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Complement activating galactan chains in a pectic arabinogalactan (AGIIb-1) from the roots of *Angelica acutiloba* Kitagawa*

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The complement activating (anti-complementary) arabinogalactan (AGIIb-1), isolated from the roots of *Angelica acutiloba* Kitagawa, was digested with exo-β-D-(1→3)-galactanase after a preceding digestion with exo-α-L-arabinofuranosidase. Gel filtration of the digested products gave a high molecular weight fraction (HMW) which could not be further digested, and an oligosaccharide fraction (LMW). The latter originated from exterior β-D-(1→3)-galactan chains of AGIIb-1. HMW showed similar potent anti-complementary activity as arabinofuranosidase-digested AGIIb-1 (AF-AGIIb-1), but LMW did not show any activity. HMW was composed mainly of Ara and Gal in addition to small proportions of Rha, Xyl, GlcA and GalA. Methylation analysis indicated that HMW comprised terminal Ara*f*, terminal Gal and 3,6-linked Gal as well as 4-linked Gal, 4-linked GalA and terminal GlcA. Further digestion of HMW with endo-β-D-(1→4)-galactanase gave a polymeric fraction (GN-1) and oligosaccharidefraction (GN-2). Although most of β-D-(1→4)-galactosyl chains were liberated from HMW by this galactanase digestion, anti-complementary activity of GN-1 was not changed significantly in comparison with that of HMW.

Controlled Smith degradation of HMW gave four kinds of fractions. Among them, the fraction (CSD-1) having the largest molecular-weight showed a relatively potent anti-complementary activity, and it was mainly composed of a (1→3)-galactan. Exo-β-D-(1→3)-galactanase digestion of CSD-1 gave six fractions (CSD-1-1~CSD-1-6). The polymeric fraction (CSD-1-1) and the oligosaccharide fractions (CSD-1-4 and 1-5) had potent anti-complementary activity. Methylation analysis indicated that CSD-1-1, 1-4 and 1-5 consisted mainly of terminal, 6-linked and 3,6-linked Gal. CSD-1-4 and CSD-1-5 were also mainly composed of 3-linked Gal. These results indicates that complement activating ability of AGIIb-1 is expressed by the inner galactan chains. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The arabinogalactan (AGIIb-1), isolated from the roots of *Angelica acutiloba* Kitagawa (Japanese name, Yamato-Tohki), is a complex pectic arabinogalactan (Kiyohara *et al.*, 1986; Yamada *et al.*, 1987). AGIIb-1 showed anti-complementary activity due to activation of complement system through classical and alternative pathways (Kiyohara *et al.*, 1989b). Structural analysis has revealed that AGIIb-1 comprises a rhamnogalacturonan core possessing at least four kinds of arabinogalactan side chains. Some preparations of AGIIb-1 also contain α-L-(1→3,5)-arabinan side chains (Kiyohara & Yamada, 1989a). It has been suggested that these side chains are directly attached to Rha in the rhamnogalacturonan core or through 4-linked GalA. One of the arabinogalactan side chains has been proposed to be a typical neutral arabino-3,6-galactan, which was named as N-I unit (Kiyohara *et al.*, 1987; Kiyohara & Yamada, 1989a). It has been shown that trimming of Ara*f* side chains from these arabinogalactan side chains increased the anti-complementary activity of AGII-1, suggesting that galactan moieties are responsible for expression of the activity (Yamada *et al.*, 1987). Comparison of anti-complementary activities of

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the N-I unit and other arabinogalactan side chains released from AGIIb-1 showed that the N-I unit possessed the most potent anti-complementary activity. This suggests that N-I unit plays an important role in the expression of the activity of AGIIb-1 among arabinogalactan and arabinan side chains in AGIIb-1 (Kiyohara *et al.*, 1989b). Recently we have found that exo-β-D-(1→3)-galactanase digestion decreases the anti-complementary activity of N-I unit and that galactosyl side chains in N-I unit contribute to the activity of N-I (Kiyohara *et al.*, 1996). However, it was not clarified whether the activity of AGIIb-1 was mainly expressed by the action of N-I.

In the present paper, we report on our studies of the contribution of galactan side chains in the expression of anti-complementary activity of AGIIb-1 by using sequential enzymic digestions and controlled Smith degradation.

