Carbohydrate Research 344 (2009) 105–112

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Structure and chain conformation of five water-soluble derivatives of a β-D-glucan isolated from *Ganoderma lucidum*

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ARTICLE INFO

Article history:
Received 30 June 2008
Received in revised form 10 September 2008
Accepted 22 September 2008
Available online 30 September 2008

Keywords:
Ganoderma lucidum
Derivatives of glucan
Structure
Chain conformation
Molecular mass
NMR spectroscopy

ABSTRACT

A linear water-insoluble (1→3)-β-D-glucan, coded as GL-IV-I, was isolated from the fruit body of *Ganoderma lucidum* by extracting with NaOH solution. Its derivatives were prepared by using sulfation, carboxymethylation, hydroxyethylation, hydroxypropylation, and methylation, respectively, and these were labeled as S-GL, CM-GL, HE-GL, HP-GL and M-GL. Five derivatives exhibited good water solubility. Their structures and chain conformations were investigated with infrared spectroscopy, elemental analysis (EA), one- and two-dimensional NMR spectroscopy, laser light scattering (LLS), and size-exclusion chromatography combined with LLS (SEC–LLS). The reactivity of the hydroxyl group of GL-IV-I was ordered as C-6 > C-4 > C-2 for the five derivatives. The degree of substitution (DS) of the derivatives was calculated from EA and NMR spectroscopy to be from 0.32 to 1.18. The weight-average molecular mass (M_w) of GL-IV-I, S-GL, CM-GL, HE-GL, HP-GL, and M-GL was 13.3×10^4, 10.1×10^4, 6.3×10^4, 7.2×10^4, 5.1×10^4, and 14.1×10^4, respectively. The conformation analysis studies revealed that GL-IV-I exists as a compact coil in dimethyl sulfoxide, whereas the five derivatives are slightly expanded flexible chains in 0.9% aqueous NaCl solution.

1. Introduction

Ganoderma lucidum (Leyss, ex Fr.) Karst (Gl), a basidiomycete belonging to the Polyporaceae, is a mushroom known in Chinese traditional medicine as '*Lingzhi*'. Its fruiting bodies have been long used to promote health and longevity. It was regarded as an elixir of youth by ancient emperors. The earliest record of '*Lingzhi*' was in the 'Shen Nong's Materia Medica' in Han Dynasty of China about two thousands years ago.[1] Nowadays, it is still used to treat gastric ulcers, chronic hepatitis, nephritis, hypertension, hyperlipemia, arthritis, insomnia, bronchitis, asthma, arteriosclerosis, leukopenia, diabetes, and anorexia.[2–4] It has even been called a therapeutic fungal biofactory.[5] Recently, polysaccharides extracted from *G. lucidum* and other fungal mushrooms have attracted much attention because of their antitumor activities which arise by mediating mechanisms in the immune system.[6–16] Water-insoluble polysaccharides extracted from the fruit body of *G. lucidum* have been reported by Sone et al. and Chen et al. for a (1→3)-β-D-glucan with a low degree of branching and a (1→3)-α-D-glucan, respectively.[17,18] Polysaccharides from the mycelium of *G. lucidum* have also been studied in our laboratory.[19] However, a linear β-D-glucan from *G. lucidum* obtained by cultivation has been never reported. It is noted that the water-soluble derivatives prepared from the water-insoluble polysaccharide through sulfation and carboxymethylation reactions have shown relatively higher antitumor and antiviral activities.[20–27] Moreover, the molecular mass, chain conformation, and chemical structure of the polysaccharides can significantly affect their antitumor activities and immunomodulatory activities.[28,29,12] Usually, these bioactivities are related to the chain conformations and molecular mass of the polysaccharides. However, the chain conformation and bioactivities of the glucan isolated from *G. lucidum* and its derivatives are still unclear. In this work, we extracted a linear water-insoluble (1→3)-β-D-glucan from *G. lucidum* by using an alkaline solution, and then we prepared five water-soluble derivatives from it. Their structure, molecular mass, and chain conformation of the various derivatives were studied with laser light scattering (LLS), size-exclusion chromatography (SEC), and viscometry. This work could provide derivatization conditions for water-insoluble linear polysaccharides, as well as the important information on the molecular mass and chain conformations of the water-soluble derivatives.

2. Experimental

2.1. Materials

Dried *G. lucidum* was a kind gift of Longyan College in Fujian, China. The fruit body was powdered, then defatted by using a Soxhlet extractor with EtOAc for 8 h and then with acetone for

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0008-6215/$ - see front matter © 2008 Published by Elsevier Ltd.
doi:10.1016/j.carres.2008.09.024

8 h. The resultant residue was immersed in 0.9% aq NaCl solution at 25 °C, 40 °C, and 95 °C, respectively, and then stirred three times for 24 h to remove the water-soluble polysaccharide. The residue mentioned above was extracted with 1 M aq NaOH solution for 8 h at 40 °C. The resulting extract was centrifuged at 7000 rpm and then the supernatant was neutralized by HOAc. The deposition was collected and washed with distilled water ten times, then lyophilized to get a white powder, coded as GL-IV-I. To get a more purified sample, the polysaccharide was dissolved in Me_2SO with stirring at 25 °C overnight and then centrifuged to obtain the supernatant. It was dialyzed (regenerated cellulose tubing: M_w cut-off 8000, USA) with water for five days and with distilled water for three days to get a white precipitate, which was lyophilized to get the pure white powder GL-IV-I in a maximum yield of 7.6%.

