J Food Sci Nutr
Vol 9, p 21~28 (2004)

J Food Science and Nutrition

Characterization and Purification of Anti-Complement Polysaccharide from *Spirodela polyrhiza*

Jin-Gi Min†, Doo-Seog Lee, Jeong-Heum Park, Moon-Soo Heo[1], Tae-Jin Kim[2], Kil-Bo Shim[2] and Young-Je Cho[2]

Sanitation and Processing Research Division, National fisheries R & D Institute, Busan 619-900, Korea
[1]Faculty of Marine Science, Cheju National University, Jeju 690-756, Korea
[2]Faculty of Food Science and Biotechnology, Food Science and Technology Major, Pukyong National University, Busan 608-737, Korea

Abstract

We purified and characterized a crude polysaccharide from *Spirodela polyrhiza* with anti-complement activities. The crude polysaccharide fraction (SP-0) which had potential anti-complement activity was extracted in hot water for 4 hrs at 100°C. The ethanol-precipitate, the crude polysaccharide fraction (SP-1), showed a potent anti-complement activity. Further purification of the crude polysaccharide (SP-1) was carried out by cetavlon, ion exchange chromatography and gel column chromatography. Among cetavlon fractions, SP-4 showed the most potent anti-complement activity. When 100 μg/mL of SP-4 was incubated with an equal volume of normal human serum (NHS), the TCH_{50} was reduced by about 78%. When the SP-4 fraction was futher purified by DEAE-Sepharose (Cl^-), the SP-4IIa, SP-4IIb and SP-4IIc, absorbed fractions, were almost the same as the anti-complement activities of SP-4. SP-4IIc, having the greatest potential activation and the highest yield by ion exchange chromatography, was further purified by gel column chromatography on a Sepharose CL-6B column. Four polysaccharide fractions of SP-4IIc-1, SP-4IIc-2, SP-4IIc-3 and SP-4IIc-4 were obtained, consisting mainly of arabinose, rhamnose, galactose and glucose, with approximate molecular weights of about 305,000, 132,000, 64,000 and 12,000, respectively. Among these subfractions, SP-4IIc-1 had the most potent anti-complement activity. When the SP-4IIc-1 aggregate was applied to a gel column chromatography in 10 mM and 50 mM NaCl solution, the position of the peak fractions shifted to a low molecular weight region, and the molecular weight of SP-4IIc-1 decreased with increased NaCl concentration in the gel column chromatography. It was found that the self-aggregation formed spontaneously in void volume by gel column chromatography using Sepharose CL-6B in water and the self-aggregation significantly affected the anti-complement function.

Key words: anti-complementary, polysaccharide, *Spirodela polyrhiza*

INTRODUCTION

The complement system consists of over 20 serum proteins including nine complement components of C1 to C9 and their regulators. It is important in initiating inflammation, and its activation might result in opsonization, activation of leukocytes, mast cell degranulation or lysis of target cells by the end product C5b-9 of the cascade. The complement system is a major complement of the immune system response to pathogens, with or without the involvement of antibodies (1,2).

In general, the complement system is activated in two ways. The first activation way is the classical pathway which is mediated by an immune complex including IgM or IgG. The second pathway is the alternative pathway, which does not require antibodies and directly activates C3.

The classical pathway begins with interaction with the C1q subcomponent formed by complement activator (antibody-antigen complex). Binding of C1q in turn converts C1r in the C1 complex into its proteolytically active form C1r. C1r activates the serine proteinase proenzyme C1s, to form C1s, which in turn cleaves and activates C4 and C2 which form the complex proteinase C4b2a2b, the 'C3 convertase' of this pathway. Since activated C4, like C3, can undergo covalent binding reactions, the convertase enzyme becomes localized on the surface of the complement activator. C3 is cleaved by the proteolytically active subunit, C2a, of the convertase. C3b, which is deposited covalently on the complement activator

†Corresponding author. E-mail: jkmin@nfrda.re.kr
Phone: +82-51-720-2623, Fax: +82-51-720-2619

surface close to the site of attachment of the convertase, this serves as a binding site for C5, which is then activated by the convertase. Activation of C5 is followed by non-enzymatic assembly of the C5b6789 lytic complex. Initial events in the activation of the alternative pathway are less well understood, but early events include the assembly of a C3bB complex on the surface of the activator. This is in turn activated by factor D to form the C3bBb complex, which is homologous to C4b2a2b and is the 'C3 convertase' of the alternative pathway (3). In order to activate the complement system through the above pathways, various polysaccharides have recently been purified from natural plant materials. In particular, there are 2 kinds of arabibogalactans and 4 kinds of pectic arabinogalactans fractionated from root of *Angelica acutiloba* (4-6), acidic heteroglycan from leaves of *Artemisia princeps* (7) and stem of *Coix lachryma-jobi* var. *ma-yuen* (8), acidic heteroglycan of three kinds from leaves from *Panax ginseng* (9,10), and heterogalactan from *Arcae pericarpium* (11).

The materials, which have been demonstrated to activate the complement system, hint at the possibility that they can be developed into biological response modifiers (BRM) for preventing and treating diseases (12-14).

