Carbohydrate Research, 193 (1989) 193–200
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

RELATIONSHIP BETWEEN STRUCTURE AND ACTIVITY OF AN ANTI-COMPLEMENTARY ARABINOGALACTAN FROM THE ROOTS OF *Angelica acutiloba* KITAGAWA*

HIROAKI KIYOHARA, JONG-CHOL CYONG, AND HARUKI YAMADA†
Oriental Medicine Research Center of the Kitasato Institute, Minato-ku, Tokyo 108 (Japan)
(Received December 9th, 1988; accepted for publication, May 2nd, 1989)

ABSTRACT

An anti-complementary arabinogalactan (AGIIb-1), isolated from the roots of *Angelica acutiloba* Kitagawa, comprised one neutral (N-I) and two acidic arabinogalactan (A-I and A-II) units and one neutral arabinan unit (N-II). N-I showed the most potent anti-complementary activity, AGIIb-1, A-I, and A-II had similar moderate activities, but N-II had weak activity. The product (AF-AGIIb-1) of digestion of AGIIb-1 with exo-α-L-arabinofuranosidase had markedly increased anti-complementary activity, as did that (AF-N-I) of N-I. Degradation of the rhamnogalacturonan core in AGIIb-1 slightly decreased the anti-complementary activity, whereas the high-molecular-weight neutral arabinogalactan and galacto-oligosaccharide side-chains in A-I and A-II showed potent activities. When AF-AGIIb-1 was digested with endo-arabinanase, the activity decreased slightly. Partial elimination of the (1→6)-β-D-galactosyl side-chains from AF-N-I by digestion with exo-β-D-galactosidase did not affect the activity. AGIIb-1 reacted weakly with the β-D-glucosyl-Yariv antigen, but AF-AGIIb-1 and AF-N-I had increased reactivity with the antigen. The anti-complementary activity of AGIIb-1 was expressed mainly through the classical pathway, whereas AF-AGIIb-1 and AF-N-I had markedly increased activity through the alternative pathway.

INTRODUCTION

The anti-complementary arabinogalactan (AGIIb-1[2]), isolated from the roots of *Angelica acutiloba* Kitagawa (Japanese name, Yamato-Tohki), is a complex pectic arabinogalactan[3,4]. The structural features of the carbohydrate units of AGIIb-1 are summarised in Table I. The units A-I and A-II are linked through Rha in the core. N-I is attached to the rhamnogalacturonan core or to the galactosyl oligosaccharide chain in A-I through Rha or Gal, and N-II is attached to the Ara*p*-rich oligosaccharide chain in A-I through Ara. It has been suggested[1,3] also that the

*Studies on Polysaccharides from *A. acutiloba*, Part XI. For Part X, see ref. 1.
†Author for correspondence.

0008-6215/89/$ 03.50

TABLE I

NEUTRAL CARBOHYDRATE CHAIN MOIETY IN CARBOHYDRATE UNITS OF AGIIb-1

Carbohydrate unit	*Neutral carbohydrate side-chains*[1]	*Structure*[1]
N-I		Arabino-3,6-galactan [(1→3)-linked galactan backbone branched at positions 6, (1→6)-linked galactosyl side-chains with Ara*f* side-chains attached at positions 3]
N-II		(1→3,5)-α-L-Arabinan [(1→5)-linked L-arabinan with Ara*f* side-chains attached at positions 3]
A-I	Oligo-1	Galactosyl chain [6-, 3,6-, and 4,6-linked Gal-rich chain with Ara side-chains]
	Oligo-2	Arabinosyl chain [terminal, 3-linked Ara*p*, and 4- or 5-linked Ara-rich chain]
	H-N_I-1	High-molecular-weight arabinogalactan consisting mainly of an arabino-3,6-galactan skelton
A-II	H-N_{II}-1	High-molecular-weight arabinogalactan consisting mainly of (1→3,6)-linked galactan framework with branched arabinosyl side-chains
	H-N_{II}-2	High-molecular-weight complex galactan with branched arabinosyl side-chains

acid-labile glycosidic linkages in AGIIb-1 and Ara*f* side-chains are involved in the expression of the anti-complementary activity.

We now report on the relationship between structure and anti-complementary activity of AGIIb-1.

