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Molecular weight and aggregation behaviour in solution of β-D-glucan from *Poria cocos* sclerotium

Lina Zhang [a,*], Qiong Ding [a], Pingyi Zhang [a], Rongping Zhu [b], Yihua Zhou [b]

[a] *Department of Chemistry, Wuhan University, Wuhan 430072, China*
[b] *National Research Center for Certified Reference Materials, Beijing 100013, China*

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Abstract

The weight-average molecular weights M_w and intrinsic viscosities $[\eta]$ of linear β-(1 → 3)-D-glucan (PC3), a major polysaccharide in *Poria cocos* sclerotium were studied by light scattering and viscometry. The M_w values of glucan PC3 fractions in 20% cadoxen solution and in cadoxen were 20.6×10^4 and 8.93×10^4, respectively. The $[\eta]$ values of glucan PC3 in water–cadoxen mixtures underwent an almost discontinuous decrease in the range from 20 to 30% cadoxen. The data (M_w, $[\eta]$) of the unfractionated PC3 and of two fractions in 20% cadoxen and in cadoxen showed that glucan PC3 forms aggregates in 20% cadoxen solution, and dissociates to single chains in cadoxen. The change of aggregation and disaggregation in water–cadoxen mixtures are reversible. © 1997 Elsevier Science Ltd.

Keywords: *Poria cocos*; Polysaccharide; Molecular weight; Light scattering; Intrinsic viscosity; Aggregation

1. Introduction

Poria cocos Wolf has been long used as traditional Chinese herb with diuretic and sedative activities. It is known by the name of Fuling in Chinese and grows under ground on the roots of pine trees. Recently, polysaccharides from the sclerotium or mycelia of *Poria cocos* have been studied from the viewpoint of their antitumor effect [1–4]. It has been found that the bioactivity of the polysaccharides is related to the chemical structure and molecular weight [5].

In the previous paper [6], we identified three heteropolysaccharides (PC1, PC2, PC2-A) and two β-D-glucans (PC3 and PC4) from a fresh sclerotium of *Poria cocos* by using a fractionation procedure that involved extraction and separation with 0.9% NaCl aqueous solution, hot water, a DEAE–Sephadex column, 0.5 M NaOH aqueous solution and 88% formic acid. The analytical results by IR, GC, HPLC, and ^{13}C NMR indicated that glucan PC3 obtained from 0.5 M NaOH aqueous extract is the major polysaccharide component of *Poria cocos* sclerotium and its constitution mainly a linear β-(1 → 3)-D-glucan. It is therefore identified with the polysaccharide named pachyman. Pachyman itself shows no antitumor activ-

* Corresponding author.

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ity, whereas activity is shown after treatment with periodate or urea, or following carboxymethylation [1,3]. The common change, besides some chemical modification, seems to be a degradation of the molecular weight of the glucan during these treatments. Therefore, elucidation of the molecular weight of the β-(1 → 3)-D-glucan is very important in order to establish some correlation to the bioactivity of *Poria cocos*.

Studies on the molecular weight of *Poria cocos* polysaccharides have already seen reported [2,7–9], but there are some discrepancies among the literature results. The number-average degree of polymerization (DP) of polysaccharide, extracted from *Poria cocos* with aqueous 0.5 M NaOH, was estimated to be 255 (corresponding to $M_n = 4.1 \times 10^4$) by the procedure of Smith [7]. The nitric ester derivative of pachyman has been studied by osmotic pressure in acetone, and a DP of 690, corresponding to $M_n = 11.2 \times 10^4$, was obtained [8]. A weight average molecular weight of 37.0×10^4 was determined by light scattering for pachyman extracted from deoiled *Poria cocos* with 2 g/dL NaOH solution [2]. Finally, it is puzzling that the molecular weight of β-(1 → 3)-D-glucan from the mycelia of *Poria cocos* Wolf was calculated to be 5×10^6 by the gel-filtration method with CPG-1000 Å [9].

The purpose of this study was to resolve the discrepancies in the molecular characteristics reported, and to clarify the molecular weights of linear β-(1 → 3)-D-glucans from *Poria cocos* sclerotium. The unfractionated sample of PC3 previously isolated, and two fractions obtained by selective precipitation, were used in this work. The molecular weights and intrinsic viscosities of the polysaccharides were determined by light scattering and viscometry, and aggregation process in solution are discussed.

2. Experimental

Preparation of samples and of cadoxen solution.—The isolation and characterization of the sample PC3 were described in reference [6]. The polydisperse glucan PC3 dissolved in aqueous 0.2 M NaOH was fractionated by addition of EtOH as precipitant at 25 °C according to the non-solvent addition method. Eight fractions were obtained, which were redissolved and dialyzed against distilled water for a week at 25 °C, and then rotary evaporated at reduced pressure below 50 °C. Here the fourth and seventh fraction (PC3-F4 and PC3-F7) were used.

