Available online at www.sciencedirect.com
SCIENCE DIRECT
Carbohydrate Polymers 59 (2005) 257–263
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Comparison on chain stiffness of a water-insoluble (1→3)-α-D-glucan isolated from *Poria cocos* mycelia and its sulfated derivative

Lina Zhang[a,*], Li Chen[a], Xiaojuan Xu[a], Yulu Lin[b], Peter Chi Keung Cheung[c], John F. Kennedy[d,e]

[a]*Department of Chemistry, Wuhan University, Wuhan 430072, China*
[b]*College of Chemistry and Environment Engineering, Jianghan University, Wuhan 430056, China*
[c]*Department of Biology, The Chinese University of Hong Kong, Hong Kong, China*
[d]*Birmingham Carbohydrate and Protein Technology Group, School of Chemical Sciences, The University of Birmingham, Birmingham BI5 2TT, UK*
[e]*Chembiotech Laboratories, University of Birmingham Research Park, Vincent Drive, Birmingham BI5 2SQ, UK*

Received 28 July 2004; revised 22 September 2004; accepted 28 September 2004
Available online 2 November 2004

Abstract

A water-insoluble (1→3)-α-D-glucan (ab-PCM3-I) isolated from the *Poria cocos* mycelia by extracting with 0.5 sodium hydroxide was fractionated into ten fractions by non-solvent addition method in 0.25 M lithium chloride in dimethylsulfoxide. Seven fractions were treated with chlorosulfonic acid-pyridine complex to synthesis water-soluble sulfated derivatives (S-ab-PCM3-I) with degrees of substitution in the range 0.35–0.81. The solution behaviours of the α-D-glucan and its derivatives were studied by laser light scattering (LLS), size exclusion chromatography combined with laser light scattering (SEC-LLS) and viscometry at 25 °C. The dependences of intrinsic viscosity ([η]) on weight-average molecular mass (M_w) for the native glucan and the sulfated derivatives were found to be $[\eta]=9.92\times10^{-3}\ M_w^{0.77}$ (cm^3 g^{-1}) in the range of M_w from 1.08 to 23.1×10^4 and $[\eta]=2.92\times10^{-4}\ M_w^{1.09}$ (cm^3 g^{-1}) in the range of M_w from 0.74 to 5.80×10^4, respectively. On the basis of current theories for a wormlike chain model, the conformation parameters of the samples ab-PCM3-I and S-ab-PCM3-I were calculated to be 584 and 834 nm^{-1} for the molar mass per unit contour length (M_L), 3.7 and 6.8 nm for the persistence length (q), 11.6 and 18.1 for characteristic ratio (C_∞), respectively. The results indicated that the α-D-glucan ab-PCM3-I exists as an extended random coil chain in 0.25 M lithium chloride in dimethylsulfoxide, and that the sulfated derivatives S-ab-PCM3-I has good water-solubility and exist as semi-stiff chains in 0.9% w/v aqueous sodium chloride, owing to the steric hindrance and electrostatic repulsion of the sulfate groups.

Keywords: *Poria cocos* mycelia; (1→3)-α-D-glucan; Sulfated derivative; Conformation; Molecular mass

1. Introduction

Recently, it has been found that polysaccharides play roles that are essential to all biological systems, although no single common theory has emerged to explain the remarkable complexity and diversity of these molecules (Striegel & Timpa, 1995). Most antitumor polysaccharides have a basic (1→3)-β-D-glucan structure (Bohn & BeMiller, 1995). However, other polysaccharides including a α-D-glucan (Tanigami, Kusumoto, Nagao, Kokeguchi, Kato, Kotani, & Shiba, 1991), polysaccharide-protein complexes and heteropolysaccharides have also exhibited antitumor activities. Young and Jacobs (Young & Jacobs, 1998) have indicated that molecular conformation is an important factor in determining the biological activity of glucans. It has been reported that (1→3)-α-D-glucan chain conformation is nearly completely extended, close to a 2/1 helix in the solid state by an X-ray diffraction (Ogawa, Misaki, Oka, & Okamura, 1979), and (1→3)-α-D-glucan is more expanded (Rees & Scott, 1971) or stiffer (Burton & Brant, 1983) than (1→3)-β-D-glucans. Some (1→3)-α-D-glucan samples are water-insoluble, but the introduction of a charged group at hydroxyl groups on the glucan chain could improve the water solubility and enhance the antitumor activities

* Corresponding author. Tel.: +86 27 87219274; fax: +86 27 687562661.
E-mail address: lnzhang@public.wh.hb.cn (L. Zhang).

0144-8617/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2004.09.017

(Adachi, Ohno, Ohsawa, Sato, Oikawa, & Yadomae, 1989). Ukai et al. (Kiho, Yoshida, Nagai, Ukai, & Hara, 1989) have reported that native (1→3)-α-D-glucan from *Agrocybe cylindracea* had little antitumor effect but that the water-soluble carboxymethyl derivative showed potent antitumor activity against the solid form of Sarcoma 180 in mice. Moreover, some modified derivatives of (1→3)-α-D-glucan had stimulating effects on lymphocyte proliferation and antibody production, and the introduction of carboxymethyl groups at low degrees of substitution (DS < 0.28) could improve the immunostimulating activities (Bao, Duan, X. Fang, & J. Fang, 2001). Therefore, more attention has been attracted toward modified polysaccharides, with especial focus on the sulfated derivatives because of their obvious bioactivities (Katsuraya, Shoji, Inazawa, Nakashima, Yamamoto, & Uryu, 1994; Katsuraya, Shibuya, Inazawa, Nakashima, Yamamoto, & Uryu, 1995; Katsuraya, Nakashima, Yamamoto, & Uryu, 1999). Unfortunately, the solution properties and conformation of such polysaccharide derivatives have been scarcely reported but it is our case that the effects of the secondary structure (molecular mass and conformation, etc.) of the polysaccharides on bioactivities should not be neglected.

