J. Microbiol. Biotechnol. (1999), **9**(4), 450–456

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Anti-Complementary Properties of Polysaccharides Isolated from Fruit Bodies of Mushroom *Pleurotus ostreatus*

KWEON, MEE-HYANG[1], HYO JANG, WANG-JIN LIM, HYO-IHL CHANG, CHAN-WHA KIM, HAN-CHUL YANG, HAN-JOON HWANG, AND HA-CHIN SUNG*

[1]Institute of Biotechnology, Graduate School of Biotechnology, Korea University, Seoul 136-701, Korea
Graduate School of Biotechnology, Korea University, Seoul 136-701, Korea

Received: April 2, 1999

Abstract A high molecular-weight water-soluble fraction (PO) obtained by the ethanol precipitation of 0.1 N NaOH extracts of the mushroom *Pleurotus ostreatus* showed 82% anti-complementary activity for complement consumption hemolysis. The PO consisted of 42% carbohydrate (w/w), 50% protein (w/w), and 3% uronic acid (w/w). Fifty-eight percent of the anti-complementary activity decreased by periodate oxidation and 22% by protease digestion, suggesting that the sugar and protein moieties are essential for this activity. Two polysaccharide fractions, PO-IIIa-1 and PO-IIIa-2, with anti-complementary activity were isolated from the PO using DEAE-Sepharose FF followed by Sephadex G-75 and Sepharose CL-6B gel permeation chromatographies. The PO-IIIa-2 was found by HPLC to be nearly homogeneous, with the molecular mass of 531 kDa, and showed 96% $ITCH_{50}$ (inhibition against the total complement hemolysis of deionized water as the control) at a concentration of 1 mg/ml. This fraction contained galactose, mannose, fucose, and glucose with molar ratios of 1.75:1:0.65 and 0.59, respectively. The majority of galactose and mannose units in the PO-IIIa-2 were composed of TGal*p*1→, →6Gal*p*1→, →2,6Gal*p*1→, and →Man*p*1→. The PO-IIIa-1 (molecular mass of 2000 kDa), exhibiting higher activity than the PO-IIIa-2, was further purified into two fractions, unbound proteoglycan (PO-IIIa-1A) and bound glucomannan (PO-IIIa-1B), by affinity chromatography using ConA-Sepharose CL-4B. The anti-complementary activity of each affinity purified fraction decreased as compared to that of the native PO-IIIa-1 fraction, indicating that the formation of complex between both polysaccharide fractions was necessary for full anti-complementary activity.

Key words: *Pleurotus ostreatus*, anti-complementary, polysaccharides, aggregation

*Corresponding author
Phone: 82-2-3290-3418; Fax: 82-2-927-9028;
E-mail: hcsung@kuccnx.korea.ac.kr

Polysaccharides are essential constituents for all living organisms to sustain their lives, because they are associated with a variety of vital functions which sustain life. They are found most abundantly in seaweeds, fungi and higher terrestrial plants. In recent years, polysaccharides of natural origin have emerged as an important class of bioactive materials. For example, several essential biological activities, such as antitumor [19], immunological [14], anti-complementary [24], anti-inflammatory [3], anticoagulant [16], hypoglycemic [22], and antiviral activities are known to be associated with a wide range of polysaccharides.

In particular, an activation of the complement system by some anti-complementary polysaccharides was reported to enhance macrophage activation, cytolysis, and opsonization against the tumor cell. Similarly, a correlation between the activation of the complement system and anti-tumor effect by polysaccharides was observed. This fact suggested that the anti-complementary polysaccharides were expected to be immunopotentiators or immunomodulators, and the anti-complementary polysaccharides became the focus of interest. The term "anti-complementary activity" means inhibition hemolysis by the remaining complement in the complement fixation test. Therefore, the complement activating polysaccharide can also be referred to as an anti-complementary polysaccharide. Polysaccharides possessing anti-complementary activity have been isolated from bacteria [15], fungi [4], and higher plants [24]. Some of them are also known to be interferon inducers and tumor inhibitors.

It has been shown that fruit bodies and culture filtrates of various fungi contain antitumor and anti-complementary polysaccharides whose structure is 6-branched β-1,3-glucan in most cases [4, 23]. The anti-complementary activity of lentinan [2], isolated from *Lentinus edodes* and schizophyllan [8] from *Schizophyllum commune,* has already been demonstrated and these polysaccharides are now in clinical use as anticancer drugs. *Pleurotus ostreatus*

is a fungus belonging to the family of *Polyporaceae* (Basidiomycetes), and its fruiting bodies are used as one of the most favorite edible mushrooms.

Most of the previous studies [4, 17] on the physiological activity of *Pleurotus ostreatus* have been performed by characterizing the water insoluble β-glucan isolated from liquid cultured mycelia. In the present paper, however, the heteropolysaccharides, PO-IIIa-1 and PO-IIIa-2, were purified from the water-soluble fraction of alkali extracts of the mushroom *Pleurotus ostreatus*. The anti-complementary activities of the newly identified polysaccharides and their structural features are identified.