We attempted to purify and characterize anti-complement polysaccharides from *Spirodela polyrhiza,* which is used for oriental medical purposes in Korea.

MATERIALS AND METHODS

Materials

Spirodela polyrhiza, a freshwater aquatic plant also known in English as giant duckweed, was purchased for experimental use at Pusan-Jin market in Pusan, Korea. DEAE-Sepharose Fast Flow, Sepharose CL-6B, EDTA-gelatin veronal buffered saline (EDTA-GVB^{++}, pH 7.4), pronase, and sodium periodate were obtained from Sigma Co., Ltd., Dialysis membranes (MWCO: 1,000) were purchased from Spectrum Co.

General method

The total carbohydrate and uronic acid contents were determined by the phenol-sulfuric acid method (15) and the *m*-hydroxydiphenyl method (16), respectively, using glucose and galacturonic acid as the respective standards. The amount of protein was assayed by the method of Lowry et al. (17) with bovine serum albumin as the standard.

Purification of crude polysaccharide

The dried leaves of *Spirodela polyrhiza* (600 g) were decocted with hot water and lyophilized to make a dried extract. The lyophilized extract (SP-0) was refluxed with methanol (3 L) for 2h, and then the methanol insoluble precipitate was dissolved in distilled water. The addition of ethanol (5 volumes) gave a precipitate that was collected by centrifugation and redissolved in distilled water. The distilled water soluble fraction as dialysed against running distilled water through Visking cellophane tubing, and the crude polysaccharide (SP-1) was obtained as the lyophilisate of the internal solution.

Crude polysaccharide (SP-1) was fractionated into three fractions (SP-2, SP-3, SP-4) as 8% solution of cetyltrimethylammonium bromide (cetavlon) by the method of Yamada et al. (5). SP-4 was applied to a DEAE-Sepharose Fast Flow column (3.2×45 cm) with chloride as the counter-ion. The column was first eluted with water at 1 mL/min, followed by NaCl gradient (0～1 M), and then the carbohydrate profile was determined using phenol-sulfuric acid. When the column was eluted with distilled water, the first fraction, non-binding fraction (SP-4I) from the column was obtained. And when eluted by NaCl solution, binding fractions (SP-4IIa, SP-4IIb, SP-4IIc, SP-4IId) were fractionated.

For further purification, the SP-4IIa was purified as SP-4IIc-1, SP-4IIc-2, SP-4IIc-3 and SP-4IIc-4, respectively, according to molecular weight by gel filtration (Fig. 1).

Methanolysis and GC

The polysaccharide samples (100 g) and standards (100 nmol) together with myo-inositol were dried in a vacuum over at P_2O_5 for 24 h, and then subjected to methanolysis with methanolic HCl for 16 h at 70°C (18). The samples were dried under nitrogen at room temperature, 2-methyl-2-propanol was added and the samples were dried again. Prior to trimethylation, the samples were vacuum dried at P_2O_5 for 4 h. The samples were subjected to gas chromatographic analysis on a Varian 3400 instrument equipped with a flame ionization detector and a split-spilitless injector. The column was a DB-1 fused silica capillary column (30 m, 0.32 mm i.d) with a film thickness of 0.25 m. Helium was used as carrier gas at a flow rate of 3.0 mL/min. Both injector and detector temperatures were 280°C. The column temperature was initially 140°C (15 min), then increased at a rate of 15°C/min to 170°C (1 min), followed by an increase at 10°C/min to 250°C (6 min) and then 15°C/min to 290°C (10 min).

Pronase digestion of the crude polysaccharide

SP-1 (200 mg) was dissolved in 50 ml of 50 mM Tris-HCl, pH 7.9, containing 10 mM $CaCl_2$, and then 50 mg of pronase was added. The reaction mixture was incubated at 37°C for 48 h with a small amount of toluene. The reation was terminated by boiling for 5 min. The