MATERIALS AND METHODS

Materials

The roots of *A. acutiloba* Kitagawa were purchased from Uchida-Wakan-yaku (Tokyo, Japan). Bio-gel P-2, P-10, and P-30 were obtained from Bio-Rad, DEAE-Sepharose CL-6B from Pharmacia, and Sep-pak C_{18} cartridges from Waters Assoc. Exo-α-L-arabinofuranosidase and endo-α-L-(1→5)-arabinanase (Megazyme, Australia) from *Aspergillus niger* were purified by FPLC (Lerouge *et al.*, 1993). Driselase (*Irpex lacteus*) was obtained from Kyowa-Hakko Kogyo Co. Ltd, Japan, and exo-β-D-(1→3)-galactanase from Driselase was purified by the procedure of Tsumuraya *et al.* (1990). Endo-β-D-(1→4)-galactanase from *A. niger* was purchased from Megazyme. Contaminating enzyme activities in the β-D-(1→4)-galactanase were checked by using each *p*-nitrophenyl derivatives of (α-L-Ara*f*, α-D-Glc, β-D-Glc, β-D-Gal, α-L-Rha, β-D-GlcA and α-D-Man, and α-L-(1→5)-arabinan as the substrates, and no other enzyme activities were observed.

General method

Total carbohydrate, pentose and uronic acid were determined by phenol-H_2SO_4 (Dubois *et al.*, 1956), phloroglucinol-AcOH (Dische & Borenfreund, 1957) and *m*-hydroxybiphenyl methods (Blumenkrantz & Asboe-Hansen, 1973), respectively, by using Gal, Ara and GalA as the respective standards. Polysaccharides were hydrolysed with 2 M TFA at 121°C for 1.5 h. TLC of hydrolysates was performed on cellulose using AcOEt–pyridine–AcOH–water (5:5:1:3) as the developing solvent. Reducing sugars were detected by alkaline $AgNO_3$ (Trevelyan *et al.*, 1950), and uronic acid with *p*-anisidine HCl (Hough *et al.*, 1950). Sugars were converted into TMS methyl glycoside derivatives (York *et al.*, 1986) and analysed by GLC using a DB-1 capillary column (0.20 μm film, 0.25 mm i.d. × 30 m, Supelco). GLC was performed on a HP-5890 Series II gas chromatograph (Hewlett-Packard, USA) and the programme was: 60°C for 1 min, 60→170°C (30°C/min), 170→190°C (1°C/min), and 190→300°C (30°C/min). High performance anion-exchange chromatography using pulsed electrochemical detector (HPAEC-PED) was performed on a Dionex Bio LC equipped with a CarboPak PA1 column (4 × 250 mm), and monosaccharides were analysed by the method of De Ruiter *et al.* (1992). HPLC was performed on a Shimadzu LC-6A equipped with combined columns of Asahi-pak GS-510 and 320 (Asahi Chemical Industry Co., Ltd, Japan) and a 0.2 M NaCl solution as eluent. Molecular weights of polysaccharides were estimated from a calibration curve constructed for standard pullulans (P-400, 200, 100, 50, 20, 10 and 5, Showa Denko Co. Ltd, Japan). Single radial gel diffusion by using the β-glucosyl-Yariv antigen was performed in according to the method of Holst and Clarke (1985).

Preparation of AGIIb-1

A neutral polysaccharide fraction (AR-4) was prepared from *A. acutiloba* by hot water extraction and precipitation with ethanol and Cetavlon (cetyltrimethylammonium bromide) (Yamada *et al.*, 1984). AGIIb-1 was purified from AR-4 by anion-exchange chromatography and gel filtration (Kiyohara *et al.*, 1986). AGIIb-1 preparation used in the present study did not comprise a neutral arabinan unit (N-II) as indicated by methylation analysis.

Enzymic digestion of AGIIb-1

Exo-α-L-arabinofuranosidase

AGIIb-1 (540 mg) was digested with exo-α-L-arabinofuranosidase (0.01 U) in 50 ml of 200 mM acetate buffer (pH 4.5) at 40°C. Every hour, an aliquot (10 μl) of the mixture was analysed by HPAEC-PED in order to monitor the liberation of Ara. The enzyinic digestion was stopped after 7 h by neutralization with 3 M NaOH. The digest was fractionated on a column (2.5 × 50 cm) of Bio-gel P-2 at 4°C with water as eluent. The carbohydrate containing fractions in the void volume (AF-AGIIb-1) and in the included volume were collected. TLC and GLC showed that the included volume fraction consisted of Ara.

