

Transition from Triple Helix to Coil of *Lentinan* in Solution Measured by SEC, Viscometry, and ^{13}C NMR

Lina ZHANG,[†] Xuelian LI, Qi ZHOU, Xufeng ZHANG, and Ruiqiang CHEN*

Department of Chemistry, Wuhan University, Wuhan 430072, China

*Laboratory of Cellulose and Lignocellulosic Chemistry of Chinese Academy Sciences, Guangzhou 510650, China

(Received January 4, 2002; Accepted April 15, 2002)

ABSTRACT: *Lentinan*, β -(1 \rightarrow 3)-D-glucan with (1 \rightarrow 6) branching, was isolated from *Lentinus edodes*. Weight-average molecular weight M_w , radius of gyration $\langle s^2 \rangle_z^{1/2}$ and intrinsic viscosity $[\eta]$ of *Lentinan* in 0.2 M NaCl aqueous solution, dimethylsulfoxide (DMSO) and water/DMSO mixtures were measured by light scattering (LS), size exclusion chromatography (SEC) combined with LS, and viscometry. The results indicated that the glucan exists mainly as triple-helical chains in 0.2 M NaCl aqueous solution and water/DMSO mixtures with over 20 wt% water content, and as single-flexible chain in DMSO. The data from SEC-LS, viscosity and ^{13}C NMR measurements proved strongly that the helix-coil conformation transition occurred in a narrow range from 80 to 85 wt% DMSO aqueous solution, accompanying with obvious changes of M_w , $\langle s^2 \rangle_z^{1/2}$, $[\eta]$ as well as signals of C6 and C6_s. The transition of *Lentinan* in water/DMSO mixture was irreversible. The difference in ^{13}C NMR spectra for the triple-helical and coil conformations was the disappearance of the signals of C3 in β -(1 \rightarrow 3)- linked backbone and the enhancement in relative intensities of glucose substituted C6_s in the helix state, as well as the appearance of an asymmetric and broad peak of C6 in the intermediate of the conformation change. This suggests that the immobilization of the backbone by binding with intra- and intermolecular hydrogen bonds resulted in the loss of the signals of its carbon atoms in the triple helix state. An overcoating cylinder model composed of the β -(1 \rightarrow 3)- linked backbone as helix core and the side chains as rotatable overcoat was proposed to illustrate the triple-helical conformation and its transition in the solution.

KEY WORDS *Lentinus edodes* / β -(1 \rightarrow 3)-D-glucan / Triple-helical Chain / Conformation Transition / ^{13}C Nuclear Magnetic Resonance (NMR) / Viscometry / Light Scattering /

Chihara *et al.* have reported that the β -(1 \rightarrow 3)-D-glucan, *Lentinan*, obtained from *Lentinus edodes* has a strong antitumour activity against sarcoma 180 implanted subcutaneous in mice.¹ Thereby, the importance of the polysaccharides has provided a major impetus for increasing attention. Particularly mushroom polysaccharides have been used as functional food, and are developed in the field of new drugs.² The structural analysis may offer the most fundamental knowledge to understand the functions of polysaccharide, but the diversity and irregularity of polysaccharide chain make the structural analysis a formidable task.³ Although a monosaccharide unit is common to many polysaccharides, its linkage mode varies and characteristic functions properties will appear accordingly. It is worth noting that polysaccharides in aqueous solution exhibit different chain conformation such as single helix,⁴ triple helix,⁵ random coil,⁶ and aggregate,⁷ even if their backbone all are β -(1 \rightarrow 3)-D-glucan. However, conformation and its transition from an ordered state to a disordered for β -(1 \rightarrow 3)-D-glucan in solution have not been fully investigated.

Helix conformations of β -(1 \rightarrow 3)-D-glucan with two (1 \rightarrow 6)-glucosyl side groups for every five residues iso-

lated from *Lentinus edodes* have been reported, based on analysis from the results of X-Ray diffraction⁸ and ^{13}C NMR.^{9–12} However, it has long been suspected to be a triple helix of the β -D-glucan in aqueous solution without strong evidence. In our recent work,¹³ experimental results from light scattering have indicated that the predominant species of the β -D-glucan in 0.5 M NaCl aqueous solution exist as triple-helical chains with high rigidity, and in dimethylsulfoxide (DMSO) as single-flexible chains. The single or multiple helices of biopolymers in aqueous solution aggregate easily to form gel. The gelation and the helical state may be induced by lowering the temperature, by changing the solvent, or by adding salt.¹⁴ The gel-forming ability of polysaccharide is of considerable importance in view of their biological functions.¹⁵ Moreover, helix-coil transitions are important in biology, as they appear in proteins and DNA.¹⁶ In this work, the conformation transition for the β -(1 \rightarrow 3)-D-glucan (*Lentinan*) from *Lentinus edodes* in water/DMSO mixture solution was investigated by laser light scattering, size exclusion chromatography, viscometry and ^{13}C NMR. The changes of weight-average molecular weight M_w , radius of gyration $\langle s^2 \rangle_z^{1/2}$, intrinsic viscosity $[\eta]$ and