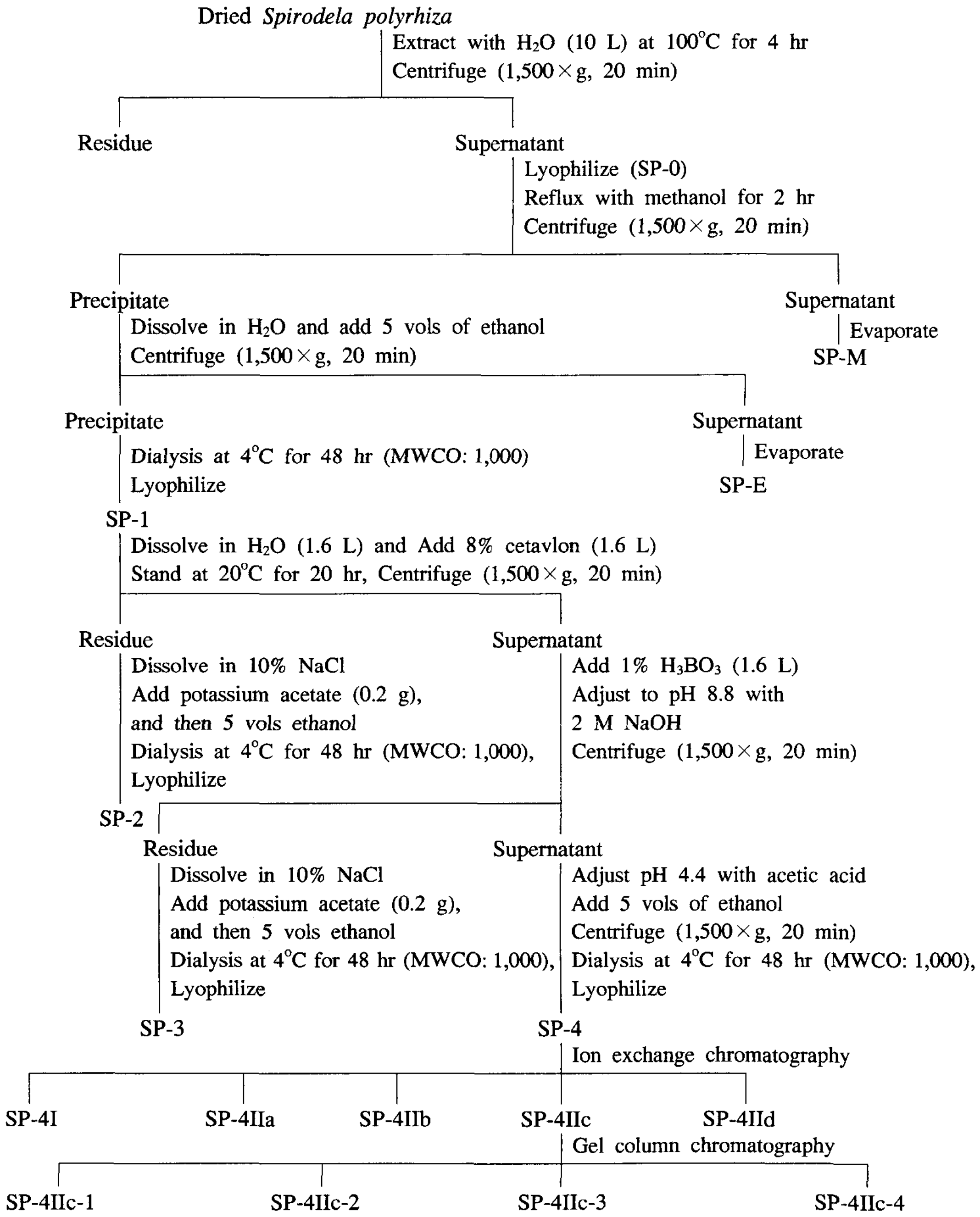

Fig. 1. Preparation of crude polysaccharide from *Spirodela polyrhiza*.

mixture was then dialysed against distilled water for 2 days, and the non-dialysable portion was lyophilized to obtain the SP-1 pronase digest.

Periodate oxidation of the crude polysaccharide

SP-1 (50 mg) was dissolved in 30 mL of 50 mM acetate buffer, pH 4.5, and then 50 mM $NaIO_4$ (10 mL) was added. The reaction mixture was incubated at 4°C in the dark for 3 days. Ethylene glycol (5 mL) was added to destroy the excess periodate, and the mixture was dialysed against distilled water for 2 days. The non-dialysable solution was concentrated while being continuously stirred for 12 h at room temperature. After the neutralization of the reaction mixture with acetic acid, the H_3BO_3 in the sample was removed by the repeated addition and evaporation of methanol. Finally, the oxidized SP-1 was obtained as the lyophilisate after dialysis

Anti-complement activity

Gelatin-veronal-buffered saline (pH 7.2) containing 500 M Mg^{++} and 150M Ca^{++} (GVB^{++}) was prepared as previously described (19). Normal human serum (NHS) was obtained from a healthy adult. Various dilutions of polysaccharide fractions in distilled water, polysaccharides

(50 μL) were incubated with 50 μL of NHS and 50 μL of GVB^{++}. The mixtures were incubated at 37°C for 30 min and the residual total haemolytic complement (TCH_{50}) was determined by a method using IgM haemolysin-sensitized sheep erythrocytes (EA) at 1×10^8 cell/mL. NHS was incubated with distilled water and GVB^{++} to provide a control. The anti-complement activities of the polysaccharide fractions were expressed as the percent inhibition from TCH_{50} of the control.

RESULTS AND DISCUSSION

Isolation of water-soluble polysaccharide

The first crude polysaccharide fraction obtained from hot water extraction of *Spirodela polyrhiza* exhibited a potent anti-complement activity. We compared extractions with different pH and extraction times. Most of the anti-complement activity was extracted after 1 hour (data not shown). In case of extracting with 10 and 100 mM HCl and NaOH, the anti-complementary activities showed lower than that of SP-0 (data not shown).

As shown in Fig. 2, the SP-1, which had the methanol soluble and the ethanol soluble materials removed from SP-0, showed much higher anti-complement activity in comparison with methanol (SP-M) and ethanol (SP-E) materials. In order to further characterize the components with anti-complement activity, SP-1 was treated with periodate and pronase. The anti-complement activity of the deprotainized polysaccharide did not change significantly contrasting with that of SP-1, but the activity was decreased by the periodate oxidation of polysaccharide (Fig. 3). These results indicate that the carbohydrate moiety is responsible for the anti-complement activity, which is not surprising since other immunomodulatory activities have been reported for crude polysaccharide fractions of the higher plants (4,7,8,20).

