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Correlation of structure to antitumor activities of five derivatives of a β-glucan from *Poria cocos* sclerotium

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Abstract—A water-insoluble (1→3)-β-D-glucan isolated from fresh sclerotium of *Poria cocos* was, respectively, sulfated, carboxymethylated, methylated, hydroxyethylated, and hydroxypropylated, to afford five water-soluble derivatives. Their weight-average molecular masses (M_w) and intrinsic viscosities ([η]) were determined by size-exclusion chromatography combined with laser light scattering (SEC-LLS), LLS, and viscometry in phosphate buffer solution (PBS) at 37 °C. The antitumor activities, against Sarcoma 180 tumor cell (S-180) and gastric carcinoma cell strain (MKN-45 and SGC-7901) of the native β-glucan and the five derivatives, were tested in vitro and in vivo. The M_w values of the five derivatives in PBS were determined to be 3.8×10^4, 18.9×10^4, 16.0×10^4, 76.8×10^4, and 224.3×10^4, respectively. The high M_w values of the hydroxyethylated and hydroxypropylated derivatives in aqueous solution resulted from aggregation, and their true M_w values obtained in dimethyl sulfoxide were 20.1×10^4 and 19.1×10^4. The sulfated and carboxymethylated derivatives having DS of 1.0–1.3 show good water solubility, and exist as relatively expanded chains in aqueous solution. Interestingly, the native β-glucan did not show antitumor activity, whereas the sulfated and carboxymethylated derivatives exhibit significant antitumor activities against S-180 and gastric carcinoma tumor cells. This work showed that good water solubility, relatively high chain stiffness, and moderate molecular mass of the derivatives in aqueous solution contribute beneficial to enhancement of antitumor activity.

Keywords: Polysaccharide; Antitumor activity; Light scattering; Molecular mass; Sulfation; Carboxymethylation; Methylation; Hydroxyethylation; Hydroxypropylation; *Poria cocos* sclerotium

1. Introduction

The commercial importance of polysaccharides have attracted much attention in the field of functional foods and new drugs.[1,2] Some natural polysaccharides show significant antibacterial,[3] antiviral,[4] antitumor,[5,6] and immune activities,[7] but others are completely devoid of bioactivities.[8] It is noteworthy that water-insoluble polysaccharides show little bioactivity, whereas such glucan derivatives such as dextran sulfate, lentinan sulfate, and pullulan sulfate exhibit high anti-HIV activities and low anticoagulant activities.[9,10] Interestingly, carboxymethylated derivatives from both α- and β-D-glucans show higher water solubility along with antitumor activity against Sarcoma 180.[11,12] Moreover, hydroxyethylation, hydroxypropylation, and methylation can also increase the water solubility and antitumor activity of certain polysaccharides.[13,14] In addition, the introduction of suitable ionic groups with appropriate degrees of substitution (DS) can also cause the polymer chain to adopt certain conformations in aqueous solution.[15] The effects of different substitution groups, their positions, and DS, on the bioactivities of polysaccharides have been reported.[16,17] *Poria cocos* is one of the most important traditional medicines in China and Japan, and exhibits various biological activities.[18–20] However, the bioactivities of various derivatives of the

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water-insoluble β-D-glucan from *Poria cocos* have still not been fully studied.

In our previous work, six polysaccharides were isolated from the sclerotium of *Poria cocos*, the main component being a water-insoluble linear β-D-glucan termed PCS3-II,[21] which was devoid of antitumor activity. In a quest for antitumor activity, five water-soluble derivatives were prepared, and their chemical structures determined by ^{1}D and ^{2}D NMR spectra, infrared spectroscopy (IR), and elementary analysis.[22] In this work, the weight-average molecular masses (M_w) and chain conformations of these derivatives were determined by size-exclusion chromatography combined with laser light scattering (SEC-LLS), LLS, and viscosity in phosphate buffer solution (PBS). The effects of different groups, degree of substitution (DS), water solubility, molecular mass, and chain conformation, on the antitumor activities against Sarcoma 180 tumor cell and a gastric carcinoma cell strain were investigated. Although there have been several reports on the antitumor activities of polysaccharides against gastric carcinoma,[23,24] this is the first integrated report on the antitumor activities against gastric carcinoma of chemically modified β-glucans from *Poria cocos* sclerotium.

