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Chain conformation of sulfated derivatives of β-glucan from sclerotia of *Pleurotus tuber-regium*

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Abstract

Six water-insoluble (1 → 3)-β-D-glucan fractions TM8-1 to TM8-6 with weight-average molecular mass M_w ranging from 5.76 to 77.4×10^4 obtained from the sclerotia of *Pleurotus tuber-regium* were sulfated to produce the water-soluble fractions S-TM8-1 to S-TM8-6 with M_w from 6.0 to 64.8×10^4. The degree of substitution (DS) of S-TM8 fractions was analyzed by elemental analysis (EA) to be 1.14–1.74. The ^{13}C NMR results indicated that the C-6 was fully substituted, and C-2, C-4 were partially substituted by the sulfo-groups. The M_w and the intrinsic viscosity $[\eta]$ of the S-TM8 fractions were measured, respectively, by size-exclusion chromatography combined with laser light scattering (SEC-LLS), LLS and viscometry in phosphate buffer solution (PBS) at 37 °C. The dependences of $[\eta]$ and radius of gyration $\langle s^2\rangle_z^{1/2}$ on M_w for the S-TM8 samples were found to be $[\eta] = 1.89 \times 10^{-2}\ M_w^{0.70}$ (cm^3/g) and $\langle s^2\rangle_z^{1/2} = 1.12 \times 10^{-4} M_w^{0.81}$ (nm) in the M_w range tested. Based on current theories for a wormlike chain model, the molar mass per unit contour length M_L and persistence length q of the S-TM8 were calculated to be 990 nm^{-1} and 8.5 nm, respectively. The relatively higher q value suggested a more expanded flexible chain of S-TM8 in PBS. The water-solubility and relatively expanded chain conformation of the STM8 fractions were considered to be significant to their antiviral activity.

Keywords: Sulfonation; *Pleurotus tuber-regium*; β-D-Glucan; Conformation; Molecular mass

1. Introduction

The sclerotia of *Pleurotus tuber-regium*, are edible and extremely rich in non-starch polysaccharides, composed mainly of β-glucan.[1] *P. tuber-regium* forms very hard and solid sclerotia, which have many culinary and medical uses and have attracted much recent attention.[2] Our previous studies showed the polysaccharide TM8, extracted from the sclerotia of *P. tuber-regium* with hot alkali to consist of a main chain of (1 → 3)-β-D-glucopyranosyl units with every third unit having on average a (1 → 6)-β-D-glucopyranosyl branch.[3] The results from in vivo and in vitro experiments indicate that the TM8 fractions have immunomodulatory and direct cytocidal antitumor activity.[4] The introduction of suitable ionic groups with appropriate degrees of substitution should not only enhance the water solubility of the β-D-glucan, but also change the intra- and inter-molecular hydrogen bonding and strengthen the effect of electrostatic repulsion, enabling the polymer chain to adopt a certain conformation in aqueous solution.[5,6] Although the antiviral properties of sulfated polysaccharides have been known for 30 years,[7] interest in the antiviral potential of sulfated polysaccharides has regained attention by recent studies showing that several polyanionic substances are effective inhibitors of human immunodeficiency virus replication.[8] Sulfated polysaccharides are widespread in nature, occurring as components of the extracellular matrix, on the cell surface of vertebrates, and are produced by marine organisms. However, most natural sulfated polysaccharides are complex mixtures of macromolecules showing wide variations in their structure and activities, which complicates further studies correlating structure and activity, as well as the development of such polymers into new drugs. The activity of these sulfated polysaccharides also depends

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on such structural parameters as the degree of substitution (DS),[9] the weight-average molecular mass M_w,[10] the position of sulfation,[11] and glycosidic branching.[12] However, little information on the conformation of these sulfated polysaccharides in aqueous solution has been reported. Here we aimed to partially synthesize sulfated polysaccharides with potential antiviral activity from the β-D-glucans extracted from the sclerotia of *P. tuber-regium*, and to characterize their M_w, DS, and conformation in solution; essential information for correlating their structures to their antitumor and antiviral activities.