Exo-β-D-(1→3)-galactanase digestion

AF-AGIIb-1 (330 mg) was incubated with exo-β-D-(1→3)-galactanase (O.1 U) in 50 mM acetate buffer (pH 4.6, 30 ml) at 37°C for 96 h. After neutralization, the digestion mixture was desalted by using an electrophoretic dialyzer (Microacylizer, Asahi Chemical

Industry Co. Ltd, Japan) giving GT-AF-AGIIb-1. GT-AF-AGIIb-1 was fractionated on a column (2.6 × 96 cm) of Sepharose CL-6B with 0.2 M NaCl, and the fractions eluted in a higher (HMW) and lower-molecular-weight (LMW) fractions were obtained.

Endo-β-D-(1→4)-galactanase

HMW (6 mg) was incubated with endo-β-D-(1→4)-galactanase (4.4 U) in 50 mM acetate buffer (pH 4.0, 1 ml) at 37°C for 48 h. After neutralization with 1 M NaOH, the digestion products were fractionated on a column (2.2 × 50 cm) of Bio-gel P-10 at 4°C using water as eluent. The void volume (GN-1) and inner volume (GN-2) fractions each were collected.

Controlled Smith degradation of HMW

HMW (50 mg) was incubated with 50 mM $NaIO_4$ in 50 mM acetate buffer (pH 4.0, 6.5 ml) at 4°C in the dark for 96 h. After reduction with $NaBH_4$, the product was hydrolysed with 1 M TFA at room temperature for 48 h, and fractionated on Bio-gel P-30 with 50mM acetate buffer (pH 5.5) to obtain four fractions (CSD-1~CSD-4). CSD-1 was further digested with exo-β-D-(1→3)-galactanase as described above, and the digestion product was fractionated on Bio-gel P-30 with 50 mM acetate buffer (pH 5.5).

Methylation analysis

Each sample was methylated according to the Hakomori method (Hakomori, 1964) and the methylated products were recovered using a Sep-pak C_{18} cartridge by the procedure of Waeghe *et al.* (1983) except that samples were eluted by only EtOH. Uronic acids in the methylated polysaccharides were reduced by $LiB(C_2H_5)_3D$ in THF (Super-Deuteride®, 1 ml, room temperature, 1 h, Sigma), and the reduced product was recovered by using a Sep-pak C_{18} cartridge. The methylated products were hydrolysed with 2 M TFA at 121°C for 1.5 h, and converted into partially methylated alditol acetates. The resulting partially methylated alditol acetates were analysed by GLC and GLC-EIMS. GLC was performed on a Hewlett-Packard model 5890 gas chromatograph equipped with a SP-2380 capillary column, and EIMS was done on a Hewlett-Packard model 5970B mass spectrometer. The GLC conditions were as described by Zhao *et al.* (1991). Methylated alditol acetates were identified by their fragment ions in EIMS and relative retention times in GLC, their molar ratios were estimated from the peak areas and response factors (Sweet *et al.*, 1975).

Anti-complementary activity

Anti-complementary substances express their activity by either inhibition or activation of the complement system. AGIIb-1 has been found to express the anti-complementary activity only by activation of the complement system (Kiyohara *et al.*, 1989b). Therefore, complement activating activity of samples were expressed as anti-complementary activity in the present work.

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 500 µM $MgCl_2$ and 150 µM $CaCl_2$ (GVB^{2+}). The mixture was pre-incubated at 37°C for 30 min and the residual hemolytic complement (TCH_{50}) was determined using IgM-hemolysin-sensitized sheep erythrocytes (Meyer, 1964). NHS (50 µl) was incubated with water (50 µl) to provide a control.

RESULTS

Effect of digestion of AF-AGIIb-1 with exo-β-D-(1→3)-galactanase on the anti-complementary activity