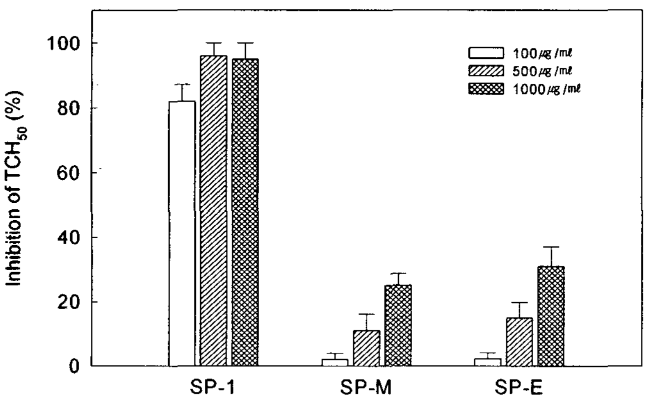

Fig. 2. Anti-complementary activities of several extracts obtained from *Spirodela polyrhiza*. SP-1, ethanol precipitate; SP-M, methanol soluble component; SP-E, ethanol soluble component.

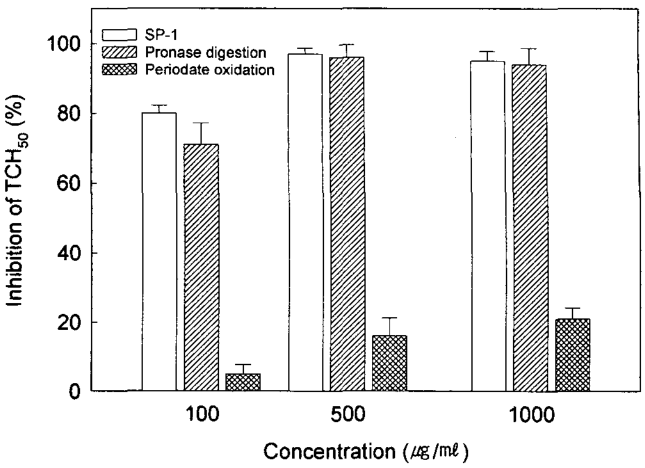

Fig. 3. Ant-complementary acitvities of polysaccharides treated by periodate oxidation and pronase digestion.

Purification of polysaccharide by cetavlon and ion chromatogrphy

The crude polysaccharide, SP-1, was further fractionated by the addition of cetyltrimethylammonium bromide (cetavlon) into three polysaccharide fractions designated SP-2, SP-3 and SP-4. SP-2 and SP-3 contained arabinose, rhamnose, galactose and glucose as the major neutral sugars (Table 1). In particular, SP-2 contained high concentrations of galacturonic acid and glucuronic acid, 20.6 and 10.8 mol%, respectively. Furthermore, SP-2 was not very soluble in distilled water. This was consistent with earlier reports that insoluble polysaccharide complexs are precipitated when the acidic polysaccharides react with ammonium in cetyltrimethylammonium (21).

SP-4 also contained the similar sugars as SP-2 and SP-3, except that its uronic acid content was the lowest among the polysaccharide fractions (Table 1). Among

Table 1. Chemical components and yield of polysaccharide fractions on cetyltrimethylammonium bromide treatment of SP-1

Items	SP-1	SP-2	SP-3	SP-4
Total sugar (%, w/w)	43.0	56.4	50.6	65.0
Uronic acid (%, w/w)	8.8	21.3	12.4	8.2
Protein (%, w/w)	5.1	2.3	5.6	6.6
Yield[1)] (%)	65.2	20.4	9.4	14.8
(Component of sugar)		(Mol.%)		
Rhamnose	10.7	9.5	11.3	10.6
Arabinose	18.3	13.1	18.7	19.9
Xylose	7.6	6.8	7.8	7.2
Fucose	6.5	5.3	6.9	6.9
Mannose	5.2	3.9	5.7	4.8
Galactose	19.7	18.7	19.5	23.6
Glucose	13.2	11.3	11.7	15.5
Galacturonic acid	12.0	20.6	13.0	7.7
Glucuronic acid	6.8	10.8	5.4	3.8

[1)]From the SP-0.

these fractions, SP-4 showed the most potent anti-complement activity. When 100 μg/mL of SP-4 was incubated with an equal volume of normal human serum (NHS), the TCH_{50} was reduced by about 78% (Fig. 4).

SP-4, the most active fraction, was further separated into unabsorbed fraction (SP-4I) and absorbed fractions (SP-4IIa, -IIb, -IIc, -IId) by elution with a linear gradient of NaCl (0~1 M) on a DEAE-Sepharose (Cl^-) column (Fig. 4). Among the absorbed fractions, SP-4IIa, SP-4IIb, SP-4IIc and SP-4IId were eluted in 0.10~0.20 M, 0.20~0.28 M, 0.28~0.34 M and 0.37~0.49 M NaCl, respectively (Fig. 5).

