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RELATIONSHIP BETWEEN CHEMICAL STRUCTURE AND ANTI-COMPLEMENTARY ACTIVITY OF PLANT POLYSACCHARIDES

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ABSTRACT

Seventeen water-soluble polysaccharides obtained from various plants were tested for anti-complementary activities. Considerable activity was observed for Zizyphus-arabinan [(1→5)-linked α-L-arabinofuranosyl main-chain having (1→2)-linked α-L-arabinofuranosyl side-chains] from the dried fruit of *Zizyphus jujuba* var. *inermis*, paniculatan [highly branched, partially *O*-acetylated, acidic mucous polysaccharide composed of (1→2)-linked α-L-rhamnopyranosyl residues having branches composed of *O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-(1→4)-β-D-galactopyranose at position 4 and (1→4)-linked α-D-galactopyranosyluronic acid residues having β-D-glucosyluronic acid groups at position 3] from the inner bark of *Hydrangea paniculata*, and Plantago-mucilage A [highly branched, partially *O*-acetylated, acidic mucous polysaccharide with a main chain of (1→4)-linked β-D-xylopyranosyl residues having other β-D-xylopyranosyl groups and branches composed of *O*-(α-D-glucopyranosyluronic acid)-(1→3)-α-L-arabinofuranosyl and *O*-(α-D-galactopyranosyluronic acid)-(1→3)-α-L-arabinofuranosyl groups at position 3 as side chains] from the seed of *Plantago asiatica*. The majority of the mucous acidic polysaccharides from the plants belonging to *Malvaceae* had weak activities. These polysaccharides commonly contained (1→2)-α-L-rhamnopyranosyl-(1→4)-α-D-galactopyranosyluronic acid residues as the main chain. Glucomannans and pectin did not show any significant activity. The results of tests on C4 titration, anti-complementary activity in the absence of Ca^{2+}, crossed immunoelectrophoresis, and activity of the alternative complement pathway using rabbit erythrocytes indicated that the mode of complement activation by paniculatan occurred mainly *via* the classical pathway, whereas activation by Zizyphus-arabinan and Plantago-mucilage A were both *via* the alternative and classical pathways. The mode of complement activation by paniculatan did not depend on its particulate state.

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INTRODUCTION

The complement system plays an important role in host defence, inflammation, or allergic reactions, and activation occurs *via* both the classical and alternative pathways. The classical pathway is activated by an immune complex containing IgM and IgG antibodies, the acute phase protein, C-reactive protein, and RNA tumor viruses. The alternative pathway does not require antibodies and is directly activated by polysaccharides, certain immunoglobulins, viruses, fungi, bacteria, certain animal cells, and parasites. The common denominator of these activators is still unknown, although carbohydrate has been found to be a constituent of most of them. Some anti-complementary polysaccharides, for example, lipopolysaccharide[1-3], (1→3)-β-D-glucan[4-5], 6-branched (1→3)-β-D-glucan[4-5], and inulin[6], have already been isolated from bacteria, fungi, and plants, but there is little information on other types of anti-complementary polysaccharides. Potent anti-complementary activity has been observed in extracts of some Chinese herbs[7] and it was suggested[7] that this may have been caused by polysaccharide(s), because treatment with periodate inhibited the activity but digestion with pronase did not. Recently, Yamada *et al.*[8,9] isolated a new type of anti-complementary polysaccharide from a Chinese herb, the root of *Angelica acutiloba* Kitagawa, and reported that the active principle was an arabinogalactan[10].

In order to clarify the relationship between the chemical structure of the polysaccharide and its anti-complementary activity, several kinds of plant polysaccharides, characterised by Tomoda *et al.*[11-27], have been investigated.

