Journal of Biochemistry and Molecular Biology, Vol. 36, No. 2, March 2003, pp. 230-236

JOURNAL OF Biochemistry and Molecular Biology

Purification and Characterization of Complement-activating Acidic Polysaccharides from the Fruits of *Capsicum annuum*

Soon-Young Paik[†], Kyung Soo Ra[‡], In Seop Chang[#], Yoon Chang Park[§], Hee Sung Park[?], Hyung Suk Baik[††], Jong Won Yun[‡‡] and Jang Won Choi[§,*]

[†]Department of Microbiology, College of Medicine, The Catholic University of Korea, Seoul 137-701, Korea
[‡]Department of Food and Nutrition, Daegu Technical College, Daegu 704-721, Korea
[#]Water Environment Research Center, Korea Institute of Science and Technology, Seoul 136-791, Korea
[?]Department of Plant Genetic Engineering, Catholic University of Daegu, Kyungbuk 712-714, Korea
[††]Department of Microbiology, Pusan National University, Pusan 609-735, Korea
[‡‡]Department of Biotechnology, College of Engineering, Daegu University, Kyungbuk 712-714, Korea
[§]Division of Life Resources, College of Natural Resources, Daegu University, Kyungbuk 712-714, Korea

Received 28 November 2002, Accepted 24 December 2002

Hot water-soluble crude polysaccharide (HCAP-0) that was obtained from the fruits of *Capsicum annuum* showed potent anti-complementary activity. The activity was unchanged by pronase digestion, but decreased by periodate oxidation. The HCAP-0 was fractionated by DEAE ion-exchange chromatography to give two major fractions, HCAP-II and III. These two fractions were finally purified by gel filtration to give HCAP-IIa, HCAP-IIIa1, and IIIa2 fractions that had high anti-complementary activities. The HCAP-IIIa1 and IIIa2 consisted of homogeneous polysaccharides. The anti-complementary activities were unaffected by treatment with polymyxin B, indicating that the modes of complement activation were not due to preexisting lipopolysaccharide. The molecular weight and sugar content of HCAP-IIIa2 had potent anti-complementary activity. The highest yields were 55 kDa and 75.9%, and the molar ratio of galactose (Ara:Gal, 1.0:4.6) was higher than other sugars. The crossed immuno-electrophoresis showed that both classical and alternative pathways were activated by HCAP-IIIa2.

Keywords: Acidic polysaccharide, *Capsicum annuum*, Complement

*To whom correspondence should be addressed.
Tel: 82-53-850-6756; Fax: 82-53-850-6769
E-mail: chjawo@daegu.ac.kr

Introduction

Several immunomodulatory activities were observed in the high molecular weight fractions of the cold- and hot-water extracts of edible plants, hot-water extracts of Chinese herbs, and alkali extracts of edible mushrooms, including fruiting bodies and mycelium. These include the followings; interferon inducing activity (Kojima *et al*., 1980), polyclonal B-cell activator activity (Kumazawa *et al*., 1982), anti-inflammatory activity (Ukai *et al*., 1983), anti-tumor activity (Iino *et al*., 1985), mitogenic activity for lymphocytes (Zhao *et al*., 1991), anti-ulcer activity (Yamada *et al*., 1991), anti-coagulant activity (Ra *et al*., 1997), anti-oxidative activity (Lee and Lim, 2001), and anticarcinogenic effect by dietary garlic powder (Park *et al*., 2002). Various anti-complementary (complement activating) polysaccharides from Chinese herbs and other plants were particularly identified, and their structures were analyzed as follows; for example, four kinds of anti-complementary pectins from *Angelica acutiloba* (Kiyohara *et al*., 1989), neutral and acidic polysaccharides from *Bupleurum falcatum* (Yamada *et al*., 1988, 1989), four acidic heteroglycans from *Lithopermum euchromum* (Zhao *et al*., 1993), and heteroxylan polysaccharide fractions from the seeds of *Plantago major* L (Samuelsen *et al*., 1999).

The genus *Capsicum* has a variety of species and adds color, pungency, and aroma to the cuisine of most countries in the world. The main component of the fruits of *Capsicum annuum* (hot red pepper) is capsaicin, which plays an important role in the physiological and pharmacological effects on the sensory and cardiovascular systems (Toda *et al*., 1972). Although the fruits of *C. annuum* are also very important food stuffs that are used as spices for many Korean

cuisines, most of the studies for *C. annuum* were limited to capsaicin (Gonzalez *et al*., 1998). Previously, we found the polysaccharide that has a potent anti-complement activity in cold-water extracts from the fruits of *C. annuum*, and we reported about the isolation, purification of polysaccharide, and its chemical properties (Ra *et al*., 1989).

