Polymer Bulletin 41, 471–478 (1998)

Polymer Bulletin

Viscosity behavior and chain conformation of a (1→3)-α-glucan from *Ganoderma lucidum*

Jinghua Chen[1], Lina Zhang[1], Yo Nakamura[2], Takashi Norisuye[2]

[1] Department of Chemistry, Wuhan University, Wuhan 430072, China
[2] Department of Macromolecular Science, Osaka University, Toyonaka, Osaka 560-0043, Japan

Received: 28 July 1998/Accepted: 18 August 1998

Summary

Seven fractions of a (1→3)-α-D-glucan from *Ganoderma lucidum* have been studied by light scattering, sedimentation equilibrium, and viscometry in dimethylsulfoxide (DMSO) containing 0.25 M lithium chloride at 25°C. The intrinsic viscosity $[\eta]$ - molecular weight relation for this glucan in the mixed solvent is found to be represented approximately by $[\eta] = 0.071\ M_w^{0.60}$ cm^3 g^{-1} in the range of weight-average molecular weight M_w studied, i.e., from 8 x 10^3 to 4.4 x 10^5. Its analysis based on current theories for wormlike chains shows that, without excluded volume effect, the (1→3)-α-D-glucan chain is characterized by a linear mass density of 380 nm^{-1}, a Kuhn segment length of about 3 nm, and a diameter of 1.2 nm and is somewhat more extended but more flexible than amylose, a (1→44)-α-glucan, in DMSO.

Introduction

Experimental exploration of the relationship between the glycosidic bonding geometry and the chain conformation is basic to our systematic understanding of physical properties of linear homoglucans, but dilute-solution data available to date are yet limited by and large to (1→4)-α-, (1→4)-β-, and (1→3)-β-D-glucans (1-6). The lack of solution data for (1→3)-α-D-glucans may be attributed to the unavailability of test samples.

Very recently, we found from liquid chromatography, NMR, infrared spectroscopy, and other techniques that the water-insoluble polysaccharide extracted from the fruiting body of *Ganoderma lucidum* (*Rheishi*) is a linear (1→3)-α-D-glucan and dissolves in 0.25 M LiCl/DMSO, i.e., dimethylsulfoxide (DMSO) containing 0.25 M lithium chloride, at room temperature (7). Thus, in the present work, we prepared (1→3)-α-glucan fractions of different molecular weights and studied them by light scattering, sedimentation equilibrium, and viscomery in 0.25 M LiCl/DMSO, hoping to deduce some conformational characteristics of the glucan. The intrinsic viscosity ($[\eta]$) data obtained as a function of weight-average molecular weight M_w are analyzed below on the basis of the wormlike chain (8) or more generally the helical wormlike chain (9).

Experimental

Samples

The (1→3)-α-glucan samples GL4-1 and GL4-2 (7) previously extracted from *Rheishi* with 1 N aqueous NaOH at 25 and 65°C, respectively, were fractionated as follows. A mixture of acetone and 0.25 M LiCl/DMSO (4: 1 by volume) was slowly added to a 0.25 M LiCl/DMSO solution of each sample (about 1% polymer concentration) at 25°C until the solution became turbid. The liquid was then heated (or cooled), but its turbidity remained unchanged. After being brought to 25°C and allowed to stand for several hours, the turbid solution was centrifuged at about 25°C to separate the concentrated phase. The supernatant was subjected to further fractionation. In this way, samples GL4-1 and GL4-2 were divided into 13 and 8 parts, respectively. Some of the products were further fractionated by repeating the above procedure once or twice to extend the molecular weight range of the study as wide as possible.

From a number of fractions thus obtained, seven middle ones sufficient in quantity for molecular weight determination were chosen and designated A-1, A-2, A-3 (from GL4-2), B-1, B-2, B-3, and B-4 (from GL4-1). These fractions were reprecipitated from 0.25 M LiCl/DMSO solutions into 80% aqueous acetone, washed with 50% aqueous acetone three times and anhydrous acetone six times, and dried in vacuum for seven days.