EXPERIMENTAL

Purification of plant polysaccharides. — Bletilla-glucomannan[11] was isolated from the tuber of *Bletilla striata,* Lilium-A-glucomannan[12] from the bulb of *Lilium auratum,* Lilium-S-glucomannan[13] from the bulb of *Lilium speciosum,* Lilium-Ma-glucomannan[14] from the bulb of *Lilium maculatum,* Lilium-J-glucomannan[15] from the bulb of *Lilium japonicum,* Narcissus-T-glucomannan[16] from the bulb of *Narcissus tazetta,* Lycoris-R-glucomannan[17] from the bulb of *Lycoris radiata,* Lycoris-S-glucomannan[18] from the bulb of *Lycoris squamigera,* Zizyphus-arabinan[19] from the fruit of *Zizyphus jujuba* var. *inermis,* paniculatan[20] from the inner bark of *Hydrangea paniculata,* Althaea-mucilage O[21] from the root of *Althaea officinalis,* Abelmoschus-mucilage M[22] from the root of *Abelmoschus manihot,* Abelmoschus-mucilage G[23] from the root of *Abelmoschus glutinotextilis,* Althaea-mucilage OL[24] from the leaf of *Althaea officinalis,* Althaea-mucilage R[25] from the root of *Althaea rosea,* Plantago-mucilage A[26] from the seed of *Plantago asiatica,* and Zizyphus-pectin A[27] from the fruit of *Zizyphus jujuba* var. *inermis.* AR-Arabinogalactan[8-10], acidic heteroglycans AAF-IIb-2 and IIb-3[28], and LR-polysaccharide IIa[29] were isolated according to the method of Yamada *et al.*[10,28,29] from the root of *Angelica acutiloba* Kitagawa, the leaf of *Artemisia princeps* PAMP, and the root of *Lithospermum euchromum* ROYLE, respectively.

Anti-complementary activity. — The anti-complementary activity was measured as described previously[7-8]. Gelatin-veronal-buffered saline (pH 7.4) containing 500μM Mg^{2+} and 150μM Ca^{2+} (GVB^{2+}) was prepared as previously described, and normal human serum (NHS) was 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^{2+}. The mixtures were incubated at 37° for 30 min and the residual total hemolytic complement (TCH_{50}) was determined by a method using IgM-hemolysin-sensitised sheep erythrocytes (EA) at 1×10^8 cells/mL. NHS was incubated with water and GVB^{2+} 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[30] in 10mM EGTA containing 2mM $MgCl_2$ in GVB^{2-} (Mg^{2+}–EGTA–GVB^{2-}). A sample of the anti-complementary polysaccharide was incubated with Mg^{2+}–EGTA–GVB^{2-} 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^{2-}.

Crossed immunoelectrophoresis. — NHS was incubated with an equal volume of the solution of the anti-complementary polysaccharide with Mg^{2+}–EGTA–GVB^{2-} for 30 min at 37°. The serum was then subjected to crossed immunoelectrophoresis to locate the C3 cleavage products[31]. 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 (1.5-mm layer) containing 0.5% of a rabbit anti-human serum to C3 at a potential gradient of 1 mA/cm for 10 h. After the electrophoresis, the plate was fixed and stained with Ponceau 3R.

Determination of C4. — Titration of C4 was performed[32] 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.

RESULTS

Anti-complementary activity of plant polysaccharides. — Various water-soluble plant polysaccharides, including glucomannans, arabinan, and four types of acidic heteroglycan, were tested. Some data on these polysaccharides are given in Table I. The anti-complementary activities of these polysaccharides are shown in Fig. 1. Zizyphus-arabinan, paniculatan, and Plantago-mucilage A had potent activities, which were almost at the same level as that of the positive control (AR-arabinogalactan mixture from *A. acutiloba* Kitagawa). Abelmoschus-mucilage M, Althaea-mucilage O, Althaea-mucilage R, and Althaea-mucilage OL had weak activities. These polysaccharides were isolated from plants belonging to *Malvaceae,* and each contained a (1→2)-α-L-rhamnopyranosyl-(1→4)-α-D-galactopyranosyl-uronic acid residue as the main chain[21,22,24,25]. Zizyphus-pectin A and the gluco

