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Microwave-assisted solubilization and solution properties of hyperbranched polysaccharide

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ABSTRACT

A water-insoluble hyperbranched β-D-glucan (TM3a), extracted from sclerotia of *Pleurotus tuber-regium*, was treated by microwave exposure to improve its solubility in water. This method led to complete dissolution of the TM3a polysaccharide in 0.02 wt % aqueous NaN_3. Various treatment periods were tested, and optimal conditions corresponded to 35 s at 765 W. The solution properties of TM3a in water were studied systematically by using size-exclusion chromatography combined with laser light scattering, viscometry, and dynamic light scattering at 25 °C. The dependences of intrinsic viscosity ($[\eta]$), radius of gyration ($\langle S^2\rangle_z^{1/2}$), and hydrodynamic radius (R_h) on weight average molecular weight (M_w) for TM3a in 0.02 wt % aqueous NaN_3 at 25 °C were found to be $[\eta] = 3.02 \times 10^{-2} M_w^{0.44\pm0.1} (cm^3 g^{-1})$, $\langle S^2\rangle_z^{1/2} = 8.32 \times 10^{-2} M_w^{0.38\pm0.2}$ (nm), and $R_h = 4.37 \times 10^{-2} M_w^{0.46\pm0.08}$ (nm) in the M_w range from 8.20×10^5 to 4.88×10^6. The fractal dimension, ratio of $\rho = \langle S^2\rangle_z^{1/2}/R_h$, and the $<r^2>_o/M_w$ value of TM3a were calculated and discussed. The results indicated that TM3a existed in a sphere-like conformation in 0.02 wt % aqueous NaN_3. Furthermore, by using transmission electron microscopy, we observed directly the spherical molecules of TM3a. This work gave valuable information on improvement of solubility and chain conformation characterization of the water-insoluble polysaccharide in water.

1. Introduction

Fungal polysaccharides exhibit significant antiviral, antitumor, and immune activities.[1–3] Recently, the potential commercial importance of polysaccharides has been focused on fungi as a functional food and as a source for the development of drugs.[4–6] It is noteworthy that water-insoluble polysaccharides are the main products in fungi extractive. However, the poor solubility of the polysaccharides in water hinders their potential clinical applications since intravenous administration of the micro-particulate form of insoluble glucans have been associated with a number of undesirable side effects including hepatosplenomegaly, microembolization, and enhanced endotoxin sensitivity.[7]

As it is known, the multiplicity of inter-chain hydrogen bonds is the origin of the aggregation and insolubility of polysaccharides in aqueous solutions. A number of approaches have been adopted to reduce this effect. A common technique involves the use of solvent such as alkali or LiCl/Me_2SO to break the intermolecular hydrogen bonds of the polysaccharides, resulting in dissolution.[8,9] However, such solvent systems are not applicable in bioactivity assays. Additionally, chemical modifications have been used to improve the solubility of polysaccharides. It is known that native water-insoluble polysaccharides are readily solubilized by sulfation,[10] carboxymethylation,[11] hydroxyethylation, hydroxypropylation, and methylation.[12]

Recently, 'physical' methods have been developed to disperse the supramolecular aggregates by increasing the energy of the polymer chains, and homogeneous aqueous solutions of polysaccharides have been prepared successfully. Such techniques include sonication,[13] γ-irradiation,[14] autoclaving,[15] pressure/heating,[16,17] and microwave.[18–20] The technique of the microwave oven heating has advantages including savings in time and energy, and ease of use. The heating of samples in a microwave oven is caused by interaction of an electromagnetic field with the chemical constituents of solutions. These interactions immediately generate heat because of molecular friction and excitation.[21] This approach was employed originally to produce starch solutions. Delgado et al. have used microwave energy to solubilize maize starch in water for subsequent size-exclusion chromatography (SEC) analysis.[22] Since antitumor and immunomodulatory activities are related to the molecular weight and chain conformation of polysaccharides,[23] it is essential to understand their solution properties for further research related to biochemistry or medical application.

The sclerotia of *Pleurotus tuber-regium* are edible and extremely rich in non-starch polysaccharides that are mainly composed of β-glucan.[24] These sclerotia are used as folk medicine to treat

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asthma, stroke, and breast cancer as well as to promote the development of fetus.[25,26] In our previous work,[9,27,28] two kinds of hyperbranched polysaccharides (TM3a and TM3b) were extracted from the sclerotia of *P. tuber-regium* by hot water. TM3b is water-soluble, and shows potent antitumor activities in vivo and in vitro. TM3a is a water-insoluble polysaccharide, the yield of which (7.8%) is higher than that of TM3b (1.3%). In the present project, the water-insoluble TM3a hyperbranched polysaccharide was treated by microwave heating for solubilization. The effect of microwave exposure time upon the molecular weight and molecular weight distributions was determined in order to obtain optimal settings. Furthermore, we attempted to study the solution properties of TM3a in aqueous solution using SEC combined with laser light scattering (SEC-LLS), dynamic light scattering (DLS), and viscometry. With applying the theory of polymer solution, we analyzed the molecular parameters of the hyperbranched polysaccharide in aqueous solution. Transmission electron microscopy (TEM) was used to observe the shape of the polysaccharide molecules. This work provides valuable information to further understand solubilization and chain conformation properties of water-insoluble biopolymers.

