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Physicochemical characterization of grifolan: Thixotropic properties and complex formation with Congo Red

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Abstract

Grifolan is a branched (1,3)-β-glucan extracted from the fungus *Grifola frondosa*. Its physicochemical properties were investigated by studying the visible spectrum of Congo Red–glucan complexes and the rheological behavior. The analysis of the visible spectrum of Congo Red–glucan complexes indicated that the alkali-extracted grifolan was in a triple-helix conformation. The triple-helix structure remained unaltered in the temperature range studied (5–25 °C) or when varying the sucrose content (0–20%), but disappeared at a urea concentration of 5.0 M. The alkali-extracted grifolan formed a gel at subambient temperatures. The thixotropic viscosity profile of grifolan gels was fitted by a structural kinetics model, and the thixotropy was attributed to the breakdown of aggregates of triple helices under shear. The rate of structure breakdown was found to increase both with increasing shear rate and temperature. The amount of gel structure available for shear-induced structure breakdown was shear-rate independent, but decreased with increasing temperature. The presence of sucrose or urea in grifolan gels resulted in significant changes in thixotropy, which were attributed to the enhancing effect of sucrose on the strength of aggregates of triple helices and the loss of triple-helix structure due to the breaking of intermolecular hydrogen bonds by urea.

Keywords: Grifolan; Triple helix; Congo Red complex; Thixotropy

1. Introduction

Grifolan is the polysaccharide extracted from the fruit body or the mycelium of the fungus *Grifola frondosa*. It has been reported that grifolan acts as a biological response modifier to enhance the immune system and exhibits anti-tumor activity (Ohno et al., 1984; Ohno et al., 1985; Ohno et al., 1986a). The active component in grifolan has been isolated and identified as (1,6)-branched (1,3)-β-D-glucan. This structure is similar to other branched (1,3)-β-glucans, such as schizophyllan and scleroglucan (Bohn & BeMiller, 1995), which also show significant antitumor activity.

Branched (1,3)-β-glucans are known to adopt a triple-helix conformation (Norisuye, Yanaki, & Fujita, 1980; Yanaki & Norisuye, 1983) similar to that found in curdlan (an unbranched (1,3)-β-glucan). The physicochemical properties of these glucans are related to their structural features. The branches are extruded from the helix, thus preventing the formation of large aggregates of triple helices. Nevertheless, at high concentrations of these glucans, associations between triple helices lead to gelation (Bot, Smorenburg, Vreeker, Pâques, & Clark, 2001; Grassi, Lapasin, & Pricl, 1996). It was also reported that the immune stimulating capability of these glucans is strongly influenced by their conformation (Falch, Espevik, Ryan, & Stokke, 2000).

The conformation of grifolan has been investigated using cross polarization-magic angle spinning NMR spectroscopy (Ohno et al., 1986b; Ohno, Ohsawa, Sato, Oikawa, & Yadomae, 1987). Two types of conformations were identified in the NMR spectra: the helix (curdlan) type and the native (laminaran) type. Laminaran is a

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low-molecular-weight (1,3)-β-glucan with a random-coil conformation. The grifolan in the liquid culture adopts the laminaran-type conformation and is transferred into the helix form during purification by treatment with urea and NaOH.

The triple-helix conformation of (1,3)-β-glucans can be detected simply by examining the visible spectrum of its complex with Congo Red, which shows a red shift of the maximum absorption wavelength (λ_{max}) (Ogawa, Tsurugi, & Watanabe, 1972). Grifolan has been reported to form complexes with Congo Red, indicating the presence of triple-helix conformation (Iino et al., 1985; Ohno et al., 1985). Iino et al. (1985) reported that under physiological conditions, a soft gel of grifolan is formed via the association between triple helices. They also reported that the gel-forming ability of grifolan is associated with its antitumor activity.

The physicochemical properties of grifolan reflect its conformation and secondary structure. To the best of our knowledge, no report has been published on systematic studies of the physicochemical properties of grifolan in aqueous solutions. In this study, the complex formation of grifolan with Congo Red in dilute solutions was investigated to verify the presence of triple helices. The thixotropic behavior of grifolan gels was then examined to elucidate the aggregate nature of triple helices. A viscometer was used to obtain the time-dependent rheological profile as a function of temperature, shear rate, sucrose content, and urea concentration. An attempt was made to model the thixotropy using the structural kinetics approach, which has been shown to be able to successfully describe the structure breakdown of weak gels in a shear field (Mao & Chen, 2006).