In the previous study, we have reported that Ara*f* side chains, which could be liberated from AGIIb-1 by exo-α-L-arabinofuranosidase digestion, did not contribute to expression of anti-complementary activity of AGIIb-1 (Yamada *et al.*, 1987). Therefore, after the Ara*f* chains were removed from AGIIb-1 by the arabinofuranosidase digestion, the remaining fraction (AF-AGIIb-1) was further digested with exo-β-D-galactanase in order to clarify the contribution of β-D-(1→3)-galactan moiety in outer chains of AF-AGIIb-1 on the anti-complementary activity. AF-AGIIb-1 reacted strongly with β-D-glucosyl-Yariv antigen on single radial gel diffusion whereas the mixture of the galactanase-digested products (GT-AF-AGIIb-1) did not react with the antigen (Fig. 1). However, GT-AF-AGIIb-1 had similar anti-complementary activity as AF-AGIIb-1 (Fig. 2A). When GT-AF-AGIIb-1 was fractionated on Sepharose CL-6B in 0.2 M NaCl, the higher (HMW) and lower-molecular-weight fractions (LMW) were obtained (Fig. 3B). Molecular weight of HMW (8.3×10^4) was lower than that of AF-AGIIb-1 (9.6×10^4) (Figs. 3A and B). LMW seemed to contain various kinds of oligosaccharides (data not shown). Component sugar analysis indicated that HMW and LMW consisted of Ara, Rha, Xyl, Gal, Glc, GalA and GlcA in molar ratios of 3.0:1.0:0.1:9.3:0.2:0.8:0.3 and 16.6:1.0:0.5:21.6:0.3:0.9:0.8, respectively. Methylation analysis indicated that HMW mainly comprised terminal Ara*f*, terminal Gal, 6-linked Gal and 3,6-linked Gal in addition to small proportions of 4- or 5-linked Ara, 2,4-linked Rha, 3-linked Gal, 4-linked Gal, 3,4,6-linked Gal, 4-linked GalA and terminal GlcA (Table 1). LMW was mainly composed of terminal Ara*f*, 4- or 5-linked Ara, terminal Gal, 4-linked Gal and 6-linked Gal (Table 1). Base-catalysed β-elimination of methylated HMW indicated that 2,4-linked Rha was decreased significantly by the β-elim-

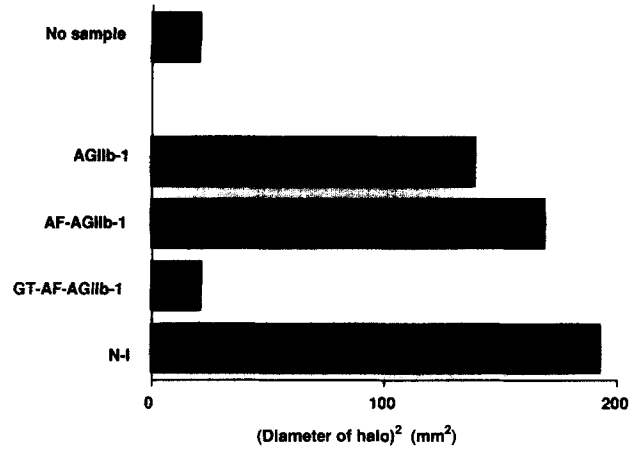

Fig. 1. Reactivity to the β-glucosyl-Yariv antigen of enzymic digestion products from AGIIb-1. N-I; Neutral arbinogalactan unit of AGIIb-1 (positive control).

ination (data not shown), suggesting that HMW comprised a rhamnogalacturonan backbone carrying β-D-(1→3)-galactanase-resistant side chains. It was also indicated that LMW was derived from β-D-(1→3)-galactan chains in the exterior of AF-AGIIb-1. It was noticed that HMW had a similar anti-complementary activity as AF-AGIIb-1 and the neutral arabinogalactan unit (N-I) of AGIIb-1 (Fig. 2B and 2C), LMW, however, did not show any activity (Fig. 2B). These results suggest that β-D-(1→3)-galactans in the exterior chains of AGIIb-1 do not contribute to the expression of the activity of AGIIb-1.

Effect of endo-β-D-(1→4)-galactanase digestion on the anti-complementary activity of HMW

Methylation analysis indicated that HMW also contained (1→4)-linked galactosyl residues (Table 1). In order to investigate the contribution of (1→4)-linked galactosyl sequences to the activity of HMW, HMW was digested with endo-β-D-(1→4)-galactanase.

When the digested products were fractionated on Bio-gel P-10, two fractions, one eluting in the void (GN-1) and one in the inner volume (GN-2) were obtained (Fig. 4A). GN-1 consisted of Ara, Rha, Xyl, Gal, Glc, GalA and GlcA in molar ratios of 2.2:1.0:0.1:6.8:0.1:1.0:0.3. In comparison to HMW, 30% of Gal was removed by enzymic digestion. GN-2 was composed only of Gal. Methylation analysis showed that the proportion of 4-linked Gal (77%) and 4,6-linked Gal (~100%) in HMW decreased by the galactanase digestion. GN-2 mainly comprised terminal and 4-linked Gal (Table 1). When anti-complementary activity of GN-1 was compared with HMW, GN-1 seemed to show slightly more potent activity than HMW (Fig. 4B). This result suggests that β-D-(1→4)-galactosyl chain in HMW does not contribute to the activity of HMW.