In the previous work, we have extracted from *Poria cocos* mycelia, water-insoluble polysaccharide (ab-PCM3-I), which was determined by FTIR spectra, gas chromatography and ^{13}C NMR to be a linear (1→3)-α-D-glucan (Lin, Zhang, Jin, Chen, & Zhang, 2003). In this work, we prepared its fractions of different molecular mass and the corresponding sulfated derivatives, then studied the molecular paramaters by size exclusion chromatography combined with laser light scattering (SEC-LLS) and viscometry to deduce the conformation of the glucan and its derivatives in solution. The intrinsic viscosities obtained as a function of weight-average molecular mass were analyzed on the basis of the wormlike cylinder model (Yamakawa & Fujii, 1974; Yamakawa & Yoshizaki, 1980), It is our hope that this work may contribute some meaningful information to aid a deeper understanding of the correlation of physicochemical properties to bioactivities of the polysaccharides.

2. Experimental

Samples. (1→3)-α-D-glucan ab-PCM3-I previously extracted from *Poria cocos* mycelia with 0.5 M sodium hydroxide was fractionated by the non-solvent addition method as follows. A mixture of acetone and 0.25 M lithium chloride in dimethylsulfoxide (4:1 by volume) was slowly added to a 0.25 M lithium chloride in dimethylsulfoxide (DMSO) solution of the sample (about 0.5% polymer concentration) at 25 °C until the solution became turbid. The liquid was heated to 50 °C, and then allowed to stand for 12 h at 25 °C. The turbid solution was centrifuged at about 25 °C to separate the concentrated phase as the first fraction. The supernatant was subjected to further fractionation. In this way, the sample ab-PCM3-I was divided into 10 fractions designated as F-1, F-2, …, F-10. The fractions were reprecipitated from 0.25 M lithium chloride in dimethylsulfoxide solutions into 80% aqueous acetone, washed with anhydrous acetone four times, and then dried in vacuum for seven days to white powders.

Sulfation. Seven of the fractions (F-1∼F-7, 100 mg each) were sulfated according to the previous method (P. Zhang, L. Zhang, & Cheng, 2002). Polysaccharide (100 mg) was soaked in dry 0.25 M lithium chloride in dimethylsulfoxide (5 mL) to obtain a suspension/solution, which was kept at ambient temperature overnight with stirring, and then pyridine was added with stirring for 30 min. Chlorosulfonic acid (1 mol for every 2 mol of pyridine) was added drop to the solution with stirring, and then the solution was heated to 60 °C, with a reaction time for 120 min. The molar ratio of chlorosulfonic acid/glucopyranosyl units was kept at 5:1. After cooling to ambient temperature, distilled-water (25 mL) was added with stirring to the reaction mixture, and then the solution was adjusted to pH 10 by addition of 1 M sodium hydroxide. The sulfated derivatives of the polysaccharides were dialyzed against slightly alkaline water (pH 9) to remove pyridine. Finally, the sulfated derivatives were dialyzed against distilled water for 5 days, then freeze-dried to obtain white powder samples S-1 to S-7.

Characterization. The IR spectra were recorded with a Nicolet Fourier transform infrared (FTIR) spectrometer (Spectrum One, Perkin–Elmer Co., USA). Samples were prepared by using the KBr-disk method. The elemental compositions of C, H, O and S in the sulfated samples were determined by an elemental analyzer (EA, Heraeus Co., Germany).

Solution preparation. Dry lithium chloride was added to distilled dimethylsulfoxide to afford a 0.25 M lithium chloride in dimethylsulfoxide solution which was treated with molecular sieves for further dehydration. To minimize undesired effects on the determination of molecular mass and viscosity, the mode of preparation of the polysaccharide solutions was strictly the same in all steps. A relatively concentrated stock solution was carefully prepared by completely dissolving the appropriate amount of polysaccharide in the desired solvent for 24 h under stirring, and a series of concentrations were obtained by successive dilution. Finally, each solution was further filtered through a 0.45 μm filter (PTFE, Puradisc 13 mm Syringe Filters, Whatman, England) three times into the scintillation vial to be used for the parallel light scattering and viscosities measurements.

Viscometry. The viscosity of the fractions of native glucan in 0.25 M lithium chloride in dimethylsulfoxide and the sulfated derivatives in 0.9% w/v sodium chloride aqueous solution were measured at 25 °C using an Ubbelohde viscometer. The kinetic energy correction was negligible. Huggins and Kraemer equations were used to

estimate the intrinsic viscosity ([η]) 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, the reduced specific viscosity; $(\ln \eta_r)/c$, inherent viscosity.

Laser light scattering. The light-scattering intensities of polysaccharides solution were determined with a multi-angle laser light scattering (LLS) instrument equipped with a He–Ne laser (λ=633 nm; DAWN® DSP, Wyatt Technology Co., USA) in the angles of 43, 49, 56, 63, 71, 81, 90, 99, 109, 118, 127, 136, and 152° at 25 °C. The optical clarification of the polysaccharide solutions of desired concentrations was achieved by filtration through a 0.2 μm pore size filter (Whatman, England) into the scattering cell. The refractive index increments (d*n*/d*c*) were determined using an Optilab refractometer (DAWN®DSP, Wyatt Technology Co., USA) at 633 nm and 25 °C. The d*n*/d*c* value of the water-soluble samples in 0.9% w/v aqueous sodium chloride was determined to be 0.136 $cm^3\ g^{-1}$. Astra software (Version 4.70.07) was utilized for data acquisition and analysis.

