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Characteristics of anti-complementary biopolymer extracted from *Coriolus versicolor*

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Abstract

Activation of complementary system (anti-complementary activity) was studied under the influence of biopolymers extracted from fruiting bodies of *Coriolus versicolor*. The anti-complementary activities of the water-soluble extract (CV-S1) and the ethanol precipitate of the water-soluble extract (CV-S2) were 71.8 and 98.1%, respectively. Activated pathway of complement system was found to be occurred through both the classical and alternative pathways as evidenced by crossed immunoelectrophoresis, where the major pathway was detected to be the classical one. The CV-S2 was fractionated into CV-S2-Fr.I, II and III by gel chromatography. The anti-complementary activities were retained mainly in CV-S2-Fr.I and II. The molecular weights of CV-S2-Fr.I and II were estimated to be about 1200 and 150 kDa, respectively. The structures of CV-S2-Fr.I and II were characterized as 2,3,4,6-tetra-O-methyl, 2,4,6-tri-O-methyl, 2,3,4-tri-O-methyl, 2,3,6-tri-O-methyl, 2,4-di-O-methyl and 2,3-di-O-methyl-D-glucitol in different proportions.

Keywords: Anti-complementary activity; Biopolymer; *Coriolus versicolor*

1. Introduction

Coriolus versicolor, a mushroom of important medicinal value has already been recorded in the *Compedium of Materia Medica* by Li Shi Zhen during the Ming Dynasty in China (Ng, 1998). This mushroom has historically attracted attention as a health oriented food not only in China but also worldwide. A variety of compounds have been isolated from this mushroom and some of them are commercially available. The polysaccharopeptide (PSP) obtained from this mushroom possesses immunomodulatory (Liu, Ng, Sze, & Tsui, 1993), antitumor (Xu, 1993) and hepatoprotective (Yeung, Chiu, & Ooi, 1994) activities, and has been used as an immunomodulatory and antitumor drug for the cancer patients (Liao & Zhao, 1993). Another polysaccharide, very closely related to PSP, is PSK (Krestin), which has been isolated from *C. versicolor* by Japanese investigators has been shown to have immunostimulatory activity (Hirase et al., 1970). However, research is still being continued with this mushroom species in order to explore other medicinal activities.

The complement system is a major effector of the humoral immunity involved in the host defense. A number of substances from chemicals, plants or microbial origins have been reported to modulate the complement cascade (Hildebert & Jordan, 1988). The anti-complementary activities of biopolymers extracted from fruiting bodies, mycelium and culture precipitate (Song, Ra, Yang, & Jeon, 1998) of various mushrooms have previously been investigated in our laboratory.

In the present investigation, the anti-complementary activities of biopolymers from *C. versicolor* fruiting bodies were studied and the effort was made to purify and fractionate from the crude extract in order to obtain the pure component and chemical compositions.

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2. Materials and methods

2.1. Material and chemicals

The fruiting bodies of *C. versicolor* were purchased from the local market. Goat anti-human C3 was purchased from Sigma Co., and IgM hemolysin sensitized sheep erythrocyte (EA) from Lyophilization Laboratory Inc. (Japan) were used in this study. 5.5′-Barbituric acid sodium salt was purchased from Merck Co. The other chemicals were reagent grade and obtained from commercial source. Normal human serum (NHS) was obtained from healthy adults.

2.2. Preparation and purification of biopolymer

The fruiting bodies of *C. versicolor* after cutting into small pieces were smashed and autoclaved for 2 h. The preparation of biopolymers from the *C. versicolor* is shown in Fig. 1.

The biopolymer solution was applied to a column (2.4 × 99 cm) of Sepharose CL-6B, which had been equilibrated with 0.2 M NaCl and eluted with the same solution at 4 °C.

2.3. Anti-complementary activity assay and determination of the complement activated pathway

The anti-complementary activity was measured by the complement fixation test based on complement consumption and degree of red blood cell lysis by the residual complement (Kabat & Mayer, 1964). Fifty microliter of biopolymer solution in water was mixed with equal volumes of NHS and gelatin veronal buffered saline (GVB, pH 7.4) containing 500 μg Mg^{++} and 150 μg Ca^{++}. The mixtures were incubated at 37 °C for 30 min and the residual total complement hemolysis (TCH_{50}) was determined by using IgM hemolysin sensitized sheep erythrocytes at 1×10^8 cells/ml. The NHS was incubated with deionized water (DIW) and GVB^{++} to provide a control. The anti-complementary activity of biopolymer was expressed as the percentage inhibition of the TCH_{50} of control.