The sugars comprising SP-4I were mainly glucose, galactose, arabinose and mannose; with glucose accounting for about 39% of the total sugars. SP-4IIa primarily contained galactose, arabinose and glucose at the ratio of 27.5 : 22.1 : 13.1 mol%. SP-4IIc was mostly

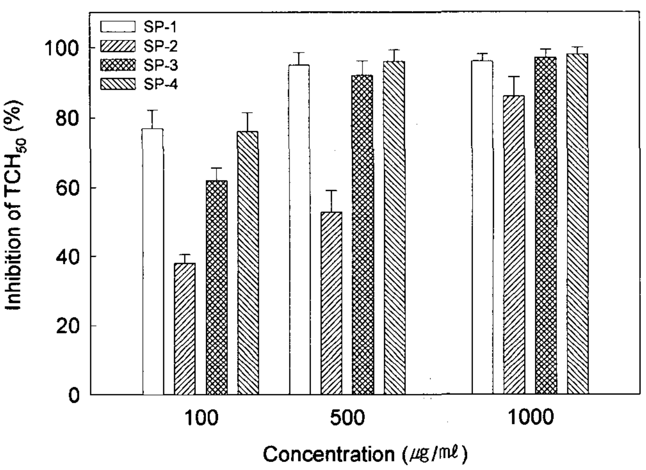

Fig. 4. Anti-complementary activities of the polysaccharide fractions obtained by cetyltrimethyl ammonium bromide treatment of SP-1.

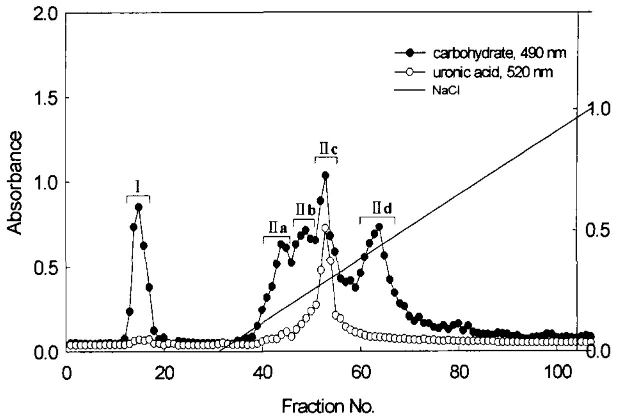

Fig. 5. DEAE-Sepharose Fast Flow (Cl^- form) ion exchange chromatography of SP-4. SP-4 was dissolved in distilled water. Linear gradient elution was carried out with 0~1 M NaCl, column size (3.2×40 cm).

glucose, galactose and arabinose, but also had substantial amounts of rhamnose as well as galacturonic acid and glucuronic acid at 8.6 and 5.0 mol% (Table 2). The anti-complement activities of SP-4IIa, SP-4IIb and SP-4IIc were similiar to that of SP-4, but those of SP-4IId and SP-4I were very low (Fig. 6).

Purification of polysaccharide by gel chromatography

SP-4IIc, the most active fraction obtained by ion exchange chromatography, was further purified by gel column chromatography on a Sepharose CL-6B, giving rise to broad carbohydrate peaks that co-eluted with compounds containing uronic acids. Four polysaccharide fractions of SP-4IIc-1, SP-4IIc-2, SP-4IIc-3 and SP-4IIc-4, were obtained (Fig. 7). These consisted mainly of arabinose, rhamnose, galactose and glucose, with lesser amounts of xylose, fucose, mannose, galacturonic acid

Table 2. Chemical properties and yield of SP-4 subfractions obtained from DEAE-Sepharose Fast Flow (Cl^-) ion exchange chromatography

Items	SP-4I	SP-4IIa	SP-4IIb	SP-4IIc	SP-4IId
Total sugar (%, w/w)	57.9	64.0	52.7	59.8	13.3
Uronic acid (%, w/w)	0.4	2.9	5.4	7.1	0.2
Protein (%, w/w)	0.5	1.7	2.5	2.3	2.4
Yield[1] (%)	16.5	10.1	10.5	15.8	21.5
(Component of sugar)			(Mol.%)		
Rhamnose	-[2]	7.5	7.0	10.1	13.6
Arabinose	21.7	22.1	18.5	15.3	15.9
Xylose	-	9.9	7.4	7.5	11.3
Fucose	-	8.2	7.3	7.2	-
Mannose	15.7	5.5	5.7	4.6	9.2
Galactose	24.1	27.5	33.7	29.1	24.0
Glucose	38.5	13.1	12.9	12.6	19.1
Galacturonic acid	-	6.2	4.4	8.6	4.9
Glucuronic acid	-	-	4.1	5.0	2.0

[1]From the SP-4.
[2]Not detected.

