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Antitumor Activities of *O*-Sulfonated Derivatives of (1 → 3)-α-D-Glucan from Different *Lentinus edodes*

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Four water-insoluble (1 → 3)-α-D-glucans, coded L-II$_1$, L-II$_2$, L-II$_3$ and L-II$_4$, with different molecular weights were isolated from four kinds of fruiting bodies of *Lentinus Edodes*. The four α-D-glucans were *O*-sulfonated to obtain derivatives (SL-II) having degrees of substitution (DS) from 0.9 to 2.1 respectively. The structure of the samples was analyzed by infrared spectra, elemental analysis, and ^{13}C NMR. The weight-average molecular weight (M_w), radii of gyration ($\langle s^2 \rangle_z^{1/2}$) and intrinsic viscosity ([η]) of the native α-D-glucans and *O*-sulfonated derivatives were measured by size-exclusion chromatography combined with laser light scattering (SEC-LLS), LLS, and viscometry in 0.2 M aqueous NaCl and in dimethyl sulfoxide (DMSO) containing 0.25 M LiCl at 25 °C respectively. The M_w values of the *O*-sulfonated derivatives were much lower than those of the native α-D-glucans. The experimental results indicate that the *O*-sulfonated derivatives are water-soluble and exist as an expanded flexible chain in aqueous solution owing to intramolecular hydrogen bonding or interaction between charge groups. The *in vivo* and *in vitro* antitumor activities of the native α-D-glucans and their *O*-sulfonated derivatives against solid tumor Sarcoma 180 cells were evaluated and compared. Interestingly, all of the *O*-sulfonated derivatives exhibited higher antitumor activities than those of the native glucans. The results reveal that the effect of *O*-sulfonation of the α-D-glucan on the improvement of their antitumor activities was considerable.

Key words: (1 → 3)-α-D-glucans; *Lentinus edodes*; *O*-sulfonation; antitumor activity

Lentinus edodes is one of the most common edible mushrooms in China and Japan, and grows naturally on fallen wood in broadleaf forests. According to a Chinese physician of the Ming Dynasty (1368–1644), Wu Juei, it preserves health, improves stamina and circulation, cures colds, and lowers blood cholesterol.[1)] Chihara *et al.*[2)] have isolated and purified *lentinan*, a polysaccharide possessing significant antitumor activity, from hot-water extracts of *Lentinus edodes*. Sasaki and Takasuka[3)] have reported that *lentinan* is a branched (1 → 3)-β-D-glucans with (1 → 3)- and (1 → 6)-linked D-glucose residues, and that only the hydrolyzed fractions (molecular weight 1.6×10^4) show antitumor activity. Normally, most of the antitumor polysaccharides have the basic β-glucan structure such as (1 → 3)-β-linkages in the main chain of the glucan. However, obvious variations of antitumor polysaccharides have also been reported, such as α-glucan,[4,5)] and α-glucan protein.[6)] Shida[7)] *et al.* have isolated an α-heterogalactan and (1 → 3)-α-D-glucan from the fruiting bodies of *Lentinus edodes* by extraction with 3% trichloroacetic acid and 1 M aqueous NaOH respectively. To improve bioactivity, *O*-sulfonation of the (1 → 3)-β-D-glucan from *Lentinus edodes*, showing considerable anti-HIV activity as well as low anti-coagulant activities, has been investigated.[8)] In addition, a water-soluble product obtained by *O*-carboxymethylation of a linear (1 → 3)-α-D-glucan ($M_w = 5.6 \times 10^5$) has shown antitumor activity against Sarcoma 180 in mice.[9)] But the effects of α-glucan and its derivatives from *Lentinus edodes* with different strain species on bioactivity have scarcely been published.

In our laboratory, we have investigated the molecular weight and conformation of (1 → 3)-α-D-glucan from different *Lentinus edodes*.[10)] Moreover, the solution properties of *O*-sulfonated derivatives of (1 → 3)-α-D-glucan from *Lentinus edodes* have been studied in our laboratory, indicating stiffer chains than the native glucan.[11)] In this study, we attempted to investigate the antitumor activities of the four water-insoluble (1 → 3)-α-D-glucans with different molecular weight isolated from four kinds of fruiting bodies of *Lentinus edodes* and their water-soluble sulfated derivatives. The content of protein, weight-average molecular weight (M_w), and intrinsic viscosity ([η]) of the native α-D-glucans and *O*-sulfonated derivatives were measured by the Kjeldhal method, size-exclusion chromatography combined with laser light scattering (SEC-LLS), LLS, and viscometry respectively. The antitumor activities of the samples against the growth of Sarcoma 180 (S-180) solid tumor implanted in mice were tested, in the hope that our work

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might contribute meaningful information to understand the correlation of the molecular structure to the bioactivity of polysaccharides.

