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Rhamnogalacturonan I domains from ginseng pectin

Li Yu [a,1], Xu Zhang [a,1], Shanshan Li [a], Xiaoyu Liu [a,b], Lin Sun [a], Haibo Liu [a], Jeff Iteku [a], Yifa Zhou [a,*], Guihua Tai [a,*]

[a] Laboratory of Molecular Epigenetics of MOE, School of Life Sciences, Northeast Normal University, 5268 Renmin Street, Changchun 130024, PR China
[b] College of Sciences, Shenyang Agricultural University, Shenyang 110161, PR China

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ABSTRACT

Five rhamnogalacturonan I (RG-I) domains RG-I-1, RG-I-2, RG-I-3A, RG-I-3B and RG-I-4 were isolated from ginseng pectin by endo-polygalacturonase hydrolysis and a combination of ion-exchange and gel-permeation chromatography. The five domains all contain galacturonic acid, rhamnose, galactose and arabinose as main components and their rhamnose/galacturonic acid is from 0.26 to 0.64, among the range of RG-I. The molecular weights of RG-I-1 (5 kDa), RG-I-2 (4 kDa) and RG-I-3B (6 kDa) are smaller than those of RG-I-3A (45 kDa) and RG-I-4 (60 kDa). ^{13}C NMR spectra of all domains showed RG-I features. RG-I-2 and RG-I-3B contained RG-I domains linked with highly methyl-esterified and acetylated homogalacturonan domains and the side chains probably belonging to type I and type II arabinogalactans, while RG-I-3A and RG-I-4 may have the side chains of type I arabinogalactans. 4-O-methyl-β-D-glucuronic acid residues were present at non-reducing terminals of RG-I-2.

1. Introduction

Pectin is a family of complex polysaccharides present in all plant primary cell walls (Voragen, Coenen, Verhoef, & Schols, 2009). Pectin usually contains four domains: homogalacturonan (HG), rhamnogalacturonan I (RG-I), rhamnogalacturonan II (RG-II) and xylogalacturonan (Ridley, O'Neill, & Mohnen, 2001; Schols & Voragen, 1996). HG domain is a linear chain of 1,4-linked α-D-galacturonic acid (Gal*p*A) residues whose carboxyl groups are often partially methyl-esterified (Ridley, O'Neill, & Mohnen, 2001). RG-I is composed of a backbone of alternating α-L-rhamnose (Rha*p*) and α-D-Gal*p*A residues (McNeil, Darvill, & Albersheim, 1980). α-L-Arabinose (Ara*f*)- and β-D-galactose (Gal*p*)-rich side chains are attached via C-4 of the Rha residues to this backbone (Ridley, O'Neill, & Mohnen, 2001). Occasionally the side chains are terminated by α-L-fucose (Fuc*p*), β-D-glucuronic acid (Glc*p*A) and 4-O-methyl-β-D-glucuronic acid (4-O-Me Glc*p*A) residues (Albersheim, Darvill, O'Neill, Schols, & Voragen, 1996). RG-II has a homogalacturonan backbone composed of 9–10 α-1,4-linked D-Gal*p*A units and four different oligosaccharide chains attached to the uronic acid backbone via 2- or 3-O of GalA (Ridley, O'Neill, & Mohnen, 2001). It has been reported that the pectic polysaccharides from plants have many pharmaceutical activities such as anti-ulcer, anti-tumor, radiation protectant, immuno-modulation, and complement activation (Inngjerdingen et al., 2008; Subramanian, Chintalwar, & Chattopadhyay, 2002; Yamada, Hirano, & Kiyohara, 1991; Zhang, Kiyohara, Sakurai, & Yamada, 1996).

Panax ginseng C.A. Meyer (*P. ginseng*) has been used as an herb in Asian countries for over 2000 years (Attele, Wu, & Yuan, 1999). Ginseng polysaccharides are active components of ginseng roots and composed of neutral starch-like polysaccharide and pectin (Ovodov & Solov'eva, 1966). The ginseng starch-like polysaccharide consists of a group of glucans structurally similar to starch and exhibits anti-cancer, anti-oxidant and hypoglycemic activities (Fu et al., 1994; Konno, Sugiyama, Kano, Takahashi, & Hikino, 1984; Luo & Fang, 2008). The ginseng pectin contains GalA, Gal, Ara and Rha as major composition. In addition, it contains minor mannose (Man) and GlcA. Comparing to the studies on the starch-like polysaccharide from ginseng, there are more reports about the activity and structure of ginseng pectin. It has been reported that the ginseng pectin has immuno-modulating, anti-cancer, anti-oxidant, hypoglycemic activities and anti-adhesion against pathogenic bacteria (Gao, Hiyohara, Cyong, & Yamada, 1989; Konno et al., 1984; Lee et al., 2006; Tomoda et al., 1993). Structural analyses have revealed that ginseng pectin contains β-(1,3)-, β-(1,4)- and β-(1,6)-linked galactopyranosyl residues and α-(1,3)- and (1,5)-linked arabinofuranosyl residues (Solov'eva, Arsenyuk, & Ovodov, 1969; Tomoda, Hirabayashi, Shimizu, Gonda, & Ohara, 1994; Tomoda, Hirabayashi et al., 1993; Tomoda et al., 1993). But it still remains unclear the detailed structural information about ginseng pectin, which makes it difficult to study structure–activity relationship.