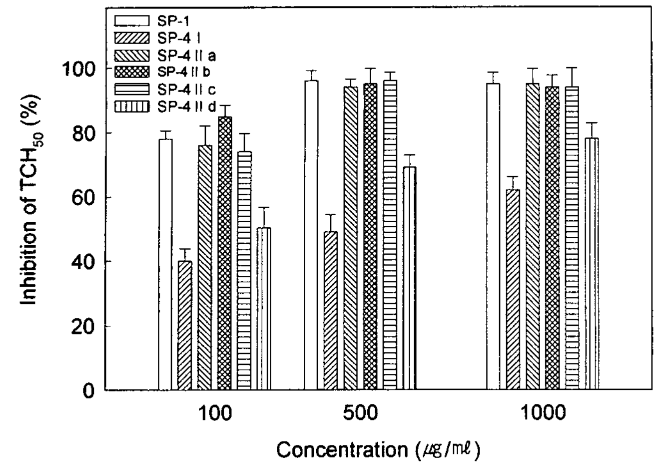

Fig. 6. Anti-complementary activities of SP-4 subfractions obtained from DEAE-Sepharose Fast Flow (Cl⁻) ion exchange chromatography.

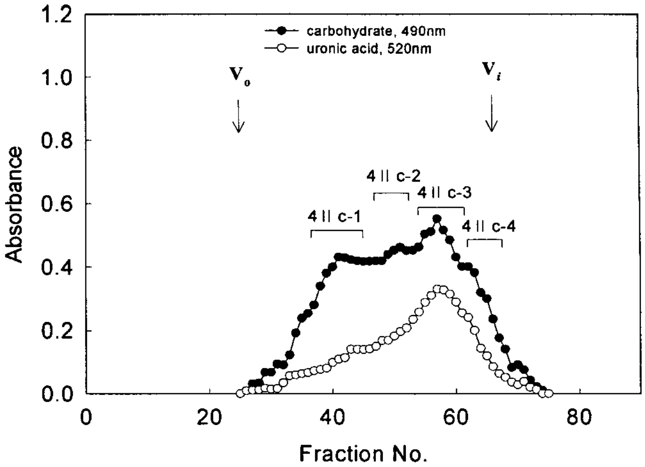

Fig. 7. Gel column chromatography of SP-IIc on Sepharose CL-6B. SP-IIc was chromatography by a column (3.2×87 cm) of Sepharose CL-6B eluted with 250 mM NaCl. The effluent was collected in 4.2 mL fractions. V_o, Void volume; V_i, inner volume.

and glucuronic acid (Tabe 3). Among these subfractions, SP-4IIc-1 had the most potent anti-complement activity with less activity for SP-4IIc-2, SP-4IIc-3, SP-4IIc-4, in that order (Fig. 8).

Yamada et al. (22,23), Kiyohara et al. (24), and Samuelsen et al. (25,26) all reported that the anti-complement polsaccharides which they separated consisted mainly of arabinose and galactose. SP-4IIc-1 purified in our study also was similar to the anti-complement polysaccharides they identified. We suggest that the anti-complementary polysaccharides in *Spirodela polyrhiza* are not simple polysaccharides such as lectinan (β(1→3) glucan) but complex polysaccharides having arabinose, rhamnose, glucose and galactose.

Average molecular weights of SP-4IIc-1, 2, 3 and 4

We used a Sepharose CL-6B gel column in order to determine molecular weight of the subfractions.

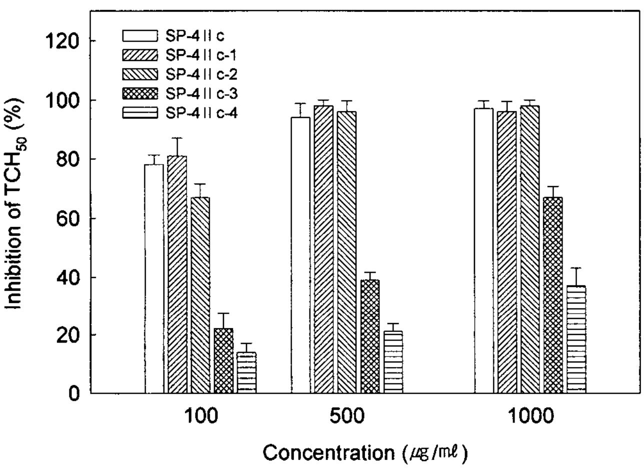

Fig. 8. Anti-complementary activites of SP-4IIc subfractions separated by gel column chromatography on Sepharose CL-6B.

Table 3. Chemical components and yield of SP-4IIc subfractions separated by gel column chromatography on Sepharose CL-6B

Items	SP-4IIc-1	SP-4IIc-2	SP-4IIc-3	SP-4IIc-4
M_r	305,000	132,000	64,000	12,000
Total sugar (%, w/w)	79.5	83.4	80.2	81.8
Uronic acid (%, w/w)	12.6	16.3	22.0	17.3
Protein (%, w/w)	1.7	2.1	2.4	3.1
Yield[1)] (%)	22.4	19.6	25.3	13.9
(Component sugar)		(Mol.%)		
Rhamnose	11.6	10.7	9.2	11.0
Arabinose	16.3	13.9	14.8	15.9
Xylose	8.1	7.6	6.5	9.1
Fucose	5.6	6.0	5.7	8.0
Mannose	5.9	5.5	4.4	6.2
Galactose	27.2	27.7	24.5	23.3
Glucose	12.4	11.0	10.2	13.2
Galacturonic acid	9.3	12.4	18.3	7.2
Glucuronic acid	3.6	5.2	5.4	6.1

[1)]From the SP-4IIc.

