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Shrinking factors of hyperbranched polysaccharide from fungus

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ABSTRACT

The branched structure properties of hyperbranched polysaccharides (TM3a and TM3b), extracted from sclerotia of *Pleurotus tuber-regium*, were studied by using laser light scattering and viscometry. The configurational shrinking factor (g) and viscometric shrinking factor (g') of TM3a and TM3b were discussed, where curdlan and pullulan were taken as the linear references for derivation of g and g'. The dependences of g factor, g' factor, and Flory factor (Φ_{branched}) on weight average molecular weight (M_w) were established to be $g = 1.07 \times 10^2\ M_w^{-0.48\pm0.09}$, $g' = 3.63 \times 10^1\ M_w^{-0.43\pm0.01}$, and $\Phi_{\text{branched}} = 7.08 \times 10^{20}\ M_w^{0.39\pm0.1}$ for TM3a in 0.25 M LiCl/DMSO at 25 °C, when curdlan acted as the linear reference. A power law relationship $g' = 2.71 \times 10^{-1}\ g^{-0.61\pm0.1}$ for TM3a was found, and the exponent was approximately same to 0.60 established by Kurata et al. for polystyrene star molecules. The dependence of g factor on M_w for TM3b was found to be $g = 1.99 \times 10^2\ M_w^{-0.53\pm0.02}$, when pullulan was used as the linear reference. On the basis of Zimm–Stockmayer equation for tetrafunctional units, molecular weight of branching unit (M_0) deduced from nonlinear curve fitting of g versus M_w was 8739 ± 564 g/mol and 3961 ± 1245 g/mol for TM3a and TM3b, respectively. The effect of different linear reference curves and polydispersity was discussed. This work gave valuable information on branched structure characterization and insights into the biosynthetic pathways of the hyperbranched polysaccharide from fungus.

1. Introduction

The study of the polymers with hyperbranched structure has become a topic of principal interest in recent activities of polymer research.[1–3] Highly branched macromolecules exhibit spherical architectures, which have attracted much attention from the viewpoint of materials.[4] The presence of a large number of end-groups at the periphery of the hyperbranched macromolecules reduces steric hindrance, resulting in high reactivity of functionalization.[5] Furthermore, the high branching density is an efficient measure to keep the energy, charge, or gene information to store in a rather compact, space-saving structure.[6–8] For example, glycogen and amylopectin are hyperbranched energy storage polysaccharides for most animals and higher plants, respectively. Glycogen has essentially the same chemical structure as amylopectin, and these biopolymers have been used as ideal models for the study of hyperbranching. Different methods have been described to study the branching characteristics of amylopectin and glycogen, such as enzymatic hydrolysis,[9] methodology,[10] asymmetrical flow field flow fractionation coupled with multi-angle laser light scattering,[11] size-exclusion chromatography combined with different detectors,[12,13] and molecular modeling,[14] in order to get the information on the biosynthetic pathways of hyperbranched biopolymers.

The values of radius of gyration ($\langle S^2\rangle_z^{1/2}$) and intrinsic viscosity ($[\eta]$) of branched polymers are smaller than those of linear ones with the same weight average molecular weight (M_w). An important way to characterize branching structures is via the configurational shrinking factor (g) and the viscometric shrinking factor (g'). Shrinking factors are defined as the ratio of the branched to the linear quantities at the same M_w[15]

$$g = \frac{\langle S^2\rangle_{z,\text{branched}}}{\langle S^2\rangle_{z,\text{linear}}} \tag{1}$$

$$g' = \frac{[\eta]_{\text{branched}}}{[\eta]_{\text{linear}}} \tag{2}$$

Zimm and Kilb found a simple relationship $g' = g^{1/2}$ for star-branched macromolecules,[16] Kurata et al. expanded this relationship to a power law $g' = g^{b_\eta}$, and estimated the exponent from literature data of star and comblike molecules to $b_\eta = 0.60$.[17] The value of b_η is sensitive to structure and chain conformation. Berry reported that it tended to a theoretical limit of 1.5 for combs having long backbones and short branches.[18] Furthermore, Zimm and Stockmayer have described the branching theory as[19]

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$$g = \left[\left(1+\frac{n}{7}\right)^{1/2} + \frac{4n}{9\pi}\right]^{-1/2} \quad \text{for trifunctional units} \tag{3}$$

$$g = \left[\left(1+\frac{n}{6}\right)^{1/2} + \frac{4n}{3\pi}\right]^{-1/2} \quad \text{for terafunctional units} \tag{4}$$

where $n(=M/M_0)$ is the number of branching points in the macromolecules, and M_0 is the molecular weight of branching unit. M_0 and number of monomer units between two branching point can be derived from nonlinear curve fitting of g versus M.[6]

Recently, the hyperbranched polysaccharides have been synthesized by acid-catalyzed ring-opening polymerization and cationic polycondensation.[20,21] At the same time, various highly branched polysaccharides have been extracted from natural resource, and exhibit significant bioactivities.[22,23] In our previous work, chemical structures and solution properties of the TM3a and TM3b hyperbranched polysaccharides, extracted from sclerotia of *Pleurotus tuber-regium*, have been studied in detail.[24–26] However, the characterization of the samples is not complete because we avoided the evaluation of the shrinking factors.