* Corresponding authors. Address: School of Life Sciences, Northeast Normal University, 5268 Renmin Street, Changchun 130024, Jilin Province, PR China. Tel./fax: +86 431 85098212.
E-mail addresses: zhouyf383@nenu.edu.cn, zhouyf383@yahoo.cn (Y. Zhou), taigh477@nenu.edu.cn (G. Tai).
[1] These authors contributed equally to this paper.

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In our previous study, we totally fractionated ginseng polysaccharides and found that ginseng pectin contained both RG-I-rich and HG-rich domains (Zhang et al., 2009). In the present paper, we report the isolation and structural features of the RG-I domains from ginseng pectin.

2. Materials and methods

2.1. Materials

The chromatographic supports DEAE–Sepharose Fast Flow, Sepharose CL-6B, Sephadex G-75 and Sephadex G-25 were purchased from Sigma–Aldrich, endo-polygalacturonase (Endo-PG, EC 3.2.1.15 from *Aspergillus niger)* from Sigma–Aldrich. All other reagents and chemicals were commercially available reagents of analytical grade produced in China.

2.2. Analytical methods

Total carbohydrate contents were determined by the phenol–sulphuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The standard was the mixture of monosaccharides that constituted the polysaccharide to be tested. Uronic acid contents were determined by the m-hydroxydiphenyl method (Blumenkrantz & Asboe-Hansen, 1973), using galacturonic acid as standard. Gel-permeation and anion-exchange chromatographies were monitored by assaying the total sugar and uronic acid contents.

2.3. Purification of RG-I domains

Dried water-soluble ginseng polysaccharide (WGP) from ginseng roots was a gift from Hongjiu Company (Jilin, China). WGP was fractionated according to the procedure established in our lab (Zhang et al., 2009). In brief, WGP was applied to DEAE–Cellulose, eluting with distilled water to give a neutral fraction (WGPN) and then with 0.5 M NaCl to give an acid fraction (WGPA). WGPA was then hydrolyzed by Endo-PG and the fractionation of the hydrolysates was performed by a combination of anion-exchange and gel-permeation chromatography as shown in Fig. 1.

2.3.1. Enzymatic hydrolysis

WGPA (47 g) was dissolved in sodium acetate buffer (470 mL, 25 mM, pH 4.2) and incubated with Endo-PG (2068 U) at 50 °C for 2 h. This procedure was monitored by an increase of the reducing sugars in the reaction mixture by the method of Nelson (1944).

2.3.2. Separation of the hydrolysate

The hydrolysates were applied to a Sephadex G-25 (4 × 14 cm) column eluted with distilled water at flow rate of 2 mL/min. The fraction WGPA-UD (un-digested fraction) was collected in void volume and another fraction WGPA-D (digested fraction) was collected in the volume close to total volume.

2.3.3. DEAE–Sepharose Fast Flow chromatography of WGPA-UD

2.3.3.1. Analytical chromatography. A water solution of WGPA-UD (1 mL, 5 mg/mL) was loaded onto a column of DEAE–Sepharose Fast Flow (1.0 × 25 cm, Cl^-), pre-equilibrated with distilled water at 0.5 mL/min. After washing with distilled water, the bound material was eluted with a linear NaCl gradient (0–0.5 M NaCl, 150 mL). The eluates were collected at 4-mL per tube and analyzed for the distribution of total sugar and uronic acids.

2.3.3.2. Preparative chromatography. WGPA-UD (7 g in 210 mL distilled water) was loaded onto a preparative DEAE–Sepharose Fast Flow column (6 × 20 cm, Cl^-) pre-equilibrated with distilled water. The column was eluted sequentially with distilled water and a NaCl gradient (0.07, 0.16, 0.22, and 0.30 M) at 6 mL/min. The eluates were collected at 15-mL per tube and assayed for distribution of total sugar. The appropriate collections were combined, concentrated, dialyzed and freeze-dried to give five fractions: WGPA-UDN (distilled water), WGPA-UD1 (0.07 M NaCl), WGPA-UD2 (0.16 M NaCl), WGPA-UD3 (0.22 M NaCl), and WGPA-UD4 (0.30 M NaCl).

2.3.4. Gel-permeation chromatography

The bound fractions (WGPA-UD1, WGPA-UD2, WGPA-UD3 and WGPA-UD4) were purified by a Sephadex G-75 (1.5 × 90 cm) or Sepharose CL-6B (1.5 × 90 cm) column, eluted with a 0.15 M NaCl solution at 0.15 mL/min. The eluates (3-mL per tube) were collected and assayed for distribution of total sugar and uronic acids. The appropriate collections were combined, dialyzed and freeze-dried to give the corresponding fractions.

