Carbohydrate Polymers 86 (2011) 844–851

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Structure and chain conformation of water-soluble heteropolysaccharides from *Ganoderma lucidum*

Jianguo Wang[a,b], Zhaocheng Ma[c], Lina Zhang[a,*], Yapeng Fang[b], Fatang Jiang[b], Glyn O. Phillips[b]

[a] Department of Chemistry, Wuhan University, Wuhan 430072, China
[b] Glyn O. Philips Hydrocolloid Research Centre, School of Bioengineering, Hubei University, China
[c] National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan 430070, China

ARTICLE INFO

Article history:
Received 2 April 2011
Received in revised form 16 May 2011
Accepted 18 May 2011
Available online 26 May 2011

Keywords:
Ganoderma lucidum
Polysaccharide
Water-soluble
Structure
Anti-tumor activities
Degree of branching

ABSTRACT

Five water-soluble heteropolysaccharides designated GL-I to GL-V, were isolated from the cultured fruit body of *Ganoderma lucidum*. Using a range of techniques the samples are shown to be heteropolysaccharides, mainly composed of glucose, galactose, mannose, arabinose with the relatively low weight-average molecular weights of 6.1×10^4 to 18.4×10^4. The amount of uronic acid present is low. As successive isolations proceed to give GL-I to GL-V, the content of glucan increased, the content of galactan and mannan decreased, and the branching decreased from high to near linear. GL-I is most branched of the heteropolysaccharides with a 27.0% degree of branching, whereas GL-V is mainly a linear glucan. Due to their branched chains samples GL-I to GL-III are in a compact coil conformation in 0.9% NaCl at 25 °C, whereas GL-V has a slightly flexible chain. Atomic force microscopy images confirmed the spherical shape of samples GL-I to GL-III in water.

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

Ganoderma lucidum (Leyss, ex Fr.) Karst (Gl), a therapeutic fungal bio-factory, is named *Lingzhi* in China, and is used in traditional Chinese medicine and in Asian countries to promote health and longevity (Paterson, 2006). The earliest record of Lingzhi is in "Shen Nong's Materia Medica" (*Shen Nong Ben Cao Jing*), from the Han Dynasty of China about two thousands years ago. *G. lucidum* has been used to treat various human diseases such as hypertension, bronchitis, arthritis, neurasthenia, hepatopathy, chronic hepatitis, nephritis, gastric ulcer, tumorigenic diseases, hypercholesterolemia, immunological disorders, and scleroderma (Arisawa, 1986; El-Mekkawy, Meselhy, Nakamura, & Kakiuchi 1998; Kim, 2000; Su, Shiao, & Wang, 2000; Wasser, 1999; Yang, Ke, & Kuo, 2000; Yen & Wu, 1999). Polysaccharides isolated from natural plants have been regarded as an important class of biological response modifiers (Wasser, 2002; Yang, Zhao, & Lv, 2007). The polysaccharide from *G. lucidum mycelium* has been shown to have potential biological effect on the immune system and cancer cells (Park, Lai, & Kim, 2004). These polysaccharides stimulate the proliferation of T lymphocytes and the humoral immune function; inhibit tumor growth and metastasis without any side effects on normal cells (Wang, Hsu, Tzeng, & Ho, 1997). Its medicinal effects on cancer, hypertension, hepatitis and hypercholesterolemia have also been studied (Baea, Janga, Yimb, & Jinc, 2005; Cao & Lin, 2006; Mizuno, Ito, Mayuzmi, Okamoto, & Li, 1994; Sun, He, & Xie, 2004; Zhang & Lin, 1999). The indication is that the polysaccharides can significantly inhibit the growth of locally implanted sarcoma, melanoma, and colon cancer (Lavia, Friesemb, Gereshc, Hadarb, & Schwartz, 2006; Ohta et al., 2007; Tommonaro et al., 2007). Polysaccharides from *Cordyceps militaris*, *Passiflora liguralis*, *Gynostemma pentaphyllum* Makino, *Camellia sinensis* also possess bioactivities such as anti-oxidative effects, immunostimulatory activity and antivirus activity (Liu, Yuan, Chung, & Chen, 2002; Monobe, Ema, Kato, & Maeda-Yamanoto 2008; Pugh, Ross, ElSohly, & Pasco, 2001; Yang, Zhao, Yang, & Ruan, 2008). More attention has been recently given to the relationship between polysaccharide structure and biological activity. Polysaccharides from *G. lucidum* mycelium have been studied in our laboratory and also heteropolysaccharides from spores (Chen, Zhang, Yu, & Zhu, 2000). But there is very little information about the structure of polysaccharides from the natural fruit body of *G. lucidum*. In this work, we have isolated five water-soluble polysaccharides from the untreated natural fruit body of *G. lucidum* using 0.9% sodium chloride aqueous solution and 1 M sodium hydroxide. The chemical structures were determined by FT-IR, elementary analysis, gas chromatography, gas chromatography/mass spectrometry and ^{13}C NMR. The molecular mass, and chain stiffness were determined using laser light scattering (LLS), size exclusion chromatograph (SEC-LLS) and viscometry. The results provide

* Corresponding author. Tel.: +86 27 87219274; fax: +86 27 68754067.
E-mail addresses: lnzhang@public.wh.hb.cn, linazhangwhu@gmail.com (L. Zhang).