MATERIALS AND METHODS

General Procedure

The total carbohydrate and uronic acid contents were determined by phenol-sulphuric acid and *m*-hydroxydiphenyl methods [9], respectively, using glucose and galacturonic acid as the respective standards. Protein was assayed by the method of Lowry *et al.* [13] using bovine serum albumin as the standard. For thin layer chromatography (TLC), polysaccharides were hydrolyzed with 2 M trifluoroacetic acid at 121°C for 1.5 h, and applied on cellulose coated plastic sheets (Merck). The TLC sheets were developed with a solvent system, ethyl acetate-pyridine-acetic acid-water (5:5:1:3). The resulting reducing sugars and uronic acids were detected with alkaline silver nitrate. For GLC analysis, the neutral sugars were converted into the corresponding alditol acetates, applied onto a packed stainless column (OV-225 Uniport, 0.2 i.d. ×200 cm), and detected with a flame ionization detector (FID) [6].

Glycosidic Linkage Analysis

Exhaustive methylation of polysaccharide was performed with methylsulfinyl carbanion and methyl iodide in dimethyl sulfoxide using the method of Hakomori [5]. Complete methylation of the polysaccharide was examined using triphenylmethane, and the methylated polysaccharides were recovered by a Sep-Pak C_{18} cartridge. They were then hydrolyzed with 2 M trifluoroacetic acid, followed by reduction and acetylation, as previously described [10]. The partially methylated alditol acetates obtained were analyzed by GC/MS on a fused silica capillary column (0.32 mm × 30 m) of SP-2380 (Supelco Co. Bellefonte, U.S.A.) with a programmed temperature; one minute at 60°C, a linear gradient to 150°C at a rate of 18°C/min (at 150°C held for 5 min), and a subsequent linear gradient to 210°C at a rate of 2°C/min (at 210°C held for 6 min). The compound at each peak was charaterized by an interpretation of their characteristic mass spectra and retention times relative to the standard sugar derivatives and by comparison with library spectra. A calibration of the molar ratio for each sugar derivative was performed using the peak areas and response factors.

Assay of Anti-Complementary Activity

The anti-complementary activity was measured by the complement fixation test based on complement consumption and a degree of red blood cell lysis by residual complement protein [9]. Briefly, each sample was diluted at various ratios in deionized water (DIW) and incubated with normal human serum (NHS) and gelatin-veronal-buffered saline (pH 7.4) containing 500 μM Mg^{2+} and 150 μM Ca^{2+} (GVB^{2+}). The residual total hemolytic complement (TCH_{50}) was determined by using immunoglobulin M-sensitized sheep erythrocytes. As a control, NHS was incubated with DIW and GVB^{2+}. The activity of each sample was expressed as % $ITCH_{50}$, a percentage inhibition of 50% total complement hemolysis of the control. For a consistency of the assay, HPA-0 (1 mg/ml), a hot water extract of *Pteridium aquilinum* L. [11], was used as a positive control.

Separation of the PO Fraction

Fruiting bodies of *Pleurotus ostreatus* (10 kg) were freeze-dried and extracted with 5 volumes of 80% ethanol at 70°C for 3 h. The soluble substances were removed by repeating this extraction process five times. The residue was then extracted with 0.1 N NaOH containing 5% urea at 37°C for 24 h (twice), and then the extract was neutralized and centrifuged at 5000×g for 30 min. Ethanol was added to the supernatant up to a concentration of 70% (v/v), and the mixture was centrifuged. The precipitate was dissolved in 1000 ml deionized water and dialyzed against distilled water at 4°C for 3 days. The insoluble material which remained inside the dialysis bag was filtered off through a Whatman GF/D filter and the filtrate was lyophilized (fr. PO, 4.1 g).

Purification of PO-IIIa-1 and PO-IIIa-2

One gram of the crude PO fraction was applied onto a DEAE-Sepharose Fast Flow column (Pharmacia Co., Cl^- form, 3.5 × 48 cm) equilibrated with distilled water. The column was initially washed with distilled water (total *ca.* 800 ml) at a flow rate of 1.2 ml/min and each 7-ml fraction was collected. The unabsorbed fractions, which were positive in both total carbohydrate and protein assays, were then combined (PO-I; 260 mg). The bound materials to the column were eluted stepwisely with NaCl gradient solutions containing 0.15, 0.3, 0.5, and 2.0 M NaCl. The eluates were designated as PO-II (156 mg),PO-III (185 mg), PO-IV (60 mg), and PO-V (136 mg), respectively. The PO-III fraction (40 mg) was dissolved in 0.2 M NaCl (1.5 ml) and applied to a column (2.5×100 cm) of Sephadex G-75 (Pharmacia Co., U.S.A.), which had been pre-equilibrated and eluted with the same buffer at a flow rate