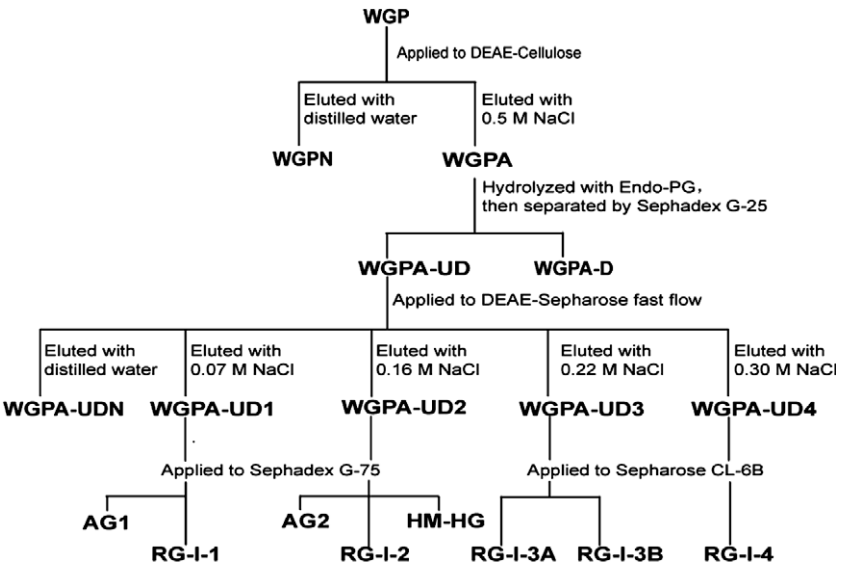

Fig. 1. Fractionation scheme for RG-I domains by anion-exchange and gel-permeation chromatography.

Table 1
Yields and sugar compositions of ginseng pectin and fractions on DEAE–Sepharose Fast Flow.

Fraction	Yield (% w)[a]	Monosaccharide composition (mol%)							
		GalA	Rha	Gal	Ara	Man	Glc	GlcA	Fuc
WGPA[b]	17.1	44.2	2.5	18.0	15.5	–	18.5	1.3	–
WGPA-UD	23.7	32.4	12.2	20.7	13.6	1.8	10.9	3.3	1.4
WGPA-UDN	16.7	–	1.0	8.7	31.5	1.9	54.5	–	–
WGPA-UD1	3.3	18.4	9.7	29.4	13.9	4.2	14.1	3.3	1.1
WGPA-UD2	21.7	35.8	12.3	23.6	13.8	1.5	3.3	4.4	2.0
WGPA-UD3	16.3	34.1	14.8	24.2	14.7	1.5	3.0	3.2	1.8
WGPA-UD4	16.3	33.8	21.8	19.5	9.2	0.4	3.0	2.2	1.5

[a] Yields in relation to fraction applied into column.
[b] Values for WGPA are from Zhang et al. (2009).

2.4. Sugar composition analysis

Sugar composition analysis was performed as described by Zhang et al. (2009). Each of polysaccharide samples (2 mg) was hydrolyzed first with anhydrous methanol containing 1 M HCl at 80 °C for 16 h and then with 2 M TFA at 120 °C for 1 h. The released monosaccharides were derived by 1-phenyl-3-methyl-5-pyrazolone (PMP) and the derivatives were analyzed by high performance liquid chromatography (HPLC).

2.5. Homogeneity and molecular weight distribution

The molecular weight was estimated by gel-permeation chromatography on a TSK-gel G-3000PW$_{XL}$ column (7.8 × 300 mm, TOSOH, Japan) coupled to a Shimadzu HPLC system as described by Zhang et al. (2009). The column was pre-calibrated by standard dextrans (50 kDa, 25 kDa, 12 kDa, 5 kDa and 1 kDa) using linear regression.

2.6. NMR spectra

The ^{13}C NMR spectra were obtained on a Bruker AV600 spectrometer at 150 MHz. The sample (20 mg) was dissolved in deuterated water (1 mL, 99.8%) with overnight stirring at room temperature. The spectra were recorded at 25 °C after 57,000 scans.

3. Results and discussion

3.1. Preparation of RG-I domains from ginseng pectin

Our previous study showed that ginseng pectin contained arabinogalactan (AG), RG-I-rich and HG-rich polysaccharide molecules (Zhang et al., 2009). Endo-PG is capable of degrading the un-esterified HG regions (Daas, Voragen, & Schols, 2001). Thus, when ginseng pectin fraction WGPA was treated by Endo-PG, theoretically, un-esterified HG regions should be hydrolyzed to GalA or small oligomers, while AG, RG-I and highly methyl-esterified HG domains remained. The hydrolysates were separated on a Sephadex G-25 column to give two fractions referred as WGPA-UD (in void volume) and WGPA-D (close to total elution volume). Sugar composition analysis (Table 1) indicated that the amount of Rha in WGPA-UD (12.2%) is more than that in precursor fraction WGPA (2.5%) and, in contrast, the amount of GalA in WGPA-UD (32.4%) is lower than the precursor (44.2%). According to the findings that Rha content increases and GalA decreases in WGPA-UD, we could deduce that un-esterified HG regions might be hydrolyzed and the RG-I domains remained in WGPA-UD.