2. Experimental

2.1. Materials

A water-insoluble (1→3)-β-D-glucan termed, PCS3-II extracted with 0.5 M NaOH aqueous solution from the sclerotium of *Poria cocos* was used.[21] The β-glucan was, respectively, sulfated, carboxymethylated, methylated, hydroxyethylated, and hydroxypropylated to obtain five water-soluble derivatives[22] coded as Samples 1–5 here. The DS from elemental analysis, NMR spectra, and the solubilities of the derivatives are listed in Table 1.

2.2. Viscometry

Intrinsic viscosities [η] of the polysaccharide derivatives were measured at 37 ± 0.1 °C using an Ubbelohde capillary viscometer. A phosphate buffer solution (8.812 g NaCl, 0.201 g KCl, 0.204 g KH_2PO_4, and 1.150 g Na_2HPO_4 were dissolved in 1 L of distilled water), and also dimethyl sulfoxide (Me_2SO) were used as solvents for the derivatives. Huggins and Kraemer equations were used to estimate the [η] value by extrapolation to concentration (c) to be zero as follows:

$$\eta_{sp}/c = [\eta] + k'[\eta]^2 c, \quad (1)$$

$$(\ln \eta_r)/c = [\eta] - k''[\eta]^2 c, \quad (2)$$

where k' and k'' are constants for a given polymer at a given temperature in a given solvent; η_{sp}/c is the reduced specific viscosity and $(\ln \eta_r)/c$ is the inherent viscosity.

2.3. LLS measurement

The light-scattering intensities of sample solutions were measured with a multi-angle laser light scattering instrument equipped with a He–Ne laser ($\lambda = 633$ nm; DAWN® DSP, Wyatt Technology Co., USA) at angles of 42°, 49°, 56°, 63°, 71°, 81°, 90°, 99°, 109°, 118°, 127°, 136°, and 152° at 37 °C. The sample solutions of desired concentrations were prepared, and optical clarification was achieved by filtration through a sand filter followed by a 0.2 μm pore-size filter (Whatman, England) into the scattering cell (SV mode). The refractive index increments (dn/dc) were determined using an Optilab refractometer (DAWN® DSP, Wyatt Technology Co., USA) at 633 nm. The dn/dc values of samples in 0.2 M NaCl and Me_2SO were determined to be 0.140 and 0.060 mL/g, respectively. Astra software (Version 4.70.07) was utilized for data acquisition and analysis.

2.4. SEC-LLS measurement

Size-exclusion chromatography combined with laser light scattering measurements were performed on the DAWN® DSP multi-angle laser photometer already mentioned, combined with a P-100 pump (Thermo Separation Products, San Jose, USA) equipped with a TSK-GEL G5000 and G3000 PWXL column (7.8 mm × 300 mm) in PBS at

Table 1. Solubility in water and Me_2 SO, and degree of substitution calculated from element analysis and ^{13}C NMR of the samples PCS3-II and the five derivatives[22]

Sample	Substituted ionic groups	Solubility[a]		DS from ^{13}C NMR				DS from EA results
		Water	Me_2SO	C-6	C-4	C-2	Total DS	
PCS3-II	N	–	+	N	N	N	N	N
Sample 1	Sulfation	++	–	0.44	0.24	0.34	1.02	1.21
Sample 2	Carboxymethylation	++	–	0.71	0.35	0.21	1.27	1.34
Sample 3	Methylation	++	+	0.33	0.21	0.15	0.69	0.82
Sample 4	Hydroxyethylation	+	+	0.25	0.24	*	0.49	0.60
Sample 5	Hydroxypropylation	+	+	0.23	*	*	0.23	0.3

N: not substitution.

[a] Determined by naked eye; ++: highly soluble; +: soluble; –: insoluble.

37 °C, and with a G4000 H_6 column (7.5 mm × 300 mm) in Me_2SO. A differential refractive index detector (RI-150) was simultaneously connected. The PBS and Me_2SO were used, respectively, as eluents with a flow rate of 1.0 mL/min. All solutions, having a sample concentration of 1.0×10^{-3}–2.0×10^{-3} g/mL, were filtered first with a sand filter followed by a 0.20 μm filter (Whatman, England), and then kept in sealed glass bottles before injection onto the SEC column. Astra software (Version 4.70.07) was utilized for the data acquisition and analysis.