In this study, polysaccharides that were extracted with hot water out of the residues after a cold water extraction from the fruits of *C. annuum* were purified and tested for anti-complementary activity. Among them, polysaccharides that have a potent anti-complementary activity with high yields were further fractionated, and their chemical properties and activation mode were investigated.

Materials and Methods

Materials The powder of the fruits of *C. annuum* was used for the extractions of the cold and hot water-soluble polysaccharides. DEAE Sepharose CL-6B for ion-exchange chromatography and Sepharose CL-4B, CL-6B, Sephadex G-75, G-100, G-200 for gel filtration were purchased from Amersham Pharmacia Biotech. (Buckinghamshire, England). Polymyxin B, lipopolysaccharide of *E. coli* 0127:B8, and rabbit anti-human C3 serum were from Sigma Co. (St Louis, USA). The normal human serum that was used in the anti-complementary assay was supplied fresh from healthy adults. IgM hemolysin-sensitized sheep erythrocytes were purchased from Nippon Biotest Laboratory Inc. (Tokyo, Japan). All of the other materials were reagent grade.

Analysis of sugar content and composition Total carbohydrate and uronic acid content were determined by the phenol-sulfuric acid (Dubois *et al*., 1956) and *m*-hydroxydiphenyl (Blumenkrantz and Asboe-Hansen, 1973) methods using arabinose and galacturonic acid as standards. Protein was assayed by the Lowry method using bovine serum albumin as an internal standard. Polysaccharides were hydrolyzed with 2 M TFA at 121°C for 1 h. The depolymerized polysaccharides that contained neutral sugar and uronic acid were reduced with $NaBH_4$ (sodium borohydride) to alditol and aldonic acid, which were then separated by Dowex-1 (CH_3COO^-). The aldonic acids that were bound to the resin were eluted with 1 N HCl, followed by evaporation of HCl. The dried residues were reduced with $NaBH_4$. The alditols that were formed from neutral sugar and uronic acid were acetylated to form alditol acetates (Jones and Albersheim, 1972). They were then analyzed by GC (Shimadzu GC-14A) using a stainless column (OV-225, 0.2 × 200 cm) and flame ionization detector (FID).

Isolation, fractionation, and purification of crude polysaccharide The powder of the fruits of *C. annuum* (4 kg) was extracted 5 times with methanol (16 l). Cold water (16 l) was added to the residue, and it was stirred in the cold room (4°C) for 24 h. After 5 extractions with cold water, the residue was finally boiled at 100°C in order to obtain a hot water extract. Hot water-soluble crude polysaccharide (HCAP-0) was obtained as lyophilisate of the non-dialyzable portion after performing with ethanol-precipitation, resuspension with water, and dialysis. HCAP-0 (500 mg) was applied to a column (3.2 × 32 cm) of DEAE Sepharose CL-6B (Cl^-) that was equilibrated with H_2O. The column was first eluted with H_2O until sugar was no longer detected, then eluted with 0.1-2.0 M NaCl (stepwise elution). The unadsorbed fractions and six adsorbed fractions were obtained as lyophilisate after dialysis. Among them, HCAP-II and HCAP-III were applied to a column (2.6 × 90 cm) of Sephadex G-100 that was equilibrated with 0.2 M NaCl. In order to purify further, the major fraction of HCAP-IIIa was applied to a column (2.6 × 90 cm) of Sepharose CL-6B that was equilibrated with 0.2 M NaCl. For identifying homogeneity, HCAP-IIIa2 was applied to Sephadex G-200 (0.6 × 70 cm). To determine the molecular weight, standard dextrans of T-2000 (2×10^6), T-500 (5×10^5), T-70 (7×10^4), T-40 (4×10^4), and T-10 (1×10^4) were applied to a column (1.3 × 95 cm) of Sepharose CL-4B that was equilibrated with 0.2 M NaCl.

Determination of anti-complementary activity Various concentrations of polysaccharide in water (50 µl) were mixed with 50 µl each of normal human serum and gelatin veronal-buffered saline that contained 500 µM Mg^{2+} and 150 µM Ca^{2+} (GVB^{++}, pH 7.4). The mixtures were pre-incubated at 37°C for 30 min and 350 of GVB^{++} was added. IgM-hemolysin-sensitized sheep erythrocyte (250 of 1×10^8 cells/ml) was added to the serially-diluted mixtures (10-160 fold), then incubated at 37°C for 1 h. After the addition of phosphate-buffered saline (PBS, pH 7.2), the absorbance of the supernatant was detected at 412 nm. Normal human serum was incubated with PBS and GVB^{++} to provide a control. The anti-complementary activity was expressed as the percentage inhibition of the total complement hemolysis (TCH_{50}) of the control (Kabat and Mayer, 1964).