TABLE I

PLANT POLYSACCHARIDES

Polysaccharides	$[\alpha]_D$ *(water, degrees)*	*Molar ratio of sugars*	*O-Acetyl (%)*	*Protein (%)*	*Ref.*
Glucomannans					
Bletilla	−31.6	Man/Glc 3:1	4.2		11
Lilium-A	−37.9	8:3	5.1		12
Lilium-S	−29.5	2:1	3.3		13
Lilium-Ma	−32.4	7:4	4.7		14
Lilium-J	−40.7	5:2	5.0		15
Narcissus-T	−24.3	5:1	22.7		16
Lycoris-R	−28.5	12:1	15.5		17
Lycoris-S	−23.1	7:2	16.7		18
Arabinan					
Zizyphus	−142.2	Ara/Gal 30:1			19
Acidic polysaccharides					
Paniculatan	+80.0[a]	Rha/Gal/GalA/GlcA/4MeGlcA 4:4:3:2:5	2.0		20
Althaea-mucilage O	+50.5	Rha/Gal/GalA/GlcA 3:2:3:3	0.7		21
Abelmoschus-mucilage M	+51.7[b]	Rha/GalA/GlcA 1.1:1:1		17.0	22
G	+53.3	Rha/GalA/GlcA 4:4:3		18.0	23
Althaea-mucilage OL	+61.6[b]	Rha/GalA/GlcA 1.5:1.1:1	1.0	3.3	24
R	+51.7	Rha/Gal/Glc/GalA/GlcA 6:2:1:4:6	9.8	8.3	25
Plantago-mucilage A	−38.1	Xyl/Ara/GalA/GlcA 21:6:1:5	4.8		26
Zizyphus-pectin A	+201.2	Rha/Gal/Ara/GalA 1:1:4:35	2.3		27

[a]0.05% Ammonia. [b]0.1% Ammonia.

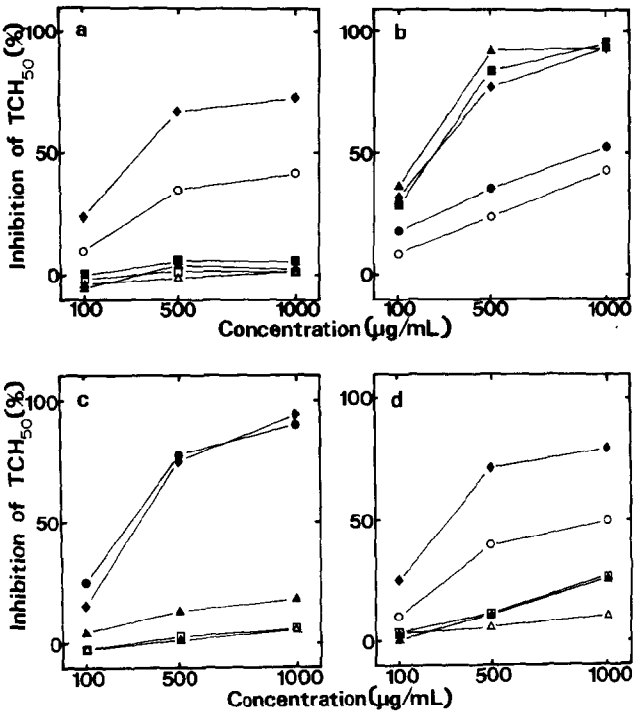
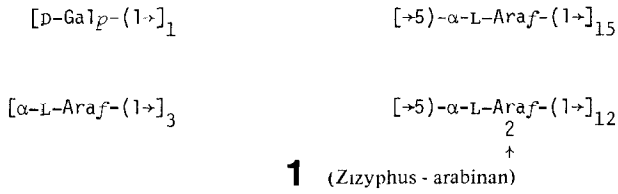


