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Correlation between antitumor activity, molecular weight, and conformation of lentinan

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Abstract—A (1→3)-β-D-glucan having (1→6) branching (L-FV-IB) from *Lentinus edodes* in water was degraded into seven fractions of different molecular weights by ultrasonic irradiation, and each was further fractionated into three parts, by precipitation from water into acetone at room temperature. The weight-average molecular weight (M_w), radius of gyration ($<S^2>_z^{1/2}$), and intrinsic viscosity ($[\eta]$) of lentinan and its fractions in 0.9% NaCl aqueous solution and dimethyl sulfoxide (Me_2SO) were determined by size-exclusion chromatography combined with multi-angle laser light scattering (SEC-LLS), LLS, and viscometry. Analysis of M_w, $[\eta]$, and $<S^2>_z^{1/2}$ in terms of known theory for worm-like chains yielded $2240 \pm 100\,\text{nm}^{-1}$, and $100 \pm 10\,\text{nm}$ for molar mass per unit contour length (M_L), and persistence length (q), respectively, corresponding with theoretical data for triple-helical chains. The $[\alpha]_D$ of lentinan in water–Me_2SO mixtures indicated an order–disorder transition. The results indicated that lentinan exists as a triple helix in 0.9% NaCl aqueous solution and as a single flexible chain in Me_2SO. Assays in vivo and in vitro against the growth of Sarcoma 180 solid tumor as well as the colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method for lentinan showed that the triple-helix sample exhibited a relatively high inhibition ratio. Interestingly, the triple-helix lentinan with M_w of 1.49×10^6 exhibited the highest antitumor activity in vivo, having an inhibition ratio (ξ) of 49.5%, close to that of 5-fluorouracil ($\xi = 50.5\%$), whereas the bioactivity ($\xi = 12.3\%$) of its single flexible chains almost disappeared. The triple-helix conformation plays an important role in enhancing the antitumor effects of lentinan.

Keywords: Lentinan; Antitumor activity; Molecular weight; Light scattering; Size-exclusion chromatography; Triple helix

1. Introduction

Fungal polysaccharides of the (1→3)-β-D-glucan type, such as schizophyllan from *Schizophyllum commune*,[1,2] scleroglucan from *Sclerotium*,[3,4] and grifolan from *Grifola frondosa*,[5,6] belong to the group of so-called biological-response modifiers, many of which have antitumor properties.[7–10] These effects are believed to arise from stimulation of a cell-mediated immune response rather than a direct cytotoxic action,[11,12] but much controversy still surrounds the relationship between structure and antitumor activity. For instance, ultrastructure, including triple-helix conformation and high molar mass has been considered to be important.[13,14] In contrast, Demleitner et al.[15] have proposed that the (1→3)-β-glucosidic linkage is the essential structural feature for immunostimulatory and antitumor effects, and no high molar mass is required. However, there are some antitumor polysaccharides having other chemical structures, such as hetero-β-glucan,[16] heteroglycan,[17] α-glucan,[18] and α-glucan–protein.[19]

Maeda and Chihara have reported that lentinan, the (1→3)-β-D-glucan, obtained from *Lentinus edodes*, has a strong antitumor activity against sarcoma 180 implanted subcutaneously in mice.[20] Lentinan has been demonstrated by high-performance liquid chromatography (HPLC), infrared (IR), and ^{13}C NMR spectroscopy to be a (1→3)-β-D-glucan having two (1→6)-glucosyl side groups for every five residues.[21] Our previous work, employing light scattering indicated that lentinan in

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0.5 M NaCl aqueous solution exists predominantly as triple-helical chains with high rigidity, and in dimethyl sulfoxide (Me_2SO) as single-flexible chains.[22] The triple-helical structure can be dissociated (denatured) by increasing the temperature in aqueous solution,[23] by dissolving the molecules in Me_2SO with a water weight fraction (W_H) less than 0.18 at 25 °C,[24] or in aqueous hydroxide.[25] Although the molecular weight, conformation, and solubility of the polysaccharides significantly affect antitumor and immunomodulatory activity,[26–28] there is lack of strong evidence. It is thus important to investigate the influence of M_w and high-order structure on the antitumor activity of lentinan. In the present work, lentinan was degraded by ultrasonic irradiation to obtain samples having different molecular weights while retaining the native chemical structure and conformation.[29,30] The conformations of lentinan and its fractions were investigated by SEC-LLS, viscometry, and optical rotation in 0.9% NaCl aqueous solution and in Me_2SO. Moreover, the antitumor activities both in vivo and in vitro were tested to assess the correlation of molecular weight and conformation to antitumor activities.