3.2. Fractionation of RG-I domains by ion-exchange chromatography

Before fractionated on a preparative scale, WGPA-UD was applied to an analytical DEAE–Sepharose Fast Flow column, eluted with distilled water and a linear gradient of NaCl. As shown in Fig. 2a, the bound molecules were present in three major fractions at the elution concentration of 0.07, 0.16 and 0.22 M NaCl. As the linear gradient elution could not efficiently separate WGPA-UD for a practical pooling strategy, we fractionated WGPA-UD on a preparative DEAE–Sepharose Fast Flow (Fig. 2b), eluted with H_2O, 0.07 M, 0.16 M, 0.22 M and 0.3 M NaCl to give five fractions WGPA-UDN (16.7%), WGPA-UD1 (3.3%), WGPA-UD2 (21.7%), WGPA-UD3 (16.3%) and WGPA-UD4 (16.3%), respectively.

The yields and sugar compositions of the collected fractions were listed in Table 1. WGPA-UDN contains Glc (54.5%), Ara (31.5%), Gal (8.7%) and a little Rha and Man, but no GalA. It might be a mixture of glucans, arabinans and arabinogalactans which were released during enzymatic hydrolysis of WGPA. In this paper, WGPA-UDN was not further studied. WGPA-UD1, WGPA-UD2, WGPA-UD3 and WGPA-UD4 were rich in GalA, Rha, Gal and Ara. The ratios of Rha/GalA for WGPA-UD1–4 were 0.53, 0.34, 0.43 and 0.64, respectively, which are among the RG-I range from 0.05 to 1.0 defined by Schols and Voragen (1996). These four RG-

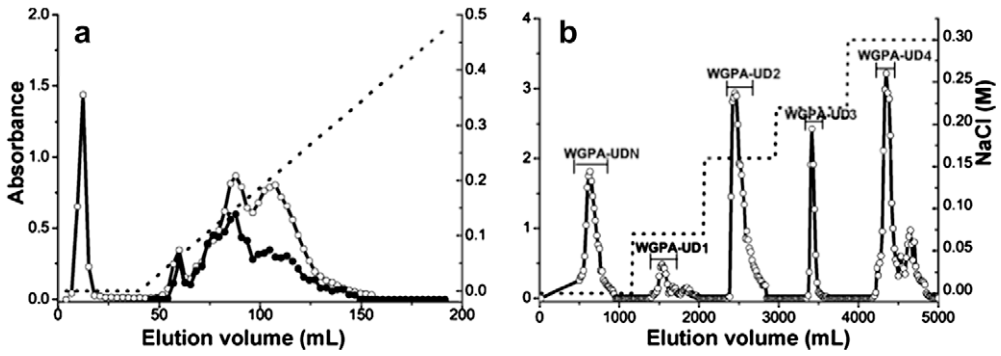

Fig. 2. Elution profiles of WGPA-UD on DEAE–Sepharose Fast Flow column, (a) analytical chromatography eluted by a linear gradient of NaCl and (b) preparative chromatography eluted by a stepwise gradient of NaCl (total sugars, -○-; uronic acid, -●-).

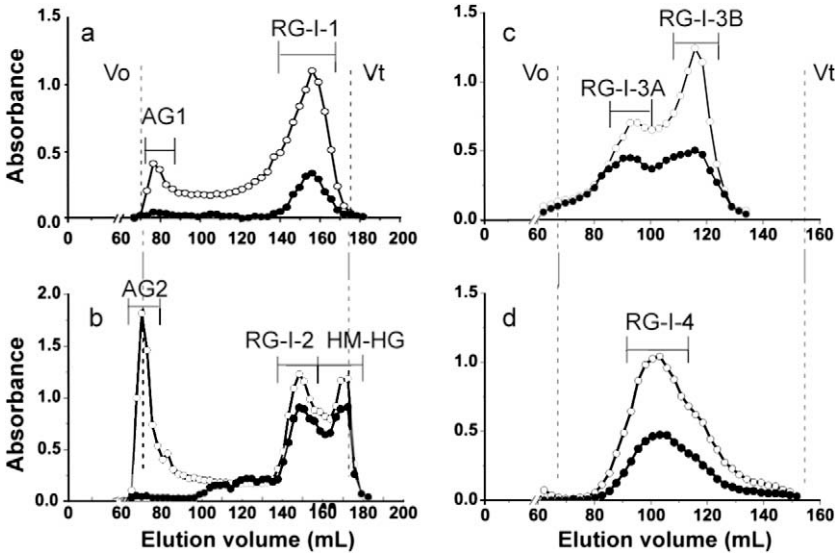

Fig. 3. Elution profiles of collected fractions on Sephadex G-75 column, (a) WGPA-UD1, (b) WGPA-UD2; and on Sepharose CL-6B column, (c) WGPA-UD3, (d) WGPA-UD4 (total sugars, -○-; uronic acid, -●-).