The Ca^{++} ion is required for the activation of complement via the classical pathway but not the alternative pathway, the activation through the alternative pathway was measured in the Ca^{++} free condition. The alternative complement pathway was determined in 10 mM EGTA containing 2 mM $MgCl_2$ in GVB^{--} (Mg^{++}–EGTA–GVB^{--}) by a modified method of Platt-Mills and Ishizaka (1974). A sample was incubated with Mg^{++}–EGTA–GVB^{--} and NHS at 37 °C for 30 min, and the residual complement mixtures were measured by hemolysis of rabbit

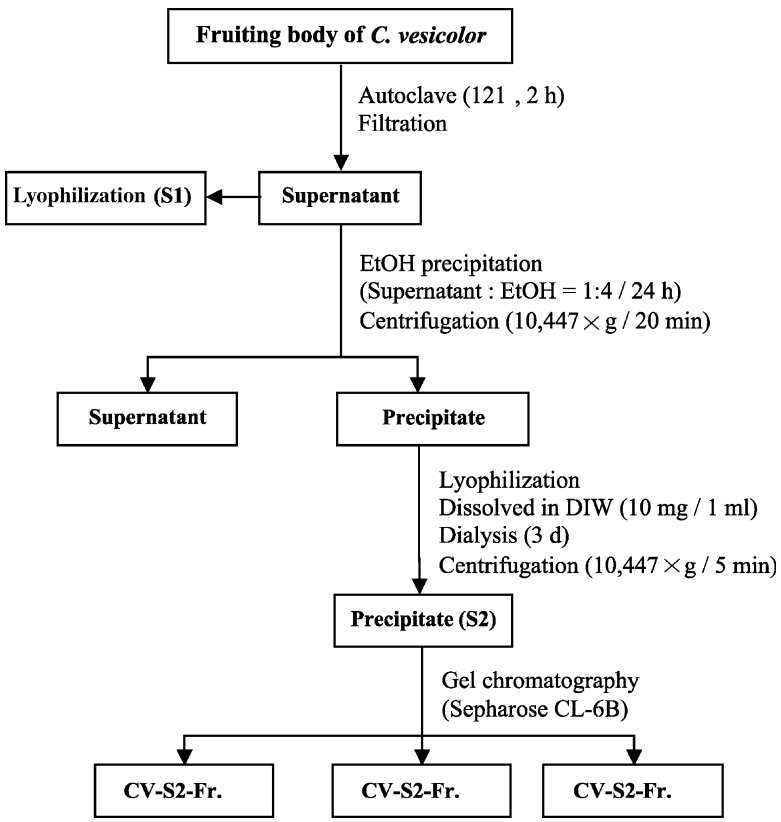

Fig. 1. A schematic diagram depicting the recovery process of biopolymers from fruiting bodies of *Coriolus versicolor*.

erythrocytes (5×10^7 cells/ml) incubated with Mg^{++}–EGTA–GVB^{--}.

2.4. Crossed immunoelectrophoresis

The specific activation of C3 complement component by biopolymer in NHS was assessed by comparative measurements of C3 cleavage. The NHS was incubated with an equal volume of the biopolymer solution in GVB^{++}, GVB^{--} containing 10 mM EDTA (EDTA–GVB^{--}) or Mg^{++}–EGTA–GVB^{--} at 37 °C for 30 min. The mixture was then subjected to crossed immunoelectrophoresis to locate the C3 cleavage product. All the samples (10 μl) were subjected to isoelectric focusing on 1% agarose gel. After the first run in barbital buffer pH 8.6, ion strength 0.025 with 1% agarose for 2 h, the second run was carried out in a gel plate containing an anti-human complement C3, which recognizes both C3 and C3b (complement C3 antiserum raised in goat, Sigma Co., USA), at a potential gradient of 15 mA/plate for 15 h. After the electrophoresis, the plate was fixed and stained with 0.2% bromophenol blue. The ratio between the heights of the C3 and C3b peaks was calculated (Cyong et al., 1982).

2.5. Pronase digestion of biopolymer

The biopolymer (40 mg) was dissolved in 40 ml of 50 mM Tris–HCl buffer, pH 7.9, containing 10 mM $CaCl_2$, and then 10 mg pronase was added. The reaction mixture was incubated at 37 °C for 48 h with a small amount of toluene. The reaction was terminated by boiling for 5 min. The mixture was then dialyzed against DIW for 2 days, and the non-dialyzable portion was lyophilized (Yamada et al., 1985a; Yamada, Ohtani, Cyong, & Otsuka, 1985b).

