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RELATIONSHIP BETWEEN CHEMICAL STRUCTURE AND ACTIVATING POTENCIES OF COMPLEMENT BY AN ACIDIC POLYSACCHARIDE, PLANTAGO-MUCILAGE A, FROM THE SEED OF *Plantago asiatica*

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ABSTRACT

The structure–complement activation relationship of Plantago-mucilage A, a partially *O*-acetylated glucuronoarabinoxylan isolated from the seed of *Plantago asiatica,* has been studied. The anti-complementary activity was markedly enhanced when the polysaccharide was deacetylated, but carboxyl-reduction and partial acid hydrolysis had little effect. Periodate oxidation decreased, but subsequent reduction restored, the activity; the activity of this polyalcohol was not changed in the absence of Ca^{2+}. When serum was incubated with the deacetylated polymer, more marked C4 consumption was observed in the presence of Ca^{2+} than with Plantago-mucilage A, but the polyalcohol did not show any C4 consumption. After incubation of the serum with the polyalcohol in the absence of Ca^{2+}, a cleavage of C3 in the serum was detected by immunoelectrophoresis and by the consumption of complement when rabbit erythrocytes were used in the assay system, and these effects were much higher than for the original polysaccharide. Although the product of carboxyl-reduction did not contain *O*-acetyl groups, its C4 consumption was almost the same as that of Plantago-mucilage A. Thus, the *O*-acetyl groups in Plantago-mucilage A prevent the activation of complement *via* the classical pathway but the carboxyl groups are essential for the activation. The polyalcohol increases the activation of complement *via* the alternative pathway, but decreases the activation *via* the classical pathway.

INTRODUCTION

Various anti-complementary polysaccharides have been isolated from bacteria, fungi, and plants; for example, lipopolysaccharides[1–3], water-insoluble 6-branched (1→3)-β-D-glucans from *Lentinas edodes*[4,5] and *Poria cocos*[4,5], zymosan from yeast cell-wall preparations[6], inulin[7], AR-arabinogalactan IIa[8,9] and IIb-1[10] from *Angelica acutiloba* KITAGAWA, acidic heteroglycans, AAF-IIb-2 and IIb-3[11,12], from *Artemisia princeps* PAMP, and an acidic polysaccharide, LR-polysaccharide

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IIa[13], from *Lithospermum euchromum* ROYLE. However, little is known about the structure–activity relationships. Considerable anti-complementary activity has been observed[14] in Plantago-mucilage A [**1**; a partially *O*-acetylated acidic polysaccharide; (1→4)-β-D-xylopyranan with branches composed of *O*-(α-D-glucopyranosyluronic acid)-(1→3)-α-L-arabinofuranose and *O*-(α-D-galactopyranosyluronic acid)-(1→3)-α-L-arabinofuranose at position 3 as side chains][15] from the seed of *Plantago asiatica*. The complement activation by **1** is *via* both the alternative and classical pathways[14]. We now report on the activation activities of the chemically modified products of this polysaccharide.

EXPERIMENTAL

Purification of Plantago-mucilage A (**1**). — The mucilage was isolated[15] from the seed of *Plantago asiatica*.

General methods. — Optical rotations were determined at 20° with a JASCO DIP-140 polarimeter. Viscosity was determined with an Ubbelohde-type viscosimeter. Carbohydrate was determined by the phenol–sulfuric acid method[16]. The molecular weights of the deacetylated and carboxyl-reduced products were determined by gel filtration on a column (2.6 × 92 cm) of Sepharose CL-4B by elution with 0.1M Tris-HCl buffer (pH 7) in comparison with dextran standards. The molecular weight of the product obtained by partial acid hydrolysis at 80° was determined by the use of a column (2.6 × 96.5 cm) of Sephacryl S-300 with the above buffer, in comparison with pullulan standards. Sugars in hydrolysates were determined by g.l.c. of the derived alditol acetates[15]. *O*-Acetyl groups in hydrolysates were determined[17] by g.l.c.

Modification of Plantago-mucilage A (**1**). — *(a) Deacetylation*. To a stirred solution of **1** (500 mg) in water (100 mL) was added 0.2M NaOH (100 mL). The solution was stirred at 26° for 10 min, then neutralised with M HCl, dialysed against water overnight, concentrated, applied to a column (5 × 80 cm) of Sephadex G-25, and eluted with water (20-mL fractions). Fractions 27–49 were combined and concentrated to give the deacetylation product (61.8%).