EXPERIMENTAL

Materials and methods. — The source of the *A. acutiloba* roots and the isolation of AGIIb-1 have been reported[1,2]. Exo-α-L-arabinofuranosidase-digested AGIIb-1 (AF-AGIIb-1) was prepared as described[3], and the units, N-I, N-II, A-I, and A-II from AGIIb-1, and AF-N-I, AF-N-II, AF-A-I, and AF-A-II from AF-AGIIb-1, were purified as described[4]. Endo-arabinanase[5] was purified[6] from *Bacillus subtilis F-11. B. subtilis* F-11, exo-α-L-arabinofuranosidase *(Rhodotolura flava),* and α- and β-D-glucosyl-Yariv antigens were gifts from Dr. A. Kaji, Dr. N. Shibuya (National Food Research Institute, Ministry of Agriculture, Forestry and Fisheries, Japan), and Dr. A. E. Clarke (Plant Cell Biology Research Center, School of Botany, University of Melbourne), respectively. Exo-β-D-galactosidase (jack bean) was purchased from Seikagaku Kogyo.

Total carbohydrate in column eluates was assayed by the phenol–sulfuric acid method[7].

Preparation of the neutral side-chains from A-I and A-II. — H-N_I-1, H-N_{II}-1, H-N_{II}-2, Oligo-1, and Oligo-2 were prepared as described[1], and their structures are shown in Table I.

Degradation of the rhamnogalacturonan core in AGIIb-1. — The core was degraded[1] by base-catalysed β-elimination in the presence of sodium borohydride[8].

Enzymic digestion. — (*a*) *Exo-β-D-galactosidase.* AF-N-I was digested with exo-β-D-galactosidase in 50mM acetate buffer (pH 4.0) at 37° for 4 days. The digest was eluted (water) from Sephadex G-10 to give Gal-AF-N-I in the void volume.

(*b*) *Endo-arabinanase.* AF-AGIIb-1 was incubated with endo-arabinanase[5,6] in 10mM phosphate buffer (pH 6.0) at 30° for 4 days. The digest was eluted (water) from Sephadex G-10 to give endo-arabinanase-digested AGIIb-1 in the void volume.

Single radial gel-diffusion of polysaccharides using the Yariv antigen. — Polysaccharides were tested for reactivity against the Yariv antigen by single radial gel-diffusion[9]. β-D-Glucosyl-Yariv antigen [1,3,5-tri-(4-β-D-glucopyranosyloxyphenylazo)-2,4,6-trihydroxybenzene] was used as a positive Yariv antigen, and α-D-glucosyl-Yariv antigen as a negative antigen. The samples (15 μg of each) were applied to an agarose plate containing the Yariv antigen (10 μg/mL), and incubated overnight at room temperature.

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 $MgCl_2$ and 150 μM $CaCl_2$ (GVB^{2+}), and the mixtures were pre-incubated at 37° for 30 min. The residual hemolytic complement (TCH_{50}) was determined[10] using IgM-sensitised sheep erythrocytes, and the total anti-complementary activities were calculated as described[2,3].

In order to determine the anti-complementary activity through the alternative pathway, the mixture of the polysaccharide solution and NHS was pre-incubated with Gelatin–veronal-buffered saline (pH 7.4) containing 10mM ethylenebis(oxyethylenenitrilo)tetra-acetic acid (EGTA) and 2mM $MgCl_2$ (Mg^{2+}–EGTA–GVB^{2-}). TCH_{50} was then measured in GVB^{2+}.

Determination of C4. — Titration of C4 was performed[11] using intermediate cells $EAC1^{gp}$ for C4. $EAC1^{gp}$ cells were prepared from EA (10^8 cells/mL) incubated with C1 solution (10^{12} SFU/mL) in the ratio of 28:1 at 4° for 1 h.

RESULTS

Anti-complementary activity of the carbohydrate units. — The activities are shown in Table II. Exo-α-L-arabinofuranosidase-digested AGIIb-1 (AF-AGIIb-1) was more potent than AGIIb-1[3]. Of the carbohydrate units from AGIIb-1, N-I was slightly more active than A-I and A-II, which had moderate activities, and N-II had weak activity. AF-N-I from AF-AGIIb-1 was the most potent of all the units. The activities of AF-N-II and N-II were similar, as were those of AF-A-I and A-I, whereas that of AF-A-II was less than that of A-II.