2.2. Preparation of derivatives

2.2.1. Sulfation

According to the procedures of Yoshida et al.,[30] 500 mg of GL-IV-I was dissolved in 20 mL of Me_2SO with stirring at room temperature overnight to get a bright solution, and then pyridine and chlorosulfonic acid were sequentially added to the solution to prepare the sulfated glucan. The resultant solution of sulfated glucan was dialyzed against water for 5 days, and then distilled water for 3 days to remove the pyridine. Finally, the solution was lyophilized using a lyophilizer (CHRIST Alpha 1-2, Germany) to get the light-yellow sample, coded as S-GL.

2.2.2. Carboxymethylation

According to the procedures of Bao et al.,[31] 500 mg of the polysaccharide was suspended in 15 mL of 2-PrOH at room temperature, and 10 mL of 30% aq NaOH solution and 3 g of $ClCH_2CO_2H$ were sequentially added and allowed to react for 2 h at 50 °C. The reaction mixture was then separated into two layers. The top 2-PrOH layer contained very little carboxymethylated glucan, while the bottom layer contained the carboxymethylated derivatives in aq NaOH solution. The whole mixture was neutralized with HCl to give a homogeneous solution consisting of carboxymethylated glucan, NaCl, 2-PrOH, and water. The resultant solution was dialyzed against water for 5 days and then against distilled water for 3 days, at the end of which time it was lyophilized to get a white scale-like sample of carboxymethylated glucan, coded as CM-GL.

2.2.3. Hydroxyethylation and hydroxypropylation

Two derivatives were prepared by using 2-chloroethanol and 1,2-epoxypropane via the same procedure as that used for CM-GL. 2-Chloroethanol (5 mL) was subsequently added and stirred for 8 h at 45 °C, and then 1,2-epoxypropane (20 mL) was added to the mixture in an ice bath with stirring for 24 h at 60 °C. The reacted mixture was neutralized, dialyzed and lyophilized as in the forgoing procedure to get a white scale-like sample, coded as HE-GL for hydroxyethylated glucan and HP-GL for hydroxypropylated glucan, respectively.

2.3. Methylation

Methylation of the sample was performed according to the classical method of Needs and Selvendran.[32] A 500-mg sample of polysaccharide was suspended in 20 mL of dry Me_2SO with stirring at 30 °C overnight to get a homogenous solution. NaOH powder was added to the reaction mixture, and under N_2 and in an ice bath, iodomethane was added. Water was added to quench the reaction, and the solution was dialyzed and filtrated to get a clear solution that was then lyophilized to get a white scale-like methylated glucan, coded as M-GL.

3. Characterization

Infrared spectra of the samples were recorded with a Nicolet Fourier transform infrared (FTIR) spectrometer (Spectrum One, Thermo Nicolet Co., Madison, WI, USA) in the range of 4000–400 cm^{-1} using the KBr disk method. High-resolution ^{13}C NMR spectroscopy was carried out with a Varian Mercury 500 NMR spectrometer (Varian Inc., Palo Alto, CA, USA) at room temperature. The concentrations of the GL-IV-I and its derivatives were adjusted to 100 mg/mL for the NMR experiments. Me_2SO-d_6 was used as solvent for GL-IV-I, and 99.96% D_2O was used as solvent for the derivatives.

Intrinsic viscosities ($[\eta]$) of the polysaccharides were measured in 0.9% aqueous NaCl solution and in Me_2SO at 25 ± 0.1 °C by using an Ubbelohde capillary viscometer. The kinetic energy correction was negligible. Huggins and Kraemer equations were used to estimate the $[\eta]$ value 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 certain conditions in a given solvent, η_{sp}/c is the reduced specific viscosity, and $(\ln \eta_r)/c$ is the inherent viscosity.

Weight-average molecular mass (M_w) and radius of gyration ($\langle S^2 \rangle_z^{1/2}$) of samples in 0.9% aqueous NaCl solution were determined with a multi-angle laser light-scattering (MALLS) instrument equipped with a He–Ne laser (MALLS, λ = 633 nm; DAWN-DSP,

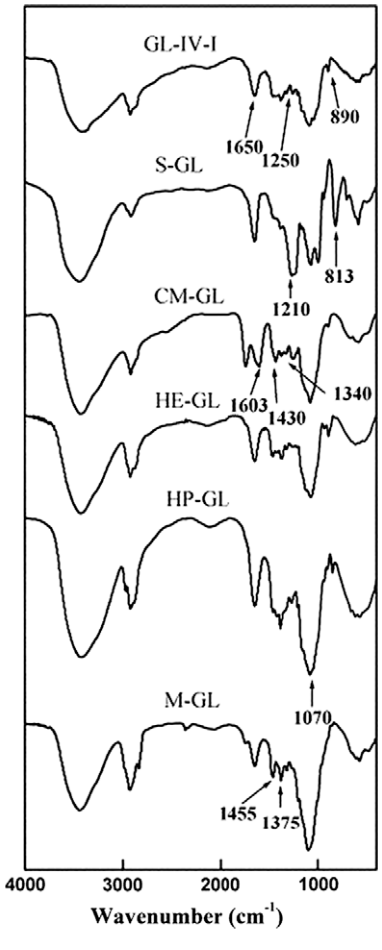

Figure 1. FTIR spectra of the native GL-IV-I and its derivatives.