(b) Periodate oxidation. To a solution of **1** (100 mg) in water (25 mL) was added 0.1M sodium metaperiodate (25 mL), and the mixture was stored at 3° for 7 days in the dark (IO_4^- consumption, 0.79 mol/component mol). Ethylene glycol (0.5 mL) was then added and, after storage at 3° for 1 h, the mixture was dialysed against water. The product was purified by gel filtation on Sephadex G-25 (yield, 75%).

(c) Periodate oxidation–borohydride reduction. After the addition of ethylene glycol as in (*b*), sodium borohydride (500 mg) was added and the solution was stored at 3° for 16 h. The pH of the mixture was then adjusted to 5 with acetic acid and the mixture was dialysed against water. The non-dialysable portion was concentrated, applied to a column (5 × 80 m) of Sephadex G-25, and eluted with water (20-mL fractions). Fractions 29–34 were combined and lyophilised to give the polyalcohol (70%).

(d) Carboxyl-reduction. Mucilage 1 was reduced[15] with carbodiimide and sodium borohydride.

(e) Partial acid hydrolysis. To a solution of **1** (208 mg) in water (30.4 mL) was added 2M trifluoroacetic acid to 0.1M. The mixture was stirred at 80° for 1 h, the acid was then evaporated, and methanol was repeatedly evaporated from the residue, a solution of which in water was applied to a column (5 × 80 cm) of Sephadex G-25 eluted with water (20-mL fractions). Fractions 29–33 were combined and lyophilised (51.4% yield).

Anti-complementary activity[8,9]. — Gelatin–veronal–buffered saline (pH 7.4) containing 500μM Mg^{2+} and 150μM Ca^{2+} (GVB^{++}) was used together with normal human serum (NHS) obtained from a healthy adult. Various dilutions of polysaccharides in water (50 μL) were incubated with 50 μL of NHS and 50 μL of GVB^{++}. The mixtures were incubated at 37° for 30 min and the residual total hemolytic complement (TCH_{50}) was determined by using IgM-hemolysin-sensitised sheep erythrocytes (EA) at 1×10^8 cells/mL. NHS was incubated with water and GVB^{++} to provide a control. The anti-complementary activity of the polysaccharide was expressed as the percentage inhibition of the TCH_{50} of the control.

Determination of the complement hemolysis through the alternative complement pathway (ACH_{50}). — ACH_{50} was determined[18] in 10mM EGTA containing 2mM $MgCl_2$ in GVB^{--} (Mg^{2+}–EGTA–GVB^{--}). A sample of the anti-complementary polysaccharide was incubated with Mg^{2+}–EGTA–GVB^{--} and NHS at 37° for 30 min, and the residual complement of the mixtures was measured by the hemolysis of rabbit erythrocytes (5×10^7 cells/mL) incubated with Mg^{2+}–EGTA–GVB^{--}.

Crossed immunoelectrophoresis. — NHS was incubated with 0.5 vol. of the solution of the anti-complementary polysaccharide with Mg^{2+}–EGTA–GVB^{--} for 30 min at 37°. The serum was then subjected to crossed immunoelectrophoresis to locate the C3 cleavage products[19]. Shortly after the first run (barbital buffer, pH 8.6; ionic strength, 0.025; with 1% of agarose), the second run was carried out on a gel plate (2.0-mm layer) containing 3% of a rabbit anti-human serum to C3 at a potential gradient of 0.8 mA/cm for 15 h. After the electrophoresis, the plate was fixed and stained with Ponceau 3R.