0144-8617/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2011.05.031

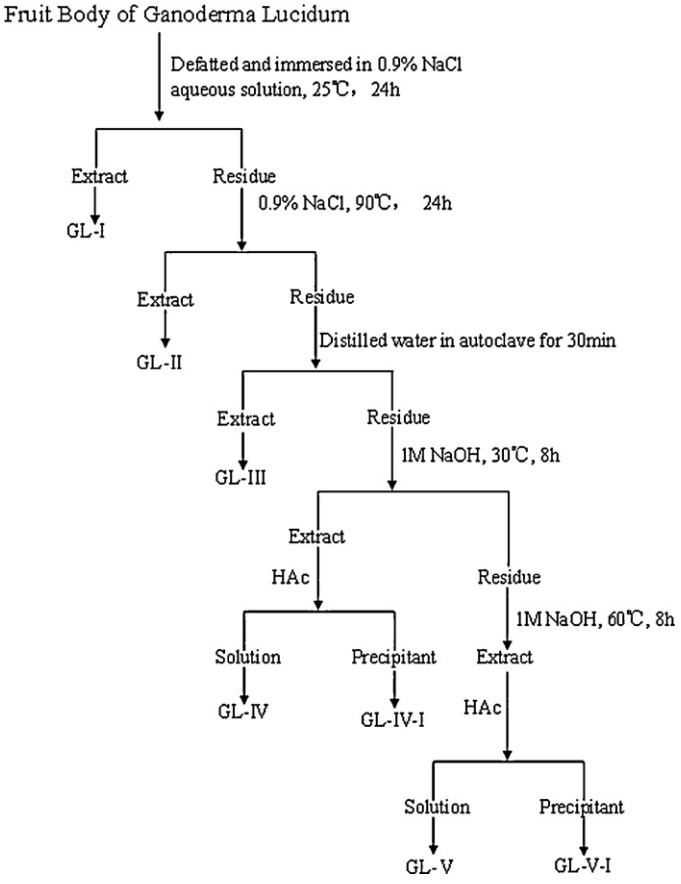
Fig. 1. Photographs of the fruit body of *G. lucidum*.

information about the chain conformation and branched structure of these heteropolysaccharides.

2. Experimental

2.1. Preparation of polysaccharides

Cultured *G. lucidum* fruit body (Fig. 1) was a gift from *Longyan* Colledge of Wuping (Fujian, China). It was dried and powdered. Scheme 1 shows the procedure for isolation of the water-soluble polysaccharides. The powdered fruit body was defatted with ethyl acetate for 8 h, followed by acetone extraction for 8 h. The residue was immersed in 0.9% aqueous sodium chloride at 25 °C and stirred for 24 h. The process was repeated 3 times. Subsequently, the mixture was centrifuged and the supernatant retained, from which the free protein was removed using the Sevag method (Staub, 1965)



Scheme 1. Isolation procedure of polysaccharides from the fruit body of *G. lucidum*.

several times. After decolorizing using 30% H_2O_2 at 40 °C, the supernatant was dialyzed using a regenerated cellulose tube (M_w cut-off 8000, Union Carbide USA) against tap water for 7 days and distilled water for 3 days. The resulting solution was concentrated by rotary evaporator at 44 °C under reduced pressure and lyophilized (CHRIST Alpha 1-2, Germany) to give a colorless sample designated GL-I. This process was repeated at 90 °C to isolate the sample, coded GL-II. The sample designated GL-III was obtained by subjecting the solid residue in an autoclave with distilled water for 30 min. After isolating GL-III, the resultant residue was treated successively with 1 M sodium hydroxide at 30 °C and 60 °C. The resulting supernatants were neutralized with 1 M acetic acid to precipitate the polysaccharides, which were coded GL-IV and GL-V, respectively. The samples were purified with distilled water (5 times) and then lyophilized to give a colorless powder. This powder was dissolved in distilled water and centrifuged to give a supernatant, which was successively dialyzed and lyophilized to get pure GL-IV and GL-V.

2.2. Characterization

Infrared spectra of the samples were recorded with Nicolet Fourier transform infrared (FTIR) spectrometer (Spectrum One, Thermo Nicolet Co., Madison, WI, USA) in the range 4000–400 cm^{-1} using the KBr disk method. Gas chromatography (GC) of alditol acetates derivatives of the samples were performed as described by Yasuyuki, Kiyokazu, Mitsuyoshi, and Tohru (1987). GC analysis was carried out with an Agilent 6820 gas chromatograph system (Agilent, Palo Alto, CA, USA) equipped with a flame ionization detector using a DB-5MS capillary column (30 m × 0.32 mm × 0.25 μm) programmed from 180 °C to 220 °C at 4 °C/min and held at 220 °C for 30 min. The injection volume was 3 μL, the carrier gas was helium. The contents of glucuronic acid in the samples were measured by the sulfuric acid-carbazole colorimetry method and the total sugar contents were determined by phenol-sulphuric acid method (Dubois, 1956). High resolution ^{13}C NMR was recorded with a Mercury 600 NMR spectrometer (Varian Inc., Palo Alto, CA, USA) at room temperature. The concentrations of the samples were adjusted to 100 mg/mL, and 99.96% D_2O were used as solvent. The protein contents were analyzed by amino acid auto-analyzer (HitachiL 8880) and confirmed by calculating from the content of N (EA, Heraeus Co., Hanau, Germany).

Gas chromatography–mass spectrometry (GC/MS) was carried out according to John, Kim, Reuhs, Raymond, and Rasappa (2006) on a GCT system (Macro-Mass, Waters, USA) with a capillary GC column (HP-5MS, 30 m × 0.32 mm ID × 0.25 μm, Agilent, USA) using He as carrier gas. The oven temperature was set to increase from 180 °C to 220 °C at a rate of 4 °C/min and the detector temperature was set at 250 °C. The polysaccharide samples were first methylated by CH_3I, and then extracted with $CHCl_3$. These extracts were washed with distilled water to remove the unmethylated

water-soluble polysaccharide. The methylated polysaccharide was hydrolyzed in 12 M H_2SO_4 at 20 °C for 1 h, followed by 2 M H_2SO_4 at 90 °C for 2 h, and then acetylated with acetic anhydride (Ac_2O) in pyridine at 90 °C for 3 h. The samples were directly hydrolyzed and acetylated. The standard monosaccharides were also acetylated.

2.3. Intrinsic viscosity measurement

The intrinsic viscosities ([η]) of the polysaccharides in 0.9% NaCl aqueous solution were measured at 25 ± 0.02 °C using an Ubbelohde capillary viscometer. Huggins and Kraemer equations were used to estimate the [η] values by extrapolation to infinite dilution as follows (Huggins, 1942; Kraemer, 1938):

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

$$\frac{(\ln \eta_r)}{C} = [\eta] - \beta[\eta]^2 C \quad (2)$$

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.