Table 1
^{13}C NMR chemical shifts for native sample GL-IV-I and its derivatives

Sample	δ (ppm)											
	C-1	C-1'	C-2	C-2s	C-3	C-3'	C-4	C-4s	C-5	C-5'	C-6	C-6s
GL-IV-I	103.7		73.5		86.7		69.1		76.9		61.5	
S-GL	103.7	101.2	73.6	79.7	86.8		69.0	76.2	77.0	72.8	61.4	67.9
CM-GL	102.9		73.5	82.0	86.2	85.2	68.9	74.5	76.5	74.1	61.3	68.9
HE-GL	102.7		73.8	82.1	86.1		68.3	76.0	76.6	73.9	60.9	70.0
HP-GL	102.3		73.8	81.8	85.8	84.5	69.5	74.2	77.0	74.1	60.7	66.9
M-G-	101.6		73.6	80.2	86.0	84.1	69.6	74.3	77.8	74.0	60.1	68.4

Table 2
The yield, solubility and degree of substitution (DS) for the derivatives

Sample	Yield (%)	Solubility[a]		DS from	DS from ^{13}C NMR			
		0.9% NaCl	Me_2SO	EA result	C-6	C-4	C-2	Total DS
GL-IV-I	7.6	–	+					
S-GL	86.3	++	–	0.94	0.68	0.12	0.08	0.88
CM-GL	95.1	++	–	1.18	0.62	0.28	0.19	1.09
HE-GL	74.8	++	+	0.53	0.23	0.15	0.11	0.49
HP-GL	65.4	++	+	0.44	0.21	0.13	0.07	0.41
M-GL	82.7	++	+	0.74	0.28	0.22	0.19	0.69

[a] ++: Good solubility; +: soluble; –: insoluble.

Wyatt Technology Co., Santa Barbara, CA) at various angles. The basic light scattering equation is as follows:

$$\frac{Kc}{R\theta}=\frac{1}{M_{\mathrm{w}}}\left(1+\frac{16\pi^2<S^2>}{3\lambda^2}\mathrm{Sin}^2(\theta/2)\right)+2A_2c+\cdots \qquad (3)$$

where K is an optical constant equal to $[4\pi^2n^2(\mathrm{d}n/\mathrm{d}c)^2]/(\lambda^4N_{\mathrm{A}})$, c is the polymer concentration in mg/mL, R_θ is the Rayleigh ratio, λ is the wavelength, n is the refractive index of the solvent, dn/dc is the refractive index increment, N_{A} is Avogadro's number, and A_2 is the second virial coefficient. The polysaccharide solutions achieved by filtration through a 0.2-μm filter (PTFE, Puradisc 13-mm Syringe Filters, Whatman, Kent, UK) into the scattering cell. The dn/dc values were measured using an interferometric refractometer (Optilab, DAWN® DSP, Wyatt Technology Co., Santa Barbara, CA, USA) at a wavelength of 633 nm at 25 °C. The experimental values of dn/dc at 633 nm were 0.147 cm^3/g, 0.141 cm^3/g, 0.143 cm^3/g, 0.146 cm^3/g, and 0.144 cm^3/g for S-GL, CM-GL, HE-GL, HP-GL, and M-GL, respectively. ASTRA software (Version 4.70.07) was utilized for data acquisition and analysis. A size-exclusion chromatograph combined with multi-angle laser light scattering (SEC–LLS) combined with UV and differential refractive index (DRI) detectors has been demonstrated to be a convenient method for characterization of polymers.[33] SEC–LLS measurements of the samples were carried out by a P100 pump (RI-150 Thermo separation products, San Jose, CA, USA) equipped with columns of G4000PWXL (MicroPak, TSK) in 0.9% aqueous NaCl solution. A column of G4000HXL in Me_2SO at 25 °C was also used for GL-IV-I. The carrier solutions were 0.9% aqueous NaCl solution for the five derivatives and Me_2SO for GL-IV-I. The injection volume was 200 μL with a concentration of 3 mg/mL for the sample, and the flow rate was 0.5 mL/min in 0.9% aqueous NaCl solution and in Me_2SO. ASTRA software (version 4.90.07) was utilized for data acquisition and analysis.

4. Results and discussion

4.1. Chemical structure

The FTIR spectra of the native GL-IV-I and its derivatives are shown in Figure 1. All samples exhibited the characteristic IR absorption of the polysaccharide at 1250 and 1650 cm^{-1}. The native GL-IV-I exhibited absorption peaks at 890 cm^{-1}, which is the absorption peak of a β-glucan.[34] Compared to GL-IV-I, the characteristic peak of the β-configuration of S-GL at 890 cm^{-1} disappeared, and two new absorption peaks at 813 and 1210 cm^{-1} appeared for a sulfated derivative, which corresponds to the C–S–C symmetrical vibration and S=O asymmetrical stretching.[35] This indicated that a sulfated derivative had been successfully synthesized from GL-IV-I. For CM-GL, the characteristic peak of the native polysaccharide also weakened, and two new absorption peaks appeared at 1603 and 1430 cm^{-1}, assigned to the asymmetrical –COO stretching vibration. The symmetrical –COO stretching and the new peak at 1340 cm^{-1} were assigned to the –CH_2– group.[36–38]

