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RELATIONSHIP BETWEEN STRUCTURE AND ACTIVITY OF THE "RAMIFIED" REGION IN ANTI-COMPLEMENTARY PECTIC POLYSACCHARIDES FROM *Angelica acutiloba* KITAGAWA*

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ABSTRACT

One of the anti-complementary pectic polysaccharides (AR-2IIa) isolated from the root of *Angelica acutiloba* Kitagawa gives the "ramified" region (PG-1a, rhamnogalacturonan with neutral side-chains) in addition to oligogalacturonides on digestion with endo-α-D-(1→4)-polygalacturonase. When the neutral side-chains in PG-1a were digested with both exo-α-L-arabinofuranosidase and exo-β-D-galactosidase, ~70% of the arabinosyl chains and ~30% of the galactosyl chains were released. The resistant product E-PG-1a had the same anti-complementary activity as PG-1a. E-PG-1a gave long (d.p. ≥5) and short (d.p. ≤4) neutral galactosyl chains after degradation of the GalA moiety by base-catalysed β-elimination in the presence of sodium borodeuteride followed with lithium-mediated degradation. Methylation analysis showed that the long galactosyl chains consisted mainly of terminal, 6-linked and 3,6-disubstituted Gal, and that the short chains were rich in 6-linked Gal. Degradation of the GalA moieties in PG-1a markedly decreased the anti-complementary activity, but the long and short galactosyl chains still expressed ~50 and ~20%, respectively, of the anti-complementary activity of E-PG-1a.

INTRODUCTION

Four anti-complementary pectic polysaccharides (AR-2IIa–IId)[2] isolated from the root of *Angelica acutiloba* Kitagawa consist of large proportions of polygalacturonan regions and small proportions of "ramified" regions. The anti-complementary activities of AR-2IIa–IId are expressed[2] mainly by their "ramified" regions, the activities of which are regulated by the polygalacturonan regions. The "ramified" regions consist[3] of rhamnogalacturonan cores with galactosyl and arabinosyl side-chains, some of which possess common structures and play a role in the expression of the anti-complementary activity.

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We now report on the relationship between structure and activity of the "ramified" regions.

EXPERIMENTAL

Materials and methods. — The root of *A. acutiloba* was purchased from Tochimoto Tenkaidoh (Japan), and AR-2IIa–IId were prepared[2] by hot-water extraction, precipitations with ethanol and Cetavlon (cetyltrimethylammonium bromide), and anion-exchange chromatography. Sephadex G-10 and DEAE-Sephadex A-25 were purchased from Pharmacia and Bio-gel P-6 (−400 mesh) from Bio-Rad. Pectinase from *Aspergillus niger* was purchased from Sigma, endo-α-D-(1→4)-polygalacturonase was purified[4] by Q.-P. Gao in our laboratory, exo-β-D-galactosidase from jack bean was purchased from Seikagaku Kogyo, and exo-α-L-arabinofuranosidase from *Rhodotolura flava* was a gift from Dr. N. Shibuya (National Food Research Institute).

Total carbohydrate in column eluates was assayed by the phenol–sulfuric acid method[5]. Samples were hydrolysed with 2M trifluoroacetic acid at 121° for 1.5 h, and t.l.c. of the products in the hydrolysates was performed on cellulose, using ethyl acetate–pyridine–acetic acid–water (5:5:1:3). Reducing sugars were detected with alkaline silver nitrate[6]. Neutral sugars and uronic acids, which were converted[7] into the corresponding reduced products, were analysed[8] by g.l.c. as the alditol acetates.

Preparation and modification of the "ramified" region (PG-1a) from AR-2IIa. — AR-2IIa was de-esterified in 0.2M sodium hydroxide for 2 h at room temperature, then digested with endo-α-D-(1→4)-polygalacturonase in 50mM acetate buffer (pH 4.2) at 37° for 4 days, and the products were fractionated on Sephadex G-50. PG-1a, eluted in the void volume, was digested either with exo-α-L-arabinofuranosidase or exo-β-D-galactosidase in 50mM acetate buffer (pH 4.0) at 50° or 37° for 4 days, to give AF-PG-1a or Gal-PG-1a, respectively, each of which was eluted from Sephadex G-10 in the void volume. PG-1a was also digested with both the arabinofuranosidase and galactosidase at 37° for 4 days, to give E-PG-1a.

Digestion of AR-2IIa–IId with enzyme. — Each polysaccharide was incubated with exo-α-L-arabinofuranosidase in 50mM acetate buffer (pH 4.0) at 50° for 4 days. The products were fractionated on Sephadex G-10, and AF-AR-2IIa–IId were each eluted in the void volume.