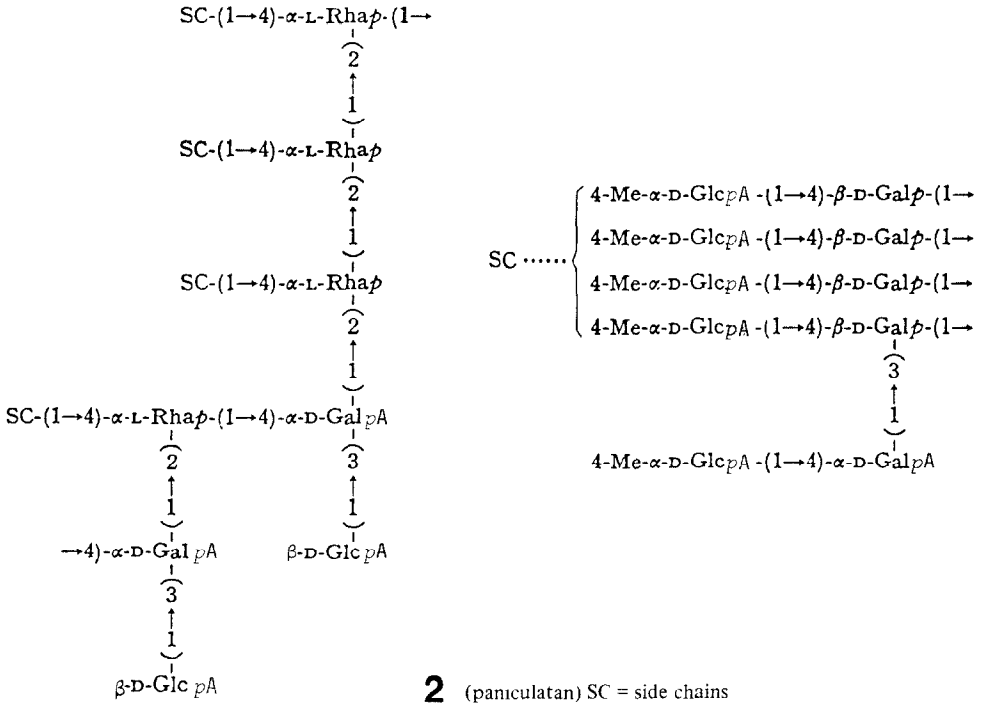
Fig. 1. Anti-complementary activity of plant polysaccharides. (*a*) ◆, AR-arabinogalactan mixture; ○, Abelmoschus-mucilage M; ▲, Lilium-Ma-glucomannan; △, Lilium-J-glucomannan; ■, Abelmoschus-mucilage G; □, Narcissus-T-glucomannan; (*b*) ◆, AR-arabinogalactan mixture; ▲, paniculatan; ●, Althaea-mucilage O; ■, Plantago-mucilage A; ○, Althaea-mucilage R; (*c*) ◆, AR-arabinogalactan mixture; ●, Zizyphus-arabinan; □, Bletilla-glucomannan; △, Lilium-A-glucomannan; ▲, Lilium-S-glucomannan; (*d*) ◆, AR-arabinogalactan mixture; △, Lycoris-R-glucomannan; ▲, Zizyphus-pectin A; □, Lycoris-S-glucomannan; ○, Althaea-mucilage OL.

mannans showed insignificant activity. Although Abelmoschus-mucilage G was isolated from a *Malvaceae* plant, it was also inactive. A significant degree of activity was found in Zizyphus-arabinan, paniculatan, and Plantago-mucilage A; in the same test system, paniculatan was the most active. Details of the structures of Zizyphus-arabinan[19], paniculatan[20], and Plantago-mucilage A[26] are shown in **1–3**. Each polysaccharide has a highly branched structure.