2.4. Laser light scattering measurements

Weight average molecular mass (M_w) and radius of gyration ($\langle S^2 \rangle^{1/2}$) of the polysaccharides in 0.9% NaCl aqueous solution were determined with a multi-angle laser light scattering instrument equipped with a He–Ne laser (MALLS, λ = 633 nm; DAWN-DSP, Wyatt Technology Co., Santa Barbara, CA) in various angles. The basic light scattering equation is as

$$\frac{Kc}{R_\theta} = \frac{1}{M_w}\left(1 + \frac{16\pi^2\langle S^2\rangle}{3\lambda^2}\sin^2\left(\frac{\theta}{2}\right)\right) + 2A_2c + \ldots \quad (3)$$

where K is an optical constant equal to $[4\pi^2 n^2 (dn/dc)^2]/(\lambda^4 N_A)$, 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' number, and A_2 is the second virial coefficient. The polysaccharide solution with desired concentrations was prepared, and optical clarification of the polysaccharide solutions of desired concentrations was achieved by filtration through a 0.2 μm pore size filter (PTFE, Puradisc 13 mm Syringe Filters, Whatman, Kent, U.K.) into the scattering cell. The refractive index increment (dn/dc) was measured with a double-beam differential refract meter (DRM-1020, Otsuka Electronics Co., Tokushima, Japan) at a wavelength of 633 nm at in NaCl solution 25 °C. The dn/dc values at 633 nm obtained were 0.149 cm^3/g, 0.145 cm^3/g, 0.141 cm^3/g, 0.140 cm^3/g, 0.144 cm^3/g for GL-I, GL-II, GL-III, GL-IV and GL-V samples, respectively. Astra software (Version 4.70.07) was utilized for data acquisition and analysis.

2.5. SEC-LLS measurements

Size exclusion chromatography combined with laser light scattering (SEC-LLS) measurements were carried out using the multi-angle laser photometer combined with a P100 pump equipped with TSK-GEL G4000PWXL column (7.8 mm × 300 mm) and differential refractive index detector (RI-150) at 25 °C. The eluent was 0.9% NaCl aqueous solution at a flow rate of 1.0 mL/min. Optical clarification of the solutions was achieved by filtration through 0.2 μm pore size filter (PTFE, Puradisc 13 mm Syringe Filters, Whatman, Kent, U.K.). Astra software was utilized for data acquisition and analysis.

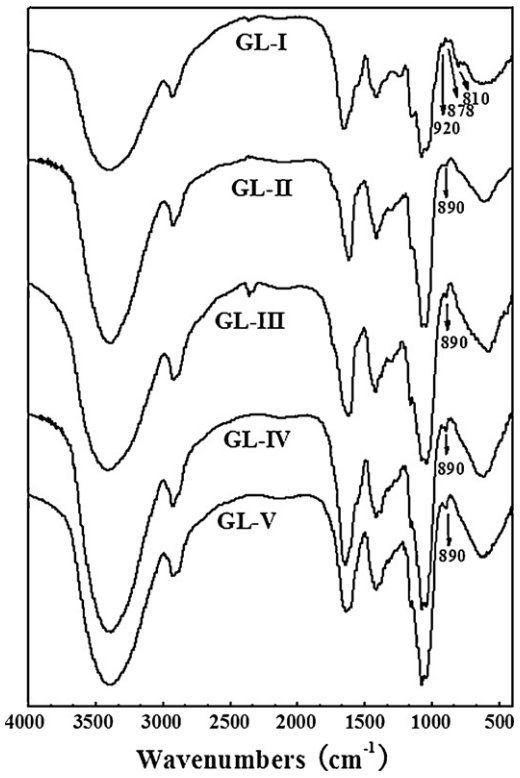


Fig. 2. IR spectra of water soluble polysaccharides from the fruit body of *G. lucidum*.

2.6. Characterization using atomic force microscopy (AFM)

Samples were dissolved at approximately 1 mg/mL in water by energetic stirring for 24 h, then diluted with distilled water to a polymer concentration of 20 μg/mL, then filtered through a 0.45 μm filter (NYL, 13 mm Syringe filter, Whatman, Inc., USA). A 10 μL drop was deposited on to freshly cleaved mica and left to dry in air for 1.5 h at room temperature in a small covered Petri dish prior to imaging with magnetically AC (MAC) mode AFM. The specimen was examined using a Picoscan atomic force microscope (Molecular Imaging, USA) in a MAC mode with commercial MAClever II tips (Molecular Imaging, USA), with a spring constant of 0.95 N/m. A piezoelectric scanner with a range up to 6 μm was used for the image. The scanner was calibrated in the x–y directions using a 10 μm diffraction grating and in the z direction using several conventional height standards. The measurement was performed in air at ambient pressure and humidity and the image was stored as 256 × 256 point arrays.

3. Results and discussion

3.1. Chemical structure

The FTIR spectra of polysaccharides GL-I to GL-V are shown in Fig. 2. All of the samples had the characteristic absorption peaks of polysaccharides at near 1650, 1400 and 1250 cm^{-1}. The peaks at 810 cm^{-1} and 920 cm^{-1} for GL-I were assigned to mannose and α-D-glucans, respectively. GL-II, GL-III, GL-IV and GL-V had peaks at 890 cm^{-1}, the characteristic band of β-D-glucans (Wang, Zhang, Li, Hou, & Zeng, 2004). The sugar components and the yield and protein content of the samples are listed in Table 1. The total water soluble polysaccharide yield was 1.32%, which was higher than the yield reported previous (Chen et al., 2000). The protein content is comparable for the two methods used. The Sevag method needed to be repeated several times to ensure complete removal of free

Table 1
Monosaccharides and protein content of polysaccharide from *G. lucidum*.

