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Antigenic epitope for polyclonal antibody against a complement activating pectin from the roots of *Angelica acutiloba* Kitagawa

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Abstract

A polyclonal antibody (anti-AR-2IIb-IgG) was developed against a complement activating pectin (AR-2IIb) which was purified from the roots of *Angelica acutiloba* Kitagawa. Neutral oligosaccharide–alditols were released from the "ramified" region (rhamnogalacturonan core possessing side chains rich in neutral sugars, PG-1) by lithium-degradation. The mixture significantly inhibited the reactivity of anti-AR-2IIb-IgG to PG-1 or AR-2IIb. Gel filtration and HPLC analysis indicated that the mixture contained two kinds of long oligosaccharide–alditols in addition to several kinds of shorter oligosaccharide–alditols, and both the long oligosaccharide–alditols had significant inhibitory activity against the reactivity of the antibody to PG-1. However, the short oligosaccharide–alditols had no effect. Methylation analysis suggested that one of the long oligosaccharide–alditols consisted of a β-(1 → 3,6)-galactan-like structure. The inhibitory activity of PG-1 against the reactivity of the antibody to PG-1 decreased significantly on *exo*-α-l-arabinofuranosidase digestion followed with *exo*-β-d-(1 → 3)-galactanase digestion, but not by *exo*-α-l-arabinofuranosidase or *exo*-β-d-galactosidase digestion. Although β-d-(1 → 6)-galacto-di- to tetra-saccharides showed no inhibitory activity, a mixture of oligosaccharides liberated from PG-1 by *exo*-β-d-(1 → 3)- galactanase digestion, still showed inhibitory activity. The oligosaccharide consisting mainly of terminal, 3- and 4-linked and 3,6-branched Gal, terminal Ara*f* and 4-OMe-GlcA inhibited the reactivity of the antibody to PG-1.

Keywords: *Angelica acutiloba*; Pectin; Antibody; Epitope

1. Introduction

In Japanese herbal (Kampo) medicines, the roots of *Angelica acutiloba* Kitagawa (Japanese name, Yamato-Tohki) is a well-known component herb used in the treatment of gynaecological diseases and arthritis. We isolated an acidic arabinogalactan (AGIIa), a pectic arabinogalactan (AGIIb-1) and four pectins (AR-2IIa, IIb, IIc and IId) as complement activating polysaccharides and one pectic arabinogalactan (AR-4E-2) as an anti-tumor polysaccharide (Kiyohara, Yamada, Cyong & Otsuka, 1986; Kiyohara, Cyong & Yamada, 1988; Yamada, Kiyohara, Cyong & Otsuka, 1985; Yamada, Komiyama, Kiyohara, Cyong, Hirakawa & Otsuka, 1990). Studies on structure–activity relationship for these complement activating pectic polysaccharides have clarified that β-(1 → 6)-linked galacto-oligosaccharides, which are attached to rhamnogalacturonan core or to β-(1 → 3)-galactan backbone, play important roles in the expression of the complement activating activity of AGIIb-1 and AR-2IIa-IId (Kiyohara, Cyong & Yamada, 1989a,b). The polyclonal antibody (anti-AR-2IIb-IgG) against the major complement activating pectin (AR-2IIb) of *A. acutiloba* was developed, and the "ramified" region (rhamnogalacturonan core possessing side chains, PG-1) of AR-2IIb, which was characterized as the active site for expression of the complement activating activity, are suggested to contain antigenic epitope for the antibody (Wang, Kiyohara, Matsumoto, Otsuka, Hirano & Yamada, 1994).

The present study deals with the clarification of the major antigenic epitope in the "ramified" region of AR-2IIb for anti-AR-2IIb-IgG.

2. Materials and methods

2.1. Materials

The roots of *A. acutiloba* Kitagawa were purchased from Uchida Wakanyaku Co. Ltd., Japan. The major complement activating pectin (AR-2IIb) was purified from the acidic polysaccharide fraction (AR-2) by anion-exchange

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chromatography as described previously (Kiyohara et al., 1986). IgG class antibody (anti-AR-2IIb-IgG) against AR-2IIb was prepared as described previously (Wang et al., 1994). *Endo*-α-d-(1 → 4)-polygalacturonase [(1 → 4)-α-d-galacturonan glycanohydrolase; EC 3.2.1.15] was purified from pectinase (*Aspergillus niger*, Sigma) by the procedure of Thibault and Mercier (1977). *Exo*-β-d-(1 → 3)-galactanase was purified from Driselase (*Irpex lacteus*, Kyowa-Hakko Co. Ltd., Japan) according to the procedure of Tsumuraya, Mochizuki, Hashimoto & Kovac (1990). *Exo*-α-l-arabinofuranosidase (*A. niger*) was purchased from Megazyme (Australia), and purified according to the method of Lerouge, O'Neil, Darvill & Albersheim (1993). β-d-(1 → 3)-galactan was prepared by controlled Smith degradation using the method of Tsumuraya et al. (1990). β-d-(1 → 6)-galacto-di- to tetrasaccharides were gifts from Dr. Hashimoto and Dr. Tsumuraya (Saitama University).