Light Scattering

Weight-average molecular weights for five higher molecular weight fractions, A-1, A-2, A-3, B-1, and B-2, were determined by static light scattering (Fica-50 photometer) in 0.25 M LiCl/DMSO at 25°C using vertically polarized incident light of 436-nm wavelength (see ref. 10 for the experimental procedures). The intensity data obtained were analyzed by the square-root plots of $(Kc/R_\theta)^{1/2}$ vs $\sin^2(\theta/2)$ and $(Kc/R_\theta)^{1/2}$ vs c, where K is the optical constant, c the polymer mass concentration, and R_θ the excess reduced scattering intensity (for vertically polarized incident light without analyzer) at scattering angle θ. The estimated molecular weights and second virial coefficients were corrected for optical anisotropy by the conventional method (11), since appreciable depolarized scattering was observed when an analyzer was set in the horizontal direction. The optical anisotropy factor δ appearing in the relation, $R_{\theta,\mathrm{Hv}}$ (the depolarized component) = $3\delta KcM_w$ at $c = 0$ and $\theta = 0$, increased up to 0.03 with decreasing molecular weight, but its molecular weight dependence was not analyzed.

Test solutions were made optically clean by filtration through Teflon Millipore filters followed by centrifugation at about 2.5×10^4 gravities for 2 h. Prior to this optical clarification, they had been heated at 65°C for 20 min to minimize aggregation effects on scattering intensities at low θ. This procedure was essential to obtain reproducible data. Virtually no change in $[\eta]$ at 25°C was observed before and after the heating.

The specific refractive index increment $(\partial n/\partial c)_\mu$ in 0.25 M LiCl/DMSO at 25°C was determined for dialyzed solutions of fraction B-2 to be 0.056_0 and 0.058_0 cm^3 g^{-1} at 436 and 546 nm, respectively, using a modified Schulz-Cantow type differential refratometer. The subscript μ attached to $(\partial n/\partial c)$ signifies the condition that the chemical potentials of all diffusible components are held constant. The $(\partial n/\partial c)_\mu$ value of 0.056_0 cm^3 g^{-1} at 436 nm is slightly smaller than that (0.058_0 cm^3 g^{-1}) determined previously (7) for a purified GL4-2 fraction at the same wavelength. In the present work, we used the former for B-1 and B-2 and the latter for A-1, A-2, and A-3.

Sedimentation Equilibrium

The molecular weights of fractions B-3 and B-4 were determined by sedimentation equilibrium in a Beckman Model E ultracentrifuge with 0.25 M LiCl/DMSO at 25°C as the solvent. A Kel-F 12-mm double sector cell was used and the liquid column was adjusted to about 2 mm. The rotor speed was chosen to be 24000 or 28000 rpm.

Densities ρ were measured as a function of c for dialyzed solutions of B-2 using a bicapillary pycnometer of 30-cm^3 capacity. The value of $(\partial\rho/\partial c)_\mu$ in 0.25 M LiCl/DMSO at 25°C was 0.234.

Viscometry

Viscosities of 0.25 M LiCl/DMSO solutions of all fractions were measured at 25°C using a conventional viscometer of the Ubbelohde type. For the two lowest molecular weight fractions B-3 and B-4, the difference between solvent and solution densities was taken into account in the evaluation of the relative viscosity; the partial specific volume in 0.25 M LiCl/DMSO at 25°C determined for undialyzed solutions was 0.663 cm^3 g^{-1}.

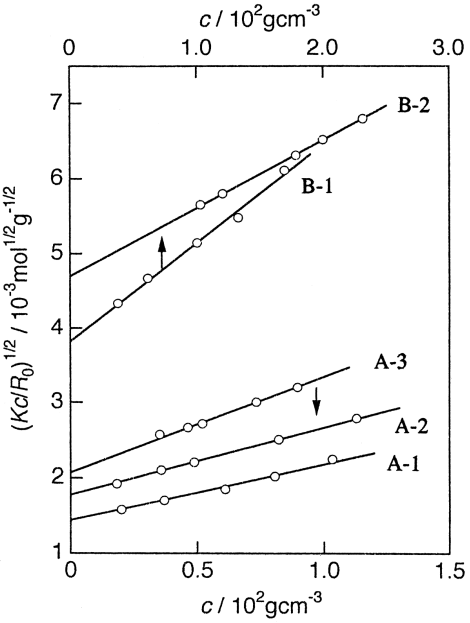

Figure 1. Plots of $(Kc/R_0)^{1/2}$ vs. c for indicated fractions of the *Rheishi* (1→3)-α-D-glucan in 0.25 M LiCl/DMSO at 25°C.