2.5. In vivo antitumor test

Sarcoma 180 tumor cells, provided by Pharmacy Laboratory of Tongji Medical College, Huazhong University of Science and Technology, were subcutaneously inoculated (5×10^6 cells/mouse) into 8-week-old female BALB/c mice weighing 17 ± 1 g. 5-Fluorouracil (5-FU) and the tested samples were dissolved in PBS, and then injected intraperitoneally (ip) once a day for 7 days, starting 24 h after tumor inoculation. The same volume of PBS was injected ip into the control mice. The tumor was allowed to grow in the mice for 7 days before being removed from the animal and weighed. The inhibition ratio (ξ) and enhancement ratio of body weight (f) were calculated as follows:

$$\xi = [(W_c - W_t)/W_c] \times 100\%, \quad (3)$$

$$f = [(W_a - W_b)/W_b] \times 100\%, \quad (4)$$

where W_c is the average tumor weight of the control group, W_t is the average tumor weight of the tested group; and W_b and W_a are the body weight of mice before and after the assay. Statistical evaluations in all experiments were performed by a student's t-test. A P value of less than 0.05 was considered significant.

2.6. In vitro antitumor activity assay against Sarcoma 180 tumor cell

Colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method was used for measuring the proliferation of adherent tumor cells. The Sarcoma 180 tumor cells were inoculated on a 96-well cultivation plate at a concentration of 1×10^4 cells/mL. Each well was inoculated with 100 μL Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% fetal bovine serum solution containing the tumor cells and 20 μL samples (at concentrations of 0.005, 0.05, 0.5, and 5 mg/mL in PBS, respectively) under an atmosphere of 5% CO_2 at 37 °C for 24 h. The tumor cells were continuously inoculated for another 4 h after 10 μL MTT (5 mg/mL) had been added. The supernatant was removed by centrifuging, and then 100 μL Me_2SO was added to terminate the reaction. The survival rate of the tumor cells was assayed by measuring the optical intensity by an auto enzyme-labeled meter (CliniBio 128, Australia) at 550 nm. The sample groups were compared with control group in the absence of the tested samples. All in vitro results were expressed as the inhibition ratio (Φ) of tumor cell proliferation as follows:

$$\Phi = [(A - B)/A] \times 100\%, \quad (5)$$

where A and B are the average number of viable tumor cells of the control group and test group, respectively. All assays were made in triplicate.

2.7. In vitro antitumor activity assay against gastric carcinoma cell strain

The colorimetric MTT method was used for measuring the proliferation of tumor cells. The gastric carcinoma tumor cell strains MKN-45 and SGC-7901, provided by Union Hospital of Tongji Medical College, Huazhong University of Science and Technology, were inoculated with 180 μL RPMI 1640 medium supplemented with 10% fetal bovine serum solution and 20 μL sample solutions at different concentrations in PBS under an atmosphere of 5% CO_2 at 37 °C for 24 h. The tumor cells were continuously inoculated for another 4 h after 20 μL MTT (5 mg/mL) had been added. The supernatant was removed by centrifuging, and then 150 μL Me_2SO was added to terminate the reaction. The survival rate of tumor cells was assayed by an auto enzyme-labeled meter (Bio-Tek EX-800, USA) at 570 nm. The inhibition ratio of tumor cell proliferation was calculated as in Eq. 5.

3. Results and discussion

3.1. Chemical structure

The solubilities, and DS calculated from elemental analyses and ^{13}C NMR of polysaccharide PCS3-II and the five derivatives are summarized in Table 1. Table 1 shows that the substitution of Samples 1–3 occurred mainly at C-6 position and secondly at C-4 and C-2, position. The substitution in Sample 4 occurred mainly on C-6 and secondly on C-4, and that in Sample 5 was almost entirely on C-6. The DS and ease of substitution of the five derivatives are in the order Sample 2 > Sample 1 > Sample 3 > Sample 4 > Sample 5. All substitutions were nonselective, and the DS values from ^{13}C NMR range from 0.23 to 1.27.