2. Materials and methods

2.1. Materials

The fruiting bodies of *L. edodes* were cultivated in Shen Longjia of Hubei in China. All chemical reagents were of analytical grade, and were purchased from Tianjing Fucheng Chemical Reagent Corporation (China). Me_2SO was distilled, and treated with molecular sieves for further dehydration.

2.2. Sample preparation

Native lentinan, coded as L-FV-IB, was isolated from fruiting bodies of *L. edodes* by extraction with 5% NaOH–0.05% $NaBH_4$ twice, and precipitation with 36% AcOH to remove (1→3)-α-D-glucan, according to a previously reported method.[21] The supernatant was deproteinized (Sevag method), and decolorized with 30% H_2O_2. An aqueous solution of the lentinan was dialyzed against distilled water for 5 days, and concentrated by rotary evaporation below 45 °C, and finally lyophilized to afford colorless flakes.

The (1→3)-β-D-glucan solution in distilled water (0.2 wt %, 600 mL) was exposed to 33 kHz ultrasonic irradiation by an ultrasonic cleaner (MUS-1004, Shenzhen Modern Ultrasonic Industrial Co., Ltd., China) for 1–24 h afford the fractions having different molecular weights, coded as L-FV-IB1, L-FV-IB2, L-FV-IB3, L-FV-IB4, L-FV-IB5, L-FV-IB6, L-FV-IB7. The jacket of the sonication vessel was maintained below 35 °C. The seven sonicated solutions were further fractionated, and each separated into three parts by precipitation from water by acetone at room temperature, and coded as L-FV-IBX1, L-FV-IBX2, and L-FV-IBX3 (X represents sonicated fractions from 1 to 7), respectively. The fractions were dissolved in distilled water and dialyzed against distilled water for 4 days. The resulting transparent solutions were concentrated by rotary evaporation under reduced pressure below 45 °C, and lyophilized to afford colorless flakes.

The fractions coded as L-FV-IB21 and L-FV-IB31 were dissolved in Me_2SO and then precipitated by acetone at room temperature after dissolution for 24 h to obtain single glucan chain. The samples were purified by acetone three times and lyophilized to obtain colorless flakes, coded as L-FV-IB21D and L-FV-IB31D. Elemental analysis of the samples showed no sulfur, indicating that Me_2SO had been completely removed.

2.3. SEC-LLS measurements

Size-exclusion chromatography combined with multi-angle laser light scattering (SEC-LLS) is a convenient method for the determination of true molecular weight and distribution without standard samples. SEC-LLS measurements were carried out on a multi-angle laser photometer (DAWN® DSP, Wyatt Technology Co. Santa Barbara, CA, USA) at 633 nm combined with a P100 pump (Thermo Separation Products, San Jose, USA) equipped with TSK-GEL G6000 PWXL and a G4000 PWXL column (7.8 mm × 300 mm) for an aqueous solution, and with G4000-H8 and G3000H8 column for Me_2SO, and a differential refractive index detector (RI-150, Japan) at 25 °C. The eluent was a 0.9% aqueous NaCl and Me_2SO at a flow rate of 1.00 mL min^{-1}. The samples were dissolved in 0.9% NaCl and Me_2SO overnight with stirring. All solutions were filtered through a 0.45-μm filter (CA, Puradisc™ 13 mm Syringe Filters, Whatman, England) for 0.9% NaCl and a 0.45-μm filter (PTFE, Puradisc™ 13 mm Syringe Filters, Whatman, England) for Me_2SO, and then degassed before use. The injection volume was 200 μL with concentrations of 0.1–0.2% for each sample. Astra software was utilized for data acquisition and analysis.