Degradation of the GalA moieties in PG-1a. — PG-1a was treated[9] with lithium in ethylenediamine and the reaction was stopped by adding water. The products were desalted with AG50W-X8 (H^+) resin, to obtain lithium-treated PG-1a.

Preparation of the neutral side-chains from PG-1a and E-PG-1a. — PG-1a and E-PG-1a were esterified with diazomethane and subjected[10] to base-catalysed β-elimination (0.1M NaOH, 100°, 3 h) in the presence of sodium borodeuteride. The procedure was repeated five times. The products were fractionated on DEAE-

Sephadex A-25 ($HCOO^-$ form) and neutral (*Na* and *E-Na*) and acidic fractions were obtained[10]. Each acidic fraction was treated with lithium in ethylenediamine as above, then with sodium borohydride, and the products were desalted with AG50W-X8 (H^+) resin, to give the neutral fractions *Aa* and *E-Aa*.

Methylation analysis. — Each sample was methylated[11], and the products were purified[12] using a Sep-pak C_{18} cartridge (Waters Assoc.), then hydrolysed with 2M trifluoroacetic acid at 121° for 1 h. The products were converted into the alditol acetates, and analysed[2] by g.l.c. and g.l.c.–e.i.-m.s. (70 eV) on an SPB-1 capillary column (SUPELCO) with splitless injection. G.l.c.–m.s. was performed on a Hewlett–Packard model 5890 gas chromatograph equipped with a Hewlett–Packard model 5970B mass spectrometer.

G.l.c.–m.s. of methylated oligosaccharide-alditols. — The methylated oligosaccharide-alditols were analysed by g.l.c.–m.s. on an SPB-1 capillary column. G.l.c.–c.i.(isobutane)-m.s. was performed on a JEOL DX-300 mass spectrometer with splitless injection, and g.l.c.–e.i.-m.s. (70 eV) on a Hewlett–Packard model 5970B mass spectrometer with on-column injection. C.i.-[13] and e.i.-m.s. fragment ions [A, J and alditol (ald)][14] were used to determine the structures of the methylated oligosaccharide-alditols.

Anti-complementary activity. — Various dilutions of the sample in water (50 μL) were mixed with 50 μL each of normal human serum (NHS) and gelatin–veronal-buffered saline (pH 7.4) containing 500μM magnesium chloride and 150μM calcium chloride (GVB^{2+}). The mixtures were pre-incubated at 37° for 30 min and the residual hemolytic complement (TCH_{50}) was determined using IgM-hemolysin-sensitised sheep erythrocytes. NHS was incubated with water to provide a control. The anti-complementary activity of the sample was expressed as inhibition of TCH_{50} (%) given by

$$\frac{TCH_{50}\text{ of control} - TCH_{50}\text{ treated with polysaccharides}}{TCH_{50}\text{ of control}} \times 100.$$

RESULTS

Effects on anti-complementary activity of treatment of PG-1a with enzymes. — When PG-1a was digested with either exo-α-L-arabinofuranosidase or exo-β-D-galactosidase in order to remove some neutral side-chains, 68.2% of Ara or 30.6% of Gal was released, but the products (AF-PG-1a and Gal-PG-1a) had the same anti-complementary activity as PG-1a (Table I). Likewise, digestion of PG-1a with the arabinofuranosidase and galactosidase gave a product (E-PG-1a) with the same activity as PG-1a (Table I).

Fragmentation of the neutral side-chains of E-PG-1a. — Base-catalysed β-elimination[10] of E-PG-1a gave a small proportion of a neutral fraction (*E-Na*) and a large proportion of an acidic fraction. Treatment[9] of the acidic fraction with lithium in ethylenediamine decomposed all the GalA and gave a neutral side-chain

TABLE I

EFFECTS OF CHEMICAL AND ENZYMIC TREATMENTS ON THE ANTI-COMPLEMENTARY ACTIVITY OF PG-1a

Treatment		*Concentration (μg/mL)*		
		1000	*500*	*100*
		Inhibition of TCH_{50} (%)		
1	Original	88.9	87.5	76.8
	Araf-ase[a]	89.7	86.4	78.3
	Gal-ase[b]	91.0	90.4	80.3
2	Original	79.9	77.9	71.9
	Araf-ase–Gal-ase[c]	82.1	80.4	70.9
3	Original	84.3	77.7	76.0
	Lithium-treated	37.5	31.5	10.8

[a]Exo-α-L-arabinofuranosidase. [b]Exo-β-D-galactosidase. [c]Exo-α-L-arabinofuranosidase and exo-β-D-galactosidase.

fraction (*E-Aa*). The ratio of *E-Na* and *E-Aa* was 1.1:1.0. *E-Na* and *E-Aa* contained Ara and Gal in the molar ratios 0.1:1.0 and trace:1.0, respectively, and, when fractionated on Bio-gel P-6, gave (Fig. 1) fractions (E-Na-1 and E-Aa-1) eluted in the void volume, intermediate fractions (E-Na-2 and E-Aa-2), and fractions (E-Na-3 and E-Aa-3) of low molecular weight. E-Na-2 and E-Aa-2 were eluted in the region of tri- and tetra-saccharides, and E-Na-3 and E-Aa-3 in the region of mono- and di-saccharides.