The solvent cadoxen was freshly prepared at once. A 29 wt% aqueous solution of ethylenediamine was saturated with CdO at 0 °C under vigorous stirring, and kept for 8 h below 5 °C. The solution was centrifuged at 8000 rpm for 20 min, and then the supernatant was filtered through a sand filter. The cadoxen so prepared, which was transparent and stable at 25 °C, was refrigerated until use. The 20% cadoxen was prepared by mixing the appropriate volume ratio of cadoxen and distilled water (2:8 v/v). The refractive index n_0 of 20% cadoxen and cadoxen at 633 nm at 25 °C were 1.340 and 1.388, respectively.

Viscometry.—Viscosities of the polysaccharide solutions were measured at 25 ± 0.05 °C by using a modified capillary viscometer supplied by the Institute of Industrial Science, Tokyo University. The 20% cadoxen and cadoxen were used as solvents for samples PC3, PC3-F4, and PC3-F7, respectively. The kinetic energy correction was always negligible. Huggins and Kraemer plots were used to estimate the intrinsic viscosity $[\eta]$ and the Huggins constant k'.

Light scattering measurements.—The light-scattering intensities were observed with a dynamic light scattering spectrophotometer (DLS-700, Otsuka Electronics Co.) at 633 nm in an angular range from 30 to 150° at 15° intervals at 25 °C. The polysaccharide solutions were prepared with 20% cadoxen and cadoxen solvent, and used within 24 h after preparation. Optical clarification of the solution was achieved by using a sand filter, with subsequent filtration through a 0.2-μm pore-size filter (M-HJV) for 20% cadoxen or a 0.45-μm pore-size filter (Nylon Acrodisc, Gelman Sci. Inc.) for cadoxen solution into the scattering cell. The refractive index increments (dn/dc) were measured with a double-beam differential refractometer (DRM-1020, Otsuka Electronics Co.) at 633 nm and 25 °C. The values of dn/dc of PC3 and its fractions PC3-F4 and PC3-F7 were 0.120 $cm^3\ g^{-1}$ in 20% cadoxen, and 0.161 $cm^3\ g^{-1}$ in cadoxen.

3. Results and discussion

Molecular weights.—Figs. 1 and 2 illustrate the Zimm plots for glucan PC3 in 20% cadoxen and for PC3-F4 in cadoxen, respectively. Here K is the light-scattering constant, R_θ is the reduced Rayleigh ratio at angle $\theta°$, and c is polysaccharide concentration. The weight-average molecular weight M_w and root-mean-square radii of gyration $\langle s^2 \rangle^{1/2}$ of the glucans PC3, PC3-F4, and PC3-F7 are summarized in

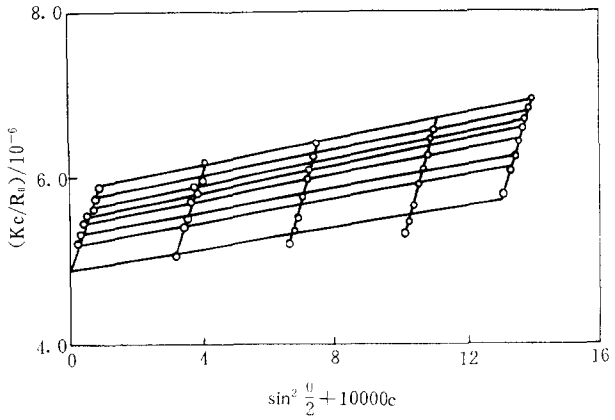

Fig. 1. Zimm plot of glucan PC3 in 20% cadoxen at 25 °C.

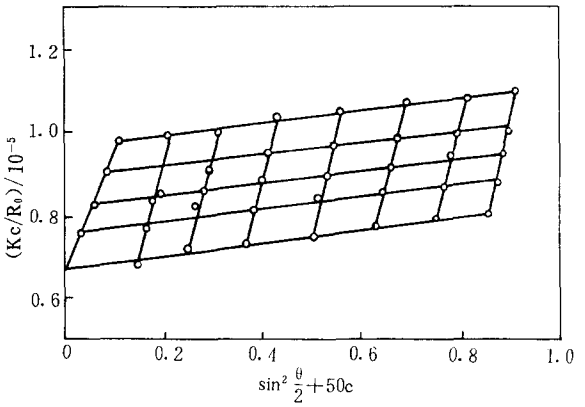

Fig. 2. Zimm plot of glucan PC3-F4 in cadoxen at 25 °C.