SEC-LLS measurements. Size exclusion chromatography combined with static laser light scattering (SEC-LLS) measurements were carried out on a laser photometer (DAWN®DSP Wyatt Technology Co., Santa Barbara, CA, USA) at 633 nm in an angular range from 26 to 142° combined with a P100 pump (Thermo Separation Products, San Jose, Japan) equipped with a TSK-GEL G4000 H_6 column (7.5×300 mm) for 0.25 M lithium chloride in dimethylsulfoxide and TSK-GEL G6000 PWXL (7.8×300 mm) combined with G4000 PWXL (7.8×300 mm) for 0.9% w/v aqueous sodium chloride at 25 °C. A differential refractive index detector (RI-150, Japan) was simultaneously connected. The injection volume was 200 μL with the concentration of 2–3 mg cm^{-3} for each fraction, and the flow rate was 1.0 $cm^3\ min^{-1}$. The calibration of the photometer was done with ultra pure toluene, and the normalization of the RI detector was done with pullulan standards. The values of d*n*/d*c* of samples in 0.25 M lithium chloride in dimethylsulfoxide and in 0.9% w/v aqueous sodium chloride were determined to be 0.055 and 0.136 $cm^3\ g^{-1}$, respectively. Astra software (Version 4.70.07) was utilized for the data acquisition and analysis.

3. Results and discussion

Chemical structure. Compared with the native α-glucan, two new absorption peaks appeared in the IR spectra of the sulfated derivative (Fig. 1) at 820 and 1240 cm^{-1} characteristic of the sulfate acid group

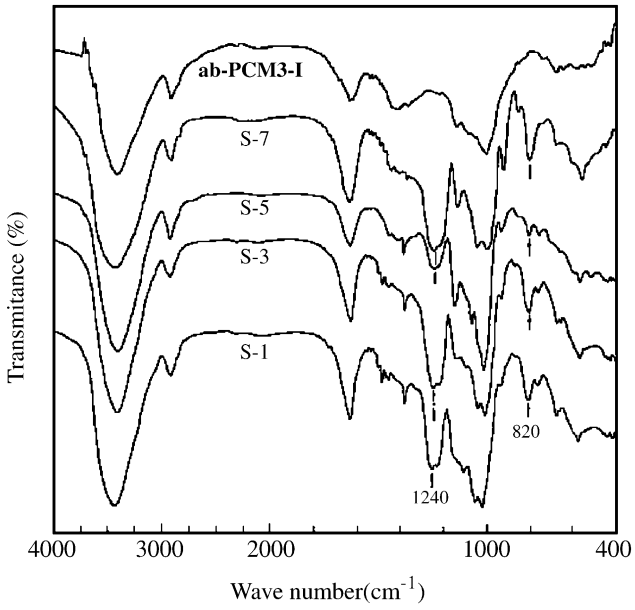

Fig. 1. Infrared spectra for samples ab-PCM3-I and S-ab-PCM3-I.

(Katsuraya, Nakashima, Yamamoto, & Uryu, 1999) for the derivatives as a result of the presence of the bonds of C–S–O and S=O, respectively, indicating that the sulfated reaction has occurred. The degrees of substitution (DS), which designates the average number of sulfur groups on each sugar residue, was estimated on the basis of the sulfur content (S%) by the following formula (M. Zhang, L. Zhang, Wang, & Cheung, 2003)

$$DS = (162 \cdot S\%)/(32 - 80 \cdot S\%). \quad (3)$$

The DS values for the sulfated derivatives from elemental analysis are summarized in Table 2. The chemically modified derivatives S-1～S-7 can all dissolve in 0.9% w/v aqueous sodium chloride, indicating that the derivatives with the DS from 0.34 to 0.81 are water-soluble. The water-solubility of the (1→3)-α-D-glucan was greatly enhanced by sulfation, enabling investigation of the solution properties.

Table 1
SEC-MALLS, molecular weight and intrinsic viscosity data for ab-PCM3-I in 0.25 M lithium chloride in dimethylsulfoxide at 25 °C

Sample	$M_w \times 10^{-4}$	M_w/M_n	[η] ($cm^3\ g^{-1}$)
ab-PCM3-I	5.91	3.64	46.5
F-1	23.1	3.19	128.9
F-2	20.3	2.64	127.1
F-3	13.0	1.54	126.8
F-4	7.53	1.76	116.7
F-5	7.10	1.76	54.1
F-6	4.72	1.32	38.1
F-7	2.39	1.21	23.1
F-8	1.58	1.43	15.9
F-9	1.36	1.21	15.5
F-10	1.08	1.54	13.1

Table 2
SEC-LLS, molecular weight, viscosity and degree of substitution data for S-ab-PCM3-I at 25 °C in 0.9% w/v aqueous sodium chloride

Sample	$M_w \times 10^{-4}$	M_w/M_n	$[\eta]$ ($cm^3\ g^{-1}$)	DS
S-1	5.80	1.6	52.0	0.38
S-2	4.18, 4.16[a]	1.2	34.8	0.49
S-3	3.24	1.6	24.3	0.44
S-4	2.68	1.3	19.6	0.34
S-5	1.88	1.8	13.1	0.35
S-6	1.14	2.0	7.65	0.51
S-7	0.74	1.2	4.68	0.81

[a] Data obtained from LLS.