of 0.2 ml/min. Five-ml fractions were collected, and aliquots were assayed for sugar and protein contents, producing three fractions (PO-IIIa, PO-IIIb, PO-IIIc). Fraction PO-IIIa (20 mg) was re-chromatographed using a column of Sepharose CL-6B (2×70 cm) (Pharmacia Co.) in the same manner as the above. The eluates, pooled from fraction numbers 25 through 32 (PO-IIIa-1) and 33 through 45 (PO-IIIa-2) were dialyzed and lyophilized. PO-IIIa-1 (9 mg) was further purified using a column of Sepharose CL-4B (2.5×47 cm) (Pharmacia Co.), while PO-IIIa-2 (7 mg) was fractionated by a column of Sepharose CL-6B. All fractionations were based on the peaks of neutral sugar, uronic acid, and protein determined by the methods described in Materials and Methods. The purity of each fraction was confirmed by HPLC and the average molecular weight was determined by gel permeation chromatography in comparison with standard dextrans (Sigma, St. Louis, U.S.A.).

Periodate Oxidation and Pronase Digestion of PO-IIIa

Ten ml of 50 mM sodium periodate ($NaIO_4$) was added to PO-IIIa (30 mg) dissolved in 50 ml of a 50 mM acetate buffer (pH 4.5), and the reaction mixture was then incubated at 4°C for 3 days in the dark. To inactivate the residual periodate, the mixture was treated with ethylene glycol (5 ml). The solution was reduced by sodium borohydride ($NaBH_4$, 20 mg), neutralized, and dialyzed. Protein-free PO-IIIa was then obtained as follows: PO-IIIa (30 mg) was dissolved in 50 ml of a 50 mM Tris-HCl buffer (pH 7.9) containing 10 mM $CaCl_2$, and 10 mg of proteinase (*ca.* 200 unit) was added to the solution. The reaction mixture was incubated at 37°C for 48 h, and boiled at 100°C in order to precipitate the enzyme protein which was then removed by centrifugation. The treated PO-IIIa contained less than 5% (w/w) Lowry-positive material, which was enough to be used for the anti-complementary activity assay.

HPLC

HPLC was performed with a Waters, Model 410 (Water Assoc., U.S.A.) instrument equipped with an RI (refractive index) detector and a shodex OH-pak KB805 (SEC type, 8 × 300 mm) column equilibrated with 0.2 M NaCl. The flow rate for the chromatography was 1.0 ml/min.

Affinity Chromatography of PO-IIIa-1

Polysaccharide PO-IIIa-1 (9 mg) was affinity-purified by applying to a column of concanavalin A (Con A)-Sepharose CL-4B (1×10 cm) (Pharmacia Co.), equilibrated with a 20 mM Tris-acetate buffer containing 0.1 M NaCl. The unabsorbed fraction (A) was collected by eluting the column with the same buffer, and the absorbed material (B) was eluted with a Tris-acetate buffer containing 0.3 M methyl-α-D-glucoside at a flow rate of 0.15 ml/min.

Results and Discussion

Anti-Complementary Activity of PO

Anti-complementary activity in the alkali extracts of the mushroom *Pleurotus ostreatus* was identified. The extract was further separated to obtain a highly active fraction PO, a high molecular weight compound which contained 42% carbohydrate (w/w), 50% protein (w/w), and 3% uronic acid (w/w). A sugar composition analysis of the anti-complementary PO fraction (Table 1) was performed using GLC and it identified galactose, mannose, glucose, and fucose in a molar ratio of 2.3:1.0:0.8:0.1. To investigate which moieties were essential for the anti-complementary activity, the PO was oxidized by sodium periodate and then hydrolyzed with pronase. The anti-complementary activity of the PO (85% $ITCH_{50}$, 1 mg/ml) was drastically decreased to 38% by periodate oxidation and slightly decreased to 68% by protease digestion (Fig. 1), indicating that the sugar moiety was more essential for the anti-complementary activity than the protein moiety.

Purification of Anti-Complementary Polysaccharides

The fr. PO was fractionated by DEAE-Sepharose FF chromatography into two major fractions, a non-binding fraction (PO-I, *ca.* 26.0% of fr. PO by weight) and a binding fraction which was further separated into 4 different fractions with an increasing concentration of a NaCl solution (PO-II to PO-V) (data not shown). The anti-complementary activity of each fraction (1 mg/ml) is shown in Fig. 2. The highest activity was found in fr. PO-III (*ca.* 18.5% of fr. PO by weight), which consisted of 58.4% (w/w) total carbohydrates, 5.9% uronic acid (w/w), and 39.2% (w/w) Lowry-positive materials (data not shown). Further purification of the PO-III was attempted by a gel permeation chromatography using Sephadex G-75 (Fig. 3). The fraction that eluted at a void volume (fr. PO-IIIa, fraction numbers 30 through 40) exhibited the highest activity. This fraction was further separated into four fractions (PO-IIIa-1, PO-IIIa-2, PO-IIIa-3, and PO-IIIa-4) on a Sepharose CL-6B column (Fig. 4).