To study the shrinking factors of TM3a and TM3b, a linear polysaccharide reference curve must be needed. Curdlan is a linear polysaccharide consisting of (1→3)-β-glucose residues. Its conformation changes from triple helical structure to random coil with increasing alkali concentration from 0.19 to 0.24 M, or in different solvent.[27–29] It takes up a single flexible chain conformation in DMSO, but exists as a triple helix conformation in water.[30,31] This restricts the application as a reference standard for the study of water-soluble hyperbranched polysaccharide in an aqueous solution. Pullulan is a water-soluble linear (1→6)-α-glucan, and takes up a single flexible chain conformation in water or DMSO.[32] Nevertheless, the TM3a and TM3b hyperbranched polysaccharides are of β-glucans, and the glycosidic residues connect through different linkage types.[24,26] Rees and his coworkers simulated linear and branched pyranosic glucans, and delineated the conformations of glucan with various glycosidic linkages.[33–35] (1→3)-β-Glucan has the flexible and helical conformation; (1→4)-β-glucan has the extended and ribbon-like conformation; (1→2)-β-glucan has rigid and crumpled conformation. (1→6)-β- and (1→6)-α-glucans are quite different from glucans in the other linkages because they have many possible conformations. This is mainly due to the fact that residues connected through the (1→6) linkage are separated by three bonds rather than two, so that its freedom of rotation is much higher than that of other linkages.

In the present project, the shrinking factors of TM3a and TM3b were studied by using curdlan and pullulan as the linear reference curves. The shrinking factors as functions of M_w were evaluated, and the dependence between g' and g was checked. Moreover, we attempted to clarify the effect of the different linear reference curves and polydispersity on the shrinking factors, and attempted to study whether and how the Flory factor Φ_{branched} varied with g and M_w for the TM3a hyperbranched polysaccharide. This work provides valuable information to further understand branched structure properties of hyperbranched polysaccharide.

2. Experimental

2.1. Preparation of sample

The samples were the same as given in Refs. 24,26 The preparation of the samples was described there in detail. Curdlan (Wako Pure Chemical Industries. LTD. Japan) was fractionated by nonsolvent addition to give 3 fractions with different M_w, coded as CF1, CF2, and CF2.

2.2. Characterization

$[\eta]$ of TM3a, the curdlan fractions, and the pullulan standard samples (P100, P200, P400, P800, and P1600) in 0.25 M LiCl/DMSO were measured at 25 ± 0.1 °C by using an Ubbelohde capillary viscometer. The kinetic energy correction was assumed to be negligible. Huggins and Kraemer equations were used to estimate the $[\eta]$ value by extrapolating to an infinite dilution formulated as

$$\eta_{sp}/c = [\eta] + k'[\eta]^2 c \tag{5}$$

$$(\ln \eta_r/c = [\eta] - \beta[\eta]^2 c \tag{6}$$

Both k' and β are constants for a given polymer at a given temperature in a given solvent; η_{sp}/c is reduced specific viscosity; $(\ln \eta_r)/c$ is inherent viscosity.

M_w and $\langle S^2\rangle_z^{1/2}$ of the TM3a fractions, the curdlan fractions, and the pullulan standard samples in 0.25 M LiCl/DMSO were measured with a laser light scattering instrument equipped with a He–Ne laser (MALLS, λ = 633 nm, Wyatt Technology Co., Santa Barbara, CA, USA) at multiple angles (θ) in the range from 26° to 142° at 25 °C. The basic light scattering equation is as following:

$$\frac{Kc}{R_\theta} = \frac{1}{M_w}\left(1 + \frac{16\pi\langle S^2\rangle_z}{3\lambda^2}\cdot \sin^2\left(\frac{\theta}{2}\right)\right) + 2A_2c + \cdots \tag{7}$$

where K is an optical constant equal to $[4\pi^2 n^2(\mathrm{d}n/\mathrm{d}c)^2]/(\lambda^4 N_A)$; c, the polymer concentration in mg/mL; R_θ, the Rayleigh ratio; λ, the wavelength; n, the refractive index of the solvent; dn/dc, the refractive index increment; N_A, the Avogadro number; A_2, the second virial coefficient. The polysaccharide solutions with desired concentrations were prepared, and optical clarification of the solutions was achieved by filtration through a 0.2 μm pore size filter (PTFE, Puradisc 13-mm Syringe Filters, Whatman, England) into a scattering cell. The refractive index increment (dn/dc) was measured with a double-beam differential refractometer (DRM-1020, Otsuka Electronics Co. Japan) at the wavelength of 633 nm. The dn/dc values of TM3a, curdlan, and pullulan in 0.25 M LiCl/DMSO were 0.031 cm^3 g^{-1}, 0.058 cm^3 g^{-1}, and 0.055 cm^3 g^{-1}, respectively. Astra software V4.90.07 was utilized for data acquisition and analysis.

SEC-LLS measurements were carried out on size-exclusion chromatograph combined with multi-angle laser photometer (MALLS, λ = 633 nm; Wyatt Technology Co., Santa Barbara, CA, USA). A P100 pump (Thermo separation products, San Jose, USA) equipped with G4000HXL (MicroPak, TSK) column was used as SEC instrument. An Optilab refractometer (Wyatt Technology Co., Santa Barbara, CA, USA) was simultaneously connected. The solvent was purified by a 0.2 μm filter and degassed before use. The polysaccharide solution with desired concentration was prepared, and optical clarification of the solution was achieved by filtration through a 0.2 μm pore size filter (PTFE, Puradisc 13-mm Syringe Filters, Whatman, England) into a scattering cell. The injection volume was 200 μL, and the flow rate was 0.5 mL/min. The dn/dc value was determined to be 0.030 mL g^{-1} for TM3b in DMSO by using a double-beam differential refractometer at 633 nm.[24]

3. Results and discussion

3.1. Shrinking factor of TM3a

In this part, curdlan was used as a required linear reference to study the shrinking factors of TM3a. To get the information on M_w, $\langle S^2\rangle_z^{1/2}$, and $[\eta]$ of the single chains, the polysaccharide should not be aggregated in the solvent. Figure 1 shows the intrinsic viscosity results of the curdlan samples with different M_w. Good linear