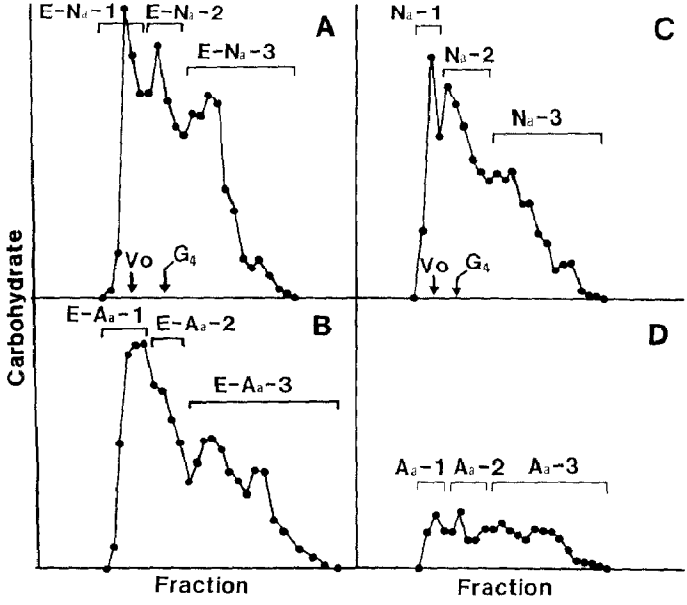

Fig. 1. Gel filtration on Bio-gel P-6 of **A**, fraction *E-Na*; **B**, fraction *E-Aa*; **C**, fraction *Na*; **D**, fraction *Aa*: ●, carbohydrate (490 nm); Vo, void volume; G_4, glucotetraose.

Structure of the neutral side-chains. — Methylation analysis (Table II) showed that E-Na-1 and E-Aa-1 mainly contained terminal, 3- and 6-linked, and 3,6-di-substituted Gal, and E-Na-2, E-Aa-2, E-Na-3, and E-Aa-3 contained mainly terminal and 6-linked Gal. E-Na-3 also contained terminal Ara*f*. Traces of 4-linked rhamnitol-*1-d* were present in E-Na-2, E-Aa-2, E-Na-3, and E-Aa-3; 2-linked rhamnitol-*1-d* in E-Na-2, E-Aa-2, and E-Aa-3; and 2-linked rhamnitol in E-Aa-2 and -3. E-Na-3 also contained a trace of 6-linked galactitol-*1-d*. Small proportions of terminal and 4-linked GalA were detected as Gal-*6,6-d*$_2$ in E-Na-1 and -2, and E-Aa-1–3, respectively, probably formed[10] during the β-elimination reaction.