2.4. Viscometry

Viscosity of the samples in 0.9% aqueous NaCl and in Me_2SO was measured at 25 ± 0.1 °C using a low-shear four-bulb capillary viscometer and Ubbelohde viscometer, respectively. All solutions were filtered through a sand filter. The kinetic energy correction was negligible. Huggins and Kraemer equations were used to estimate the intrinsic viscosity ($[\eta]$) by extrapolation to concentration (c) of zero 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 at given temperature in given solvent, η_{sp}/c is the reduced specific viscosity, and $(\ln \eta_r)/c$ is the inherent viscosity. From the dependence of intrinsic viscosity on shear rate ($\dot{\gamma}$), the zero shear-rate viscosity ($[\eta]$) for the samples in 0.9% NaCl aqueous solution was determined.

2.5. Optical rotation

Lentinan was dissolved directly in the desired water–Me_2SO mixtures for 12 h, and the specific optical rotation at 589 nm was measured on a Perkin–Elmer 341 polarimeter in a jacketed standard cell (10 cm/6.2 mL, Perkin–Elmer). All solutions were kept at 20 °C during the measurements.

2.6. Assay of the antitumor activity

2.6.1. In vivo antitumor test. Sarcoma 180 (S-180) tumor cells (1×10^5 cells/mouse) were subcutaneously inoculated into 8-week-old male BALB/c mice. 5-Fluorouracil (5-Fu) and the tested samples were dissolved in 0.9% aqueous NaCl, and injected intraperitoneally once a day for 10 days, starting 24 h after tumor inoculation. The same volume of 0.9% aqueous NaCl was injected intraperitoneally into the control mice. The mice were killed on the next day after the last injection, and the tumors were excised. 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 weight of mice before and after the assay. Complete regression is indicated as the ratio of the number of tumor-free mice to the number of mice tested. Statistical evaluations in all experiments were performed by a Student's *t*-test. A P value of less than 0.05 was considered significant.

2.6.2. In vitro antitumor test. A colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method was used for measuring the proliferation for adherent tumor cells. The S-180 tumor cells (1×10^5 cells/mL) were grown in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% fetal bovine serum under an atmosphere of 5% carbon dioxide at 37 °C for 72 h, containing tested samples at concentrations of 0.005, 0.05, and 0.5 g/L in 0.9% aqueous NaCl. 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 Mosmann.[31] 5-Fu and the tested samples were compared with a control sample. All in vitro results were expressed as the ratio of inhibition (ξ) of tumor cell proliferation calculated as follows:

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

where A and B are the average number of viable tumor cells of the control and experimental samples, respectively.

3. Results and discussion

3.1. Molecular weight

SEC chromatograms for the fractions in 0.9% aqueous NaCl are shown in Figure 1. 'LS, AUX (volts)' represents an arbitrary unit of scattering intensity. The values of weight-average molecular weight (M_w), radius of gyration ($<S^2>_z$),[1,2] and $[\eta]$ for the samples are listed in Table 1. The error margin of measurement for M_w and $<S^2>_z$ by SEC-LLS is <5%. The values of M_w and $[\eta]$ for the fractions decrease with increase of ultrasonic time, and the polydispersity (M_z/M_w) of the fractions mainly lies in the range from 1.07 to 1.48, indicating a relatively narrow distribution of M_w. SEC chromatograms of the ultrasonic glucans in 0.9% aqueous NaCl all show only a sharp peak, indicating that pure triple-helical chains, without fragments of single chains with lower molecular weight, had been obtained

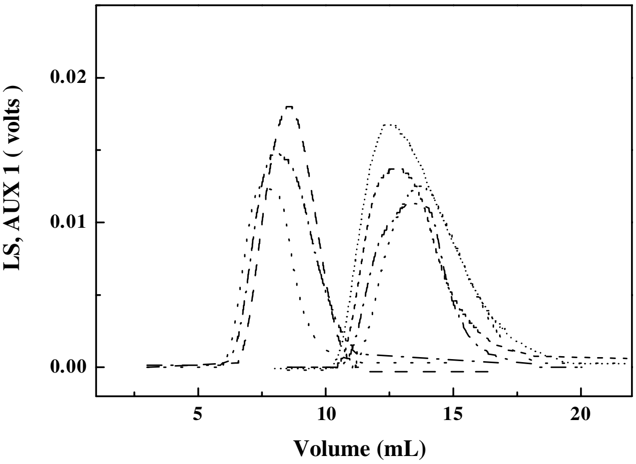

Figure 1. SEC chromatograms of the lentinan fractions L-FV-IB11, L-FV-IB21, L-FV-IB31, L-FV-IB41, L-FV-IB32, L-FV-IB52, L-FV-IB62, L-FV-IB73 (from left to right) in 0.9% aqueous NaCl at 25 °C by SEC-LLS.