2.6. Periodate oxidation of biopolymer

The biopolymer (40 mg) was dissolved in 40 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 was added to destroy the excess periodate, and the mixture was dialyzed against DIW for 2 days. The non-dialyzable solution was concentrated 10 ml, and 20 mg sodium borohydride was added while being continuously stirred for 12 h at room temperature. After the neutralization of reaction mixture with acetic acid, the boric acid contained in the sample was removed by the repeated addition and evaporation of methanol. Finally, the oxidized sample was obtained as the lyophilizate after the dialysis (Yamada et al., 1985a,b).

2.7. Determination of molecular weight

Molecular weight of biopolymer was determined by HPLC using the Shodex GS520, GS320, and GS220 packed column. Standard pullulans (P1600, 800, 400, 200, 100, 50, 20, 10 and 5) were used for calibration.

2.8. SEC-MALLS analysis

Size exclusion-multi-angle laser light scattering (SEC-MALLS; DAWN DSP; Wyatt Technology, Santabara, CA, USA) was used to confirm the molecular weight of the biopolymer. The biopolymer was dissolved in a phosphate/chloride buffer (ionic strength 0.1, pH 6.8) containing 0.04% ethylenediaminotetra–acetic acid disodium salt (Na_2EDTA) and 0.01% sodium azide and filtered through 0.22 μm (if necessary 0.025 μm) filter membranes (Millex HV type; Millipore Corp., Bedford, MA, USA) prior to injection into the SEC-MALLS system (Jumel, Fiebrig, & Harding, 1996). The chromatographic system consisted of a high performance pump (Model 590 Programmable solvent delivery module; Waters Corp., Milford, MA, USA), a degassing system (Degasys, DG-1200, Uniflows; HPLC Technology, Macclesfield, UK), an injection valve (Rheodyne Inc., Cotati, CA, USA) equipped with a 100 μl sample loop, three SEC columns connected in series and housed in a column oven with a Dawn-DSP multi-angle laser light scattering detector (Wyatt Technology, USA) and a refractive index detector (Waters 410). The SEC columns were Shodex PROTEIN KW-802.5, 803 and 804 (Shodex PROTEIN KW-802.5, 803, 804; Showa Denko K.K., Tokyo, Japan) with pore sizes of 150, 300 and 500 Å, respectively. Chromatography was performed at room temperature with a flow rate of 0.8 ml/min. Injection volume was 100 μl with a biopolymer concentration of 3 mg/l. During the calculation of molecular weights of each biopolymer, the value of d*n*/d*c*, so-call 'specific refractive index increment' was used according to guide from the Wyatt Technology and data in literature (Jumel et al., 1996) after modification to take into account the carbohydrate and protein contents of the biopolymers. Calculations of molecular weight and root mean square (RMS) radius of gyration for each biopolymer were performed by Astra 4.72 software (Wyatt Technology). The RMS radii of each biopolymers were determined from the slope by extrapolation of the first-order Debye plot (Astafieva, Eberlein, & Wang, 1982; Wyatt, 1933).

2.9. Analysis of protein and sugar composition of biopolymer

Total protein content of the biopolymer was determined by the method of Lowry, Rosebrough, Farr, and Randill (1951) with bovine serum albumin (BSA) as a standard. The protein was hydrolyzed and the amino acid composition was analyzed by a Biochrom 20 (Pharmacia Biotech. Ltd, USA) amino acid auto analyzer with a Na-form column. A total sugar content was determined by phenol sulfuric acid method (Dubios, Gilles, Hamilton, Rebers, & Smith, 1964) using glucose and arabinose mixture (1:1) as a standard.

The sugar composition was analyzed by a Varian GC3600 gas chromatography equipped with a flame-ionization detector on a SP™-2380 capillary column (15 m × 0.25 mm i.d., 0.2-μm film: SUPELCO) based on the hydrolysis and acetylation method (Jones & Albersheim, 1972).

2.10. Methylation of biopolymer

Each polysaccharide was methylated by method of Hakomori (1964). The bioploymer (2 mg) was dissolved in dimethyl sulfoxide (0.1 ml) by ultrasonication in a nitrogen atmosphere. The solution was treated with methylsulfinyl carbanion (0.1 ml) for 4 h at room temperature, and then with methyl iodide (0.1 ml) for 12 h at room temperature. Each methylated biopolymer was purified by using a Sep-pak C_{18} cartridge (Waters Assoc.). The permethylated biopolymer was hydrolyzed with 2 M trifluoroacetic acid (1.5 ml) for 1 h at 121 °C, and reduced with sodium borohydride and acetylated. The resulting methylated alditol acetates were analysed by gas liquid chromatography (GLC) and gas liquid chromatography–mass spectrometry (GLC–MS). GLC was performed on a Varian model STAR 3600CX gas chromatography equipped with a flame-ionization detector on a SP™-2380 capillary column (30 m × 0.25 mm i.d., 0.2-μm film: SUPELCO). GLC-MS (70 eV) was performed on a Shimadzu QP5050 instrument equipped with same capillary column. Peaks were identified on the basis of relative retention time and fragmentation patterns. The mol% for each sugar were calibrated using the peak areas.