Molecular mass and viscosity. The values of DS, M_w, polydispersity index (M_w/M_n) and $[\eta]$ of the fractions of native glucans and the sulfated derivatives are summarized in Tables 1 and 2, respectively. It is clear that the average values of M_w of sulfated derivatives are much lower than those of the native glucans, suggesting that the sulfation process causes the decrease of M_w, and also that the glycosidic linkage is comparatively weak (P. Zhang, L. Zhang, & Cheng, 2000). The SEC patterns of the fractions for ab-PCM3-I in 0.25 M lithium chloride in dimethylsulfoxide and of S-ab-PCM3-I in 0.9% w/v aqueous sodium chloride at 25 °C are shown in Figs. 2 and 3, respectively. Obviously, each fraction of different M_w was eluted at different elution volume. The results from Tables 1 and 2 indicate that the molecular mass distribution of the fractionated samples is relatively narrow. The single peak of each sample detected by LLS in Fig. 4 shows that there is no aggregation of the glucan in the solution. Therefore, the samples are suitable for investigating the solution properties. Fig. 5 shows the Zimm plot for the fraction S-2 in 0.9% w/v aqueous sodium chloride at 25 °C. Here K is the light scattering constant, R_θ is the reduced Rayleigh ratio at angle θ, and c is polysaccharide concentration. The M_w value of S-2 obtained from LLS is 4.16×10^4, which is in good agreement with the result of SEC-LLS (4.18×10^4). Therefore, the SEC-LLS was used to measure M_w and M_w/M_n of all fractions of native glucan and sulfated ones.

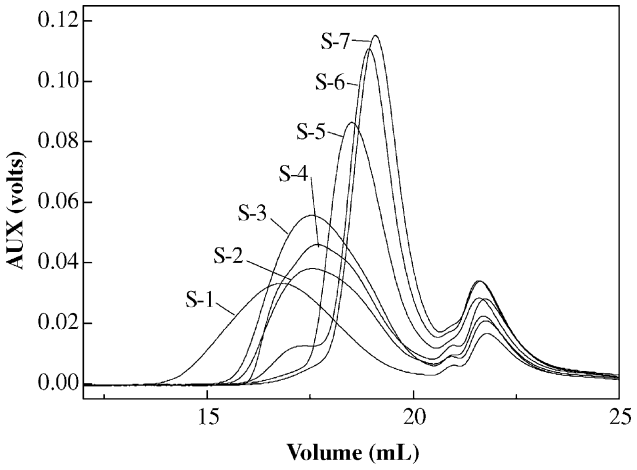

Fig. 3. Size exclusion chromatogram of the fractions of S-ab-PCM3-I determined by interferometric refractometer in 0.9% w/v aqueous sodium chloride at 25 °C.

The M_w dependence of $[\eta]$ for the (1→3)-α-D-glucan in 0.25 M lithium chloride in dimethylsulfoxide at 25 °C and for the sulfated derivatives in 0.9% w/v aqueous sodium chloride are shown in Fig. 6. Although, the DS values of S-ab-PCM3-I are different, the influence of DS on the $[\eta]$-M_w relationship could be negligible over a small range of DS. The Mark-Houwink equations for the fractions of ab-PCM3-I with M_w ranged from 1.08 to 23.1×10^4 and for S-ab-PCM3-I with M_w ranged from 0.74 to 5.80×10^4, are represented by

$$[\eta] = 9.92\times10^{-3}\, M_w^{0.77} (cm^3\ g^{-1},\ \text{ab-PCM3-I}) \quad (4)$$

$$[\eta] = 2.92\times10^{-4}\, M_w^{1.09} (cm^3\ g^{-1},\ \text{S-ab-PCM3-I}) \quad (5)$$

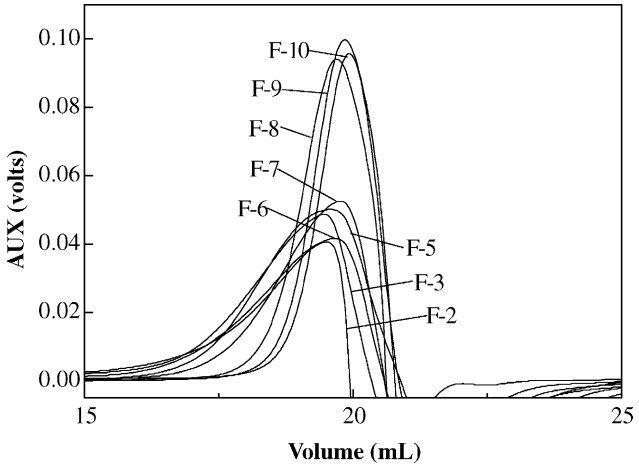

Fig. 2. Size exclusion chromatogram of the fractions of ab-PCM3-I determined by interferometric refractometer in 0.25 M lithium chloride in dimethylsulfoxide at 25 °C.