The native β-glucan PCS3-II is water insoluble, in contrast to the derivatives, and it has a tendency to aggregation caused by intermolecular hydrogen bonds.[25] Thus, Me_2SO was used as the solvent for determining molecular mass of the samples. The high water solubility of the derivatives results from the introduction of ionic groups. The water solubility of the derivatives is

in the order Sample 1 > Sample 2 > Sample 3 > Sample 4 > Sample 5, similar to the order of their DS.

3.2. Molecular mass and intrinsic viscosity

Figure 1 shows the SEC-LLS chromatograms of the derivatives Samples 1–3 in PBS at 37 °C. The single peak for Samples 1–3, as detected both by refractometry and LLS indicate that there is no aggregation in aqueous solution, similar to the behavior of a single-stranded chain of the β-glucan from *Poria cocos* sclerotium at 80 °C, giving one peak in SEM.[26] Figure 2 shows the SEC-LLS chromatograms of Samples 4 and 5 in PBS (A) and in Me_2SO (B) at 37 °C, respectively. There is only one peak for Samples 4 and 5 in Me_2SO, but more than two peaks in PBS. From the peak shapes and different M_w values in PBS solution and Me_2SO, we can deduce that the two derivatives formed aggregates in aqueous solution, whereas the aggregates were resolved into single-stranded form in Me_2SO.[27]

By working in batch mode with the LLS detector, the M_w and $\langle s^2\rangle^{1/2}$ values of polymers can be obtained directly by computing a classical Zimm plot from light-scattering data collected at various angles (θ) for each concentration (c). In practice, the scattering intensity is converted into an excess Rayleigh ratio, R_θ, and the quantity Kc/R_θ is plotted versus $\sin^2(\theta/2) + \text{Const.} \times c$, with K being an optical constant. The constant is arbitrary and a stretch factor is selected to obtain a well-defined plot. The collected data for Kc/R_θ at different angles are extrapolated to 0° for each concentration. The common intercept of the extrapolated curves yields M_w, and the slope of the $c = 0$ curve yields $\langle s^2\rangle^{1/2}$. Figure 3 shows the angular dependences of $(Kc/R_\theta)_{c=0}$ for the five derivatives in PBS solution at 37 °C. The measured data of $[\eta]$, M_w radii of gyration ($\langle s^2\rangle^{1/2}$) and polydispersity index (M_w/M_n) of the samples in PBS and Me_2SO from viscometry, LLS, and SEC-LLS are summarized in Table 2.

The M_w values of Samples 1–3 measured by LLS are similar to those measured by SEC-LLS. As compared to

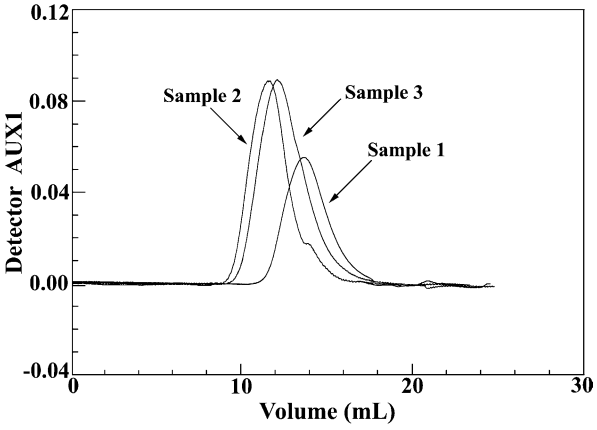

Figure 1. SEC-LLS chromatograms of sulfated, carboxymethylated, and methylated derivatives in PBS aqueous solution at 37 °C.