3. Result and discussion

3.1. Anti-complementary activity of biopolymer

The yields of water-soluble extract (CV-S1) of the *C. versicolor* fruiting bodies and its dialyzed ethanol precipitate (CV-S2) were 89.9 and 57.5 g/kg, respectively. Their anti-complementary activities were compared at the concentrations of 100, 500 and 1000 μg/ml (Fig. 2). It was found that the anti-complementary activities of both CV-S1 and CV-S2 were increased in accordance with the increase of concentration. However, CV-S2 exhibited higher activity than CV-S1 at all the concentration levels tested. At concentrations of 1000 μg/ml, the anti-complementary activities of CV-S1 and CV-S2 were 71.8 and 98.1%, respectively.

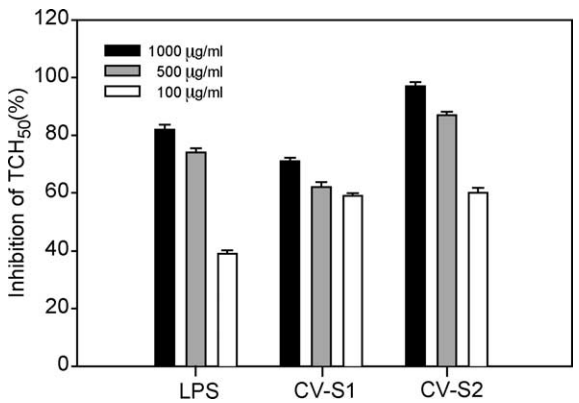

Fig. 2. Anti-complementary activities of each fractions obtained from *Coriolus versicolor*. CV-S1: water-soluble extract. CV-S2: ethanol precipitate of water-soluble extract. LPS: positive control (Lipopolysaccharide from *E.coli Serotype* 0127:B8).

The protein-bounded polysaccharide produced from mycelia of *C. versicolor* is defined as an agent capable of modifying the host biological response by stimulating the immune system and thereby augmenting various therapeutic effects (Yang et al., 1993a). In addition, it increases the production of complement C3 in sarcoma bearing mice (Yang et al., 1993b).

3.2. Activation mode of complementary system by biopolymer

It is known that both Mg^{++} and Ca^{++} ions are needed for the activation of the classical pathway but only Mg^{++} is needed for the activation of the alternative pathway (Law & Reid, 1988). In order to evaluate the complement activated pathway, CV-S2 fraction was studied in different buffer system. In GVB^{++} condition, the anti-complementary activity detected was about 98%, which is the outcome of the participation of both complement activated pathways leading to cellular lysis. However, when the anti-complementary activity was performed in Ca^{++} depleted experimental condition, which acts only in the alternative pathway, CV-S2 showed only 28% activity. There was very little anti-complementary activity detected in the EDTA–GVB^{--} system (Table 1).

This result suggests that the complement system was activated via both the classical and alternative pathway by CV-S2. To confirm the above results, the crossed immunoelectrophoresis was carried out after incubation of NHS with CV-S2 in both GVB^{++} and Mg^{++}–EGTA–GVB^{--} to determine whether C3 activation occurred. The C3

Table 1
Anti-complementary activities of biopolymers obtained from the *Coriolus versicolor* in the presence or absence of Ca^{++} and Mg^{++}

	Inhibition of TCH_{50} (%) ± SD[a]	
	CV-S1	CV-S2
GVB[b]	73.1 ± 1.5	98.0 ± 1.7
Mg^{++}–EGTA–GVB^{--}[c]	18.5 ± 1.2	28.1 ± 1.3
EDTA–GVB^{--}[d]	4.9 ± 0.9	4.1 ± 1.0

[a] Three replicated.
[b] Activated the both pathway.
[c] Activated the alternative pathway.
[d] Blocked the both pathways.