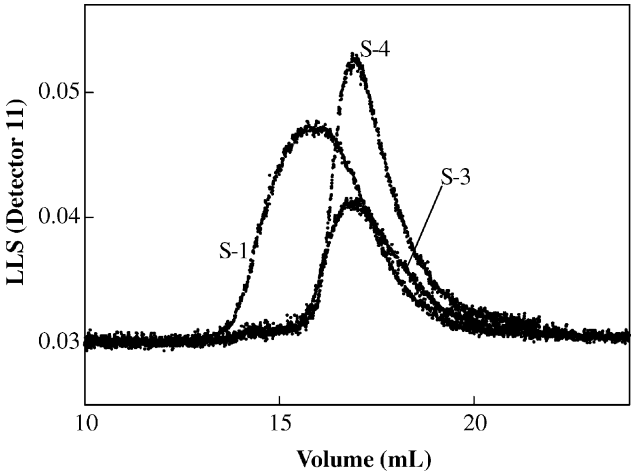

Fig. 4. Size exclusion chromatogram of the fractions of S-ab-PCM3-I determined by laser light scattering photometry at 90° light scattering angle (detector 11).

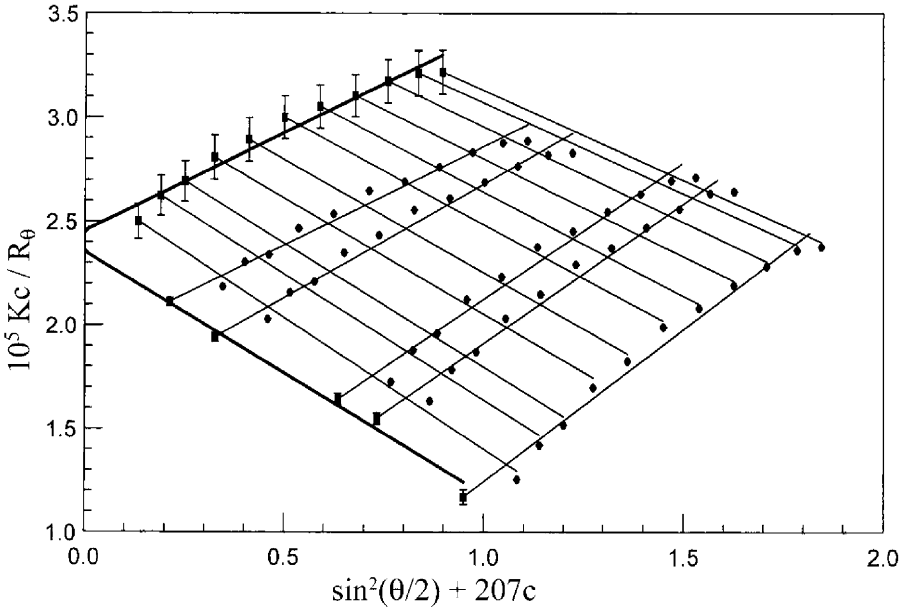

Fig. 5. Zimm plots for S-2 in 0.9% w/v aqueous sodium chloride at 25 °C.

The exponent α value is related to the shape of the macromolecule and nature of the solvent. For flexible linear polymer in good solvent the α value is in the range from 0.5 to 0.8. The polymer chain is more extended or semi-stiff when the α value is above 0.8 (Shukla, Cotts, Miller, Russell, Smith, Wallraff, & Baier, 1991). Therefore, the α value of 0.77 for the (1→3)-α-D-glucan in 0.25 M lithium chloride in dimethylsulfoxide lies in the range of an random coil chain polymer, similar to schizophyllan, (1→3)-β-D-glucan, (the point line in Fig. 6) having the α value of 0.68, which in DMSO exists as random coil conformation (Norisuye, Yanaki, & Fujita, 1980). On the other hand, the high exponent α value (1.09) of S-ab-PCM3-I indicated that the sulfated α-D-glucan exhibits as more extended molecular chains such as semi-flexible or stiff chain than the original.

Conformation parameters. On the basis of the data of M_w and [η], a wormlike cylinder model can be used for conformational characterization of the (1→3)-α-D-glucan fractions. Bohdanecky (Bohdanecky, 1983) independently has indicated that the Yamakawa–Fujii–Yoshizaki (Y–F–Y) theory (Yamakawa & Fujii, 1974; Yamakawa & Yoshizaki, 1980) for [η] of an unperturbed wormlike cylinder can be represented approximately by

$$(M^2/[\eta])^{1/3} = A_\eta + B_\eta M^{1/2} \tag{6}$$

$$A_\eta = \phi_{0,\infty}^{-1/3} A_0 M_L \ (g^{1/3}\ cm^{-1}) \tag{7}$$

$$B_\eta = \phi_{0,\infty}^{-1/3} B_0 (2q/M_L)^{-1/2} (g^{1/3}\ cm^{-1}) \tag{8}$$

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 (Bohdanecky, 1983), and $\phi_{0,\infty}$ is 2.87×10^{23} mol^{-1}. The plots of $(M_w^2/[\eta])^{1/3}$ vs. $M_w^{1/2}$ of the two series of polysaccharides are shown in Fig. 7. Substituting the intercept and slope of this plot into Eqs. (6)–(8) yielded 584 nm^{-1} for M_L and 3.7 nm for q of the native (1→3)-α-D-glucan ab-PCM3-I. Usually, the q value of flexible synthetic polymers in organic solvents ranges from 0.5 to 1.0 nm, but that of natural polysaccharide cannot reach so small value, such as (1→3)-α-D-glucan from *Ganoderma lucidum* (q = 1.5) (Chen, Zhang, Nakamura, & Norisuye, 1998). Therefore, ab-PCM3-I is regarded as a flexible chain characteristic. It was also found that the values of M_L and q for the sulfated polysaccharides are 834 nm^{-1} and 6.8 nm, respectively. Compared with