TABLE II

ANTI-COMPLEMENTARY ACTIVITY OF POLYSACCHARIDES AND CARBOHYDRATE UNITS THROUGH CLASSICAL AND/OR ALTERNATIVE PATHWAYS

Polysaccharide	*Buffer of pre-incubation*[a]	*Anti-complementary activity (%)*		
		Concentration (μg/mL)		
		1000	*500*	*100*
AGIIb-1	A	79.0	63.5	100.0
	B	18.5	10.6	
AF-AGIIb-1	A	93.0	86.2	41.5
	B	70.0	34.0	11.0
N-I	A	80.2	76.5	42.5
	B	17.0	6.0	
AF-N-I	A	100.0	87.0	74.5
	B	40.0	26.8	14.5
N-II	A	40.0	27.5	11.0
	B	2.0		
AF-N-II	A	42.5	27.5	4.0
	B	9.0		
A-I	A	65.0	47.0	34.0
	B	15.0	12.0	
AF-A-I	A	65.0	42.0	25.0
	B	14.0	4.6	
A-II	A	80.0	60.0	33.5
	B	27.0	14.2	7.0
AF-A-II	A	54.0	37.0	22.0
	B	n.d.[b]	9.0	5.0

[a]A, GVB^{2+} + NHS for anti-complementary activity through both classical and alternative pathways; B, Mg^{2+}–EGTA–GVB^{2-} + NHS for activity through the alternative pathway. [b]Not determined.

Contribution of the rhamnogalacturonan core and the neutral side-chains to the anti-complementary activity. — When the rhamnogalacturonan core in AGIIb-1 was degraded[1] by base-catalysed β-elimination in the presence of sodium borohydride[8], the anti-complementary activity of the product (Table III) was decreased slightly. A-I and A-II (Table I) gave[1] neutral side-chains on degradation[8] of the rhamnogalacturonan core. H-N_I-1 and Oligo-1 from A-I had anti-complementary activities higher than that of A-I, whereas Oligo-2 had lower activity (Table IV). The activities of H-N_{II}-1 and -2 were similar to that of A-II.

Effects of enzymic digestion on the anti-complementary activity of AGIIb-1 and N-I. — AF-AGIIb-1 was further digested with endo-arabinanase[5,6] in order to cleave the arabinan moiety, obtained as AF-N-II after the mild acid treatment of AF-AGIIb-1, and the products were fractionated on Sephadex G-10 to give endo-arabinanase-digested AF-AGIIb-1, the anti-complementary activity of which was slightly less than that of AF-AGIIb-1 (Table III). When AF-AGIIb-1 was mildly treated[3] with acid (10mM hydrochloric acid, 100°, 10 min) in order to cleave the acid-labile linkages, the activity of the product was lower than that of endo-arabinanase-digested AF-AGIIb-1 (Table III).

TABLE III

EFFECTS OF ENZYMIC AND CHEMICAL DEGRADATION ON ANTI-COMPLEMENTARY ACTIVITY OF AGIIb-1 AND N-I

Polysaccharide	*Treatment*	*Anti-complementary activity (%)*		
		Concentration (μg/mL)		
		1000	*500*	*100*
AF-AGIIb-1[a]	None	89.0	78.5	70.0
	Endo-arabinanase-digested	81.0	70.5	64.0
	10mM HCl, 100°, 10 min	54.4	46.2	28.2
AGIIb-1	None	80.0	69.0	33.5
	Rhamnogalacturonan-degraded[b]	70.0	63.0	26.5
AF-N-I[c]	None	86.9	85.6	76.2
	β-D-Galactosidase-digested	88.5	89.1	83.8

[a]Exo-α-L-arabinofuranosidase-digested AGIIb-1. [b]AGIIb-1 treated with 0.1M sodium hydroxide containing 0.2M sodium borohydride at 100° for 3 h after methyl-esterification. [c]Obtained as the neutral arabinogalactan unit from exo-α-L-arabinofuranosidase-digested AGIIb-1 by treatment with a weak acid.

AF-N-I from AF-AGIIb-1 was digested further with exo-β-D-galactosidase, and the products were fractionated on Sephadex G-10 to give Gal-AF-N-I. Although ~35% of the Gal was released from AF-N-I by the digestion, the anti-complementary activity was not changed (Table III).

Mode of action of the polysaccharides. — AGIIb-1, AF-AGIIb-1, and their units were incubated with NHS in GVB^{2+}, and the residual activity of C4, involved in the classical pathway of the complement system, was measured by C4 titration (Table V). When 1000 μg/mL of AGIIb-1 was used for the titration, 94% of the

TABLE IV

ANTI-COMPLEMENTARY ACTIVITY OF A-I AND A-II AND THEIR NEUTRAL SIDE-CHAINS

Acidic unit	*Neutral carbohydrate side-chains*	*Anti-complementary activity (%)*		
		Concentration (μg/mL)		
		1000	*500*	*100*
A-I		32.0	26.0	17.0
	Oligo-1	45.0	37.5	24.0
	Oligo-2	29.0	21.0	6.0
	H-N_I-1	59.0	49.0	36.5
A-II		50.0	43.5	25.4
	H-N_{II}-1	54.0	46.0	39.0
	H-N_{II}-2	40.5	29.5	11.4