Table 1. Chemical composition of alkali extract and fr. PO from *P. ostreatus*.

	Carbohydrate (%)	Uronic acid (%)	Protein (%)	Composition of carbohydrates					
				Fucose	Arabinose	Xylose	Mannose	Galactose	Glucose
Alkali extract	61	6	32	0.1	0.1	0.1	1.0	0.8	2.4
fr. PO	42	3	50	0.1	<0.1	<0.1	1.0	2.3	0.8

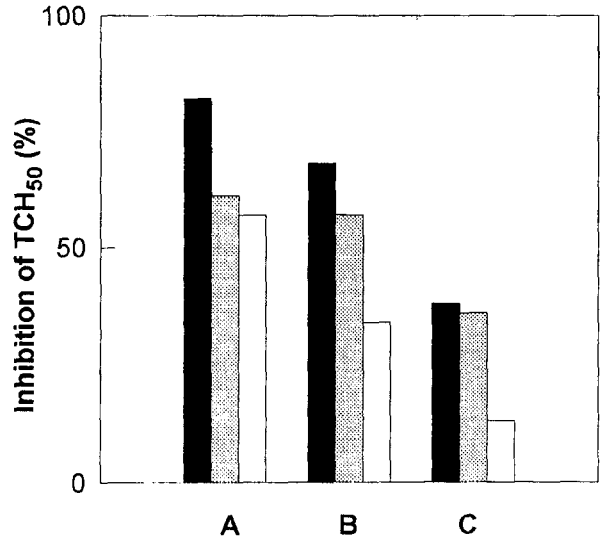

Fig. 1. Anti-complementary activity of protease digested or periodate oxidized fr. PO.
A: Native fr. PO; B, Protease-digested fr. PO; C, Periodate-oxidized fr. PO. ■, 1,000 μg/ml; □, 500 μg/ml; □, 100 μg/ml.

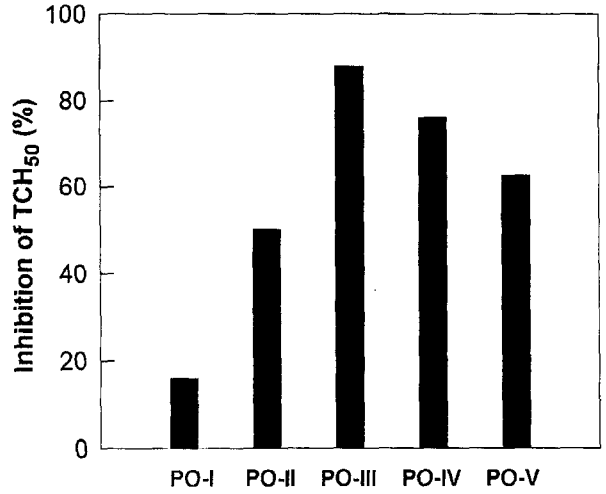

Fig. 2. Anti-complementary activity of the subfractions separated by DEAE-Sepharose FF chromatography.
Concentration of each fraction was 1 mg/ml.

The anti-complementary activity of each fraction is presented in Fig. 5. The fraction with the highest molecular weight (PO-IIIa-1, fraction numbers 25 through 31) was composed of almost an equal amount of sugar and protein, and significantly increased in anti-complementary activity by more than 80% $ITCH_{50}$ at a concentration of 100 μg/ml. Another major fraction (PO-IIIa-2, fraction numbers 32 through 47) containing *ca.* 88% neutral sugar showed a relatively high anti-complementary activity of more than 96% $ITCH_{50}$ at a concentration of 1,000 μg/ml. After re-chromatographies of the PO-IIIa-1 and the PO-IIIa-2 on Sepharose CL-4B and Sepharose CL-6B to remove minor contaminants, each polysaccharide fractions

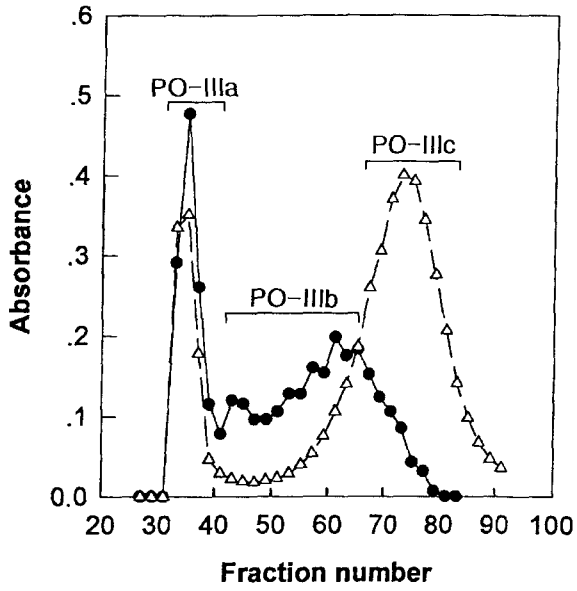

Fig. 3. Chromatogram of the fr. PO-III on a Sephadex G-75 column.
Each fraction was monitored using the phenol-sulfuric acid method at $A_{490\,nm}$ (-●-) and protein absorbance at $A_{280\,nm}$ (-△-). The column of Sephadex G-75 (2 × 70 cm) was eluted with 0.2 M NaCl at a flow rate of 0.2 ml/min.