2. Experimental

2.1. Preparation of samples

Dried sclerotia powder of *P. tuber-regium* was defatted sequentially by using the Soxhlet extraction method with EtOAc and Me_2CO as solvents over 6 h, respectively. The residue was immersed stepwise in 0.15 M aq NaCl at 20, 80, and 120 °C. In each step, the mixture was stirred overnight, and then centrifuged at the speed of 9045 g for 20 min. The supernatants were labeled as TM1, TM2, and TM3, respectively. TM3 supernatant formed two phases during the cooling process to room temperature. The centrifugation gave two types of crude polysaccharide fractions, namely TM3a (water-insoluble) and TM3b (water-soluble). TM3a was then dissolved in 0.25 M LiCl/Me_2SO, and was fractionated by non-solvent addition to give white powders with different average molecular weights.[9]

2.2. Microwave heating

The solubilization procedure involved addition of water (15 mL), containing 0.02% sodium azide as bactericide, to a dried and dispersed TM3a sample (30 mg), which had been weighted into the home-made glass bottle (total volume, 60 mL). This assembly was centered inside a 850 W microwave oven VIP273F (Whirlpool, China). The sample was heated for 35 s, 45 s, 60 s, 75 s, and 90 s at 765 W, respectively. The treated solns were filtered through a 0.2 μm pore size filter (PTFE, Puradisc 13-mm Syringe Filters, Whatman, England), and then were analyzed using several techniques as follows.

2.3. Characterization

^{13}C NMR spectra of TM3a were recorded on a Mercury 600 NMR spectrometer (Varian Inc., USA) at 20 °C. TM3a was dissolved in D_2O by microwave heating for 35 s and in Me_2SO, respectively, to obtain a concentration of 30 mg/mL.

The optical absorption (Abs) of the treated solns was measured by using an ultraviolet–visible spectrophotometer (UV-160, Shimadzu, Japan) at 200 nm.

Intrinsic viscosity ($[\eta]$) of the TM3a polysaccharide in 0.02 wt % aq NaN_3 was measured at 25 ± 0.1 °C by using an Ubbelohde capillary viscometer. The kinetic energy correction was always negligible. Huggins equation was used to estimate the $[\eta]$ value by extrapolation to infinite dilution as follows

$$\eta_{sp}/c = [\eta] + k'[\eta]^2 c \tag{1}$$

where k' is constant for a given polymer at a given temperature in a given solvent, and η_{sp}/c is the reduced specific viscosity.

SEC-LLS measurements were carried out on size-exclusion chromatograph combined with multi-angle laser photometer (MALLS, λ = 633 nm; DAWN®DSP, Wyatt Technology Co., Santa Barbara, CA, USA). A P100 pump (Thermo separation products, San Jose, USA) equipped with G4000PWXL (MicroPak, TSK) and G6000PWXL (MicroPak, TSK) columns was used as SEC instrument. An Optilab refractometer (Dawn-DSP, Wyatt Technology Co., Santa Barbara, CA, USA) was simultaneously connected. The solvent, 0.02 wt % aq NaN_3, was purified by a 0.2 μm filter and degassed before use. The polysaccharide solns with desired concentrations were prepared, and optical clarification of the solns 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 vol was 200 μL, and the flow rate was 1.0 mL/min. The refractive index increment (dn/dc) value of the TM3a sample in 0.02 wt % aq NaN_3 was determined by using an Optilab refractometer (Dawn-DSP, Wyatt Technology Co., Santa Barbara, CA, USA) at 633 nm and 25 °C to be 0.133 mL g^{-1}.

The basic light scattering equation is as follows

$$\frac{Kc}{R_\theta} = \frac{1}{M_w}\left(1 + \frac{16\pi^2 < S^2 >_z}{3\lambda^2} \cdot \sin^2\left(\frac{\theta}{2}\right)\right) + 2A_2c + \ldots \tag{2}$$

where K is an optical constant equal to $[4\pi^2 n^2 (dn/dc)^2]/(\lambda^4 N_A)$; 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. As the column separates the polymer according to molecular weight, each fraction is led to the light scattering detector for instantaneous measurement of the scattering intensities. The refractive index detector connected in series gives the estimate of the polymer concentration. In chromatography mode, we have a single and sufficiently low concentration at a particular slice because of the further dilution by the SEC column of the already dilute injected soln. Then with the pre-input data of dn/dc, a Zimm plot, which is for one concentration only, is prepared for each fraction. Molecular weight and radius of gyration for each fraction are obtained from the Zimm plot. Furthermore, since chromatographic concentrations are typically very small, the effect of A_2 is usually negligible, and it can be set to zero in the Peak information dialog. ASTRA software V4.90.07 was utilized for data acquisition and analysis by following

$$\text{Number-average molecular weight: } M_n = \frac{\sum c_i}{\sum \frac{c_i}{M_i}} \tag{3}$$

$$\text{Weight-average molecular weight: } M_w = \frac{\sum (c_i M_i)}{\sum c_i} \tag{4}$$

$$z\text{-Average molecular weight: } M_z = \frac{\sum (c_i M_i^2)}{\sum (c_i M_i)} \tag{5}$$

The quantities c_i and M_i in the above equations are the mass concentration and molecular weight of the ith fraction, respectively.

DLS measurements were carried out using an ALV photogoniometer (ALV, Langen, Germany) equipped with an ALV 5003 correlator. The solns of the TM3a polysaccharide fractions in 0.02 wt % aq NaN_3 were prepared at a concentration of 0.1 mg/mL, and all the measurements were carried out at 25 °C.

Molecular morphology of the TM3a specimen was performed on a high-resolution transmission electron microscope (JEM-2010,