Table 1. Experimental results from SEC-LLS and viscometry for the β-D-glucan L-FV-IB and fractions in 0.9% aqueous NaCl solution at 25 °C

Samples	$M_w \times 10^{-5}$	$[\eta] \times 10^{-2}$ (g^{-1} cm^3)	k'	$<S^2>_z^{1/2}$ (nm)	M_z/M_w
L-FV-IB11	28.3	21.4	0.40	160	1.45
L-FV-IB21	18.4	16.3	0.46	132	1.10
L-FV-IB22	16.5	14.8	0.39	—	1.26
L-FV-IB31	14.9	11.7	0.43	115.1	1.10
	12.2[a]			137.8[a]	
L-FV-IB41	11.4	9.50	0.42	97.0	1.07
L-FV-IB32	9.79	7.25	0.37	87.7	1.26
L-FV-IB52	5.71	4.05	0.38	60.5	1.48
L-FV-IB62	5.27	3.36	0.37	60.0	1.17
L-FV-IB43	4.2	—	—	45.0	1.24
L-FV-IB73	3.57	2.02	0.42	39.9	1.10
L-FV-I6	2.73[b]	1.07[b]	0.46[b]	—	1.17
L-FV-I7	1.87[b]	0.71[b]	0.44[b]	—	1.23

[a] Data from Zimm plot of LLS.

[b] Data taken from Ref. 22.

by ultrasonic irradiation and by fractionating with precipitation from water to acetone.

The M_w dependences on $[\eta]$ for the fractions in 0.9% aqueous NaCl and in Me_2SO at 25 °C are illustrated in Figure 2. From the data points for M_w and $[\eta]$ in the M_w range from 1.87×10^5 to 1.84×10^6, the Mark–Houwink equation of lentinan in 0.9% aqueous NaCl at 25 °C are established as the following:

$$[\eta] = 2.94 \times 10^{-7} M_w^{1.58} \quad (M_w < 6.0 \times 10^5), \tag{6}$$

$$[\eta] = 2.30 \times 10^{-5} M_w^{1.25} \quad (M_w > 6.0 \times 10^5). \tag{7}$$

The high exponent of the $[\eta]$–M_w relationship reflects the character of the stiff chains, and is similar to triple-helix schizophyllan.[32,33] An approximated slope of the $[\eta]$–M_w relationship was estimated from five samples in Me_2SO to be 0.54. Usually, the exponent of a flexible polymer in a good solvent is from 0.5 to 0.8. This suggests that lentinan exists as flexible chains in Me_2SO.

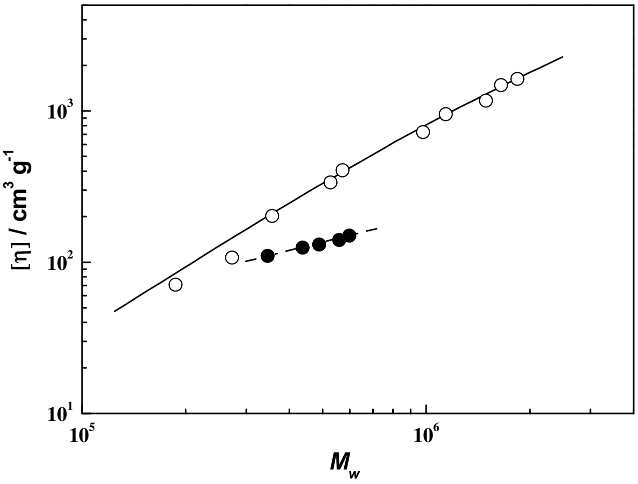

Figure 2. M_w dependence of $[\eta]$ for the lentinan fractions in 0.9% aqueous NaCl (○) and Me_2SO (●) at 25 °C. The full line shows values calculated from Yamakawa–Yoshizaki theory for a wormlike cylinder[35] for q = 100 nm and with M_L and d fixed at 2240 nm^{-1} and 2.2 nm, respectively.