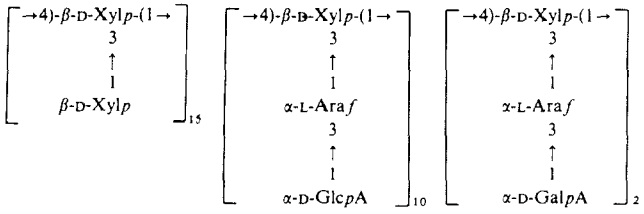
Mode of action of anti-complementary polysaccharides. — NHS was incubated with paniculatan, Plantago-mucilage A, or Zizyphus-arabinan in GVB^{2+} at 30° for 30 min, and the residual activity of C4 was estimated by hemolytic assay (Fig. 2). Paniculatan decreased the C4 content of NHS drastically. When NHS incubated with 100 μg/mL of paniculatan was used for C4 titration, ~50% of the hemolytic titer of C4 was consumed similar to that of AAF-IIb-3, an anti-complementary polysaccharide from *A. princeps* PAMP. Zizyphus-arabinan and Plantago-mucilage A also decreased the C4 content of NHS significantly. The anti-complementary activity caused by these polysaccharides also diminished in the



1 (Zizyphus - arabinan)



2 (paniculatan) SC = side chains



The terminal xylosyl groups consist of D-Xyl*p* 2-Ac-D-Xyl*p* (3 . 2), the intermediate xylosyl residues consist of D-Xyl*p* 2-Ac-D-Xyl*p* (8 1), and the intermediate arabinosyl residues consist of L-Ara*f* 2-Ac-L-Ara*f* (3 1)

3 (Plantago - mucilage A)

absence of Ca^{2+} with surplus Mg^{2+} (Fig. 3). In the case of paniculatan, the activity was completely reduced. These results show that the classical pathway of the complement was activated by these polysaccharides. Furthermore, when these polysaccharides were incubated with NHS in Mg^{2+}–EGTA–GVB^{2-} at 37° for 30 min and a hemolytic assay (ACH_{50}) was carried out using rabbit erythrocytes, Plantago-mucilage A and Zizyphus-arabinan showed a dose-dependent anti-complementary activity on ACH_{50} (ACP activity) (Fig. 4). Therefore, crossed immunoelectrophoresis was carried out after the incubation of NHS with these polysaccharides in Mg^{2+}–EGTA–GVB^{2-} to determine whether C3 activation had occurred (Fig. 5). A cleavage of the C3 precipitin line was obtained in the serum treated with these polysaccharides. Potent ACP-active Plantago-mucilage A caused the highest C3 cleavage. However, although paniculatan showed potent anti-complementary activity *via* the classical pathway, it also caused slight C3 cleavage in serum. These results indicate that Zizyphus-arabinan and Plantago-mucilage A also activate complement *via* the alternative pathway, whereas paniculatan mainly activates complement *via* the classical pathway.

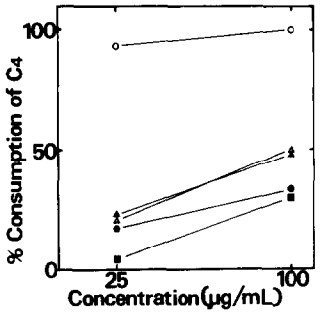


Fig. 2. Consumption of C4 by anti-complementary polysaccharides: ●, Zizyphus-arabinan; ▲, paniculatan; ■, Plantago-mucilage A; △, AAF-IIb-3; ○, AAF-IIb-2.

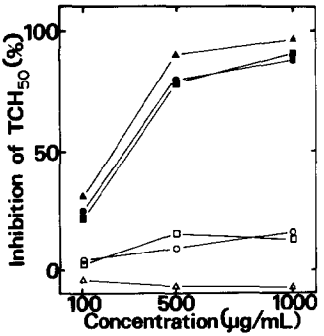


Fig. 3. Change of TCH_{50} by incubation with the anti-complementary polysaccharides in the presence or absence of Ca^{2+}: ▲, paniculatan in GVB^{2+}; △, paniculatan in Mg^{2+}–EGTA–GVB^{2-}; ●, Zizyphus-arabinan in GVB^{2+}; ○, Zizyphus-arabinan in Mg^{2+}–EGTA–GVB^{2-}; ■, Plantago-mucilage A in GVB^{2+}; □, Plantago-mucilage A in Mg^{2+}–EGTA–GVB^{2-}.