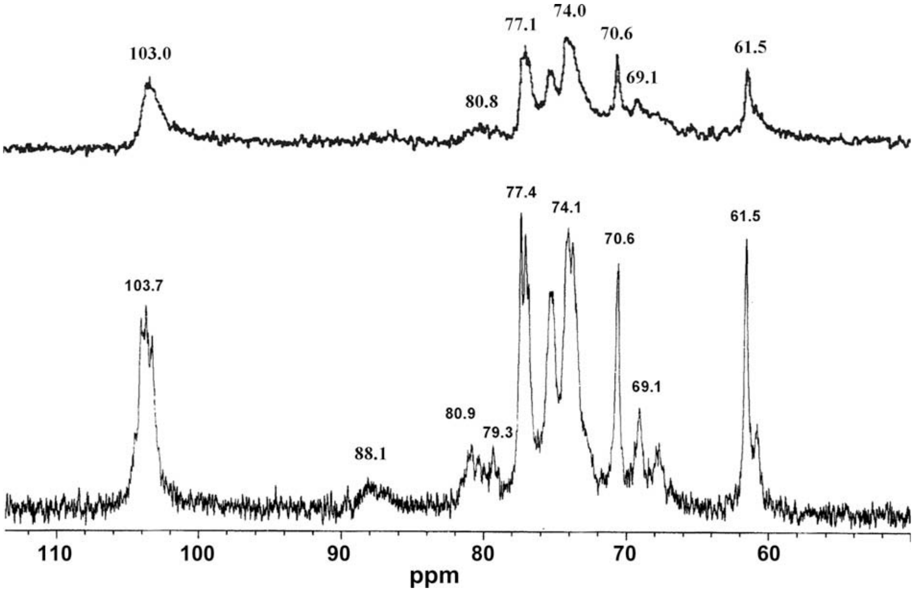

Figure 1. ^{13}C NMR spectra of TM3a in Me$_2$SO-d_6 (bottom) and in D$_2$O after microwave heating for 35 (top).

JEOL, Tokyo, Japan). The objective lens raster was added to enhance the contrast, and the images were recorded by CCD digital camera. The sample was prepared by dissolving 30 mg of TM3a in 15 mL of 0.02 wt % aq NaN$_3$ by microwave heating for 35 s. After purification through a 0.2 μm filter, a droplet of the sample soln was deposited on a holy carbon film, which was supported by a copper grid. A thin layer was suspended over the holes of the grid. The specimen was finally dried in air at ambient temperature and pressure, and then TEM images were taken at an accelerating voltage of 200 kV.

3. Results and discussion

3.1. Chemical structure

^{13}C NMR spectra of the TM3a polysaccharide before and after microwave heating are shown in Figure 1. For the TM3a polysaccharide after microwave heating, the major signals were found at 103.0 ppm for C-1, 77.1 ppm for C-3 and C-5, 74.0 ppm for C-2, 70.6 ppm for C-4, and 61.5 ppm for C-6, similar to those for methyl β-D-glucopyranoside.[29] Thus, these peaks should be assigned to the carbons of the non-reducing β-D-glucopyranosyl terminals of TM3a. Similar peak positions are found for TM3a in Me$_2$SO-d_6.[9] This result indicates that TM3a is still a kind of polysaccharides with numerous terminal units after microwave exposure, and allows to characterize it as a hyperbranched polysaccharide as reported.[30] Furthermore, the signals at 80.8 and 69.1 ppm represent internal residues substituted at C-2 and C-6 positions, respectively.

3.2. Microwave heating efficiency

SEC has become a mature and well-accepted technique for characterization of synthetic polymers and biopolymers.[31] The technique of SEC-LLS, as an absolute method, is used to determine weight-average molecular weight (M_w), number-average molecular weight (M_n), z-average molecular weight (M_z), radius of gyration ($\langle S^2 \rangle_z^{1/2}$), and polydispersity ($M_w/M_n$) of polymers. The effect of microwave exposure time upon the molecular weight and molecular weight distributions was determined in order to ascertain an appropriate treatment for subsequent assays. The TM3a polysaccharide was heated for 35 s, 45 s, 60 s, 75 s, and 90 s, respectively. Figure 2 shows the molecular weight distributions of the TM3a polysaccharide treated with different microwave times. All the samples treated by selected microwave exposure time show more or less the same shape. Figure 3 presents the changes in M_w and M_n as a function of microwave exposure times. The values of M_w and M_n were found to shift to lower values when the heating periods were increased during the dissolution process. The results are listed in Table 1. The reduction in molecular weight is rapid for short microwave time periods. The magnitude of molecular mass reduction agrees well with that for autoclaving studies of oat β-glucan,[15] guar,[32] and konjac glucomannan.[33] The kinetics of molecular weight reduction is consistent with those observed for microwave of other systems, such as starches in water.[18,34] The TM3a polysaccharide solution heated for 35 s has a higher M_w than that heated for 90 s. This observed behavior is interpreted as a result of two effects. The first one is that the aggregates are broken up on heating. The second effect is degradation of the polymer (breaking of covalent bonds) in the harsh dissolution process. It is believed that the disaggregation is accompanied by degradation of the TM3a polysaccharide, such as hydrolysis of the side chains and backbones. Therefore, a heating time of 35 s

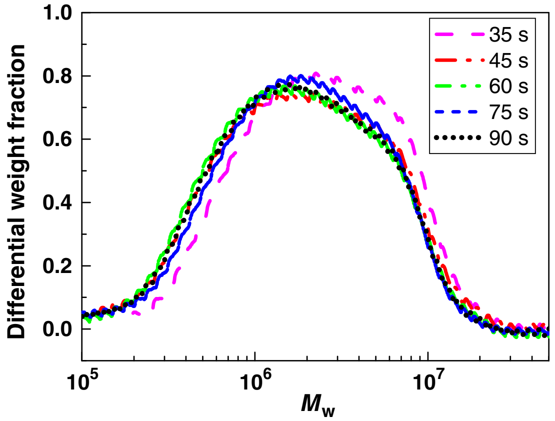

Figure 2. Evolution of the molecular weight distribution of the TM3a polysaccharide dissolved by microwave heating for different times.

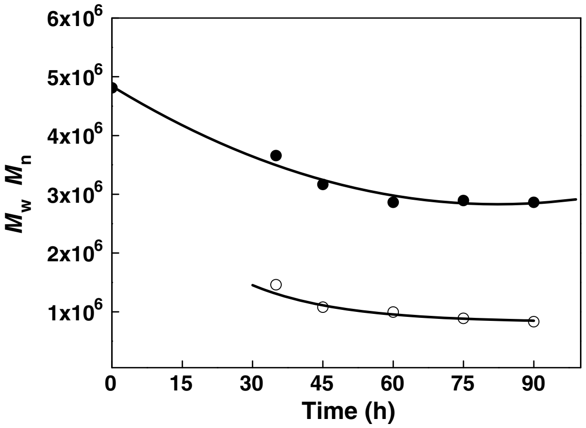

Figure 3. M_w (●) and M_n (○) of the TM3a polysaccharide dissolved by microwave heating for different times.