Experiments

Materials. Four kinds of *Lentinus edodes* with different strains (Hua 4, FL 66, Fcro 2, and FJ 1), coded as nos. 2, 36, 39, and 50, cultivated in bags filled with log pieces, were supplied by the Laboratory of Applied Mycology at Huazhong Agricultural University. Dimethylsulfoxide (DMSO) and pyridine were dehydrated with molecular sieves before use. The other reagents were analytical grade and were used without further purification.

Preparation of samples. The four kinds of (1 → 3)-α-D-glucans used in this study were isolated from different fruiting bodies of *Lentinus edodes* (154–189 g) by extraction with 5% NaOH/0.05% $NaBH_4$ two times, and neutralization with 36% acetic acid to remove β-glucan.[12] The precipitates containing α-glucan were washed with water five times, and finally lyophilized using lyophilizer (CHRIST Alpha 1–2, Osterode am Harz, Germany) to afford colorless flakes. The yields of the L-II_1, L-II_2, L-II_3, and L-II_4 samples, which were isolated from nos. 2, 36, 39, and 50 *Lentinus edodes* in order, were 2.7 g, 8.5 g, 8.4 g, and 14.2 g respectively, and were chosen for this study.

The sulfating agent was prepared using dry pyridine and chlorosulfonic acid on the basis of the CSA method described by Yoshida *et al.*[13] The L-II_1, L-II_2, L-II_3, and L-II_4 α-glucans (500 mg) were suspended in 50 ml DMSO at room temperature with stirring for 2 h, and then pyridine was added to the suspended solution with stirring for 30 min. Chlorosulfonic acid (for every 2 mol of pyridine, 1 mol chlorosulfonic acid) was added dropwise to the resulting solution with stirring, and then was heated to 60 °C for 3 h. After the reaction finished, the mixture was cooled, and the solution was adjusted to pH 10 with 10% aqueous NaOH, then the mixed solution was dialyzed against slightly alkaline water (pH 9) to remove pyridine. Finally the *O*-sulfonated derivatives were dialyzed in distilled water for 7 d, and the products were concentrated and freeze-dried to afford light yellow powder products, coded as SL-glucan. The yields of the SL-II_1, SL-II_2, SL-II_3, and SL-II_4 samples were 400 mg, 584 mg, 576 mg, and 571 mg respectively.

Analysis of structure. Infrared (IR) spectra of the native α-glucans and their *O*-sulfonated derivatives were recorded with a Nicolet Fourier transform infrared (FTIR) spectrometer (Spectrum One, Thermo Nicolet, Madison, WI, U.S.A.) in the range 4000–500 cm^{-1} using the KBr-disk method. The protein content in each polysaccharide was measured with a KJELETC 1030 semimicro Kjeldhal self-analyzer (Switzerland) according to the semi-micro Kjeldahl principle. Elemental compositions were determined for C, H, O, and S in the *O*-sulfonated derivatives with an elemental analyzer (EA) (Heraeus, Hanau, Germany). The ^{13}C NMR spectra were recorded on an INOVA-600 spectrometer (Varian, Palo Alto, CA, U.S.A.) at ambient temperature. The polymer concentration was adjusted to 5 wt % in all experiments. Dimethyl sulfoxide-d_6 (DMSO-d_6) was used as a solvent for native α-glucans, and D_2O for *O*-sulfonated derivatives. Chemical shifts were expressed relative to that of tetramethylsilane.

LLS measurement. The light scattering intensities of native sample solutions were determined with a multi-angle laser light scattering instrument (MALLS) equipped with a He–Ne laser ($\lambda = 633$ nm; Dawn® DSP, Wyatt Technology, Santa Barbara, CA) at the angles of 42, 49, 63, 71, 81, 90, 99, 109, 118, and 127° at 25 °C. The solutions of desired polysaccharide concentrations were prepared, and optical clarification of the solutions was achieved by filtration through a sand filter followed by a 0.2 μm pore-size filter (Whatman, Clifton. NJ, U.S.A.) into the scattering cell. Refractive index increments (dn/dc) were determined using an Optilab refractometer (DAWN® DSP, Wyatt Technology) at 633 nm. The dn/dc values of samples in 0.2 M aqueous NaCl and in 0.25 M LiCl/DMSO were determined to be 0.140 and 0.060 ml g^{-1} respectively. Astra software (Version 4.70.07) was utilized for data acquisition and analysis.