3.2. Chain conformation

The rigidity of a wormlike chain is defined by the molar mass per unit contour length (M_L) and the persistence length (q). The Benoit–Doty expression[34,40] for $<S^2>$ of the Kratky–Porod wormlike chain is as follows:

$$<S^2> = (qM_w/3M_L) - q^2 + (2q^3M_L/M_w)[1 - (qM_L/M_w)(1 - e^{-M_w/qM_L})] \tag{8}$$

and can be approximated by[35,41]

$$(M_w^2/12 <S^2>)^{2/3} = M_L^{4/3} + (2/15)(M_L^{1/3}/q)M_w. \tag{9}$$

Eq. 9 is suitable for $M_w/2qM_L < 2$. Thus M_L and q were calculated as 2180 ± 100 nm^{-1} and 100 ± 10 nm, respectively, according to Eq. 9. As shown in Figure 3, the dashed line represents values computed from Eq. 8 with M_L = 2180 nm^{-1} and q = 100 nm. Obviously, the data points of M_w and $<S^2>_z$ of lentinan agree most satisfactorily with the theoretical curve at a M_w range from 1.87×10^5 to 18.4×10^5 g mol^{-1}.

The Yamakawa–Yoshizaki theory[36] for $[\eta]$ of the worm-like cylinder chain can be approximated by Bohdanecky's equation[37,38]

$$(M_w^2/[\eta])^{1/3} = I + SM_w^{1/2}, \tag{10}$$

$$I = 1.516 \times 10^{-8} A_0 M_L \quad (g^{1/3}\ cm^{-1}), \tag{11}$$

$$S = 1.516 \times 10^{-8} B_0 (2q/M_L)^{-1/2} \quad (g^{1/3}\ cm^{-1}), \tag{12}$$

where the values of A_0 and B_0 depend on q and d (the hydrodynamic diameter of cylinder), and can be ob-

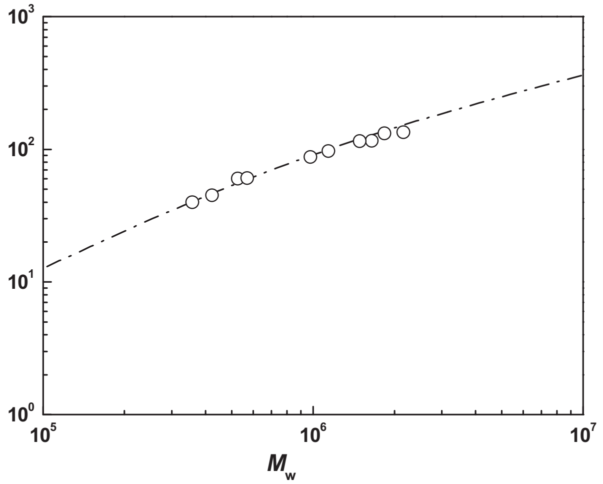

Figure 3. M_w dependence of $<S^2>_z^{1/2}$ for the lentinan fractions in 0.9% aqueous NaCl at 25 °C. Dashed lines depict theoretical values calculated from Eq. 8 with $M_L = 2180\ \mathrm{nm}^{-1}$ and $q = 100$ nm.

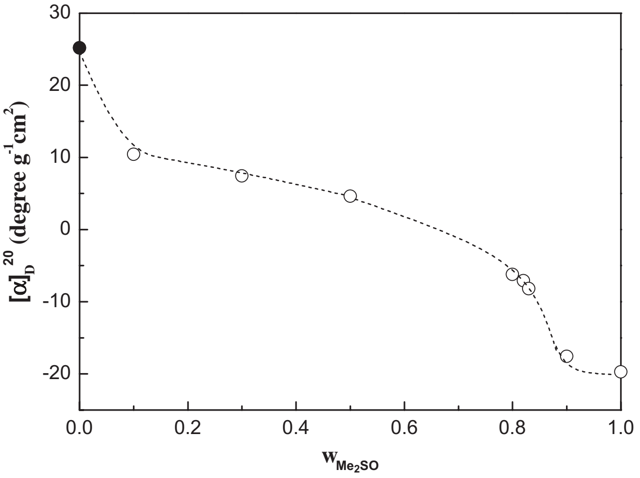

Figure 4. w_{Me_2SO} dependence of $[\alpha]_D$ for lentinan in water–Me_2SO mixtures at 20 °C. Filled circle represent the data of lentinan in 0.9% aqueous NaCl.