Table 1

M_w, $\langle S^2\rangle_z^{1/2}$, and M_w/M_n of the TM3a polysaccharide dissolved by microwave heating for different times

Sample	Treatment time (s)	$M_w \times 10^{-6}$	$\langle S^2\rangle_z^{1/2}$ (nm)	M_w/M_n
TM3a	0	4.81	42.5	–
	35	3.66	40.9	2.5
	45	3.17	40.1	2.9
	60	2.86	38.9	2.9
	75	2.89	40. 5	3.2
	90	2.86	35.8	3.5

was used as the reference heating time to dissolve the samples in all experiments discussed later.

3.3. Effect of storage time after microwave heating

The stability of solutions obtained by microwave heating was tested using UV after storing them for different times. Figure 4 shows the storage time dependence of the UV absorption value of the TM3a polysaccharide solutions, which were prepared by microwave heating for different time. No significant differences were found for the UV absorption of the TM3a polysaccharide solutions during the storage time. It demonstrates high stability of the TM3a solution for at least 95 h. Autoclave heating method was applied to dissolve the TM3a water-insoluble hyperbranched polysaccharide in order to compare with the microwave heating method. Different exposure periods, for example 10, 20, 30, 45, 60, and 90 min, were used, respectively. TM3a can be dissolved in water to form a transparent solution by autoclave heating for more than 20 min, but the transparent solution became gradually turbid during the cooling process to room temperature. However, TM3a can be dissolved in water to form a transparent solution by microwave heating for more than 35 s, and the solution can hold transparent for at least 95 h at room temperature. The differences between the microwave heating and classical conductive heating methods are as follows. Microwave heating is caused by the disruption of weak hydrogen bonds resulting from dipole rotation of free water molecules and by electrophoretic migration of dissolved ions in an electrical field of rapidly changing polarity. As microwaves can transfer energy throughout the volume of the material, the potential exists to reduce processing time and enhance overall quality.[35] Microwave utilizes three ways of heat transfer within the sample: irradiation, conduction, and convection. As a result, with microwave oven, heat is produced more quickly within the material as well as from the outside. With classical conductive heating methods, heat transfer can occur through conduction and convection only.[36] Microwave heating has many advantages over conventional heating including even more distribution of heat and better control over the heating process. Sources of microwave radiation allow high temperatures and high rates of heating to be obtained and show excellent efficiencies both for conversion of electrical energy into heat (80–85%) and for heat transfer to the load.[37] In comparison to conventional heating processes, microwave heating has inherent advantages in that it can be selective, direct, and efficient. Therefore, microwave irradiation to efficiently distribute thermal energy is becoming a standard technique in various fields of chemistry.

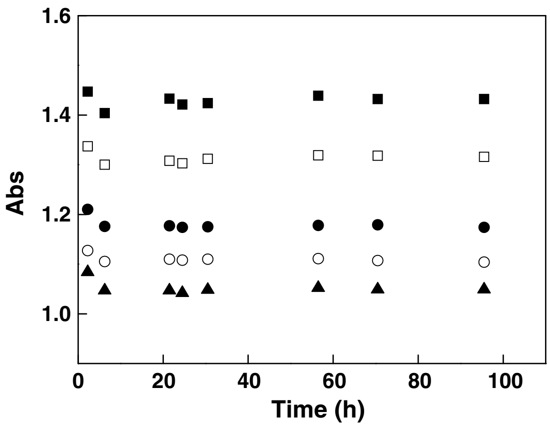

Figure 4. Storage time dependence of the UV absorption value of the TM3a polysaccharide in 0.02 wt % aqueous NaN_3 dissolved by microwave heating for 35 s and c=2.0521 × 10^{-3} (■), 45 s and c = 2.0264 × 10^{-3} (□), 60 s and c = 2.0910 × 10^{-3} (●), 75 s and c = 2.0302 × 10^{-3} (○), and 90 s and c = 2.0258 × 10^{-3} (▲), respectively.

3.4. Fractal dimension

The molecular weight distributions of the TM3a polysaccharide fractions with different M_w treated for 35 s are shown in Figure 5. A broad polydispersity is evident. The values of the polydispersity index lie in the range from 1.5 to 2.5. The M_w values of the TM3a fractions dissolved in 0.02 wt % aqueous NaN_3 by microwave heating for 35 s are listed and compared with the previous results of the TM3a fractions dissolved in 0.25 M LiCl/Me_2SO in Table 2. The latter solvent system is known to break intermolecular hydrogen bonds of polysaccharides, leading to dissolution of the aggregates by the way of single chains. This suggests that the M_w values in 0.25 M LiCl/Me_2SO correspond to individual molecules

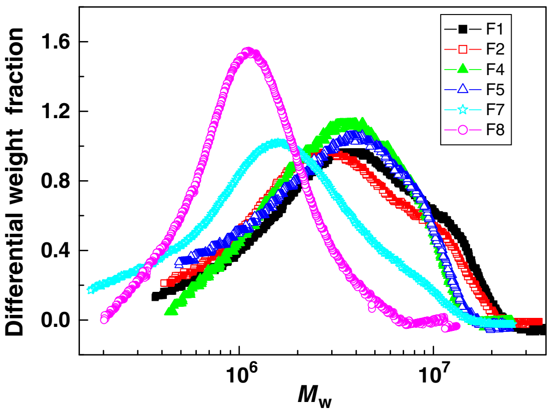

Figure 5. Evolution of the molecular weight distribution of the TM3a fractions with different M_w dissolved by microwave heating for 35 s.