2. Materials and methods

2.1. Materials and preparation

Grifolan was extracted from the pulverized mycelium of *G. frondosa* (Geneferm Biotechnology, Tainan, Taiwan). Before extraction, the mycelium was heated in 10 volumes of 80% ethanol at 60 °C for 4 h to remove fatty materials. The defatted mycelium was suspended in 20 volumes of distilled water and autoclaved for 20 min. The extraction was repeated three times. The extract was concentrated to a small volume and dialyzed against de-ionized water. The polysaccharide fraction was precipitated by adding 4 volumes of 95% ethanol at 4 °C for 24 h. The precipitate was then freeze-dried and stored in a desiccator (denoted as MHW). The hot water extract residue was further extracted in a 10% sodium hydroxide solution containing 4% urea at 4 °C for 24 h. The extraction was repeated three times. The extract was then neutralized with acetic acid. The precipitate produced by neutralization was discarded while the supernatant was dialyzed against de-ionized water, and precipitated by adding 4 volumes of 95% ethanol at 4 °C for 24 h. The precipitate was then freeze-dried and stored in a desiccator (denoted as MCA). Curdlan in the Congo Red–glucan complex formation experiment was purchased from Sigma (St. Louis, MO) and used without further purification.

Grifolan gels were prepared by first dispersing samples at room temperature in de-ionized water. The solutions were then homogenized at 3000 rpm for 5 min, autoclaved for 20 min, and stored at 5 °C. 0.01% of sodium azide was added to prevent bacterial degradation.

2.2. Chemical analysis

Total sugar was determined by the phenol-sulfuric acid method using glucose as a standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956), total protein by the Lowry's method using bovine serum albumin as a standard (Lowry, Rosebrough, Farr, & Randall, 1951), and moisture by loss of water in a thermogravimetric analyzer from room temperature to 220 °C at a heating rate of 10 °C/min.

The component sugar analysis was performed using the alditol acetate method (Blakeney, Harris, Henry, & Stone, 1983). The hydrolysis was performed in 2 M trifluoroacetic acid for 1.5 h at 121 °C, followed by reduction in dimethyl sulfoxide with sodium borohydride at 100 °C. Acetylation was performed with acetic anhydride using 1-methylimidazole as catalyst for 10 min at room temperature. The alditol acetate derivatives were then separated by a gas chromatograph (Agilent 6890) using a capillary column (SP-2380, 30 m × 0.32 mm ID, Supelco) at a constant temperature of 220 °C.

The molecular weight was measured using size-exclusion chromatography equipped with three SEC columns (TSK-GEL $GMPW_{XL}$, Tosoh) connected in series. The columns were calibrated using dextran standards.

2.3. Complex formation with congo red

The Congo Red–glucan complex was prepared by mixing the grifolan solution (1 mg/mL) with the Congo Red solution (26 μM) in equal volumes. The visible absorption spectrum was recorded using a Perkin Elmer Lambda 25 UV–vis spectrophotometer. The effects of temperature, urea concentration and sucrose content on the shift in λ_{max} were examined. The complex formation of curdlan with Congo Red was also examined for comparison.

2.4. Rheological measurements

The rheological properties of grifolan gels were determined by using a cone-and-plate viscometer (Brookfield, Model HBDV-III+) equipped with a sample cup connected to a thermostatically controlled water bath. A spindle with a radius of 12 mm and a cone angle of 3°, and a cup with a radius of 27 mm were used. Grifolan gels of 0.5 cm^3 were introduced in the gap between the cone and the cup, and then were allowed to equilibrate for 10 min prior to measurement.

In the transient rheological measurements, the sample was sheared at a constant shear rate and the shear stress was recorded as a function of time until a steady value of stress was reached. The apparent viscosity was calculated as the ratio of shear stress to shear rate. Fresh samples were loaded for each measurement. The transient viscosity profile was modeled by the structural kinetics expression developed by Nguyen, Jensen, and Kristensen (1998):

$$\left(\frac{\eta}{\eta_\infty}-1\right)^{1-n}=[(n-1)kt+1]\left(\frac{\eta_0}{\eta_\infty}-1\right)^{1-n} \quad (1)$$

where η_0 and η_∞ are the asymptotic values of the apparent viscosity at $t=0$ and $t\to\infty$, respectively, n is the kinetics order for the structure breakdown process, and k is the shear-rate dependent rate constant. In this equation there are three adjustable parameters, n, k, and the ratio of η_0 to η_∞, and the η_∞ value is assigned equal to the equilibrium viscosity in the transient viscosity profile.

In the steady-state rheological measurements, a pre-shearing period of 1 h at a shear rate of 400 s^{-1} was performed prior to measurement, and then a shear rate sweep from 1 to 400 s^{-1} was made. The pre-shearing procedure allows samples to achieve an equilibrium structure and eliminates the thixotropic effects.

3. Results and discussion

3.1. Chemical characterization of grifolan

The results of the chemical analysis of grifolan samples are listed in Table 1. Both MHW and MCA contain significant amounts of protein, while the MCA has a higher carbohydrate level. The component sugar analysis shows that the MHW and MCA have different sugar compositions. The MHW contains considerable amounts of mannose, galactose, fucose, and xylose in addition to glucose, indicating that it is a heteropolysaccharide rather than a glucan. The MCA, on the other hand, is composed mainly of glucose residues.