[†]To whom correspondence should be addressed (E-mail: lnzhang@public.wh.hb.cn).

signals of ^{13}C NMR for *Lentinan* in an intermediate of the helix-coil transition were examined and discussed in water/DMSO mixtures by changing DMSO content.

EXPERIMENTAL

Preparation of Sample and Solutions

Lentinan coded as L-FV-IA was isolated from fruiting bodies of *Lentinus edodes* cultivated in Suixian of Hubei in China by extraction with 5% NaOH/0.05% NaBH_4 two times, and precipitation with 36% acetic acid to remove α -(1 \rightarrow 3)-D-glucan, according to previously reported method.¹³ The supernatant was subjected to the Sevag method to remove proteins, and treated with 30% H_2O_2 to decolorize. Aqueous solution of the *Lentinan* was dialyzed against distilled water for 4 days, and concentrated by rotary evaporator at reduced pressure below 45°C, and finally lyophilized to obtain colorless flakes.

DMSO was distilled, and treated with a molecular sieve to further dehydrate. By changing the weight fraction of DMSO in the mixture (w_{DMSO}), a series of mixed aqueous solutions with different w_{DMSO} were obtained. The *Lentinan* L-FV-IA was dissolved, respectively, in 0.2 M NaCl aqueous solution, DMSO and the water/DMSO mixtures to prepare the polymer solution. In addition to clarify the conformation transition process, the glucan was dissolved in pure DMSO firstly, and then diluted with water to obtain the polysaccharide aqueous solution with different w_{DMSO} .

Relatively concentrated stock solution was carefully prepared by completely dissolving the proper amount of the L-FV-IA in solvent for over 24 h with stirring. A series of polymer concentrations were obtained by successive dilution of the clarified stock solution. Finally, each solution was filtered with sand filter for viscosity measurement. For light scattering and size exclusion chromatography measurements, the solutions were filtered with sand filter, and then with 0.45 μm filter (CA, PuradiscTM 13 mm Syringe Filters, Whatman, England) for 0.2 M NaCl and 0.45 μm filter (PTFE, PuradiscTM 13 mm Syringe Filters, Whatman, England) for DMSO and water/DMSO mixtures.

Light Scattering

Scattering light intensity was measured by multi-angle laser light scattering instrument (MALLS) equipped with a He-Ne laser ($\lambda = 632.8$ nm) (DAWN[®] DSP, Wyatt Technology Co., USA) in the angles of 42°, 49°, 63°, 71°, 81°, 90°, 99°, 109°, 118°, and 127° at 25°C. Refractive indexes of 0.2 M NaCl aqueous solution and DMSO were determined by an Abbe refractometer respectively. Measurements of the specific re-

fractive index increments (dn/dc) were performed using an Optilab refractometer (Wyatt Tech. Co., USA) at 632.8 nm and 25°C. Astra software was utilized for data acquisition and analysis. The determination of weight-average molecular weight M_w and radius of gyration $\langle s^2 \rangle_z^{1/2}$ was based on Zimm fit method.

SEC-LS Measurement

Size exclusion chromatography (SEC) combined light scattering (SEC-LS) is convenient for determination of true molecular weight and its distribution. SEC-LS measurement of the samples was performed on the multi-angle laser photometer mentioned above with a pump P100 (Thermo Separation Products, San Jose, USA) equipped with TSK-GEL column (7.8 mm \times 300 mm) and differential refractive index detector (RI-150) at 25°C. The fluent was 0.2 M NaCl aqueous solution for a G6000PWXL and a G4000PWXL columns as well as DMSO and water/DMSO mixture solution for G4000-H8 column, respectively, at a flow rate of 1.0 mL min^{-1} . Astra software was utilized for data acquisition and analysis.