Pronase digestion and periodate oxidation of crude polysaccharide Crude polysaccharide, HCAP-0 (20 mg), was dissolved in 50 ml of 50 mM Tris-HCl buffer (pH 7.9) that contained 10 mM $CaCl_2$, and was treated with 50 mg of pronase. The reaction mixture was incubated at 37°C for 48 h. The reaction was stopped by boiling for 5 min, followed by dialysis. The non-dialyzable portion was lyophilized in order to obtain the pronase-digested product. For periodate oxidation, HCAP-0 (20 mg) was dissolved in 30 ml of a 50 mM acetate buffer (pH 4.5), and sodium periodate ($NaIO_4$) was added to 50 mM. The mixture was reacted at 4°C in the dark for 3 days. Ethylene glycol (5 ml) was added in order to remove excess periodate. The non-dialyzable solution was reduced with $NaBH_4$ (20 mg) for 24 h at room temperature. The mixture was dialyzed after decomposition of excess $NaBH_4$ with acetic acid. Finally, the oxidized HCAP-0 was obtained as lyophilisate.

Effect of polymyxin B on the anti-complementary activity The effect of hemolysis by polymyxin B was examined by the procedure of Morrison and Jacobs (1976). Lipopolysaccharide and HCAP-polysaccharide fractions that were obtained from gel chromatography were treated with an equal weight of polymyxin B and GVB^{++} (1 ml). Fifty µl of the solution was used for the assay of anti-complementary activity, while the control sample was not treated with polymyxin B.

Crossed immuno-electrophoresis Normal human serum (50 µl)

was incubated with a solution of the anti-complementary polysaccharides (50 μg) and 50 μl of 2 mM Mg^{2+}-EGTA-GVB^{-}, 10 mM EDTA-GVB^{-}, or GVB^{++} , separately, for 30 min at 37°C. The serum was then subjected to crossed immuno-electrophoresis in order to confirm the position of the C3 cleavage products. After the first run was carried out in a barbital buffer, pH 8.6, with 1% agarose, the second run was carried out on a gel plate that contained 0.5% of a rabbit anti-human to C3 at 1 A/cm for 12 h. After the electrophoresis, the plate was fixed and stained with Ponceau 3R (Yamada *et al*., 1985).

Results and Discussion

Isolation of crude polysaccharide from *Capsicum annuum*

The hot water-soluble crude polysaccharide (HCAP-0) was prepared as a lyophilisate of the non-dialyzable portion from the ethanol-precipitation of a supernatant that was obtained by boiling the residues after methanol-extraction and five time extractions with cold water for the *Capsicum annuum* powder (Fig. 1). As seen in Table 1, the yield (0/16%) of the HCAP-0 fraction had a low level when compared with those of five crude polysaccharides (1^{st}-5^{th} CAP-0, average 0.72%) that were obtained by cold water. However, the HCAP-0 fraction showed potent anti-complementary activity of 85% at a concentration of 1 mg/ml (Fig. 2). The HCAP-0 consisted of 74.7% total sugar, 18.5% uronic acid, and 25.3% protein. The sugar components were composed of rhamnose, arabinose, xylose, mannose, galactose, and glucose in the molar ratios of 1.08: 1.0: 0.53: 0.94: 2.04: 3.95 by a GC analysis. The uronic acid turned out to be galacturonic acid by TLC. The uronic acid content in the CAP-0 fractions (average 44%) was higher, but the molar ratio of glucose was much lower than that of HCAP-0. This indicates that the sugar content and composition can be altered by differences in extraction methods or solvents.

Methanol and ethanol-soluble portions that were obtained during the preparation of crude polysaccharides, CAP-0 and

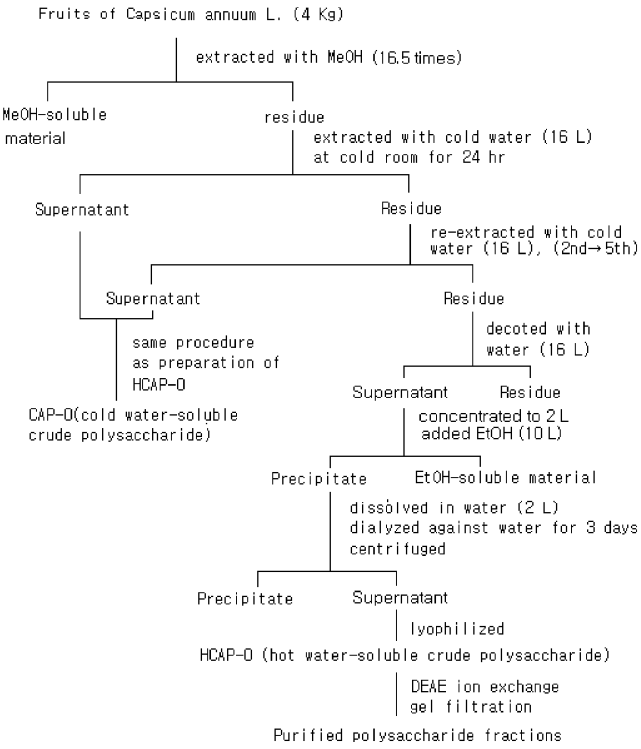

Fig. 1. Purification of polysaccharide fractions from the fruits of *Capsicum annuum* L.