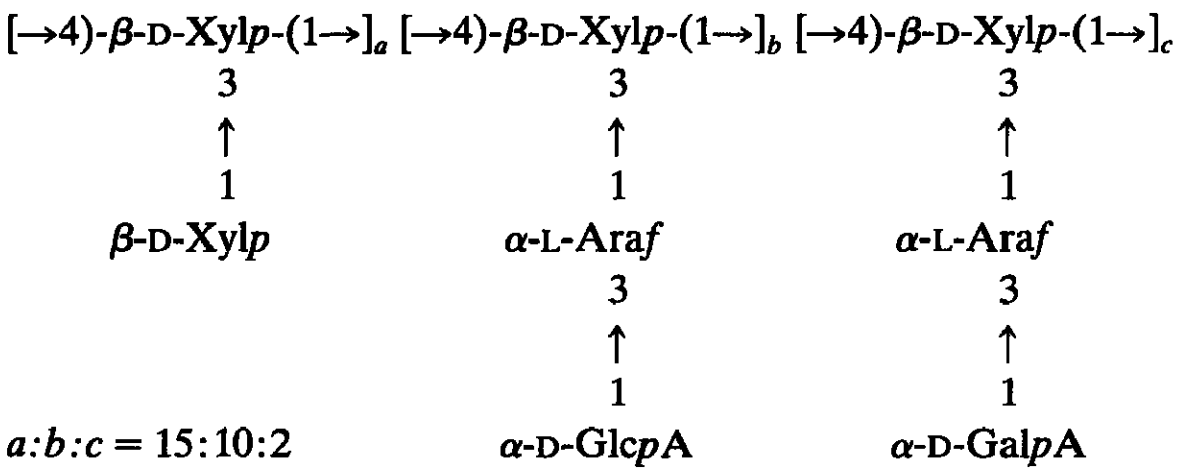
Determination of C4. — Titration of C4 was performed[10,19] using intermediate cells $EAC1^{gp}$ for C4. $EAC1^{gp}$ cells were prepared from EA (1×10^9 cells/mL) incubated with C1 solution (1×10^{12} SFU/mL) in the ratio of 28:1 at 4° for 1 h.

Materials. — Sephadex G-25, Sepharose CL-4B, and Sephacryl S-300 were obtained from Pharmacia. Human C3 and guinea pig C1 and C2 were obtained from Cordis Lab. Anti-human C3 serum was obtained by immunisation of rabbits with human C3. AR-Arabinogalactan[8–10] and acidic heteroglycans, AAF-IIb-2 and IIb-3, were isolated[11] from the root of *Angelica acutiloba* KITAGAWA and the leaf of *Artemisia princeps* PAMP, respectively. These polysaccharides were used as positive controls of anti-complementary activity, ACP activity, and C4 consumption.

RESULTS

Anti-complementary activity of Plantago-mucilage A (**1**) *and its chemical modification products.* — The results of anti-complementary activity are shown in Figs. 1a and b. Periodate oxidation of **1** decreased the activity to a negligible level, but subsequent reduction with sodium borohydride (to give the polyalcohol) restored the anti-complementary activity to the original level. The 4.8% of *O*-acetyl groups in **1** were located on the terminal xylopyranosyl groups and internal xylopyranosyl residues and the side-chain arabinofuranosyl residues[15], and *O*-deacetylation gave a more active product. In order to determine the importance of the D-glucopyranosyluronic acid and D-galactopyranosyluronic acid terminal groups[15] in the anti-complementary activity, **1** was carboxyl-reduced with the carbodiimide–sodium borohydride system. The product had decreased anti-complementary activity at 500 μg/mL compared to that of **1**. When **1** was partially hydrolysed (0.1M trifluoroacetic acid, 80°, 1 h), the activity was slightly enhanced. Table I shows physicochemical properties of the chemical modifications of **1** none of which contained *O*-acetyl groups, and only the product of partial acid hydrolysis had a decreased molecular weight. The anti-complementary activities of **1** and the products obtained by periodate oxidation, deacetylation, partial acid hydrolysis, and carboxyl-reduction diminished in the absence of Ca^{2+} with surplus Mg^{2+}, whereas that of the polyalcohol decreased only slightly (Fig. 2). These results suggested that deacetylated **1** activates complement *via* the classical pathway, but that the polyalcohol activates complement *via* the alternative pathway (Table II).

In order to confirm this speculation, the polyalcohol and the deacetylated product were subjected to C4 titration. NHS was incubated with the polyalcohol,



Terminal Xyl units consist of D-Xyl*p*:2-Ac-D-Xyl*p* (3:2).
Intermediate Xyl units consist of D-Xyl*p*:2-Ac-D-Xyl*p* (8:1).
Intermediate Ara units consist of L-Ara*f*:2-Ac-L-Ara*f* (3:1).