2.2. General

Carbohydrate and uronic acids in column eluates were assayed by phenol–H_2SO_4 (Dubois, Gilles, Hamilton, Rebers & Smith, 1956) and *m*-hydroxybiphenyl methods (Blumenkrantz and Asboe-Hansen, 1973), respectively. Polysaccharides were hydrolyzed with 2 M TFA at 121°C for 1.5 h, and monosaccharides in hydrolyzates were converted into the corresponding alditol acetates, and analyzed by GLC as described previously (Hirano, Kiyohara, Matsumoto & Yamada, 1994).

2.3. Enzyme-linked immunosorbent assay using anti-AR-2IIb-IgG

The procedure was performed as described previously (Wang et al., 1994). Briefly, the "ramified" region (PG-1) of AR-2IIb (125 ng/well) was incubated in phosphate-buffered saline (pH 7.2, PBS) on microtiter plates (Sumitomo, MS-3596 F/H) at 37°C overnight, and unbound PG-1 was removed by washing four times with PBS containing 0.05% Tween 20 (PBS–Tween, 250 μl). The plate was blocked by incubation with 0.8% gelatin in PBS at 37°C for 1 h. Anti-AR-2IIb-IgG in PBS–Tween containing 0.5% bovine serum albumin (BSA, PBS–Tween–BSA, 250 μl) was pre-incubated with various amounts of polysaccharide or oligosaccharides at 37°C for 1 h, and the plate was further incubated with these pre-incubated antibodies at 37°C for 1 h. After the plate was washed five times with PBS–Tween–BSA, it was incubated with alkaline phosphatase-labelled anti-rabbit IgG (Kappel, USA) diluted with PBS–Tween–BSA (1:1000) at 37°C for 2 h. After washing the plate five times with PBS–Tween–BSA, the enzymic activity was measured by incubation of the plate with chromogenic substrate solution (1 mg of *p*-nitrophenyl phosphate disodium salt in 1 ml of M diethanolamine buffer, pH 9.8) at room temperature. The reaction was stopped by the addition of 3 M NaOH, and absorbance at 405 nm was measured by a Microplate Reader Model 450 (Bio-Rad).

2.4. Preparation of "ramified" region from AR-2IIb

AR-2IIb (200 mg) was digested with *endo*-α-(1 → 4)-polygalacturonase in 50 mM acetate buffer (pH 4.5) after de-esterification of AR-2IIb as described previously (Kiyohara et al., 1988). The products were fractionated on Bio-gel P-30 with 50 mM acetate buffer (pH 5.5) as the eluent and the "ramified" region (PG-1) was obtained as the fraction eluted in the void volume.

2.5. Preparation of neutral oligosaccharide–alditols from "ramified" region of AR-2IIb

2.5.1. Treatment of "ramified" region with lithium metal in ethylene diamine

In order to degrade the uronic acids in the "ramified" region of AR-2IIb, PG-1 was treated with lithium metal in ethylene diamine by the method of Lau, McNeil, Darvill and Albersheim (1987). After the reduction of the products with $NaBH_4$, the products were fractionated on a column of DEAE-Sephadex A-25 ($HCOO^-$), and neutral fraction was obtained as the unabsorbed fraction (yield of neutral fraction; 51.2%).

2.5.2. Fractionation of neutral oligosaccharide–alditols derived from "ramified" region

The neutral fraction (65 mg) obtained was fractionated on a column (2.5 × 50 cm) of Bio-gel P-30 with distilled water, and the fractions eluted in the void volume (NS-1), intermediate fraction and the excluded volume were obtained. The fractions eluted in the intermediate fraction and the excluded volume were separately fractionated on a column (2.6 × 95 cm) of Bio-gel P-2 with distilled water. The intermediate fraction gave two fractions (NS-2 and NS-3), whereas the fraction eluted in the excluded volume gave two fractions (NS-4 and NS-5) which were eluted near and in the excluded volume, respectively (yield ratios, NS-1:NS-2:NS-3:NS-4:NS-5 = 1.0:1.0:4.6:8.5:4.7).

2.5.3. Purification of long neutral oligosaccharide–alditols by HPLC

NS-1 was fractionated by HPLC on combined columns of Asahi-pak G-320 and G-220 (Asahi Chemical Industry, Japan) with 0.2 M NaCl, and the fractions eluted in the void volume (NS-1-1) and intermediate fractions (NS-1-2) were obtained (yield ratio, NS-1-1:NS-1-2 = 1.0:1.4).

2.6. Enzymic treatments of "ramified" region of AR-2IIb

2.6.1. Digestion with *exo*-β-d-*galactosidase*

To a solution of PG-1 (2.1 mg) in 50 mM acetate buffer (pH 4.0, 2 ml), *exo*-β-d-galactosidase (jack bean, 0.05 U, Sigma) was added, and the mixture was incubated at 37°C for four days. After neutralization, the mixture was fractionated on a column (2.5 × 50 cm) of Bio-gel P-10 with distilled water. The fraction eluted in the void volume was further digested with the galactosidase in the same manner,

and the enzyme-resistant fraction (Gal-PG-1) was recovered as the void volume fraction by gel filtration on Bio-gel P-10.