2. Experimental

2.1. Preparation of sulfated derivatives

The TM8 sample and several fractions thereof having different M_w values (TM8-1 to TM8-6), previously isolated from the sclerotia of *P. tuber-regium*,[3] were used. The samples were sulfated individually by the method of Paper et al.[9] The polysaccharide was soaked in dry *N*,*N*-dimethylformamide (DMF), the suspension was kept at 50 °C overnight with stirring, and then pyridine (1.5 mL) was added and the solution was heated to 80 °C, and stirred for another 30 min. Chlorosulfuric acid (1.25 mL) was added dropwise to the solution at 80 °C with stirring, and then the reaction was stopped after 60 min. After cooling to room temperature, distilled water (25 mL) was added with stirring and then the pH was adjusted to 10 by 1 M NaOH. The resulting sulfated polysaccharide was precipitated with EtOH, redissolved in water, and dialyzed against slightly alkaline water (pH 9) to remove pyridine. Finally the sulfated polysaccharide was dialyzed against distilled water, until the contents of the dialysis tube were slightly acidic. The retentate was then made neutral and freeze-dried to afford samples labeled as S-TM8 and S-TM8-1, -2, -3, -4, -5, and -6. The water solubility of sulfated β-D-glucans is largely dependent on the molar ratio of chlorosulfuric acid:glucopyranosic unit, the reaction temperature, and the time of reaction.[13] In this research, different reaction conditions were conducted on the unfractionated TM8 samples. From their SEC patterns (data not shown), the molar ration of 5:1 for chlorosulfuric acid:glucopyranosic unit and the reaction time of 60 min were found optimum for producing totally water-soluble sulfated fractions without obvious degradation of the polymer chains. Therefore, six TM8 fractions having different M_w values from the sclerotia of *P. tuber-regium* were reacted under these optimum conditions to yield the water-soluble samples S-TM8-1 to -6, as shown in Table 1.

2.2. Structural analysis

The IR spectra were recorded with a Nicolet Fourier-transform infrared (FTIR) spectrometer (Spectrum One, Perkin–Elmer Co., USA). Test specimens were prepared by the KBr-disk method. The elemental compositions for C, H, O, and S in the S-TM8 fractions were determined by an elemental analyzer (EA) (Heraeus Co., Germany). The ^{13}C NMR spectra of the sulfated glucan were recorded on a 600 MHz NMR spectrometer (Varian Inova) by using a standard 5-mm probe and D_2O as the solvent at 60 °C.

2.3. Viscometry

The viscosity of S-TM8 fractions was determined with an Ubbelohde viscometer in phosphate buffer solution (PBS; 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 ultra pure water) at 37 °C. The kinetic energy correction was negligible. Huggins and Kraemer equations were used to estimate the intrinsic viscosity $[\eta]$ by extrapolation to infinite dilution 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 under given conditions in a given solvent; η_{sp}/c, the reduced specific viscosity; and $\ln \eta_r/c$, inherent viscosity.

2.4. Light scattering measurements

The light scattering intensities of the samples were measured with a laser light scattering (LLS) instrument (DAWN DSP, Wyatt Technology Co., USA) at 633 nm in an angular range from 26° to 142° at 37 °C. Samples were dissolved in PBS to obtain clear aqueous solutions. Optical clarification of the solution was made by filtration through 0.45 μm Millipore filter (PTFE, Puradisc 13 mm Syringe Filters, Whatman, England) into the scattering cell. The specific refractive index increment (dn/dc) at 633 nm and 37 °C was determined using an interferometric refractometer (Optilab/903, Wyatt Technology, USA). The dn/dc value was averaged to 0.136 mL/g and was assumed to be constant over the sample elution. Astra software was utilized for the data acquisition and analysis. The plot of angular dependence on $(Kc/R_\theta)_{c=0}$ was used to obtain M_w and the radius of gyration $\langle s^2 \rangle_z^{1/2}$.