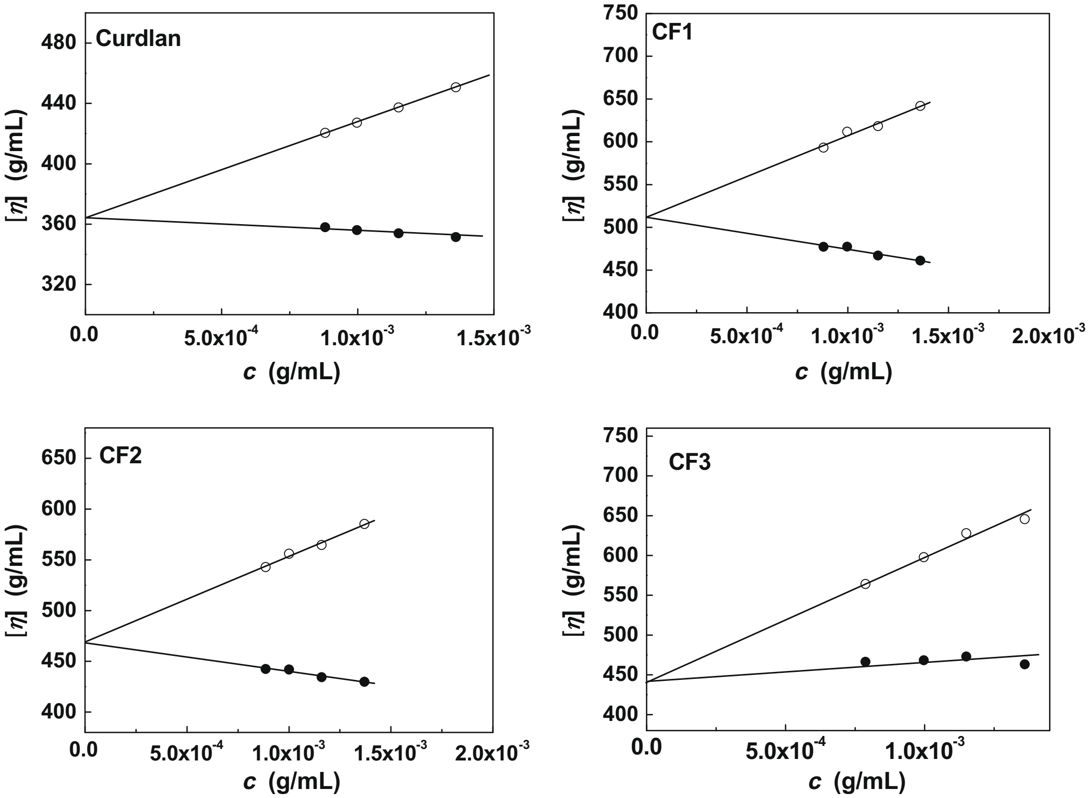

Figure 1. $\eta_{sp}/c - c$ and $(\ln \eta_r)/c - c$ relationships for curdlan fractions in 0.25 M LiCl/DMSO at 25 °C.

relationships of $\eta_{sp}/c - c$ and $(\ln \eta_r)/c - c$ for the curdlan fractions have been obtained. This indicates that curdlan is completely dissolved in 0.25 M LiCl/DMSO, and exhibits the normal solution behavior. Zimm plots for curdlan in 0.25 M LiCl/DMSO at 25 °C are shown in Figure 2. From the intercept of two straight lines and the slope of the angular dependence, the values of M_w and $\langle S^2\rangle_z^{1/2}$ are calculated, respectively. The angular dependences of $(Kc/R_\theta)_{c=0}$ for the curdlan fractions in 0.25 M LiCl/DMSO at 25 °C are shown in Figure 3. The good linear relationships further indicate that 0.25 M LiCl/DMSO is a good solvent for curdlan, and no existence of aggregates in the used solvent. The experimental results of the curdlan fractions in 0.25 M LiCl/DMSO are listed in Table 1. Figures 4 and 5 show the plots of $\langle S^2\rangle_z^{1/2}$, and $[\eta]$ versus M_w for the curdlan samples in 0.25 M LiCl /DMSO at 25 °C, in the M_w range from 1.34×0^6 to 2.14×10^6, respectively. The resulting relationships are expressed as

$$\langle S^2\rangle_z^{1/2} = 7.59 \times 10^{-3} M_w^{0.64\pm0.1} \tag{8}$$

$$[\eta] = 1.23 \times 10^{-2} M_w^{0.73\pm0.1} \tag{9}$$

The previous data of M_w, $\langle S^2\rangle_z^{1/2}$, and $[\eta]$ for the TM3a hyperbranched β-D-glucan in 0.25 M LiCl/DMSO are also compared in Figures 4 and 5, respectively. The relationships for TM3a are found as[26]

$$\langle S^2\rangle_z^{1/2} = 4.79 \times 10^{-2} M_w^{0.43\pm0.04} \tag{10}$$

$$[\eta] = 0.46 M_w^{0.30\pm0.01} \tag{11}$$

With these relationships established for the hyperbranched and the linear polysaccharides, we can obtain the values of the shrinking factors g, for the radius of gyration, and g' for the intrinsic viscosity. The g and g' values of TM3a are listed in Table 2. The corresponding

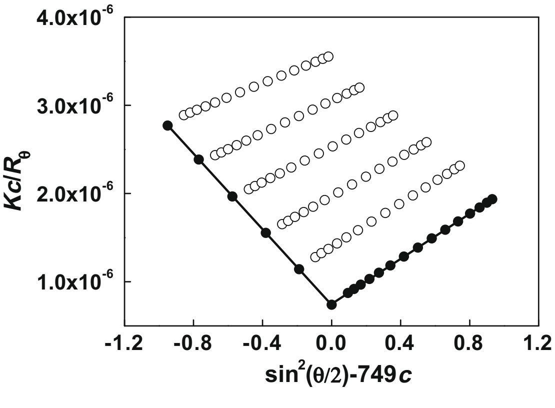

Figure 2. Zimm plots for curdlan in 0.25 M LiCl/DMSO at 25 °C.