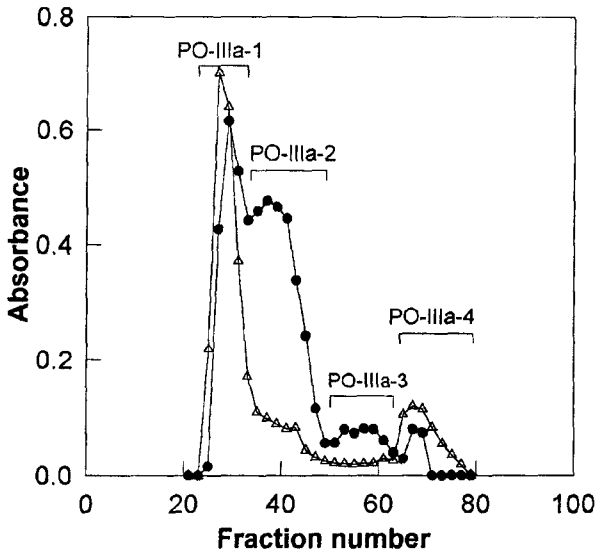

Fig. 4. Elution profile of the fr. PO-IIIa on gel permeation Sepharose CL-6B.
Each fraction was monitored using the phenol-sulfuric acid method at $A_{490\,nm}$ (-●-) and protein absorbance at $A_{280\,nm}$ (-△-). The column (2 × 70 cm) of Sephadex G-75 was eluted with 0.2 M NaCl at a flow rate of 0.2 ml/min.

exhibited symmetrical peaks on gel permeation HPLC (Fig. 6).

Chemical Properties of Anti-Complementary Polysaccharides

The chemical composition and molecular mass of the polysaccharides, PO-IIIa-1 and PO-IIIa-2, are shown in

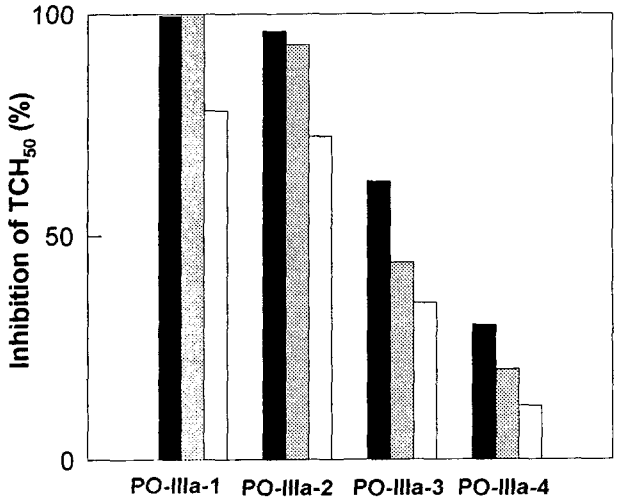

Fig. 5. Comparison of the anti-complementary activity of the subfractions obtained by Sepharose CL-6B chromatography of fr. PO-IIIa.
■: 1,000 g/ml; ▨, 500 g/ml; □, 100 g/ml.

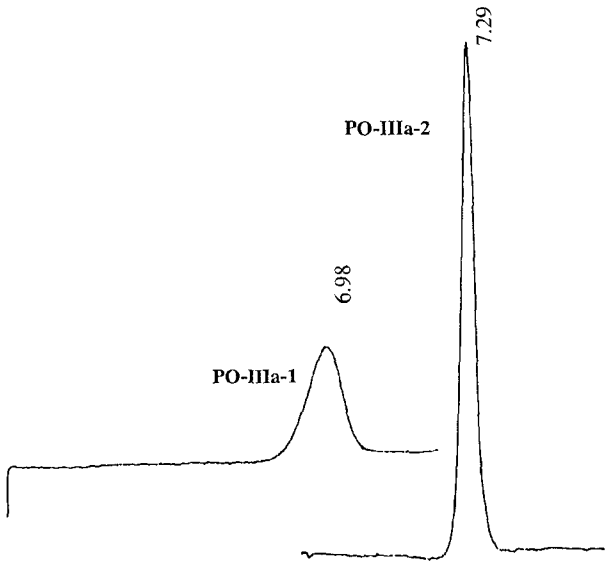

Fig. 6. Gel permeation HPLC profiles of PO-IIIa-1 and PO-IIIa-2.

