chylinska et al., 2016). A band at 1613 cm^{-1} coupled with another weaker band at 1417 cm^{-1} were from the asymmetric and symmetric stretching vibration of the carboxylate groups, respectively. (Nejatzadeh-Barandozi & Enferadi, 2012), confirming the presence of uronic acid. The bands observed in the region from 1000 to 1200 cm^{-1} were assigned to the absorption of skeletal C—O and C—C vibration of glycosidic bonds and pyranoid ring, which is unique to pectic polysaccharide (Liu et al., 2016).

3.3. Monosaccharide composition of PPc

The monosaccharide composition of PPc was determined following hydrolysis and pre-column derivatization by RP-HPLC (Fig. 2). Eight standard monosaccharides were separated within 50 min on the XDB-C18 column. The monosaccharide species in PPc were identified by matching their retention times with those of standard monosaccharides. The results showed that PPc was composed of rhamnose, galacturonic acid, galactose, and arabinose with a molar ratio of 7.4: 25: 28: 2.6. The proportion was calculated using the peak area of each monosaccharide, corrected by corresponding standards (Table 1.). Galactose was the most abundant monosaccharide in PPc, followed by galacturonic acid and rhamnose. The monosaccharide composition of PPc was qualitatively similar but

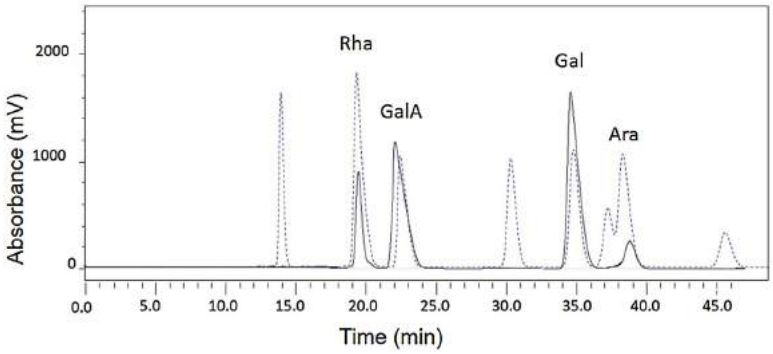

Fig. 2. Monosaccharide composition of PPc. The dash line was HPLC chromatogram of eight standard monosaccharides. The solid line was HPLC chromatogram of PPc.

Table 1
Molar ratio of rhamnose, galacturonic acid, galactose, and arabinose in PPc.

	Rha	GalA	Gal	Ara
Peak area of Standards	50280153	38004948	42759181	40726952
Peak area of PPc	37321268	94868514	120092468	10764194
Peak area (PPc/Standards)	0.7423	2.4962	2.8086	0.2643
Molar ratio	7.4: 25: 28: 2.6			

quantitatively different from that of other pectic polysaccharides isolated from various pumpkin cell wall materials (Košťálová et al., 2010; Nosál'ová et al., 2011; Yang et al., 2007), which typically have a higher amount of glucose. The high galactose content of PPc suggested that it might possess RG-I domain with long galactan side chain. The DE of PPc was 5.4% determined by titrimetric method, which was quite low due to the deesterification effect of alkali treatment.

3.4. Structure of PPc confirmed by NMR spectroscopy

A combination of 1D and 2D NMR was applied to characterize the main structural feature to further confirm the RG-I structure of PPc. The ^{1}H NMR spectrum indicated that PPc mainly contained five signals at anomeric protons region (4.50–5.20 ppm), other proton peaks were found in the region of 3.30–4.30 ppm (Fig. 3A). The prominant peaks at the high field (1.23–1.17 ppm) were assigned to the $-CH_3$ (C6) of rhamnose (de Bruyn, Anteunis, de Gussem, & Dutton, 1976), demonstrating a high content of rhamnose in PPc. In the ^{13}C NMR spectrum (Fig. 3B), PPc gave several anomeric carbon signals from 97.50 ppm to 108.00 ppm and multiple non-anomeric carbon signals in a broad region from 60.00 ppm to 84.00 ppm. The signal present at low field 174.15–173.75 ppm were assigned to the carboxyl carbons of Gal*p*A, similar to pectin extracted from other plants. (Gao et al., 2013; Panda et al., 2015). A small peak at 107.48 ppm and corresponding peak at 82.36 ppm indicated the presence of furan conformation, of arabinose. In addition, rhamnose gave the clear resonances for $-CH_3$ (C6) at 16.73 and 16.49 ppm. (Yu et al., 2010). Signals for O—CH3 group, which often appear at ~52.80 ppm in the spectra of pectins (Wang et al., 2014), were not observed due to hydrolysis occurring on alkali treatment (Renard, & Thibault, 1996).