TABLE V

ANTI-COMPLEMENTARY ACTIVITY OF ARABINOGALACTANS AND THEIR UNITS THROUGH THE CLASSICAL PATHWAY

Polysaccharide	*Consumption of C4 (%)*	
	Concentration (μg/mL)	
	1000	*500*
AGIIb-1	94.0	44.5
AF-AGIIb-1[a]	96.0	81.8
N-I[b]	97.2	92.4
AF-N-I[c]	97.2	97.8
N-II[b]	25.4	18.5
AF-N-II[c]	55.8	29.8
A-I[b]	87.8	65.0
AF-A-I[c]	90.5	64.0
A-II[b]	93.4	85.0
AF-A-II[c]	80.8	53.2

[a]Exo-α-L-arabinofuranosidase-digested AGIIb-1. [b]Obtained from AGIIb-1. [c]Obtained from AF-AGIIb-1.

hemolytic titre of C4 was consumed. N-I, A-I, and A-II also consumed most of C4 (97–98% at 1000 μg/mL), whereas ~80% of C4 remained after incubation of N-II. These results showed that AGIIb-1, N-I, A-I, and A-II strongly activated the complement system through the classical pathway. AF-AGIIb-1 consumed more C4 (82%) at 500 μg/mL than AGIIb-1, but AF-N-I and AF-A-I consumed C4 to extents similar to those of N-I and A-I. AF-N-II also consumed more C4 than N-II, whereas AF-A-II had a less increased consumption than A-II.

When the polysaccharides were pre-incubated with NHS in Mg^{2+}–EGTA–GVB^{2-} in order to measure the extent of the alternative pathway of the complement system, AGIIb-1, N-I, A-I, and A-II showed weak activities (15–27% at 1000 μg/mL) through the alternative pathway, and N-II had negligible activity (Table II). AF-AGIIb-1 had increased activity through the alternative pathway compared with AGIIb-1, whereas only AF-N-I among the units from AF-AGIIb-1 had increased activity compared to the corresponding units from AGIIb-1 (Table II).

Reactivity of the polysaccharides with the Yariv antigen. — Since β-D-glucosyl-Yariv antigen reacts[12] with (1→3,6)-β-D-galactan to form a red dye, the reactivity of AGIIb-1, AF-AGIIb-1, and their units to the antigen were tested by the single radial gel-diffusion[9]. As shown in Fig. 1, AGIIb-1 reacted with the antigen, whereas AF-AGIIb-1 had markedly increased reactivity. A-I, AF-A-I, A-II, and AF-A-II reacted with the antigen similarly, whereas AF-N-I had markedly increased reactivity compared with N-I. N-II and AF-N-II did not react with the antigen. AGIIb-2, which was isolated[2] as an anti-complementary inactive arabinogalactan from the arabinogalactan fraction of *A. acutiloba*, also did not react with the antigen. These polysaccharides did not react with α-D-glucosyl-Yariv antigen as negative control[12] (data not shown).

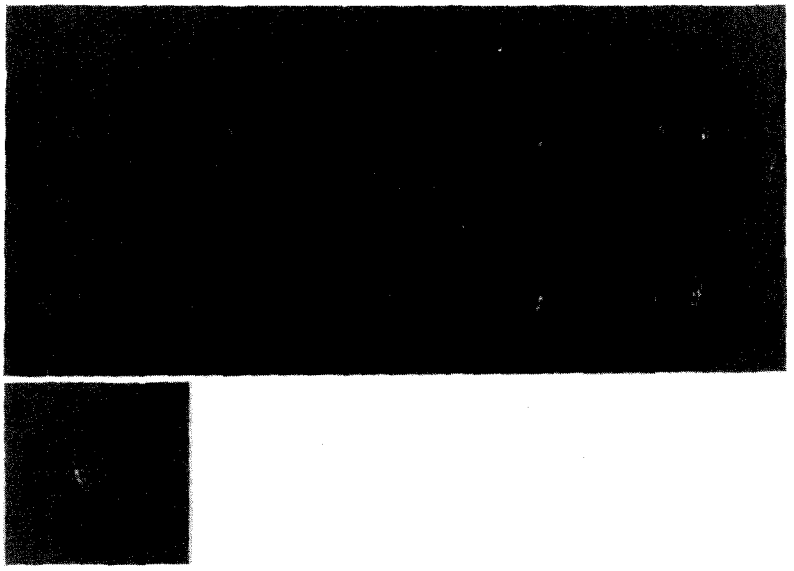

Fig. 1. Single radial gel-diffusion, using β-D-glucosyl-Yariv antigen, of polysaccharides: a, AGIIb-1; b, AF-AGIIb-1; c, AGIIb-2; d, N-I; e, AF-N-I; f, N-II; g, AF-N-II; h, A-I; i, AF-A-I; j, A-II; k, AF-A-II.