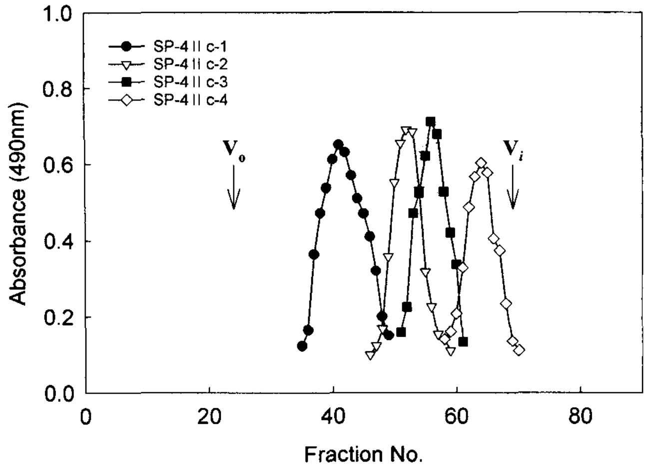

Fig. 9. Gel column chromatography of SP-4IIc-1, SP-4IIc-2, SP-4IIc-3 and SP-4IIc-4 on Sepharose CL-6B. This was chromatography by a column (3.2×87 cm) of Sepharose CL-6B eluted with 250 mM NaCl. The effluent was collected in 4.2 mL fractions. V_o, void volume; V_i, inner volume.

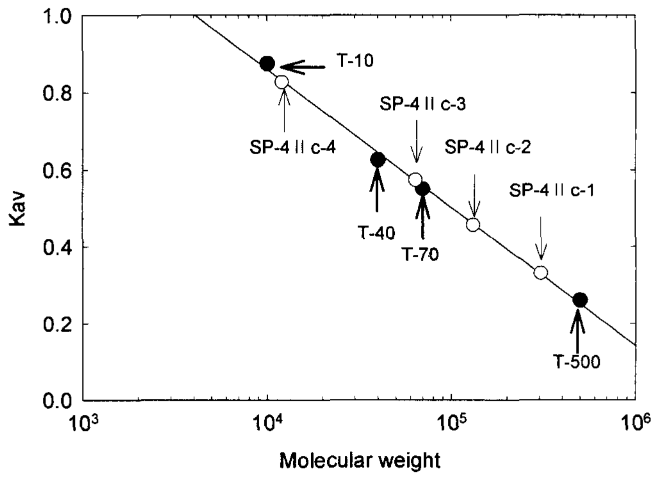

Fig. 10. Determination of molecular weights of SP-4IIc-1, SP-4IIc-2, SP-4IIc-3 and SP-4IIc-4 by gel column chromatography on Sepharose CL-6B. T-500, T-70, T-40 and T-10 are standard dextrans of 5×10^5, 7×10^4, 4×10^4 and 1×10^4 molecular weight respectively. Kav = (Ve - Vo)/(Vt - Vo), Vo: void volume, Vt: total volume, Ve: elution volume.

Because the subfractions all appeared a single peak, we could know that the subfractions are uniform (Fig. 9). The average molecular weights of SP-4IIc-1, 2, 3 and 4 were about 305,000, 132,000, 64,000 and 12,000, respectively (Fig. 10). When we measured their anti-complement activities, it was revealed that the higher molecular weight molecules had higher activities. Therefore, the anti-complement activity was increased with the molecular weight. Kweon et al. (27), Ukai et al. (28) and Shin et al. (11) reported that there is no correlation between activity and molecular weight, but the results of this study were similar to those of Kiyohara et al. (24) that the anti-complement activity of polysaccharides from the root of *Angelica acutiloba* are size dependent.