By combining 2D COSY, TOCSY, HSQC and HMBC NMR spectroscopy, the sugar ring carbon/hydrogen signals could be identified (Table 2). Gal*p*A gave two anomeric carbon/hydrogen signals at 99.33/4.99 ppm and 97.54/4.93 ppm for 1,4-α-Gal*p*A and 1,4-α-Gal*p*A connected with 1,2-α-Rha*p*, respectively (Fig. 4A). The α-1,4-linkage between Gal*p*A in the main chain was confirmed by a cross peak of H-1 and C-4 at 4.99/77.97 ppm in HMBC spectrum (Fig. 4D). Rha*p* gave clear resonance signals of C-1/H-1 and C-6/H-6. The

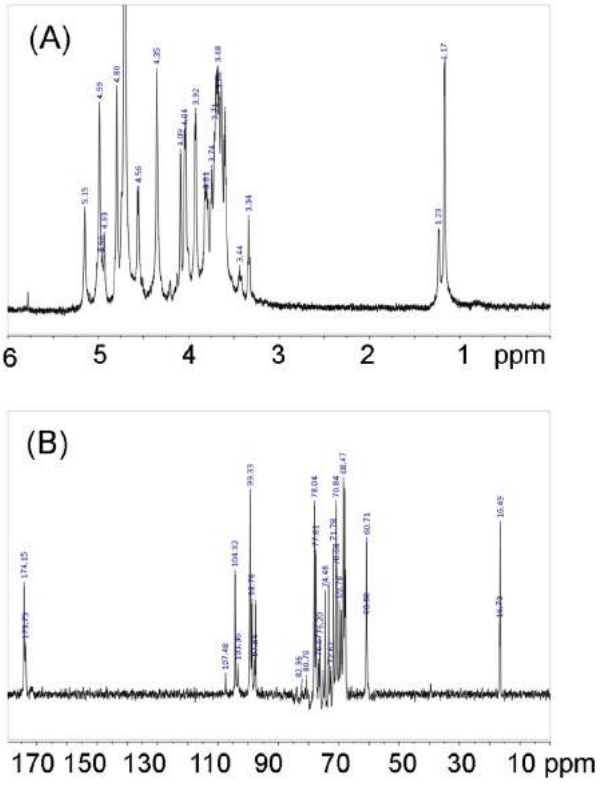

Fig. 3. 1D NMR spectrum of PPc. (A) ^{1}H NMR spectrum of PPc. (B) ^{13}C NMR spectrum of PPc.

C-1/H-1 signal for 1,2-linked α-Rha*p* and 1,2,4-linked α-Rha*p* appeared at 98.76/5.15 ppm and 97.84/4.96 ppm (Fig. 4A), respectively, as did their C-6/H-6 chemical shifts at 16.49/1.17 ppm and 16.73/1.23 ppm, respectively. Based on the ratio of these two H-6 peaks, the ratio for α −1,2,4-Rha*p*/(α-1,2-Rha*p* + α-1,2,4-Rha*p*) was estimated to be 0.29 for PPc. The value represents the substitution degree at the C-4 of Rha*p* in relation to total