3.2. Complex formation with Congo red

The triple-helix conformation of a glucan can be identified by a red shift in the λ_{max} value of the Congo Red–glucan complex in a visible spectrum. Fig. 1 shows the effect of alkaline concentration on the λ_{max} value of the Congo Red–glucan complex. Curdlan is known to adopt a triple-helix conformation at low alkaline concentrations, thus exhibiting a large red shift in λ_{max} as compared to a pure Congo Red solution. As the alkaline concentration

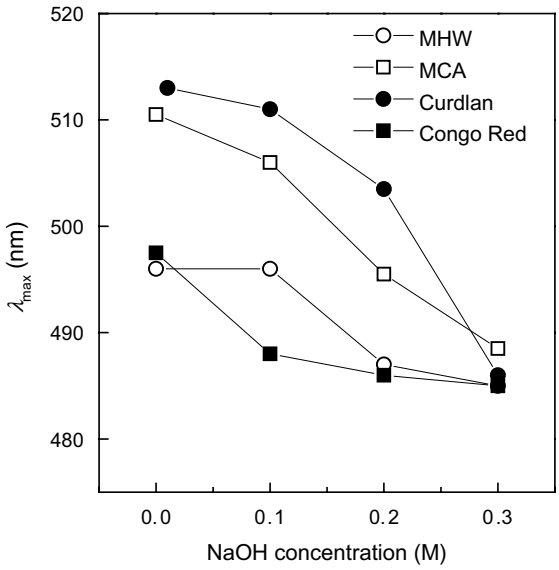

Fig. 1. Plot of the absorption maximum of Congo Red–glucan complexes at various concentrations of NaOH. The Congo Red solution is served as a blank for comparison.

increases, curdlan undergoes a helix-coil transition and the λ_{max} value approaches that of a Congo Red solution. For the MCA, the Congo Red–glucan complex results in a red shift in the λ_{max} value similar to that for the curdlan, indicating the existence of triple helix, whereas for the MHW, no significant shift in λ_{max} can be observed. This result is consistent with the finding in the component sugar analysis that the MHW is a heteropolysaccharide, which cannot form a triple-helix structure. Consequently, in what follows we focus only on the properties of the MCA.

Fig. 2 shows the effect of urea concentration on the λ_{max} value of the Congo Red–glucan complex. It can be seen that for the Congo Red solution, the λ_{max} value increases slightly with the urea concentration, whereas for the grifolan and curdlan, the λ_{max} value decreases with increasing urea concentration. When the urea concentration reaches 5.0 M, the λ_{max} values of the Congo Red–glucan complex are almost identical to that of the Congo Red solution, reflecting the loss of triple-helix structure.

Two mechanisms have been proposed to explain the effect of urea on biopolymer conformation (Bennion & Daggett, 2003). The first involves the interaction of urea with the polar groups of biopolymers to stabilize the denatured conformation. The second involves an indirect effect of urea on the promotion of the interaction between water and biopolymers via altering the water structure. Zhang, Zhang, and Cheng (2000) reported that urea changes the

Table 1
Molecular weight, protein, sugar, and moisture contents and component sugar analysis of grifolans obtained from the mycelium of *G. frondosa*

Samples	M_n ($\times 10^6$)	Protein (%)	Sugar (%)	Moisture (%)	Sugar composition
MHW	9.0	3.6	63	11.4	Glc:Man:Gal:Fuc:Xyl (1:1.03:0.46:0.30:0.29)
MCA	25.0	6.1	83	10.4	Glc:Man:Xyl (1:0.05:0.06)

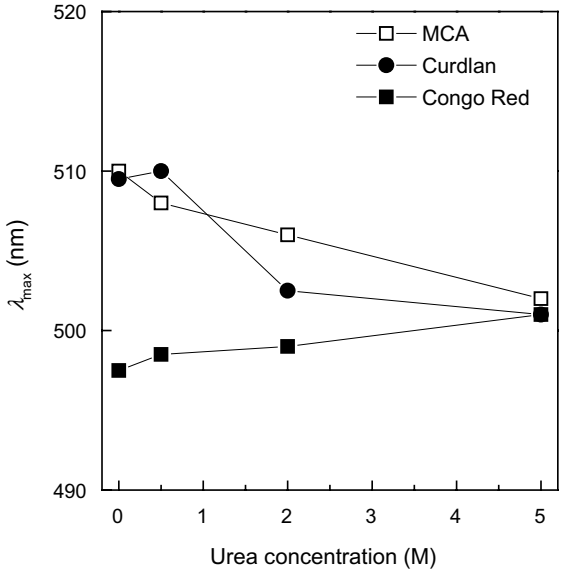

Fig. 2. Plot of the absorption maximum of Congo Red–glucan complexes at various concentrations of urea. The Congo Red solution is served as a blank for comparison.

glucan conformation as well as its water solubility by breaking intermolecular hydrogen bonds. In the case of grifolan, the triple-helix conformation of (1,3)-β-glucan is lost at high urea concentrations, possibly due to the breakage of intermolecular hydrogen bonds.