Results

Figure 1 illustrates the concentration dependence of $(Kc/R_0)^{1/2}$, i.e., the zero-angle value of $(Kc/R_\theta)^{1/2}$, for the

indicated fractions of the *Rheishi* glucan. The plotted points for each fraction follow a straight line, whose intercept and slope give apparent values of M_w and A_2 (the second virial coefficient). The true values obtained by correction for optical anisotropy are summarized in Table I, along with the data of M_w and A_2 for fractions B-3 and B-4 from sedimentation equilibrium and those of $[\eta]$ and k' (the Huggins constant) for the seven fractions.

Table I. Results from light scattering, sedimentation equilibrium, and viscosity measurements on (1→3)-α-D-glucan fractions in 0.25 M LiCl/DMSO at 25°C

fraction	M_w x 10^{-4}	A_2 x 10^4 [c]	$[\eta]$ / cm^3 g^{-1}	k'
A-1	44.5[a]	1.25[a]	154	0.39
A-2	29.0[a]	1.84[a]	133	0.38
A-3	20.8[a]	3.26[a]	122	0.38
B-1	5.69[a]	6.79[a]	54.0	0.38
B-2	3.73[a]	6.31[a]	37.0	0.45
B-3	1.19[b]	30[b]	19.7	0.51
B-4	0.82[b]	30[b]	16.1	0.50

[a] from light scattering.
[b] from sedimentation equilibrium.
[c] in units of mol g^{-2} cm^3.

The molecular weight dependence of $[\eta]$ for the (1→3)-α-glucan in 0.25 M LiCl/DMSO is shown in Figure 2, which includes our previous data (7) for a purified GLA-2 sample (M_w = 1.95 x 10^5 and $[\eta]$ = 102 cm^3 g^{-1}). The solid curve fitting the plotted points is represented by $[\eta] = 0.071 M_w^{0.60}$ cm^3 g^{-1}. The viscosity exponent 0.6 suggests that the glucan chain be flexible.

The dashed, dotted, and dot-dash lines in Figure 2 represent the published log $[\eta]$ - log M_w relations for cellulose [(1→4)-β-D-glucan] in water-diluted cadoxen (3,4), amylose [(1→4)-α-D-glucan] in DMSO (1), and curdlan [(1→3)-β-D-glucan] in water-diluted cadoxen (5), respectively. The line for the *Rheishi* glucan appears far below that for cellulose but appreciably above those for amylose and curdlan in the region of M_w below 10^5, implying that, when compared at the same but not-too-high molecular weight, the

(1→3)-α-glucan chain is more extended than the (1→4)-α-and (1→)-β-glucan molecules. This is in line with the early computer modeling by Rees and Scott (12), who predicted on the basis of hard-core potentials that, while the most stable conformations of (1→4)-α- and (1→3)-β-glucans are helical, that of (1→3)-α-glucan is ribbon-like and extended. X-ray diffraction analysis by Ogawa et al. (13) shows the (1→3)-α-glucan chain in the cryslalline state to be nearly completely extended with a fiber repeat of 0.844 nm (the contour length per glucose residue equals 0.422 nm). The point is that, though none of these single polysaccharide chains can maintain such regular structure in solution, the magnitude of $[\eta]$ of each glucan at very low M_w reflects the geometry of glycosidic linkage and hence the local chain conformation. Note that at high M_w, $[\eta]$ is strongly affected by chain flexibility (or stiffness) and intramolecular excluded-volume effect.

Discussion

Data Analysis

We analyze the viscosity data for the *Rheishi* glucan in Figure 2 on the basis of the Yoshizaki - Nitta - Yamakawa theory (14) for the intrinsic viscosity $[\eta]_0$ of an unperturbed helical wormlike (HW) chain (9) combined with the quasi-two-parameter (QTP) theory (9,15,16) for excluded-volume effects, confining ourselves to the wormlike chain limit of the HW chain. The former theory in this limit contains three parameters, L (the contour length of the chain), λ^{-1} (Kuhn's segment length or more generally the stiffness parameter in the HW chain), and d (the chain diameter in the bead model). The first parameter is related to the molecular weight M by $L = M/M_L$, with M_L being the molar mass per unit contour length. In the QTP scheme, the cube of the viscosity expansion factor, α_η^3 ($\equiv [\eta]/[\eta]_0$), may be expressed as (9)