Table 2. The molecular mass of the PO-IIIa-1 was estimated to be about 2,000 kDa calculated from the K_{av} value on GPC based on the standard dextran molecular markers. It contained 49.2% (w/w) carbohydrates and 52.3% proteins. The molecular weight of the PO-IIIa-2 which did not aggregate was estimated to be 531 kDa by the same procedure (data not shown). It contained D-galactose, D-mannose, L-fucose, and D-glucose in a molar ratio of 1.75:1:0.65:0.59, respectively.

The two polysaccharides produced in this study had a high molecular weight compared to other water-solube anti-complementary polysaccharides, which generally ranged in size from 10 kDa to 1,000 kDa [12]. The high molecular size of the PO-IIIa-1 and the PO-IIIa-2 was believed to be important for the relatively higher anti-complementary activity, in agreement with that reported by other researchers [22]. Most anti-complementary polysaccharides isolated from fungi were known to contain arabinose and galactose as a component sugar together with a significant amount of glucose [18]. The sugar composition of the PO-IIIa-2 was different from those of other anti-complementary [18] or antitumor polysaccharides isolated from the Basidiomycetes. Notably, the presence of fucose was rather similar to that of the fucoidan, an anti-coagulant polysaccharide from a dietary seaweed, *Ecklonia kurome* [16]. Recently, fucose was reported as a sugar component of exopolysaccharides produced by *Bacillus* sp. [1].

To determine the glycosidic linkages of PO-IIIa-2, it was permethylated, hydrolyzed, and acetylated, and, subsequently, the partially methylated alditol acetates were analyzed by GLC and GC/MS. The identification of each methylated alditol acetate was deduced on the basis of the relative retention time and mass fragment patterns (data not shown). The position of glycosidic linkages in the monosaccharides corresponded to the position of the methoxy group in the methylated sugars [22]. Table 3 shows that the majority of galactose and mannose units in PO-III-2 were located in TGal*p*1→ (13.6%), →6Gal*p*1→ (25.4%), →2,6Gal*p*1→ (16.3%), and →2Man*p*1→ (24.8%). The polysaccharide moiety in PO-IIIa-2 was thought to be a galactomannan containing a backbone chain composed of 1,6-linked and 2,6-branched galactosyl residues. The fundamental structure of PO-IIIa-2 was unique compared to that of other anti-complementary polysaccharides, the fungal 6-branched β-1,3-glucans, such as lentinan [2] isolated from *Lentinus edodes* and schizophyllan [8] from *Schizophyllum commune*, scleroglucan, etc [2, 8, 16].

The homogeneity of the PO-IIIa-1 was not further confirmed due to the apparent large molecular size and

Table 2. Properties of the anti-complementary polysaccharides, PO-IIIa-1 and PO-IIIa-2.

	M.W.[a] (kDa)	Carbohydrate (%)	Protein (%)	Composition of carbohydrates (molar ratio)			
				Galactose	Mannose	Fucose	Glucose
PO-IIIa-1	2,000	49.2	52.3	0.85	1.00	<0.01	2.62
PO-IIIa-2	531	88.0	7.9	1.75	1.00	0.65	0.59

[a]Molecular weights were determined on the basis of elution volume on GPC (see Materials and Methods).

Table 3. Glycosidic linkage composition of the PO-IIIa-2 from *P. ostreatus*.

Methylated alditol acetate	RT[a]	Major mass spectral fragments (m/e)[b]	Linkage	Abundance (%)[c]
1.5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl galacitol	23.0	71, 101, 117, 129, 161,	TGalp1→[d]	13.6
1.5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methyl galacitol	29.4	117, 101, 159, 161, 189	→6Galp1→	25.4
1.2,5-tri-*O*-acetyl-3,4,6-tri-*O*-methyl mannitol	33.9	129, 161, 189, 215, 233	→2Manp→	16.3
1,2,5,6-tetra-*O*-acetyl-3,4-di-*O*-methyl galacitol	36.8	129, 259	→2,6Galp1→	24.8

[a]Retention time of each methylated alditol acetate from the PO-IIIa-2 in TIC.
[b]The fragments were obtained from the EI-MS spectra of methylated alditol acetates which recorded on a Hewlett-Packard 5988 MS.
[c]Calculated from the abundance of the total ion of each sugar derivative obtained from the EI-MS spectra.
[d]Nonreducing terminal residues are indicated by T.

high amount of protein. In particular, the definite antitumor or anti-complementary activity observed in the proteoglycan molecules, which were obtained by the dissociation of the aggregate, indicated that the aggregation was not a prerequisite for these activities [17]. It was also reported previously that a molecular aggregation in a polysaccharide could increase the biological activities [6]. Therefore, an attempt was made to examine whether the protein in the PO-IIIa-1 was bound to the polysaccharide moiety in a proteoglycan, or whether it formed a simple aggregation with other polysaccharides. As shown in Fig. 7, the PO-IIIa-1 was separated by affinity chromatography on ConA-Sepharose CL-4B into the unbound proteoglycan (PO-IIIa-1A) and the bound glucomannan (PO-IIIa-1B) fractions.