I domains containing fractions were further fractionated by following gel-permeation chromatographies.

3.3. Fractionation of RG-I domains by gel-permeation chromatography

WGPA-UD1 and WGPA-UD2 were relative small molecules and were applied to Sephadex G-75, while WGPA-UD3 and WGPA-UD4 were quite large molecules and were fractionated on Sepharose CL-6B. As seen in Fig. 3a, WGPA-UD1 showed two populations in Sephadex G-75 column and was fractionated into two fractions AG1 and RG-I-1. WGPA-UD2, showing three populations on Sephadex G-75 column, was fractionated into three fractions AG2, RG-I-2 and HM-HG (Fig. 3b). On Sepharose CL-6B column as shown in Fig. 3c and d, WGPA-UD3 showed two major peaks and was separated into RG-I-3A and RG-I-3B, and WGPA-UD4 showed one major population and was purified to be RG-I-4.

3.4. The yields and sugar compositions of collected fractions

The yields and sugar compositions of the collected fractions were listed in Table 2. The total carbohydrate contents of all the collected fractions were more than 85%. AG1 and AG2 were mainly composed of Gal and Ara, suggesting that they were arabinogalactan. HM-HG contained more than 80% GalA, suggesting it was a highly methyl-esterified HG-rich domain. RG-I-1, RG-I-2, RG-I-3A, RG-I-3B and RG-I-4 contained Rha and GalA in a ratio range of 0.26–0.64, suggesting they were RG-I domains. In present paper, the research work is focused on the collected RG-I domains. The investigation for other fractions will be discussed in future papers.

The recoveries of RG-I-1, RG-I-2, RG-I-3A, RG-I-3B and RG-I-4 related to WGPA were 0.35%, 1.63%, 1.15%, 2.53% and 3.72% (Table 2), respectively, and the recovery ratio was 1.0: 4.7: 3.3: 7.2: 10.6. The content of RG-I-1 was the lowest among the five RG-I domains, lower than 5% of all RG-I domains. RG-I-4 was the highest, more than 40% of all RG-I domains. The RG-I domains accounted for 9.38% of WGPA, which is similar to the RG-I content (10%) in the pectin from citrus peels (Yapo, Lerouge, Thibault, & Ralet, 2007).

The main monosaccharides constituents of the five RG-I domains are GalA, Rha, Gal and Ara. In addition, they contain small amounts of Fuc, Glc, GlcA and Man. The composition characterizations are consistent with those of RG-I domain. As seen in Table 2, GalA content ranges from 26.8% to 44.6% among these RG-I domains. GalA content in RG-I-2 (44.3%) is similar to that in RG-I-3B (44.6%), about 10% more than those in RG-I-1 (26.8%), RG-I-3A (32.2%) and RG-I-4 (33.8%). Rha content in RG-I-1–3 varies little, from 11.1% to 14.1%. But in RG-I-4, it rises up to 21.8%.

The values for Rha/GalA in all obtained RG-I domains are in the range of 0.26–0.64, lower than 1 (Table 3), which indicated that GalA oligomeric units might be present in each RG-I domain. The experimental results (see Section 3.6: structural analysis) indicated that the GalAs in all collected RG-I domains were partially methylated and highly acetylated, which might prohibit the GalA oligomeric units to be digested by Endo-PG. These findings are consistent with the observations in literatures (Chen & Mort,

Table 2
Yields and sugar compositions of AG, RG-I and HG fractions.

Fraction	Yield (% w)[a]	Yield (% w)[b]	Sugar content (% w)	Monosaccharide composition (mol%)							
				GalA	Rha	Gal	Ara	Man	Glc	GlcA	Fuc
AG1	37.6	0.29	94.8	4.7	6.3	48.9	12.8	1.9	15.3	1.0	0.7
RG-I-1	45.0	0.35	89.0	26.8	12.8	21.2	13.0	5.9	7.2	7.4	2.0
AG2	17.2	0.89	92.4	5.0	9.5	55.9	18.6	2.1	4.0	3.8	–
RG-I-2	31.6	1.63	87.2	44.3	11.7	12.4	14.5	1.0	4.4	5.8	3.8
HM-HG	25.0	1.29	86.3	84.5	1.7	1.3	1.6	0.4	2.1	0.6	–
RG-I-3A	29.8	1.15	91.0	32.2	11.1	31.6	16.3	2.1	1.9	3.0	0.7
RG-I-3B	49.1	2.53	89.5	44.6	14.1	13.7	11.9	1.3	2.5	3.7	2.3
RG-I-4	96.2	3.72	86.9	33.8	21.8	19.5	9.2	0.4	3.0	2.2	1.5

[a] Yields in relation to fraction applied into column.
[b] Yields in relation to WGPA.

Table 3
Structural features of RG-Is.