Viscosity Measurement

Viscosity of the samples in 0.2 M NaCl aqueous solution, DMSO and the water/DMSO mixture was measured at $25 \pm 0.1^\circ\text{C}$ using a low-shear four-bulb capillary viscometer. Kinetic energy correction was always negligible. Huggins and Kraemer plots were used to get intrinsic viscosity $[\eta]$. From the dependence of intrinsic viscosity on shear rate $\dot{\gamma}$, zero shear-rate viscosity $[\eta]$ was determined.

Nuclear Magnetic Resonance Spectroscopy

^{13}C NMR spectra of the glucan were recorded with AVANCE DRX-400 spectrometer (Bruker Co., Germany-Switzerland) at 25°C with TMS as the internal standard. The sample was dissolved in mixture solution of D_2O with 30 wt%, 80 wt%, and 85 wt% DMSO- d_6 , named as w_{DMSO} of 0.3, 0.8, and 0.85, as well as in pure DMSO- d_6 , respectively, to prepare 10 mg mL^{-1} polymer solution.

RESULTS AND DISCUSSION

NMR is an effective technique, which records transitions between the energy levels of magnetic nuclei in an external magnetic field, and can be used to study on the molecular interactions, conformation and helix-coil transitions of polymers in solution. Figure 1 shows ^{13}C NMR spectra for *Lentinan* (L-FV-IA) in the water/DMSO mixtures with different w_{DMSO} . The chemical shifts for the L-FV-IA in solution state are summa-

Table I. Comparison of ^{13}C NMR Chemical Shifts for the Polysaccharides in Solution State

Sample	Solvent	Chemical shift (ppm)								Source
		C1,C1'	C2	C3	C3'	C4	C5	C6	C6 _s	
β -(1 \rightarrow 3)-D-glucan	DMSO- <i>d</i> ₆	103.0	72.8	86.2		68.4	76.3	60.9	—	ref 15
<i>C. Albicans</i> glucan	D ₂ O	104.2	74.5	No ^a	76.9	70.8	76.2	62.3	70.1	ref 15
Lentinan fraction	D ₂ O	104.2	74.4	No ^a	76.9	70.4	76.1	61.9	70.9	ref 9
<i>Lentinan</i>	0.5 M NaOD/D ₂ O	103.3	73.5	86.8		68.5	76.3	60.5	70.2	ref 22
Glucan L-FV-IA	D ₂ O/DMSO- <i>d</i> ₆ (70:30)	104.1	74.5	No ^a	77.1	70.2	76.3	62.2	71.0	This work
Glucan L-FV-IA	D ₂ O/DMSO- <i>d</i> ₆ (20:80)	103.7	73.9	No ^a	76.7	69.1	75.9	61.6	70.3	This work
Glucan L-FV-IA	D ₂ O/DMSO- <i>d</i> ₆ (15:85)	103.7	73.9	S ^b	76.7	69.0	75.9	61.4	70.3	This work
Glucan L-FV-IA	DMSO- <i>d</i> ₆	103.1	72.9	86.3	76.8	68.5	76.2	61.0	70.1	This work

^a“No” means the peak could not be observed. ^b“S” means the peak was small.

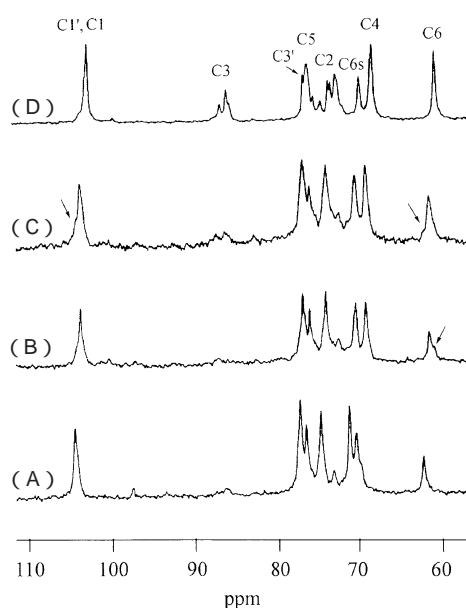


Figure 1. ^{13}C NMR spectra for glucan L-FV-IA in water/DMSO mixtures with different w_{DMSO} of 0.3 (A), 0.8 (B), 0.85 (C), and in DMSO (D).