For the temperature- and sucrose concentration-dependent studies, no distinct difference in the λ_{max} value of the Congo Red–glucan complex was observed by varying temperature from 5 to 25 °C or sucrose content from 0% to 20%. These results are not shown for brevity.

3.3. Thixotropic behavior of grifolan gels

The aqueous solution of the MCA with a concentration of 4.0% by weight formed a gel at 5 °C. The gel gradually melted when placed at room temperature. The MHW solution, in contrast, was a viscous fluid at 5 °C. This difference in gelling ability is due to whether the triple-helix structure exists or not. Schizophyllan possessing a similar structure to grifolan, has been reported to form a gel, which has a melting transition at subambient temperatures (Bot et al., 2001; Tako, 1996). These branched glucans all have a common feature that the single glucosyl branches on the outside of the helix prevent the association between triple helices at room temperature. However, at subambient temperatures, weak aggregates of triple helices can be formed, thus resulting in a weak gel.

The grifolan gel is weak and easily broken by shear forces. In this section, the breakdown of gel network under shear for the MCA gel is investigated by studying its time-dependent thixotropic behavior. The transient viscosity profiles are fitted by the structure breakdown kinetics (Eq. (1)). The thixotropic study is divided into four parts by considering separately the effect of shear rate, temperature, sucrose, and urea on the breakdown kinetics. It is noted that though the triple-helix structure does not change over the temperature and sucrose content range examined, the thixotropic behavior is influenced significantly by these factors.

3.3.1. Effect of shear rate on thixotropy

When the grifolan gel is subjected to a shear force, its network structure breaks down, leading to a gradual decrease in viscosity over time. Fig. 3 shows that the viscosity change versus time at different shear rates for the 4% MCA gel. It can be seen that the viscosity decreases more rapidly at higher shear rates and the equilibrium viscosity at high shear rates is lower than that at low shear rates.

The transient viscosity profiles in Fig. 3 can be modeled using the structural kinetics expression. Normally, the data fitting is performed by assuming a kinetics order (n) and examining the linearity of the relationship between $(\eta/\eta_\infty - 1)^{1-n}$ and time (t) (Mao & Chen, 2006; Nguyen et al., 1998). However, it is found that for grifolan gels, these profiles cannot be described by a single kinetics order. The kinetics order is shear-rate dependent and has a non-integral value. A nonlinear regression code based on the Levenverg–Marquardt method was thus used to obtain the parameters of Eq. (1) for each viscosity profile. The resulting parameter values are listed in Table 2. It can be seen that the kinetics order as well as the rate constant increases with increasing shear rate and the η_0/η_∞ value is roughly shear-rate independent.

The varying kinetics order suggests that the kinetics order here bears no physical significance like that in a chemical reaction, but can be treated as an empirical

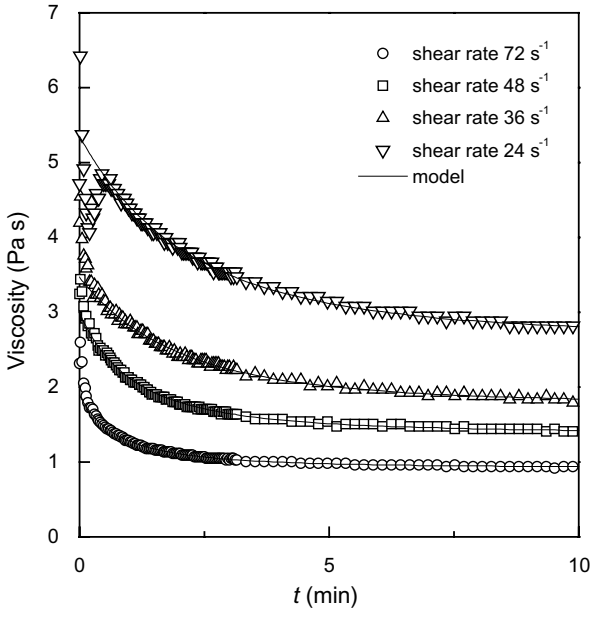

Fig. 3. Effect of shear rate on the transient velocity profiles for the 4% grifolan MCA gel at 5 °C. The solid curves are derived using the structural model.