Fraction	Mw (kDa)	DM[a] (%)	DAc[b] (%)	Rha/GalA	A/B[c]	(Gal + Ara)/Rha	Gal/Ara	AG as side chains
RG-I-1	5	ND	ND	0.48	ND	2.70	1.63	ND
RG-I-2	4	60.0	30.0	0.26	0.51	2.33	0.86	AG-I, AG-II
RG-I-3A	45	0.0	~100	0.34	0.71	2.86	1.94	AG-I
RG-I-3B	6	40.0	50.0	0.32	0.75	1.82	1.15	AG-I, AG-II
RG-I-4	60	5.0	~100	0.64	0.67	1.25	2.12	AG-I

ND: not determined.
[a] DM: degree of methyl-esterification.
[b] DAc: degree of acetyl-esterification.
[c] A/B = 1,2,4-Rha/(1,2,4-Rha + 1,2-Rha).

1996; Ralet, Crépeau, & Bonnin, 2008; Ralet et al., 2005; Yapo et al., 2007). As seen in Table 3, the ratio of (Gal + Ara)/Rha for RG-I-1, RG-I-2, RG-I-3A, RG-I-3B and RG-I-4 is 2.70, 2.33, 2.86, 1.82 and 1.25, respectively, which roughly indicates the length of the side chains. The lower the ratio of (Gal + Ara)/Rha, the shorter the side chains attached to 4-O-Rha in RG-I backbone (Ferreira, Mafra, Soares, Evtuguin, & Coimbra, 2006; Sengkhamparn, Verhoef, Schols, & Sajjaanantakul, 2008). Another sugar composition feature for RG-I domain is the ratio of Gal/Ara, of which the collected five RG-I domains is from 0.86 to 2.12. Gal/Ara for RG-I-2 is less than 1, while others are more than 1 (Table 3).

3.5. Molecular weight distribution (Mw)

The molecular weight distribution of RG-I fractions were analyzed using HPGPC. As seen in Fig. 4, the elution profile of RG-I-2 shows a single and symmetrical narrow peak with Mw of 4 kDa. But the elution profiles of other RG-I fractions show broad and asymmetric peaks, suggesting there are several different molecular weight populations in each RG-I domain. However, it is clear that each RG-I domain contains a main molecule population. Based on the curve calibrated by standard dextrans, the Mw of the major populations of the RG-I domains were estimated (Table 3). RG-I-1 and RG-I-3B have relative small molecular weight, about 5 kDa and 6 kDa, respectively, while RG-I-3A and RG-I-4 are relative big molecule, with molecular weight about 45 kDa and 60 kDa, respectively.

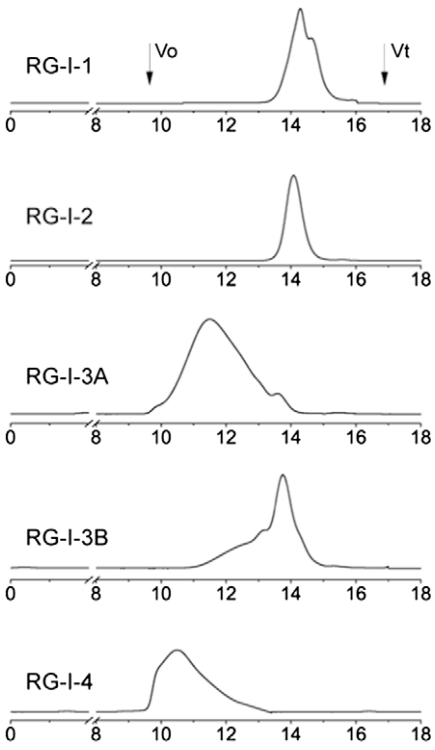

Fig. 4. HPGPC elution profiles of purified RG-I domains.

3.6. Structural features of the isolated RG-I domains

The structural features of main RG-I domains RG-I-2, RG-I-3A, RG-I-3B and RG-I-4 were analyzed by ^{13}C NMR (Fig. 5). The structures of RG-I domains are very complex which resulted in much complicated NMR spectra. Most of the signals from C-2 to C-5 cannot be exactly assigned. But some characteristic peaks from C-1 and C-6 can be clearly identified. RG-I-1 was isolated from ginseng pectin in very low yield and was not applied to NMR analysis.

Rha*p* gave the clear resonance signals of C-1 and C-6 in all NMR spectra of the four RG-I domains. The peak of C-1 appeared at 98.7 ppm and the peaks of C-6 to α-1,2-Rha and α-1,2,4-Rha appeared at 18.2 and 17.7 ppm, respectively (Polle, Ovodova, Chizhov, Shashkov, & Ovodov, 2002). Based on the ratios of these two C-6 peaks, the values for α-1,2,4-Rha/(α-1,2-Rha + α-1,2,4-Rha) were estimated to be 0.51, 0.71, 0.75 and 0.67 for RG-I-2, RG-I-3A, RG-I-3B and RG-I-4, respectively. These values represent the substitute degrees at C-4 of Rha in relation to total Rha residues in corresponding RG-I domain (Westereng, Michaelsen, Samuelsen, & Knutsen, 2008). The degree of branching was deduced to be RG-I-3B > RG-I-3A > RG-I-4 > RG-I-2.