tained from the literature.[38] The M_L, q, and d values for lentinan in aqueous solution were calculated as $2240 \pm 100\ \mathrm{nm}^{-1}$, 100 ± 10 nm, and 2.2 ± 0.6 nm, respectively. As shown in Figure 2, the line represents the Yamakawa–Yoshizaki theory curve for a triple-helix chain[37] with $M_L = 2240\ \mathrm{nm}^{-1}$, $q = 100$ nm, and $d = 2.2$ nm. The data points of M_w and $[\eta]$ of lentinan are in agreement with the theoretical curve at the M_w range from 1.87×10^5 to 1.84×10^6. The values of M_L and q of the glucans are close to the values determined from $<S^2>_z$, and both are in good agreement with theoretical data for triple-helical chains, and close to that of triple-helix schizophyllan.[32,33] Therefore, it is been strongly confirmed that lentinan exists as a triple-helix conformation in 0.9% aqueous NaCl.

The order–disorder transitions of a helix are often accompanied by a change, that may be monitored by optical rotation.[39] The Me_2SO weight fraction (w_{Me_2SO}) dependence of $[\alpha]_D$ for lentinan in water–Me_2SO mixtures at 20 °C is shown in Figure 4. The values of $[\alpha]_D$ decrease with an increase of w_{Me_2SO}. When w_{Me_2SO} increases from 0.80 to 0.90, the $[\alpha]_D$ sharply decreases, accompanying a conformational transition from triple helix to single flexible chains, being thus similar to the results from viscosity and ^{13}C NMR.[24]

3.3. Antitumor activity

The results of antitumor activities of the fractions from L-FV-IB11 to L-FV-IB73 against Sarcoma 180 tumor in mice are summarized in Table 2, which also lists the results obtained with 5-fluorouracil (5-Fu) in parallel tests. The differences in antitumor activity for the samples having a triple helix are considered to be an effect of the molecular weight. Obvious antitumor activities are observed in all eight samples, and the samples having a relatively high M_w of $1.49 \times 10^6\ \mathrm{g\ mol}^{-1}$ for L-FV-IB31 at high dose and with a lower M_w of $5.71 \times 10^5\ \mathrm{g\ mol}^{-1}$ for L-FV-IB52 exhibit higher inhibition ratios than the other samples. It is noteworthy that L-FV-IB31 shows high activity equivalent to that of 5-Fu. Interestingly, the enhancement ratios of body weight for the eight samples are much more than that for 5-Fu group, especially for L-FV-IB31 at a dose of 60 mg/kg. This implies that the lentinan samples do not have the same toxicity as 5-Fu, which kills normal cells as well as cancer cells.

To clarify the effect of the chain conformation on bioactivity, the triple-helix lentinan was dissolved in Me_2SO to break it into single random-coil chains, and then the resulting L-FV-IB21D and L-FV-IB31D samples were tested for antitumor activity. The results for the L-FV-IB21D and L-FV-IB31D samples in vivo are summarized in Table 2. Both L-FV-IB21D and L-FV-IB31D exhibit far lower in vivo antitumor activities than the original samples. The results indicate that the triple-helix conformation is an important structural requirement for the antitumor effects of lentinan.

In the MTT assay, the inhibition ratios of Sarcoma 180 tumor-cell growth by lentinan samples having various M_w are shown in Figure 5. All samples show relatively strong inhibition ratios against tumor-cell growth at concentrations of 500 and 50 μ/mL. Interestingly, the samples having relatively low M_w exhibit higher activities than those having higher M_w and L-FV-IB52 exhibits higher activity than others, whereas its in vivo bioactivity is lower than that of L-FV-IB31. However, L-FV-IB31, with relatively lower activity in vitro exhibits the highest antitumor activity in vivo. Therefore, the bioactivities are related to the molecular weight of the glucan, but the correlation of M_w to the activities is different in vivo and in vitro. The forgoing results