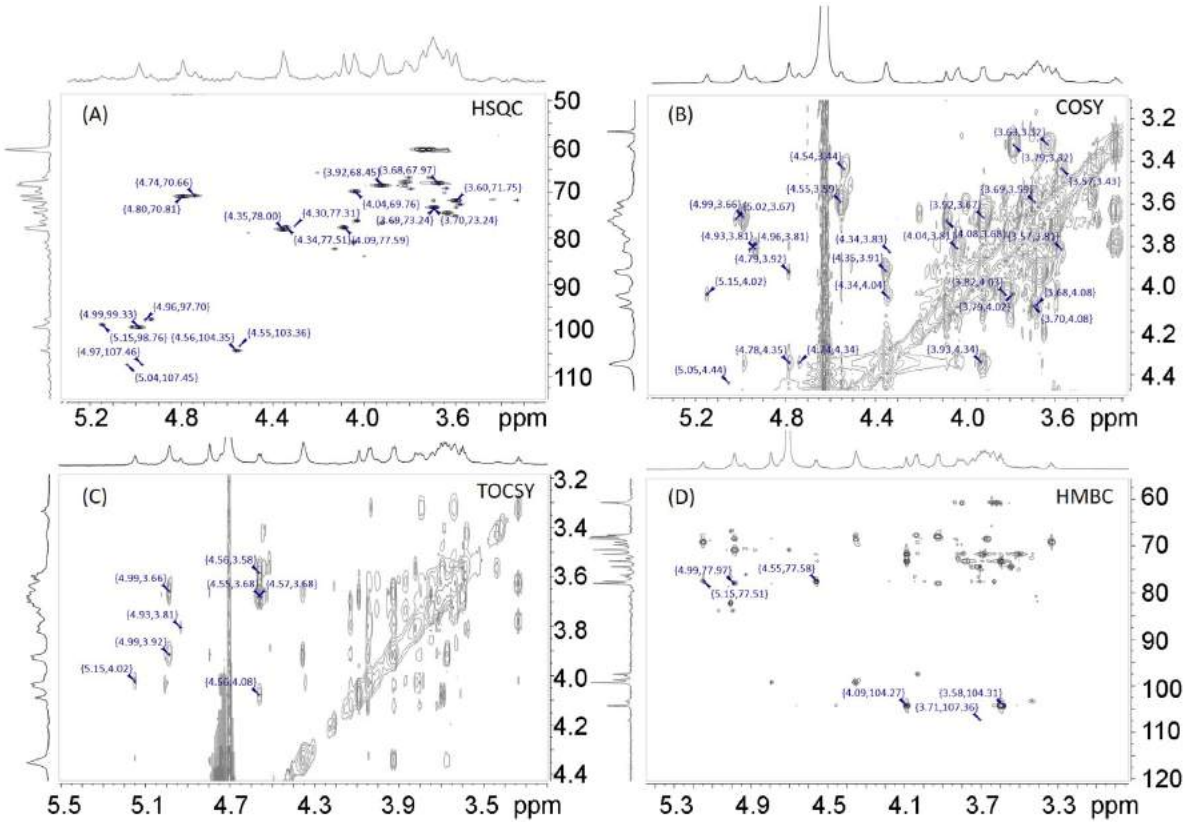

Fig. 4. 2D NMR spectrum of PPc. (A) HSQC. (B) COSY. (C) TOCSY. (D) HMBC.

Table 2
Assignment of sugar ring carbon/hydrogen signals of PPc.

Residue		Atom	^{13}C/ppm	^{1}H/ppm
Gal*p*A	1,4-α-Gal*p*A-	1	99.33	4.99
		2	67.97	3.66
		3	68.45	3.92
		4	78.00	4.35
		5	70.81	4.80
		6	174.15	–
	1,4-α-Gal*p*A-	1	97.54	4.93
	(1,2-α-Rha*p*)	2	66.79	3.81
		3	69.76	4.04
	–	4	77.51	4.34
		5	70.66	4.74
		6	173.75	–
Rha*p*	1,2-α-Rha*p*	1	98.76	5.15
		2	76.17	4.03
		3	69.25	3.80
		4	71.71	3.33
		5	69.10	3.63
		6	16.49	1.17
	1,2,4-α-Rha*p*	1	97.84	4.96
		2	96.18	4.54
		3	71.59	3.43
		4	72.60	3.58
		6	16.73	1.23
Gal*p*	β-1,4-Gal*p*	1	104.32	4.55
		2	71.75	3.59
		3	73.24	3.69
		4	77.59	4.09
		6	60.71	3.73
	t-β-Gal*p*	1	103.36	4.56
		6	60.88	3.64
Ara*f*	α-Ara*f*	1	107.48	4.97

–, Not determined.

Rha*p* residues, providing the degree of branching in corresponding RG-I domain (Westereng, Michaelsen, Samuelsen, & Knutsen, 2008). The two cross peaks in HMBC spectrum (Fig. 4D) at 5.15/77.51 ppm (H-1 of Rha*p* and C-4 of Gal*p*A) and 4.93/76.17 ppm (H-1 of Gal*p*A and C-2 of Rha*p*) confirmed the linkage between Rha*p* and Gal*p*A, which is →4)-α-Gal*p*A-(1→2)-α-Rha*p*-(1→4)-α-Gal*p*A-(1→. Gal*p* gave two anomeric carbon/hydrogen signals at 104.32/4.55 and 103.36/4.56 ppm (Fig. 4A). According to literature, the C-1/H-1 signal at 104.32/4.55 ppm and the matching signal for C-6/H-6 at 60.71/3.73 ppm indicate the presence of β-1,4-D-Gal*p* (Seymour et al., 1990; Yu et al., 2010), and the C-1/H-1 signal at 103.36/4.56 ppm and the matching signal at 60.88/3.64 ppm for C-6/H-6 indicate the presence of non-reducing terminal β-D-Gal*p* (Zhang et al., 2012). The β-1,4-linkage between Gal*p* in the side chain was confirmed by the cross peak at 4.56/77.58 ppm (H-1/C-4) and 4.09/104.27 ppm (H-4/C-1) in HMBC spectrum (Fig. 4D). Another cross peak at 3.58/104.31 ppm (H-4 of Rha*p* and C-1 of Gal*p*) proves that the side chain Gal*p* is linked to Rha*p* at C-4 position. The C-1/H-1 signal of Ara*f* appeared at 107.48/4.97 ppm, which indicated that the Ara*f* residues present in PPc is in the form of α-Ara*f* (Habibi, Heyraud, Mahrouz, & Vignon, 2004; Yu et al., 2010). The C-1 of α-Ara*f* was attached to C-3 of Gal*p* as indicated by a cross peak at 3.71/107.36 ppm (H-3 of Gal*p* and C-1 of Ara*f*) in the HMBC spectrum (Fig. 4D). These results suggest that the side chain of PPc is a type I arabinogalactan (AG-I).