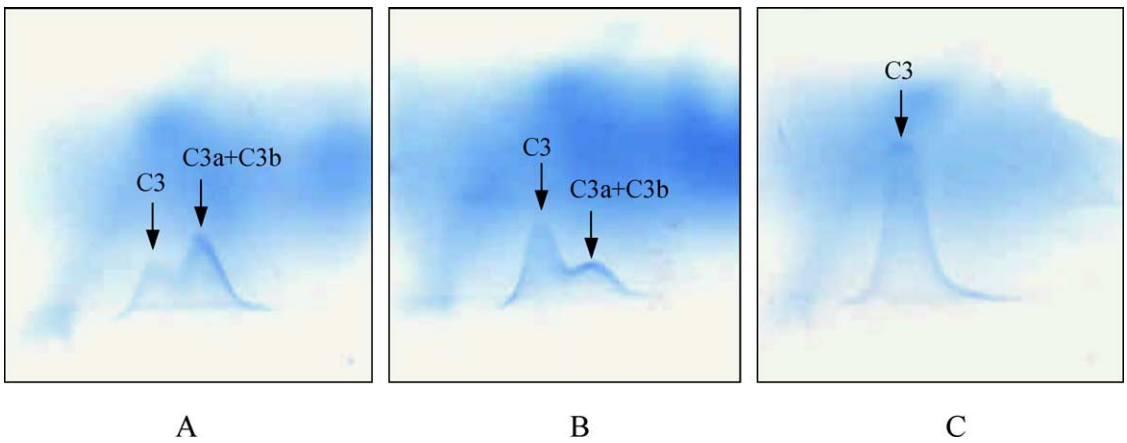

Fig. 3. Crossed immunoelectrophoresis patterns of C3 converted by CV-S2 from *Coriolus versicolor* in condition of Ca^{++}-free or divalent metal ion-free condition. Normal human serum was incubated with GVB^{++}, Mg^{++}–EGTA–GVB^{--} or EDTA–GVB^{--} at 37 °C for 30 min. The sera were then subjected to immunoelectrophoresis using anti-human C3 sera to locate C3 cleavage products. Anode is to the right. (A) GVB^{++}, presence of Ca^{++} and Mg^{++}. (B) Mg^{++}–EGTA–GVB^{--}, presence of Mg^{++} and absence of Ca^{++}. (C) EGTA-GVB^{--}, absence of Ca^{++} and Mg^{++}.

cleavage product C3a and C3b were obtained in the serum treated with CV-S2 in both the buffer system, and the height of C3a and C3b precipitin line in GVB^{++} (Fig. 3A) was higher than in Mg^{++}–EGTA–GVB^{--} (Fig. 3B). No C3a and C3b precipitin line was observed in the EDTA–GVB^{--} buffer system (Fig. 3C). These results indicated that the mode of complement activation by CV-S2 fraction from *C. vesicolor* was activated not only via the classical pathway but also the alternative pathway, although at the lesser extent. This pattern of complement activation by CV-S2 was similar to that of AR-arabinogalactan (Yamada et al., 1985b) and LPS, as they appear to activate both the pathways (Polley & Müller-Eberhad, 1967).

3.3. Purification of crude biopolymer

A crude biopolymer fraction (CV-S2) was obtained from the fruiting bodies of *C. versicolor* extracted with hot water followed by precipitation with ethanol and dialysis. As the CV-S2 showed higher anti-complementary activity as compared to the CV-S1, it was fractionated by Sepharose CL-6B gel chromatography, which yielded the fractions, CV-S2-Fr.I, Fr.II and III (Fig. 4).

The anti-complementary activities of these three fractions are shown in Fig. 5. The highest anti-complementary activities were exhibited by CV-S2-Fr.I, which was followed by CV-S2-Fr.II and CV-S2-Fr.III. CV-S2-Fr.I was composed of 95.9% neutral sugar and 4.1% protein. It showed a high anti-complementary activity of about 66% $ITCH_{50}$ at a concentration of 100 μg/ml. The CV-S2-Fr.II containing 87.8% sugar showed relatively lower anti-complementary activity (58% $ITCH_{50}$) at the same concentration (Fig. 5). These two active fractions were found as a single symmetrical peak by gel filtration of Sepharose CL-6B (data not shown). This result suggested that two of the fraction were homogeneous and pure enough for structural analysis.

The molecular weights of CV-S2-Fr.I and CV-S2-Fr.II were determined by HPLC and were found to be about 1200 and 150 kDa, respectively (Fig. 6). These two biopolymers had higher molecular weight compared to CV-S2-Fr.III (15 kDa), which showed very poor anti-complementary activity. It has been stated that the anti-complementary activity is highly dependent on the molecular weight of the compound concerned. The high molecular weight of the CV-S2-Fr.I and CV-S2-Fr.II were believed to be responsible for the relatively higher anti-complementary activity, in agreement with that reported by other investigator (Yamada et al., 1985a).