HCAP-0, showed low anti-complementary activity beyond 25% at a concentration of 1 mg/ml. When the HCAP-0 was treated with pronase, the activity was unchanged. However, HCAP-0 activity sharply decreased with periodate oxidation (Fig. 2). Generally, it was reported that the low molecular materials that were soluble in methanol or ethanol had low anti-complementary activity. Also, the pronase treatment of crude polysaccharide did not affect activity in many cases. However, both the pronase treatment and periodate oxidation of crude polysaccharide (obtained from 'Juzen-Taiho-To' that

Table 1. Chemical analysis and yields of cold water (1^{st}-5^{th} CAP-0) and hot water-soluble crude polysaccharides from the fruits of *Capsicum annuum*

	CAP-0 (%)					HCAP-0 (%)
	1^{st}	2^{nd}	3^{rd}	4^{th}	5^{th}	
Total sugar	89.5	84.4	74.5	71.5	60.4	74.7
Uronic acid	67.9	61.0	35.6	34.2	19.4	18.5
Protein	10.5	15.6	25.5	28.5	33.6	25.3
Yield	1.7	1.4	0.3	0.15	0.07	0.16
Sugar components (Molar ratio)						
Rhamnose	0.73	1.49	1.40	1.26	0.89	1.08
Arabinose	1.0	1.0	1.0	1.0	1.0	1.0
Xylose	0.15	0.16	0.20	0.21	0.21	0.53
Mannose	0.18	0.13	0.23	0.26	0.53	0.94
Galactose	1.20	1.07	1.0	1.19	1.62	2.04
Glucose	0.08	0.1	0.20	0.24	0.35	3.95

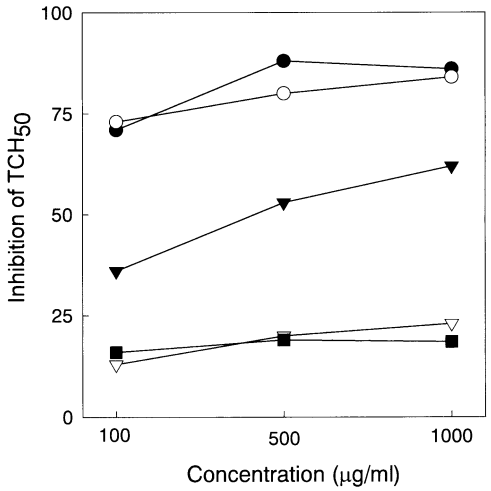

Fig. 2. Comparison of anti-complementary activities between the methanol, ethanol-soluble materials, pronase digest, and periodate oxidate of crude polysaccharide: ●, HCAP-0; ○, pronase digestion; ▼, periodate oxidation; ■, ethanol soluble material, ▽, methanol soluble material.

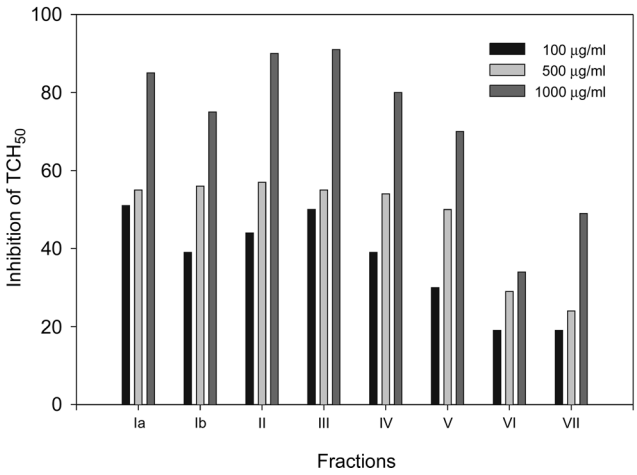

Fig. 3. Anti-complementary activities of polysaccharide fractions from DEAE Sepharose CL-6B (Cl^-) of crude polysaccharide (HCAP-0). The column (3.2 × 32 cm) was eluted by stepwise gradient with NaCl (0.1, 0.2, 0.3, 0.4, 0.5, and 2 M) to collect adsorbed fractions (HCAP-II~VII). Fractions Ia and Ib are unadsorbed neutral fractions that were eluted with H_2O.

was used as Japanese herbal medicine containing anti-complementary activity and mitogenic activity) decreased the anti-complementary activity. This indicates that crude polysaccharide is a proteoglycan, and anticomplementary activity exists in the peptide portion of the polysaccharide (Yamada *et al.*, 1990). Accordingly, our results from *C. annuum* indicate that the carbohydrate moiety might contribute to the activity, and the resistant glycosidic linkages to periodate oxidation exist because the activity did not completely vanish.