alized in Table I. The data for β -(1 \rightarrow 3)-linked backbone D-glucosyl units, triple-helical *C. Albicans* glucan composed of β -(1 \rightarrow 3)-D-linked backbone with branching points in position 6 of glucosyl units,¹⁵ and *Lentinan* fraction IV⁹ were also listed in the table. Clearly, in spite of different nature of the side chains, the chemical shifts of the carbon atoms in main chain of the β -(1 \rightarrow 3)-D-glucan with and without side groups in DMSO-*d*₆ are quite similar. The signals at 103.1, 86.3, and 61.0 for the L-FV-IA in DMSO-*d*₆ were assigned to C1, C3, and C6 of β -(1 \rightarrow 3)-D-linked backbone, and the multiplicity and broad signal of C3 can be ascribed to the presence in the glucans of linear β -(1 \rightarrow 3)-D, branched β -D-(1 \rightarrow 6) and terminal β -D-glucosyl residues, corresponding with branched β -(1 \rightarrow 3)-D-glucan structure.¹⁷ The chemical shifts of the L-FV-IA in DMSO-*d*₆ were very similar to those of the glucan L-FV-I from *Lentinus edodes* in Fangxian of China in 0.5 M NaOD/D₂O.¹⁸ This implies that intra- and intermolecular hydrogen bonds, which maintained triple he-

lix structure, were broken in 0.5 M NaOH aqueous solution, and the conversion from triple helix to random coil occurred. The disordered conformation of the L-FV-IA in DMSO is also similar to schizophyllan and *Lentinan* in 0.38 M NaOH aqueous solution.^{10, 19} The ^{13}C NMR results indicated that the L-FV-IA sample exists as a random coil form in DMSO.

However, obvious difference, which resulted from conformation transition of the L-FV-IA in D₂O/DMSO mixture, was observed. The C3 signal at 86.3 ppm for the L-FV-IA in the backbone significantly decreased with an increase of water content, and disappeared in D₂O/DMSO (70:30). Noted that the relative intensities of C3' signal of the side chain at 76.7–77.1 ppm in D₂O/DMSO mixture increased with an increase of water content. The information from C3 and C6 signals is in agreement with that of triple-helical *C. Albicans*¹⁵ and *Schizophyllan* in 0.06 M NaOD/D₂O.¹⁰ Moreover, with an increase of water content the relative intensities of C6 signal at 61 ppm significantly decreased and shifted to downfield by 1.2 ppm. The cause of reducing C6 and C3 signals can be interpreted in terms of the restriction of the backbone chain due to intra- and intermolecular hydrogen bonds. This implies that the L-FV-IA in water/DMSO mixture with w_{DMSO} of 0.3 exists as a triple helix.

Interestingly, the C6 peak-profiles in D₂O/DMSO mixtures with w_{DMSO} of 0.85 and 0.80 exhibited an asymmetrical and broad nature, suggesting the coexistence of triple helix and single flexible chains in an intermediate of the conformation change. The signal at 70.1–71.0 ppm for the L-FV-IA was assigned to glucose substituted C6_s.^{17, 20} The relative intensity of C6_s signal significantly increased with an increase of water content, and the area ratio of C6_s to C6 changed from 0.64 in DMSO to 2.10 in D₂O/DMSO (70:30), suggesting a conformation change. Usually, the area ratio should be a constant in homogenous system. In addition, the signals of C1, C2, and C4 shifted to downfield with increasing the water content. The loss of the peak areas and downfield displacement of the carbon

Table II. Experimental results from SEC-LS, LS and viscometry for the L-FV-IA in water/DMSO mixtures at 25°C

Solvent system	$M_w \times 10^{-5}$		$\langle s^2 \rangle_z^{1/2}/\text{nm}$		$[\eta]/\text{cm}^3 \text{g}^{-1}$	$\frac{M_w (0.2 \text{ M NaCl})}{M_w (\text{DMSO})}$	
0.2 M NaCl aqueous solution	7.79	8.80 ^a	75.6	92.2 ^a	508	3.03	3.16 ^a
Water/DMSO (70:30)	—	—	—	—	687		
Water/DMSO (20:80)	—	—	—	—	544		
Water/DMSO (18:82)	6.28	—	50.0	—	406		
Water/DMSO (15:85)	—	—	—	—	173		
DMSO	2.57	2.78 ^a	30.7	43.3 ^a	63		

^aThe data from Zimm plot of LS.

atom signals in the backbone can be explained in terms of restricted mobility, intermolecular interaction and ordered-disordered conformation.²¹ These results suggest that the immobilization of the main chain by binding with intra- and inter-molecular hydrogen bonds resulted in loss of the signals of β -(1 \rightarrow 3)-D-linked backbone and in relative enhancement of the signals of the side chain as well as glucose substituted carbon atom in the triple helix state.