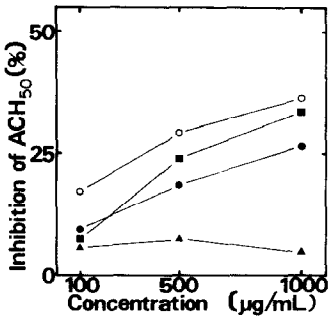


Fig. 4. ACP activity of anti-complementary polysaccharides: ●, Zizyphus-arabinan; ▲, paniculatan; ■, Plantago-mucilage A; ○, AAF-IIb-2.

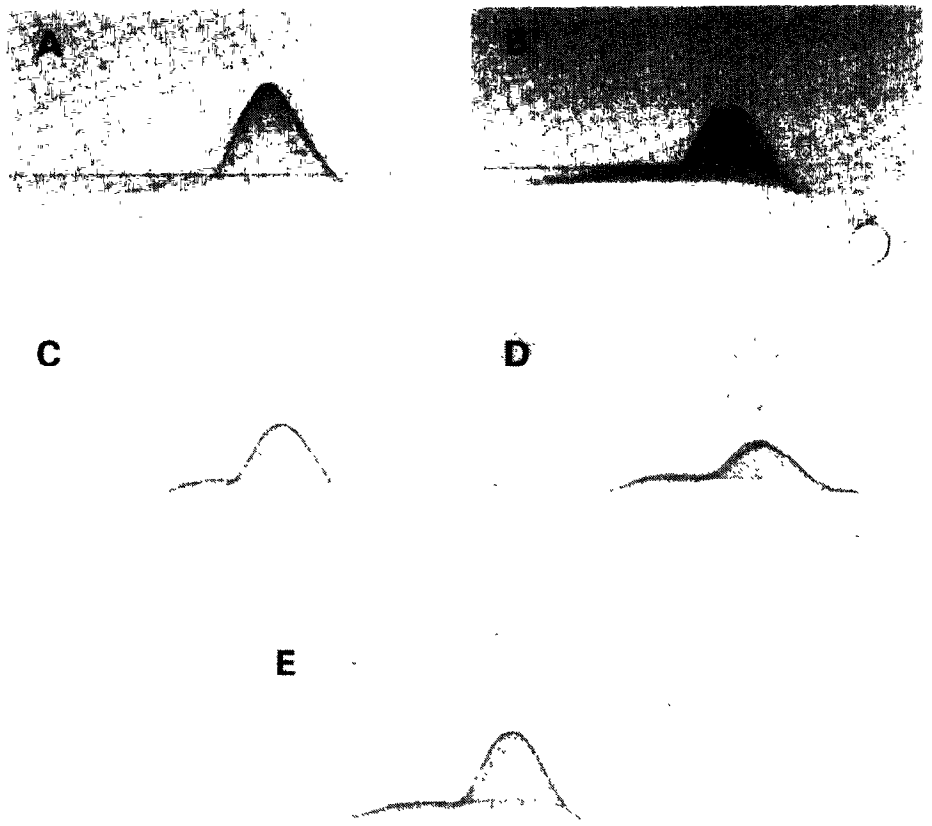


Fig. 5. C3 Activation by anti-complementary polysaccharides. NHS was incubated with an equal volume of (A) PBS^-, (B) LR-polysaccharide IIa, (C) paniculatan, (D) Plantago-mucilage A, or (E) Zizyphus-arabinan solution (1 mg/mL) with Mg^{2+}–EGTA–GVB^{2-} at 37° for 30 min. The sera were then subjected to crossed immunoelectrophoresis, to locate C3 cleavage products. The anode is to the left.