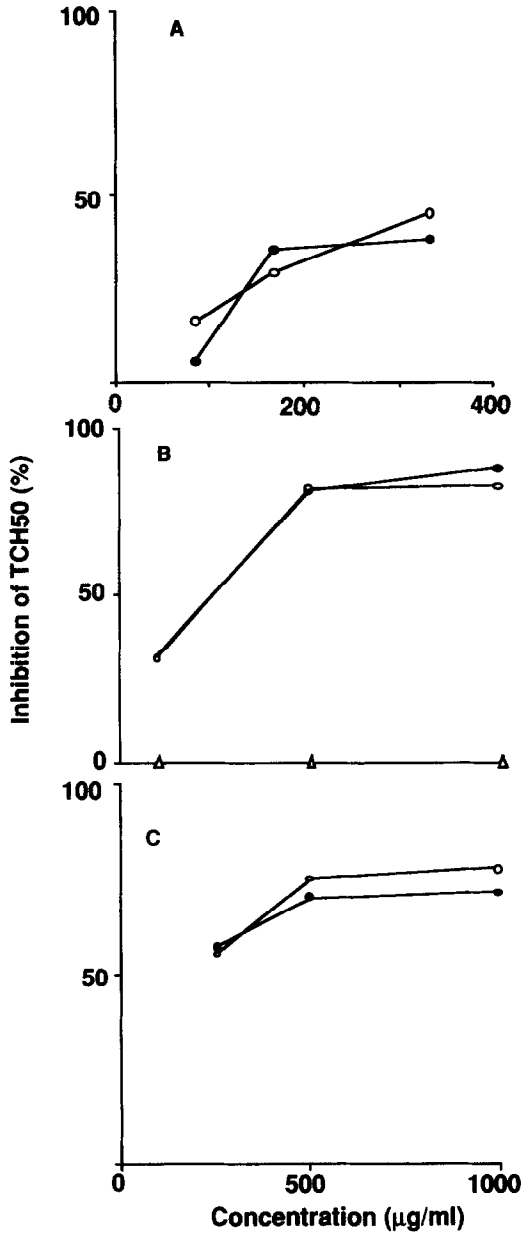

Fig. 2. Anti-complementary activity of products derived from AF-AGIIb-1 by exo-β-D-galactanase digestion. (A) ●, AF-AGIIb-1; ○, GT-AF-AG11b-1. (B) ●, AF-AGIIb-1; ○, HMW; △, LMW. (C) ○, (N-I); ●, HMW

Controlled Smith degradation of HMW

Although we attempted to remove most of the galactan chains from HMW by further digestion with several kinds of glycosidases, these digestions were not effective. Since galactan chains were attached to a rhamnogalacturonan core, some steric hindrance of galactan chains in HMW might be prevented from the enzymic digestion. In order to identify the carbohydrate chains in HMW responsible for expression of anti-complementary activity, HMW was subjected to controlled Smith degradation. The degradation products were fractionated on Bio-gel P-30, and the fractions eluting in

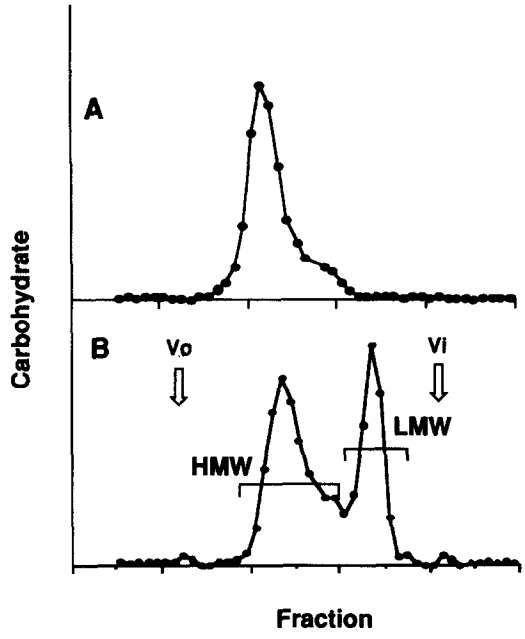

Fig. 3. Gel filtration patterns on Sepharose CL-6B of (A) AF-AGIIb-1, and of (B) GT-AF-AGIIb-1 derived from AF-AGIIb-1 by exo-β-D-(1→3)-galactanase digestion. V_o, void volume; V_i, inner volume.

the void volume, intermediate fraction (CSD-2) and lower-molecular-weight fractions (CSD-3 and CSD-4) were collected (weight ratios; CSD-1:CSD-2:CSD-3:CSD-4 = 9.0:4.3:4.4:0.2, Fig. 5A). When the void volume fraction was further fractionated on Sepharose CL-6B, CSD-1 was eluted as a single peak, and its average molecular weight was estimated to be 4.0×10^4 on HPLC. When anti-complementary activities of CSD-1~4 were compared with HMW, CSD-1 and 4 showed a reduced but still observable activity (Fig. 6A). However, CSD-2 and 3 had weak activities. CSD-4 could not be further analysed because too small amounts were available.