Table 2
Shear-rate and temperature dependence of the fitting parameters for the 4% grifolan MCA gel

$\dot{\gamma}$ (s^{-1})	T (°C)	n	k (min^{-1})	η_0/η_∞
72	5	1.5	1.30	2.06
48	5	1.5	0.93	2.17
36	5	1.2	0.51	1.93
24	5	1.2	0.46	1.99
36	0	1.4	0.11	1.96
36	10	1.4	1.22	1.65
36	15	2.2	7.32	1.59

parameter, along with the rate constant, representing the rate of structure breakdown. The structure of a complex fluid can be simply described by a structure parameter ψ, which is defined in terms of apparent viscosities:

$$\psi = \frac{\eta - \eta_\infty}{\eta_0 - \eta_\infty} \tag{2}$$

As can be seen from the definition, the structure parameter has a value of unity at $t = 0$ and a value of zero at $t \to \infty$. Consequently, the ψ value represents the fraction of unbroken gel networks. The change in ψ with respect to time can be calculated from Eq. (1) with known kinetic parameters. Fig. 4 illustrates the decay of ψ over time at different shear rates for the 4% MCA gel. It can be seen that the ψ value decreases more rapidly at higher shear rates. The result indicates that the rupture rate of the gel structure under shear proceeds faster at high shear rates, as suggested by the increase in kinetics order and rate constant.

The η_0/η_∞ value, on the other hand, is not directly related to the rate of structure breakdown, but is used as a measure of thixotropy, relating to the amount of gel networks that can be ruptured by a shear force. The shear-rate independence of η_0/η_∞ value thus implies that the amount of gel networks available for shear-induced structure breakdown remains fixed regardless of the change of shear rate.

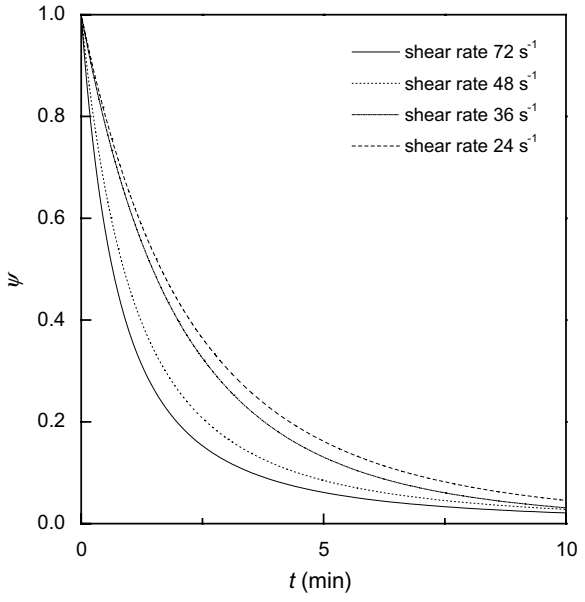

Fig. 4. Plot of the structure parameter against time t for the 4% grifolan MCA gel at different shear rates.

3.3.2. Effect of temperature

Fig. 5 shows the effect of temperature on the transient viscosity profiles for the 4% MCA gel. It can be seen that the viscosity reaches a steady value in 5 min when temperatures are higher than or equal to 10 °C. For temperatures higher than 20 °C, thixotropic behavior was not observed (not shown in the figure). At lower temperatures, the viscosity decreases at a much slower rate. It is also noted that at 0 °C, an initial fluctuation in viscosity occurs, probably caused by the slip at the contact surfaces due to an increase in sample elasticity.

The large variation of the thixotropy of grifolan gels in Fig. 5 indicates that the gel network is strongly temperature-dependent. Since the triple-helix structure does not change over this temperature range, the gel structure can be attributed to aggregates of triple helices. For temperatures higher than 20 °C, thermal energy overcomes the intermolecular forces holding the aggregates, thus preventing the formation of a gel; while at lower temperatures, the gel structure is formed through the aggregation of triple helices, thus exhibiting significant thixotropic behavior in a shear field.

The viscosity profiles in Fig. 5 can also be described by the structural kinetics model. The resulting fitting parameters are given in Table 2. The kinetics order and rate constant roughly increase with increasing temperature, indicating that the rate of structure breakdown becomes higher at higher temperatures. The η_0/η_∞ value, on the

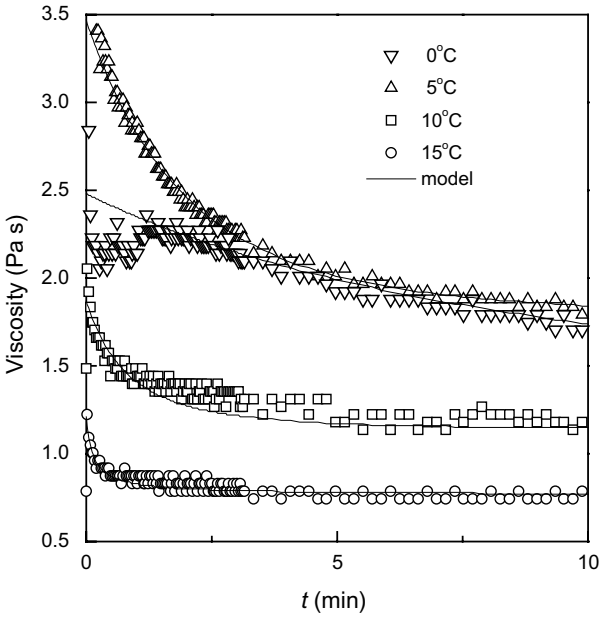

Fig. 5. Effect of temperature on the transient velocity profiles for the 4% grifolan MCA gel at a constant shear rate (36 s^{-1}). The solid curves are derived using the structural model.