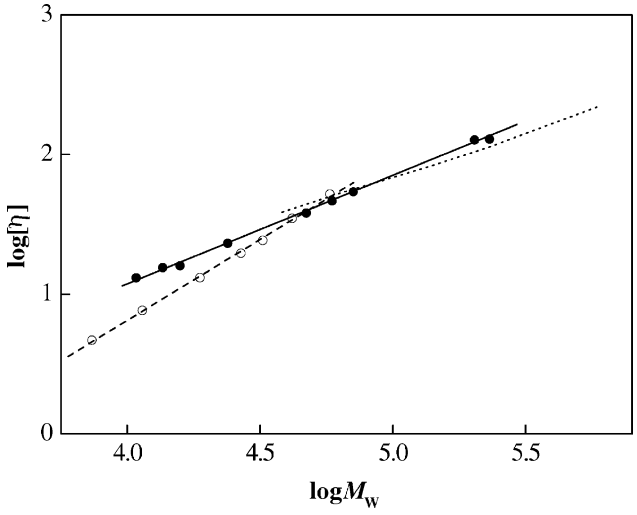

Fig. 6. The double-logarithmic plot of [η] against M_w for ab-PCM3-I fractions in 0.25 M lithium chloride in dimethylsulfoxide (●), S-ab-PCM3-I in 0.9% w/v aqueous sodium chloride (○) at 25 °C and schizophyllan (···) in DMSO.

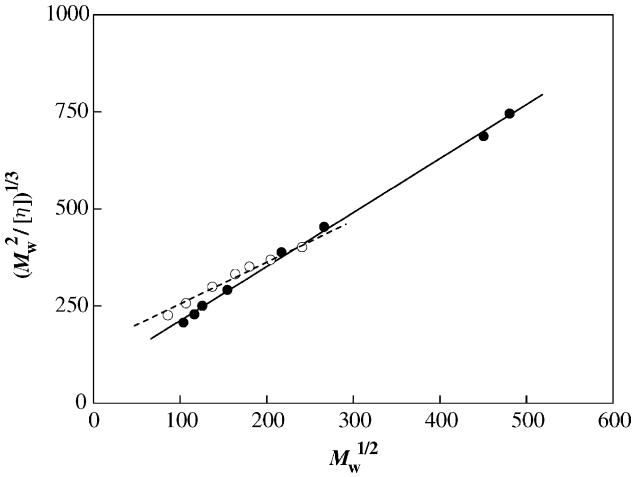

Fig. 7. The plot of $(M_w/[\eta])^{1/3}$ against $M_w^{1/2}$ for ab-PCM3-I fractions in 0.25 M lithium chloride in dimethylsulfoxide (●) and S-ab-PCM3-I in 0.9% w/v aqueous sodium chloride (○) at 25 °C.

the data of ab-PCM3-I, the relatively larger values of M_L and q for the S-ab-PCM3-I further provide the more extended chain conformation than the original. This indicates that the substituted sulfate group can enhance the chain stiffness from the q values of the former (3.7) and the latter (6.8).

The characteristic ratio (C_∞) can represent how much the chain is extended by steric hindrance. The C_∞ is defined as follow (Chen et al., 1998)

$$C_\infty = M_0/(\lambda M_L l^2) \tag{9}$$

where M_0 is the average molar mass of a glucose residue in repeat unit, λ^{-1} is the Kuhn's segment length ($\lambda^{-1} = 2q$), and l is the virtual bond length equal to the distance between two successive glycosidic oxygens O(3) and O(3′) in the present case. For ab-PCM3-I, assuming that l may be 0.42 nm as that of (1→3)-α-D-glucan (Chen et al., 1998), together with M_0 = 162, λ^{-1} = 7.4 nm, M_L = 584 nm^{-1}, C_∞ of α-D-glucan in 0.25 M lithium chloride in dimethylsulfoxide was calculated to be 11.6. It has been reported (Kato, Okamoto, Tokuya, & Takahashi, 1982) that pullulan, a linear polysaccharide polymerized from maltotriose as the repeating unit through the (1→6)-α-glucosidic linkage, has C_∞ value of 4.3 calculated from the data of M_w and [η] in 0.02% w/v aqueous sodium azide solution at 25 °C and exists as an slightly extended flexible coil. In this work, the C_∞ value of ab-PCM3-I was higher than that of pullulan, indicating that ab-PCM3-I exists as an extended flexible chain in 0.25 M lithium chloride in dimethylsulfoxide. For S-ab-PCM3-I, assuming that M_0 is above 162 considering the effect of the substituted group on the glucose residue, together with l = 0.42, λ^{-1} = 13.6 nm, M_L = 834 nm^{-1}, then the C_∞ value of the sulfated derivatives is calculated to be 18.1. The C_∞ value of S-ab-PCM3-I is much higher than that of native ab-PCM3-I, indicating that the chain density and chain stiffness has increased after sulfation, resulted from the substituted sulfate groups on the backbone that intensifies the steric hindrance. Remarkably, the C_∞ value of S-ab-PCM3-I lies in the range from 15 to 24, similar to that of cellulose that has a semi-stiff chain conformation in different solvent (Saalwalchter, Burchard, Klufers, Kettenbach, Mayer, Klemm, & Dugarmaa, 2000). This suggests that S-ab-PCM3-I exhibits a semi-stiff chain in aqueous solution.