2.6.2. Digestion with exo-α-l-arabinofuranosidase

PG-1 (2.15 mg) of AR-2IIb was digested with the purified *exo*-α-l-arabinofuranosidase (0.7 U) from *A. niger* in 50 mM acetate buffer (pH 4.0, 2 ml) at 37°C for four days. The products were fractionated on a column (2.57 × 50 cm) of Bio-gel P-10 with distilled water. The fraction eluted in the void volume was re-digested with the arabinofuranosidase in a similar manner, and the enzyme-resistant fraction (Ara-PG-1) recovered as the void volume fraction by gel filtration on Bio-gel P-10.

2.6.3. Exo-β-d-(1 → 3)-galactanase digestion

To a solution of PG-1 or Ara-PG-1 (2 mg) in 50 mM acetate buffer (PH 4.6, 2 ml), *exo*-β-d-(1 → 3)-galactanase (0.1 U) was added, and the mixture was incubated at 37°C for three days. The products were fractionated in a column (2.5 × 50 cm) of Bio-gel P-30 with distilled water, and the fractions eluted in the void volume (GN-1 from PG-1 or GN-Ara-PG-1 from Ara-PG-1) and near the inner volume (GN-2 from PG-1) were obtained.

2.7. Purification of oligosaccharides from digestion products of "ramified" region obtained by exo-β-d-(1 → 3)-galactanase

2.7.1. Gel filtration and anion-exchange chromatography

After PG-1 (30 mg) was digested with *exo*-β-d-(1 → 3)-galactanase as mentioned earlier, the digestion products were fractionated on Bio-gel P-2 (2.6 × 95 cm) with 50 mM acetate buffer (pH 5.5). Six oligosaccharide fractions (Oligo-1–Oligo-6) and the fraction eluted in the void volume were obtained. Oligo-3 was further fractionated by DEAE-Sephadex A-25 ($HCOO^-$). After the column was eluted with distilled water to obtain a neutral oligosaccharide fraction (Oligo-3N), an acidic oligosaccharide fraction (Oligo-3A) was obtained by eluting with 2 M NaCl, and desalted by Microacylizer (Asahi Chemical Industry Co. Ltd., Japan).

2.7.2. HPAEC-PAD

HPAEC-PAD was performed with a Dionex metal-free BioLC. Acidic oligosaccharides in Oligo-3A were separated on a Carbopac PA-1 column (0.4 × 25 cm, Dionex), and detected using a pulsed electrochemical detector equipped with a gold working electrode. To facilitate the detection of carbohydrates, 400 mM NaOH was added post-column at a flow rate of 0.5 ml/min. Oligosaccharides were eluted at 1 ml/min with 100 mM NaOH for 1 min, then with a 60 min linear gradient to 500 mM CH_3COONa in 100 mM NaOH.

2.8. Methylation analysis

The sample was methylated by the method of Hakomori (Hakomori, 1964), and the methylated product was recovered by a Sep-pak C_{18} cartride (Waters Asocc.) in accordance with the procedure of Waeghe, Darvill, McNeil & Albersheim (1983) except that the sample was eluted with EtOH. Uronic acids in the methylated sample were reduced by $LiB(C_2H_5)_3D$ in THF (Super-Deuteride®, 1 ml, room temperature, 1 h, Aldrich), and the reduced product was recovered by a Sep-pak C_{18} cartridge. Methylated sample was hydrolyzed with 2 M TFA at 121°C for 1 h, and the products were converted into alditol acetates. The resulting methylated alditol acetates were analyzed by GLC and GLC-MS using an SP-2380 capillary column (30 m × 0.25 mm i.d., 0.20-μm film thickness, Supelco) as described previously (Zhao, Kiyohara, Yamada, Takemoto & Kawamura, 1991). Methylated alditol acetates were identified by their fragment ions and relative retention times in GLC, and their molar ratios were calculated from the peak areas and response factors (Sweet, Shapiro & Albersheim, 1975).

2.9. Single radial gel diffusion by using β-glucosyl-Yariv antigen

Samples were tested for reactivity against the Yariv antigen by single radial gel diffusion (Holst and Clarke, 1985). β-d-glucosyl-Yariv antigen [1,3,5-tri-(4-β-d-glucopyranosyloxyphenylazo)-2,4,6-trihydroxybenzene (Biosupplies, Australia)] was used as a positive Yariv antigen, and α-d-glucosyl-Yariv antigen as a negative antigen. The samples (15 μg each) were applied to an agarose plate containing the Yariv antigen (10 μg/ml), and incubated overnight at room temperature).

3. Results

3.1. Contribution of neutral oligosaccharide side chains in "ramified" region on reactivity of "ramified" region to anti-AR-2IIb-IgG

Previous study has indicated that the "ramified" region (PG-1, rhamnogalacturonan core possessing side chains) of AR-2IIb contains antigenic epitopes for anti-AR-2IIb-IgG, but that the antibody could not recognize a rhamnogalacturono-tetrasaccharide which has resemblance to that in the rhamnogalacturonan core (Wang et al., 1994). PG-1 was digested either with *exo*-α-l-arabinofuranosidase or *exo*-β-d-galactosidase. The products were separately fractionated on Bio-gel P-10, and gave the fraction (Ara-PG-1 and Gal-PG-1, respectively) eluted in the void volume in addition to Ara and Gal, respectively (data not shown). Component sugar analysis indicated that 82.9% of Ara and 40% of Gal were released from PG-1 by the arabinofuranosidase and galactosidase digestions, respectively. The reactivity of Ara-PG-1 and Gal-PG-1 with β-glucosyl-Yariv antigel did not change compared to that of PG-1, and their inhibitory activities for the reactivity of PG-1 to anti-AR-2IIb-IgG were similar to that of PG-1 (data not