Purification of the anti-complementary polysaccharide from crude polysaccharide

(1) Ion-exchange chromatography of HCAP-0: The crude polysaccharide, HCAP-0, was further fractionated on the column of the DEAE Sepharose CL-6B into two unadsorbed (HCAP-Ia and Ib; eluted with H_2O) and six adsorbed fractions (HCAP-II~VII; eluted with 0.1, 0.2, 0.3, 0.4, 0.5 and 2 M NaCl, respectively). As shown in Figure 3, the HCAP-II and III showed high activity (above 90%). Its unadsorbed neutral fractions also had approximately 80% activity, but the yields of HCAP-Ia (9.8%) and Ib (9.6%) were lower than those of HCAP-II (25.4%) and III (20.0%). The total sugar content of HCAP-II and III were 81.1% and 54.6%, respectively, but the uronic acid content of HCAP-III (35.5%) was higher than that of HCAP-II (12.1%). The sugar components of HCAP-II consisted of rhamnose, arabinose, mannose, galactose, and glucose (molar ratio, 1.1:1.0:1.4:2.8:1.6), but HCAP-III contained no glucose (data not shown). The unadsorbed neutral fractions, HCAP-Ia and Ib, primarily consisted of glucose (Ara:Glc: Ia; 1.0:11.5, Ib; 1.0:9.8). This indicates that most of the glucose in crude polysaccharide is significantly present in neutral fractions that are eluted with H_2O. Among the polysaccharide fractions that were extracted from the Chinese herbs, high anti-complementary activity appeared in more of the acidic polysaccharide fractions than in the neutral fractions. However, the neutral fraction that was obtained by the DEAE ion-exchange chromatography of crude polysaccharide from *Bupleurm falcatum* showed high anti-complementary activity (Yamada *et al*., 1988).

(2) Gel filtration of HCAP-II and HCAP-III: The most active polysaccharide fractions, HCAP-II and HCAP-III, were further fractionated by gel filtration on Sephadex G-100 to obtain HCAP-IIa, IIb, HCAP-IIIa, and IIIb, respectively. The major fractions, HCAP-IIa and IIIa, that were eluted in the void volume had larger molecular weight and higher anti-complementary activity (above 90%) than HCAP-IIb and IIIb. The total sugar content of HCAP-IIa and IIIa was 89.4% and 63.1%, respectively. The uronic acid contents of HCAP-IIIa (19.2%) and IIIb (32.0%) were higher than HCAP-IIa (7.0%) and IIb (13.6%). HCAP-IIa consisted of rhamnose, arabinose, xylose, mannose, galactose, and glucose in the molar ratio of 1.82:1.0:0.78:3.90:4.05:3.87, but glucose was undetected in HCAP-IIIa and IIIb. It was reported that among the polysaccharides that were obtained from Chinese herbs, edible plants, and food stuffs, the polysaccharides with a high molecular weight generally had a higher anti-complementary activity, although the relationship between the activity and sugar content or composition is not yet precisely known. Although the attempt for further fractionation of active fractions, HCAP-IIa and IIIa, was carried out on all gel filtrations (including Sepharose CL-6B), further fractionation of HCAP-IIa was impossible because broad peaks were not distinctly separated; whereas, HCAP-IIIa was fractionated to two distinctive peaks, HCAP-IIIa1 eluted in void volume and HCAP-IIIa2, on Sepharose CL-6B. The anti-complementary

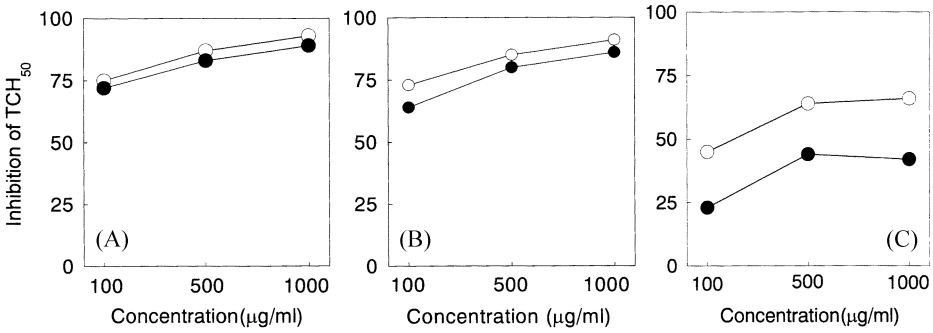

Fig. 4. Effect of polymyxin B on the anti-complementary activities of bacterial lipopolysaccharide and polysaccharide fractions from *Capsicum annuum*. Panel A, HCAP-IIIa1; Panel B, HCAP-IIIa2; Panel C, LPS. ○, before treatment of polymyxin B; ●, after treatment of polymyxin B.