3.5. Proposed structure of PPc

Based on all the results provided above, a structure of PPc is proposed (Fig. 5). PPc is a RG-I-like polysaccharide with a molecular weight of 22.6 kDa. The main chain is composed of a backbone

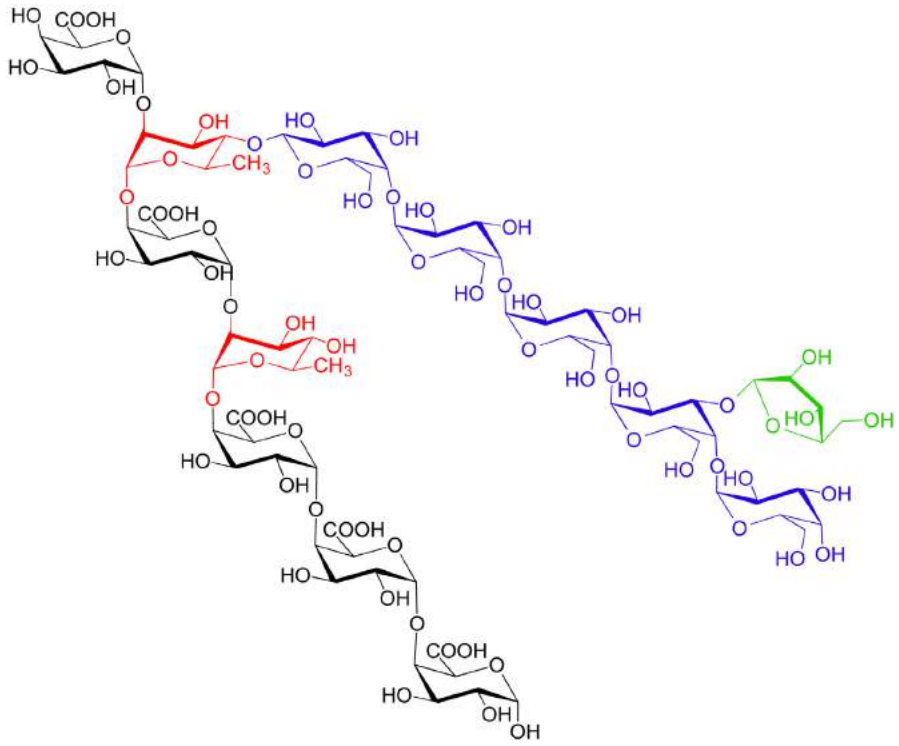

Fig. 5. Proposed structure of PPc. The glycoside units were illustrated to be α-D-Gal*p*A (black), α-L-*Rha*p (Red), β-D-Gal*p* (blue) and α-L-Ara*f* (green). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