Sample	Monosaccharide relative content in polysaccharide (%)						Protein (%)		Glucuronic acid (%)	Yield (%)	Water solubility
	Ful	Ara	Xyl	Man	Glu	Gala	Amino acid auto-analyzer	Element analyzer			
GL-I	–	10.4	0.8	12.2	24.7	52.0	14.6	15.1	0.765	0.17	++
GL-II	–	3.8	–	6.7	81.2	8.3	5.7	5.8	1.676	0.14	++
GL-III	–	3.2	3.2	4.4	82.7	6.5	3.6	3.7	1.447	0. 12	++
GL-IV	–	6.1	–	5.6	88.4	0.9	–	–	1.123	0.24	+
GL-V	–	3.1	1.5	2.4	92.2	–	–	–	1.309	0.65	+

++: easily dissolved in 0.9% sodium chloride aqueous solution. +: dissolved in 0.9% sodium chloride aqueous solution.

protein. Thus, it can be concluded that the protein remained in GL-I, GL-II, GL-III is bound. No residual protein was found in the GL-IV and GL-V samples, probably due to the strong alkaline conditions used. Clearly, as the separation progressed, the relative content of both galactose and mannose in polysaccharides decreased from 52.0% to 0.9% and 12.2% to 2.4% respectively. Moreover, no trace of galactose was found in the GL-V sample. Arabinose also decreased as the extraction progressed (Table 1). In contrast, the relative content of glucose increased markedly from 24.7% to 92.2%, shown also by the results from IR. The total sugar and glucuronic acid contents are also shown in Table 1. The amount of glucuronic acid in GL-I to GL-V is small.

Fig. 3 shows the total ion current (TIC) chromatograms of GC/MS from methylation analyses of the five polysaccharides. The relatively intense peak at retention time 13.8 min for GL-I was 1,4,6-galactan, indicating the branched structure of GL-I. The most intense peaks at retention time 13.8 min for GL-II to GL-V were assigned to 2,4,6-tri-O-methyl-D-glucose, indicating a (1 → 3)-glucan linkage. Peaks at 12.9 min were assigned to 2,4,6-tri-O-methyl-D-man, indicating a (1 → 3)-mannan linkage. The peak at 13.6 min was assigned to 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-D-glucitol, indicating a 1-glucan terminal residue. The less intense peaks indicated that there were additional 1,4,6-glucan, (1 → 3)-galactan, (1 → 6)-galactan, (1 → 4)-grabinan, (1 → 3)-mannan, and/or (1 → 4)-xylan linkages in the polysaccharides.

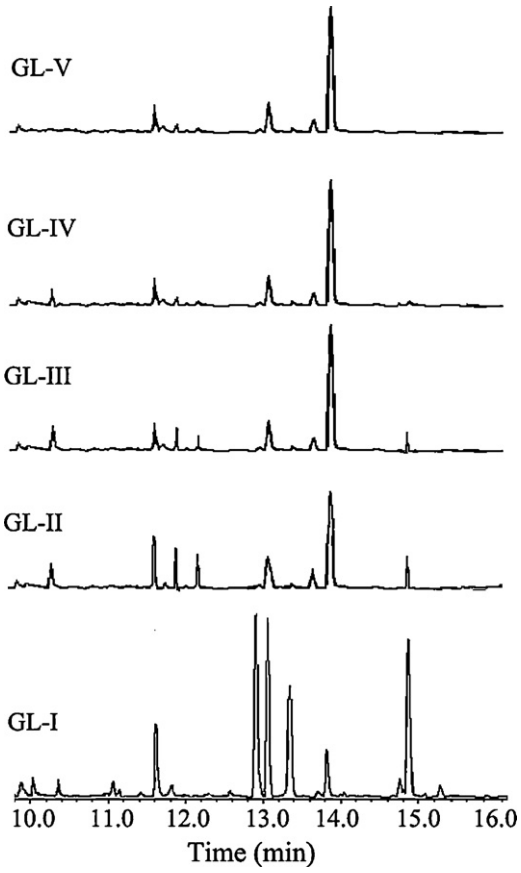


Fig. 3. GC/MS TIC chromatograms of the methylation analyses for polysaccharides.

The results show that GL-I is a branched (1 → 4)-galactan heteropolysaccharide whereas GL-II to GL-V are mainly composed of (1 → 3)-glucan as the main chain and contain arabinose, galactose and mannose. The position of the branch position in GL-I is at O-6 as shown by the presence of 1,4,6-galactan linkages. Thus, the main chain of GL-I is (1 → 4)-galactan with the branch linkage chains at O-6 position. The molar ratios of glucose determined by GC/MS were in good agreement with the results obtained from GC. The degree of branch (DB) can be calculated from the equation of Tao, Yan, and Zhang (2007):

$$\mathrm{DB} = \frac{N_T + N_B}{N_T + N_B + N_L} \tag{4}$$

where N_T, N_B, and N_L are the numbers of the terminal residues, branch residues, and linear residues, respectively. Using the data from GC/MS, the DB values of the heteropolysaccharides were 27%, 13%, 10%, 9% and 8% for GL-I to GL-V.

The ^{13}C NMR spectra of the five samples are shown in Fig. 4. The strong signals at 98.2, 70.1, 69.1, 69.8 and 61.3 ppm in the spectrum of GL-I were assigned to C-1, C-3, C-2, C-5 and C-6 of α-D-galactose, and the signals at 101.7, 78.1, 72.0 and 61.0 to C-1, C-4, C-3 and C-6 to mannose. The signals at 102.9, 73.4, 66.8, 75.1, and 61.1 can be ascribed as C-1, C-2, C-4, C-5 and C-6 of β-1,3-D-glucose. The results show that GL-I is mainly α-D-galactose, and contained also the other sugar units, mannose and glucose. The strong signals of GL-II at 102.9, 73.5, 84.8, 69.9, 75.9 and 61.1 can also be assigned to C-1, C-2, C-3, C-4, C-5 and C-6 of β-1,3-D-glucose (Sone, Okada, Wada, Kisguda, & Misaki, 1985). From analysis of the signals in GL-I, the comparative content of glucose was much higher for GL-II. This is in good agreement with the results of IR and GC. The comparative weak peaks at 98.2, 69.9, 68.5, and 61.1 could be ascribed to C-1, C-3, C-5 and C-6 of α-D-galactose, indicating that GL-II mainly contained β-1,3-D-glucose.

For GL-III, the ^{13}C NMR spectra signals are quite similar to GL-II. The components analysis gave results identical to that from GC. Signals at 175.4, 174.8 and 172.7 for GL-I GL-II and GL-III separately in the ^{13}C NMR were ascribed to the –CONH– group of proteins (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956).