CSD-1 is composed mainly of Gal (92.6 mol.%) in addition to small amounts of Glc, GalA and GlcA (1.3~5.4 mol.%). Methylation analysis indicated that CSD-1 was composed mainly of terminal, 3-linked, 3,4-linked and 3,6-linked Gal (Table 1). In addition, CSD-1 comprised a large proportion of 3,4,6-1inked Gal, however, this linkage might be due to under-methylation. Since CSD-1 contained large proportions of 3-linked, 3,4-linked and 3,6-linked Gal, CSD-1 was further digested with exo-β-D-(1→3)-galactanase. Although CSD-1 reacted with the β-D-glucosyl-Yariv antigen, mixtures of the digestion products (GT-CSD-1) could not react with the antigen (data not shown), suggesting that most of β-D-(1→3)-galactan chains in CSD-1 were cleaved by the galactanase digestion. GT-CSD-1 was fractionated on Bio-gel P-30 and 6 fractions (CSD-1-1~1-6) were obtained as shown in Fig. 5C. When anti-complementary activities of CSD-1-1~1-6 were compared with CSD-1, CSD-1-1 showed an increased potent activity, and its activity seemed to be similar to that of HMW (Fig. 6B). It was also suggested that CSD-1-4 and CSD-1-5 had a potent activity. However, CSD-1-2 and CSD-1-6 showed weak activity (Fig. 6B).

The major active fraction, CSD-1-1 was eluted as a single peak on HPLC, and its molecular weight was estimated to be 2.0×10^4. CSD-1-1 was composed mainly of Gal (89.0 mol%) in addition to small

Table 1. Methylation analysis of products from AGIIb-1

Residues	Linkage	Mol.% AF-AGIIb-1	HMW	LMW	GN-1	GN-2	CSD-1	CSD-1-1	CSD-1-4	CSD-1-5
Ara	terminal (*f*)	6.7	9.7	11.3	10.9			4.5		
	2	0.3	0.4	0.2	0.4			1.4		
	3	0.6	0.5	1.0	0.4					
	4 or 5	17.2	7.1	11.6	1.9			1.5		
Rha	terminal	0.5	0.7	1.2	0.7					
	2	0.7	0.6	0.6	0.6					
	4	trace	0.7	0.5	0.6					
	2,4	4.2	6.4	1.7	5.9					
Gal	terminal	8.7	12.1	19.9	15.5	65.4	14.3	22.0	18.9	18.1
	3	5.8	5.5	0.9	6.5	5.1	26.4	27.9	25.7	25.5
	4	6.6	8.3	11.1	1.9	14.3		2.0	6.7	10.4
	6	14.8	15.4	17.4	19.9	8.6	6.0	4.6	8.1	10.2
	2,6	trace	0.2	0.3	0.3					
	3,4	1.1	1.4	0.4	1.6		19.8	8.7	9.6	8.0
	3,6	15.8	12.5	1.4	13.4		17.3	14.6	21.7	18.8
	4,6	1.7	1.8	7.4	n.d.[a]	6.6		2.2	5.2	4.9
	3,4,6	4.0	4.4	1.6	4.5		14.8	7.1		
Glc	6				n.d.			0.9		
GalA	4	4.1	5.6	1.9	7.6		1.4	0.8	1.8	1.7
GlcA	terminal	6.1	5.7	8.5	8.5			2.0	2.2	2.5
	3	1.0	1.0	0.9	0.9					

[a] Not detected.