Table 1. The M_w value of glucan in cadoxen is close to that estimated from the glucan nitric ester in acetone by osmometry [8], and lower than those determined from the glucan in aqueous solution by light scattering and GPC [2,9]. It is worth noting that the values of M_w, $\langle s^2\rangle^{1/2}$ of PC3 glucan and its fractions in 20% cadoxen are markedly higher than those in cadoxen. As shown in Table 1, the values of M_w (in 20% cadoxen)/M_w (in cadoxen) are not constant, and are higher for the fractionated samples. The high value of $\langle s^2\rangle^{1/2}$ for the fraction PC3-F7 in 20% cadoxen is clearly due to some aggregation process with a concentration dependence. The aggregation phenomenon is also apparent by the large value of the ratio (5.93) of the molecular weight in cadoxen and that in 20% cadoxen.

It would be easy to assume that PC3 is a linear β-(1 → 3)-D-glucan as the pachyman polysaccharide, and could adopt a triple-stranded helix conformation in non-denaturing conditions, as etc. curdlan, paramylon, and laminaran. However, it should also be noted that curdlan usually has high molecular weight and that the triple-stranded helix becomes a random coil under alkaline conditions, whereas it can form resilient gels upon heating its neutral aqueous solutions [10]. Laminaran and other linear β-(1 → 3)-D-glucans characterized by a DP < 200 cannot gel, although regularity of structures in the triple-stranded helix produces crystalline, insoluble material [11]. The solution behaviour of this class of polysaccharides differs substantially from that of the triple-stranded helix of schizophyllan [12], the double-stranded helix of xanthan [13], and the single helix *Auricularia auricula-judae* β-(1 → 3)-D-glucan (glucan A) [14]. In these latter systems, the values of M_w decrease in Me_2SO or cadoxen by a factor of ~ 3, 2, and 1, respectively.

It is likely that, to a large extent, the PC3 polysaccharide and its fractionated samples exist in aque-

Table 1
Comparison of molecular weight and intrinsic viscosities of unfraction and fractions for glucan PC3 in 20% cadoxen and in cadoxen

Sample	Solvent	$M_w \times 10^{-4}$	$\langle s^2\rangle^{1/2}$ (nm)	$[\eta]$ (cm^3 g^{-1})	k'	$\frac{M_w(\text{in 20\% cadoxen})}{M_w(\text{in cadoxen})}$	$\frac{[\eta](\text{in 20\% cadoxen})}{[\eta](\text{in cadoxen})}$
PC3	20% cadoxen	20.6	32.4	61.6	0.41	2.31	1.59
	cadoxen	8.93	26.7	38.7	0.44		
	0.5 M NaOH	14.3 [a]		41.7			
PC3-F4	20% cadoxen	51.8	55.0	103.2	0.45	3.50	1.98
	cadoxen	14.8	31.3	52.0	0.79		
PC3-F7	20% cadoxen	27.8	62.8			5.93	
	cadoxen	4.69	21.2	29.2	0.65		

[a] The value was measured by an LLS spectrometer (ALV/SD-150) at the Chinese University of Hong Kong.

ous solution as aggregates (even insoluble) and that the increasing cadoxen concentration leads to chain solubilization by destruction of the aggregating interactions.

Viscosity behaviour.—The intrinsic viscosities $[\eta]$ and Huggins constants k' of the glucans PC3, PC3-F4, and PC3-F7 are summarized in Table 1. The $[\eta]$ values of glucan PC3 and its fractions in 20% cadoxen are higher than those in cadoxen, thus resembling the molecular weight results. However, the ratios of the values of $[\eta]$ in 20% cadoxen to those of $[\eta]$ (in cadoxen) are much lower than those of the corresponding M_w. It can be explained that the disruption of the glucan aggregates in cadoxen leads to an extensive decrease in $[\eta]$, but the solvation in cadoxen causes the increase in chain dimension. For example, $[\eta]$ of amylose drops by a factor ~ 2 on passing from Me_2SO to water [15]. The paucity of data does not allow us to formulate any quantitative analysis within a theoretical framework of the dependence of the hydrodynamic properties on the molecular weight. However, the approximated value of this exponent of the Mark–Houwink equation can be obtained for the two fractions in cadoxen: the value of about 0.6 is smaller than that it would have been expected for an expanded worm-like chain, but is more conceivable for an expanded random coil.