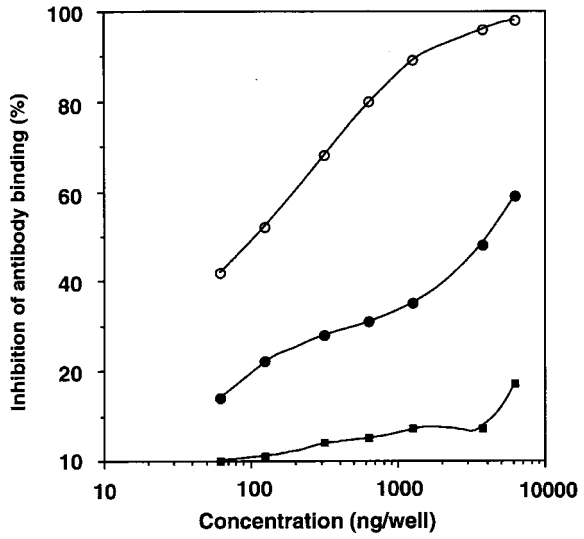

Fig. 1. Comparison of the inhibitory activity of neutral oligosaccharide–alditols and rhamnogalacturonotetrasaccharide on the reactivity of PG-1 to anti-AR-2IIb-IgG. ○, "ramified" region of AR-2IIb; ●, mixture of neutral oligosaccharide–alditols derived from "ramified" region by lithium degradation; ■, α-l-Rha-(1 → 4)-α-d-GalA-(1 → 2)-α-l-Rha-(1 → 4)-α-d-MeGalA.

shown). The inhibitory activity of PG-1 also did not change even when PG-1 was digested with both arabinofuranosidase and galactosidase (data not shown).

In order to know whether the carbohydrate side chains in PG-1 participates as the epitope, PG-1 was subjected to lithium degradation, and gave a mixture of liberated neutral oligosaccharide–alditols after anion-exchange chromatography. The mixture of neutral oligosaccharide–alditols showed weak, but significant inhibitory activity for reactivity of PG-1 with anti-AR-2IIb-IgG although rhamnogalacturono-tetrasaccharide did not show the inhibitory activity (Fig. 1). Therefore, the mixture of the liberated neutral oligosaccharide–alditols was further fractionated on Bio-gel P-30, and the fractions eluted in the void volume (NS-1), intermediate fraction and the lowest molecular weight oligosaccharide–alditol fraction were obtained (Fig. 2A). The intermediate and the lowest molecular weight oligosaccharide–alditol fractions were fractionated on Bio-gel P-2, and four fractions (NS-2–NS-5) were obtained (Fig. 2B and C). When NS-1–NS-5 were tested, the inhibitory activity for the reactivity of PG-1 with anti-AR-2IIb-IgG, only NS-1 had similar inhibitory activity as the mixture of neutral oligosaccharide–alditols (Fig. 3A). NS-1 was further purified on HPLC using combined columns of Asahi-pak GS-320 and GS-220, and two kinds of long oligosaccharide–alditols (NS-1-1 and NS-1-2) were obtained (Fig. 2D). Both