SEC-LLS was performed on the multi-angle laser photometer already mentioned combined with a p100 pump (Thermo Separation Products, San Jose, Japan) equipped with columns of PSW5000 and PSW3000 (TSK) and the interferometric refractometer at 37 °C. The carrier solution was PBS, and the samples were

Table 1
Sulfur content and DS of the sulfated TM8 fractions having different M_w values

Sample name	Time (min)	–OH:$ClSO_3H$	Yield (%)	Sulfur content (%)	DS
S-TM8-1	60	1:5	95.4	14.41	1.14
S-TM8-2	60	1:5	100.3	15.12	1.23
S-TM8-3	60	1:5	120.2	16.69	1.45
S-TM8-4	60	1:5	115.3	16.62	1.44
S-TM8-5	60	1:5	128.7	18.08	1.67
S-TM8-6	60	1:5	140.4	18.49	1.74

dissolved in PBS with stirring. The solution and PBS were made dust-free by passing through a 0.45 μm filter and were degassed before use. The injection volume was 200 μL, and the flow rate was 1.0 mL/min. The calibration of the photometer was done with ultra pure toluene, and the normalization of the RI detector was done with bovine albumin monomer (Sigma A-1900). Astra software was utilized for the data acquisition and analysis.

3. Results and discussion

3.1. Chemical structure of sulfated β-glucan

The sulfur content and the DS values of the sulfated TM8 fractions are summarized in Table 1. The DS which designates the average number of sulfo-groups on each sugar residue, was established on the basis of the sulfur content determined after Schoniger[14] by the following formula.

$$DS = (162 \times S\%)/(32 - 80 \times S\%) \qquad (3)$$

The estimation errors for DS were calculated to be around 0.6% from the experimental error of the instrument. The chemically modified S-TM8 fractions were all soluble in aqueous PBS, indicating that the sulfated polysaccharides having DS values from 1.14 to 1.74 are water-soluble. The IR spectra of the native TM8 and its sulfated derivatives S-TM8-2, S-TM8-4, and S-TM8-6 showed characteristic absorptions at 890 cm^{-1} (data not shown), assigned to be the β configuration that had also been confirmed by our previous study.[3] Compared with the IR spectrum of the native TM8, two new absorption peaks appeared at 817 and 1260 cm^{-1} for the S-TM8-2, -4, and -6 (data not shown), due to the presence of the C–S–O and S=O bonds, respectively, indicating that sulfation had actually occurred.

The ^{13}C nuclear magnetic resonance (NMR) spectra of the samples S-TM8-2, -4, and -6 in D_2O are shown in Fig. 1. Comparing with the chemical shifts of the native TM8 from reference[3] listed in Table 2, there are five new peaks appearing in the S-TM8 spectra, namely C-1′ (101.5), C-2s (79.4), C-2′s (80.3), C-4s (77.4), and C-6s (67.7 ppm), resulting from sulfonation of the hydroxyl groups at positions 6, 4 and 2. The peak appearing at 60.9 ppm, which is the chemical shift of C-6 for TM8, disappeared in the ^{13}C NMR spectra of S-TM8. A new peak appeared at 67.7 ppm in S-TM8, assigned to the substituted C-6, because the downfield shift of a carbon atom linked by a sulfate group is ~7–10 ppm.[15] This indicates that C-6 was fully substituted by sulfo-groups and the C-6 signal was shifted downfield to 67.7ppm. The chemical shifts of 72.4, 73.7, and 68.4 ppm were designated as the signals of C-2, C-2′ and C-4,[3] as is also observed in the spectra of S-TM8. According to Ref. 15, the new peaks at 79.4 and 80.3 ppm for the S-TM8 fractions may be assigned to C-2 and C-2′ which are partially substituted by sulfo groups. The new peak at 77.4 ppm was assigned to the substituted C-4. Moreover, a new peak at 101.5 ppm could be assigned to C-1 because C-2 was partially substituted and thus influenced the chemical shift of the adjacent C-1.[15] From the results of ^{13}C NMR, it may be concluded that non-selective sulfonation of β-glucan occurred, with C-6 being fully substituted and C-2, -4 being partially substituted. The intensity of the C-6 signals was more evident than that of C-2s and C-4s, owing to steric hindrance, as reported by Katsuraya[16].