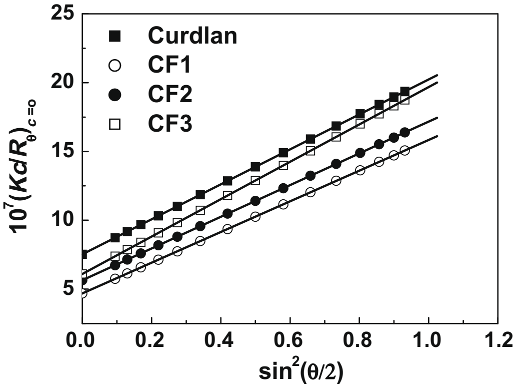

Figure 3. Angular dependences of $(Kc/R_\theta)_{c=0}^{1/2}$ for the curdlan fractions in 0.25 M LiCl/DMSO at 25 °C.

Table 1
The experimental results of M_w, $\langle S^2\rangle_z^{1/2}$, and $[\eta]$ for the curdlan and pullulan fractions in 0.25 M LiCl /DMSO at 25 °C

Sample	$M_w \times 10^{-6}$ (g/mol)	$\langle S^2\rangle_z^{1/2}$ (nm)	$\langle S^2\rangle_z^{1/2}$ [a] (nm)	$[\eta]$ (cm^3 g^{-1})
Curdlan	1.34	66.6		364.3
CF1	2.14	91.0		511.8
CF2	1.76	74.3		469.0
CF3	1.66	78.0		452.4
P1600	1.59	62.7	76.5	574.2
P800	0.95	46.1	56.2	396.7
P400	0.51	31.0	37.8	
P200	0.25	18.1	22.1	160.9
P100	0.10			81.1

[a] The $\langle S^2\rangle_z^{1/2}$values of pullulan after being multiplied by a factor of 1.22.

shrinking factors as functions of M_w are shown in Figures 6 and 7, respectively, and the relationships are represented as

$$g = 1.07 \times 10^2 M_w^{-0.48\pm0.09} \tag{12}$$

$$g' = 3.63 \times 10^1 M_w^{-0.43\pm0.01} \tag{13}$$

The configurational shrinking factor g, and the viscometric shrinking factor g' decrease with increasing M_w. This is because the values of $\langle S^2\rangle_z^{1/2}$ and $[\eta]$ for branched molecules vary less rapidly with the change of M_w than those of strictly linear molecules. A more precise estimation of the number of monomer units between two branching points can be made on the basis of modified Zimm–Stockmayer equation for the g factor, in terms of branching points.[19] For tetrafunctional units, it is given by

$$g = \left[\left(1 + \frac{M}{6M_0}\right)^{1/2} + \frac{4M}{3\pi M_0}\right]^{-1/2} \tag{14}$$

where M_0 is the molecular weight of one branching unit. In view of the result from gas chromatography–mass spectrometry (GC–MS), TM3a is constituted of terminal glucose, 1,3-linked glucose, 1,4-linked glucose, 1,6-linked glucose, 1,3,6-linked glucose, 1,2,3-linked glucose, 1,4,6-linked glucose, and 2,3,4,6-linked glucose.[26] So tetrafunctional units were chosen. A good fit can be obtained if the exponent of −0.5 is changed to a free fit parameter. The exponent and M_0 for TM3a are derived from nonlinear curve fitting of g versus M_w. An exponent of −0.47 ± 0.1 and M_0 = 8372 ± 1163 g/mol are obtained with r^2 of 0.582. Thus, the value of glucose units per branching unit, DP_0 (=M_0/162), can be calculated to 52. There are two models for estimation of chain length between two branching points (I_b). For the randomly branched materials, one has $I_{b,random}$ =2/3 DP_0, while for the hyperbranched model, one has $I_{b,hyper}$ = 1/2 DP_0. Therefore, the I_b value of the TM3a hyperbranched polysaccharide is 26. According to the cluster model structure of amylopectin proposed by French and Robin,[11] TM3a is constituted of short-chain and long-chain branching units. The chain length between two branching points of 26 anhydroglucose units (DP_0 = 52) for TM3a would be considered as effective long-chain branching, and corresponds to branching density of 3.85%.[12] In our previous work, the branching degree obtained from GC–MS is 65.5%.[26] The remaining 61.6% branching units for TM3a are attributed to short-chain branching.

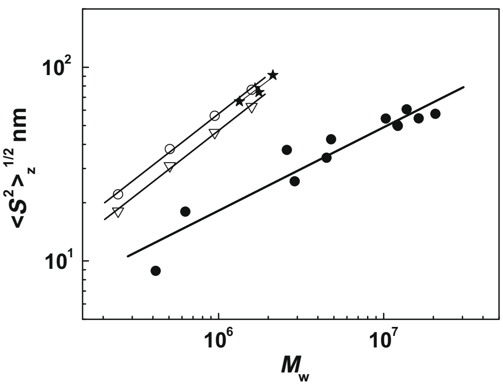

Figure 4. Plot of log $\langle S^2\rangle_z^{1/2}$ versus log M_w for curdlan (★) and pullulan (the native $\langle S^2\rangle_z^{1/2}$ data: △, and the derived $\langle S^2\rangle_z^{1/2}$ data: ○), in comparison with TM3a (●) in 0.25 M LiCl/DMSO at 25 °C.