SEC-LLS measurement. SEC-LLS is convenient for determination of true molecular mass and its molecular distribution without the use of polymeric standards. SEC-LLS measurements of the native α-glucans and *O*-sulfonated derivatives were carried out on the above-mentioned multi-angle laser photometer combined with a P 100 pump (Termo Separation Products, San Jose, CA) equipped with a TSK-GEL G5000 and G3000 PWXL column (7.8 × 300 mm) in series for aqueous solution, or with a G4000 H_6 column (7.5 mm × 300 mm) for 0.25 M LiCl/DMSO, and by using a differential refractive index detector (RI-150, Kyoto, Japan) at 25 °C. The eluent was 0.2 M aqueous NaCl or 0.25 M LiCl/DMSO with a flow rate of 1.0 ml/min. All solutions having a polysaccharide concentration of 1.0×10^{-3} to 2.0×10^{-3} g/ml were filtered with a 0.45 μm Millipore filter (PTFE, Puradise 13 mm Syringe Filters, Whatman, Clifton. NJ, U.S.A.), then kept in sealed glass bottles before injection into the SEC column. Calibration of the photometer was done with ultra-pure toluene, and the normalization of the LLS detector was done with pullulan standards. Astra software (Version 4.70.07) was utilized for data acquisition and analysis.

Viscosity measurement. The intrinsic viscosity ([η]) of the samples was measured using an Ubbelohde capillary

viscometer at 25 °C. 0.2 M aqueous NaCl and 0.25 M LiCl/DMSO were used as solvents of the samples respectively. The kinetic energy correction was always negligible. Huggins and Kraemer plots were used to estimate $[\eta]$ by extrapolation to infinite dilution, as follows:

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

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

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

In vivo *antitumor test.* S-180 cells (5×10^6 cells/mouse), provided by the Pharmacy Laboratory of Tongji Medical College, Huazhong University of Science and Technology, were transplanted subcutaneously into the right groin of 8-week-old BALB/c mice weighing 18 ± 1 g, which were divided randomly into 10 groups of 10 mice per group. 5-Fluorouracil (5-Fu) and the tested samples were dissolved or suspended in 0.2 M aqueous NaCl respectively, and then injected intraperitoneally (i.p.) once daily for 10 d at 24 h after tumor inoculation. The same volume of 0.2 M aqueous NaCl was injected i.p. into the control mice, and 5-Fu was injected i.p. into another male mouse at a dose of 20 mg kg^{-1}. For the samples, the doses were 20 mg kg^{-1} and 60 mg kg^{-1} respectively. The mice were killed on the next day after the last injection, and the tumors were removed from the animals and weighed. The tumor weights were compared with those in the control mice. 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 weights of mice before and after the assay.

In vitro *antitumor test.* A colorimetric 3-(4,5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide (MTT) (Sigma) method was used to measure the proliferation of adherent tumor cells. The S-180 tumor cells (1×10^5 cells ml^{-1}) were grown in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% fetal bovine serium under an atmosphere of 5% carbon dioxide at 37 °C for 72 h, containing tested samples at concentrations of 0.005, 0.05, 0.5, and 5 mg/ml in aqueous NaCl solution. The number of living S-180 tumor cells at the end of the 72 h incubation period was determined colorimetrically based on the tetrazolium salt MTT, as described by Moshmann.[14] 5-Fu and the tested samples were compared with a control sample. All *in vitro* results were expressed as the inhibition ratio of tumor cell proliferation as follows:

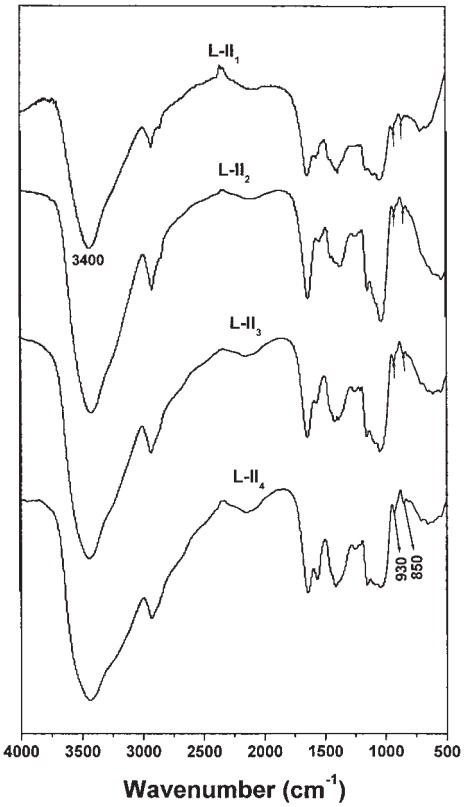

Fig. 1. FT-IR Spectra of the L-II$_1$, L-II$_2$, L-II$_3$, and L-II$_4$ Samples.

$$\text{inhibition ratio} = [(A - B)/A] \times 100\% \quad (5)$$

where A and B are the average numbers of viable tumor cells of the control and experimental samples respectively. All assays were made in triplicate.