The oligosaccharide-alditols in E-Na-2, E-Na-3, E-Aa-2, and E-Aa-3 were analysed by g.l.c.–m.s. (Tables III–V). E-Na-3 gave glycosyl-alditols (**1N–5N**) eluted in the region for disaccharide-alditols. In c.i.-m.s. (Table III), **1N** gave ions at *m/z* 442 [(M + H)$^+$], 219 (bA$_1$), and 206 (aJ$_2$); **2N–4N** gave ions at *m/z* 472 [(M + H)$^+$], 219 (bA$_1$), and 236 (aJ$_2$); and **5N** gave ions at *m/z* 474 [(M + H)$^+$], 221 (bA$_1$), and 236 (aJ$_2$), suggesting hexosyl→6-deoxyhexitol-*1-d*, hexosyl→hexitol-*1d*, and hexuronosyl→hexitol-*1-d* structures, respectively. From the e.i.-m.s. data and retention times in g.l.c., **1N–5N** were identified as Gal-(1→4)-Rha-ol-*1-d*, Gal-(1→3)-Gal-ol-*1-d*, Gal-(1→4)-Gal-ol-*1-d*, Gal-(1→6)-Gal-ol-*1-d*, and GalA-(1→6)-Gal-ol-*1-d*, respectively. E-Na-2 gave fragments eluted in the regions for tri- (**6N–9N**) and tetra-saccharide-alditols (**10N–12N**) in g.l.c.–e.i.-m.s. (Table IV). However, the fragments **6N–12N** could not be detected by g.l.c.–c.i.-m.s. E-Na-2 was subjected to g.l.c. on an SPB-1 capillary column by splitless injection in which the tri- and tetra-saccharide-alditols are usually lost, and the sensitivity of c.i.-m.s. was also lower than that of e.i.-m.s. The e.i.-m.s. data (Table IV) suggested that the fragments **6N–12N** possessed the partial structures shown in Table IV. From the methylation analysis data, most of the Gal and rhamnitol-*1-d* in **6N–12N** were indicated to be 6- and 4-linked, respectively. Therefore, **6N** was Gal-(1→6)-Gal-(1→4)-Rha-ol-*1-d*, **7N** was GalA-(1→6)-Gal-(1→4)-Rha-ol-*1-d*, **8N** or **9N** was Gal-(1→6)-Gal-(1→6)-ol-*1-d*, **10N** was Gal-(1→6)-Gal→Rha-(1→4)-Rha-ol-*1-d*, **11N** was Gal-(1→6)-Gal-(1→6)-Gal-(1→4)-Rha-ol-*1-d*, and **12N** was Gal-(1→6)-Gal-(1→6)-Gal-(1→6)-Gal-*ol-1-d*. Although E-Na-3 contained Ara (Table II), no oligosaccharide-alditols containing Ara were detected in g.l.c.–m.s. E-Aa-3 gave the fragments **1A-1–3A-2**, and E-Aa-2 gave **4A-1–6A-2** in g.l.c.–e.i.-m.s., respectively, and the fragments **1A-1–3A-2** were eluted in the region for di-saccharide-alditols and the fragments **4A-1–6A-2** in the region for trisaccharide-alditols in g.l.c. In g.l.c.–c.i.-m.s., these fragments could not be detected for the reason noted above. The e.i.-m.s. data (Table V) indicated E-Aa-3 to contain Gal-(1→2)-Rha-ol-*1-d* (**1A-1**), Gal-(1→2)-Rha-ol (**1A-2**), Gal-(1→4)-Rha-ol-*1-d* (**1A-3**), Gal-(1→4)-Rha-ol (**1A-4**), Gal-(1→3)-Gal-ol-*1-d* (**2A-1**), Gal-(1→3)-Gal-ol (**2A-2**), Gal-(1→6)-Gal-ol-*1-d* (**3A-1**), and Gal-(1→6)-Gal-ol (**3A-2**). Methylation analysis and e.i.-m.s. also suggested that E-Aa-2 contained Gal-(1→6)-Gal-(1→6)-Gal-ol-*1-d* (**6A-1**), and Gal-(1→6)-Gal-(1→6)-Gal-ol (**6A-2**). E.i.-m.s. also showed that E-Aa-2 contained GalA→Rha→Rha-ol-*1-d* (**4A-1**) and GalA→Rha→Rha-ol

TABLE II

METHYLATION ANALYSIS DATA

Glycosyl residue	*Position of OMe groups*	*Position of deuterium*	*Composition (mol %)*								
			PG-1a			*E-PG-1a*					
			Na-1	*Na-2*	*Na-3*	*E-Na-1*	*E-Na-2*	*E-Na-3*	*E-Aa-1*	*E-Aa-2*	*E-Aa-3*
Ara	2,3,5		2.8	7.4	41.4	2.6	n.d.	12.5	n.d.	n.d.	n.d.
	2,3		n.d.[a]	6.5	8.3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	2		n.d.	1.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Gal	1,2,3,4,5	1-*d*	n.d.	n.d.	n.d.	n.d.	n.d.	trace	n.d.	n.d.	n.d.
	2,3,4,6		9.7	19.0	33.1	23.6	30.0	67.1	40.2	48.2	69.7
	2,3,6		5.2	4.8	1.7	2.4	6.2	n.d.	3.9	3.9	3.4
	2,4,6		7.3	1.9	1.7	11.1	6.0	n.d.	12.9	7.7	4.1
	2,3,4		51.0	47.0	6.6	50.4	42.5	9.0	30.9	24.6	10.5
	2,3		n.d.	2.4	0.8	n.d.	1.3	n.d.	3.9	1.7	n.d.
	2,4		24.0	7.2	0.8	8.5	2.0	0.6	7.6	2.5	n.d.
	2		n.d.	0.9	n.d.	1.4	n.d.	n.d.	0.7	n.d.	n.d.
Rha	1,3,4,5		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	trace	trace
	1,3,4,5	1-*d*	n.d.	n.d.	n.d.	n.d.	trace	n.d.	n.d.	trace	trace
	1,2,3,5	1-*d*	n.d.	trace	trace	n.d.	trace	trace	n.d.	trace	trace
Glc	2,3,4,6		n.d.	1.1	3.7	n.d.	6.4	4.2	n.d.	n.d.	n.d.
	2,3,6		n.d.	n.d.	1.7	n.d.	1.1	n.d.	n.d.	1.9	2.1
GalA	2,3,4,6	6,6-d_2	n.d.	n.d.	n.d.	1.8	2.2	n.d.	n.d.	n.d.	n.d.
	2,3,6	6,6-d_2	n.d.	n.d.	n.d.	2.1	n.d.	n.d.	n.d.	7.7	8.0