These results again suggest an interpretation for the aggregation process which cannot imply an equilibrium between multiple-stranded helices of β-(1 → 3)-D-glucan and isolated single chains (expanded coils), as has been inferred for other glucans. For example, the changes of $[\eta]$ values of schizophyllan in water and in Me_2SO [12] were far more than those of M_w. It can be assumed that for glucan PC3

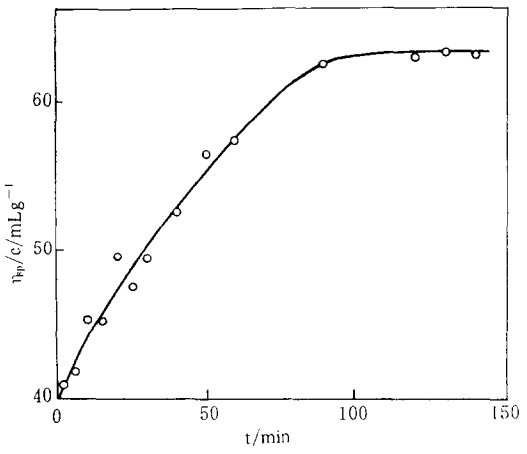

Fig. 3. Aggregation-time dependence of η_{sp}/c for glucan PC3 in 20% cadoxen at 25 °C.

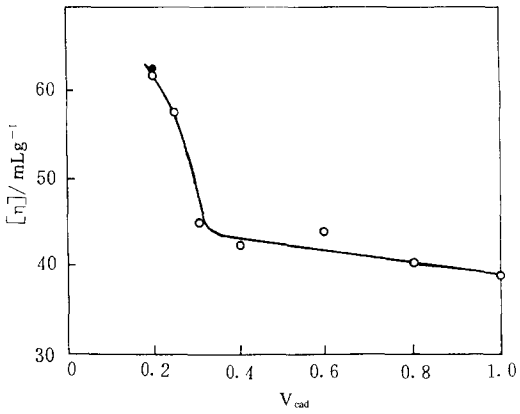

Fig. 4. Plot of $[\eta]$ against V_{cad} for glucan PC3 in water–cadoxen mixtures at 25 °C. See text for significance of solid circles.

because of the absence of cooperative intramolecular interactions (which sustain helix structure), only the intermolecular interactions are broken, resulting in the limited decrease of $[\eta]$. This provides further evidence for the absence of multiple-stranded helix chains in glucan PC3 20% cadoxen solution.

Aggregation process.—Based on the foregoing analysis of data (M_w, $[\eta]$) listed in Table 1, the chains of glucan PC3 in cadoxen can be considered as expanded flexible coils. In order to verify the reversibility of the aggregation process, the glucan PC3 was dissolved in cadoxen and then diluted by water at once to a final solvent composition of 20% cadoxen. Viscosity measurements were carried out as a function of the time and the dependence of η_{sp}/c values obtained from the glucan PC3 in water-diluted cadoxen (20%) is shown in Fig. 3. The η_{sp}/c values increase with increasing time; the leveling off of the viscosity values at time greater than about 100 min is believed to indicate a stable aggregation state.

Fig. 4 shows that the plot of $[\eta]$ for glucan PC3 in water–cadoxen mixtures against the volume fraction of cadoxen in water–cadoxen mixtures (V_{cad}). As V_{cad} increases from 0.2 to 0.3, the $[\eta]$ values decrease sharply, and then slowly decrease with continuous increase of cadoxen. This suggests that the aggregates break up to form single chains in a narrow range of solvent composition (V_{cad} between 0.2 to 0.3), while the slight decrease of $[\eta]$ above $V_{cad} = 0.3$ can be ascribed to a decrease in the chain stiffness and/or to further small changes resulted from solvation in cadoxen. The solid circle in Fig. 4 refers to the value of $[\eta]$ obtained for the glucan PC3 dissolved in cadoxen solution and then diluted to 20% cadoxen solution by water. This point agrees well with the curve, indicat-

ing that process of aggregation–disaggregation of glucan PC3 in water–cadoxen is reversible.

4. Conclusion

Glucan PC3, a major polysaccharide in *Poria cocos* sclerotium, forms aggregates in 20% cadoxen, and dissolves as a single-stranded chain in cadoxen. A stable aggregation state of the glucan PC3 in 20% cadoxen was achieved after 100 min. Aggregates can be dispersed in water–cadoxen at V_{cad} larger than 0.3. The amount and size of aggregates depend on the solvent system and mode of sample preparation. The M_w value of 8.93×10^4 obtained for glucan PC3 in cadoxen is close to the result estimated from glucan nitric ester in acetone by osmometry [8], but markedly lower than those obtained for the glucan in 2 g/dL NaOH by light scattering. The higher values reported in the literature are believed to result from an extensive aggregation that can be avoided only in such strong solvents as concentrated cadoxen.

Acknowledgements

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