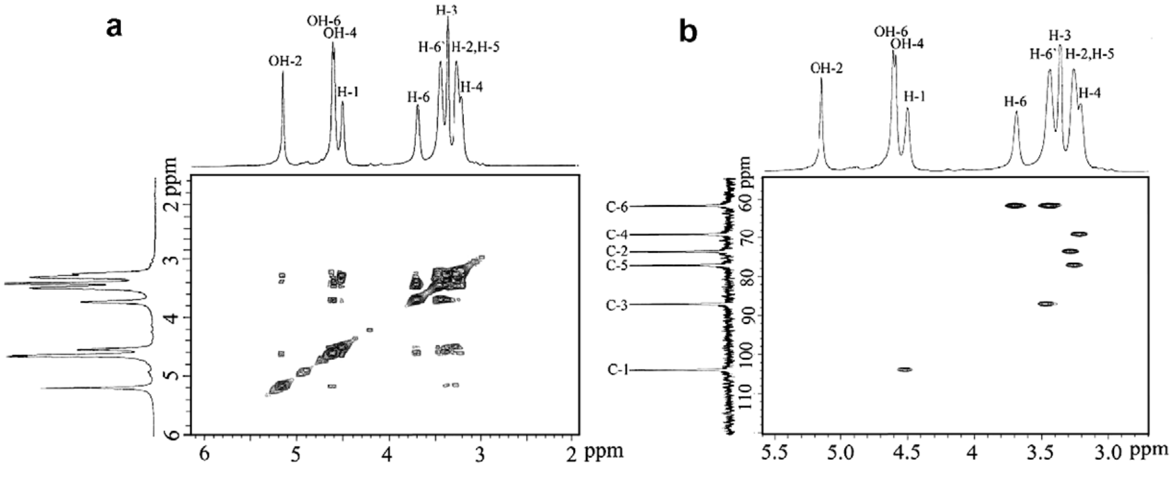

Figure 2. 2D NMR from DQF (a) and HMQC (b) experiments of GL-IV-I in D_2O at 25 °C.

The absorption peaks at 1375 and 1455 cm^{-1} in the IR spectra of M-GL indicated the presence of $-CH_3$. In the FTIR of HE-GL and HP-GL, the relatively high intensity of the C–O–C band at 1070 cm^{-1} and the –OH band at 3400 cm^{-1} suggested that the hydroxyethylated and hydroxypropylated glucan were also successfully prepared.[31,39]

The five derivatives of glucan were water soluble. The yields, water solubility and the degree of substitution (DS) of the native polysaccharides and their derivatives are summarized in Table 2. DS was calculated from the elemental composition (W%) and ^{13}C NMR spectral data.[40] DS from the elemental composition was calculated by[39]

$$DS = (162 \times W\% - M_a)/(W_a - M_s \times W\%) \tag{4}$$

where M_a is total atomic weight of the element from the original sugar unit, W_a is total atomic weight of the element from the substitution group, and M_s is the molecular mass of a substitution group.

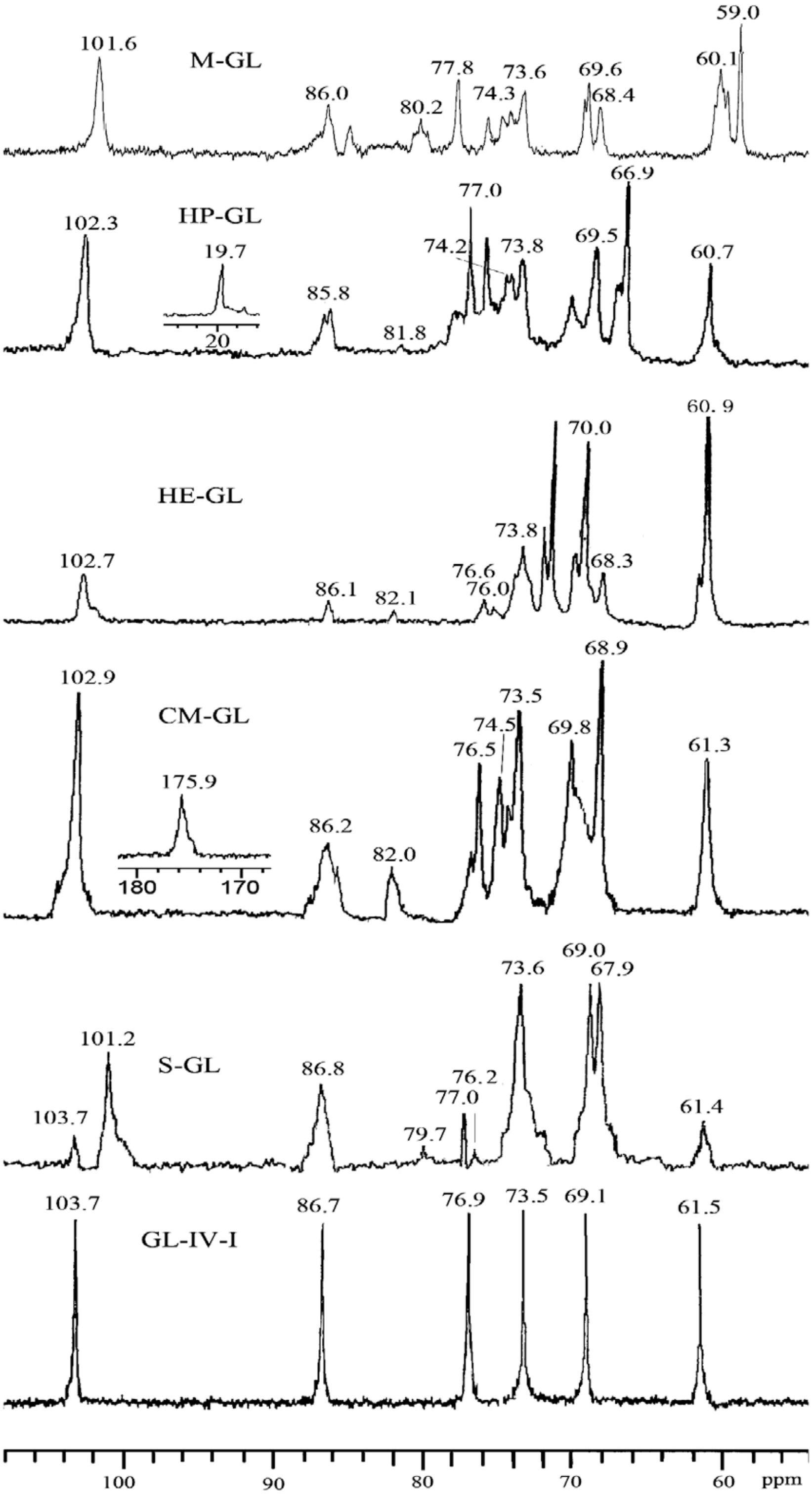

Figure 3. ^{13}C NMR spectra for native sample GL-IV-I in Me_2SO-d_6 and its derivatives in D_2O at 25 °C.