3.2. Molecular mass and viscosity

The size exclusion chromatograms of the sulfo-glucan, showing the column eluent analyzed by the LLS (90° detector) and interferometric refractometers, are presented in Fig. 2 (A) and (B). Obviously, each fraction having a different M_w was eluted at a different elution volume. Moreover, SEC-LLS, an absolute method, is basically applicable for the S-TM8 fractions over the M_w range tested in this research. The results from Fig. 2 (A) and (B) indicated that the molecular mass distributions of S-TM8 fractions are relatively narrow. The single peak of each sample detected both by LLS and refractometer showed that there was no aggregation of the sulfo-glucans in aqueous solution. Therefore, the S-TM8 fractions are suitable for investigating their solu-

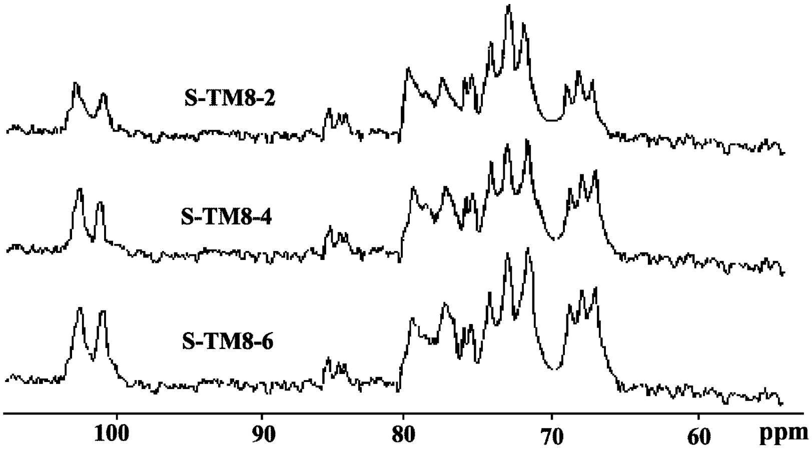

Fig. 1. The ^{13}C NMR spectra of sulfated derivatives of S-TM8-2, -4, and -6 in D_2O.

tion properties. The values of M_w, $\langle s^2\rangle_z^{1/2}$ and polydispersity index M_w/M_n obtained from SEC-LLS, and $[\eta]$ as determined by viscometry of S-TM8s are summarized in Table 3.

By working in a batch mode with the LLS detector, M_w and $\langle s^2\rangle_z^{1/2}$ of a polymer can be obtained directly by computing a classical Zimm plot from light-scattering data collected at various angles (θ) for each polymer 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 $\sin^2\theta/2+Sc$, with K being an optical constant and S a stretch factor selected to obtain a well-defined plot. The collected data of Kc/R_θ at different angles are extrapolated to 0° for each concentration. The common intercept of the extrapolated curves yields the M_w and the slope of the $c=0$ curve yields $\langle s^2\rangle_z^{1/2}$. Fig. 3 shows the angular dependences of $(Kc/R_\theta)_{c=0}$ for S-TM8 fractions in PBS at 37 °C. The M_w, and $\langle s^2\rangle_z^{1/2}$ for each fraction are also listed in Table 3. It may be noted that the values of M_w and $\langle s^2\rangle_z^{1/2}$ for S-TM8 fractions obtained from SEC-LLS and LLS are in good agreement with each other. It is known that the values of M_w and $\langle s^2\rangle_z^{1/2}$ are very important for studying the solution properties of polymers, and the molecular parameters for polymers are largely dependent on the experimental values of M_w and $\langle s^2\rangle_z^{1/2}$. The agreement of the M_w values detected by SEC-LLS and LLS justified the further analysis of these polymers.

3.3. Mark–Houwink equation

Fig. 4 shows the double-logarithmic plot of $[\eta]$ against M_w for S-TM8 fractions in PBS at 37 °C and the native TM8 fractions in Me_2SO. The Mark–Houwink equation for S-TM8 fractions with M_w ranged from 6.0 to 64.8×10^4 (the solid line in Fig. 5), may be represented by:

$$[\eta]=(1.89\pm0.05)\times10^{-2}\ M_w^{0.70\pm0.03} \tag{4}$$

The exponent α value is related to the shape of the macromolecule and the nature of the solvent. For flexible linear polymers in a good solvent, the α value is usually in the range 0.7–0.8. However, the α value of polymers, including linear and branched, ranges widely from 0.50 to 0.8. Therefore, the α value of 0.7 for the S-TM8 in PBS lies in the range of flexible polymer chains. Although the $[\eta]$–M_w curves of both S-TM8 and TM8 are within the flexible-chain range, the α value of S-TM8 (0.70) was larger than that of TM8 ($\alpha=0.51$),[3] as shown by a dashed line in Fig. 4. This implies that the sulfated β-glucan (S-TM8) has a more-expanded coil than the corresponding native TM8 fractions.