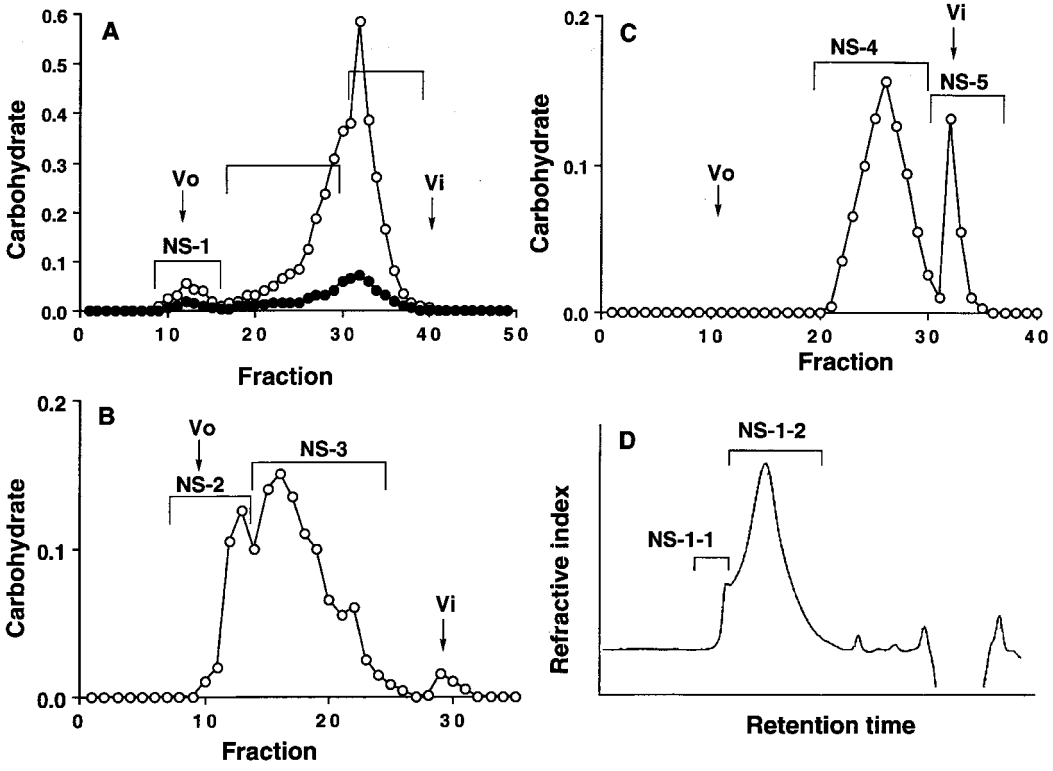

Fig. 2. (A) Gel filtration pattern on Bio-gel P-30 of a mixture of neutral oligosaccharide–alditols derived from "ramified" region of AR-2IIb by lithium degradation. ○, carbohydrate (490 nm); ●, pentose (552 nm); Vo. void volume; Vi inner volume. (B) Gel filtration pattern of intermediate fraction from (A) on Bio-gel P-2. Symbols are same as in (A). (C) Gel filtration pattern of the lowest-molecular-weight fraction from (A) on Bio-gel P-2. (D) HPLC pattern of NS-1 from (A) on combined columns of Asahi-pak GS-510 + GS-320.

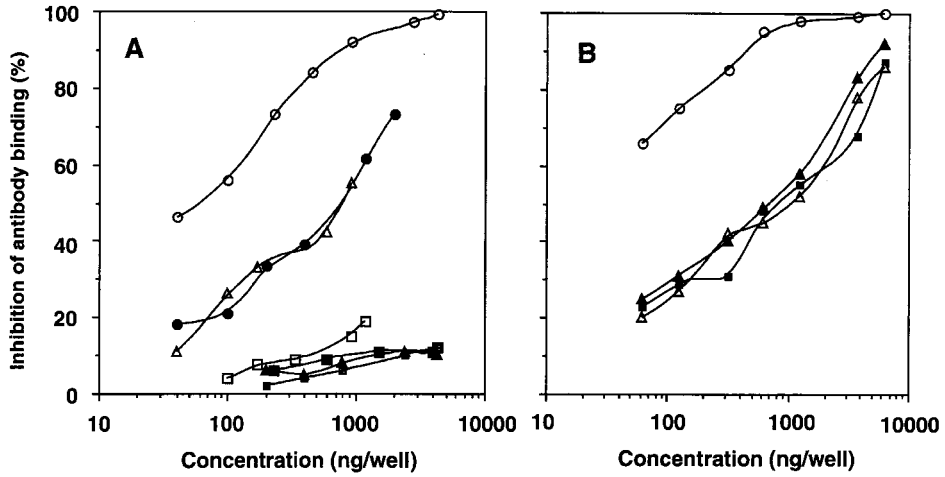

Fig. 3. Comparison of inhibitory activity of neutral oligosaccharide–alditols on interaction of "ramified" region of AR-2IIb with anti-AR-2IIb-IgG. (A) ○, "ramified" region of AR-2IIb; ●, mixture of oligosaccharide–alditols derived from "ramified" region of AR-2IIb by lithium degradation; △, NS-1; □, NS-2; ■, NS-3; ⊞, NS-4; ▲, NS-5. (B) ○, "ramified" region of AR-2IIb; ■, NS-1; △, NS-1-1; ▲, NS-1-2.

NS-1-1 and NS-1-2 exhibited similar inhibitory activities as NS-1 on the antigen–antibody interaction (Fig. 3B). NS-1-1 consisted of Rha, Man, Gal and Glc in molar ratios of 0.6:0.7:1.0:38.6 and NS-1-2 consisted of Ara, Rha, Man, Gal and Glc in molar ratios of 0.5:0.2:0.09:1.0:0.2. NS-1-1 could not be further analyzed because too little was available. Methylation analysis suggested that NS-1-2 consisted mainly of terminal, 3-linked, 6-linked and 3,6-branched Gal in addition to various linkages of Ara, Xyl, Rha, Man and Glc (Table 1).

As NS-1-2 was suggested to have β-(1 → 3,6)-galactan moiety, PG-1 was digested with *exo*-β-d-(1 → 3)-galactanase which could cleave β-(1 → 3)-galactan backbone in β-(1 → 3,6)-galactan (Tsumuraya et al., 1990). The products were fractionated on Bio-gel P-30 and gave a large amount of fraction (GN-1) eluted in the void volume and a small amount of fraction (GN-2) eluted in the excluded volume. (data not shown). Although PG-1 reacted strongly with β-glucosyl-Yariv antigen, GN-1 and GN-2 showed a weak or negligible reactivity with the antigen (data not shown). When GN-1 and GN-2 were tested for the inhibitory activity on the interaction of PG-1 with anti-AR-2IIb-IgG, GN-1 showed a decreased inhibitory activity (Fig. 4(A)). GN-2 had a weak but significant inhibitory activity. Ara-PG-1 was also further digested with *exo*-β-d-(1 → 3)-galactanase, and the enzyme-resistant fraction (GN-Ara-PG-1) was obtained by gel filtration on Bio-gel P-10 as the fraction eluted in the void volume (data not shown). When GN-Ara-PG-1 was tested for inhibitory activity on the interaction of PG-1 with anti-AR-2IIb-IgG, most of the inhibitory activity of PG-1 disappeared (Fig. 4(B)). These results suggest that anti-AR-2IIb-IgG mainly recognizes certain side chains on β-d-(1 → 3,6)-galactan-like moiety in the "ramified" region of AR-2IIb.