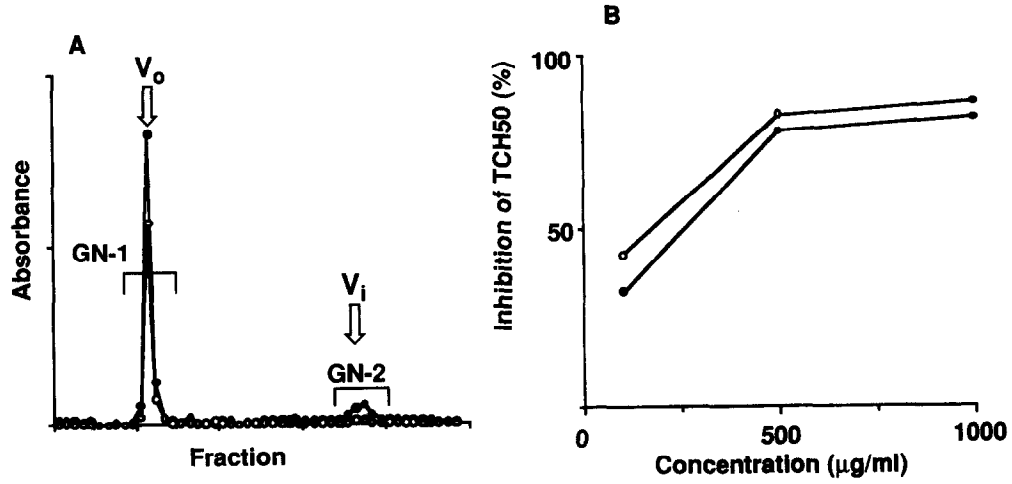

Fig. 4. (A) Gel filtration pattern on Bio-gel P-10 of products derived from HMW by endo-β-D-(1→4)-galactanase digestion. ●, carbohydrate (490 nm); ○, uronic acid (520 nm). (B) Anti-complementary activity of HMW and GN-1 from A. ●, HMW; ○, GN-1.

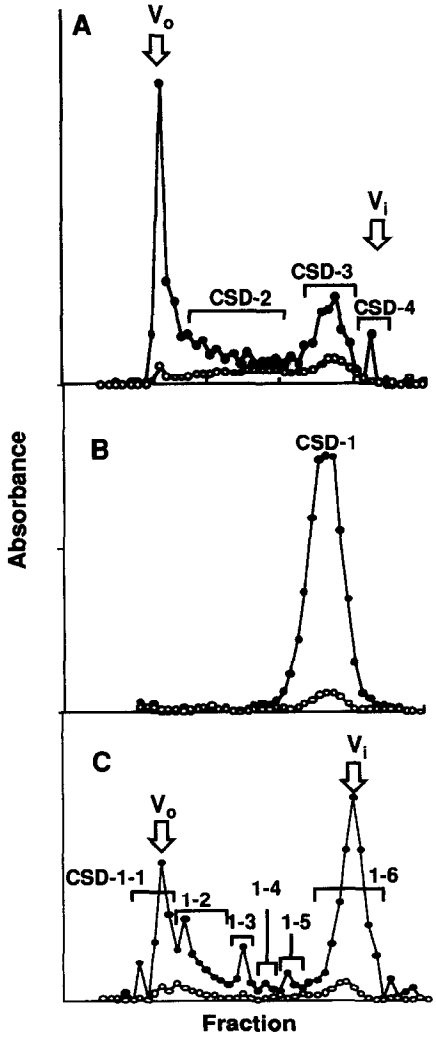

Fig. 5. (A) Gel filtration pattern on Bio-gel P-30 of products from HMW by controlled Smith degradation. (B) Gel filtration pattern on Sepharose CL-6B of the void volume fraction from A. (C) Gel filtration pattern on Bio-gel P-30 of products from CSD-1 by exo-β-D-(1→3)-galactanase digestion. ●, carbohydrate (490 nm); ○, uronic acid (520 nm). V_o, void volume; V_i, inner volume.

proportions of Ara, Rha, Glc, GalA and GlcA (0.9~3.9 mol%). Methylation analysis indicated that CSD-1-1 mainly comprised terminal, 3-linked and 3,6-linked Gal in addition to small proportions of various linkages of Ara, Gal and Glc, GalA and GlcA (Table 1). The minor active fractions (CSD-1-4 and CSD-1-5) were composed mainly of Gal (88.6 and 80.7 mol%, respectively) in addition to Glc, GalA and GlcA (2.0~8.1 mol%). Methylation analysis showed that CSD-1-4 and 1-5 also mainly comprised terminal, 3-linked and 3,6-linked Gal (Table 1).

DISCUSSION

Previously we have proposed that a neutral arabinogalactan (N-I) unit in AGIIb-1 contributes to the expression of complement-activating activity because the N-I unit showed the most potent activity among the neutral arabinogalactan and arabinan chains released from AGIIb-1 (Kiyohara *et al.*, 1989b). However, the N-I unit was present in a relatively low proportion in AGIIb-1 (Kiyohara *et al.*, 1987), therefore it is possible that other sequences of sugar residues may be responsible for expression of the activity of AGIIb-1. Structural analysis has indicated that AGIIb-1 is a kind of pectic arabinogalactan in which at least four arabinogalactan chains are attached to rhamnogalacturonan backbone. The N-I unit was suggested to be located in the exterior of one of arabinogalactan chains (Kiyohara *et al.*, 1987). Recently it has been reported that the neutral arabinogalactan unit (AF-N-I), which was derived from AF-AGIIb-1, could be digested with *I. lacteus* exo-β-D-(1→3)-galactanase which is able to cleave β-D-(1→3)-galactan backbone with or without side chains from the non-reducing terminal (Kiyohara *et al.*, 1996). The released oligosaccharide chains from AF-N-I still had significant activity (Kiyohara *et al.*, 1996).