To verify the q and M_L values calculated from the Y–F–Y theory, a trial-and-error method (Xu, Zhang, Nakamura, & Norisuye, 2002) has been used to find the suitable values of q and M_L that leads to the closest agreement between our M_w and [η] data. The theory (Yamakawa & Yoshizaki, 1980) for $[\eta]_0$ (the unperturbed intrinsic viscosity) of the wormlike chain contains one additional parameter, the chain diameter d. The solid curve in Fig. 8 represent the theoretical values computed with the resulting parameters of ab-PCM3-I, q = 3.7 nm, M_L = 584 nm^{-1}, and d = 0.9 nm. They fit the experimental data points (solid circles) of ab-PCM3-I very well. And the dash curve in Fig. 8 show the theoretical values computed with the resulting parameters of S-ab-PCM3-I, q = 7 nm, M_L = 835 nm^{-1}, and d = 1.1 nm, and also fit the experimental data points (hollow circles) of S-ab-PCM3-I quite well. Thus the resulting parameters are very close to the values calculated above, further indicating that ab-PCM3-I and S-ab-PCM3-I exist as extended random coil and semi-stiff chains in 0.25 M lithium chloride in dimethylsulfoxide and 0.9% w/v aqueous sodium chloride, respectively.

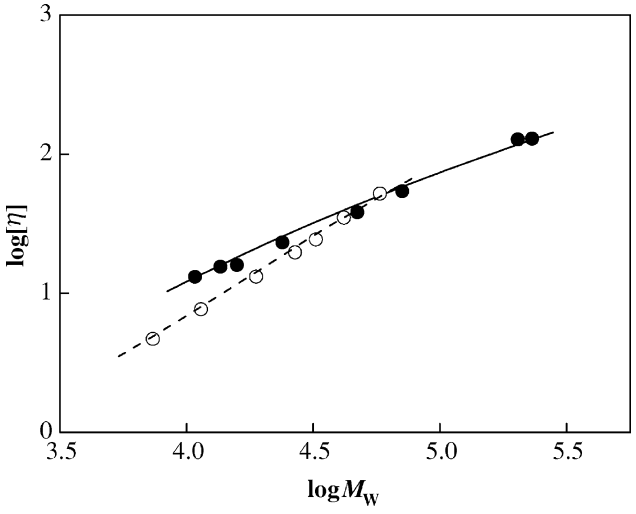

Fig. 8. Intrinsic viscosities for ab-PCM3-I fractions in 0.25 M lithium chloride in dimethylsulfoxide (●), compared with the theoretical curves for the unperturbed wormlike chain (—) with q = 3.7 nm, M_L = 584 nm^{-1}, and d = 0.9 nm, and intrinsic viscosities for S-ab-PCM3-I in 0.9% w/v aqueous sodium chloride (○), compared with the theoretical curves for the unperturbed wormlike chain (- - -) with q = 6.5 nm, M_L = 850 nm^{-1}, and d = 1.1 nm.

4. Conclusions

Water-insoluble (1→3)-α-D-glucan ab-PCM3-I was successfully sulfated by reacting with chlorosulfonic acid-pyridine complex reagent at 50 °C for 3 h to obtain water-soluble sulfated derivatives S-ab-PCM3-I having DS values ranging from 0.34 to 0.81. The dependences of intrinsic viscosity on weight-average molecular mass for the (1→3)-α-D-glucan ab-PCM3-I and the sulfated derivatives S-ab-PCM3-I determined by SEC-LLS and viscometry were found to be $[\eta]=9.92\times10^{-3}\ M_w^{0.77}$ ($cm^3\ g^{-1}$) and $[\eta]=2.92\times10^{-4}\ M_w^{1.09}$ ($cm^3\ g^{-1}$). On the basis of the current theories for a wormlike chain model, the conformation parameters of the glucan ab-PCM3-I and S-ab-PCM3-I were calculated to be 584 and 834 nm^{-1} for M_L, 3.7 and 6.8 nm for q, 11.6 and 18.1 for C_∞, respectively. The results revealed that ab-PCM3-I is water-insoluble and exists as a slightly extended random coil chain in 0.25 M lithium chloride in dimethylsulfoxide, whereas the sulfated derivative S-ab-PCM3-I has good water-solubility and exists as semi-stiff chain in aqueous solution. The relatively high chain stiffness of S-ab-PCM3-I can be attributed to the steric hindrance and electrostatic repulsion of the sulfate groups. Therefore, sulfation can transform water-insoluble polysaccharides into water-soluble one and increase its chain stiffness.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (20074025), the RGC Earmarked Research Grant (CUHK 4161/99M) and UGC-AoE Plant and Fungal Biotechnology Project of Hong Kong.

References

Adachi, Y., Ohna, N., Ohsawa, M., Sato, K., Oikawa, S., & Yadomae, T. (1989). Physicochemical properties and antitumor activities of chemically derivatives of antitumor glucan 'Grifolan LE' from *Grifola frondosa*. *Chemical and Pharmaceutical Bulletin*, *37*, 1838–1843.

Bao, X., Duan, J., Fang, X., & Fang, J. (2001). Chemical modifications of the α-(1→3)-D-glucan from spores of *Ganoderma lucidum* and investigation of their physicochemical properties and immunological activity. *Carbohydrate Research*, *336*, 127–140.

Bohdanecky, M. (1983). New method for estimating the parameters of the wormlike chain model from the intrinsic viscocity of stiff-chain polymers. *Macromolecules*, *16*, 1483–1489.

Bohn, J., & BeMiller, J. N. (1995). (1→3)-α-D-Glucans as biological response modifiers: a review of structure-functional activity relationships. *Carbohydrate Polymers*, *28*, 3–14.