Results and Discussion

Chemical structure

The IR spectra for the L-II$_1$, L-II$_2$, L-II$_3$, and L-II$_4$ samples are shown in Fig. 1. All samples exhibit the characteristic IR absorption of polysaccharide at 1650 cm^{-1} and clearly showed IR absorption at 850 and 930 cm^{-1}, characteristic of an α-D-glucan.[15] The spectrum of the SL-II$_3$ sample (Fig. 2) showed two new characteristic of absorption bands, one at 1240 cm^{-1} describing an asymmetrical S=O stretching vibration, and the other at 820 cm^{-1} indicating symmetrical C–O–S vibration. This suggests that the *O*-sulfonation reaction actually occurred.[16] The data for the element content in the *O*-sulfonated derivatives are listed in Table 1. The degree of substitution (DS), which designates the average number of *O*-sulfo groups on each sugar residue, was calculated from the results of EA on the basis of the determined sulfur content (S%) by the following formula:

$$DS = (162 \times S\%)/(32\text{–}102 \times S\%) \quad (6)$$

The water-soluble *O*-sulfonated derivatives (SL-II) possessed degrees of substitution (DS) from 0.9 to 2.1.

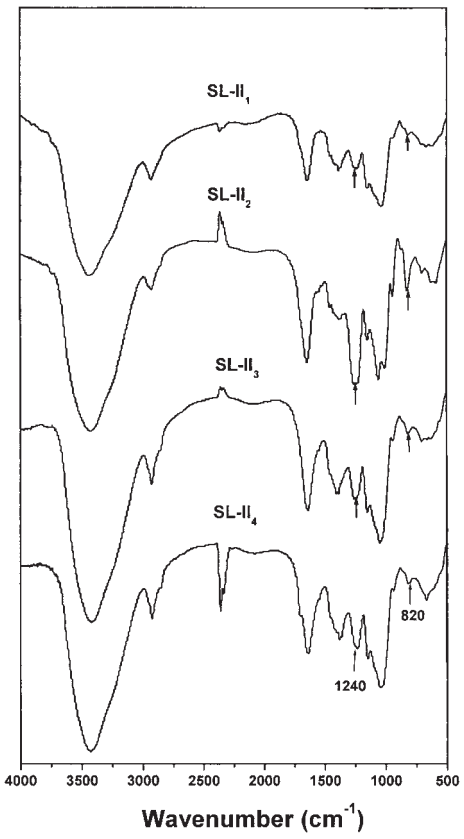

Fig. 2. FT-IR Spectra of the SL-II_1, SL-II_2, SL-II_3, and SL-II_4 Samples.

Table 1. Results of Elemental Analyses and Yields of the *O*-Sulfonated Derivatives

Sample	C (%)	N (%)	S (%)	DS	Yield (%)
SL-II_1	37.3	n.d	11.4	0.91	80.1
SL-II_2	29.0	n.d	15.0	1.46	~100
SL-II_3	22.9	0.7	17.8	2.08	~100
SL-II_4	34.5	n.d	11.5	0.92	~100

[a] not detected.

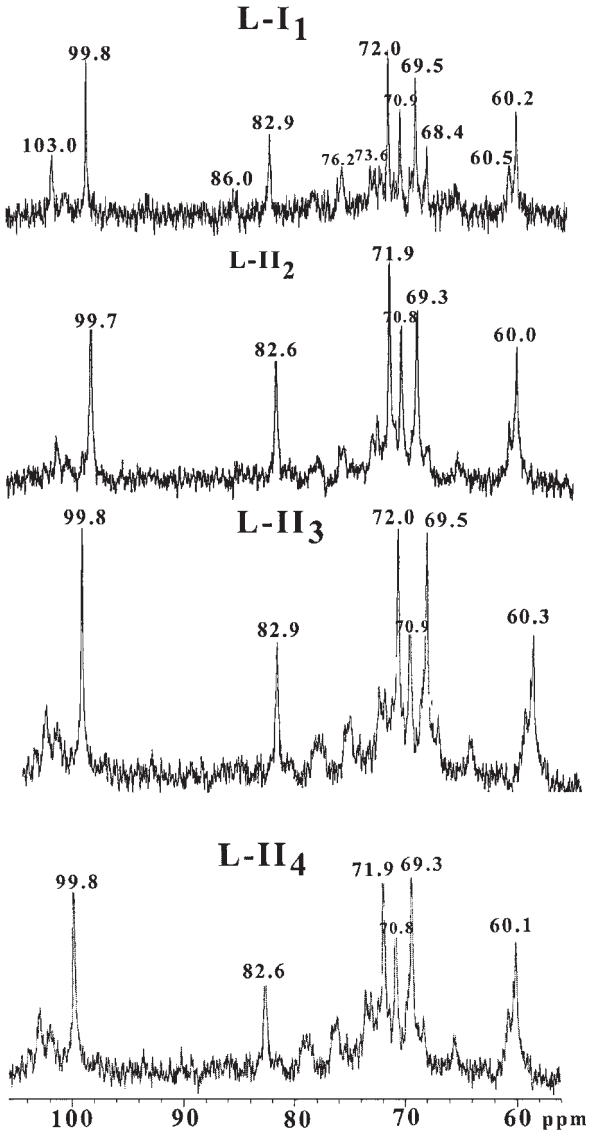

Fig. 3. ^{13}C NMR Spectra of the L-II_1, L-II_2, L-II_3, and L-II_4 Samples in DMSO-d_6 at 25 °C.