The ^{13}C NMR spectra for native sample GL-IV-I and its derivatives are shown in Figure 3, and their chemical shifts are listed in Table 1. The ^{13}C NMR spectrum of the native GL-IV-I exhibits six signals around δ 103.7, 86.9, 77.0, 73.5, 69.1, and 61.5, attributed to C-1, C-3, C-5, C-2, C-4, and C-6, respectively, indicating the characteristic of a β-(1→3)-D-glucan.[41] The chemical structure of GL-IV-I was analyzed further by heteronuclear multiquantum coherence (HMQC) and double quantum filter (DQF) spectroscopy as shown in Figure 2. The HMQC spectrum for GL-IV-I indicated the correlation between the directly bonded C and the directly bonded H atoms with distinguishing the protons directly and indirectly linked to a C atom. The DQF spectrum indicated the correlation among the H atoms connected within three chemical bonds. The combined spectral data allow us to assign the peaks to H and C.[42] The peaks of the proton at δ 4.51 correlated to the carbon at δ 103.8 and could be assigned to the pair of H-1/C-1. The protons at δ 3.27, 3.37, 3.22, 3.22, and 3.69 correlated to the carbon at δ 73.6, 86.9, 69.1, 77.1, and 61.6 for H-2/C-2, H-3/C-3, H-4/C-4, H-5/C-5 and for H-6/C-6, respectively.[43] The signals in the ^{1}H NMR spectrum at δ 5.16, 4.62, and 4.60 were assigned to OH-2, OH-6, and OH-4. This conformed that GL-IV-I was a linear β-(1→3)-D-glucan.[44]

Figure 3 shows the ^{13}C NMR spectra of S-GL. Compared with the native GL-IV-I sample, there were several new signals caused by sulfated groups in S-GL. The peak at δ 67.9 was assigned to the signal of the C-6s, δ 79.7 for the C-2s, and δ 76.2 for the C-4s. The peak at δ 61.6 was weakened, indicating that C-6 had been substituted by sulfur-containing group, whereas the C-2 and C-4 have been partially substituted. The strong peaks were present in the NMR spectrum and the peaks at δ 79.7 for C-2s and at δ 76.2 for the C-4s were much weaker than that of the C-6s at δ 67.9. We concluded that the C-6 position was more active than the others as a result of steric hindrance.[45] The downfield shift of the carbon atoms linked with the sulfur groups was about 6–7 ppm.[42] Furthermore, a new peak appeared at δ 101.2, and the original C-1 peak at δ 103.7 became weaker in the NMR spectrum of S-GL. This could be explained by the fact that as the C-2 and C-6 had been substituted, the influence on the adjacent C-1 was sufficient to split the peak into two peaks. The up field shift from C-1 to C-1′ was about 2.5 ppm, which is somewhat in line with the description of Gorin.[42] The same situation exists for C-5, resulting in the peak at δ 72.8 being assigned to C-5′ of S-GL.

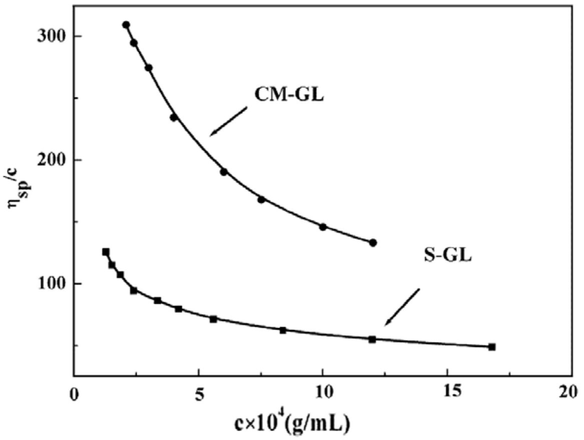

Figure 4. Reduced viscosity (η_{sp}/c) against concentration(c) of CM-GL and S-GL in pure water at 25 °C.

Compared to the native GL-IV-I, a new strong peak at δ 175.9 for CM-GL could be attributed to the C=O carbon of carboxymethyl group, indicating the successful carboxymethylation of GL-IV-I. Peaks for the C-6s, the C-4s, and the C-2s were at δ 68.9, 74.5, and 82.0, respectively, and the peak intensity was in the order of C-6s > C-4s > C-2s. Therefore, the C-6 position was more active than that of either C-4 or C-2 for the carboxymethylation. Compared to the sulfation and carboxymethylation of GL-IV-I, the hydroxyethylation of GL-IV-I at C-6 showed a weak peak for the C-6s at δ 70.0, and the peaks for the C-2s and the C-4s at δ 82.1 and 76.0, respectively, were also very weak, indicating that hydroxyethylation had occurred.