3.4. Chain conformation

Based on the data of M_w, $\langle s^2\rangle_z^{1/2}$, and $[\eta]$, a wormlike cylinder model can be used for conformational characterization of the S-TM8 fractions. Bushin et al.[17] and

Table 2
The chemical shifts of the native and sulfated β-glucan from the sclerotia of *P. tuber-regium*

Chemical shift	C-1	C-2	C-3	C-4	C-5	C-6	C-1′	C-2s	C-2′s	C-4s	C-6s
Native glucan (TM8)	103.0	72.5 (73.7)	86.3 (76.8, 86.0, 87.0)	68.5	76.1	60.9					
Sulfated glucan (S-TM8)	103.1	72.4 (73.7)	86.1 (76.8, 85.7, 86.9)	68.4	76.2	$^{-}$	101.5	79.4	80.3	77.4	67.7

$^{-}$ Not observed.

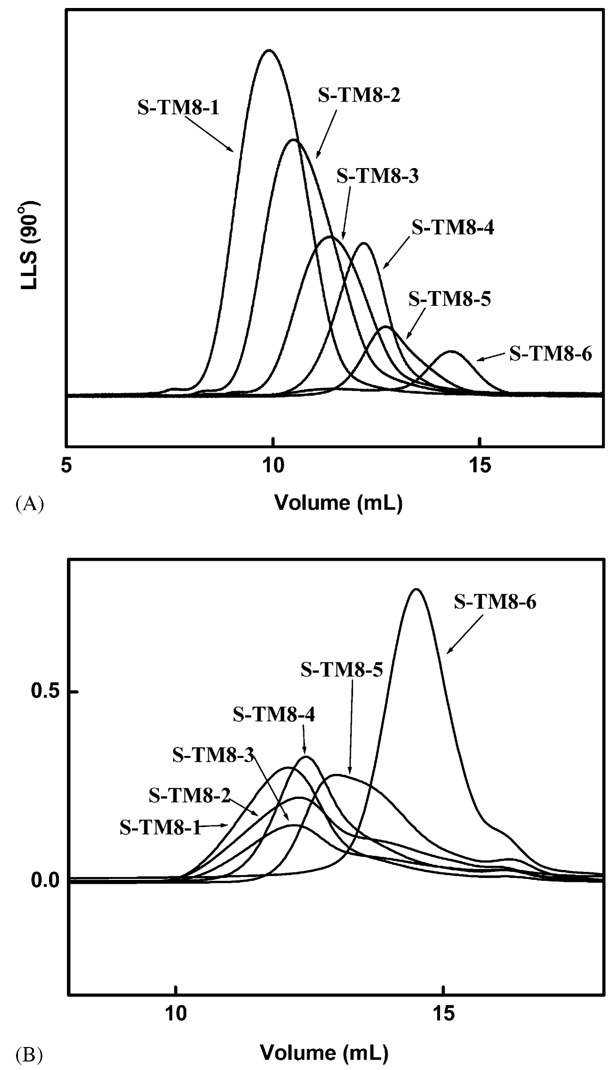

Fig. 2. Size-exclusion chromatogram of glucan sulfates S-TM8-1 to S-TM8-6 determined by multi-angle LLS photometry (A: LLS) and interferometric refractometer (B). The LLS data represents the 90° light scattering angle (detector 11).