For GL-IV, signals at 102.7, 73.3, 84.3, 69.7, 75.8, and 60.8 were the characteristic peaks of C-1, C-2, C-3, C-4, C-5 and C-6 for β-1,3-D-glucose. For GL-V, signals at 102.8, 73.2, 84.4, 69.7, 75.9, 60.8 could be ascribed to C-1, C-2, C-3, C-4, C-5 and C-6 of β-1,3-D-glucan (Gorin, 1981). From the ^{13}C NMR spectra, we can also calculate the DB value of the samples from the peak distribution. All of the samples have peaks around 67.0 ppm, which were assigned to the C′-6. The contribution of C-6 to the total DB is 13.3%, 9.7%, 7.3%, 6%

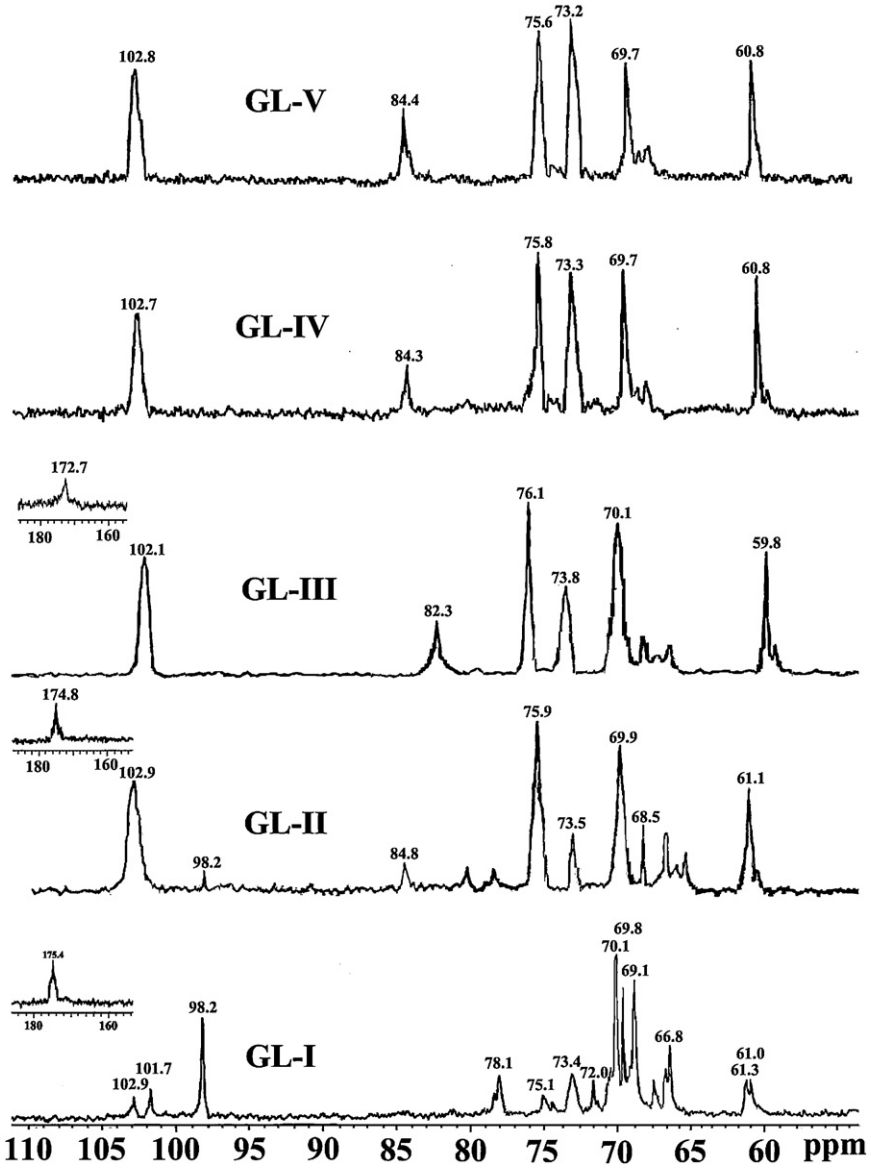


Fig. 4. ^{13}C NMR spectra of GL-I to GL-V in D_2O at 25 °C.

and 6.3%. The data from ^{13}C NMR are in agreement with the results calculated from the GC/MS.

The ^{13}C NMR and GC/MS data show that the content of galactose and mannose in the samples GL-I to GL-V decreased, while the content of glucose increased, as extraction proceeded to higher temperature. The GC/MS and ^{13}C NMR also showed that the DB of the samples decreased, indicating that GL-V is mainly composed of β-(1 → 3)-D-glucan with very little branch chains. Usually, the linear β-(1 → 3)-D-glucan, such as curdlan (Needs & Selvendran, 1993) and *G. lucidum* polysaccharides (Wang & Zhang, 2009), are water-insoluble, so the presence of branched chains play important role in improving water solubility of polysaccharides.

3.2. Molecular mass and chain conformation

The M_w and $\langle S^2\rangle^{1/2}$ values of polymers were calculated from the classical Zimm light scattering plot collected at various angles (θ) at each concentration (c). The Kc/R_θ was plotted versus $\sin^2(\theta/2)$ + Const. × c, and extrapolated to 0 for both θ and polymer concentration. The common intercept of the extrapolated curves yields M_w, and the slope of the $c = 0$ curve yields $\langle S^2\rangle^{1/2}$. Fig. 5 shows the Zimm plots for GL-III and GL-IV (others are not shown here). Fig. 6 shows the angular dependences of $(Kc/R_\theta)_{c=0}$ and the concentration dependences $(Kc/R_\theta)_{\theta=0}$ of the five polysaccharides in 0.9% NaCl at 25 °C, they all show good linear relationship. The M_w values measured for the samples by SEC-LLS were comparable to those by light scattering. The values of $[\eta]$ and $\langle S^2\rangle^{1/2}$ reflect the expanded extent and the stiffness of the polysaccharide chains. The M_w, $[\eta]$, A_2 data are given in Table 2. For polymers with the same M_w and different DB, the $[\eta]$ values are different, because of the different chain conformation. The linear polysaccharides have higher $[\eta]$ values than the branching polysaccharides with the same M_w. The $[\eta]$ value of GL-IV and GL-V was much higher than that of the other three samples with similar M_w. This again supported the view that the degree of branching of GL-IV and GL-V was much lower than the other three samples. The A_2 value reflects the extent of solubility. Low A_2 value samples reflect low solvation and poor water-solubility. The A_2 values of GL-IV (1.45) and GL-V (0.59) were much lower than those of GL-I to GL-III (2.01, 2.93 and 4.11), indicating that the water-solubility of GL-IV and GL-V is relatively poor. As might be predicted, therefore, the linear β-1,3-D-glucan is water-insoluble and the branched GL-V readily soluble in water.