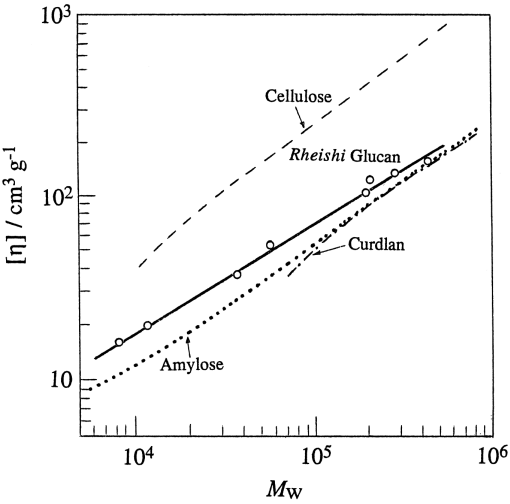

Figure 2. Molecular weight dependence of $[\eta]$ for the *Rheishi* (1→3)-α-D-glucan in 0.25 M LiCl/DMSO at 25°C (circles), compared with the published relations for cellulose in water-diluted cadoxen (3,4), amylose in DMSO (1), and curdlan in water-diluted cadoxen (5).

$$\alpha_\eta^3 = (1 + 3.8\tilde{z} + 1.9\tilde{z}^2)^{0.3} \quad (1)$$

if the Barrett function (17) is adopted. Here, $\tilde{z}$ is the scaled excluded-volume parameter defined by

$$\tilde{z} = (3/4)K(\lambda L)z \quad (2)$$

with

$$z = (3/2\pi)^{3/2}(\lambda B)(\lambda L)^{1/2} \quad (3)$$

In eqs 2 and 3, $K(\lambda L)$ is a known function of λL (16) and B is the excluded-volume strength defined for the wormlike chain by $B = \beta/a^2$, with β and a being the binary cluster integral and the bead spacing, respectively. Thus $[\eta]$ for a given M is determined by M_L, λ^{-1}, d, and B. Equation 1 is known to be a good approximation to linear homopolymers, both flexible (9) and stiff (18).

We may take M_L of the *Rheishi* glucan to be 380 nm^{-1}, a value obtained from the monomeric length 0.422 nm along the chain contour of the (1→3)-α-glucan in the crystalline state (13). With this M_L, we estimated λ^{-1}, d, and B so that the theory of Yoshizaki et al. with eq 1 gives the best agreement with our $[\eta]$ data. Equally close agreements were found for a number of parameter sets within the ranges of λ^{-1} from 2.6 and 3.4 nm, d from 1.1 to 1.3 nm, and B from 0.10 to 0.31 nm. The large uncertainty in each parameter arose not only from scatter of data points but also from the relatively narrow range of molecular weight studied, and λ^{-1}, d, and B for the α-glucan chain were determined only with moderate accuracy as 3.0 (± 0.4) nm, 1.2 (± 0.1) nm, and 0.21 (± 0.11) nm, respectively. Figure 3 shows that our data are fitted by the solid curve computed with M_L = 380 nm^{-1}, λ^{-1}, = 3.0 nm, d = 1.2 nm, and B = 0.21 nm. The dashed line in the figure represents the theoretical values for $[\eta]_0$. A point to note is that, though this unperturbed line somewhat varies depending on the choice of parameter sets of λ^{-1} and d within the above uncertainty, excluded-volume effects on $[\eta]$ are significant, at least, for M_w above 4 x 10^4.

Conformational Characteristics

The λ^{-1} value of 3.0 (± 0.4) nm estimated above leads to the conclusion that the (1→3)-α-glucan chain in 0.25 M LiCl/DMSO is very flexible. In fact, this value is even smaller than that (4.0 nm) for amylose in DMSO (at 25°C), which is locally helical but randomly coiled as a whole (1,19). Although the bead diameter of 1.2 (± 0.1) nm for our glucan is considerably larger than what is expected from the chemical structure (about 0.7 nm), it is comparable to that (1.4 nm) of solvated amylose molecules in DMSO (1). Thus the

(1→3)-α-glucan chain in 0.25 M LiCl/DMSO should also be solvated by DMSO molecules or their complexes with LiCl.