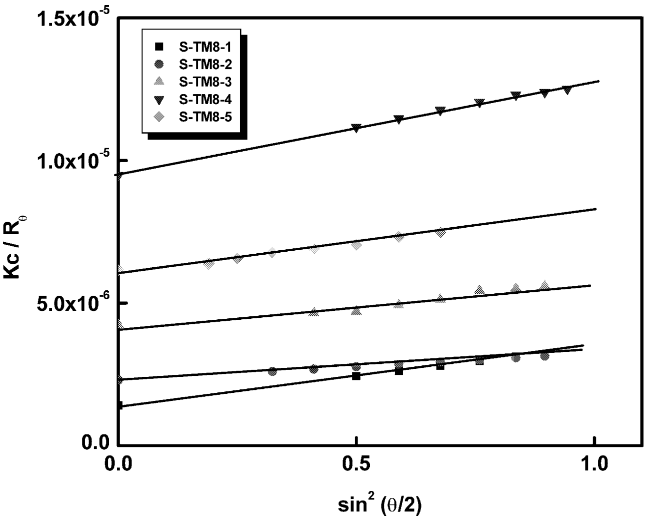

Fig. 3. The angular dependences of $(Kc/R_\theta)_{c=0}$ for the S-TM8 fractions in PBS at 37 °C.

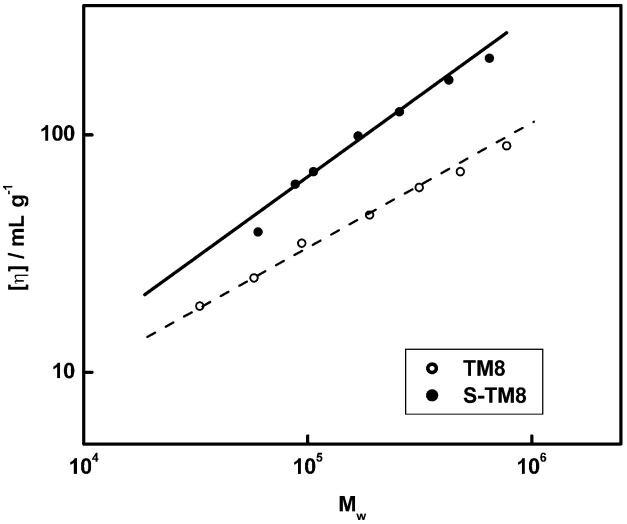

Fig. 4. The double-logarithmic plots of $[\eta]$ against M_w for the S-TM8 fractions in PBS at 37 °C and the native TM8 fractions in Me_2SO.

Table 3
Results of SEC-LLS, LLS and intrinsic viscosity for S-TM8 fractions

Sample	$M_w \times 10^{-4}$[a]	$M_w \times 10^{-4}$[b]	M_w/M_n	$[\eta]$ (mL/g)	$\langle s^2 \rangle_z^{1/2}$ (nm)[a]	$\langle s^2 \rangle_z^{1/2}$ (nm)[b]	Native samples	$M_w \times 10^{-4}$[c]
S-TM8-1	64.8 ± 0.05	65.8 ± 0.62	1.62	210	79 ± 3.2	68 ± 4.1	TM8-1	77.4
S-TM8-2	42.7 ± 0.41	55.9 ± 0.05	1.55	170	48 ± 2.8	42 ± 6.4	TM8-2	42.2
S-TM8-3	25.7 ± 0.08	28.3 ± 0.34	1.83	125	31 ± 6.3	39 ± 1.5	TM8-3	20.3
S-TM8-4	16.8 ± 0.26	21.0 ± 0.08	1.62	99	20 ± 5.5	23 ± 0.9	TM8-4	17.1
S-TM8-5	10.6 ± 0.09	11.0 ± 0.04	1.60	70	13 ± 2.9	17 ± 1.4	TM8-5	10.3
S-TM8-6	6.0 ± 0.71	7.1 ± 0.07	1.73	39	–	–	TM8-6	5.76

[a] Obtained from SEC-LLS.
[b] Obtained from LLS.
[c] Previous results quoted for comparison.
– Not determined.

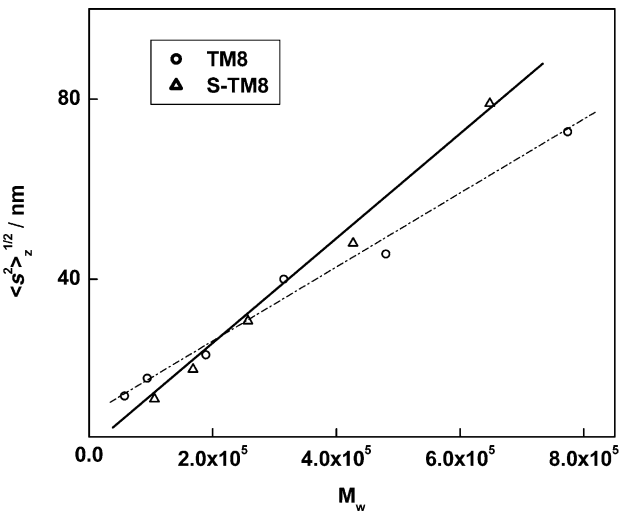

Fig. 5. The double-logarithmic plots of $\langle s^2\rangle_z^{1/2}$ against M_w for S-TM8 in PBS at 37 °C and native TM8 fractions in Me_2SO.