For HP-GL, a new peak at δ 19.7 could be attributed to the C atom of $-CH_2-$, indicating that hydroxypropylation occurred. The peak for the C-6s for HP-GL at δ 66.9 was much stronger than that of the C-4s at δ 74.2, and the C-2s at δ 81.8. One could conclude that the C-6 position was more active than either C-4 or C-2 for the hydroxypropylation reaction, which is similar to the situation of carboxymethylation. For the ^{13}C NMR spectrum of M-GL, the peaks around δ 59.0–60.1 could be assigned to the $O-CH_3$ resonances, and the new peaks at δ 68.4, 74.3, and 86.0 could be attributed to the C-6s, the C-4s, and the C-2s, respectively. Details of the chemical shifts of HE-GL, HP-GL, and M-GL are compiled in Table 1.[39]

From the ^{13}C NMR results, that non-selective substitution occurred on the active hydroxyl groups of GL-IV-I, and the

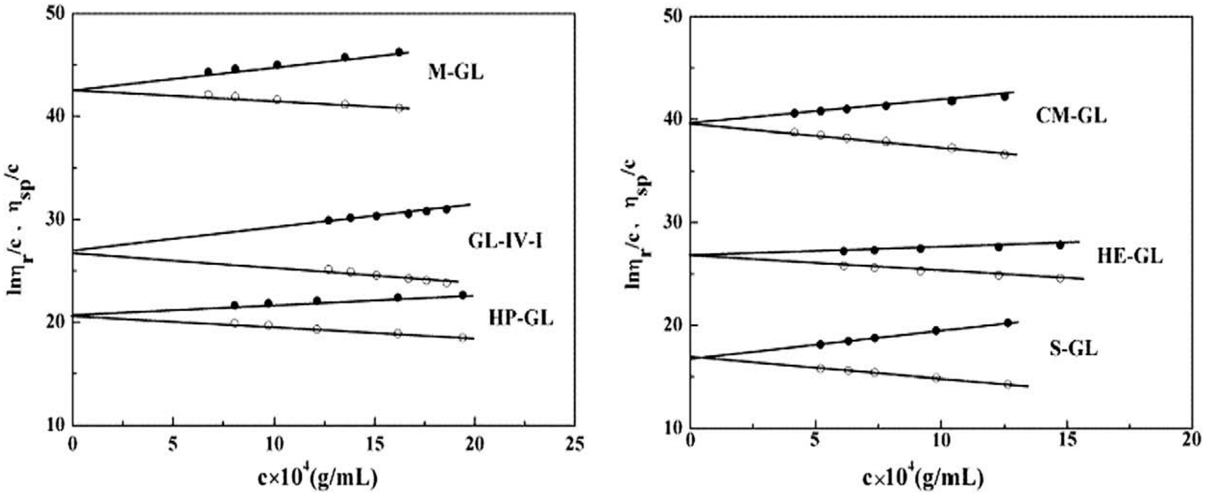

Figure 5. Plots of $\eta_{sp}/c - c$ and $\ln\eta_r/c - c$ of GL-IV-I in Me_2SO and its derivatives in 0.9% aqueous NaCl solution at 25 °C.

Table 3
Experiment results from LLS, SEC–LLS, and viscosity for GL-IV-I and its derivatives

Sample	Solvent	LLS			SEC–LLS			$[\eta]$ (cm^3 g^{-1})
		$M_w \times 10^{-4}$ (g mol^{-1})	$A_2 \times 10^4$ (mol mL g^{-2})	$\langle S^2 \rangle_z^{1/2}$ (nm)	$M_w \times 10^{-4}$ (g mol^{-1})	M_w/M_n	α	
GL-IV-I	Me_2SO	12.4	3.67	53.5	13.3	1.37	0.47	27.8
S-GL	0.9% NaCl	12.5	2.14	56.6	10.1	1.86	0.61	16.7
CM-GL	0.9% NaCl	5.2	3.56	35.7	6.3	1.62	0.56	39.7
HE-GL	0.9% NaCl	7.9	3.31	46	7.2	1.65	0.54	26.8
HP-GL	0.9% NaCl	5.6	3.12	38	5.1	1.76	0.51	21.2
M-GL	0.9% NaCl	15.9	0.14	60.6	14.1	1.68	0.58	43.2

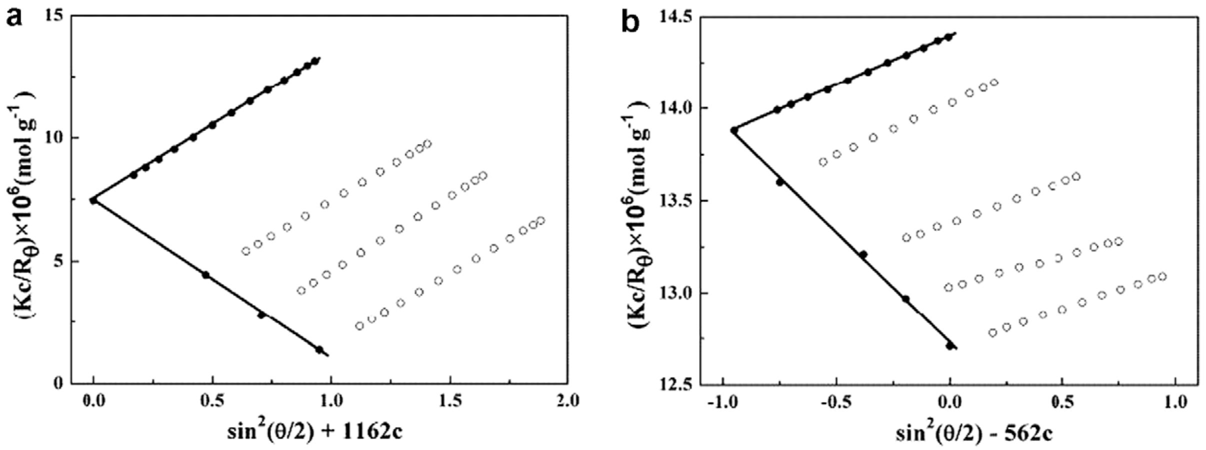

Figure 6. Zimm plots for GL-IV-I (a) in Me_2SO and HE-GL (b) in 0.9% aqueous NaCl solution at 25 °C.