Table 2
Summary of SEC-LLS, DLS, and intrinsic viscosity results of the TM3a fractions with different M_w dissolved by microwave heating for 35 s

Sample	$M_w \times 10^{-6}$		M_z[a] $\times 10^{-6}$	$\langle S^2\rangle_z^{1/2}$[a] (nm)	M_w/M_n[a]	$[\eta]$[a] (cm^3g^1)	R_h[a] (nm)	$\frac{\langle S^2\rangle_z^{1/2}}{R_h}$[a]
	Microwave exposure[a]	Native[b]						
TM3a	3.66	4.81	7.48	40.9	2.5	19.4	59.6	0.69
F1	4.88	20.6	8.33	30.2	2.2	35.0	43.3	0.70
F2	4.23	16.3	7.49	28.7	2.1	29.3	46.3	0.62
F4	3.95	12.2	5.90	15.5	1.7	19.8	46.6	0.33
F5	3.93	12.1	6.11	19.0	1.9	19.6	45.1	0.42
F7	2.27	4.51	4.40	24.2	2.3	14.6	46.0	0.53
F8	1.36	2.89	1.98	10.9	1.5	12.5	24.3	0.45
F9	0.82	2.59	2.70	17.1	2.5	14.4	—	—

[a] The results of the TM3a polysaccharide fractions dissolved by microwave heating.

[b] Previous results quoted for comparison.[9]

of TM3a. The results in Table 2 indicate that M_w values of TM3a fractions heated in microwave oven were lower than those of the native ones due to the degradation of the polysaccharides during the microwave heating process. According to Galema,[38] microwave heating results in polarization of polar bonds (such as the C–O–C glycosidic linkages), increasing molecules reactivity. Therefore, microwave heating may cause hydrolytic cleavage of polysaccharide chains as well as breaking intermolecular hydrogen bonds.

Relevant structural informations and further insight into the nature of the hyperbranched polysaccharide can be obtained by investigating the fractal dimension. For a rigid rod, the value of d_f is 1, and linear polymers with Gaussian coil nature has d_f value ranging from 5/3 to 2. A three-dimensional object with a homogeneous density has a mass fractal dimension of 3. The fractal dimension (d_f) of monodisperse polymers can be extracted directly from the angular dependence of the scattered light or neutron intensity.[39,40] However, to use light scattering for polysaccharides or other water-soluble polymers is not an ideal approach since the $q\langle S^2\rangle_z^{1/2}$ values of these macromolecules are generally not high enough. This approach has been successfully applied to highly branched polysaccharides with high molecular size such as amylopectin,[41,42] and other synthetic branched polymers.[43] Alternatively, the fractal dimension can also be determined from the M_w dependence of $\langle S^2\rangle_z^{1/2}$, and is defined as the inverse of the exponent ν[41]

$$\langle S^2\rangle_z^{1/2} \sim M^{\nu} \tag{6}$$

$$d_f = 1/\nu \tag{7}$$

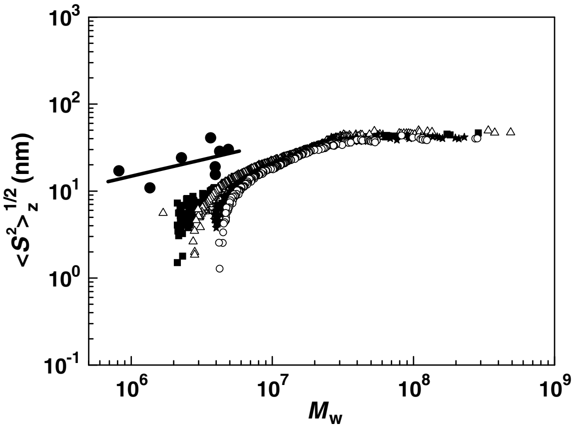

Figure 6. Plot of log $\langle S^2\rangle_z^{1/2}$ versus log M_w for TM3a in 0.02 wt % aqueous NaN_3 at 25 °C a series of non-fractionated samples (●); SEC fractionated samples with $M_w = 4.88 \times 10^6$ (■), with $M_w = 4.23 \times 10^6$ (△), with $M_w = 3.95 \times 10^6$ (*), and with $M_w = 2.27 \times 10^6$ (○).

Figure 6 shows the plot of $\langle S^2\rangle_z^{1/2}$ versus M_w of the TM3a polysaccharide fractions dissolved in 0.02 wt % aqueous NaN_3 by microwave heating for 35 s. The resulting relation is expressed as

$$\langle S^2\rangle_z^{1/2} = 8.32 \times 10^{-2} M_w^{0.38\pm0.2}\ (\text{nm}) \tag{8}$$

The d_f value was calculated to be 2.63 for TM3a according to Eqs. 7 and 8. The value of 2.63 is characteristic of a particle having an internal structure between the hard sphere ($d_f = 3.0$) and the fully swollen branched macromolecule in a thermodynamically good solvent ($d_f = 2.0$).[41] A fractal dimension of $d_f = 2.5$ is predicted for branched clusters, which are not swollen, either for thermodynamic reasons or because of steric hindrances.[44] Scherrenberg et al. have found that the value of d_f for some dendrimers is 3, suggesting a uniform distribution of segments in the space pervaded by the polymer.[45] The fractal dimension is a measure of the compactness of a polymer chain, the larger the fractal dimension, the more compact the structure. The result confirms that the TM3a polysaccharide exists as a compact chain conformation of sphere-like structure in aqueous solution.