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deacetylated **1**, or **1** in GVB^{++} at 30° for 30 min, and the residual activity of C4 was estimated by hemolytic assay (Fig. 3). The deacetylation product decreased the C4

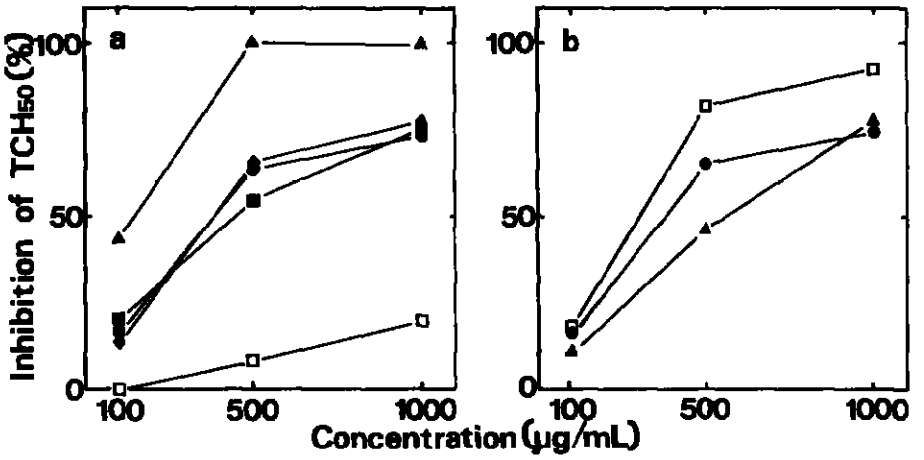

Fig. 1. Anti-complementary activity of Plantago-mucilage A (**1**) and its chemical modifications: (*a*) ●, **1**; ▲, deacetylated **1**; □, periodate-oxidised **1**; ■, polyalcohol; ◆, AR-arabinogalactan mixture; (*b*) ●, **1**; □, product of partial acid hydrolysis of **1**; ▲, carboxyl-reduced **1**.

content of NHS in comparison with **1**. When NHS incubated with 100 μg/mL of the deacetylation product was used for C4 titration, ~80% of the hemolytic titer of C4 was consumed and **1** consumed ~40% of C4 at the same dose. The polyalcohol did not consume any C4. These results show that the activation of complement *via* the classical pathway by the deacetylation product was more enhanced in comparison with the original polysaccharide, and it seems that *O*-acetyl groups in **1** prevent the activation of complement *via* the classical pathway. The carboxyl-reduced and deacetylated products had similar physicochemical properties (Table I), but the former enhanced C4 consumption slightly in comparison with **1** (Fig. 3). Thus, the carboxyl groups of the uronic acid residues are essential for the activation of complement by **1** *via* the classical pathway. Furthermore, when **1**, the deacetylated product, and the polyalcohol were incubated with NHS in Mg^{2+}–EGTA–GVB^{--}