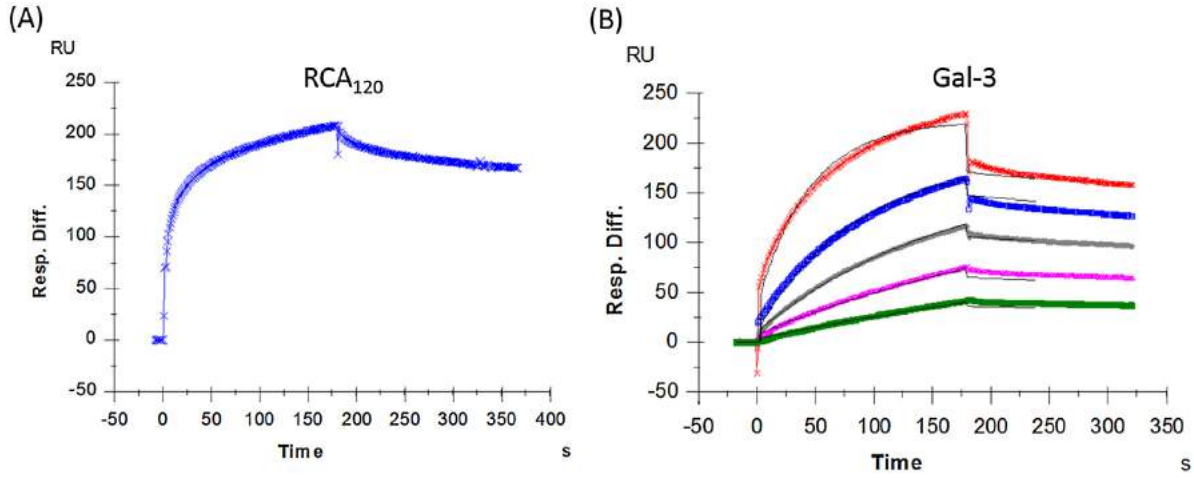

Fig. 6. RCA_{120} and Gal-3 binding character of PPc. (A) SPR sensorgrams of PPc–RCA_{120} binding. PPc concentration was 40 μM. (B) SPR sensorgrams of PPc–Gal-3 binding. PPc concentrations (from top to bottom): 40, 20, 10, 5, and 2.5 μM, respectively. The black fitting curves use models from BIAevaluation version 4.0.1.

of alternating α-L-Rha*p* and α-D-Gal*p*A residues, and β-D-Galactan side chain is attached to the main chain at C-4 position of the some of the Rha*p* residues. Occasionally, a small amount of α-L-Ara*f* is found attached to β-D-galactan side chain through the C-3 of β-D-Gal*p*.

3.6. Binding character of PPc and lectin

According to the literature, RCA_{120} can be used as a tool to detect β-D-galactose residues. The results of SPR analysis show a smooth binding curve between PPc and RCA_{120} (Fig. 6A), confirming the presence of β-D-galactose in PPc, in consistent with the NMR data. Gal-3 is a β-galactoside binding protein with roles in various cell processes. The binding between Gal-3 and pectic polysaccharide make it a potential Gal-3 inhibitory agent with applications in preventing or reducing cancer, carcinogenesis, fibrosis, and many other serious diseases. Gao et al. (2013) found that the pectin extracted from Ginseng has inhibitory effects on Galectin-3 and the galactan side chains were essential to the activity of Ginseng pectin. In the current study, the binding kinetics of PPc–Gal-3 interaction was performed by SPR using a sensor chip with immobilized Gal-3 lectin. Sensorgrams of Gal-3 binding to different PPc dilutions are

shown in Fig. 6B. Non-specific binding has been eliminated by a control flow cell without immobilized Gal-3. The specific binding curves fit well to a 1:1 Langmuir binding model, consistent with a monophasic-binding process. The apparent on (*ka*) and off (*kd*) rates for the binding are calculated as 550 (1/Ms) and 6.95×10^{-4} (1/s), respectively, suggesting quick association and slow dissociation. The binding affinity KD (*kd*/*ka*) was calculated to be 1.26 μM, indicating a moderate binding affinity for PPc to Gal-3. This Gal-3−binding affinity is higher than that of potato galactan (2.59 μM), but lower than that of an RG-I domain isolated from Ginseng pectin (22.2 nM), which were detected using a similar method by Gao et al. (2013). These data suggest a potential use of PPc from pumpkin residue as a new agent to inhibit Gal-3 binding to cell receptors, blocking its ability to send destructive molecular signal in cancer and other diseases.

4. Conclusion

In this paper, a RG-I containing pectic polysaccharide having a molecular weight of 22.6 kDa was isolated from pumpkin residue. The detailed structure of PPc was identified by chemical analysis and NMR spectroscopy as a [→4)-α-Gal*p*A-(1 → 2)-α-Rha*p*-(1 → 4)-α-Gal*p*A-(1 →] backbone with branches of β-1,4-D-galactan side chains occasionally substituted with α-L-Ara*f*. Pumpkin residue pectic polysaccharide was recognized by RCA_{120} with a binding affinity of 1.26 μM for Gal-3. Gal-3 is an important lectin implicated in a variety of biological functions, including tumor progression, cardiac fibrosis, and inflammation. The finding suggests that pectic polysaccharide from pumpkin residue might be developed as a novel, non-toxic Gal-3-inhibitor, and also serve as a potential industrial use for this agricultural waste.

Acknowledgments

This work was supported by a fund for agro-scientific research in the public interest (No. 201303112) to Q. Li, and grants from the National Institutes of Health HL062244 and HL094463 to R.J.L.

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