The results of $[\eta]$, M_w and $\langle s^2 \rangle_z^{1/2}$ for the L-FV-IA in 0.2 M NaCl aqueous solution, water/DMSO mixture with different w_{DMSO} and DMSO are summarized in Table II. The ratio of M_w for the L-FV-IA in 0.2 M NaCl aqueous solution to DMSO was approximately 3, and the data of $[\eta]$, M_w and $\langle s^2 \rangle_z^{1/2}$ in the aqueous solution agree basically with the theoretical curves for triple helix¹³ calculated from Yamakawa–Yoshizaki theory for a wormlike cylinder²² for $q = 100$ nm and from the Benoit–Doty expression for KP wormlike chain²³ for $q = 120$ (nm), respectively. It was confirmed that the L-FV-IA as triple-stranded helix in the 0.2 M NaCl aqueous solution and as single-stranded flexible chains in DMSO. SEC chromatograms for the sample L-FV-IA in 0.2 M NaCl aqueous solution, water/DMSO mixture with w_{DMSO} of 0.82 and DMSO, detected by both LS and differential refractometer are shown in Figures 2–4, respectively. “Detector: 11” and “Detector: AUX 1” represent an arbitrary unit of scattering intensity at 90° and a relative concentration, respectively. Noted that the SEC chromatogram in Figure 2 contained one peak, suggesting that the L-FV-IA in the aqueous solution exists as triple-helical chains without the obvious fragments of single chains with lower molecular weight as shown in L-FV-I.¹³ It was indicated that the sample with triple helix was prepared successfully here.

For SEC chromatogram in Figure 3, it seems that the column may be not a good condition to work for the L-FV-IA in water/DMSO because of the dead volume for high molecular weight. However, the molecular weight was detected by light scattering, an absolute method, rather than the calibration curve, so that the data of M_w and $\langle s^2 \rangle_z^{1/2}$ were not obviously affected, but the SEC peak became narrow. As shown in Figures 3 and 4, the

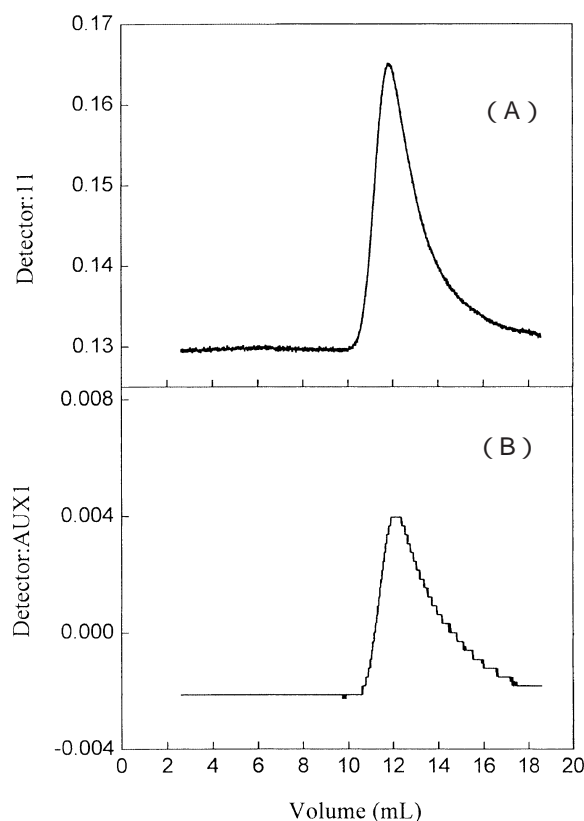


Figure 2. SEC chromatograms of the L-FV-IA in 0.2 M NaCl aqueous solution at 25°C using G6000PWXL and a G4000PWXL columns detected by LS (A) and differential refractometer (B).

SEC chromatogram of the L-FV-IA in DMSO exhibited one peak, but that in water/DMSO mixture with w_{DMSO} of 0.82 appeared a main peak of triple-helical molecules and a shoulder corresponding to dissociated chains. This indicated that with an increase of DMSO content, a sharp transition from triple helix to random coil occurred in the mixture solution, and the triple helix and flexible chain coexist in the intermediate of the conformation transition. The SEC-LS results supported strongly the deduction of triple helix-coil conformation transition by ¹³C NMR.