The ^{13}C NMR spectra of native α-glucans in DMSO-d_6 and *O*-sulfonated derivative in D_2O are shown in Figs. 3 and 4. Chemical shift data from the ^{13}C NMR spectra of all polysaccharides, together with those from the literature, are given in Table 2. The strong signals at 99.8 (α-C-1), 82.6 (α-C-3), 72.0 (α-C-5), 70.9 (α-C-2), 69.5 (α-C-4), and 60.2 (α-C-6) ppm in the native polysaccharides spectra indicate that the native polysaccharides were mostly α-(1 → 3)-D-glucans, and contained a small amount of (1 → 3)-β-D-glucans, 103.0 for C-1, 86.0 for C-3, 76.2 for C-5, 73.6 for C-2, 68.4 for C-4, and 60.5 ppm for C-6. It was very difficult completely to separate α- and β-glucans from the extract.[17] Compared with the ^{13}C NMR spectrum of the native α-glucan (L-II_3), there were new peaks, such as C-2s (80.0 ppm), C-4s (76.9 ppm), and C-6s (69.0 ppm), resulting from *O*-sulfonation at O-2, O-4, and O-6, with downfield shifts of the carbon atoms bearing *O*-sulfo groups of 8–10 ppm.[18] The position of the C-1′ signal (103.6 ppm) was influenced by the adjacent C-2's. The results indicate a nonselective *O*-sulfonation of the α-glucan.

Molecular parameters

Figure 5 shows the SEC chromatograms of the native α-glucans in 0.2 M aqueous NaCl and the *O*-sulfonated derivatives in 0.25 M LiCl/DMSO. The power law $\langle s^2 \rangle_z^{1/2} = KM_w^{\alpha}$ can be estimated from many experimental points M_w and $\langle s^2 \rangle_z^{1/2}$ in the SEC. The slopes α calculated from Fig. 5 are in the range of 0.49–0.56, indicating that the native α-glucans exist as a random coil in 0.25 M LiCl/DMSO. However, the α values (0.72–0.78) for the *O*-sulfonated derivatives are larger than those of a normal flexible chain (0.5–0.6), showing the character of a relatively stiff chain in 0.2 M aqueous

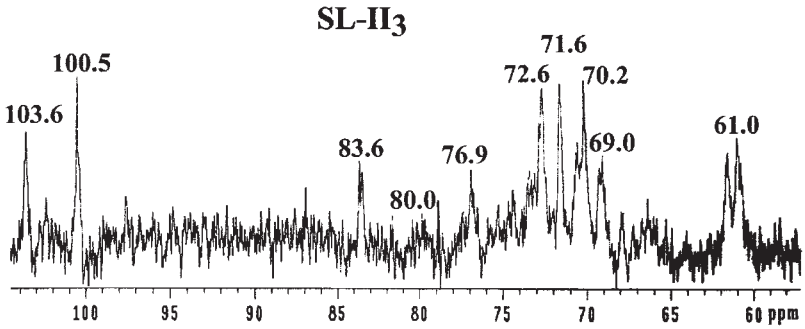

Fig. 4. ^{13}C NMR Spectrum of the SL-II$_3$ *O*-Sulfonated Derivative in D_2O at 40 °C.

Table 2. ^{13}C NMR Chemical Shifts of Native α-Glucans in D_2O and *O*-Sulfonated Derivatives in DMSO-d_6

Sample	Chemical shifts										Source
	C-1	C-2	C-3	C-4	C-5	C-6	C-1′	C-2s	C-4s	C-6s	
L-II$_1$	99.8	70.9	82.9	69.5	72.0	60.2					This work
L-II$_2$	99.7	70.8	82.6	69.3	71.9	60.0					This work
L-II$_3$	99.8	70.9	82.9	69.5	72.0	60.3					This work
L-II$_4$	99.8	70.8	82.6	69.3	71.9	60.1					This work
(1 → 3)-α-D-glucan	99.3	70.9	82.2	69.5	71.9	60.4					Ref. 18
SL-II$_3$	100.5	71.6	83.6	70.2	72.6	61.0	103.6	80.0	76.9	69.0	This work
O-sulfonated α-glucan	100.7	71.9	84.0	70.4	72.8	61.3	100.3	79.8	77.4	66.5	Ref. 11

NaCl. Figure 6 shows Zimm plots for L-II$_3$ and SL-II$_3$ samples in 0.25 M LiCl/DMSO at 25 °C. Here K is the light-scattering constant, R_θ is the reduced Rayleigh ratio at angle θ, and c is the polysaccharide concentration. From the results of LLS, SEC-LLS, and viscometry measurements, the M_w, $\langle s^2\rangle^{1/2}$ and $[\eta]$ values of the samples are summarized in Table 3. Generally, the relatively higher $[\eta]$ and $\langle s^2\rangle^{1/2}$ values reflect a relatively expanded chain of the polymer.[11] In addition, the M_w values of *O*-sulfonated derivatives are much lower than those of the original (1 → 3)-α-D-glucans, suggesting that the *O*-sulfonation process causes a decrease in M_w.[17] The relatively high values of $[\eta]$ and $\langle s^2\rangle^{1/2}$ reflect the chain stiffness of the *O*-sulfonated α-glucans. The conclusions are in good agreement with those from α in $\langle s^2\rangle_z^{1/2} = KM_w^{\alpha}$.