The resonance signals from the carboxyl carbons of un-esterified GalA residues in all RG-I domains were broad peaks at 175.5–176.6 ppm, which were also observed in other pectin (Westereng et al., 2008). The complexity of this broad peak partially reflects the esterification degree of the RG-I domains because it is influenced by the neighboring un-esterified and esterified GalA residues. As seen in Fig. 5, the sequence of the complexity of the broad peak is: RG-I-2 > RG-I-3B > RG-I-4 ≈ RG-I-3A. Correspondingly, the same order of esterification degree of uronic acid in RG-I domains is confirmed by the resonance signals from the methyl groups at 53.4 ppm and methyl ester carbonyl carbons at 172.5–174.7 ppm (Tamaki, Konishi, Fukuta, & Tako, 2008). At these regions, RG-I-2 showed relative high peaks, RG-I-3B small, RG-I-4 very little and RG-I-3A no peaks. The esterification degree of each RG-I domain can be estimated by the ratio of the signals at 100.0 and 99.7 ppm (Westerlund, Aman, Andersson, Andersson, & Rahman, 1991), assigned to C-1 of esterified (E) and un-esterified (U) GalA, respectively. E/U is about 3:2 for RG-I-2 and 2:3 for RG-I-3B, suggesting that about 60% and 40% of GalA is methyl-esterified in RG-I-2 and RG-I-3B. No signals at 100.0 ppm were identified in RG-I-4 and RG-I-3A. Based on the signal at 53.4 ppm, very little (~5%) carboxyl groups are methyl-esterified in RG-I-4 and no methyl-esterification in RG-I-3A. The results could explain why RG-I-2 and RG-I-3B contain higher GalA than RG-I-4 but were eluted with lower NaCl concentration.

In pectin molecules, O-2 or O-3 of GalA residues are often partially acetylated (Perrone et al., 2002; Ralet et al., 2005;

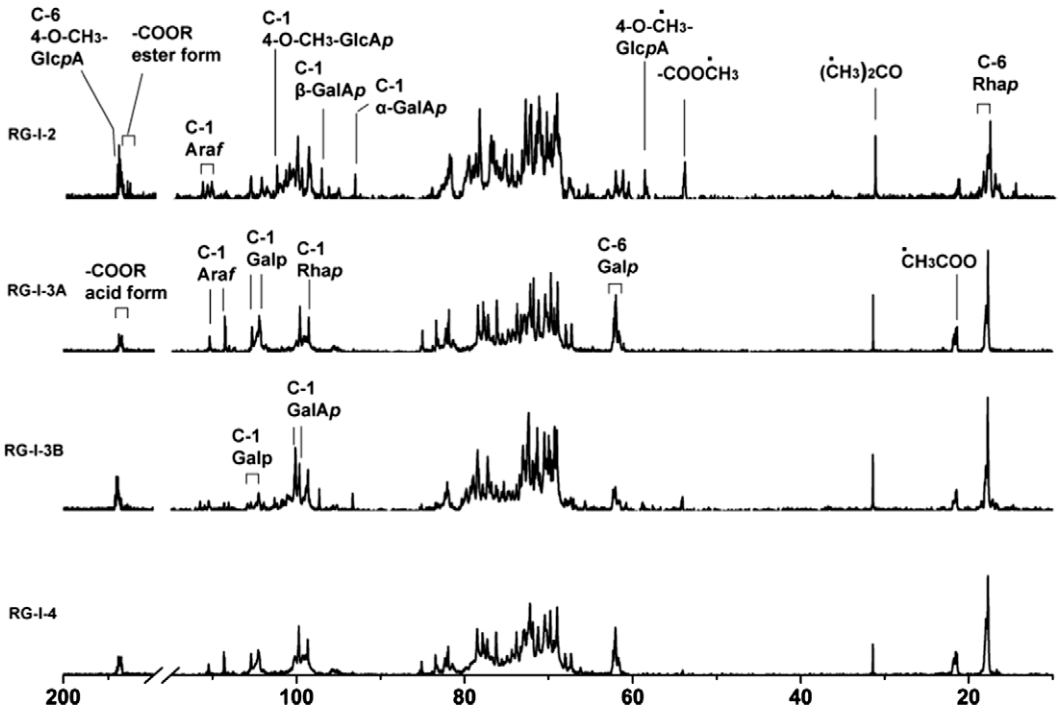

Fig. 5. ^{13}C NMR spectra of four major RG-I domains.

Schols & Voragen, 1994). In our experiments, for all NMR analyzed RG-I domains, the signals from methyl carbons of acetyl groups in O-2 or O-3 of GalA are clearly observed at 22.0 ppm. Based on the ratios of the signals from the methyl carbon of acetyl group and the carbon of carboxyl groups, the acetylation degrees (Table 3) were estimated to be ~30% (RG-I-2), ~100% (RG-I-3A), ~50% (RG-I-3B) and ~100% (RG-I-4), respectively. The methylation and acetylation prohibited the hydrolysis of GalA oligomeric units, which might be the reason that some GalA oligomeric units are present in all RG-I domains isolated from ginseng pectin.