activities were above 90% in HCAP-IIIa1 and 87% in IIIa2, but the yield of HCAP-IIIa2 (89.2%) was much higher than that of IIIa1 (10.8%). The total sugar content was 90.9% and 75.9% in HCAP-IIIa1 and IIIa2. The molar ratios of Ara:Gal were 1.0:1.8 and 1.0:4.55, respectively. HCAP-IIIa2 could not be further fractionated on Sephadex G-200. Also, according to the results of HPLC and electrophoresis on cellulose acetate membrane (data not shown), it was thought that the HCAP-IIIa2 fraction was a final product that showed high anti-complementary activity. The molecular weight of HCAP-IIIa2 was estimated to be 55 kDa by gel filtration on a Sepharose CL-4B column.

In general, it was suggested that although the overall structure of the anti-complementary polysaccharides was important to the expression of the activity, the activity was mainly present in neutral sugar residues that consisted of arabinose, galactose, and the attached main chain that contained rhamnogalacturonan (Yamada *et al*., 1989). Therefore, in acidic polysaccharides, the molecular weight and sugar composition of neutral sugar residues are more important than the total molecular weight and total sugar composition in the expression of the activity. As the HCAP-IIIa2 also had galacturonic acid (19.5%) and a large amount of galactose, a structural analysis will be needed for further study.

Effect of polymyxin B on anti-complement polysaccharides

Bacterial lipopolysaccharide (LPS) activates the complement system *via* the alternative and classical pathways (Morrison and Jacobs, 1976). Polymyxin B inhibited the complementary activity of lipopolysaccharide (Wilson and Morrison, 1982). Therefore, the possibility of contamination by LPS was examined for the purified anti-complementary polysaccharides that showed high activity from the fruits of *C. annuum*. The HCAP-IIIa1, HCAP-IIIa2, and LPS were incubated with polymyxin B at 37°C for 30 min, then the activities of the residual products were determined. As can be seen in Fig. 4, a decrement of activity was observed only in LPS, but the activities of HCAP-IIIa1 and IIIa2 were unaffected by polymyxin B. This indicates that the modes of complement activation by HCAP-IIIa1 and HCAP-IIIa2 were not due to the pre-existence of LPS or contamination during the purification process.

Activation mode of the anti-complementary polysaccharide

To study the biological activity of polysaccharide to interact with the complement cascade reaction, the anti-complementary activity was tested *in vitro*. The complement system is a part of the innate immune system that consists of a group of serum proteins which are activated in a cascade mechanism. Activation can be initiated by microorganisms or tumor cells (the alternative pathway) and by immune complexes that contain antigen, IgM, or IgG antibodies (classical pathway), or by the binding to a mannose-binding lectin that is present in serum (lectin pathway) (Samuelsen *et al*., 1999). The tracks of the classical and alternative complement pathways converge into the common track of the lytic pathway by cleaving the C5 components into C5a and C5b fragments (Rose, 1986). It was already known that Mg^{2+} and Ca^{2+} are needed for activating the classical pathway, and Mg^{2+} is needed for the alternative pathway. Also, the C3 complement is separated to C3a and C3b components by the activation *via* both pathways. To examine the activation mode of the complement by polysaccharide that is purified from *C. annuum*, the crossed immuno-electrophoresis was carried out on anti-human C3 serum after the incubation of normal human serum with HCAP-IIIa2 in EDTA-GVB^{--} (Ca^{2+}, Mg^{2+} free), Mg^{2+}-EGTA-GVB^{--} (Ca^{2+} free), and GVB^{++}. As shown in Fig. 5, C3 activation occurred with HCAP-IIIa2. That is, the cleavages of the C3 precipitin line were observed in the sera that was treated with HCAP-IIIa2 that was dissolved in the GVB^{++} and Mg^{2+}-EGTA-GVBsystems; whereas, precipitin lines were not formed in the serum and EDTA-GVB^{--}. The height of the precipitin line of the C3a and C3b mixtures in GVB^{++} was slightly higher than in Mg^{2+}-EGTA-GVB^{--}, suggesting that the activation strength was appended by the classical pathway in GVB^{++}. In the case of HCAP-IIIa1, the C3 activation occurred in the same pattern as HCAP-IIIa2 (data not shown). Therefore, these results indicate that the activation mode of the complement system by these polysaccharides occurred *via* both the classical and alternative