In vivo *results*

The antitumor activities against S-180 solid tumor of the native α-glucans and *O*-sulfonated derivatives are summarized in Table 4. All of the *O*-sulfonated derivatives exhibited higher antitumor activities than the native ones. In our previous work, the antitumor activities of (1 → 3)-β-D-glucan with triple helix extracted from the fruiting body *Lentinus edodes* were higher than others with flexible chains. Moreover, our data indicated that the α and β configuration of the *O*-sulfonated derivatives for the glucan hardly change the antitumor activity against S-180.[19] Therefore, the chain stiffness of the polysaccharides plays a role on the improvement of antitumor activity. In view of the data in Table 4, the antitumor activity of the *O*-sulfonated derivatives increases with increases in dose. It is worth noting that activities of the derivatives are slightly lower than that of 5-Fu, but their enhancement ratios of body weight are much higher than that of 5-Fu, implying that the polysaccharide derivatives might be less toxic than 5-Fu, which kills normal cells as well as cancer cells. This result is in good agreement with previous findings, which indicated the antitumor action of (1 → 3)-β-D-glucan is derived from stimulation of the immunoresponse mechanism of the host.[20] In our laboratory, two *O*-sulfonated derivatives of the (1 → 3)-α-D-glucan, extracted from both the fruiting body of *Canoderma lucidium* and the curdlan exhibited significantly higher antitumor activity against Ehrlich ascites carcinoma (EAC).[17] It is indicated that *O*-sulfonation of the (1 → 3)-α-D-glucan can improve its water solubility and antitumor activity.

In vitro *results*

Figure 7 shows the inhibition ratios against S-180 tumor cells of the native α-glucans and *O*-sulfonated derivatives *in vitro*. The *O*-sulfonated derivatives all exhibited relatively stronger inhibition ratios at all concentration levels than the native samples. In particular, the SL-II$_2$ and SL-II$_3$ samples at 0.5 mg ml^{-1} showed high activity, close to that of 5-Fu at 0.005 mg/ml, but no obvious dose-dependence relationship was observed between concentration of the derivatives and inhibition against 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-