[a]Not detected.

(**4A-2** and **5A**), but the retention time of **5A** was different from those of **4A-1** and **-2**. Although the glycosidic linkages in **4A-1** and **-2** and **5A** could not be deduced, the glycosidic linkages of fragment **5A** and fragments **4A-1** and **-2** differed. The fragments **1A-1, 1A-2, 4A-1, 4A-2**, and **5A** also were suggested to be formed from the rhamnogalacturonan core of E-PG-1a.

Contributions of neutral side-chains and GalA moieties to the anti-complementary activity of PG-1a. — Treatment[9] of PG-1a with lithium in ethylenediamine, in order to degrade the GalA, markedly decreased the anti-complementary activity (Table I). The neutral side-chains, E-Na-1 and E-Aa-1, had anti-complementary activities which were ~50% of that of E-PG-1a, whereas E-Na-2 and E-Aa-2 showed ~20% of the activity of E-PG-1a (Fig. 2). The anti-complementary activities of E-Na-3 and E-Aa-3 could not be measured because of the small amounts available.

Comparison of the structure of the neutral side-chains in PG-1a before and after the digestion with enzymes. — The neutral side-chain fractions (*Na* and *Aa*) from PG-1a (see above) consisted of Ara and Gal in the molar ratios 0.3:1.0 and trace:1.0, respectively. When fractionated on Bio-gel P-6, *Na* gave Na-1–3, and *Aa* gave Aa-1–3 (Fig. 1). Methylation analysis (Table II) indicated that Na-1 contained less terminal Gal and more 6-linked and 3,6-disubstituted Gal than E-Na-1. Na-2 and E-Na-2 contained terminal Gal and 6-linked Gal in the molar ratios 1.0:2.5 and 1.0:1.4, respectively. Na-3 contained more terminal Ara*f* and 4- or 5-linked Ara than E-Na-3. These results suggested that galactosyl chains consisting of 6-linked Gal and the arabinosyl chains in PG-1a were partially removed by the digestion with the galactosidase and arabinofuranosidase, respectively.

Because Aa-1–3 were obtained as minor fractions, further analysis could not be performed.

Effect on anti-complementary activity of treatment of the polysaccharides with enzyme. — AR-2IIa–IId were each digested with exo-α-L-arabinofuranosidase, to give AF-AR-2IIa–IId together with Ara (42.9, 43.8, 68.0, and 69.6%, respectively). Compared to the starting materials, the anti-complementary activity of AF-AR-2IIa was unchanged, that of AF-AR-2IIc was decreased by 13.5% at 1000 μg/mL, whereas those of AF-AR-2IIb and IId were increased by 9.0% at 100 μg/mL (data not shown).

DISCUSSION

The present study suggests that the product (E-PG-1a) of the enzyme-treated "ramified" region (PG-1a) possessed the structure essential for the expression of anti-complementary activity as proposed in Table VI. PG-1a contains[2] a rhamnogalacturonan core. Since the β-elimination and lithium-mediated degradation of E-PG-1a gave Gal→Rha→Rha (**4A-1, 4A-2**, and **5A**), its rhamnogalacturonan core is suggested to contain dirhamnosyl units. E-PG-1a contained mainly Gal-

TABLE III

DIAGNOSTIC IONS IN C.I.-M.S. OF METHYLATED DISACCHARIDE-ALDITOLS FROM E-Na-3

Fragment	*C.i.-m.s. fragment ions* [m/z *(relative abundance)*]							*Disaccharide-alditol*
	$(M + H)^+$	$(M + H)^+$ −MeOH	bA_1	bA_2	aJ_1	aJ_2	aJ_2OH_2	
1N	442 (36.6)	410 (100)	219 (100)	187 (100)	266 (19.2)	206 (50.2)	224 (82.2)	hexosyl→6-deoxyhexitol-*1-d*
2N	472 (13.2)		219 (100)	187 (26.1)		236 (39.7)	254 (80.1)	hexosyl→hexitol-*1-d*
3N	472 (0.7)	440 (1.1)	219 (54.0)	187 (100)		236 (1.7)	254 (2.4)	hexosyl→hexitol-*1-d*
4N	472 (23.0)	440 (3.8)	219 (50.9)	187 (100)		236 (39.4)	254 (47.4)	hexosyl→hexitol-*1-d*
5N	474 (55.7)	442 (5.5)	221 (10.1)	189 (84.5)		236 (56.6)	254 (100)	hexuronosyl→hexitol-*1-d*