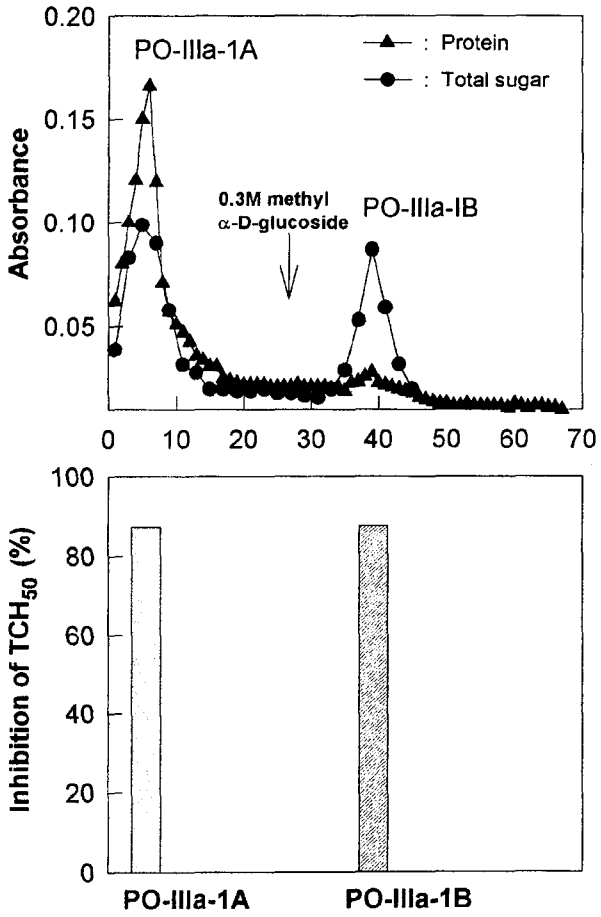

Fig. 7. Affinity chromatography of the PO-IIIa-1 on Con A-Sepharose CL-4B (top) and anti-complementary activity of each fraction (bottom).
The column of Con-A Sepharose CL-4B (1×10 cm) was eluted with a 20 mM Tris-acetate buffer and 0.3 M methyl α-D-glucoside in the buffer at a flow rate of 0.15 ml/min.

The anti-complementary activity of each isolate (1 mg/ml) was shown to be decreased as compared with the native PO-IIIa-1. Moreover, the activity in the mixture of two fractions, PO-IIIa-1A and PO-IIIa-2B, was restored to up to 90% of the native PO-IIIa-1 (data not shown). These results suggested that an aggregation and conformational change in the polysaccharides facilitated the full anti-complementary activity. The molecular aggregation was also observed in AGIIb-1, an arabino-(3→6)-galactan from *Angelica acutiloba*, although it is not yet clear whether the physical state of the arabinogalactan molecule affects the expression of its anti-complementary activity [6]. However, it is generally accepted that a steric factor in various polysaccharide molecules seems to play a key role in anti-complementary activty [19].

Further studies on the structural identification and the structure-activity relationship of the PO-IIIa-1A and the PO-IIIa-1B is in progress in order to elucidate the differences between these polysaccharides in respect to their different anti-complementary activities.

Acknowledgments

This research was supported by a research grant from the Postdoctoral Program of Graduate School of Biotechnology (1999), Korea University.

References

1. Ahn, S.-G., H.-H. Suh, C.-H. Lee, S.-H. Moon, H.-S. Kim, K.-H. Ahn, G.-S. Kwon, H.-M. Oh, and B.-D. Yoon. 1998. Isolation and characterization of a novel polysaccharide producing *Bacillus polymyxa* A 49 KCTC 4648P. *J. Microbiol. Biotechnol.* **8:** 171-177.