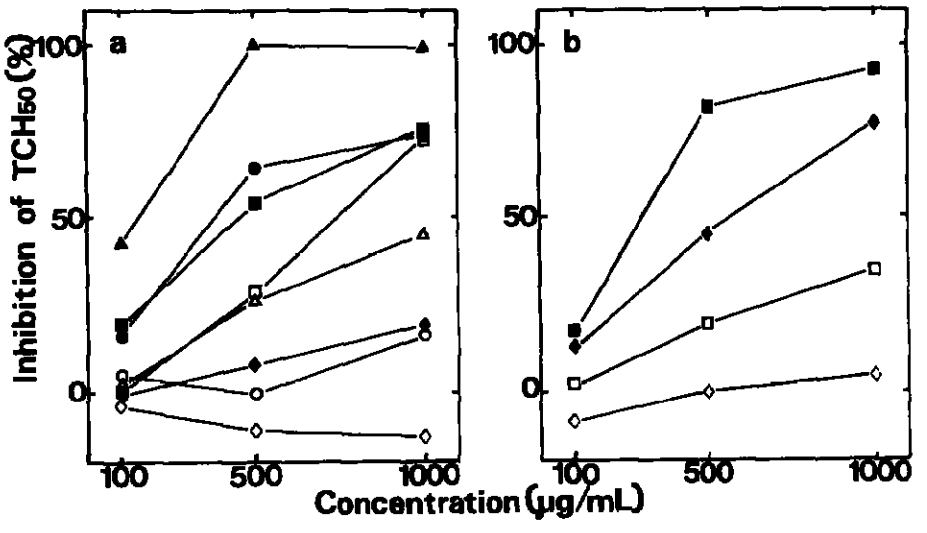

Fig. 2. Changes of TCH_{50} by incubation with Plantago-mucilage A (**1**) and its chemical modifications in the presence or absence of Ca^{2+}: (*a*) ●, **1** in GVB^{++}; ○, **1** in Mg^{2+}–EGTA–GVB^{--}; ▲, deacetylated **1** in GVB^{++}; △, deacetylated **1** in Mg^{2+}–EGTA–GVB^{--}; ◆, periodate-oxidised **1** in GVB^{++}; ◇, periodate-oxidised **1** in Mg^{2+}–EGTA–GVB^{--}; ■, polyalcohol in GVB^{++}; □, polyalcohol in Mg^{2+}–EGTA–GVB^{--}; (*b*) ■, product of partial acid hydrolysis of **1** in GVB^{++}; □, product of partial acid hydrolysis of **1** in Mg^{2+}–EGTA–GVB^{--}; ◆, carboxyl-reduced **1** in GVB^{++}; ◇, carboxyl-reduced **1** in Mg^{2+}–EGTA–GVB^{--}.

TABLE I

PHYSICOCHEMICAL PROPERTIES OF PLANTAGO-MUCILAGE A AND ITS CHEMICAL MODIFICATION PRODUCTS

Polysaccharide	$[\alpha]_D$ *(water) (degrees)*	*Component sugars (molar ratio)*	*Molecular weight*	*O-Acetyl (%)*	$[\eta]$ *(water, 30°)*
Plantago-mucilage A (**1**)	−38	Xyl/Ara/GlcA/GalA (21:6:5:1)	1.5×10^6	4.8	39.5
Deacetylated **1**	−45	Xyl/Ara/GlcA/GalA (21:6:5:1)	1.4×10^6	0	27.2
Carboxyl-reduced **1**	n.d.[a]	Xyl/Ara/Glc/Gal (21:6:5:1)	1.4×10^6	0	n.d.[a]
Periodate-oxidised **1**	n.d.[a]	Xyl/Ara (9:4)	n.d.[a]	n.d.[a]	n.d.[a]
Polyalcohol	n.d.[a]	Xyl/Ara (9:4)	n.d.[a]	n.d.[a]	n.d.[a]
Product of partial acid hydrolysis	−61	Xyl/Ara/HexA (24:6:5)	24,200	0	n.d.[a]

[a]Not determined.

TABLE II

SUMMARY OF ANTI-COMPLEMENTARY ACTIVITY OF PLANTAGO-MUCILAGE A (**1**) AND ITS CHEMICAL MODIFICATION PRODUCTS

Polysaccharide	*Anti-complementary activity (%)*[a]	
	In GVB^{++}	*In Mg^{2+}–EGTA–GVB^{--}*
1		↓
Deacetylated **1**	↑	↓
Carboxyl-reduced **1**	→	↓
Periodate-oxidised **1**	↓	↓
Polyalcohol	→	→
Product of partial acid hydrolysis	↗	↓

[a]Key: activity decreased, ↓; increased, ↑; slightly decreased or not changed, →; activity slightly increased compared with that of **1**, ↗.

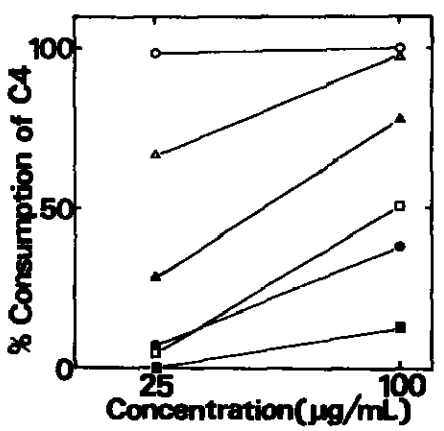

Fig. 3. Consumption of C4 by Plantago-mucilage A (**1**) and its chemical modifications: ●, **1**; ▲, deacetylated **1**; □, carboxyl-reduced **1**; ■, polyalcohol; ○, AAF-IIb-2; △, AAF-IIb-3.

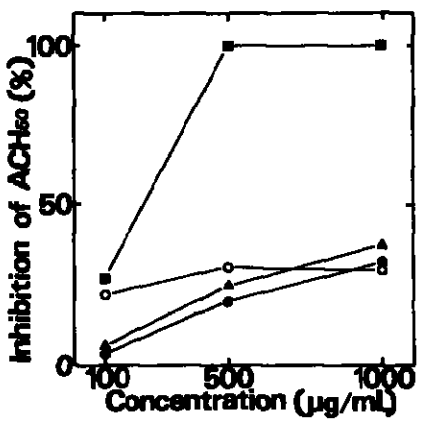

Fig. 4. ACP activity of Plantago-mucilage A (**1**) and its chemical modifications: ●, **1**; ▲, deacetylated **1**; ■, polyalcohol; ○, AAF-IIb-2.