The w_{DMSO} dependence of $[\eta]$ for the L-FV-IA in the water/DMSO mixtures is shown in Figure 5. As shown in Table II, the $[\eta]$ values in water/DMSO mixtures with w_{DMSO} between 0.1 and 0.8 were much larger than that in DMSO, and close to that in 0.2 M NaCl

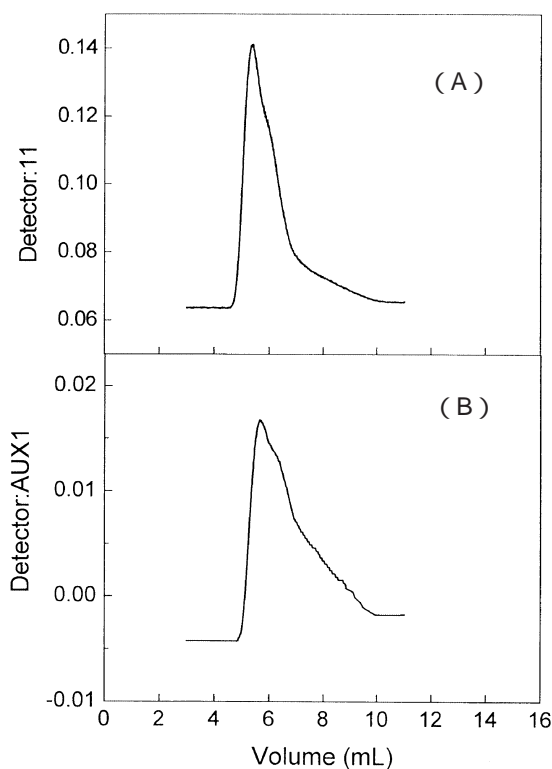


Figure 3. SEC chromatograms of the L-FV-IA in water/DMSO mixture with w_{DMSO} of 0.82 at 25°C using G4000-H8 column detected by LS (A) and differential refractometer (B).

aqueous solution. When DMSO concentration in the mixture increased from 0.80 to 0.85 of w_{DMSO} , the $[\eta]$ sharply decreased, accompanying a conformation transition from triple helix to single flexible chains. It is worth noting that in the narrow range of w_{DMSO} from 0.85 to 0.90 the $[\eta]$ decreased slightly to be near the values in pure DMSO, similar to the conformation transition of *schizophyllan*, β -(1 \rightarrow 3)-D-glucan with one β -(1 \rightarrow 6)-D-glucan side chain for every three main-chain residues, in same condition, where triple helix dissociates abruptly to single chain at $w_{\text{DMSO}} \sim 0.87$.²⁴ The sharp decrease of $[\eta]$ suggests that the triple helix of the L-FV-IA dissociated directly to single chains. However, the $[\eta]$ decreased by a factor 2 on passing from the mixture solution ($w_{\text{DMSO}} = 0.85$) to DMSO. This implies that some helical chains were not fully dissociated to random coil in water/DMSO with w_{DMSO} of 0.85.

The open triangles in Figure 5 refer to the $[\eta]$ values obtained from the L-FV-IA dissolved in DMSO and then diluted to water/DMSO mixture with desired w_{DMSO} by adding water. These experimental points were significantly lower than those from dissolved directly in water/DMSO mixture. When DMSO concentration in the mixture decreased from 1 to 0.7 of w_{DMSO} , the $[\eta]$ values hardly changed, indicating that the shape and size of the single-flexible chains remained the same

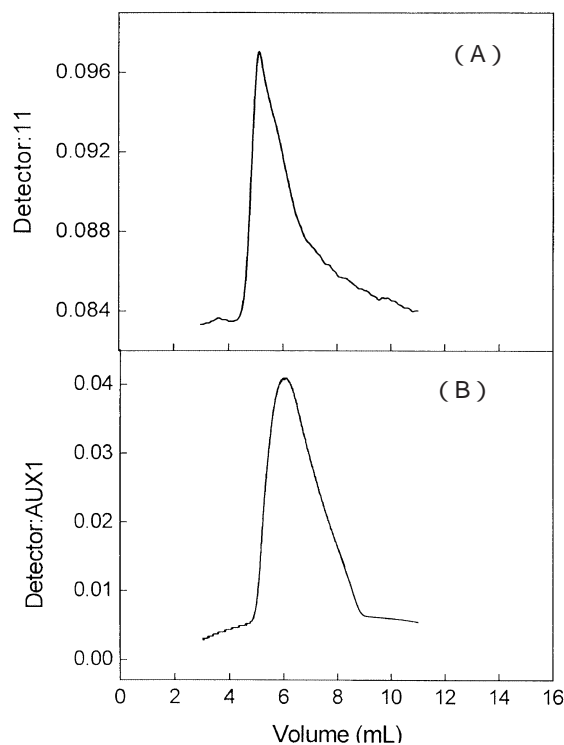


Figure 4. SEC chromatograms of the L-FV-IA in DMSO at 25°C using G4000-H8 column detected by LS (A) and differential refractometer (B).