The fractal dimension determined from Eqs. 6 and 7 is the average fractal dimension, not in all cases the true fractal dimension of the individual macromolecules. For a broad polydispersity sample, we have to take into account the effect of the polydispersity, which yields an effective fractal dimension ($d_{f,e}$).[40] 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 $(\langle S^2\rangle_z^{1/2})_j$ versus M_{wj} curve often gives s straight line in the double logarithmic plot and can be represented by $(\langle S^2\rangle_z^{1/2})_j = KM_{wj}^{\nu}$ or $M_{wj} = K'(\langle S^2\rangle_z^{1/2})_j^{d_{f,e}}$. The polydispersity in the various slices can be assumed to be very low (probably $(M_w/M_n)_j < 1.05$).[46] For such small polydispersity, the influence of the ensemble averaging is negligible. Figure 6 also shows the $(\langle S^2\rangle_z^{1/2})_j \sim f(M_{wj})$ power law for the TM3a polysaccharide fractions dissolved by microwave heating for 35 s. The straight line fitting all the experimental points from SEC chromatogram is represented by

$$(\langle S^2\rangle_z^{1/2})_j = 7.15 \times 10^{-4} M_{wj}^{0.63\pm0.08}\ (\text{nm}) \tag{9}$$

The $d_{f,e}$ value was calculated to be 1.57 for TM3a according to Eqs. 7 and 9. When comparing this effective fractal dimension ($d_{f,e}$) with the real fractal dimension (d_f), which is obtained with a series of non-fractionated samples, we can obtain the value of the exponent in the molar mass distribution (τ) according to the relationship[47]

$$d_{f,e} = d_f(3 - \tau) \tag{10}$$

The τ value was calculated to be 2.4 for TM3a according to Eq. 10. The value of τ has been predicted to be equal to 2.5 by Flory and Stockmayer,[48,49] and to be 2.2 by three-dimensional percolation theory.[50,51]

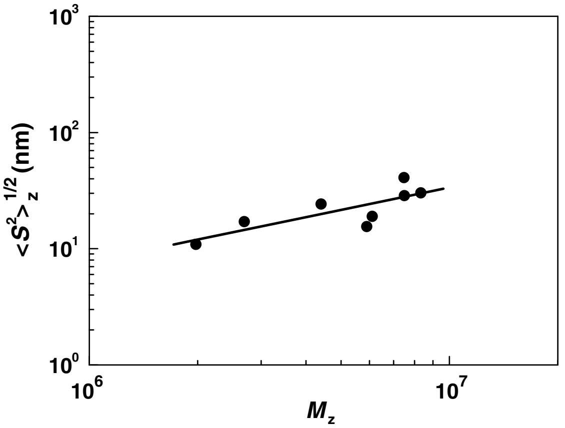

Figure 7. Plot of log $\langle S^2\rangle_z^{1/2}$ versus log M_z for TM3a in 0.02 wt % aqueous NaN_3 at 25 °C.

Furthermore, we can determine the effective fractal dimension directly from the plot of the z-average molar mass (M_z) against $\langle S^2\rangle_z^{1/2}$.[52] Figure 7 shows the plot of $\langle S^2\rangle_z^{1/2}$ versus M_z for the TM3a polysaccharide dissolved by microwave heating for 35 s. The resulting relation is expressed as

$$\langle S^2\rangle_z^{1/2} = 1.08 \times 10^{-3} M_w^{0.64\pm0.2}\ \text{(nm)} \tag{11}$$

The $d_{f,e}$ value was calculated to be 1.56 for TM3a. This result is in good agreement with that from the $(\langle S^2\rangle_z^{1/2})_j \sim f\ (M_{wj})$ power law.

3.5. Molecular parameters

The correlation functions measured by DLS at different scattering angles for TM3a after microwave heating for 35 s at c = 0.1 mg/mL are shown in Figure 8. At this low concentration, the correlation functions are dominated by a single decay mode, which is presumably related to the translational diffusion motion of the TM3a polysaccharide macromolecules. The curves for the other TM3a fractions are very similar to these in Figure 8. The hydrodynamic radius (R_h) can be obtained from the DLS measurement. Figure 9 shows the plot of the hydrodynamic radii versus M_w for the TM3a polysaccharide fractions for a microwave heating of 35 s. The resulting relation is expressed as

$$R_h = 4.37 \times 10^{-2} M_w^{0.46\pm0.08}\ \text{(nm)} \tag{12}$$

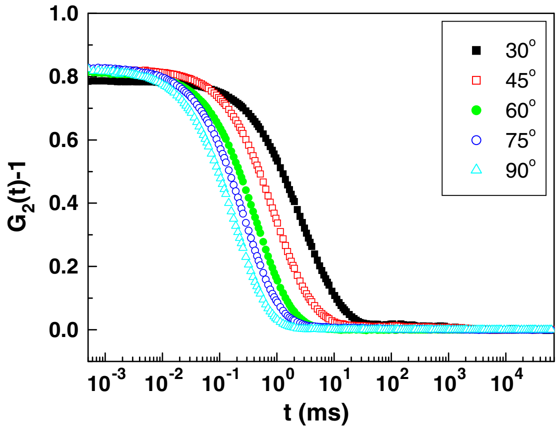

Figure 8. Intensity autocorrelation functions of TM3a in 0.02 wt % aqueous NaN_3 at different scattering angles, measured at 25 °C by DLS, c = 0.1 mg/mL.

The slope of hydrodynamic radius is larger than that of the radius of gyration. Another structural relevant parameter is obtained from the ratio $\rho = \langle S^2\rangle_z^{1/2}/R_h$. For a given polymer solution, the ratio of $\langle S^2\rangle_z^{1/2}/R_h$ depends on its chain architecture and conformation. Many previous experimental results had reported that the ρ value is in the range from 1.5 to 1.7 for flexible linear polymers in a good solvent, whereas that value is 0.78 for a homogeneous sphere. The ratio of $\langle S^2\rangle_z^{1/2}/R_h$ against M_w is plotted at the bottom of Figure 9. Within the scatter of the data, a value of $\rho = 0.53 \pm 0.14$ is obtained. This value of 0.53 is somewhat lower than that for hard spheres, corresponding to the molten globular state; that is, the surface of the globule has a lower density than the center.[53] The result further suggests that the TM3a polysaccharide exists as a sphere-like structure in 0.02 wt % aqueous NaN_3.