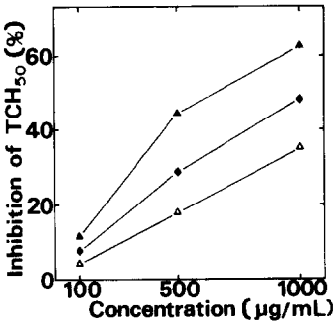


Fig. 6. Anti-complementary activity of water-soluble (▲) and -insoluble paniculatan (△), and AR-arabinogalactan mixture (◆).

Several water-insoluble polysaccharides are well-known activators of the alternative complement pathway. The question arises as to whether the activation of the alternative pathway is dependent on the solubility of the polysaccharides in water. Paniculatan, Plantago-mucilage A, and Zizyphus-arabinan are water-soluble. However, paniculatan becomes less soluble in water the longer it is stored, and water-soluble paniculatan showed much higher anti-complementary activity (Fig. 6). The activity decreased by water-insoluble paniculatan also diminished to a negligible level in the absence of Ca^{2+} (data not shown). Furthermore, the weak ACP activity caused by paniculatan did not depend on its particulate state (data not shown). Thus, the mode of complement activation by paniculatan does not depend on its particulate state.

DISCUSSION

We have been concerned to determine the structure of several anti-complementary polysaccharides that are present in hot-water extracts of such Chinese herbs as *A. acutiloba*[8–10], *L. euchromum*[29], and *A. princeps*[28]. A 3,6-arabinogalactan[10] extracted from *A. acutiloba* which has potent anti-complementary activity contains a backbone of (1→6)-linked galactopyranosyl residues and most of the arabinose was present as α-L-furanose residues in the non-reducing terminals and side chains. However, a different 3,6-arabinogalactan[10] from larch wood did not show any significant activity. Therefore, we tested the anti-complementary activity of seventeen plant water-soluble polysaccharides having different chemical structures. Potent anti-complementary activity was observed for paniculatan[20], Plantago-mucilage A[26], and Zizyphus-arabinan[19]. These polysaccharides[19,20,26] differed in component sugars, molecular weight, optical rotation, and acetyl content, although each was highly branched. Several glucomannans did not show any significant activity and all were levorotatory (Table I). Acidic polysaccharides such as Althaea-mucilage O, Abelmoschus-mucilage M, Althaea-mucilage OL, and Althaea-mucilage R from the *Malvaceae* plants had structural

units [→4)-α-GalA-(1→2)-α-Rha-(1→][21,22,24,25] in common, and each polysaccharide showed weak activity. Zizyphus-pectin A[27] also contained the same structural unit in a part of the main chain, but was inactive. Pectin isolated from *A. acutiloba* was also inactive.

Bacterial lipopolysaccharide (LPS) activates the complement *via* the alternative and classical pathways[30]. LPS is composed of lipid A, a core polysaccharide, and O-specific sugar chains, the lipid A portion activating the classical pathway and the polysaccharide moiety activating the alternative pathway[31,32]. The lipid A region of LPS can interact directly with complement[32] *via* an antibody-independent mechanism in which native C1 is bound to lipid A. Paniculatan, which activated complement mainly *via* the classical pathway, contains high proportions of 4-methylglucuronic acid, glucuronic acid, and galacturonic acid as its non-reducing terminals and side chains. It is not known whether the anionic charge due to the uronic acid residues in paniculatan activates the classical pathway, directly as does lipid A. Anti-complementary polysaccharides, for example, inulin[6], 6-branched (1→3)-β-D-glucans such as lentinan[33] and pachymaran[34], and a 6-branched (1→3)-α-D-glucan[35] from *Streptococcal* species, are insoluble activators[5] of the alternative complement pathway. However, water-insoluble paniculatan had a lower anti-complementary activity, either in the presence or absence of Mg^{2+}–EGTA, and ACP activity than water-soluble paniculatan. These results indicate that their highly branched structure could be involved in the anti-complementary activity, and that some feature such as the negative charge may determine the activation pathway. Further studies of this aspect are in progress.

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