multi-substitution at C-2, C-4, and C-6 exhibited different reactivities. The DS of the derivatives can be calculated by NMR spectroscopy according to the method reported by Zhou et al.[45] The relative DS values of derivatives at the C-2, C-4, and C-6 positions were calculated from the relative intensities of peaks C-2, C-2s, C-4, C-4s, C-6, and C-6s in the ^{13}C NMR spectra for S-GL, CM-GL, HE-GL, HP-GL, and M-GL to be 0.68:0.12:0.08, 0.62:0.28:0.19, 0.23:0.15:0.11, 0.21:0.13:0.07, and 0.28:0.22:0.19, respectively (Table 2.) Thus the reactivities of the active hydroxyl groups were in the order of C-6 > C-4 > C-2 on the whole. This could be explained in that steric hindrance led to the different reactivities of C-6, C-4, and C-2.[46] The substitution further caused the adjacent C-1, C-3, and C-5 to each split into two peaks with an upfield shift of ~2–3 ppm, as shown in Table 1.

4.2. Viscosity behavior

Figure 4 shows the dependence of the reduced viscosity (η_{sp}/c) on the concentration (c) of S-GL and CM-GL in pure water at 25 °C. There was an abnormal phenomenon for the S-GL and CM-GL samples in pure water, namely, η_{sp}/c of the derivatives displayed typical polyelectrolyte behavior, which increased remarkably with a decrease of the polymer concentration. This was a result of the electrostatic repulsion caused by the sulfate or carboxylate group, leading to the more expanded chains. However, as NaCl was added, the electrostatic repulsion could be screened out, and the Huggins and Kraemer plots became normal, showing the usual polymer solution behavior.[47] Figure 5 shows the Huggins and Kraemer plots of GL-IV-I and it derivatives in 0.9% aqueous NaCl solution at 25 °C. These plots basically indicate normal polymer solution behavior. Thus the molecular mass and chain conformation can be further studied. The $[\eta]$ value of polymers reflects the extent of expansion of the chain in solution. For macromolecules of the same molecular mass, a more compact random coil chain conformation gives a lower $[\eta]$ value. The $[\eta]$ value of GL-IV-I was much lower than the values of the glucan from *Poria cocos*[48,49] and pullulan[50], indicating that GL-IV-I in Me_2SO exists as relatively compact coils.

4.3. Molecular mass and chain conformation

The molecular mass and chain conformation parameters of GL-IV-I and its derivatives are summarized in Table 3. Figure 6 shows Zimm plots for GL-IV-I (a) in Me_2SO and HE-GL (b) in 0.9% aqueous NaCl solution at 25 °C. As shown in Table 3, the M_w values measured by SEC–LLS in 0.9% aqueous NaCl solution are in good agreement with those determined by LLS. Interestingly, the molecular mass of CM-GL, S-GL, HE-GL, and HP-GL are slightly lower than that of the native GL-IV-I sample. Usually, after derivation, the

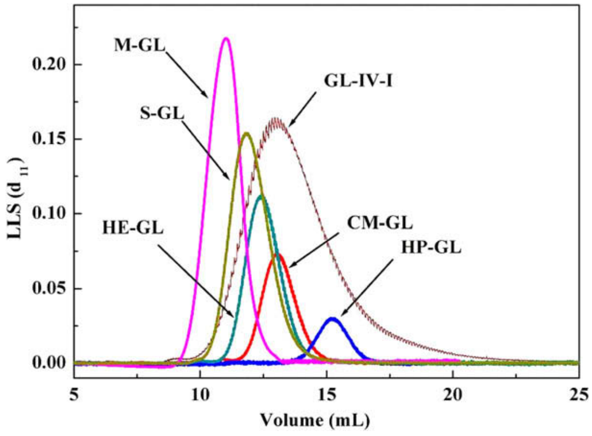

Figure 7. SEC patterns of GL-IV-I in Me_2SO and its derivatives in 0.9% aqueous NaCl solution from LLS (detector 11) at 25 °C.

molecular mass should be increased; the decrease of the molecular mass could be caused by degradation during the derivation because of the vigorous reaction condition. However, the molecular mass of M-GL was higher than that of the native sample. This suggested that there was almost no degradation of the glucan during methylation, and the introduction of the new CH_3 groups led to the increase of the M_w value.

SEC–LLS, as an absolute method, are convenient for the determination of true molecular mass and distribution without standard samples. The SEC patterns of GL-IV-I in Me_2SO and the derivatives in 0.9% aqueous NaCl solution analyzed by the LLS (detector $d_{11} = 90°$) at 25 °C are shown in Figure 7. As shown, there is a single peak with good symmetry detected by LLS for GL-IV-I and its derivatives. This suggests that there is no aggregation of GL-IV-I and its derivatives in either Me_2SO or aqueous NaCl solution. From the SEC chromatogram detected by LLS, we could obtain the M_w and $\langle S^2 \rangle_z^{1/2}$ values of numerous fractions that have been estimated from many experimental points in the SEC.[51] From data of M_w and the $\langle S^2 \rangle_z^{1/2}$, the power law describing the relationship between M_w and $\langle S^2 \rangle_z^{1/2}$ ($\langle S^2 \rangle_z^{1/2} \propto M_w$) can thus be created.[50] Usually, The chain conformation of a polymer in solution can be judged by the value of exponent α. The α value is 0.3 for a globular shape, 0.5–0.6 for a flexible chain conformation in a good solvent, and 0.6–1 for a semi-flexible chain.[52] The log–log plots of $\langle S^2 \rangle_z^{1/2} \propto M_w^{\alpha}$ for GL-IV-I in Me_2SO and its derivatives in 0.9% NaCl are shown in Figure 8. The α value for GL-IV-I was 0.47, indicating that the glucan exists as a compact random coil in Me_2SO at 25 °C. The α values for S-GL, CM-GL, HE-GL, HP-GL, and M-GL were 0.58, 0.61, 0.56, 0.54, and 0.51, respectively. The results indicate that the five derivatives exist as relatively expanded flexible chains in 0.9% aqueous NaCl