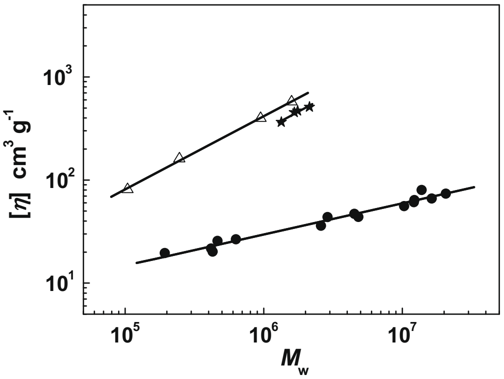

Figure 5. $[\eta]$ Dependences on the M_w for curdlan (★) and pullulan(△), in comparison with TM3a (●) in 0.25 M LiCl/DMSO at 25 °C.

3.2. Interrelation between g' and g for TM3a

The g' factor depends on the number of branching points per macromolecule, but the dependence is more involved than for the g factor because the intrinsic viscosity is in addition affected by hydrodynamic interactions. For this reason, it is of interest to establish a relationship between g and g'. Simple relationships $g' = g^{1/2}$ and $g' = g^{3/2}$ for star-branched macromolecules and combs have been found, respectively.[16,18] Subsequently, a power law $g' = g^{b_\eta}$ has been expanded, and the exponent is estimated as b_η = 0.60 from literature data of star and comblike molecules by Kurata and co-workers.[17] Similar behaviors have been obtained recently for endlinked three-arm polystyrene star molecules (b_η = 0.663),[36] and for the branched structure of commercial dextran (b_η = 0.71 ± 0.05).[12] Figure 8 shows the double logarithmic plot of g' against g for TM3a. The relationship is expressed by a power law as

$$g' = 2.71 \times 10^{-1} g^{0.61\pm0.1} \tag{15}$$

The relationship does not start at the coordinates (1, 1). It indicates that the branching process of biosynthesis is more complex than that derived from the idealized theory, which is based on a statistical process.[6] However, the exponent (b_η = 0.61) is similar to that of the star polymers estimated by Kurata.[17] This result further confirms that the TM3a polysaccharide is mainly constituted of short-chain branching structure similar to the star-branched polymers.

3.3. Flory factor $\Phi_{branched}$

The g' factor should contain the same information of the branching density, but it also depends on the Flory factor (Φ). Flory factor describes the relationships between the configurational dimensions of a polymer molecule in solution and the thermodynamic interaction between polymer segments and solvent molecules. It is defined by the relation[37]

$$[\eta] = \Phi \frac{(6\langle S^2\rangle_z)^{3/2}}{M_w} \tag{16}$$

Table 2
Some molecular parameters of the TM3a fractions in 0.25 M LiCl/DMSO at 25 °C

Sample	$M_w \times 10^{-6}$ (g/mol)	$\Phi \times 10^{-23}$	g[a] × 10^2	g[b] × 10^2	g'[a] × 10^2	g'[b] × 10^2
TM3a	4.81	1.87	8.77	11.9	4.71	3.44
F1	20.6	5.42	2.50	4.02	2.75	1.95
F2	16.3	4.53	3.03	4.66	2.93	2.15
F3	13.8	3.36	4.65	5.18	4.00	2.99
F4	12.2	4.30	3.65	5.60	3.48	2.44
F5	12.1	3.96	3.77	5.63	3.35	2.45
F6	10.3	2.45	5.39	6.23	3.45	2.56
F7	4.51	3.65	6.13	10.4	5.32	3.76
F8	2.89	5.01	6.19	13.6	6.82	4.55
F9	2.59	1.21	15.0	20.9	6.09	4.77
F10	0.63	1.95	21.2	31.9	12.6	8.72
F11	0.46	6.76	50.8	75.7	15.3	9.91
F12	0.42	—	8.82	13.2	13.9	10.4
F13	0.28	8.71	—	—	10.9	10.3
F14	0.19	0.05	426	657	19.1	14.3

[a] Data from curdlan as a reference.
[b] Data from pullulan as a reference.

With the exponent of $g' = 2.71 \times 10^{-1} g^{0.61\pm0.1}$, we obtained

$$g'(M) = \frac{\Phi_{\text{branched}}}{\Phi_{\text{linear}}} g(M)^{3/2} = 0.27 g(M)^{0.61} \tag{17}$$

$$\text{or } \Phi_{\text{branched}} = 0.27 \Phi_{\text{linear}} g(M)^{-0.89} \tag{18}$$

Both the Φ_{branched} and g factors are functions of the number of branching point. Thus, a rather defined correlation of the Flory factor Φ_{branched} with the shrinking parameter can be expected. Figure 9 shows the dependence of Φ_{branched} parameter on g for the TM3a fractions. The data follow a power law, and the line through the points corresponds to

$$\Phi_{\text{branched}} = 2.28 \times 10^{22} g^{-0.88\pm0.05} \tag{19}$$

The exponent value of −0.88 is in good agreement with that from calculation according to Eqs. 17 and 18.

As already mentioned, Flory factor describes the depth of solvent penetration into the polymer segments. This draining should be strongly reduced with increasing segment density as a result of branching, and finally should approach the limiting value of a sphere with impenetrable surface. Theoretically, for the unperturbed Gaussian chain, Φ tends asymptotically to the value 2.87×10^{23} mol^{-1} as M is increased.[38] From the data obtained from LLS and viscosity of monodisperse samples in a θ solvent, Fujita and co-workers have reported that the true value of Φ for an unperturbed linear polymer is about 2.55×10^{23} mol^{-1}.[39] M_w dependence of the Φ_{branched} parameter for TM3a is given in Figure 10, and the resulting relationship is established as

$$\Phi_{\text{branched}} = 7.08 \times 10^{20} M_w^{0.39\pm0.1} \tag{20}$$

The increase in Φ_{branched} is observed as the TM3a fractions grow in M_w, and the exponent of the power law relationship is 0.39. For the dextrans, the degraded starch, and the end-linked polystyrene stars, the exponents of 0.50, 0.31, and 0.28 have been found, respectively.[40,41] The Φ_{branched} parameters increases strongly with M_w for the TM3a hyperbranched polysaccharide, but no asymptotical value is observed in the M_w range.