DISCUSSION

H-N_I-1, H-N_{II}-1, and H-N_{II}-2, in A-I and A-II, and N-I showed potent anti-complementary activities, but the rhamnogalacturonan core did not contribute much to the activity of AGIIb-1. Therefore, the activity of AGIIb-1 is expressed by the neutral side-chains. However, the arabinan moiety contributed less to the activity of AGIIb-1 because N-II showed a weak activity. Elimination of the Ara*f* side-chains from AGIIb-1 and N-I markedly increased the anti-complementary activities and reactivities with β-D-glucosyl-Yariv antigen, suggesting that the (1→3,6)-β-D-galactan moiety of N-I makes a major contribution to the activity of AGIIb-1.

Partial removal[1] of (1→6)-β-D-galactosyl side-chains from AF-N-I did not affect the activity, and the inactive arabinogalactan AGIIb-2[2], which contained a large proportion of (1→6)-D-galactosyl linkages, did not react with β-D-glucosyl-Yariv antigen. Thus, the (1→3)-β-D-galactan backbone might be essential for the expression of anti-complementary activity.

AGIIa, isolated[13] as an anti-complementary arabinogalactan from *A. acutiloba* and proposed to be a typical arabino-3,6-galactan, reacted strongly with β-D-glucosyl-Yariv antigen (unpublished data), suggesting that AGIIa contained a galactan framework similar to that of N-I.

It has been proposed[1] that the acid-labile glycosidic linkages (Ara, Gal, and Rha), which join the four units of AGIIb-1, are involved[3] in the anti-complementary activity. However, cleavage of the arabinan moiety in AGIIb-1 did not affect the anti-complementary activity.

AGIIb-1 expresses the anti-complementary activity mainly through the classical pathway of the complement system, whereas AF-AGIIb-1 expressed its activity through both the classical and the alternative pathway. These findings suggest that the (1→3,6)-β-D-galactan moiety is involved in the expression of the anti-complementary activity through both the classical and alternative pathways, and that the Ara*f* side-chains attached strongly inhibit expression of activity through the alternative pathway.

ACKNOWLEDGMENTS

We thank Dr. A. E. Clarke for gifts of α- and β-D-glucosyl-Yariv antigen, Dr. A. Kaji for a gift of *B. subtilis* F-11, Dr. N. Shibuya for a gift of exo-α-L-arabinofuranosidase, and Dr. Y. Otsuka for encouragement. A part of this work was supported by a fund from Tsumura Co. Ltd., Japan.

REFERENCES

1 H. KIYOHARA AND H. YAMADA, *Carbohydrate Res.*, 193 (1989) 173–192.
2 H. KIYOHARA, H. YAMADA, J.-C. CYONG, AND Y. OTSUKA, *J. Pharmacobio-Dyn.*, 9 (1986) 339–346.
3 H. YAMADA, H. KIYOHARA, J.-C. CYONG, AND Y. OTSUKA, *Carbohydr. Res.*, 159 (1987) 275–291.
4 H. KIYOHARA, H. YAMADA, AND Y. OTSUKA, *Carbohydr. Res.*, 167 (1987) 221–237.
5 A. KAJI AND T. SAHEKI, *Biochim. Biophys. Acta*, 410 (1975) 354–360.
6 L. WEINSTEIN AND P. ALBERSHEIM, *Plant Physiol.*, 63 (1979) 425–432.
7 M. DUBOIS, K. A. GILLES, J. K. HAMILTON, P. A. REBERS, AND F. SMITH, *Anal. Chem.*, 28 (1956) 350–356.
8 H. KIYOHARA AND H. YAMADA, *Carbohydr. Res.*, 187 (1989) 117–129.
9 G.-J. HOLST AND A. E. CLARKE, *Anal. Biochem.*, 148 (1985) 446–450.
10 E. A. KABAT AND M. M. MAYER (Eds), *Experimental Immunochemistry*, Thomas, Illinois, 1964, pp. 133–240.
11 M. WILSON AND D. C. MORRISON, *Eur. J. Biochem.*, 128 (1982) 137–141.
12 M. A. JERMYN AND Y. M. YEOW, *Aust. J. Plant Physiol.*, 2 (1975) 501–531.
13 H. YAMADA, H. KIYOHARA, J.-C. CYONG, AND Y. OTSUKA, *Mol. Immunol.*, 212 (1985) 295–304.