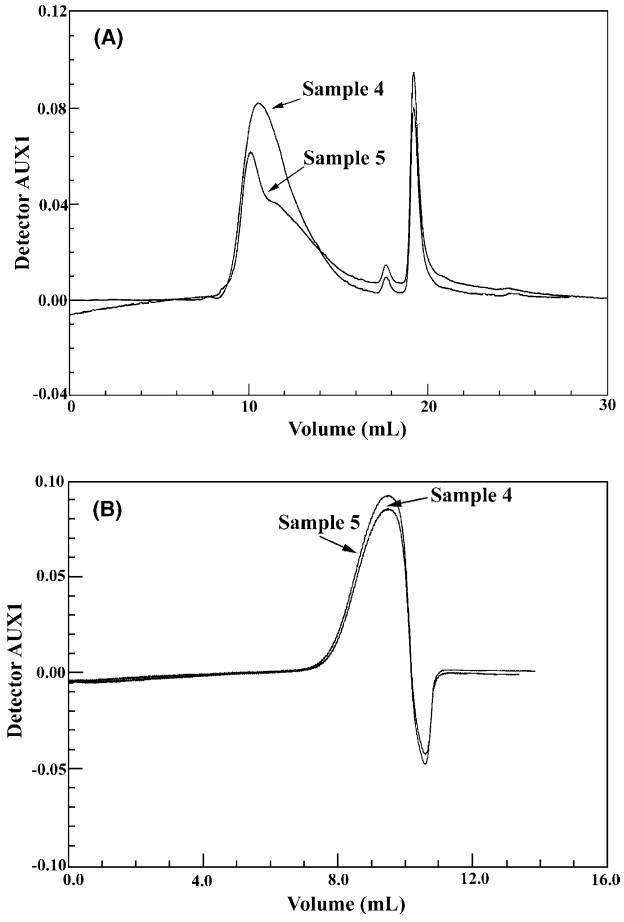

Figure 2. SEC-LLS chromatograms of hydroxyethylated and hydroxypropylated derivatives in PBS aqueous solution (A) and in Me_2SO (B) at 37 °C.

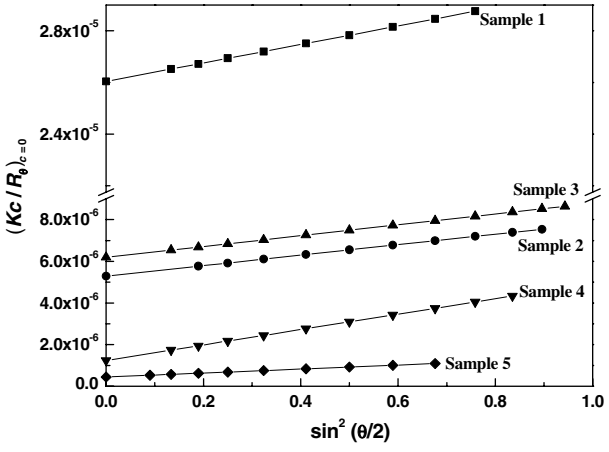

Figure 3. The angular dependences of $(Kc/R_\theta)_{c=0}$ for the five derivatives in PBS aqueous solution at 37 °C.

Table 2. Experimental results from viscosity, LLS and SEC-LLS, for PCS3-II and the five derivatives in PBS aqueous solution or Me_2SO at 37 °C

Sample	Solvents	$[\eta]$ ($cm^3 g^{-1}$)	$\langle s^2\rangle^{1/2}$ (nm)	LLS	SEC-LLS	
				$M_w \times 10^{-4}$ ($g mol^{-1}$)	$M_w \times 10^{-4}$ ($g mol^{-1}$)	M_w/M_n
PCS3-II*	Me_2SO	76.3	56.4[a]	12.3	14.0	1.7
Sample 1	PBS	20.2	—	3.8	3.5	1.6
Sample 2	PBS	167.1	45.0[a]	18.9	25.1	1.7
Sample 3	PBS	132.2	42.4[a]	16.0	21.1	2.0
Sample 4	PBS	122.5	103.0[a]	76.8	62.8	18.0
	Me_2 SO	146.2	23.9[b]	—	20.1	1.7
Sample 5	PBS	156.5	96.1[a]	224.3	297.0	2.3
	Me_2 SO	162.3	29.9[b]	—	19.1	1.6

*: Data obtained from previous work. —: data not detected.
[a] Data obtained from LLS.
[b] Data obtained from SEC-LLS.

the M_w of PCS3-II (12.3×10^4), the M_w of Sample 1 is relatively low, because the vigorous reaction conditions in its preparation caused degradation of the native glucan. However, the M_w values of Samples 2 and 3 are higher than that of PCS3-II, since carboxymethyl and methyl groups are introduced into the glucan chain with little degradation. Interestingly, the M_w of Samples 4 and 5 in PBS are much higher than those of other samples. This can be explained in terms that many OH groups remained in the derivatives of low DS (0.2–0.5), resulting in aggregation of the macromolecules in aqueous solution. Therefore, the M_w values (1.91×10^5 and 2.01×10^5) of Samples 4 and 5 measured in Me_2SO may be regarded as true molecular masses of the single-stranded chain.