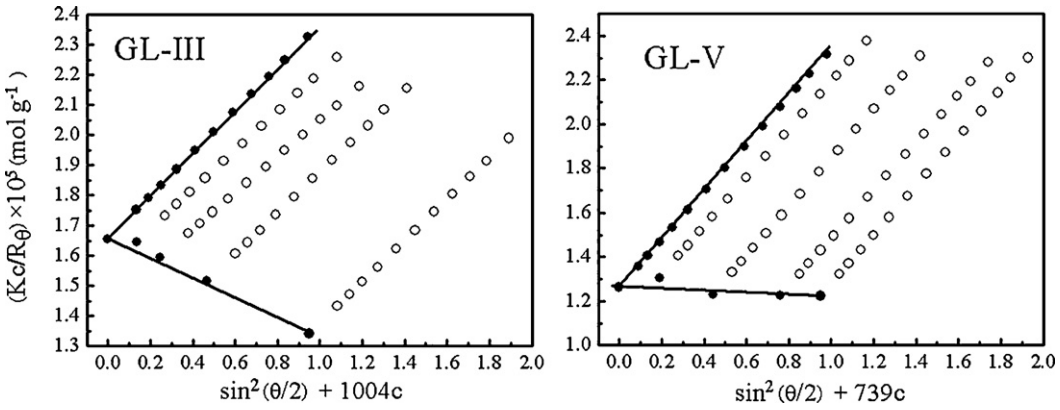


Fig. 5. Zimm plot for GL-III and GL-V in 0.9% sodium chloride aqueous solution at 25 °C.

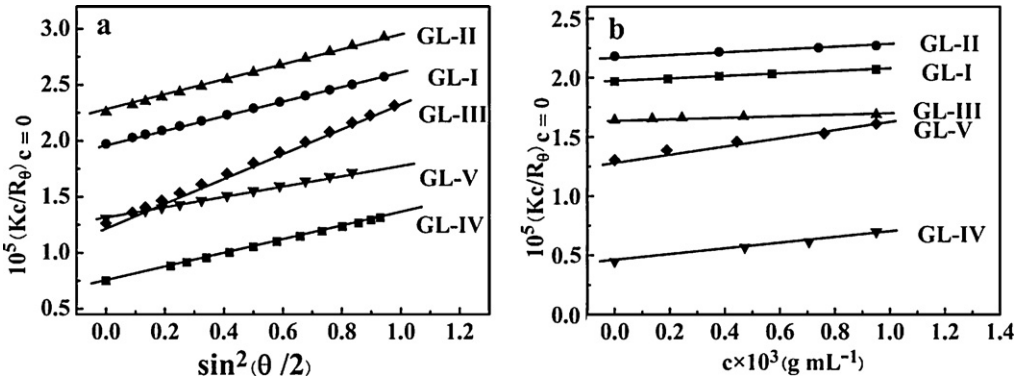


Fig. 6. Angle dependences of $(Kc/R_\theta)_{c=0}$ (a) and concentration dependences of $(Kc/R_\theta)_{\theta=0}$ (b) of polysaccharides in 0.9% sodium chloride aqueous solution at 25 °C.

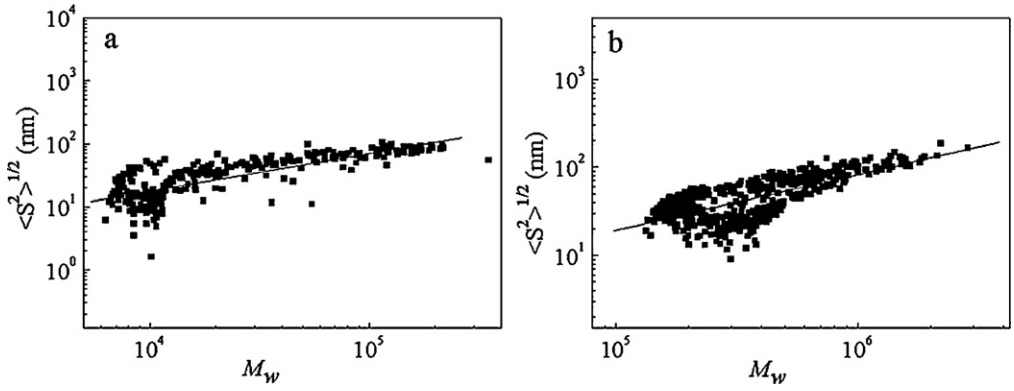


Fig. 7. Plots of $\langle S^2\rangle^{1/2}$ versus M_w for GL-I (a) and GL-IV (b) in 0.9% sodium chloride aqueous solution at 25 °C.

Additional information can also be obtained from SEC-LLS. The power law of $\langle S^2\rangle^{1/2} = f(M_w)$ can be estimated from the experimental points in the SEC chromatogram detected by LLS. Fig. 7 shows log–log plots of $\langle S^2\rangle^{1/2}$ versus M_w for GL-I and GL-IV in 0.9% NaCl aqueous solution. The values of slope (α) of GL-I to GL-V are shown in Table 2. The exponent α reflects the chain conformation of the polymers in solution. Usually, α is 0.5–0.6 for a flexible polymer in a good solvent, and 0.6–1.0 for a semi-flexible chain, 0.2–0.4

Table 2
Experiment results from LLS, SEC-LLS and viscosity for the polysaccharides from the fruit body of *G. lucidum*.