Table 1
Methylation analysis of NS-1-2 and Oligo-3A-4

Glycosyl residue	Linkage	Mol.% NS-1-2	Oligo-3A-4
Ara	Terminal (*f*)	3.7	14.9
	4 or 5	2.8	
Xyl	4	1.5	
Rha	3	3.4	
Gal	Trminal	10.4	
	3	19.5	30.7
	4	3.3	19.5
	6	9.4	
	4, 6	2.0	
	3, 6	20.1	19.7
Glc	Terminal	36.0	
	2	2.0	
	6	3.8	
	4	5.1	
	4, 6	1.2	
Man	3	2.4	
	6	2.3	
	3, 6	0.9	
4-OMe-GlcA	Terminal		15.2

3.2. Analysis of carbohydrate epitopes in side chains on β-d-(1 → 3,6)-galactan chains of "ramified" region

As GN-2 showed significant inhibitory activity against the interaction between PG-1 and anti-AR-2IIb-IgG, oligosaccharides in GN-2 seemed to have antigenicity for the antibody. It is generally considered that typical β-d-(1 → 3,6)-galactan moiety consists of β-d-(1 → 3)-galactan backbone which possesses β-d-(1 → 6)-galacto-oligosaccharide or Ara*f* side chains at position 6 of the backbone (Clarke, Anderson & Stone, 1979). β-d-(1 → 6)-galacto-di- to tetrasaccharides did not show inhibitory activity on the reactivity of PG-1 with anti-AR-2IIb-IgG (Fig. 4(C)). In order to clarify the oligosaccharides which react with the antibody, the digestion products of PG-1 by *exo*-β-d-(1 → 3)-galactanase

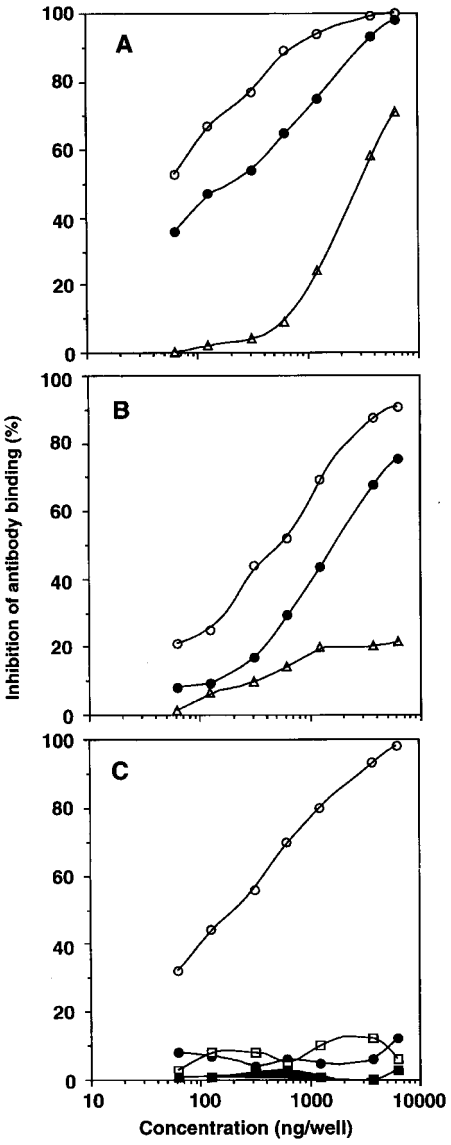

Fig. 4. Inhibitory activities of products, derived from "ramified" region of AR-2IIb by *exo*-β-d-(1 → 3)-galactanase digestion, and β-d-(1 → 6)-galacto-oligosaccharides. (A) ○, "ramified" region of AR-2IIb; ●, GN-1; △, GN-2. (B) ○, "ramified" region of AR-2IIb; ●, GN-1; △, GN-Ara-PG-1. (C) ○, "ramified" region of AR-2IIb; ●, β-d-(1 → 6)-galactobiose; □, β-d-(1 → 6)-galactotriose; ■, β-d-(1 → 6)-galactotetraose.