REFERENCES

1. Hames BD, Glover DM. 1983. The complement system. In *Molecular Immunology*. IRL press, Oxford. p 189-192.
2. Low SKA, Reid KBM. 1988. *Complement*. IRL Press, Oxford. p 1-21.
3. Sim RB, Malhotra V, Ripoche J, Day AJ, Sim E. 1986. Complement receptors and related complement control proteins. *Biochem Soc Symp* 51: 83-84.
4. Kiyohara H, Yamada H. 1989. Structure of an anti-complementary arabinogalactan from the root of *Angelica acutiloba* Kitagawa. *Carbohydr Res* 193: 173-192.
5. Yamada H, Kiyohara H, Cyong JC, Kojima Y, Kumazawa Y, Otsuka Y. 1984. Studies on polysaccharides from *Anglelica acutiloba. Planta Medica* 50: 163-168.
6. Yamada H, Kiyohara H. 1989b. Structure of the neutral carbohydrate side chains in anti-complementary acidic polysaccharides from the root of *Angelica acutiloba* Kitagawa. *Carbohydr Res* 187: 255-260.
7. Yamada H, Otsuka Y, Omura S. 1986b. Structural characterization of anti-complementary polysaccharides from the leaves of *Artemisiae princeps. Planta Medica* 52: 311-316.
8. Yamada H, Kiyohara H, Cyong JC, Otsuka Y. 1987. Characterization of anti-complementary acidic heteroglycans from the seed of *Coix lachrya jobi* var. ma-yuen. *Phytochemistry* 26: 3269-3275.
9. Gao QP, Kiyohara H, Cyong JC, Yamada H. 1988. Characterization of anti-complementary acidic heteroglycans from the leaves of *Panax ginseng* CA. METER. *Carbohydr Res* 181: 175-187.
10. Gao QP, Kiyohara H, Cyong JC, Yamada H. 1990. Further structural studies of anti-complementary acidic heteroglycans from the leaves of *Panax ginseng* CA. METER. *Carbohydr Res* 196: 111-125.
11. Shin KS, Cho HY, Sung HC, Yang HC. 1992. Action mode of the anti-complementary polysaccharide purified from *Arcae pericarpium. J Korean Agric Chem Soc* 35: 462-469.
12. Chihiro H, Tadashi K, Yushiro T, Shigeo U. 1982. Anti-complementary activity and conformational behavior of a branched (1→3)-β-D-glucan from an alkaline extract of *Dictyophora indusiata Fish. Carbohydr Res* 110: 77-82.
13. Mushel LH, Schmoker K, Webb PM. 1964. Anti-complementary action of endotoxin. *Proc Soc Exp Biol Med* 117: 639-643.
14. Usui S, Murashima K, Sakai M, Kiho T, Shigeo U. 1994. Preparation and antitumor activities of mitomycin C β-(1→6)-branched (1→6)-β-D-glucan conjugate. *Biol Pharm Bull* 17: 1165-1169.
15. Dubios MKA, Hamilton TK, Rebers PA, Sonisth F. 1956. Colorimetric method for determination of sugar and related substances. *Anal Chem* 28: 350-358.
16. Blumenkrantz N, Asboe-Hansen G. 1973. New method for quantitive determination of uronic acids. *Anal Biochem* 54: 484-489.
17. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. 1951. Protein measurement with the folin phenol reagent. *J Biol Chem* 193: 265-267.
18. Chaplin MF. 1982 A rapid and sensitive method for the analysis of carbohydrate components in glycoproteins using gas-liquid chromatography. *Anal Biochem* 123: 336-

346.
19. Min JK, Kim TJ, Lee DS, Cho SW, Yoon HD, Park JH. 2001. Characterization of anti-complementary polysaccharide from *Teucrium viscidum* var. miquelianum. *J Food Sci Nutr* 6: 137-141.
20. Yamada H, Soo KS, Kiyohara H, Cyong JC, Yang HC, Otsuka Y. 1988. Characterization of anti-complementary neutral polysaccharides from the roots of *Bupleurum falcatum* L. *Phytochemistry* 27: 3163-3168.
21. Scott JE. 1965. Fractionation by precipitation with quaternary ammonium salts. In *Method in carbohydrate chemistry*. Whistler RL, Wolfrom ML, eds. Academic press, New York. Vol 5, p 38-44.
22. Yamada H, Ohtani K, Kiyohara H, Cyong JC, Otsuka Y, Ucno Y, Omura S. 1985a. Purification and chemical properties of anti-complementary polysaccharide from the leaves of *Artemisa princeps. Planta Medica* 51: 121-125.
23. Yamada H, Kiyohara H, Cyong JC, Takemoto N, Komatsu Y, Kawamura H, Aburada M, Hosoya E. 1990. Fractionation and characterization of mitogenic and anti-complementary active fraction from Kampo (Japanese herbal) medicine "Juzen-Taiho-To". *Planta Medica* 56: 386-391.
24. Kiyohara H, Yamada H, Cyong JC, Otsuka Y. 1986. Studies on polysaccharides from *Angelica acutiloba* V. Molecular aggregation and anti-complementary activity of arabinogalactan from *Angelica acutiloba. J Pharmacobio-Dyn* 9: 339-346.
25. Samuelsen AB, Paulsen BS, Wold JK, Otsuka H, Yamada H, Espevik T. 1995. Isolation and partial characterization of biologically active polysaccharides from *Plantago major* L. *Phytother Res* 9: 211-218.
26. Samuelsen AB, Paulsen BS, Wold JK, Otsuka H, Kiyohara H, Yamada H, Knutsen SH. 1996. Characterization of a biologically active pectin from *Plantago major* L. *Carbohydr Polym* 30: 37-44.
27. Kweon MH, An HJ, Sin KS, Sung HJ, Yang HC. 1997. Purification and complement system activating polysaccharide from hot water extract of young stem of *Cinnamomum cassia Blume. Korean J Food Sci Technol* 29: 1-8.
28. Ukai S, Kiho T, Hara C, Kuruma I, Yushiro Y. 1983. Polysaccharides in fungi. XIV. Anti-inflammatory effect of the polysacchrides from the fruit bodies of several fungi. *J Pharm Dyn* 6: 983-987.

(Received October 6, 2003; Accepted February 13, 2004)