Sample	$[\eta]$ (mL/g)	LLS		SEC-LLS			$\langle S^2\rangle^{1/2}$ (nm)
		$M_w \times 10^{-4}$	$A_2 \times 10^4$ (mol mL g^{-2})	$M_w \times 10^{-4}$	M_w/M_n[a]	α	
GL-I	6.8	5.08	2.01	6.3	1.52	0.38	26.7
GL-II	7.9	4.51	2.93	6.1	1.73	0.31	32.8
GL-III	8.2	8.98	4.11	11.2	2.21	0.41	45.2
GL-IV	27.7	16.4	1.45	18.4	1.80	0.49	40.4
GL-V	13.8	7.80	0.59	10.3	2.12	0.56	37.6

[a] Calibrated on the method of He, Zhang, and Cheng (1982).

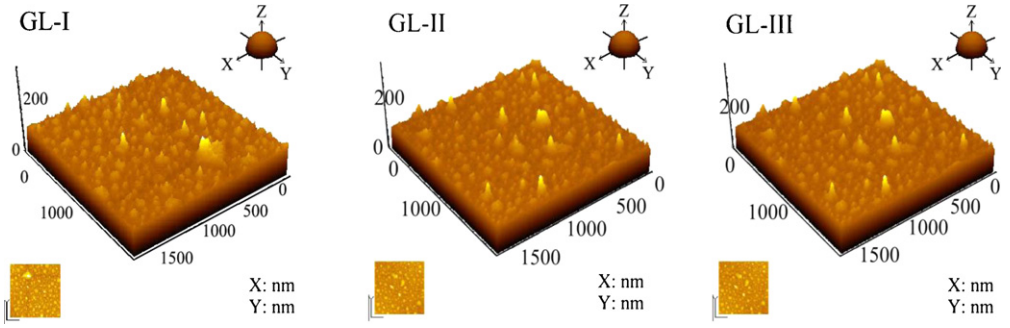


Fig. 8. AFM image of the samples from GL-I to GL-III in water (20 μg/mL).

for branching polymers with compact coil chain conformation and 0.3 for globular shape (Kogan, Alfoldi, & Masler, 1988). In view of the data, GL-V ($\alpha = 0.56$) has a flexible chain, whereas GL-I to GL-III behave as a compact coil chain conformation, with high branching.

3.3. Molecular morphology

Atomic force microscopy is useful to characterize surface topology and visualize the orientation and spatial distribution of molecules adsorbed to surfaces (Poggi et al., 2004). AFM operating in the attractive, or non-contact, mode has been used to image individual triple-helical *scleroglucan* molecules on a mica surface in air (Theresa, Reginald, & David, 1995). Fig. 8 shows the AFM image of the GL-I to GL-III samples in pure water. Monodispersed spheres were observed in the polysaccharide dilute aqueous solution and the average radii were 36 nm, 41 nm, and 60 nm, respectively. The size of the random coil chains for GL-I to GL-III was comparable with that obtained from the SEC-LLS. Therefore, GL-I to GL-III exist in aqueous solution in a compact coil chain conformation (Tao & Zhang, 2006). The morphology of the chains for GL-IV and GL-V were not observed by AFM.

4. Conclusion

GL-I to GL-III were heteropolysaccharide-protein complexes with protein contents of 14.6%, 5.7%, and 3.6%. GL-I mainly contained galactose with the relatively high content of 52.0%, as well as less glucan, mannose, xylose and arabinose. With the progress of the isolation β-1,3-D-glucan increased, whereas the degree of branch of the heteropolysaccharides decreased. The relatively high branching of the heteropolysaccharide led to the improvement of water solubility. The samples of GL-I to GL-III existed in the aqueous solution as compact coil conformation as a result of the relatively high branching.

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).