were fractionated by Bio-gel P-2. Oligosaccharide fractions (Oligo-1–6) and the resistant fraction eluted in the void volume were obtained (Fig. 5(A)). Among these fractions, Oligo-3 showed potent inhibitory activity against the interaction of PG-1 with the antibody (Fig. 5(B)). Oligo-3 was fractionated by DEAE-Sephadex to obtain neutral (Oligo-3N) and acidic (Oligo-3A) subfractions, and only Oligo-3A showed the inhibitory activity (data not shown). When oligosaccharides in Oligo-3A were further purified by HPAEC-PAD, 13 kinds of peaks were obtained (Fig. 6(A)), and Oligo-3A-1, 3–5, 9, 12 and 13 were suggested to contain carbohydrate by analyzing with phenol–H_2SO_4 method. These oligosaccharides were tested for inhibitory activity against PG-1-antibody interaction, and Oligo-3A-4 showed the most potent inhibitory activity at a concentration of 6250 ng/well (Fig. 6(B)). Methylation analysis using CD_3I indicated that Oligo-3A-4 consisted mainly of 3- and 4-linked and 3,6-branched Gal, and terminal Ara*f* and 4-OMe-GlcA at molar ratios of 1.0:1.3:2.1:1.3:1.0 (Table 1). MALDI-TOF-MS of Oligo-3A-4 failed to give a molecular ion peak because the amount was too small.

4. Discussion

Japanese herbal (Kampo) medicines have been given to patients for the treatment of several diseases, such as autoimmune diseases, allergy and inflammation which are hard to cure with modern medicines. Pectic polysaccharides such as pectins, pectic arabinogalactan and pectic heteroglycans have been considered to be essential ingredients for expression of the clinical effects of some Kampo medicines (Yamada and Kiyohara, 1989; Yamada, 1994). However, the applications of the pectic polysaccharides to quality control of Kampo medicines and their pharmacodynamics (absorption from digestive organs, tissue distribution, metabolism and excretion) have not yet been established as no specific reagent for the detection and quantification of pharmacologically active pectic polysaccharides is available. Recently, polyclonal antibody against an anti-ulcer pectin (bupleuran 2IIc), which has been purified from one of component herbs (*Bupleurum falcatum* L.) in Kampo medicines has been found to be applicable for the analysis of tissue distribution of bupleuran 2IIc on oral administration by the same group (Sakurai, Matsumoto, Kiyohara & Yamada, 1996). We have also prepared the antibody (anti-AR-2IIb-IgG) against the complement activating pectin (AR-2IIb), which is isolated from *A. acutiloba* Kitagawa, and anti-AR-2IIB-IgG has been considered to be useful for quality control of *A. acutiloba* (Kiyohara, Wang, Matsumoto, Sakurai & Yamada, 1995). However, the details of antigenic epitope for anti-AR-2IIb-IgG have not been yet clarified.

Previous results have suggested that anti-AR-2IIb-IgG cannot interact with the rhamnogalacturonan core though it recognizes the "ramified" region of AR-2IIb (Wang et al., 1994). The "ramified" region has been clarified to possess several kinds of galactosyl and arabinosyl side chains (Kiyohara and Yamada, 1989; Kiyohara et al., 1989a), and the present results indicated that Glc-rich side chain (NS-1-1) and the long galactosyl side chain (NS-1-2) consisting mainly of terminal, 3-linked, 6-linked and 3,6-branched Gal brought antigenic epitopes for the antibody. It is generally known that long β-(1 → 3,6)-galactosyl chains possess partially arabinosylated β-(1 → 6)-linked galacto-oligosaccharide and Ara*f* side chains at position 6 of the β-(1 → 3)-galactosyl backbone. The monoclonal antibody (CCRC-M7) against sycamore rhamnogalacturonan I, which consists of a rhamnogalacturonan core possessing

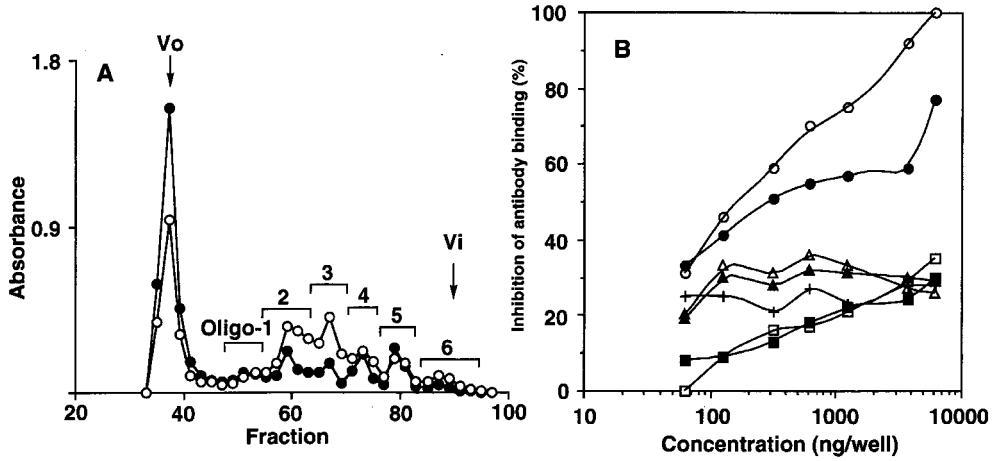

Fig. 5. (A) Gel filtration pattern of products from "ramified" region of AR-2IIb by *exo*-β-d-(1 → 3)-galactanase digestion on Bio-gel P-2. ●, carbohydrate; ○, uronic acid. (B) Inhibitory activity of oligosaccharide fractions obtained from (A) on interaction of "ramified" region of AR-2IIb with anti-AR-2IIb-IgG. ○, "ramified region; □, Oligo-1; ■, Oligo-2; ●, Oligo-3; △,Oligo-4; ▲,Oligo-5; (+) Oligo-6.