3.4. Determination of the major anti-complementary active component of biopolymer

To investigate whether the sugar or protein moiety is essential for the anti-complementary activity, CV-Fr.I, II

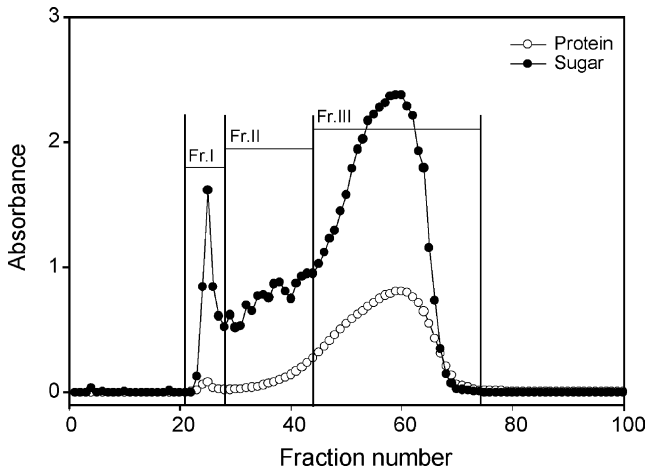

Fig. 4. Gel chromatography of the CV-S2 from *Coriolus versicolor* on Sepharose CL-6B column. CV-S2 was dissolved in 0.2 M NaCl solution. Protein (○): absorbance at 280 nm; Carbohydrate (●): absorbance at 490 nm.

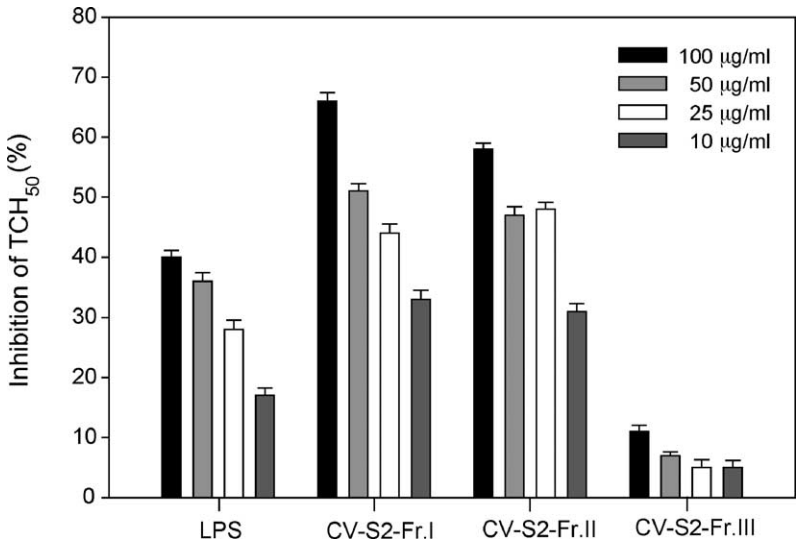

Fig. 5. Anti-complementary activities of purified biopolymers from *Coriolus vesicolor* on the Sepharose CL-6B column. LPS: positive control (Lipopolysaccharide from *E. coli* serotype 0127:B8).

and III were degraded either by oxidation with sodium periodate or hydrolysis with pronase. The anti-complementary activities of the three fractions were drastically decreased by periodate oxidation and slightly decreased after protease digestion (data not shown). When these major active compounds (CV-S2-Fr.I and CV-S2-Fr.II) were treated with pronase, the peak pattern of CV-S2-Fr.II of the digestion product was not changed in comparison to that of the native one, also, the carbohydrate peak did not shift to the lower molecular weight after gel filtration on Sepharose CL-6B. However, CV-S2-Fr.I changed its peak pattern after digestion, and the carbohydrate peak shifted to the lower molecular weight (Fig. 7B). The arrow bar in Fig. 7 shows the shift in molecular weight. These results were reconfirmed by the SEC-MALLS system (Fig. 7A). The molecular weight of pronase degradation fragment of CV-S2-Fr.I was estimated as 160 kDa by size exclusion MALLS. This molecular weight is similar to CV-S2-Fr.II. It is indicating the fact that the anti-complementary activities of those fractions were mainly attributed to the carbohydrate moiety. This result is consistent with the finding of Yamada et al. (1985b), who pointed out the involvement of the carbohydrate moiety in executing anti-complementary activity.

3.5. Chemical content and composition of biopolymer

Total sugar contents of CV-S2-Fr.I and Fr.II were 95.9 87.8%, respectively. The sugar compositions of these fractions are summarized in Table 2. The CV-S2-Fr.I and Fr.II contained mainly glucose and uronic acid was not detected in any of the fractions. Yang et al. have reported the physiochemical characteristics of the PSP isolated from deep-layer cultured mycelia of *C. versicolor*. Its polysaccharide portion is composed of glucose (74.6%) with the remainder being galactose, mannose, xylose and fucose (Yang, Yong, & Yang, 1987). Also most of the anti-complementary polysaccharides isolated from fungi were known to contain a large amount of glucose as the component sugar (Kweon et al., 1999). Total protein contents of CV-S2-Fr.I and CV-S2-Fr.II from fruiting bodies of *C. vesicolor* were 4.1 and 12.2%, respectively. The amino acid composition of these two subfractions is summarized in Table 3. The protein content of biopolymer in fruiting bodies is similar from mycelia, which was reported to be contained 10.5% protein (Lee et al., 1992). The CV-S2-Fr.I contained glycine (10.98%) and arginine (18.47%) as major amino acids whereas CV-S2-Fr.II was dominated by glycine (12.91%), valine (11.15%) and arginine (19.53%).