We finally touch on the characteristic ratio C_∞ at infinite chain length, which may be written in terms of the wormlike chain parameters as

$$C_\infty = M_0/\lambda M_L l^2) \qquad (4)$$

where M_0 is the molar mass of a glucose residue and l is the virtual bond length equal to the distance between two successive glycosidic oxgens O(3) and O(3') in the present case. According to X-ray analysis (13), l of (1→3)-α-glucan is equal to 0.42 nm, which happens to be very close to one half the fiber repeat. Introduction of this l value together with M_L = 380 nm^{-1}, λ^{-1} = 3.0 (± 0.4) nm, and M_0 = 162 into eq 4 yields 7.2 (± 1.0) for C_∞ of the (1→3)-α-glucan chain. Interestingly, this C_∞ is significantly larger than the value of 4.5 for amylose in DMSO (1), despite the above-mentioned fact that λ^{-1} for the (1→3)-α-glucan is smaller than that for the (1→4)-α-glucan. This finding gives an additional example that C_∞ is not a direct measure of chain stiffness (9).

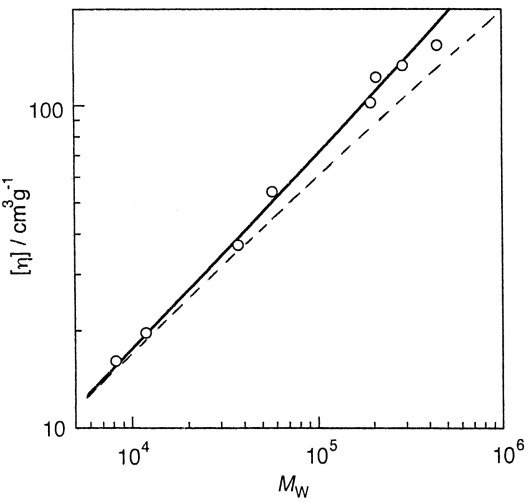

Figure 3. Comparison between the measured [η] (circles) for the *Rheishi* (1→3)-α-D-glucan in 0.25 M LiCl/DMSO at 25°C and the theoretical values (solid line) calculated from the theory of Yoshizaki et al. (14) with eq 1 for the perturbed wormlike chain with M_L = 380 nm^{-1}, λ^{-1} = 3.0 nm, d = 1.2 nm, and B = 0.21 nm. The dashed line refers to the unperturbed state.

Acknowledgment

We thank Professor K. Ogawa of Osaka Prefectural University for his interest in this work and valuable discussions on the conformation or structure of (1→3)-α-glucan. J. Chen gratefully acknowledges the Association of International Education, Japan, for the Short-Term Exchange Promotion Program, Peace and Friendship Scholarship, which enabled him to study at Osaka University for one year. L. Zhang also acknowledges with many thanks the National Natural Science Foundation of China for supporting this work.

References

1. Nakanishi Y, Norisuye T, Teramoto A, Kitamura S (1993) Macromolecules 26: 4220
2. Norisuye T (1994) Polym J 26: 1303
3. Henley D (1961) Arkiv Kemi 18: 327
4. Brown W, Wilkström R (1965) Eur Polym J 7: 1
5. Hirano I, Einaga Y, Fujita H (1979) Polym J 11: 901
6. Nakata M, Kawaguchi T, Kodama Y, Konno A (1998) Polymer 39: 1475
7. Chen J, Zhou J, Zhang L, Nakamura Y, Norisuye T (1998) Polym J in press
8. Kratky O, Porod G (1949) Rec Trav Chim (Pays Bas) 68: 1106
9. Yamakawa H (1997) Helical Wormlike Chains in Polymer Solutions. Springer Berlin
10. Tsuboi A, Yamasaki M, Norisuye T, Teramoto A (1995) Polym J 27: 1219.
11. Yamakawa H (1971) Modern Theory of Polymer Solutions. Harper & Row, New York
12. Rees DA, Scott WE (1971) J Chem Soc B 469
13. Ogawa K, Okamura K, Sarko A (1981) Int J Biol Macromol 3: 31
14. Yoshizaki T, Nitta I, Yamakawa H (1988) Macromolecules 21: 165
15. Yamakawa H, Stockmayer WH (1972) J Chem Phys 57: 2843
16. Shimada J, Yamakawa H (1986) J Chem Phys 85: 591
17. Barrett AJ (1984) Macromolecules 17: 1566
18. Norisuye T, Tsuboi A, Teramoto A (1996) Polym J 28: 357
19. Nakata Y, Kitamura S, Takeo K, Norisuye T (1994) polym J 26: 1085