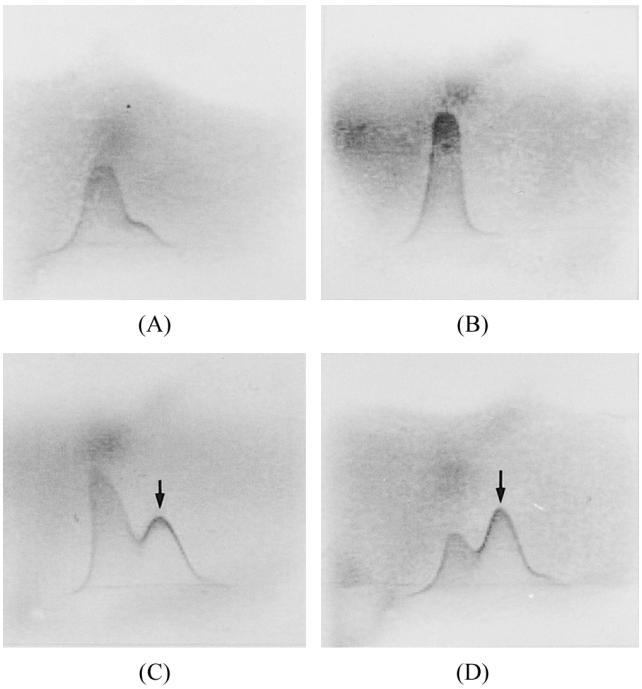

Fig. 5. Crossed immuno-electrophoresis of C3 activation by HCAP-IIIa2. Normal human serum was incubated with an equal volume of (A) GVB^{++} + PBS, (B) EDTA-GVB^{--} + HCAP-IIIa2, (C) Mg^{2+}-EGTA-GVB^{--} + HCAP-IIIa2 and (D) GVB^{++} + HCAP-IIIa2 at 37°C for 30 min. The sera were then subjected to crossed immuno-electrophoresis to locate the C3 cleavage products. The arrows indicate the mixture of C3a and C3b.

pathways.

The polysaccharides were purified from the fruits of *C. annuum* through ion exchange chromatography and gel filtrations. The sugar content, composition, and activation mode of the complement system were examined. As a result, the polysaccharides with higher anti-complementary activity were found in the hot-water extracts of *C. annuum* then in the extracts from Chinese herbs. The acidic polysaccharides with high yield and neutral polysaccharides primarily consisted of glucose that showed potent anti-complementary activities. In order to understand the biological activity of the polysaccharide on a molecular level, we will further study the relationship between the essential sugar structures of the active region and activity. Also, when these active fractions are absorbed into the body, other physiological activities will be investigated.

Acknowledgments This work was supported by Daegu University research grants, 2001.

References

Blumenkrantz, N. & Asboe-Hansen, G. (1973) New method for quantitative determination of uronic acids. *Anal. Biochem.* **54**, 484-489.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F. (1956) Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **28**, 350-365.

Gonzalez, R., Dunkel, R., Koletzko, B., Schusdziarra, V. and Allescher, H. D. (1998) Effects of capsaicin-containing red pepper sauce suspension on upper gastrointestinal motility in healthy volunteers. *Dig. Dis. Sci.* **43**, 1165-1171.

Iino, K., Ohno, N., Susuki, I., Miyazaki, T. and Yadomae, T. (1985) Structural characterization of a neutral antitumor β-D-glucan extracted with hot sodium hydroxide from cultured fruit bodies of *Grifola frondosa*. *Carbohydr. Res.* **141**, 111-119.

Jones, Y. M. and Albersheim, P. O. (1972) A gas chromatographic method for the determination of aldose and uronic acid constituents of plant cell wall polysaccharide. *Plant Physiol.* **49**, 926-936.

Kabat, E. A. and Mayer, M. M. (1964) Complement and complement fixation: in *Experimental Immunology*, Charles C. Thomas Publisher, Springfield, Illinois.

Kiyohara, H., Cyong, J.-C. and Yamada, H. (1989) Relationship between structure and activity of an anti-complementary arabinogalactan from the roots of *Angelica acutiloba* Kitagawa. *Carbohydr. Res.* **193**, 193-200.

Kojima, Y., Schibukawa, N., Mizunoe, K., Otsuka, K. and Kumazawa, Y. (1980) Screening for interferon inducers and mitogens among various medicinal plants used in traditional Sino-Japanese medicine. *Proc. Symp. WAKAN-YAKU.* **13**, 101-106.