3.3. Chain stiffness

The values of $[\eta]$ and $\langle s^2\rangle^{1/2}$ reflect the extent of chain stiffness in polymers. Generally, relatively high values of $[\eta]$ and $\langle s^2\rangle^{1/2}$ suggest a relatively expanded polymers chain. The data listed in Table 2 suggest that the chains of Samples 1 and 2 are extended in PBS. More information about chain stiffness may be obtained from SEC chromatograms. The power law of $\langle s^2\rangle^{1/2} = f(M_w)$ can be estimated from sufficient experimental points in the SEC chromatogram. Figure 4 shows log–log plots of $\langle s^2\rangle^{1/2}$ versus M_w for Sample 1 (A) and Sample 2 (B) in PBS solution at 37 °C. Usually, the exponent α is 0.5–0.6 for a flexible polymer in a good solvent, and 0.6–1.0 for a semi-flexible chain. The α values are 0.69 for Sample 1 and 0.54 for Sample 2, showing that they exist as relatively expanded flexible chains in aqueous solution.

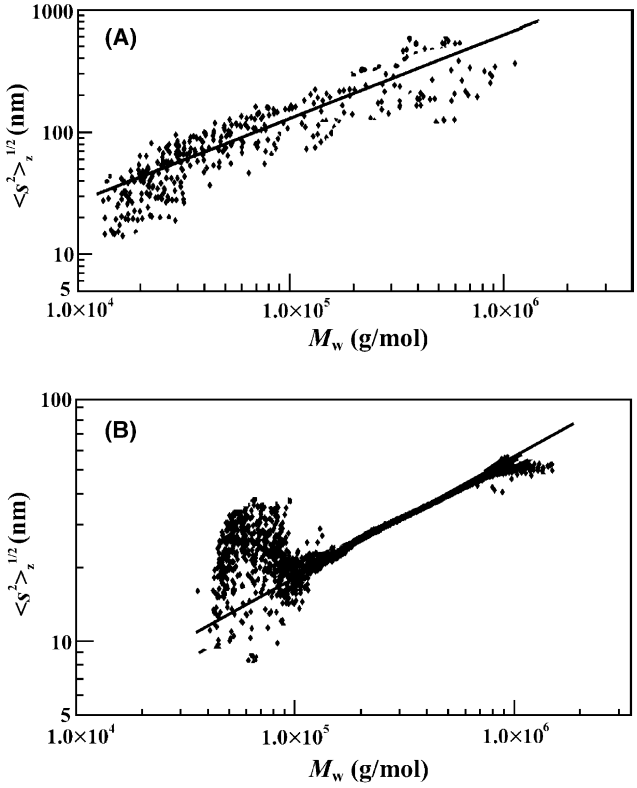

Figure 4. Dependence of $\langle s^2\rangle^{1/2}$ on M_w for sulfated (A) and carboxymethylated (B) derivatives in PBS aqueous solution at 37 °C.

3.4. Antitumor activities

The results of in vivo assay of PCS3-II and the five derivatives against Sarcoma 180 tumor cells in mice are summarized in Table 3, which also includes the results obtained with 5-fluorouracil (5-FU), a well-known anticancer chemotherapeutic agent. No obvious antitumor activity was observed with PCS3-II, indicating that it is ineffective in suppressing the growth of the S-180 tumor cells. However, all of the derivatives exhibit antitumor activities. All of them exhibited enhanced antitumor activities with increased dose in mice, and Samples 1 and 2 exhibit significant activities against S-180 at 32 mg/kg × 10 days. It is noteworthy that the antitumor activities of the five derivatives are slightly lower than that of 5-FU, but their enhancement of body weight are significantly higher than that with 5-FU, suggesting that the modified polysaccharides are not so

Table 3. Antitumor activities of PCS3-II and five derivatives against S-180 solid tumor cells in BALB/c mice