TABLE IV

DIAGNOSTIC IONS ON E.I.-M.S. OF METHYLATED OLIGOSACCHARIDE-ALDITOLS FROM E-Na-2 AND E-Na-3

Fragment	*E.i.-m.s. fragment ions* [m/z *(relative abundance)*]									*Oligosaccharide-alditol*
	bA_1	bA_2	aJ_1	aJ_2	*ald*					
1N	219 (9.2)	187 (32.7)	266 (16.8)	206 (58.1)	307 (0.4)	134 (16.8)				Gal-(1→4)-Rha-ol-*1-d*
2N	219 (18.9)	187 (44.1)	296 (4.0)	236 (80.0)	382 (1.4)	350 (1.9)	338 (2.1)	306 (1.1)	133 (6.4)	Gal-(1→3)-Gal-ol-*1-d*
3N	219 (18.3)	187 (43.9)	296 (11.2)	236 (42.1)	349 (2.8)	305 (2.1)	134 (9.4)			Gal-(1→4)-Gal-ol-*1-d*
4N	219 (25.3)	187 (37.6)	296 (0.5)	236 (53.6)	337 (2.5)	305 (0.5)	293 (0.3)	261 (0.6)	146 (17.9)	Gal-(1→6)-Gal-ol-*1-d*
5N	221 (4.8)	189 (29.8)	296 (1.0)	236 (37.8)	339 (2.4)	295 (0.4)	263 (0.7)	251 (0.4)	146 (23.2)	GalA-(1→6)-Gal-ol-*1-d*
	cA_1	cA_2	aJ_1	aJ_2	cbA_1	cbA_2	abJ_1	abJ_2	*abJ − MeOH*	
6N	219 (37.5)	187 (80.8)	266 (21.6)	206 (81.5)	423 (3.8)			410 (4.9)	378 (3.3)	Gal→Gal→Rha-ol-*1-d*
7N		189 (52.2)	266 (0.7)	206 (100)				410 (0.4)		GalA→Gal→Rha-ol-*1-d*
8N	219 (10.9)	187 (14.0)	296 (0.3)	236 (93.9)	423 (35.1)			440 (27.8)		Gal→Gal→Gal-ol-*1-d*
9N	219 (87.6)	187 (31.9)	296 (0.1)	236 (3.7)	423 (35.1)	391 (0.1)		440 (10.1)		Gal→Gal→Gal-ol-*1-d*
	dA_1	dA_2	aJ_1	aJ_2	dcA_1	dcA_2	$dcbA_1$	abJ_1	abJ_2 $abcJ_2$	
10N	219 (15.3)	187 (46.8)	266 (0.1)	206 (100)	423 (0.3)	391 (0.7)			380 (1.1)	Gal→Gal→Rha→Rha-ol-*1-d*
11N	219 (56.5)	187 (80.6)	266 (17.9)	206 (100)	423 (2.2)	391 (0.3)		470 (8.8)	410 (6.1)	Gal→Gal→Gal→Rha-ol-*1-d*
12N	219 (7.1)	187 (15.4)	296 (0.4)	236 (100)	423 (3.2)	391 (2.2)			440 (37.6)	Gal→Gal→Gal→Gal-ol-*1-d*