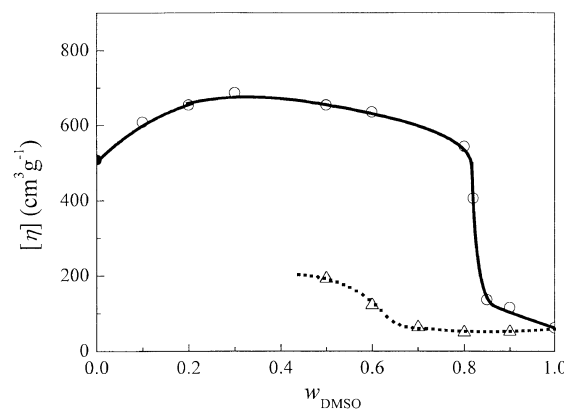


Figure 5. w_{DMSO} dependence of $[\eta]$ for the glucan L-FV-IA in water/DMSO mixtures at 25°C. Filled circle, value in 0.2 M NaCl aqueous solution; Open triangles, values obtained from the sample dissolved in DMSO then diluted to water/DMSO mixtures with desired w_{DMSO} .

as in pure DMSO. When w_{DMSO} was higher than 0.6, the $[\eta]$ increased slowly, suggesting that the aggregation or unwinding of the partial chains occurred with an increase of water content. This indicated that the helix-coil transition of the L-FV-IA in the water/DMSO mixtures was irreversible. In addition, the L-FV-IA concentration used was in the range from 2.0×10^{-4} to 5.0×10^{-3} (g cm^{-3}), and the equilibrium of the helix-coil transition is well known to depend not directly on the polymer concentration.²⁵

The results from the SEC-LS and viscosity measure-

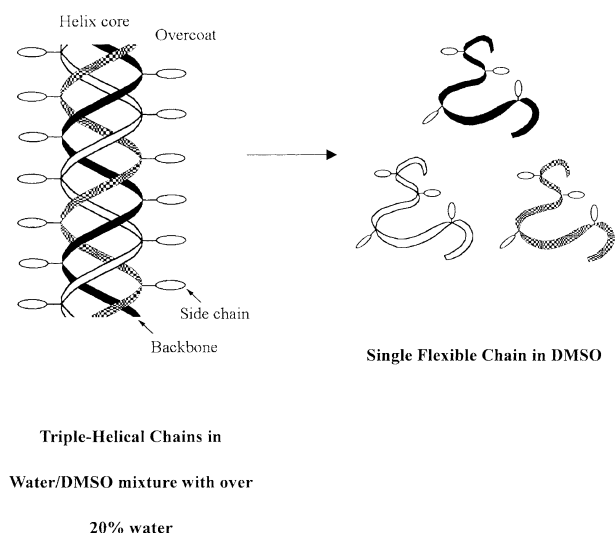


Figure 6. Schematic diagram of overcoating-cylinder model describing the chain conformations for glucan L-FV-IA in water and DMSO.

ments confirmed strongly the helix-coil transition of the L-FV-IA in the water/DMSO mixtures with w_{DMSO} from 0.80 to 0.85. On the basis of the informations obtained from ^{13}C NMR, SEC-LS and viscosity, a molecular model of the wormlike cylinder describing the triple helix conformation and its transition is shown in Figure 6. The cylinder was composed of three single-stranded helix, and assembled into an overcoating cylinder structure of the backbone as helix core and the side chain as rotatable overcoat. In the helix state, the relatively high intensities of the carbon atoms of the branched glucosyl residues may be attributable to the motion of side chains, and a possible internal rotation of the hydroxymethyl groups can be responsible for the relatively sensitive natural-abundance of the C6 signals.