at 37° for 30 min and a hemolytic titre assay (ACH_{50}) was carried out using rabbit erythrocytes, the polyalcohol dramatically enhanced the anti-complementary activity on ACH_{50} (ACP activity) in a dose-dependent manner in comparison with **1** (Fig. 4). However, the deacetylated product showed ACP activity similar to that of **1**. When crossed immunoelectrophoresis was carried out after the incubation of NHS with the polyalcohol-form product in Mg^{2+}–EGTA–GVB^{--} in order to determine whether C3 activation had been enhanced (Fig. 5), cleavage of the C3 precipitin line was obtained in the serum treated with the polyalcohol, the deacetylated product, or **1**, with different activation potency. The potent ACP-

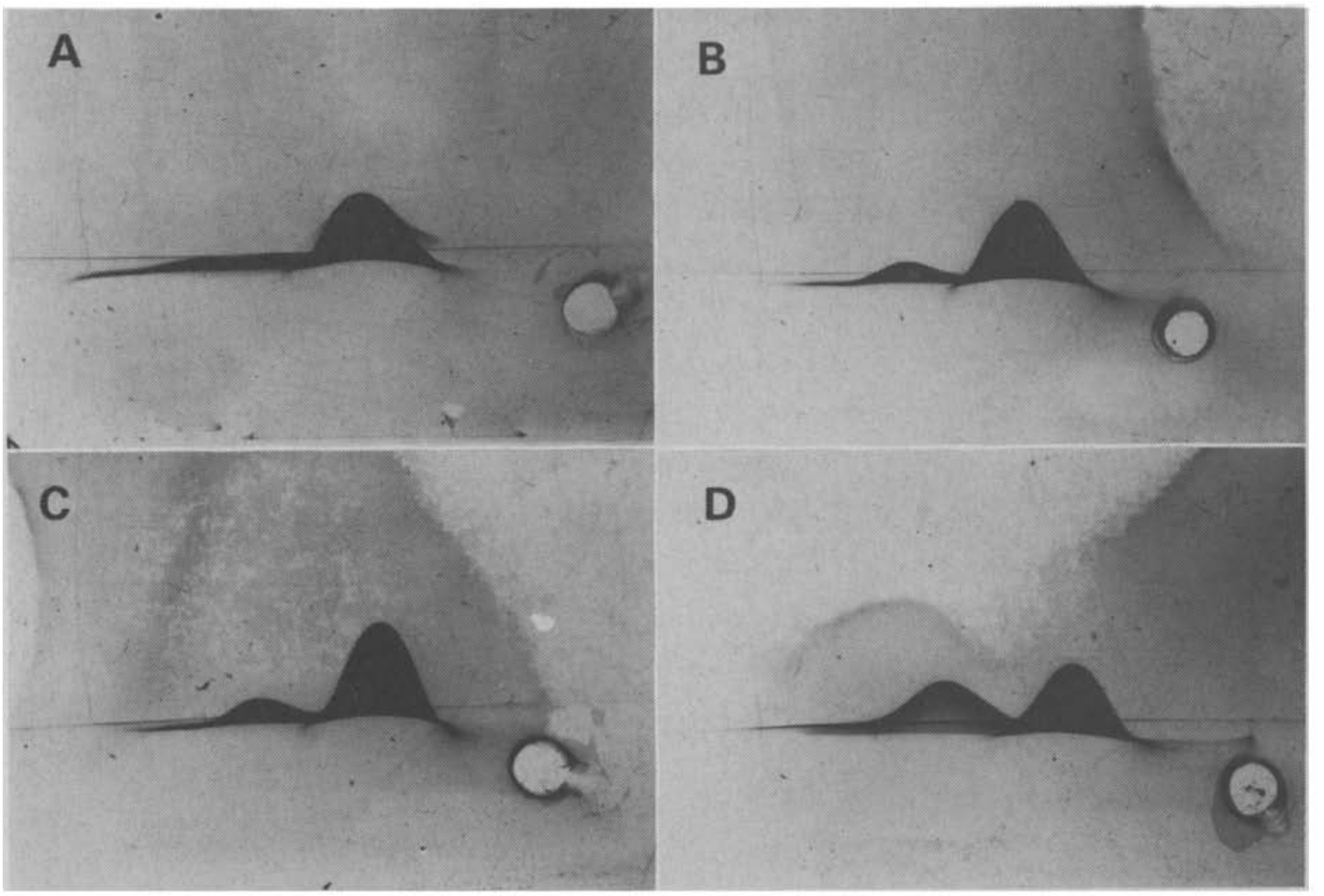

Fig. 5. C3 Activation by Plantago-mucilage A (**1**) and its chemical modifications. NHS was incubated with a half volume of (A) PBS^{-}, (B) **1**, (C) deacetylated **1**, or (D) polyalcohol solution (1 mg/mL) with Mg^{2+}–EGTA–GVB^{--} at 37° for 30 min. The sera were then subjected to crossed immuno-electrophoresis, to locate C3 cleavage products. The anode is to the left.

active polyalcohol caused the highest C3 cleavages in serum in three kinds of test samples. These results indicate that the polyalcohol mainly activates the complement *via* the alternative pathway.

DISCUSSION

Plantago-mucilage A (**1**) is a partially *O*-acetylated and highly branched glucuronoarabinoxylan[15] and we have investigated the structural features responsible for the activation mechanisms of the classical and alternative pathways. Deacetylated **1** enhanced the activation of the classical pathway, but the activation of the alternative pathway was not affected. Deacetylated **1** had almost the same molecular weight, optical rotation, and intrinsic viscosity as **1**. The polyalcohol, obtained by periodate oxidation of **1** followed by reduction with sodium borohydride, enhanced the activation of the alternative pathway, whereas activation of the classical pathway was diminished to a negligible level. Thus, *O*-acetyl groups may prevent the activation of complement *via* the classical pathway, and polyhydroxyl groups are important for the activation of complement *via* the alternative pathway.

Generally, the anti-tumor polysaccharides show anti-complementary activity[4] and activate the complement *via* the alternative pathway[5]. The anti-tumor activity of a 6-branched (1→3)-β-D-glucan was enhanced[20,21] after periodate oxidation and borohydride reduction. Misaki *et al.