TABLE V

DIAGNOSTIC IONS ON E.I.-M.S. OF METHYLATED OLIGOSACCHARIDE-ALDITOLS FROM E-Aa-2 AND E-Aa-3

Fragment	*E.i.-m.s. fragment ions* [m/z *(relative abundance)*]									*Oligosaccharide-alditol*
	bA_1	bA_2	aJ_1	aJ_2	*ald*					
1A-1	219 (9.1)	187 (34.0)	266 (10.2)	206 (41.9)	395 (0.3)	382 (0.2)	306 (0.7)			Gal-(1→2)-Rha-ol-*1-d*
1A-2	219 (9.1)	187 (34.0)	265 (4.9)	205 (24.7)	395 (0.3)	305 (1.8)				Gal-(1→2)-Rha-ol
1A-3	219 (9.1)	187 (34.0)	266 (10.2)	206 (41.9)	395 (0.3)	382 (0.2)	319 (0.7)	134 (10.9)		Gal-(1→4)-Rha-ol-*1-d*
1A-4	219 (9.1)	187 (34.0)	265 (4.9)	205 (24.7)	395 (0.3)	319 (0.7)	133 (6.1)			Gal-(1→4)-Rha-ol
2A-1	219 (21.7)	187 (53.8)	296 (3.8)	236 (13.0)	382 (2.2)	350 (4.2)	133 (10.3)			Gal-(1→3)-Gal-ol-*1-d*
2A-2	219 (21.7)	187 (53.8)	295 (15.2)	235 (41.5)	350 (4.2)	133 (10.3)				Gal-(1→3)-Gal-ol
3A-1	219 (8.1)	187 (35.3)	296 (0.4)	236 (17.8)	337 (3.7)	178 (0.9)	146 (4.4)			Gal-(1→6)-Gal-ol-*1-d*
3A-2	219 (8.1)	187 (35.3)		235 (66.6)	337 (3.7)	177 (6.6)	145 (26.5)			Gal-(1→6)-Gal-ol
	cA_1	cA_2	aJ_1	aJ_2	cbA_1	cbA_2	abJ_1	abJ_2	*abJ − MeOH*	
4A-1	221 (0.7)	189 (81.1)		206 (100)	395 (1.7)				348 (1.2)	GalA→Rha→Rha-ol-*1-d*
4A-2	221 (0.7)	189 (81.1)		205 (74.3)	395 (1.7)					GalA→Rha→Rha-ol
5A	221 (29.3)	189 (100)	265 (31.6)	206 (87.9)				379 (6.1)	348 (12.7)	GalA→Rha→Rha-ol
6A-1	219 (9.1)	187 (52.5)		236 (25.6)	423 (7.2)					Gal→Gal→Gal-ol-*1-d*
6A-2	219 (9.1)	187 (52.5)		235 (68.9)	423 (7.2)			439 (18.6)		Gal→Gal→Gal-ol

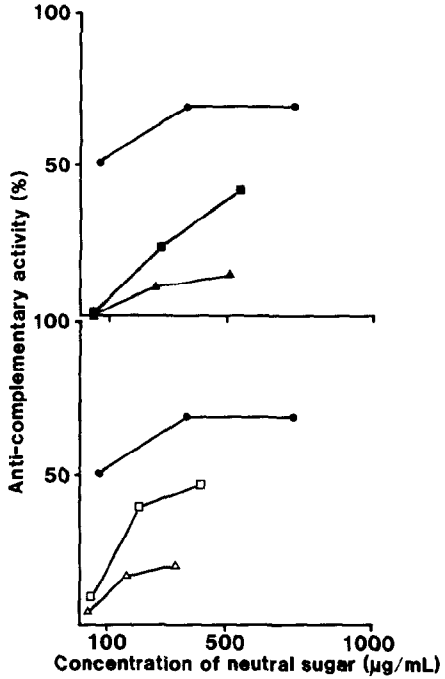

Fig. 2. Anti-complementary activity of neutral carbohydrate side-chains in enzyme-treated "ramified" region of AR-2IIa: ●, E-PG-1a; ■, E-Na-1; □, E-Aa-1; ▲, E-Na-2; △, E-Aa-2.

(1→6)-Gal-(1$\underset{0\text{–}2}{\rightarrow}$6)-Gal and galactosyl chains of d.p. ≥5 (Table VI). Since PG-1a reacted[3] strongly with the β-D-glucosyl-Yariv antigen, which has been used[15] for detection of (1→3,6)-β-D-galactans, and its long galactosyl chains comprised mainly terminal, 3- and 6-linked, and 3,6-disubstituted Gal, it might contain a (1→3,6)-β-D-galactan moiety as shown in Table VI. E-PG-1a contained galactosyl side-chains (*E-Aa*), which were obtained by both the β-elimination reaction and lithium-mediated degradation. Some of the galactosyl side-chains (**2A-1, 3A-1**, and **6A-1**) contained alditol-*1-d* units, indicating that they were attached originally to 4-linked GalA as in →4)-GalA-(1→(Gal)$_n$-(1→4)-GalA-(1→ as reported[3] (Table VI). The neutral side-chains in PG-1a are attached[3] to position 4 of 2,4-disubstituted Rha in the rhamnogalacturonan core either directly (R_2 and R_3 in Table VI) or through 4-linked GalA (R_1 in Table VI). The present results strongly suggest the Gal and di- to tetra-galacto-oligosaccharides to be attached to the rhamnogalacturonan core either directly (R_2 and R_3) or through 4-linked GalA (R_1) as shown in Table VI. However, it was not possible to determine whether the long galactosyl side-chains were attached to position 4 of 2,4-disubstituted Rha in the rhamnogalacturonan core directly (R_2 and R_3) or through 4-linked GalA (R_1). The high-molecular-weight galactan side-chains of other pectic arabinogalactans (AGIIb-1 and AR-4IIc) from *A. acutiloba*[10,16] are attached mainly to rhamnogalacturonan core