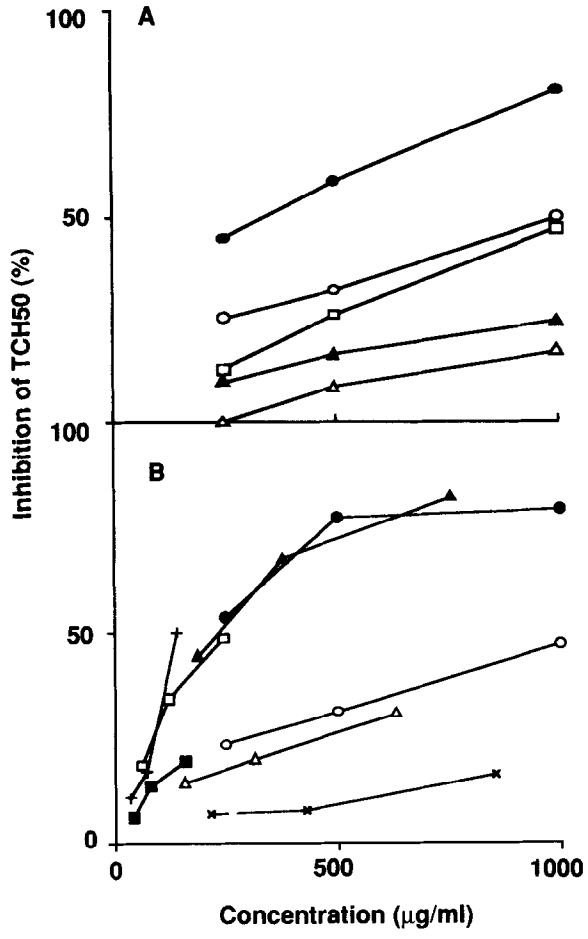

Fig. 6. Anti-complementary activity of products derived from HMW by controlled Smith degradation (A), and from CSD-1 by exo-β-D-(1→3)-galactanase digestion (B). (A) ●, HMW; ○, CSD-1; ▲, CSD-2; △, CSD-3; □, CSD-4. (B) ●, HMW; ○, CSD-1; ▲, CSD-1-1; △, CSD-1-2; ■, CSD-1-3; □, CSD-1-4; +, CSD-1-5; ×, CSD-1-6.

However, the present study indicated that exo-β-D-(1→3)-galactanase digestion of AF-AGIIb-1 did not affect the anti-complementary activity of AF-AGIIb-1. This fact indicates that the β-D-(1→3)-galactan outer chains in AGIIb-1 containing those in N-I do not contribute to expression of the activity of AGIIb-1. Methylation analysis of exo-β-D-(1→3)-galactanase-resistant portion of AF-AGIIb-1 (HMW) suggested that HMW comprised rhamnogalacturonan backbone possessing the galactanase-resistant chains. Endo-β-D-(1→4)-galactanase digestion suggested that HMW consists of galactan chains containing β-D-(1→4)-Gal, however, these galactan chains are not supposed to contribute to expression of the activity. We also attempted to degrade HMW by other exo- and endo-glycosidases, but effective degradation did not occur. The results of controlled Smith degradation indicated that the inner galactan chains in AF-AGIIb-1 also mainly consisted of a β-D-(1→3)-galactan backbone which carried some side chains at position 4 or 6 of the backbone. Controlled Smith degradation followed by exo-β-D-(1→3)-galactanase digestion also gave active fractions CSD-1-1, CSD-1-4 and CSD-1-5. Since CSD-1-1 consisted of small proportions of GalA and Rha in addition to Gal, it was also assumed that a part of the rhamnogalacturonan core still remained in CSD-1-1. Oligomeric side chains of CSD-1-4 and 1-5 were suggested to contribute to the expression of the activity of AGIIb-1. Finally, it was proposed that the anti-complementary activity of AGIIb-1 was expressed by the inner galactan chains rather than by the outer galactan chains in AGIIb-1. Details of the active oligosaccharide chains must await further study.

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