3.4. Linear reference curves for pullulan

In this part, the shrinking factors of TM3a obtained by using linear reference curve for pullulan are discussed and compared with those for curdlan. The plots of $\langle S^2 \rangle_z^{1/2}$, and $[\eta]$ versus M_w for the pullulan samples in 0.25 M LiCl /DMSO at 25 °C are also shown in Figures 4 and 5, respectively. The resulting relationships are expressed as

$$\langle S^2 \rangle_z^{1/2} = 4.79 \times 10^{-3} M_w^{0.66\pm0.02} \tag{21}$$

$$[\eta] = 2.29 \times 10^{-2} M_w^{0.71\pm0.02} \tag{22}$$

With the relationships established for the hyperbranched TM3a polysaccharide and pullulan, we can also obtain the values of the shrinking factors g and g' (listed in Table 2). The corresponding

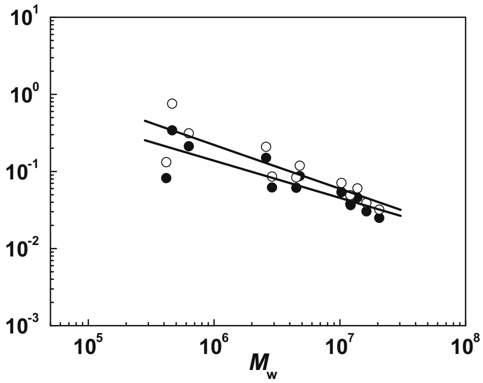

Figure 6. The configurational shrinking factor g as a function of M_w for TM3a using curdlan (●) and pullulan (○) as linear reference curves, respectively.

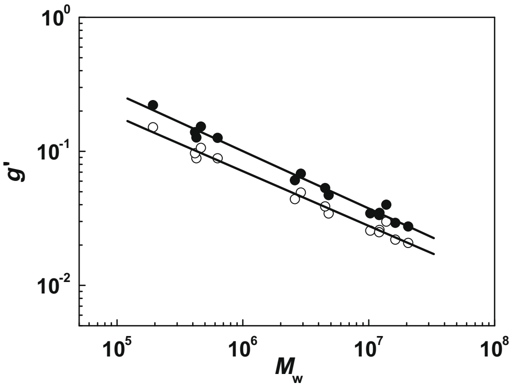

Figure 7. The viscometric shrinking factor g' as a function of M_w for TM3a using curdlan (●) and pullulan (○) as linear reference curves, respectively.

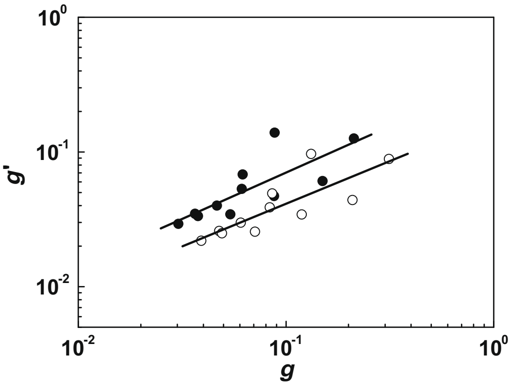

Figure 8. The viscometric shrinking factor g' as a function of the configurational shrinking factor g for TM3a using curdlan (●) and pullulan (○) as linear reference curves, respectively.

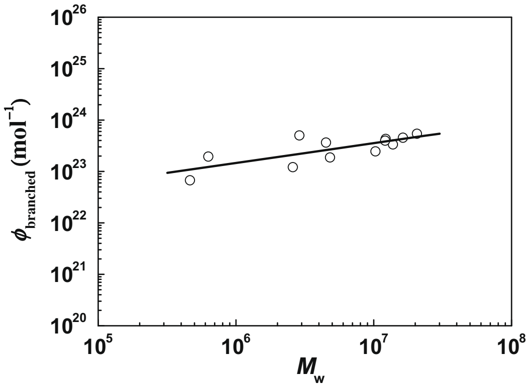

Figure 10. Flory factor Φ_{branched} dependences on the M_w for the TM3a hyperbranched polysaccharide.

shrinking factors as functions of M_w are also shown in Figures 6 and 7, respectively, and the relationships are represented as

$$g = 1.51 \times 10^3 M_w^{-0.63\pm0.07} \quad (23)$$

$$g' = 1.99 \times 10^1 M_w^{-0.41\pm0.01} \quad (24)$$

Based on the modified Zimm–Stockmayer equation, the exponent of -0.67 ± 0.1 and $M_0 = 26{,}086 \pm 12{,}066$ g/moL with r^2 of 0.895 is obtained from the best nonlinear fit when using pullulan as a linear reference curve. Figure 9 also shows the double logarithmic plot of g' against g for TM3a using pullulan as a linear reference. The relationship is expressed by a power law as

$$g' = 9.73 \times 10^{-1} g^{0.73\pm0.1} \quad (25)$$

The dependence of Φ_{branched} parameter on g for the TM3a fractions using pullulan as a reference is also shown in Figure 10. The data follow a power law, and the line through the points corresponds to