2. Chihara, G. and Y. Y. Maeda. 1971. Lentinan, a new immuno-accelerator of cell-mediated responses. *Nature* **229:** 634-640.
3. Chihiro, H., K. Tadashi, T. Yushiro, and U. Shigeo. 1982. Anti-inflammatory activity and conformational behavior of a branched (1→3)-α-D-glucan from an alkaline extract of *Dictyophora indusiata* Fish. *Carbohydr. Res.* **110:** 77-82.
4. Czop, J. K. and K. F. Austen. 1985. A β-glucan inhibitable receptor on human monocytes: Its identity with the phagocytic receptor for particulate activators of the alternative complement pathway. *J. Immunol.* **134:** 2588-2592.
5. Hakomori, S. 1964. A rapid permethylation of glycolipid and polysaccharide catalyzed by methylsulphinyl carbanion in dimethyl sulfoxide. *J. Biochem.* (Tokyo) **55:** 205-213.
6. Jones, T. M. and P. O. Albersheim. 1972. A gas chromatographic method for the determination of aldose and uronic acid constituents of plant cell wall polysaccharides. *Plant. Physiol.* **49:** 926-931.
7. Kiyohara, H., H. Yamada, J. C. Cyong, and Y. Otsuka. 1986. Studies on polysaccharides from *Angelica acutiloba* and molecular aggregation and anti-complementary activity of arabinogalactan from *Angelica acutiloba*. *J. Pharmacobio-Dyn.* **9:** 339-345.
8. Komatsu, N., S. Okubo, S. Likumoto, K. Kimura, F. Saito, and S. Sasaki. 1969. Host-mediated antitumor action of schizophyllan, a glucan produced by *Schizophyllum commume*. *Gann* **60:** 137-144.
9. Kweon, M.-H., H.-C. Sung, and H.-C. Yang. 1994. Acidic heteroglycans with anti-complementary activity from the water extract of *Pteridium aquilinum* var. *latiusculum*. *Foods Biotechnol.* **3:** 83-89.
10. Kweon, M.-H., H.-I. Kim, H.-C. Sung, and H.-C. Yang. 1994. Core structure of the anti-complementary acidic polysaccharide (PA-IIa-1) from water extract of *Pteridium aquilinum* var. *latiusculum*. *Foods Biotechnol.* **3:** 137-143.
11. Kweon, M.-H. and H.-C. Sung. 1996. Structural comparison in the four anticomplementary polysaccharides isolated from *Pteridium aquilinum* var. *latiusculum* (Bracken). *Foods Biotechnol.* **5:** 136-141.
12. Lee, J.-H., S.-M. Cho, H.-M. Kim, N.-D. Hong, and I.-D. Yoo. 1997. Immunostimulating activity of polysaccharides from mycelia of *Phellinus linteus* grown under different culture conditions. *J. Microbiol. Biotechnol.* **7:** 52-55.
13. Lowry, O. H., N. J. Rosebrough, L. Farr, and R. J. Rindall. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193:** 256-260.
14. Luettig, B., C. Steinmûller, G. E. Gifford, H. Wagner, and M.-L. Lohmann-Matthes. 1989. Macrophage activation by the polysaccharide arabinogalactan isolated from plant cell cultures of *Echinacea purpurea*. *Articles* **81:** 669-675.
15. Morrison, D. C. and L. F. Kline. 1977. Activation of the classical and properidin pathway of complement by bacterial lipopolysaccharide (LPS). *J. Immunol.* **118:** 362-366.
16. Nishino, T., H. Kiyohara, H. Yamada, and T. Nagumo. 1991. An anticoagulant fucoidan from brown seaweed *Ecklonia kurome*. *Phytochemistry* **30:** 535-540.
17. Saito, H., T. Ohki, N. Takasuka, and T. Sasaki. 1977. Isolation of antitumor β-glucan from hot water extracts of the edible mushroom *P. ostreatus*. *Carbohydr. Res.* **58:** 293-305.
18. Song, C. H., Y. J. Jeon, B. K. Yang, K. S. Ra, and J. M. Sung. 1998. The anti-complementary activity of exo-polymers produced from submerged mycelial culture of higher fungi with particular reference to *Cordyceps militaris*. *J. Microbiol. Biotechnol.* **8:** 536-539.
19. Suzuki, I., K. Hashimoto, S. Oikawa, K. Sato, M. Osawa, and T. Yadomaf. 1989. Antitumor and immunomodulating activities of a β-glucan obtained from liquid cultured *Grifola frondosa*. *Chem. Pharm. Bull.* **37:** 410-413.
20. Tanigami, Y., S. Kusumoto, S. Nagao, S. Kokeguchi, K. Kato, S. Kotani, and T. Shina. 1991. Partial degradation and biological activities of an antitumor polysaccharide from rice bran. *Chem. Pharm. Bull.* **39:** 1787-1792.
21. Waeghe, T. T., A. G. Darvill, H. McNeil, and P. Albersheim. 1983. Determination by methylation analysis of the glycosyl linkage composition of microgram quantities of complex carbohydrates. *Carbohydr. Res.* **123:** 281-289.
22. Yamada, H. and H. Kiyohara. 1989. Bioactive polysaccharides from Chinese herbal medicines. *Abs. Chinese Med.* **3:** 104-124.
23. Yoshioka, Y., R. Tabeta, H. Saito, N. Uehara, and F. Fukuoka. 1985. Antitumor polysaccharides from *P. ostreatus* (FR.): Isolation and structure of a β-glucan. *Carbohydr. Res.* **140:** 93-100.
24. Zhao, I. F., H. Kiyohar, T. Matsumoto, and H. Yamada. 1993. Anti-complementary acidic polysaccharides from roots of *Lithosperum euchromum*. *Phytochemistry* **34:** 719-723.