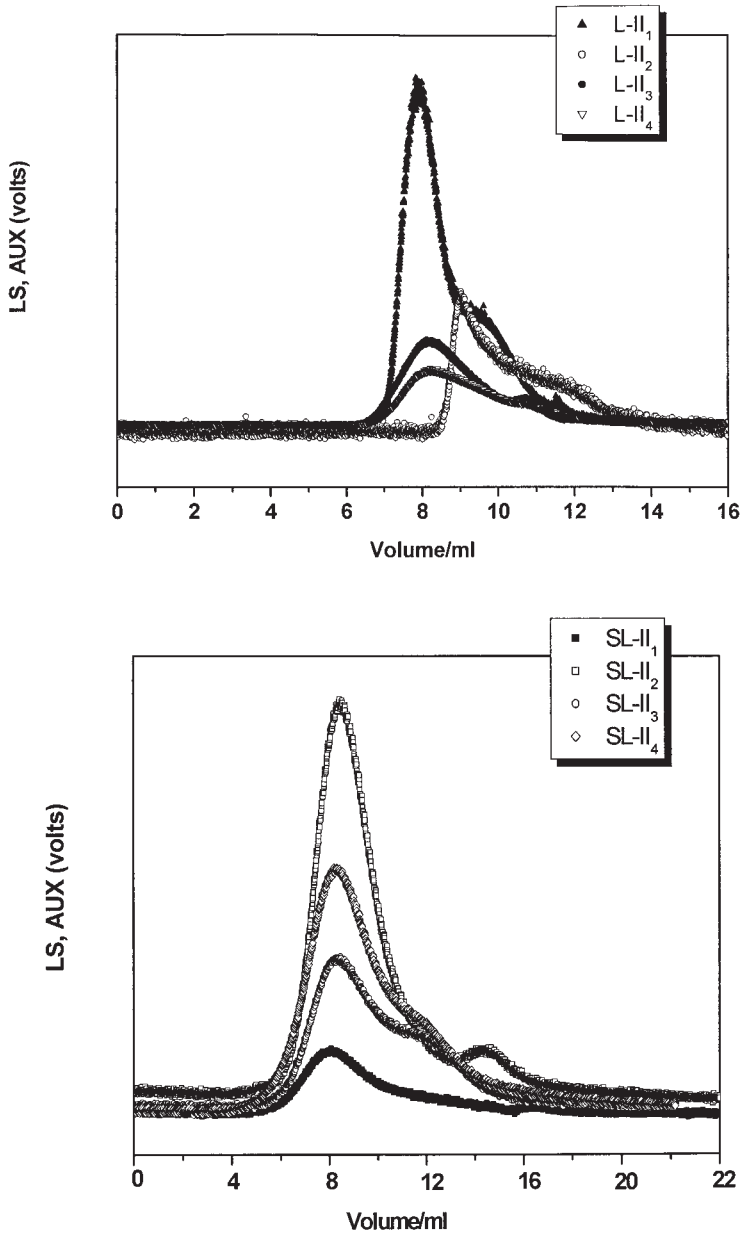

Fig. 5. SEC-LLS Chromatograms of Native α-Glucans in 0.25 M LiCl/DMSO (top) and *O*-Sulfonated Derivatives in 0.2 M Aqueous NaCl (bottom).

Table 3. Results of Viscosity and Molecular Weights of α-Glucans in 0.25 M LiCl/DMSO and Their *O*-Sulfonated Derivatives in 0.2 M Aqueous NaCl at 25 °C and Protein Content of the Samples

Sample	Solubility[a]		$[\eta]$ ($cm^3\ g^{-1}$)	k'	LLS	SEC-LLS		Protein content (%)
	Water	0.25 M LiCl/DMSO			$M_w \times 10^{-4}$	$\langle s^2\rangle^{1/2}$ (nm)	$M_w \times 10^{-4}$	
$L\text{-}II_1$	−	++	306.5	0.43	79.9	58.8	81.4	0.35
$L\text{-}II_2$	−	++	300.6	0.41	79.6	73.4	75.7	1.5
$L\text{-}II_3$	−	++	345.1	0.41	93.9	80.7	101.2	5.6
$L\text{-}II_4$	−	++	275.4	0.51	68.2	62.9	72.1	0.3
$SL\text{-}II_1$	+	+	208.9	0.48	40.8	43.3	45.3	<0.01
$SL\text{-}II_2$	+	+	200.6	0.51	37.6	65.6	40.8	<0.01
$SL\text{-}II_3$	+	+	233.4	0.51	52.0	58.5	55.3	2.9
$SL\text{-}II_4$	+	+	194.5	0.48	31.7	57.8	41.5	<0.01

[a], Determined by naked eye; ++, highly soluble; +, soluble; −, insoluble.

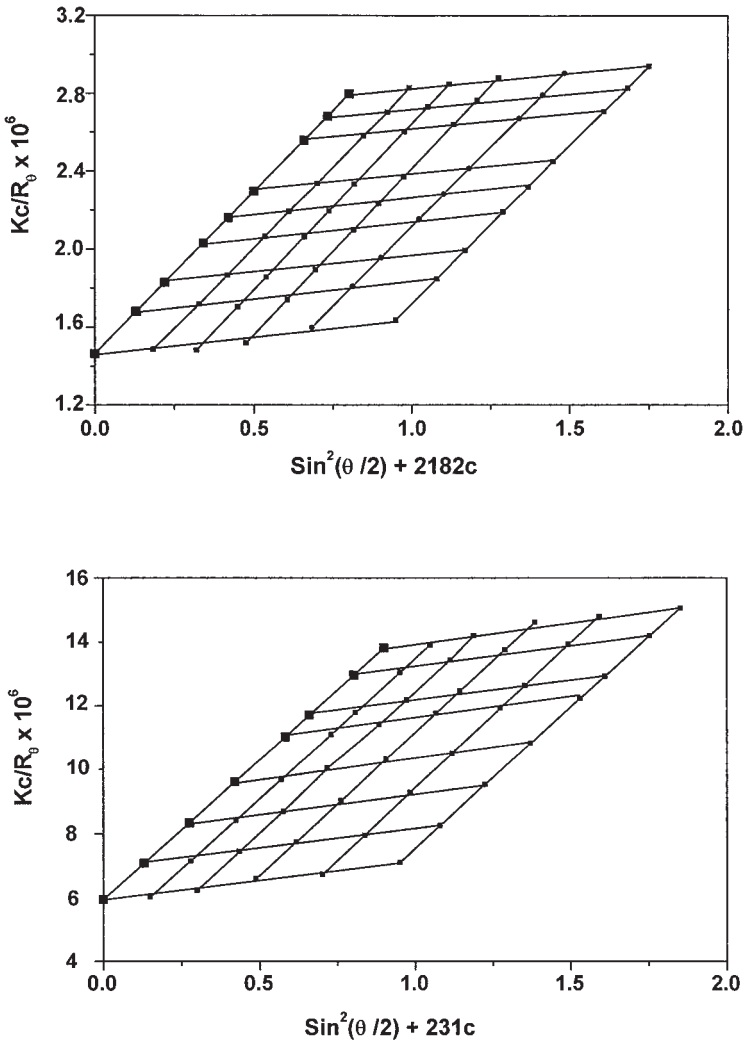

Fig. 6. Zimm Plots of the L-II$_3$ in DMSO/0.25 M LiCl (top) and SL-II$_3$ in 0.2 M Aqueous NaCl (bottom).