other hand, decreases with increasing temperature, reflecting a loss of thixotropy. This trend implies that that the network structure of the grifolan gel gradually collapses with increasing temperature, which is consistent with the observation that grifolan gels are not formed at high temperatures.

3.3.3. Effect of sucrose content

The effect of sucrose content on the transient viscosity profiles for the 4% MCA gel at 5 °C is shown in Fig. 6. It can be seen that in the presence of sucrose, the rate of viscosity decrease due to shear is significantly reduced and an initial fluctuation in viscosity occurs. These observations reflect that sucrose enhances the gel strength and consequently causes the slip at the contact surfaces during shear. Moreover, the thixotropic properties of the 4% MCA gel having a sucrose content of 10% w/v were also examined at different temperatures (figure not shown). It is found that its thixotropy is still pronounced at a temperature of 20 °C, which differs from that in the absence of sucrose. This difference demonstrates that adding sucrose to grifolan gels tends to stabilize the gel structure, which thus persists at relatively higher temperatures.

The corresponding fitting parameters at various sucrose contents are listed in Table 3. It is evident that in the presence of sucrose, the rate constant is considerably reduced, whereas the kinetics order somewhat increases. The combined effect of the rate constant and kinetics order on thixotropy can be made clear by considering the variation of the structure parameter ψ over time, as shown in Fig. 7. It can be seen that the rate of structure breakdown under shear is significantly reduced in the presence of sucrose,

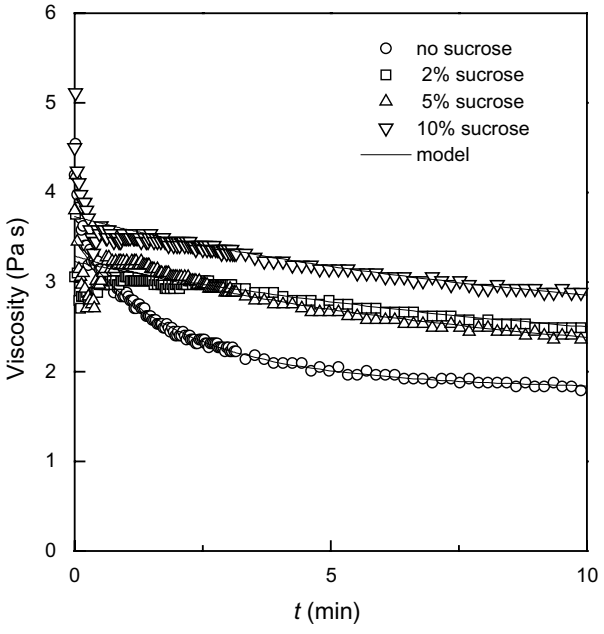

Fig. 6. Effect of sucrose content on the transient velocity profiles for the 4% grifolan MCA gel at 5 °C and a constant shear rate (36 s^{-1}). The solid curves are derived using the structural model.

Table 3
Sucrose content and urea concentration dependence of the fitting parameters at a constant shear rate (36 s^{-1}) for the 4% grifolan MCA gel at 5 °C

Sucrose content (%)	Urea (M)	n	k (min^{-1})	η_0/η_∞
2	–	1.2	0.084	1.82
5	–	1.6	0.103	1.98
10	–	1.5	0.086	1.86
–	2.0	1.4	0.153	2.14
–	5.0	–	–	–

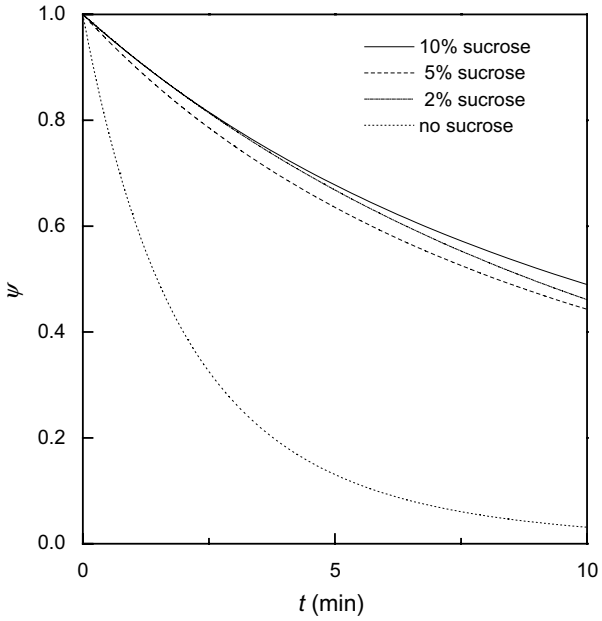

Fig. 7. Plot of the structure parameter against time t for the 4% grifolan MCA gel at different sucrose contents.