several kinds of side chains, has been found mainly to recognize neutral β-(1 → 6)-linked galacto-oligosaccharide chains with arabinosyl residue (Steffan, Kovac, Albersheim, Darvill & Hahn, 1995). However, it was indicated that anti-AR-2IIb-IgG did not recognize neutral β-(1 → 6)-linked galacto-oligosaccharide and Ara*f* chain. The present results showed that the minimum epitope for anti-AR-2IIb-IgG encompassed at least acidic galacto-oligosaccharide consisting of terminal, 4-linked, 3-linked Gal with 4-OMe-GlcA and Ara*f* residue, and suggested that rhamnogalacturonan core possessing β-(1 → 3)-galactosyl chain with the acidic galacto-oligosaccharide side chain (as mentioned earlier) is one of the major antigenic epitope for anti-AR-2IIb-IgG.

Sakurai, Kiyohara, Matsumoto, Tsumuraya, Hashimoto and Yamada. (1998) have clarified that the polyclonal antibody (anti-bupleuran 2IIc/PG-1-IgG) against the anti-ulcer pectin (bupleuran 2IIc) from *B. falcatum* recognizes β-d-(1 → 3)-galastosyl chains possessing (1 → 6)-linked galactosyl side chains containing terminal GlcA or 4-OMe-GlcA. The structure of the side chains in this antigenic epitope for anti-bupleuran 2IIc/PG-1-IgG is thought to be similar to that

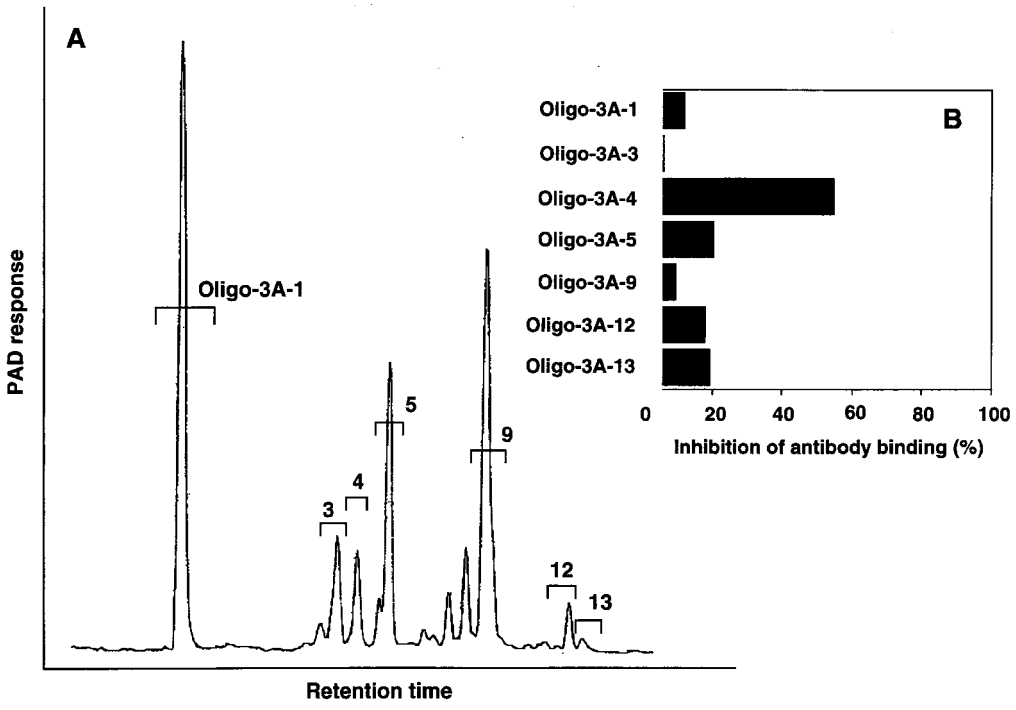

Fig. 6. (A) Elution pattern of Oligo-3 on HPAEC-PAD. (B) Inhibitory activity of oligosaccharides from (A) on interaction of "ramified" region of AR-2IIb with anti-AR-2IIb-IgG.

of the antigenic epitope for anti-AR-2IIb-IgG. However, anti-AR-2IIb-IgG and anti-bupleuran 2IIc/PG-1-IgG showed weak cross-reactivity with bupleuran 2IIc and AR-2IIb, respectively (data not shown). It is assumed that these antibodies may recognize different detailed structure in side chains of their "ramified" region of AR-2IIb and bupleuran 2IIc. Miskiel and Pazur (1991) have also clarified that polyclonal antibodies against gum arabic and gum mesquite recognize α-Ara-(1 → 4)-GlcA, β-d-GlcA-(1 → 6)-Gal and β-d-4-OMe-GlcA-(1 → 6)-Gal. These anti-polysaccharide antibody, including anti-AR-2IIb-IgG and anti-bupleuran 2IIc/PG-1-IgG were prepared by immunization of rabbits together with Freund's complete adjuvant. It is postulated that the immune system of rabbits may tend to recognize the sequences consisting of GlcA or 4-OMe-GlcA on the galactosyl chains of the polysaccharides.

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