$$\Phi_{\text{branched}} = 3.14 \times 10^{22} g^{-0.84\pm0.05} \quad (26)$$

The difference of linear reference curves affects strongly the values of the configurational shrinking factor g, resulting in obvious difference of exponent derived from g versus M_w. Nevertheless, the values of the viscometric shrinking factor g' and the exponent deduced from g' versus M_w are almost not changed with the application of different linear reference curves. The α-configuration and (1→6)-glycosidic linkages contribute to reducing the steric hindrance to free rotation of the polymer configuration. Furthermore, pullulan is narrowly distributed, but the same could not be expected for the curdlan and TM3a polysaccharides even after fractionation by nonsolvent additional method. Polydispersity causes a larger increase in $\langle S^2\rangle_z^{1/2}$ than the corresponding increase in M_w. Thus, the $\langle S^2\rangle_z^{1/2}$ value of pullulan is smaller than that of curdlan with the same M_w. This results in the drastic effect on the configurational shrinking factor g. On the other hand, $[\eta]$ is a hydrodynamic property, which represents the swollen specific volume of a macromolecule because of solvent association.[42] The polydispersity effects on $[\eta]$ is generally small and negligible.[43] Therefore, the $\langle S^2\rangle_z^{1/2}$ values of the linear pullulan samples were multiplied by a factor of 1.22. This factor corresponds to the polydispersity of a Schulz–Flory distribution.[44] The plot of the derived values of $\langle S^2\rangle_z^{1/2}$ versus M_w for pullulan is also shown in Figure 4, and compared with the dimensions of curdlan. The native $\langle S^2\rangle_z^{1/2}$ value of pullulan is smaller than that of curdlan at the same M_w (shown in Fig. 4), whereas the opposite is seen for $[\eta]$ (shown in Fig. 5) when comparing at the same M_w. The difference in order of the properties between $[\eta]$ and $\langle S^2\rangle_z^{1/2}$ disappears after using the empirical approach. The resulting relationship for the derived $\langle S^2\rangle_z^{1/2}$ values of pullulan is expressed as

$$\langle S^2\rangle_z^{1/2} = 5.83 \times 10^{-3} M_w^{0.67\pm0.02} \quad (27)$$

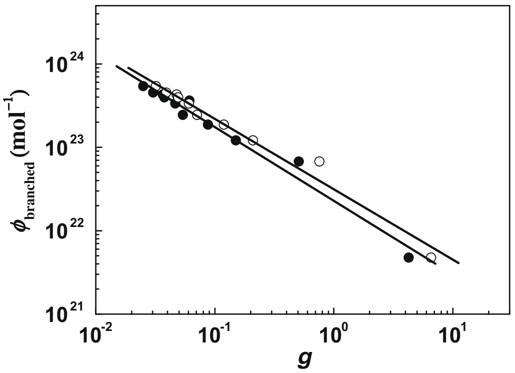

Figure 9. Dependence of Flory factor Φ_{branched} on g for TM3a using curdlan (●) and pullulan (○) as linear reference curves, respectively.

With the relationships established for the derived $\langle S^2\rangle_z^{1/2}$ values of pullulan, we recalculated the g shrinking factor for TM3a, and the corresponding relationships are represented as

$$g = 3.75 \times 10^2 M_w^{-0.59\pm0.09} \quad (28)$$

$$g' = 2.64 \times 10^{-1} g^{0.62\pm0.1} \quad (29)$$

The exponent value deduced from g' versus g is 0.62, which is in agreement with that (0.61) obtained from curdlan as a linear reference curve.

3.5. Effect of polydispersity for TM3a

As it is well known, the power law of $\langle S^2\rangle_z^{1/2} = f(M_w)$ can be estimated from many experimental points in the SEC-LLS chromatogram. The polydispersity in the various slices can be assumed to be very low (probably $(M_w/M_n)_j < 1.05$).[45] The SEC chromatograms as a function of elution volume and $(\langle S^2\rangle_z^{1/2})_j \sim f(M_{wj})$ power law of the TM3a fractions in 0.25 M LiCl/DMSO by SEC-LLS are shown in Figures 11 and 12, respectively. The straight line fitting the experimental points from SEC chromatogram is represented by

$$\langle S^2\rangle_z^{1/2} = k_\nu M_w^\nu \quad (30)$$

The exponent (ν) may provide additional insights into chain conformation. The experimental results are listed in Table 3. With the

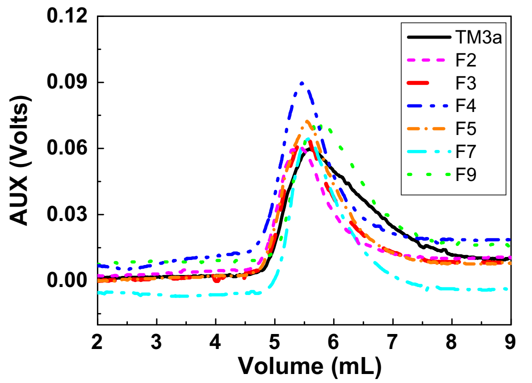

Figure 11. SEC chromatograms for the TM3a fractions in 0.25 M LiCl/DMSO at 25 °C detected by differential refractometer. The AUX values are signals from the refractive index detector.

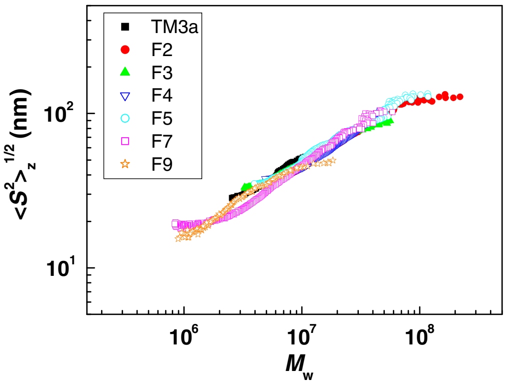

Figure 12. Plot of log $\langle S^2\rangle_z^{1/2}$ versus log M_w for the SEC fractionated TM3a samples in 0.25 M LiCl/DMSO at 25 °C.

relationships established for the SEC fractionated TM3a fractions and the native $\langle S^2\rangle_z^{1/2}$ data of pullulan, we can obtain the values of the shrinking factor g for the narrowly distributed TM3a fractions. The corresponding shrinking factor as functions of M_w is shown in Figure 13. Using all the TM3a data, an exponent of -0.46 ± 0.006 and $M_0 = 8739 \pm 564$ g/mol with r^2 of 0.885 is derived from nonlinear curve fitting of g_i versus M_{wi}. The results are in good agreement with those obtained for a series of nonfractionated samples by using curdlan as a linear reference curve.