TABLE VI

STRUCTURE OF THE NEUTRAL SIDE-CHAINS IN E-PG-1a

→2)-Rha-(1→4)-GalA-(1→2)-Rha-(1→2)-Rha-(1→4)-GalA-(1→2)-Rha-(1→4)-GalA-(1→2)-Rha-(1→4)-GalA-(1→

R_3-(1→4) *R_1-(1→4)-GalA-(1→4)* *R_2-(1→4)*

R_1	Gal-(1→3)-Gal-	**2N**
	Gal-(1→4)-Gal-	**3N**
	Gal-(1→6)-Gal-	**4N**
	Gal-[(1→6)-Gal]$_2$-	**8N** or **9N**
	Gal-[(1→6)-Gal]$_3$-	**12N**
	GalA-(1→6)-Gal-	**5N**
	→4)-GalA→Gal-(1→3)-Gal-	**2A-1**
	→4)-GalA→Gal-(1→6)-Gal-	**3A-1**
	→4)-GalA→Gal-(1→6)-Gal]$_2$-	**6A-1**
R_2	Gal-	**1N**
	Gal-(1→6)-Gal-	**6N**
	Gal-(1→6)-Gal]$_2$-	**11N**
	GalA-(1→6)-Gal-	**7N**
R_3	Gal-(1→6)-Gal-	**10N**
R_1 or R_2	[Gal-(1→6)]$_3$ \ →3)-Gal-(1→3)-Gal-(1→3)-Gal-(1→ / [Gal-(1→6)]$_4$ ± →4)-GalA-(1→	

through 4-linked GalA (R_1). Therefore, it is assumed that the long galactosyl side-chains in E-PG-1a might be linked to the rhamnogalacturonan core in the same manner. Comparison of the neutral side-chains indicated that PG-1a possessed longer galactosyl chains than E-PG-1a.

The present study suggests that the rhamnogalacturonan core in PG-1a contributed strongly to the expression of the anti-complementary activity, however, the (1→3,6)-β-D-galactan moiety of E-PG-1a still expressed anti-complementary activity. A pectin, isolated[17] from the fruit of *Zizyphus jujuba* and which showed[18] no anti-complementary activity, consisted[17] mainly of 4-linked Gal as the neutral sugar. Therefore, the neutral side-chains in PG-1a may be associated with the expression of the activity. The comparison of the neutral side-chains of PG-1a and E-PG-1a assumed the presence of arabinosyl chains in PG-1a. Since the arabinosyl chains were eluted from Bio-gel P-6 in the region for tri- and tetra-saccharides, and most of the chains could be hydrolysed by exo-α-L-arabinofuranosidase, PG-1a is suggested to contain arabinofuranosyl chains such as Ara*f*-(1→5)-Ara*f*-(1$\rightarrow_{0-2}$. Although E-PG-1a did not contain such chains, its anti-complementary activity was similar to that of PG-1a. Therefore, the anti-complementary activity of PG-1a may be due to a combination of the rhamnogalacturonan core and the long galactosyl chains.

The "ramified" regions of AR-2IIb–IId contain[3] a lower proportion of the (1→3,6)-β-D-galactan moiety than PG-1a in addition to shorter galactosyl chains as indicated by their reactivity[19] with the β-D-glucosyl-Yariv antigen; however, their anti-complementary activities were as potent as that of PG-1a. The "ramified" regions of AR-2IIb–IId contain[3] larger proportions of arabinosyl chains than PG-1a, but the present results also indicate that the arabinosyl chains in AR-2IIb–IId were less involved in the expression of the anti-complementary activities. The anti-complementary activities of "ramified" regions of AR-2IIb–IId may be expressed also by a combination of rhamnogalacturonan core and the short galactosyl chains.

The neutral arabinogalactan unit (N-I), isolated[20] from the anti-complementary pectic arabinogalactan (AGIIb-1), was[1] as potently active as PG-1a, and N-I possessed[16] the galactan framework in which (1→6)-β-D-galactosyl side-chains were attached to position 6 of the (1→3)-β-D-galactan backbone. The combination of (1→6)-β-D-galactosyl chains with either the rhamnogalacturonan core or the (1→3)-β-D-galactan might be responsible for the expression of potent anti-complementary activity.

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