The weight average molecular weight dependence of $[\eta]$ for the TM3a polysaccharide fractions dissolved by microwave heating for 35 s is shown in Figure 10. The Kuhn–Mark–Houwink–Sakurada equation of TM3a in the M_w range from 8.20×10^5 to 4.88×10^6 is established as

$$[\eta] = 3.02 \times 10^{-2} M_w^{0.44\pm0.1}\ (\text{cm}^3\ \text{g}^{-1}) \tag{13}$$

The exponent (a) is usually related to the shape of the macromolecule and the nature of the solvent. In general, the (a) exponent with a rough value of 0.5 suggests that the polymer molecules behave as a dense sphere, and a value from 0.6 to 0.8 indicates a flexible chain, while a greater value than one means an elongated rod. The experimental result of 0.44 for TM3a is noticeably low, which is ascribed to a sphere-like structure. If the structure of a polymer

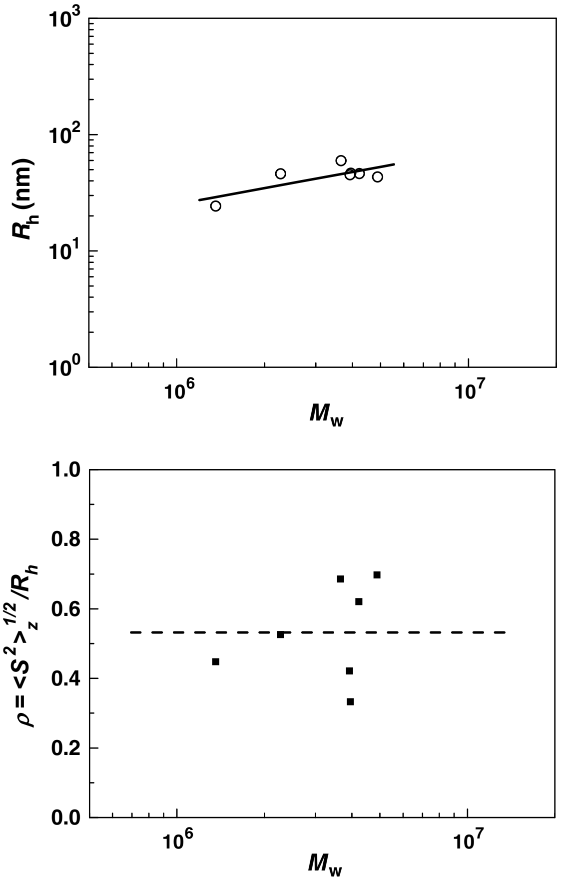

Figure 9. M_w dependencies of the hydrodynamic radius for the TM3a polysaccharide fractions dissolved in 0.02 wt % aqueous NaN_3 by microwave heating for 35 s (upper part), and the ratio of $\langle S^2\rangle_z^{1/2}/R_h$ (bottom) as a function of M_w. The dashed line corresponds to the average.

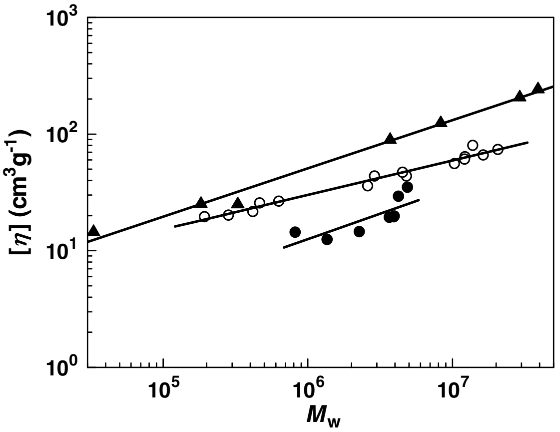

Figure 10. The $[\eta]$ dependence on the M_w for TM3a in 0.02 wt % aqueous NaN_3 at 25 °C (●), in comparison with the hyperbranched β-D-glucan extracted from the sclerotia of *Pleurotus tuber-regium* in 0.15 M aqueous NaCl at 25 °C (▲) and TM3a in 0.25 M LiCl/Me_2SO at 25 °C (○).

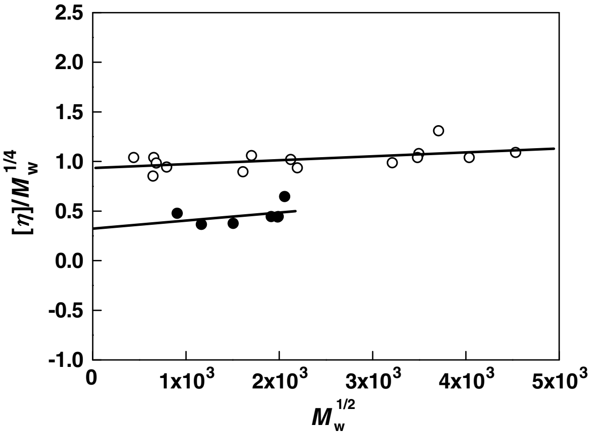

Figure 11. Modified Stockmayer–Fixman plot for hyperbranched polymers with the data for TM3a in 0.02 wt % aqueous NaN_3 at 25 °C (●), in comparison with TM3a in 0.25 M LiCl/Me_2SO at 25 °C (○).

chain is a perfect hard sphere, the exponent should be 0 theoretically.[54] The previous data of M_w and $[\eta]$ for a water-soluble hyperbranched β-D-glucan (TM3b) extracted from the sclerotia of an edible fungus (*Pleurotus tuber-regium*) in 0.15 M aqueous NaCl and the water-insoluble hyperbranched β-D-glucan TM3a in 0.25 M LiCl/Me_2SO are also compared in Figure 10.[9,28] The slope of the line for the TM3a fractions resulting from microwave heating was larger than that for the hyperbranched TM3b β-D-glucan in 0.15 M aqueous NaCl and for TM3a in 0.25 M LiCl/Me_2SO, implying a relatively expanded globular chain conformation. It can be believed that the intra- and intermolecular interactions of these hyperbranched β-D-glucans in solution are different, strongly affecting the chain flexibility.