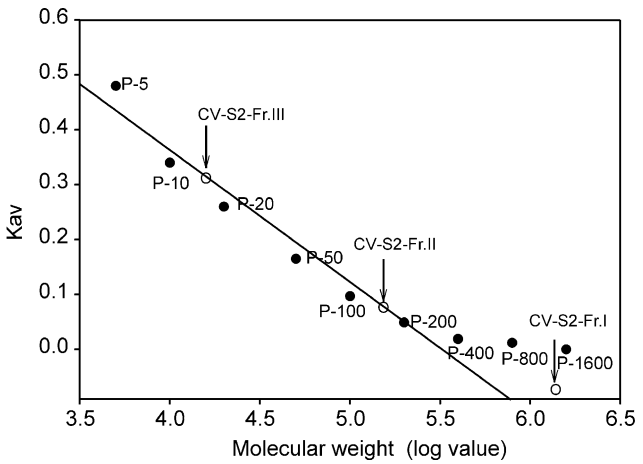

Fig. 6. Determination of molecular weight of purified biopolymers from *Coriolus versicolor*. Carbohydrate molecular weight standards consist of Pullulan series P-16000(16×105),P-800 (8×10^5), P-400 (4×10^5), P-200 (2×10^5), P-100 (1×10^5), P-50 (5×10^4), P-20 (2×10^4), P-10(1×10^4) and P-5(5×10^3). Kav = $(V_e - V_o)/(V_t - V_o)$. V_o: void volume, V_t: total volume, V_e : elution volume.

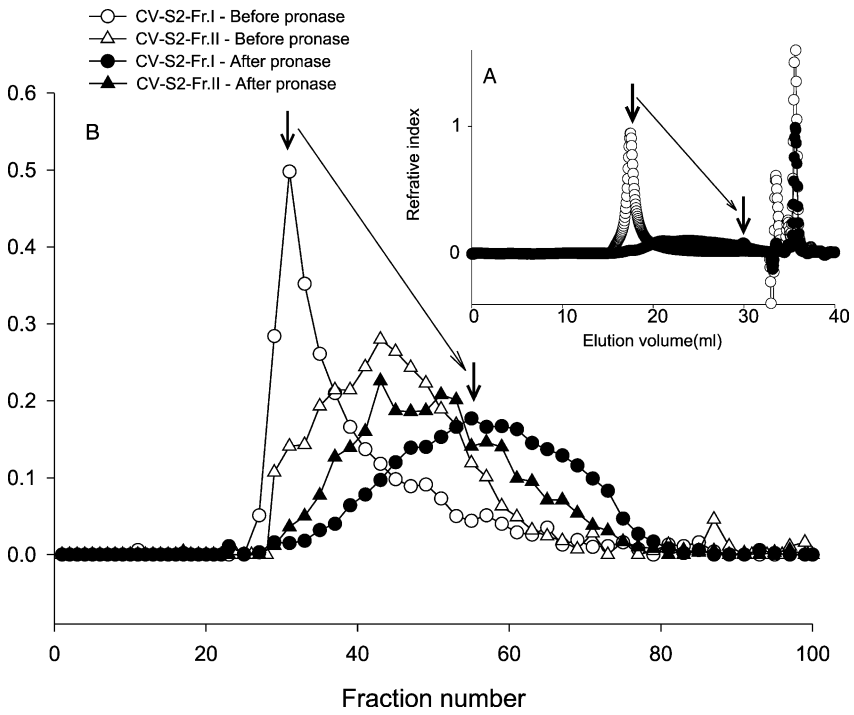

Fig. 7. The shift patterns of biopolymers from *Coriolus versicolor* of molecular weight by pronase digestion. (A) Refractive index of MALLS system. (B) Chromatogram of Sepharose CL-6B column.