The C-1 signals of Ara in the four RG-I domains appeared at the range of 111.4–108.0 ppm in NMR spectra (Habibi, Heyraud, Mahrouz, & Vignon, 2004; Polle, Ovodova, Chizhov et al., 2002), which indicated that Ara residues present in the RG-Is are in the form of α-Ara*f*. RG-I-3A and RG-I-4 gave two signals at 110.7 and 108.7 ppm corresponding to α-1,5- and non-reducing end residues, while RG-I-2 and RG-I-3B gave four signals at 111.4, 110.7, 110.3 and 108.7 ppm assigned to α-1,3-Ara*f*, α-1,5-Ara*f*, α-1,3,5-Ara*f* and non-reducing terminal α-Ara*f* (Polle, Ovodova, Shashkov, & Ovodov, 2002).

The NMR signals from C-1 of Gal in both RG-I-3A and RG-I-4 clearly show two peaks at 105.9 and 104.9 ppm (Fig. 5). According to the reports in literatures (Habibi et al., 2004; Polle, Ovodova, Shashkov et al., 2002; Ryden, Colquhoun, & Selvendran, 1989), the C-1 signal at 105.9 ppm and the matching signal for C-6 at 61.4 ppm indicated the presence of β-1,4-D-Gal*p*, and the C-1 signal at 104.9 ppm and the matching signal at 62.0 ppm for C-6 indicated the presence of non-reducing terminal β-D-Gal*p*. Therefore, the side chains of RG-I-3A and RG-I-4 might be the type I arabinogalactan (Pérez, Rodríguez-Carvajal, & Doco, 2003; Verhertbruggen & Knox, 2007).

Different from RG-I-3A and RG-I-4, the C-1 of RG-I-2 and RG-I-3B gave multiple peaks at 102.4–104.0 ppm for β-Gal*p* residues, which suggested that Gal residues have more linkage forms. The branched chains in these two RG-I domains may contain both type I and type II arabinogalactans (Pérez, Rodríguez-Carvajal, & Doco, 2003).

In the NMR spectra of RG-I-2 and RG-I-3B, two sharp signals at 93.5 and 97.4 ppm corresponded to C-1 of α- and β-GalA in reducing terminal (Perrone et al., 2002) were clearly observed, while RG-I-3A and RG-I-4 had no signals at these positions. This is consistent with the molecular weight estimation that molecular weights of RG-I-2 and RG-I-3B lower than those of RG-I-3A and RG-I-4 (Table 3), because the anomeric signals from C-1 of terminal residues can be observed only in relative low molecular weight polysaccharides.

It is noted that a clear signal at 59.0 ppm in the NMR of RG-I-2 was observed for the methyl group attached to C-4 of the GlcA (Cui, Eskin, Biliaderis, & Marat, 1996; Pinto, Martinez, Ocando, & Rivas, 2001). Correspondingly, the signals at 176.9 ppm and 102.6 ppm were assigned to the carboxyl groups and C-1 of the terminal non-reducing GlcA, respectively. This indicated that RG-I-2 contains 4-O-Me-β-D-GlcA*p* at the non-reducing terminals of branched chains, which is consistent with the sugar composition, 5.8% of GlcA in RG-I-2. In other three RG-I domains, the contents of GlcA are less than 3.7%, no signals from GlcA. The structural features of RG-I-2–4 are summarized in Table 3.

The biological activities of pectin might be related to the RG-I domains. Several recent papers have reported that some RG-I domains of the pectin have great immune-modulating activities (Inngjerdingen et al., 2007, 2008). A pectic polymer GOA1-I with RG-I properties was shown to stimulate the proliferation of B cells, the secretion of IL-1β by macrophages. In addition, it increased the amount of mRNA for IFN-γ in NK cells (Inngjerdingen et al., 2007). A RG-I domain BP1002-1 from *Biophytum petersianum* Klotzsch was shown to induce the immuno-modulating activity through the stimulation of macrophages and dendritic cells (Inngjerdingen et al., 2008). The RG-I domains from ginseng pectin have complex structures. It is reasonable to consider that those RG-I domains might play important roles on biological activities.

4. Conclusion

In previous studies, we have totally fractionated ginseng pectin into a series of RG-I-rich and HG-rich fractions (Zhang et al., 2009).

In present paper, we further isolated one minor RG-I fraction RG-I-1 and four major RG-I fractions RG-I-2, RG-I-3A, RG-I-3B and RG-I-4 from ginseng pectin by the hydrolysis of Endo-PG and a combination of ion-exchange and gel-permeation chromatography. These purified RG-I domains are quite different in their ratios of sugar composition, molecular weights, methyl-esterification degree and linkage forms. Ginseng pectin has diverse activities which could be related to these RG-I domains. The investigation about these RG-I domains on immunological activity is in progress and their structure–activity relationship would be discussed in next paper.

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