Kumazawa, Y., Mizunoe, K. and Otsuka, Y. (1982) Immunostimulating polysaccharide separated from hot water extract of *Angelica acutiloba* Kitagawa (Yamato Tohki). *Immunology* **47**, 75-81.

Lee, J. C. and Lim, K. T. (2001) Inhibitory effects of the ethanol extract of ulmus davidiana on apoptosis induced by glucose-glucose oxidase and cytokine production in cultured mouse primary immune cells. *J. Biochem. Mol. Biol.* **34**, 463-471.

Morrison, D. C. and Jacobs, D. M. (1976) Inhibition of lipopolysaccharide initiated activation of serum complement by polymyxin B. *Infect. Immunol.* **13**, 298-301.

Park, K. A., Kweon, S. and Choi, H. (2002) Anticarcinogenic effect and modification of cytochrome P450 2E1 by dietary garlic powder in diethylnitrosamine-initiated rat hepatocarcinogenesis. *J. Biochem. Mol. Biol.* **35**, 615-622.

Ra, K. S., Yamada, H., Sung, H. J., Cyong, J.-C. and Yang, H. C. (1989) Purification and chemical properties of anti-complementary polysaccharide from *Capsici fructus*. *J. Korean Agri. Chem. Soc.* **32**, 378-385.

Ra, K. S., Lee, B. L., Lee, H. S. and Kweon, M. H. (1997) Anti-coagulant polysaccharide isolated from *Ganoderma lucidum*. *Korean J. Food & Nutr.* **10**, 375-381.

Rose, G. D. (ed.) (1986) Immunobiology of the complement system. An introduction for research and clinical medicine. Academic Press, Orlando.

Samuelsen, A. B., Lund, I., Djahromi, J. M., Paulsen, B. S., Wold, J. K. and Knutsen, S. H. (1999) Structural features and anti-complementary activity of some heteroxylan polysaccharide fractions from the seeds of *Plantago major* L. *Carbohydrate Polymers.* **38**, 133-143.

Toda, N., Usui, H., Nishino, N. and Fugiwara, M. (1972) Cardiovascular effects of capsaicin in dogs and rabbits. *J.*

Pharmac. Exp. Ther. **181**, 512-521.

Ukai, S., Kiho, T., Hara, C., Kuruma, I. and Yushiro, Y. (1983) Polysaccharide in fungi. XIV. Anti-inflammatory effect of the polysaccharides from the fruit bodies of several fungi. *J. Pharmac. Dyn.* **6**, 983-990.

Wilson, M. E. and Morrison, D. C. (1982) Evidence for different requirements in physical state for the interaction of lipopolysaccharides with the classical and alternative pathways of complement. *Eur. J. Biochem.* **128**, 137-141.

Yamada, H., Kiyohara, H., Cyong, J-C., Kojima, Y., Kumazawa, Y. and Otsuka, Y. (1985) Studies on polysaccharides from *Angelica acutiloba*. *Molec. Immunol.* **22**, 295-304.

Yamada, H., Ra, K. S., Kiyohara, H., Cyong, J-C., Yang, H. C. and Otsuka, Y. (1988) Characterization of anti-complementary neutral polysaccharide from the roots of *Bupleurum falcatum*. *Phytochemistry* **27**, 3163-3168.

Yamada, H., Ra, K. S., Kiyohara, H., Cyong. J-C., Yang, H. C. and Otsuka, Y. (1989) Structural characterization of an anti-complementary pectic polysaccharide from the roots of *Bupleurum falcatum*. *Carbohydr. Res.* **189**, 209-226.

Yamada, H., Kiyohara, H., Cyong, J-C., Takemoto, N., Komatsu, Y., Kawamura, H., Aburada, M. and Hosoya, E. (1990) Fractionation and characterization of mitogenic and anti-complementary active fractions from Kampo (Japanese herbal) medicine "Juzen-Taiho-To". *Planta Med.* **56**, 386-391.

Yamada, H., Sun, X. B., Matsumoto, T., Ra, K. S., Hirano, M. and Kiyohara, H. (1991) Purification of anti-ulcer polysaccharide from the roots of *Bupleurum falcatum* L. *Planta Med.* **57**, 555-559.

Zhao, J. F., Kiyohara, H., Yamada, H., Takemoto, N. and Kawamura, H. (1991) Heterogeneity and characterization of mitogenic anti-complementary pectic polysaccharides from the roots of *Glycyrrhiza uralensis*. *Carbohydr. Res.* **219**, 149-172.

Zhao, J. F., Kiyohra, H., Matsumoto, T. and Yamada, H. (1993) Anti-complementary acidic polysaccharides from roots of *Lithospermum euchromum*. *Phytochemistry* **34**, 719-724.