Bohdanecky[18] independently showed that the Yamakawa–Fujii–Yoshizaki (Y–F–Y) theory[19,20] for $[\eta]$ of an unperturbed wormlike cylinder may be represented approximately by

$$(M^2/[\eta])^{1/3} = A_\eta + B_\eta M^{1/2} \quad (5)$$

$$A_\eta = \phi_{0,\infty}^{-1/3} A_0 M_L \ (g^{1/3}/cm) \quad (6)$$

$$B_\eta = \phi_{0,\infty}^{-1/3} B_0 \ (2q/M_L)^{-1/2} \ (g^{1/3}/cm) \quad (7)$$

where q and M_L are the persistence length and the molar mass per unit contour length, respectively. A_0 and B_0 are tabulated in Bohdanecky's paper[18], and $\phi_{0,\infty}$ is 2.87×10^{23}. The straight line of $(M_w^2/[\eta])^{1/3}$ versus $M_w^{1/2}$ can be used to yield the intercept A_η and slope B_η. Substituting the intercept and slope of this plot into Eqs. (5)–(7) yielded 990 nm^{-1} for M_L and 8.5 nm for q. Compared with the value of 3.1 nm for q of TM8, the relatively larger values of q of the S-TM8 indicate that the chain stiffness increases after sulfonation, resulting from intensified steric hindrance by the substituted sulfo groups on the backbone.

The double-logarithmic plots of $\langle s^2\rangle_z^{1/2}$ against M_w for S-TM8 and native TM8 fractions are shown in Fig. 5. For the S-TM8 fractions having M_w ranging from 6.0 to 64.8×10^4, the plot may be represented by the following equation:

$$\langle s^2\rangle_z^{1/2} = (1.12{\pm}0.04){\times}10^{-4} M_w^{0.81\pm0.02} \ (nm) \quad (8)$$

The value of the exponent (0.81) was much larger than that of native TM8 (0.56),[3] which showed a random coil conformation in Me_2SO, suggesting an expanded flexible-chain character. Based on Kratky–Porod wormlike chain model,[21] an equation, which should be suitable for the semi-flexible polymers, may be represented by:

$$(M_w/\langle s^2\rangle_z^{1/2}) = (3M_L/q)^{1/2} + 3M_L(3qM_L)^{1/2}/2M \quad (9)$$

from the straight line of $(M_w/\langle s^2\rangle_z^{1/2})$ against $1/M_w$ for S-TM8 fractions in PBS at 37 °C, the values of M_L and q were estimated to be 950 nm^{-1} and 16 nm. The values of M_L are consistent with the calculated value of 990 nm^{-1} obtained from $[\eta]$. The difference between the q values obtained from $[\eta]$ and from $\langle s^2\rangle_z^{1/2}$ may be related to the scatter of the points obtained from $\langle s^2\rangle_z^{1/2}$.

The molecular parameters of some polysaccharides and their derivatives, including S-TM8, CTM8 (carboxymethylated derivatives of TM8),[22] TM8 (native hot alkali extract from the sclerotia of *P. tuber-regium*),[3] glucan A,[23] scleroglucan,[24] lentinan,[25] and schizophyllan[26] are summarized in Table 4. The value of q for S-TM8 was much lower than that of these $(1\rightarrow3)$-β-D-glucans in water, 0.01 M NaOH, or 0.5 M aqueous NaCl from *Auricularia aurocula-judae*, scleroglucan and schizophyllan, respectively, having the same basic structure. These exhibit single- or triple-helical conformations, but the q values are higher than those of native TM8 in dimethyl-sulfoxide (Me_2SO), which exist as random coils in solution. In addition, the molecular parameters of S-TM8 are similar to those of CTM8, which exhibited a relatively expanded flexible chain in PBS.[22] Usually, a relatively large value of q reflects the enhancement of chain stiffness. Moreover, based on the analysis of the data from $[\eta]$, M_w, and $\langle s^2\rangle_z^{1/2}$, the S-TM8 exist as expanded flexible chains in PBS, and the relatively higher chain stiffness of S-TM8 is probably related to the sulfo groups. It is well known that the introduction of sulfo groups strengthens the effect of electrostatic repulsion, namely the polyelectrolytic effect, resulting in chain expansion and increased stiffness.