Samples	Dose (mg/kg × days)	Mice	W_{tumor} (g)	Inhibition ration ξ (%)	Enhancement ratio of body weight f (%)
PCS3-II	16 × 10	10/10	1.56 ± 0.42	2.46	30.3
	32 × 10	10/10	1.57 ± 0.66	3.02	49.7
Sample 1	16 × 10	10/10	1.39 ± 0.27	13.88	42.8
	32 × 10	10/10	1.21 ± 0.41	34.63[a]	49.1
Sample 2	16 × 10	10/10	1.23 ± 0.48	23.45[a]	45.5
	32 × 10	10/10	1.04 ± 0.20	35.27[b]	46.7
Sample 3	16 × 10	10/10	1.34 ± 0.46	16.65	45.9
	32 × 10	10/10	1.22 ± 0.38	24.48[a]	52.1
Sample 4	16 × 10	10/10	1.63 ± 0.36	—	60.0
	32 × 10	10/10	1.29 ± 0.29	20.20	46.0
Sample 5	16 × 10	10/10	1.45 ± 0.27	10.00	40.1
	32 × 10	10/10	1.37 ± 0.48	14.88	46.3
Control		10/10	1.61 ± 0.32	0	60.2
5-Fu	25 × 10	10/10	0.76 ± 0.16	52.76[b]	1.7

—: Negative.
[a] Compared with control group $P < 0.05$.
[b] Compared with control group $P < 0.01$.

toxic as 5-FU, which kills the normal cells as well as tumor cells. These results imply that antitumor activities of these glucan derivatives result from the stimulation of the immunoresponse mechanism of the host, which are same as the natural glucans.

Figure 5 shows the inhibition ratios growth of PCS3-II and five derivatives against of S-180 tumor cells in vitro. Samples 1–4 all exhibit relatively stronger inhibition ratios at all concentration levels than does PCS3-II. In particular, Sample 1 at 0.5 mg/mL shows activity equivalent to that of 5-FU at 0.005 mg/mL. However, no obvious dose–dependency relationship was observed between concentration of the derivatives and growth inhibition of S-180. The in vitro antitumor activities against S-180 tumor cells of the derivatives are derived from stimulation of the immunoresponse mechanism, and so they do not strictly follow the dose–dependency of chemotherapeutic anticancer agents.[28] It is surprising that Sample 5 exhibits a negative inhibition ratio at each concentration, and this phenomenon needs further investigation.

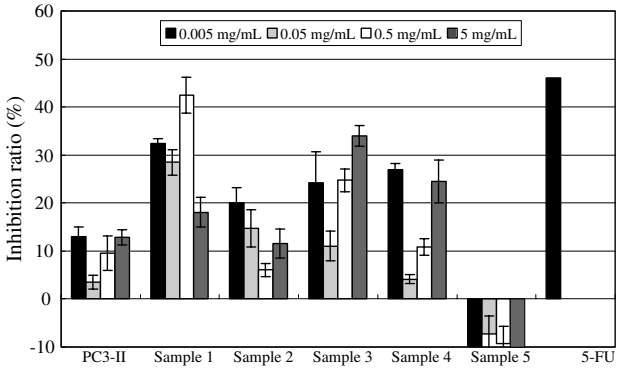

Figure 5. Inhibition ratio of proliferation of Sarcoma 180 tumor cell (S-180) in vitro by different concentrations of PCS3-II and the five derivatives.

Figures 6 and 7 show the inhibition ratios against gastric carcinoma cell strain MKN-45 and SGC-7901 growth in vitro of PCS3-II and the five derivatives. Sam-

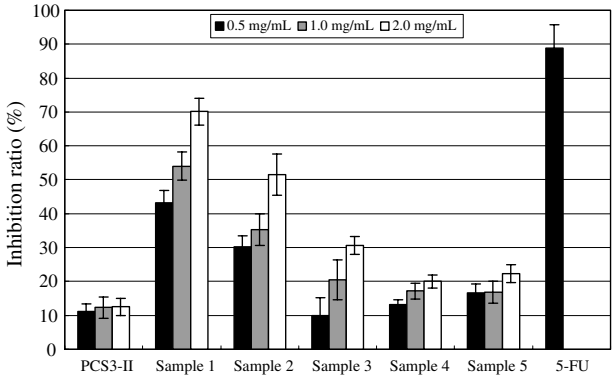

Figure 6. Inhibition ratio of proliferation of gastric carcinoma cell strain MKN-45 in vitro by different concentrations of PCS3-II and the five derivatives.