CONCLUSIONS

The predominant species of β -(1 \rightarrow 3)-D-glucan with (1 \rightarrow 6) branching (L-FV-IA) from *Lentinus edodes* exists as triple-helical chains in 0.2 M NaCl aqueous solution and water/DMSO mixtures, in which water content was higher than 20 wt%, and as single-flexible chains in DMSO. The results from SEC-LS, viscosity and ^{13}C NMR measurements indicated that the helix-coil conformation transition occurred sharply in the range from 80 to 85 wt% DMSO aqueous solution, and was irreversible. The helix and coil chains coexist in the narrow range of water/DMSO mixture with w_{DMSO} from 0.80 to 0.85, showing that obvious changes of M_w , $\langle s^2 \rangle_z^{1/2}$, $[\eta]$ as well as signals of C6 and C6_s. The obvious difference in ^{13}C NMR spectra for the triple-helix and coil conformations was that the signal at 86.3 ppm of C3 in β -(1 \rightarrow 3)-linked backbone almost completely

disappeared in the helical state; the C6 signal at 61.0–62.2 ppm exhibited an asymmetrical and broad nature in the intermediate of the conformation change; the area ratio of C6_s to C6 increased with the conformation transition from single-flexible chains to triple helix. The immobilization of the β -glucan backbone by binding with intra- and intermolecular hydrogen bonds resulted in the loss of the signals of its carbon atoms and down-field displacement in the triple helix state.

Acknowledgment. This work was supported by the National Natural Science Foundation of China (20074025), the Research Grants Council of Hong Kong Government Earmarked Grant (RGC, CUHK 4161/99 M) and Laboratory of Cellulose and Lignocellulosic Chemistry of Chinese Academy Sciences.

REFERENCES

- G. Chihara, Y. Maeda, J. Hamuro, T. Sasaki, and F. Fukuoka, *Nature*, **222**, 687 (1969).
- M. Mizuno, M. Morimoto, K. Minato, and H. Tsuchida, *Biosci. Biotechnol., Biochem.*, **62**, 437 (1998).
- K. Kajiwara and T. Miyamoto, "Polysaccharides: Structural Diversity and Functional Versatility", S. Dumitriu, Ed., Marcel Dekker, Inc., 1998, p 91.
- L. Zhang and L. Yang, *Biopolymers*, **36**, 695 (1995).
- Y. Kashiwagi, T. Norisuye, and H. Fujita, *Macromolecules*, **14**, 1220 (1981).
- F. Santi, N. Hellman, N. Ludwig, G. Babcock, R. Tokin, C. Glass, and B. Lambers, *J. Polym. Sci.*, **17**, 527 (1955).
- Q. Ding, S. Jiang, L. Zhang, and C. Wu, *Carbohydr. Res.*, **308**, 339 (1998).
- T. L. Bluhm and A. Sarko, *Can. J. Chem.*, **55**, 293 (1977).
- H. Saito, T. Ohki, N. Takasuka, and T. Sasaki, *Carbohydr. Res.*, **58**, 293 (1977).
- H. Saito, T. Ohki, and T. Sasaki, *Carbohydr. Res.*, **74**, 227 (1979).
- H. Saito, R. Tabeta, T. Sasaki, and Y. Yoshioka, *Bull. Chem. Soc. Jpn.*, **59**, 2093 (1986).
- H. Saito and M. Yokoi, *Bull. Chem. Soc. Jpn.*, **62**, 392 (1989).
- L. Zhang, X. Zhang, Q. Zhou, P. Zhang, M. Zhang, and X. Li, *Polym. J.*, **33**, 317 (2001).
- C. Viebke, L. Piculell, and S. Nilsson, *Macromolecules*, **27**, 4160 (1994).
- G. Kogan, J. Alföldi, and L. Masler, *Biopolymers*, **27**, 1055 (1988).
- M. Gawronski, H. Conrad, T. Springer, and K. P. Stahmann, *Macromolecules*, **29**, 7820 (1996).
- C. Gandon and M. Bruneteau, *Carbohydr. Res.*, **313**, 259 (1998).
- P. Zhang, L. Zhang, and S. Cheng, *Biosci. Biotechnol. Biochem.*, **63**, 1197 (1999).
- H. Saito, ACS Symp. Ser. No. 150, American Chemical Society, Washington, D.C., 1981, pp 125–147.
- H. Saito, R. Tabeta, Y. Yoshioka, C. Hara, T. Kiho, and S. Ukai, *Bull. Chem. Soc. Jpn.*, **60**, 4267. (1987).

21. J. F. Rabek, "Experimental Methods in Polymer Chemistry", John Wiley & Sons, Inc., New York, N.Y., 1980, p 206.
22. H. Yamakawa and T. Yoshizaki, *Macromolecules*, **13**, 633 (1980).
23. H. Benoit and P. Doty, *J. Phys. Chem.*, **57**, 958 (1953).
24. T. Sato, T. Norisuye, and H. Fujita, *Macromolecules*, **16**, 185 (1983).
25. K. Ueda, M. Itoh, Y. Matsuzak, H. Ochiai, and A. Imamura, *Macromolecules*, **31**, 675 (1998).