Burton, B. A., & Brant, D. A. (1983). Comparative flexibility, extension, and conformation of some simple polysaccharide chain. *Biopolymer*, *22*, 1769–1792.

Chen, J., Zhang, L., Nakamura, Y., & Norisuye, T. (1998). Viscosity behavior and chain conformation of a (1-3)-α-D-glucan from *Ganoderma lucidum*. *Polymer Bulletin*, *41*, 471–475.

Kato, T., Okamoto, T., Tokuya, T., & Takahashi, A. (1982). Solution properties and chain flexibility of pullulan in aqueous solution. *Biopolymers*, *21*, 1623–1633.

Katsuraya, K., Nakashima, H., Yamamoto, N., & Uryu, T. (1999). Synthesis of sulfated oliogosaccharides glycosides having high anti-HIV activity and the relationship between activity and chemical structure. *Carbohydrate Research*, *315*, 234–242.

Katsuraya, K., Shibuya, T., Inazawa, K., Nakashima, H., Yamamoto, N., & Uryu, T. (1995). Synthesis of sulfated alkyl malto-oligosaccharides with potent inhibitory effects on AIDs virus infection. *Macromolecules*, *28*, 6697–6700.

Katsuraya, K., Shoji, T., Inazawa, K., Nakashima, H., Yamamoto, N., & Uryu, T. (1994). Synthesis of sulfated alkyl laminara-oligosaccharides having potent anti-HIV activity and the relationship between structure and biological activities. *Macromolecules*, *27*, 6695–6699.

Kiho, T., Yoshida, I., Nagai, K., Ukai, S., & Hara, C. (1989). (1→3)-α-D-glucan from an alkaline extract of *Agrocybe cylindracea*, and antitumor activity of its O-(carboxymethyl)ated derivatives. *Carbohydrate Research*, *189*, 273–279.

Lin, Y., Zhang, L., Jin, Y., Chen, L., & Zhang, M. (2003). Isolation, chemical components and molecular weights of polysaccharides from *Poria cocos* mycelia with cultivated strain. *Acta Polymer Sinica*, *1*, 97–103.

Norisuye, T., Yanaki, T., & Fujita, H. (1980). Triple helix of a *Schizophyllum commune* polysaccharide in aqueous solution. *Journal of Polymer Science: Polymer Physics Edition*, *18*, 547–558.

Ogawa, K., Misaki, A., Oka, S., & Okamura, K. (1979). X-ray diffraction data for (1→3)-α-D-glucan. *Carbohydrate Research*, *75*, 13–16.

Rees, D. A., & Scott, W. E. (1971). Polysaccharide conformation. Part VI. Computer model-building for linear and branched pyranoglycans. Correlations with biological function. Preliminary assessment of inter-residue forces on aqueous solution. Further interpretation of optical rotation term of chain conformation. *Journal Chemical Society (B)*, 469–479.

Saalwalchter, K., Burchard, W., Klufers, P., Kettenbach, G., Mayer, P., Klemm, D., & Dugarmaa, S. (2000). Cellulose solutions in water containing metal complexes. *Macromolecules*, *33*, 4094–4107.

Shukla, P., Cotts, P. M., Miller, R. D., Russell, T. P., Smith, B. A., Wallraff, G. M., & Baier, M. (1991). Conformational transitional studies of organosilane polymers by light and neutron scattering. *Macromolecules*, *24*, 5606–5670.

Striegel, A. M., & Timpa, J. D. (1995). Molecular characterization of polysaccharides dissolved in Me_2NAc-LiCl by gel-permeation chromatography. *Carbohydrate Research*, *26*, 271–290.

Tanigami, Y., Kusumoto, S., Nagao, S., Kokeguchi, S., Kato, K., Kotani, S., & Shiba, T. (1991). Partial degradation and biological activities of an antitumor polysaccharide from rice bran. *Chemical and Pharmaceutical Bulletin*, *39*(7), 1782–1787.

Xu, X., Zhang, L., Nakamura, Y., & Norisuye, T. (2002). Dilute-solution behavior of aeromonas gum, a heteropolysaccharide. *Polymer Bulletin*, *48*, 491–498.

Yamakawa, H., & Fujii, M. (1974). Intrinsic viscosity of wormlike chains. Determination of the shift factor. *Macromolecules*, *7*, 128–133.

Yamakawa, H., & Yoshizaki, T. (1980). Transport coefficients of helical wormlike chains 3. Intrinsic viscosity. *Macromolecules*, *13*, 633–643.

Young, S. H., & Jacobs, R. R. (1998). Sodium hydroxide-induced conformational change in schizophyllan detected by the fluorescence dye, aniline blue. *Carbohydrate Research*, *310*, 91–99.

Zhang, M., Zhang, L., Wang, Y., & Cheung, P. C. K. (2003). Chain conformation of sulfated derivatives of β-glucan from sclerotia of Pleurotus tuber-regium. *Carbohydrate Research*, *338*, 2863–2870.

Zhang, P., Zhang, L., & Cheng, S. (2000). Effects of urea and sodium hydroxide on the molecular weight and conformation of α-(1→3)-D-glucan from *Lentinus edodes* in aqueous solution. *Carbohydrate Research*, *327*, 431–438.

Zhang, P., Zhang, L., & Cheng, S. (2002). Solution properties of an α-(1→3)-D-glucan from *Lentinus edodes* and its sulfated derivatives. *Carbohydrate Research*, *337*, 155–160.