indicating an increase in structure strength. The decay profile of ψ is only slightly varied with the sucrose content. It is also noted in Table 3 that the η_0/η_∞ value is not significantly affected by the presence of sucrose. These facts suggest that adding sucrose to a grifolan gel enhances its structure strength, but does not change the amount of gel structure.

The effect of low-molecular-weight carbohydrates on the gel properties of polysaccharide solutions has been addressed in the literature. Richardson and Norton (1998) have found that adding sucrose to a locust bean gum solution can decrease the solvent quality and promote the associations between polysaccharide chains. A similar effect was also observed for glucose in schizophyllan gels (Bot et al., 2001). The melting transition temperature of the gel was found to increase with increasing glucose concentration, which is attributed to a reduction in solvent quality. Apparently, the role of low-molecular-weight carbohydrates is to compete with polysaccharides for the water available for hydration, thus promoting the interchain association of polysaccharides. In this study, adding sucrose definitely promotes the strength of aggregates of grifolan triple helices, resulting in a reduction in the rate of viscosity decrease in the thixotropic measurement.

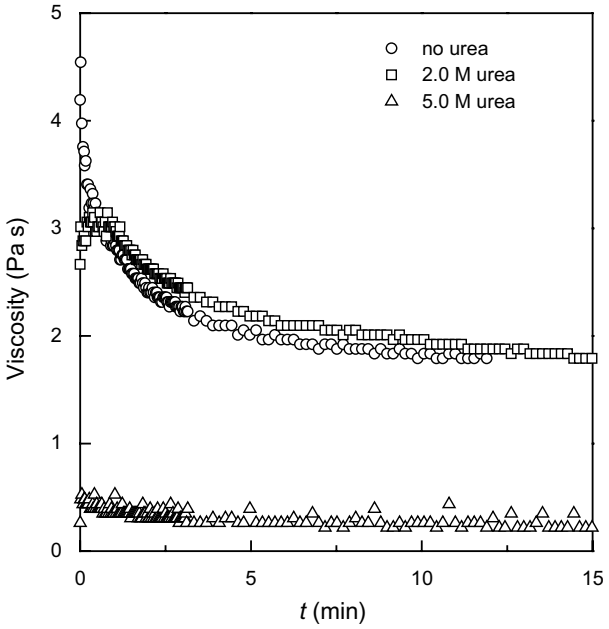

Fig. 8. Effect of urea concentration on the transient velocity profiles for the 4% grifolan MCA gel at 5 °C and a constant shear rate (36 s^{-1}).

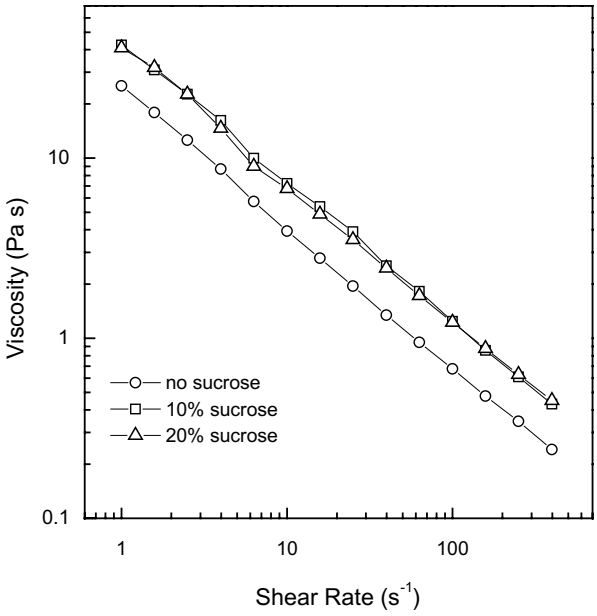

Fig. 9. Shear rate dependence of viscosity for the 4% grifolan MCA gel at 5 °C.

3.3.4. Effect of urea concentration

Fig. 8 shows the effect of urea on the transient viscosity profiles for the 4% MCA gel at 5 °C. It can be seen that the viscosity profile is slightly altered at a urea concentration of 2.0 M, whereas the thixotropic behavior is completely suppressed at a urea concentration of 5.0 M. The resulting fitting parameters for the structural kinetics model at a urea concentration of 2.0 M are listed in Table 3 for comparison.