Table 2. Antitumor activity of the L-FV-IB samples from the fruiting bodies of *Lentinus edodes* against Sarcoma 180 solid tumor grown in BALB/c mice

L-FV-IB fraction	Conformation	$M_w \times 10^{-5}$	Dose (mg/kg × days)	Inhibition ratio (%)	Enhancement ratio of body weight (%)	Complete regression
5-Fu	—		60 × 8	50.5	12.6	0/10
L-FV-IB11	Triple helix	28.3	20 × 8	19.0	36.6	0/10
			60 × 8	34.0	41.2	0/10
L-FV-IB12	Triple helix	21.6	20 × 8	23.0*	36.1	0/10
			60 × 8	34.6*	38.3	0/10
L-FV-IB21	Triple helix	18.4	20 × 8	31.2*	33.7	0/10
			60 × 8	36.3*	31.9	0/10
L-FV-IB31	Triple helix	14.9	20 × 8	28.6*	42.2	0/10
			60 × 8	49.5*	42.4	0/10
L-FV-IB41	Triple helix	11.4	20 × 8	22.1	33.4	0/10
			60 × 8	27.6	40.7	0/10
L-FV-IB32	Triple helix	9.79	20 × 8	23.5	35.3	0/10
			60 × 8	26.0	36.7	0/10
L-FV-IB52	Triple helix	5.71	20 × 8	27.2*	38.8	0/10
			60 × 8	38.2*	43.8	0/10
L-FV-IB73	Triple helix	3.57	20 × 8	27.7*	36.5	0/10
			60 × 8	35.4*	36.7	0/10
L-FV-IB21D	Single flexible chain	5.59	20 × 8	12.8	33.2	0/10
			60 × 8	16.8	33.4	0/10
L-FV-IB31D	Single flexible chain	4.37	20 × 8	8.1	41.1	0/10
			60 × 8	12.3	40.1	0/10

*: $P < 0.05$.

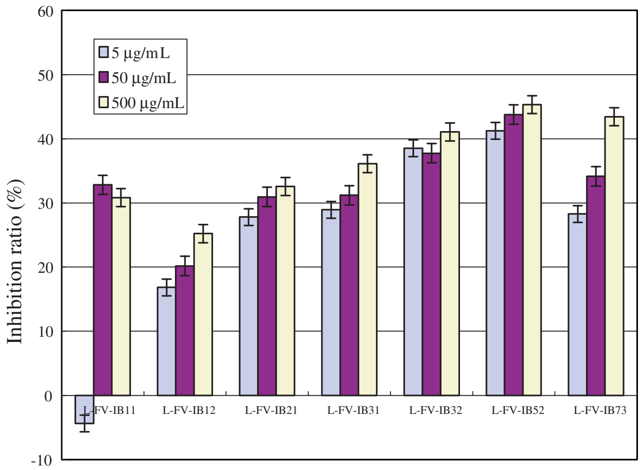

Figure 5. Inhibition ratio of proliferation of S-180 cells by different concentrations of L-FV-IB fractions.

demonstrate that the antitumor activity of lentinan may arise by stimulation of the cell-mediated immune response rather than by direct cytotoxic action.

4. Conclusion

Analysis of M_w, $[\eta]$, and $\langle S^2\rangle_z^{1/2}$ of water-soluble lentinan in 0.9% aqueous NaCl by the theory for worm-like chains yielded M_L, q, and d, indicating high rigidity of the chain and the triple-helix conformation. The lentinan molecules exist as a triple-stranded helical chain in 0.9% aqueous NaCl, and as a single-flexible chain in Me_2SO. The $[\alpha]_D$ of lentinan in water–Me_2SO mixtures revealed that order–disorder conformation transition from triple helix to single flexible chains occurred when the weight fraction of Me_2SO exceeded 0.82. The antitumor tests showed that the maximum inhibition ratio against S-180 was with triple-helical lentinan having M_w of 11.4×10^5 g mol^{-1} in vivo and that having M_w of 5.71×10^5 g mol^{-1} in vitro. The samples having single flexible chains exhibited far lower in vivo antitumor activities than the triple-helix samples. The triple-helix conformation is thus an important structural requirement for the antitumor effect of lentinan.

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