Intrinsic viscosity has been applied to evaluate unperturbed dimensions of polymers. The Stockmayer–Fixman relation[55] developed for linear polymers is unapplicable to characterize hyperbranched polymers directly. We attempted to find another method, which could be used to characterize the chain conformation of globular and hyperbranched polymers. The general relationship between $[\eta]$ and M_w for highly branched polymer proposed by Zimm and Kilb[56] is

$$[\eta]/M_w^{1/4} = K_\theta + BM_w^{1/2} \tag{14}$$

Figure 11 shows the plot of $[\eta]/M_w^{1/4}$ as a function of $M_w^{1/2}$ for the TM3a polysaccharide fractions dissolved by microwave heating for 35 s and for TM3a in 0.25 M LiCl/Me_2SO at 25 °C. The resulting relation is expressed as

$$[\eta]/M_w^{1/4} = 0.32 + 8.09 \times 10^{-5} M_w^{1/2}\ (\text{cm}^3\ \text{g}^{-1},\ \text{in 0.02 wt \% aqueous NaN}_3) \tag{15}$$

$$[\eta]/M_w^{1/4} = 0.93 + 3.94 \times 10^{-5} M_w^{1/2}\ (\text{cm}^3\ \text{g}^{-1},\ \text{in 0.25 M LiCl/Me}_2\text{SO}) \tag{16}$$

The relation for $(\frac{<r^2>_o}{M_w})^{3/2}$ is based on a constant value of Φ_b in K_θ. Due to the absence of knowledge of the Φ_b constant, we tentatively used a value for Φ_{line}, that is, 2.87×10^{23}, which give $<r^2>_o/M_w$ values of 1.075×10^{-16} cm^2 for the TM3a polysaccharide fractions dissolved by microwave heating, and 2.19×10^{-16} cm^2 for TM3a in 0.25 M LiCl/Me_2SO, respectively. The meaning of an end-to-end distance for a hyperbranched polymer is questionable, so we interpret $<r^2>_o/M_w$ as an effective value for comparison with that for pullulan, a linear polysaccharide. It has been reported that the $<r^2>_o/M_w$ value of pullulan in aqueous solution is 6.7×10^{-17} cm^2.[57] The $<r^2>_o/M_w$ value of TM3a after microwave heating is circa twice than that of the linear pullulan. This is similar to the result of hyperbranched polyester.[58] Usually, the $<r^2>_o/M_w$ value of polymers is affected by bond angle and steric hindrance including backbone chain and side chain and so much. The $<r^2>_o/M_w$ value of TM3a is much larger than that of the linear one. For an hyperbranched polymer, a Gaussian behavior is expected but with a larger $<r^2>_o/M_w$ value because of segment overcrowding in the vicinity of branching point. Therefore, it is the hyperbranched structure that accounts in low Kuhn–Mark–Houwink–Sakurada equation exponent but relatively larger $<r^2>_o/M_w$ for TM3a in 0.02 wt % aqueous NaN_3 at 25 °C. The $<r^2>_o/M_w$ value of TM3a dissolved by microwave heating is much lower than that for TM3a in 0.25 M LiCl/Me_2SO. It is probable that degradation of the side chain during the microwave heating process reduces the steric hindrance between the polymer chains.

3.6. Molecular morphology

TEM technology is widely used to observe and measure the morphology and dimension of particles including macromolecules. Figure 12 illustrates the TEM images of TM3a dissolved by microwave heating. The spherical particles of TM3a exist in the dilute solution. It provided straight evidence of the sphericity of molecules for TM3a. The result from TEM further confirms that TM3a molecules exhibit a globular molecular structure in 0.02 wt % aqueous NaN_3 after microwave heating.

4. Conclusion

The microwave heating method is an effective procedure for solubilization of the water-insoluble hyperbranched polysaccharide extracted from the sclerotia of *P. tuber-regium*. A heating time of 35 s is adequate for solubilization without drastic degradation of the polysaccharide. According to the current theory of polymer solution, the various scaling laws and molecular parameters of the TM3a polysaccharide were calculated from the experimental

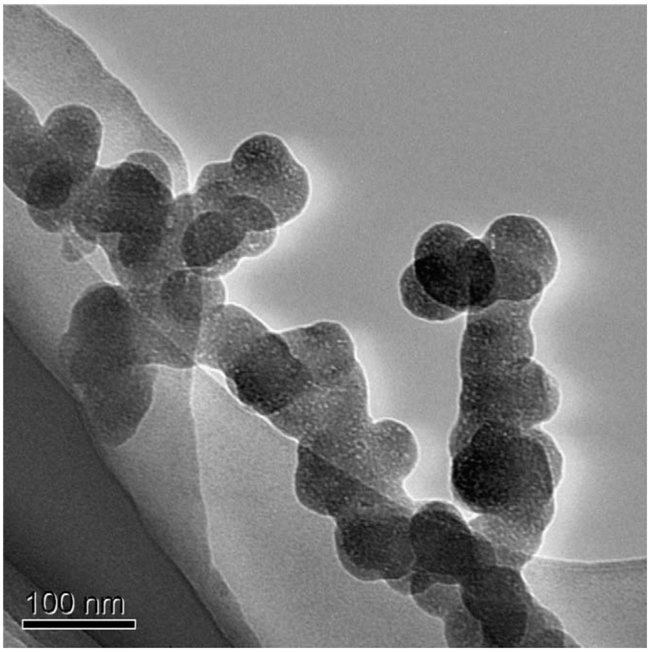

Figure 12. TEM image of TM3a in 0.02 wt % aqueous NaN_3 after microwave heating for 35 s.

data of M_w, $\langle S^2\rangle_z^{1/2}$, R_h, and $[\eta]$. The results revealed that TM3a in 0.02 wt % aqueous NaN_3 was present in a sphere-like conformation. In view of its fractal dimension, TM3a in this solvent had an internal structure between a hard sphere and a swollen branched macromolecule. Furthermore, the TEM images confirmed directly that TM3a molecules exhibited a globular molecular structure. This work provided feasible methods of solubilization and chain conformation characterization for water-insoluble polysaccharides.

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