3.6. Structural analysis of CV-S2-Fr.I and Fr.II

The CV-S2-Fr.I and Fr.II were methylated and hydrolyzed, and the products were then converted into alditol acetates, each of which was identified by GLC and by its fragmentation pattern in MS (Table 4). The predominant peaks of both CV-S2-Fr.I and Fr.II were characterized as 2,3,4,6-tetra-O-methyl, 2,4,6-tri-O-methyl, 2,3,4-tri-O-methyl, 2,3,6-tri-O-methyl, 2,4-di-O-methyl and 2,3-di-O-methyl-D-glucitol in different proportions. It indicated that CV-S2-Fr-.I and Fr.II contain non-reducing, (1 → 3) linked, (1 → 6) linked, (1 → 4) linked glucopyranosyl residue with 3,6-substituted and 4,6-substituted glucopyranosyl residues but CV-S2-Fr-I possesses more branching points than CV-S2-Fr-II.

The biologically insoluble polysaccharides purified from the fruiting bodies or mycelia of mushroom, *Ganoderma japonicum*(Ukai, Yokoyama, Hara, & Kiho, 1982), *Lentinus edodes*(Saito, Ohki, Takasuka, & Sasaki, 1977), *Grifola frondosa*(Miura et al., 1996) or *Cordyceps ophioglossoides* (Yamada et al., 1984), consisted of mainly β-D-(1 → 3) linked glucopyranosyl residue with β-D-(1 → 6) linked glucopyranosyl residue as a side chain, but an antitumor glycoprotein from mycelia of *Coriolus versicolor* consisted of α,β(1 → 3), α,β(1 → 4) and α,β(1 → 6) glucosidic linkages. Also an antitumor glucan from hot-water extract

Table 2
Sugar composition of the purified biopolymer fractions obtained from *Coriolus versicolor* by using the Sepharose CL-6B gel chromatography

Total sugar (%)	CV-S3-Fr.I 95.9	CV-S3-Fr.II 87.8	CV-S3-Fr.III 81.5
Neutral sugar (molar ratio)			
Fucose	Trace	0.52	0.34
Arabinose	1.00	1.00	0.19
Xylose	Trace	Trace	Trace
Mannose	Trace	Trace	0.48
Galactose	Trace	Trace	1.00
Glucose	4.30	3.32	25.10

Table 3
Amino acid composition of the purified biopolymer fractions obtained from *Coriolus versicolor* by using the Sepharose CL-6B gel chromatography

	CV-S2-Fr.I	CV-S2-Fr.II	CV-S2-Fr.III
Total protein (%)	4.1	12.2	18.5
Amino acid (%)			
Asp	7.08	8.43	10.09
Thr	Trace	Trace	6.77
Ser	8.83	7.73	8.18
Glu	9.07	8.96	11.44
Gly	10.98	12.91	11.11
Ala	7.48	8.43	9.59
Cys	6.51	6.72	Trace
Val	6.59	11.15	9.34
Ile	5.33	Trace	Trace
Leu	Trace	Trace	5.15
Arg	18.47	19.53	10.42

Table 4
Identification of partially methylated alditol acetates of CV-S2-Fr-I and -Fr-II from *Coriolus versicolor*

Methylated sugar	Major mass spectral fragments	Mol%[a]		Linkages
		Fr.I	Fr.II	
2,3,4,6-tetra-O-Me-D-Glc[b]	43,45,71,87,101,117,129,145,161,205	13.1	7.0	$Glc^1 \rightarrow$
2,4,6-tri-O-Me-D-Glc	43,45,87,101,117,129,161,233	17.4	16.9	$\rightarrow {}^{3}Glc^1 \rightarrow$
2,3,4-tri-O-Me-D-Glc	43,87,99,101,117,129,161,189,233	4.8	5.7	$\rightarrow {}^{6}Glc^1 \rightarrow$
2,3,6-tri-O-Me-D-Glc	43,45,87,99,101,113,117,233	46.7	51.0	$\rightarrow {}^{4}Glc^1 \rightarrow$
2,4-di-O-Me-D-Glc	43,87,117,129,189	12.6	14.0	$\rightarrow {}^{3,6}Glc^1 \rightarrow$
2,3-di-O-Me-D-Glc	43,87,101,117	5.4	5.4	$\rightarrow {}^{4,6}Glc^1 \rightarrow$

[a] Calculated from peak areas and response factors of hydrogen flame ionization detector on GLC (Sweet, Shapiro, & Albersheim, 1975).
[b] 2,3,4,6-tetra-O-Me-D-Glc = 2,3,4,6-tetra-O-methyl-D-glucitol, etc.

of *Grifola umbellata* contained (1 → 3), (1 → 4) and (1 → 6) linked glycosidic linkages with 3,6- and 4,6-branched glycosidic linkages (Miyazaki, Oikawa, Yamada, & Yadomae, 1977). From our study it is suggested that CV-S2-Fr.I and Fr.II, which were readily soluble in water, composed of similar structures as glucan from *G. umbellata*. This structure may contribute anti-complementary action as discussed above.

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