Sulfated polysaccharides offer promise as potential anti-viral candidates, being able to block HIV replication in cell culture at concentrations as low as 0.1 to 0.01 μg/mL without toxicity to the host cells at concentrations up to 2.5 mg/mL.[27] Our recent research shows that S-TM8 fractions with different M_w values are active against a wide variety of enveloped viruses, including HSV-1, HSV-2, and RSV (submitted data), and higher antiviral activity was observed when the S-TM8 fractions are present during the virus absorption period (submitted data). Therefore, a basic understanding of the conformations of the polysaccharides is essential for investigating structure–function relationships, especially the molecular mechanisms of the interactions of β-glucan with the target cells. Our previous studies showed that the relatively extended chain conformation of carboxymethylated derivatives was beneficial for enhancing the antitumor activity.[28–30] Interestingly, the most common antitumor non-starch polysaccharides, such as lentinan and schizophyllan, have a triple-helix conformation with stiff chains.[28–30] The information of the increased molecular parameters and the extended chain conformation in solution of the sulfated β-glucan from the sclerotia of *Pleurotus tuber-regium*, first reported in

Table 4
Molecular parameters for the S-TM8, CTM8, TM8, glucan A, scleroglucan, lentinan and schizophyllan

Sample	Main chain	Solvent	Conformation	q (nm)		M_L (nm^{-1})		α (the exponent of M–H equation)	Source
				From [η]	From $\langle s^2 \rangle_z^{1/2}$	From [η]	From $\langle s^2 \rangle_z^{1/2}$		
S-TM8	sulfated (1→3)-β-D-glucan	PBS	expanded coil	8.5	16	990	950	0.70	this work
CTM8	carboxymethylated (1→3)-β-D-glucan	PBS	expanded coil	9.6	14.8	790	876	0.78	Ref. 22
TM8	(1→3)-β-D-glucan	Me_2SO	random coil	3.1	–	408	–	0.51	Ref. 3
Glucan A (from *A. auricula-judae*)	(1→3)-β-D-glucan	water	single helix	90 ± 20	–	1030 ± 100	–	1.14	Ref. 23
Scleroglucan	(1→3)-β-D-glucan	0.01 M NaOH aqueous solution	triple helix	–	–	2200 ± 60	–	1.7	Ref. 24
L-FV-I (from *Lentinus edodes*)	(1→3)-β-D-glucan	0.5 M NaCl aqueous solution	triple helix	–	120 ± 10	–	2180 ± 100	1.32	Ref. 25
Schizophyllan	(1→3)-β-D-glucan	0.01 M NaOH aqueous solution	triple helix	180 ± 30	150 ± 30	2190 ± 50	2170 ± 50	1.1–1.8	Ref. 26

– Not determined.

this research, should thus give further insight on the structure–function relationship of these antiviral sulfated polysaccharides.

4. Conclusions

Sulfonation of the TM8 fractions, of the (1→3)-β-D-glucan, from the sclerotia of *P. tuber-regium* increased the water solubility and chain stiffness. The Mark–Houwink equation for the sulfated S-TM8 in PBS at 37 °C was established to be $[\eta] = (1.89 \pm 0.05) \times 10^{-2} M_w^{0.70 \pm 0.03}$. The conformational parameters of S-TM8 in PBS were 990 nm^{-1} for M_L and 8.5 nm for q, indicating a more expanded flexible chain than the native TM8 in Me_2SO. The higher chain stiffness of sulfated fractions may be attributed to the polyelectrolytic effect caused by the sulfate groups substituted on the backbone.

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