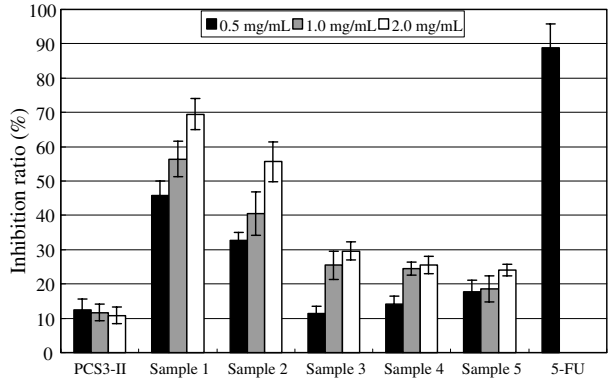

Figure 7. Inhibition ratio of proliferation of gastric carcinoma cell strain SGC-7901 in vitro by different concentrations of PCS3-II and the five derivatives.

ples 1 and 2 exhibit significantly higher inhibition ratios than PCS3-II at all concentrations, as with the results mentioned. Especially, S-PCS3-II at 2.0 mg/mL shows a high inhibition ratio of 71% against MKN-45, and 70% against SGC-7901, respectively, close to that for 5-FU. It may be noted that 5-FU is toxic, whereas polysaccharide derivatives are considered beneficial to the health. Obvious dose–dependency and time–dependency relationships are observed between the concentration of the derivatives and growth inhibition.

3.5. Correlation of structure to antitumor activity

The bioactivity results in vivo and in vitro show that chemical modification of the water-insoluble β-D-glucan from *Poria cocos* sclerotium leads to antitumor activity. The nature of the substitution group has an important effect. Introduction of sulfate and carboxymethyl groups significantly increases antitumor activity of the β-glucan. Those derivatives having relatively high DS values exhibit better antitumor activities as a result of the increased water solubility. The results in Figures 5–7, as well as Tables 1 and 2, show an increasing order of antitumor activity with increasing water solubility. The water-insoluble β-glucan PCS3-II shows almost no antitumor activity. It has been reported that periodate modification and mild hydrolysis of the β-D-glucan of *Poria cocos* sclerotium decreases the M_w and increases water solubility of the glucan, resulting in enhancement of antitumor activity.[29] Therefore, the water solubility of the samples is an important factor in the enhancement of antitumor activity. It is noteworthy that Samples 1 and 2 show stronger inhibition ratios in vivo and in vitro than the others and they exist as relatively expanded flexible chains in aqueous solution. We have shown a significant effect of expanded chain conformation of a sulfated glucan from *Ganoderma lucidum* in aqueous solution in enhancing the antitumor activity against Ehrlich ascites carcinoma.[30] Furthermore, some β-glucans having triple-helix structures show distinct antitumor activities, because of their stiff chain conformation.[31,32] It is concluded that, the relatively high chain stiffness of Samples 1 and 2 enhance their antitumor activity. In addition, Samples 4 and 5, having high M_w values in aqueous solution, exhibit lower antitumor activities than those of moderate M_w indicating the effect of molecular mass on bioactivity.

4. Conclusions

The M_w values of sulfated, carboxymethylated, methylated, hydroxyethylated, and hydroxypropylated β-D-glucan isolated from fresh *Poria cocos* sclerotium are 3.8×10^4, 18.9×10^4, 16.0×10^4, 20.1×10^4, and 19.1×10^4, respectively. However, the hydroxyethylated and hydroxypropylated glucans formed aggregates in aqueous solution, leading to high M_w. The water-insoluble native β-glucan showed no antitumor activity either in vivo or in vitro, whereas its derivatives exhibited good water solubility and manifest antitumor activities. The sulfation and carboxymethylation significantly enhanced the antitumor activities of the β-glucan against Sarcoma 180 and gastric carcinoma tumor cell in vivo and in vitro. Considering the molecular parameters and bioactivities, good water solubility, relatively high chain stiffness, and moderate molecular mass of the derivatives in aqueous solution are shown to be beneficial to enhancement of antitumor activity.

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