References

Baea, J., Janga, K., Yimb, H., & Jinc, H. (2005). Polysaccharides isolated from *Phellinus gilvus* inhibit melanoma growth in mice. *Cancer Letters*, *218*, 43–52.
Cao, Q. Z., & Lin, Z. B. (2006). *Ganoderma lucidum* polysaccharides peptide inhibits the growth of vascular endothelial cell and the induction of VEGF in human lung cancer cell. *Life Science*, *78*, 1457–1463.
Chen, J., Zhang, L., Yu, D., & Zhu, R. (2000). The chemical structure and solution properties of polysaccharides from *Ganoderma lucidum* mycelium. *Chemical Journal of Chinese University*, *21*, 961–964.
Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*, 350–356.
El-Mekkawy, S., Meselhy, M. R., Nakamura, N., Tezuka, Y., Hattori, M., & Kakiuchi, N. (1998). Anti-HIV-1 and anti-HIV-1-protease substances from *Ganoderma lucidum*. *Phytochemistry*, *49*, 1651–1657.
Gorin, P. A. (1981). Carbon-13 nuclear magnetic resonance spectroscopy of polysaccharides. *Journal of Advanced Carbohydrate Chemistry and Biochemistry*, *38*, 13–104.
He, Z., Zhang, X., & Cheng, R. (1982). Simultaneous calibration of molecular weight separation and column dispersion of GPC by coupling with lallis. *Journal of Liquid Chromatography*, *5*, 1209–1222.
Huggins, M. L. (1942). Theory of solutions of high polymers. *Journal of the American Chemistry Society*, *64*, 1712–1719.
John, S., Kim, L., Reuhs, F., Raymond, E., & Rasappa, G. (2006). Addition of glycerol for improved methylation linkage analysis of polysaccharides. *Carbohydrate Research*, *341*, 1061–1064.
Kogan, G., Alfoldi, J., & Masler, L. (1988). Carbon-13 NMR spectroscopic investigation of two yeast cell wall β-D-glucans. *Biopolymers*, *27*, 1055–1063.
Kraemer, E. O. (1938). Molecular weights of celluloses and cellulose derivatives. *Industrial and Engineering Chemistry Research*, *30*, 1200–1203.
Lavia, I., Friesemb, D., Gereshc, S., Hadarb, Y., & Schwartz, B. (2006). An aqueous polysaccharide extract from the edible mushroom pleurotus ostreatus induces anti proliferative and pro-apoptotic effects on HT-29 colon cancer cells. *Cancer Letters*, *244*, 61–70.
Liu, X., Yuan, J., Chung, C., & Chen, X. (2002). Antitumor activity of the sporoderm-broken germinating spores of *Ganoderma lucidum*. *Cancer Letters*, *182*, 155–161.
Mizuno, T., Ito, H., Mayuzmi, H., Okamoto, H., & Li, J. (1994). High dietary taurine effects on feline tissue taurine concentrations and reproductive performance. *Bioscience Biotechnology and Biochemistry*, *58*, 82–88.
Monobe, M., Ema, K., Kato, F., & Maeda-Yamanoto, M. (2008). Immunostimulating activity of a crude polysaccharide derived from green tea (*Camellia sinensis*) extract. *Journal of Agricultural and Food Chemistry*, *56*, 1423–1427.
Needs, P. W., & Selvendran, R. R. (1993). Avoiding oxidative degradation during sodium hydroxide/methyl iodide-mediated carbohydrate methylation in dimethyl sulfoxide. *Carbohydrate Research*, *245*, 1–10.
Ohta, Y., Lee, J., Hayashi, K., Fujita, A., Park, D., & Hayashi, T. (2007). In vivo anti-influenza virus activity of an immunomodulatory acidic polysaccharide isolated from *Cordyceps militaris* grown on germinated soybeans. *Journal of Agricultural and Food Chemistry*, *55*, 10194–10199.
Park, S. D., Lai, Y. S., & Kim, C. H. (2004). Immuno-potentiating and anti-tumor activities of the purified polysaccharides from Phellodendron chinense. *Life Science*, *75*, 2621–2632.
Paterson, R. R. M. (2006). Ganoderma—A therapeutic fungal biofactory. *Phytochemistry*, *67*, 1985–2001.
Poggi, M. A., Gadsby, E. D., Bottomley, L. A., King, W. P., Oroudjev, E., & Hansma, H. (2004). Scanning probe microscopy. *Analytical Chemistry*, *76*, 3432–3446.
Pugh, N., Ross, S., ElSohly, M., & Pasco, D. (2001). Evaluation of consumer-style cooking methods for reduction of *Escherichia coli* O157:H7 in ground beef. *Journal of Agricultural and Food Chemistry*, *49*, 1030–1034.
Sone, Y., Okada, R., Wada, N., Kisguda, E., & Misaki, A. (1985). Structure and antitumor activities of the polysaccharide isolated from fruiting body and growing culture of mycelium of *Ganoderma lucidum*. *Agricultural & Biological Chemistry*, *49*, 2641–2653.
Staub, A. M. (1965). Removal of protein—Sevag method. *Methods in Carbohydrate Chemistry*, *5*, 5–6.
Su, C. Y., Shiao, M. S., & Wang, C. T. (2000). Potentiation of ganodermic acid S on prostaglandin E1-induced cyclic AMP elevation in human platelets. *Thrombosis Research*, *99*, 135–145.
Sun, J., He, H., & Xie, B. (2004). Novel antioxidant peptides from fermented mushroom *Ganoderma lucidum*. *Journal of Agricultural and Food Chemistry*, *52*, 6646–6652.
Tao, Y., Yan, F., & Zhang, L. (2007). Chain conformation of water-insoluble hyperbranched polysaccharide from fungus. *Biomacromolecules*, *8*, 2321–2328.
Tao, Y., & Zhang, L. (2006). Determination of molecular size and shape of hyperbranched polysaccharide in solution. *Biopolymers*, *83*, 414–423.

Theresa, M., Reginald, M., & David, A. (1995). Triple-helical polysaccharide using noncontact atomic-force microscopy. *Macromolecules*, *28*, 6375–6377.

Tommonaro, G., Rodrguez, C., Santillana, M., Immirzi, B., Prisco, R., Nicolaus, B., et al. (2007). Solute transport in eroded and rehabilitated prairie landforms. 1. Nonreactive solute. *Journal of Agricultural and Food Chemistry*, *55*, 7427–7433.

Wasser, S. P. (2002). Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. *Applied Microbiology and Biotechnology*, *60*, 258–274.

Wang, Y., Zhang, L., Li, Y., Hou, X., & Zeng, F. (2004). Correlation of structure to antitumor activities of five derivatives of a β-glucan from Poria cocos sclerotium. *Carbohydrate Research*, *339*, 2567–2574.

Wang, J., & Zhang, L. (2009). Structure and chain conformation of five water-soluble derivatives of a β-D-glucan isolated from *Ganoderma lucidum*. *Carbohydrate Research*, *344*, 105–112.

Wang, S. Y., Hsu, M. L., Hsu, H. H., Tzeng, C. H., Lee, S. S., Shiao, M. S., et al. (1997). The anti-tumor effect of *Ganoderma lucidum* is mediated by cytokines released from activated macrophages and T lymphocytes. *International Journal of Cancer*, *70*, 699–705.

Yang, X., Zhao, Y., Yang, Y., & Ruan, Y. (2008). Isolation and characterization of immunostimulatory polysaccharide from an herb tea. *Journal of Agricultural and Food Chemistry*, *56*, 6905–6909.

Yang, X. B., Zhao, Y., & Lv, Y. (2007). Isolation and characterization of immunostimulatory polysaccharide from an Herb Tea, *Gynostemma pentaphyllum* Makino. *Journal of Agricultural and Food Chemistry*, *56*, 6905–6909.

Yang, F. C., Ke, Y. F., & Kuo, S. S. (2000). Effect of fatty acids on the mycelial growth and polysaccharide formation by *Ganoderma lucidum* in shake flask cultures. *Enzyme and Microbial Technology*, *27*, 295–301.

Yen, G. C, & Wu, J. Y. (1999). Antioxidant and radical scavenging properties of extracts from Ganoderma tsug-ae. *Food Chemistry*, *65*, 375–379.

Yasuyuki, T., Kiyokazu, I., Mitsuyoshi, O., & Tohru, C. (1987). Systematic absences are consistent with the space group. *Macromolecules*, *20*, 2413–2418.

Zhang, Q. H., & Lin, Z. B. (1999). The antitumor activity of *Ganoderma lucidum* (Curt.:Fr.) P. Karst. (Ling Zhi) (Aphyllophoromy cetideae) polysaccharides is related to tumor nerosis factor-α and interferon-γ. *International Journal of Medicinal Mushrooms*, *1*, 207–215.