*[20] suggested that the presence of numerous polyhydroxylated groups, probably arranged outside the triple-helix chains formed by the (1→3)-β-D-glucan backbone, would alter the water insolubility of the highly branched glucan. The present results suggest that the enhanced anti-tumor activity of the polyalcohol product derived from 6-branched (1→3)-β-D-glucan may modulate the anti-complementary activity. However, the polyalcohol derived from **1** was less soluble than **1** and its solubility was similar to that of periodate-oxidised **1** which failed to activate the complement. Thus, water solubility may not be related to the anti-complementary activity of **1**, and the polyalcohol may affect some other interactions such as hydrogen bonding. Although the carboxyl-reduced product contained no *O*-acetyl groups, its anti-complementary activity did not change significantly; however, the activity decreased drastically in the absence of Ca^{2+}. The carboxyl-reduced product enhanced C4 consumption slightly. These results suggest that carboxyl groups of uronic acid moieties are involved in the activation of the classical pathway, because carboxyl-reduction might diminish the enhancement of complement activation *via* the classical pathway caused by the effect of deacetylation. Although the product of partial acid hydrolysis had a decreased molecular weight, its anti-complementary activity did not change significantly. There are no *O*-acetyl groups in this product and a decreased content of terminal uronic acid groups. These facts suggest that its anti-complementary activity is caused by effects similar to that of the carboxyl-reduced product.

Molecular weight appears not to be important in the activation of complement by **1**. Paniculatan[14] from the inner bark of *Hydrangea paniculata*

activates complement mainly *via* the classical pathway and contains[22] a high proportion of 4-*O*-methylglucuronic acid, glucuronic acid, and galacturonic acid as its non-reducing terminals and side chains. These observations and the present results indicate that an anionic charge may be involved in activation of complement *via* the classical pathway, as does lipid A in LPS[23,24]. The water-insoluble glucan synthesised enzymically by *Streptococcus mutans* OMZ 176, which consists of the (1→3)-α-D-glucan backbone carrying (1→6)-α-D-glucan side-chains, was able to activate the alternative pathway of the complement system. It was concluded that both the (1→3)-α-D-glucan backbone and the (1→6)-α-D-glucan side-chains are essential for the anti-complementary activity[25]. We have reported[10] that another anti-complementary arabino-3,6-galactan (AG IIb-1) from *Angelica acutiloba* KITAGAWA tends to self-aggregate and this might be important for the expression of anti-complementary activity. These observations indicated that steric factors also seem to play an important role in anti-complementary activity.