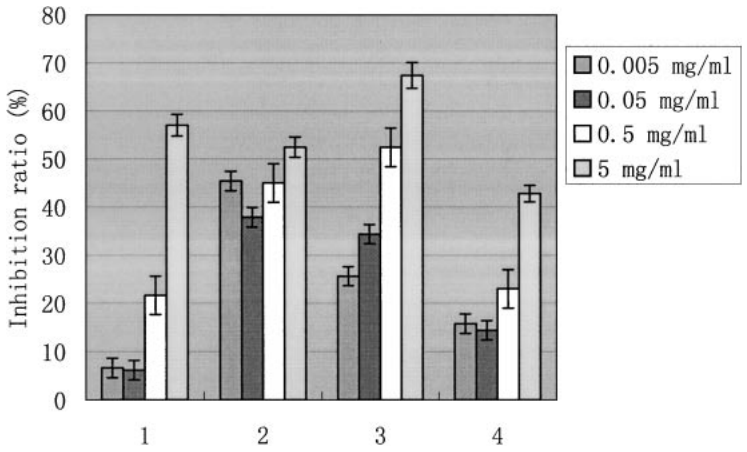

Fig. 7. Inhibition Ratios of Proliferation of Sarcoma-180 Tumor Cells *in Vitro* at Different Concentrations of SL-II$_1$ (1), SL-II$_2$ (2), SL-II$_3$ (3), and SL-II$_4$ (4) Samples.

dependency of chemotherapeutic anticancer agents.[21] The SL-II$_3$ sample exhibited significantly higher inhibition ratios than native L-II$_3$, according with the results mentioned above. Furthermore, the *O*-sulfonated derivatives of L-II glucans (M_w of 43.8×10^4 to 55.4×10^4) exhibited higher antitumor activity both *in vivo* and *in*

Table 4. *In Vivo* Antitumor Activities against Sarcoma 180 Solid Tumors of the Native α-Glucans and their *O*-Sulfonated Derivatives

Sample	Dose (mg/kg × 10 d)	Mice	W_{tumor} (g)	Inhibition ratio (%)	Enhancement ratio of body weight (%)
Control		10/10	1.26 ± 0.26		49.6
FU-5	20	10/10	0.48 ± 0.28	62.0	18.9
L-II$_1$	20	10/10	1.14 ± 0.40	9.8	46.9
	60	10/10	0.86 ± 0.32	31.7*	34.4
L-II$_2$	20	10/10	1.06 ± 0.41	15.9	50.7
	60	10/10	1.05 ± 0.41	16.7	47.8
L-II$_3$	20	10/10	0.94 ± 0.34	25.4	43.4
	60	10/10	0.88 ± 0.25	30.5	46.3
L-II$_4$	20	10/10	1.12 ± 0.40	4.0	50.5
	60	10/10	1.21 ± 0.46	11.5*	38.2
Control		10/10	1.57 ± 0.66		47.7
FU-5	20	10/10	0.76 ± 0.16	51.6	20.4
SL-II$_1$	20	10/10	1.40 ± 0.34	10.8	57.6
	60	10/10	0.69 ± 0.30	56.1*	32.6
SL-II$_2$	20	10/10	1.04 ± 0.22	33.8*	45.6
	60	10/10	0.68 ± 0.27	56.2	31.7
SL-II$_3$	20	10/10	1.22 ± 0.34	22.2	41.2
	60	10/10	1.64 ± 0.41	59.0*	36.4
SL-II$_4$	20	10/10	1.12 ± 0.35	28.6	50.7
	60	10/10	0.81 ± 0.25	48.4*	38.8

* $P < 0.05$, significant difference when compared to control.

vitro than native α-glucans. Normally, the certain degree of reduction in M_w for the sample should promote enhancement of water solubility and bioactivities.

Conclusion

Water-soluble sulfonated derivatives were satisfactorily synthesized from (1 → 3)-α-D-glucan from different *Lentinus edodes* by reaction with chlorosulfonic acid in pyridine at 60 °C for 3 h. *O*-sulfonated α-D-glucan exists as an extended flexible chain in 0.2 M aqueous NaCl, owing to intramolecular hydrogen bonding and interaction caused by *O*-sulfo groups. All the *O*-sulfonated derivatives exhibited higher *in vivo* and *in vitro* antitumor activities than native L-II glucans. The *O*-sulfonation of (1 → 3)-α-D-glucan plays an important role in the improvement of antitumor activity. The water-solubility and expanded chain conformation of the *O*-sulfonated derivatives in aqueous solution can enhance antitumor activity against S-180.

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