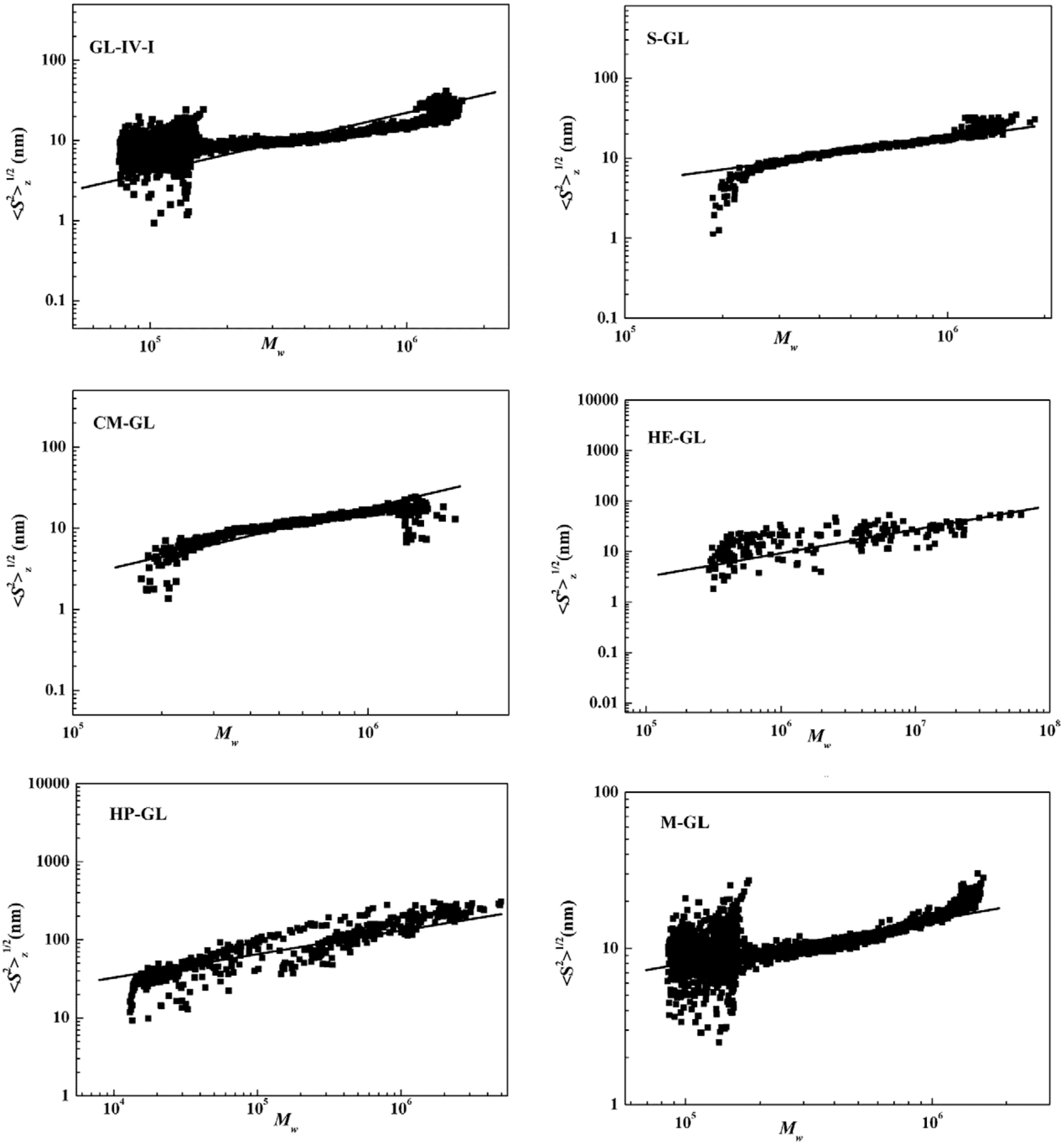

Figure 8. Dependence of $\langle S^2 \rangle_z^{1/2}$ on M_w for GL-IV-I in Me_2SO and its derivatives in 0.9% aqueous NaCl solution at 25 °C.

solution, resulting from steric hindrance of the substituted groups introduced onto the backbone.

5. Conclusion

A linear water-insoluble glucan was extracted successfully from the fruit body of *G. lucidum*. Five water-soluble derivatives were prepared from the water-insoluble glucan by sulfation, carboxymethylation, hydroxyethylation, hydroxypropylation, and methylation, respectively. Non-selectivity of substitution on the active hydroxyl groups of the GL-IV-I glucan occurred. The reactivity of the hydroxyl groups of GL-IV-I was in the order of C-6 > C-4 > C-2. S-GL and CM-GL displayed an electrostatic repulsion effect in pure water, whereas they exhibited normal polymer solution behavior in 0.9% aqueous NaCl solution, proving that an electrostatic repulsion effect could be screened out successfully by adding salts. There was no obvious aggregation for the derivatives in either Me_2SO or 0.9% aqueous NaCl solution. GL-IV-I exists as a compact coil chain in Me_2SO at 25 °C, whereas its derivatives exist as relatively expanded flexible chains in 0.9% aqueous NaCl solution at 25 °C, owing to the steric hindrance of the substituted groups.

Acknowledgments

We gratefully acknowledge the major grants of the National Natural Science Foundation of China (30530850), the National Natural Science Foundation (20874079), and the High-Technology Research and Development Program of China (2006AA02Z102).

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