REFERENCES

1 L. H. MUSCHEL, K. SCHMOKER, AND P. M. WEBB, *Proc. Soc. Exp. Biol. Med.*, 117 (1964) 639–643.
2 H. GEWURZ, H. S. SHIN, AND S. E. MERGENHAGEN, *J. Exp. Med.*, 128 (1968) 1049–1057.
3 R. L. MARCUS, H. S. SHIN, AND M. M. MAYER, *Proc. Natl. Acad. Sci. U.S.A.*, 68 (1971) 1351–1354.
4 T. OKUDA, Y. YOSHIOKA, T. IKEKAWA, G. CHIHARA, AND K. NISHIOKA, *Nature (London), New Biol.*, 238 (1972) 59–60.
5 J. HAMURO, U. HADDING, AND D. BITTER-SUERMANN, *Immunology*, 34 (1978) 695–705.
6 L. PILLEMER AND E. E. ECKER, *J. Biol. Chem.*, 137 (1941) 139–142.
7 O. GÖTZE AND H. J. MÜLLER-EBERHARD, *J. Exp. Med.*, 134 (1971) 90s–108s.
8 H. YAMADA, H. KIYOHARA, J.-C. CYONG, Y. KOJIMA, Y. KUMAZAWA, AND Y. OTSUKA, *Planta Med.*, 50 (1984) 163–167.
9 H. YAMADA, H. KIYOHARA, J.-C. CYONG, AND Y. OTSUKA, *Mol. Immunol.*, 22 (1985) 295–304.
10 H. KIYOHARA, H. YAMADA, J.-C. CYONG, AND Y. OTSUKA, *J. Pharmacobio-Dyn.*, 9 (1986) 339–346.
11 H. YAMADA, K. OHTANI, H. KIYOHARA, J.-C. CYONG, Y. OTSUKA, Y. UENO, AND S. ŌMURA, *Planta Med.*, (1985) 121–125.
12 H. YAMADA, Y. OTSUKA, AND S. ŌMURA, *Planta Med.*, (1986) 311–314.
13 H. YAMADA, J.-C. CYONG, AND Y. OTSUKA, *Int. J. Immunopharmacol.*, 8 (1986) 71–82.
14 H. YAMADA, T. NAGAI, J.-C. CYONG, Y. OTSUKA, M. TOMODA, N. SHIMIZU, AND K. SHIMADA, *Carbohydr. Res.*, 144 (1985) 101–111.
15 M. TOMODA, M. YOKOI, AND K. ISHIKAWA, *Chem. Pharm. Bull.*, 29 (1981) 2877–2884; M. TOMODA, N. SHIMIZU, K. SHIMADA, R. GONDA, AND H. SAKABE, *ibid.*, 32 (1984) 2182–2186.
16 M. DUBOIS, K. A. GILLES, J. K. HAMILTON, P. A. REBERS, AND F. SMITH, *Anal. Chem.*, 28 (1956) 350–356.
17 M. TOMODA, N. SATOH, AND C. OHMORI, *Chem. Pharm. Bull.*, 26 (1978) 2768–2773.
18 T. A. E. PLATTS-MILLS AND K. ISHIZAKA, *J. Immunol.*, 113 (1974) 348–358.
19 J.-C. CYONG, S. S. WITKIN, B. RIEGER, E. BARBARESE, R. A. GOOD, AND N. K. DAY, *J. Exp. Med.*, 155 (1982) 587–598.
20 A. MISAKI, M. KAKUTA, T. SASAKI, M. TANAKA, AND H. MIYAJI, *Carbohydr. Res.*, 92 (1981) 115–129.
21 H. YAMADA, N. KAWAGUCHI, T. OHMORI, Y. TAKESHITA, S. TANEYA, AND T. MIYAZAKI, *Carbohydr. Res.*, 125 (1984) 107–115.
22 M. TOMODA AND N. SATOH, *Chem. Pharm. Bull.*, 25 (1977) 2910–2916.
23 D. C. MORRISON AND L. F. KLINE, *J. Immunol.*, 118 (1977) 362–368.
24 M. E. WILSON AND D. C. MORRISON, *Eur. J. Biochem.*, 128 (1982) 137–141.
25 S. INAI, K. NAGAKI, S. EBISU, K. KATO, S. KOTANI, AND A. MISAKI, *J. Immunol.*, 117 (1976) 1256–1260.