The result of the thixotropic study is consistent with that in the Congo Red–grifolan complex formation experiment, where the triple-helix structure is still present at low urea concentrations but disappears at a urea concentration of 5.0 M. Apparently, the suppression of thixotropy is directly related to the loss of the triple-helix structure. This can be understood in the following way. As the intermolecular hydrogen bonding between grifolan chains is disrupted at high urea concentrations, grifolans no longer adopt the triple-helix conformation. Since the gel structure is formed through the aggregation of triple helices, lack of the triple-helix structure prevents the formation of a gel, thus resulting in a complete loss of thixotropy.

3.4. Steady rheological behavior

The steady-state viscosity as a function of shear rate for the 4% MCA gel at different sucrose contents is shown in Fig. 9. The grifolan gels exhibit a pronounced shear-thinning behavior, where the viscosity decreases with increasing shear rate. Moreover, it is noted that the Newtonian viscosity plateau was not observed in the low-shear-rate region. This feature is typical for weak gels such as xanthan, which adopts an ordered, rod-like conformation in aqueous solutions (Morris & Ross-Murphy, 1981). Since having a rigid triple-helix conformation, grifolan behaves rheologically similar to xanthan. The shear-thinning behavior of grifolan gels thus can be understood as follows. The weak association between polymer chains is readily formed for rod-like polymers since the ordered conformation confers a low entropy loss during the segment–segment interaction. Shear-thinning is the consequence of the process that the rate of dissociation due to shear is greater than the rate of formation of new associations. It is also noted that

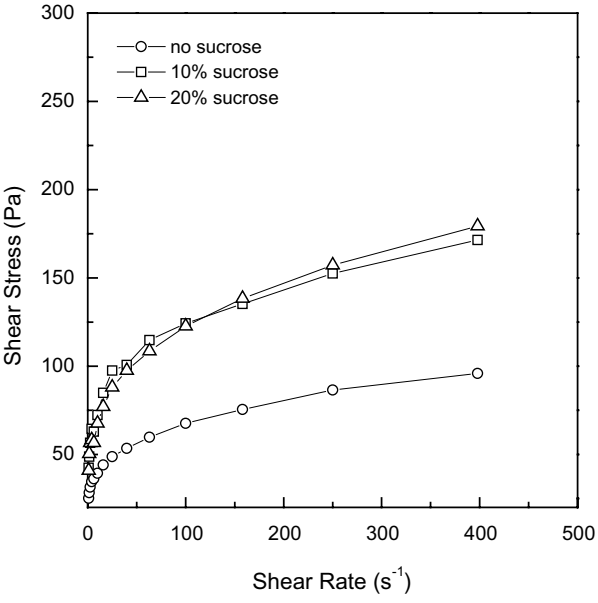

Fig. 10. Shear stress as a function of shear rate for the 4% grifolan MCA gel at 5 °C.

though the thixotropic behavior is affected by the presence of sucrose, the shear-thinning behavior seems unchanged regardless of the sucrose content.

The steady shear stress as a function of shear rate is shown in Fig. 10, typical of a shear-thinning fluid. The shear-thinning of weak gels can be best fitted to the Ostwald-de Waele power law model (Morris, 1990):

$$\tau = K_c \dot{\gamma}^{n_f} \quad (3)$$

where K_c and n_f are the consistency coefficient and the flow index of the Ostwald-de Waele model, respectively. The value of the flow index is found approximately 0.23, unaffected by the presence of sucrose. This value is comparable to that reported for xanthan solutions (Flores Candia & Deckwer, 1999).

4. Conclusions

The physicochemical properties of grifolan in aqueous solution were investigated by examining its complex formation with Congo Red and the thixotropic behavior in a shear field. The alkali-extracted grifolan is shown to adopt a triple-helix conformation and capable of forming a gel. The thixotropic behavior of the grifolan gel is attributed to the breakdown of aggregates of triple helices under shear. The transient viscosity profile, reflecting the thixotropy of the grifolan gel, can be successfully described using a structural kinetics model with three kinetic parameters – the kinetics order, the rate constant, and the ratio of the initial to equilibrium viscosity. The rate of structure breakdown under shear increases with increasing shear rate and temperature, as evidenced by the increase in the kinetics order and rate constant. The amount of gel structure, in contrast, as measured by the η_0/η_∞ value, is shear-rate independent but decreases with increasing temperature. The presence of sucrose has no significant effect on the triple-helix conformation of grifolans and the shear-thinning properties. However, it alters the thixotropic behavior by decreasing the rate of structure breakdown. This is due to a reduction in solvent quality, which consequently promotes the strength of aggregates of grifolan triple helices. Urea, on the other hand, breaks intermolecular hydrogen bonding at high concentrations, which causes dissociation of triple helices and complete suppression of thixotropy.

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