In view of the results mentioned above, the consistency of polydispersity between the branched polysaccharide and the linear reference one is crucial to the derivation of the g value.

3.6. Shrinking factor of TM3b

TM3b, a water-soluble hyperbranched polysaccharide, was extracted from the sclerotia of an edible fungus (*P. tuber-regium*). In our previous work,[24,25] chemical structure, solution properties, and antitumor activities of TM3b have been studied by different methods. However, the characterization of the sample is not complete because we avoided the evaluation of shrinking factors. The dependence of g factor on M_w for TM3b is shown in Figure 14, and the relationship is represented as

$$g = 1.87 \times 10^2 M_w^{-0.55\pm0.01} \quad \text{(curdlan as linear reference curve)} \tag{31}$$

$$g = 1.99 \times 10^2 M_w^{-0.53\pm0.02} \quad \text{(pullulan as linear reference curve)} \tag{32}$$

The slopes of -0.55 and -0.53 are apart from the theoretical value of -0.5. Based on the modified Zimm–Stockmayer equation, the best nonlinear fit is obtained with the exponent of -0.62 ± 0.04 and $M_0 = 10{,}906 \pm 2350$ g/moL with r^2 of 0.874, resulting in $DP_0 = 67$, and $I_{b,\text{hyper}} = 34$ when using curdlan as a linear reference curve. The results suggest that 1 in every 34 glucose residues in the TM3b chain is branched. It corresponds to long-chain branching density of 2.94%. When using pullulan as a linear reference curve, the best nonlinear fit is obtained with the exponent of -0.51 ± 0.05 and $M_0 = 3961 \pm 1245$ g/mol with r^2 of 0.759, resulting in $DP_0 = 24$, and $I_{b,\text{hyper}} = 12$. It corresponds to long-chain branching density of 8.33%. These two results are not in agreement with each other. The SEC-fractionated TM3b fractions can be taken as narrowly distributed samples. In this case, the structure parameters of TM3b obtained from pullulan as a linear reference curve are more reasonable than those obtained from curdlan as a linear one.

Table 3
M_w, $\langle S^2\rangle_z^{1/2}$, polydispersity indices (M_w/M_n), and ν values determined by SEC-LLS, and exponent of the power law relation between g_i and M_{wi} for the TM3a fractions in 0.25 M LiCl /DMSO at 25 °C

Sample	$M_w \times 10^{-6}$ (g/mol)	M_w/M_n	ν	Exponent of the power law relation between g_i and M_{wi}
TM3a	6.13	2.9	0.45	−0.42
F2	25.2	1.6	0.43	−0.45
F3	15.4	1.8	0.47	−0.39
F4	12.8	2.2	0.40	−0.52
F5	12.2	1.7	0.44	−0.43
F7	4.65	2.4	0.45	−0.41
F9	2.65	2.7	0.45	−0.42

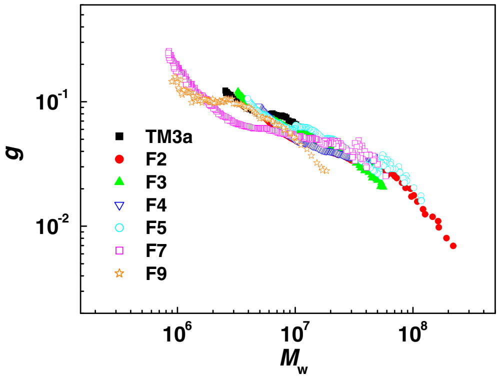

Figure 13. The configurational shrinking factor g as a function of M_w for the SEC fractionated TM3a samples in 0.25 M LiCl/DMSO at 25 °C using pullulan as linear reference curve.

4. Conclusion

The shrinking factors and Flory factor of the hyperbranched polysaccharides extracted from *P. tuber-regium* sclerotia were

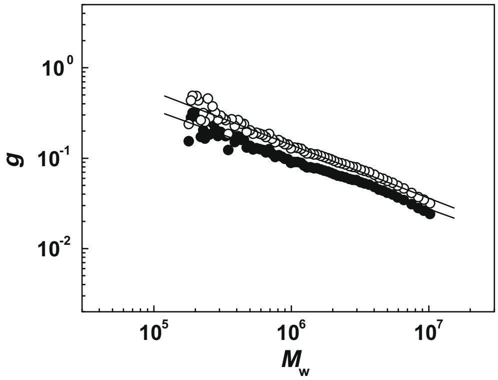

Figure 14. The configurational shrinking factor g as a function of M_w for TM3b using curdlan (●) and pullulan (○) as linear reference curves, respectively.

studied by using laser light scattering and viscometry. According to the current theory of polymer solution, the branched structure parameters and various power laws of the TM3a and TM3b polysaccharides were calculated and established from the data of M_w, $\langle S^2\rangle_z^{1/2}$, and $[\eta]$. The results indicated that the TM3a and TM3b polysaccharides were mainly constituted of short-chain branching structure. Configurational shrinking factor g was affected drastically with change of linear reference curve, whereas the viscometric shrinking factor g' hardly changed. The effect of the polydispersity on the g factor is more important than that of different linkage types between the branched polysaccharide and the linear one. Furthermore, a very strong dependence of Flory factor Φ_branched on the configurational shrinking factor g was found, and Φ_branched increased strongly with M_w for the TM3a hyperbranched polysaccharide.

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