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Identification of molecular driving forces involved in the gelation of konjac glucomannan: Effect of degree of deacetylation on hydrophobic association

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ABSTRACT

Gelation behaviors of konjac glucomannan (KGM) samples with different degree of deacetylation (DD) obtained using a heterogeneous deacetylation method were studied by rheological measurement to probe the effect of degree of deacetylation on hydrophobic association. Compared to polysaccharides whose gelation mechanism is known, we speculate hydrogen bonding and hydrophobic interaction are both presented in KGM gelation and hydrophobic interaction plays a more important role with increasing DD. Hydrophobic characteristic of partially deacetylated KGM (DD was circa 50%) was confirmed using the lyotropic series of salts, suggesting the magnitude of hydrophobic interaction is dependent on DD of KGM. Furthermore, the effect of lyotropic series of salts on gelation behavior of KGM was clarified from a molecular level.

1. Introduction

Konjac glucomannan is a water-soluble neutral polysaccharide, extracted from the tubers of *Amorphophallus konjac* C. Koch (Nishinari & Williams, 1992), composed of β-(1→4) linked D-mannose and D-glucose in a molar ratio of 1.6:1 (Kato & Matsuda, 1969) or 1.4:1 (Bewley & Reid, 1985). Acetyl groups attached to the saccharide units are scattered randomly along the molecule, with an occurrence of approximately 1 per 19 sugar residues (Maekaji, 1978). A heat stable gel is formed upon addition of a mild alkali and heat. It is suggested that the polymer is deacetylated during this process, steric hindrance is removed and the polymers are free to associate to form a gel. After excluding the possibility of covalent crosslinking, hydrogen bonding has been suggested as the mechanism which aids in stabilization of the KGM gel network for quite a long time (Maekaji, 1974). However, this theory cannot explain why KGM gels are heating irreversible and elastic modulus increases in the heating process (Tye, 1991). In our previous studies, we found, after deacetylation, KGM molecule occurred strong self-crimping from an extending semi-flexible linear chain with length of 1054.11 nm to an elastic microsphere with diameter of 40–50 nm by using atomic force microscope (Li & Xie, 2005a, 2006b), and unsymmetry of sample enhanced with increasing temperature by Circular Dichroism Spectroscopy (Li & Xie, 2004). In addition, konjac glucomannan (KGM) exhibited liquid crystalline (LC) behavior in aqueous solutions above 7% concentrations by polarized optical microscopy (Dave, Sheth, McCarthy, Ratto, & Kaplan, 1998). These research results suggest hydrogen bonding may not be the primary molecular driving forces involved in the gelation of KGM. Further, these phenomena of KGM are similar with chitosan which has been proved to aggregate by hydrophobic interaction (Olga, Evgenii, Natalia, & Alexei, 2001). Thus, we guess hydrophobic interaction may play an important role in the gelation process of KGM.

In fact, hydrophobic interaction is an important molecular driving force to promote the ordering of biomacromolecules and has been widely investigated in many polysaccharides systems, such as, curdlan (Funami, Funami, Yadaand, & Nakao, 1999; Ikeda & Shishido, 2005), methylcellulose (Desbrières, Hirrien, & Rinaudo, 1998; Desbrières, Hirrien, & Ross-Murphy, 2000), and chitosan (Amiji, 1995). Despite this, work on the role of hydrophobic interaction in the gelation of KGM is less reported possibly due to the absence of significant hydrophobic side groups of KGM backbone and the limitation of methodology. Until 1992, Case, Kropp, Hamann, and Schwartz (1992) attempted to reveal that other types of bonding or interaction, in addition to hydrogen bonding, contribute to the stabilization of KGM gels, and confirmed successfully the hydrophobic characteristics of gelation of KGM using lyotropic series of salts. In recent years, Nishinari and Zhang (2004) emphasized that the contribution of hydrophobic interaction should be studied further other than hydrogen bonding in the network formation of KGM gel in a review. By observing effects of SDS on the sol–gel transition of KGM, Yin, Zhang, Huang, and Nishinari (2008a)

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indicated that the addition of SDS promoted the gelation process of KGM in the presence of alkali in contrast with no significant change occurring in the absence of alkali and deduced that KGM backbone has weak hydrophobic properties. The gelation behavior of KGM was again studied as a function of lyotropic series of salts using rheological measurements by Yin, Zhang, Huang, and Nishinari (2008b), showing that sulfate alone was capable of inducing KGM form a thermoirreversible gel rather than via deacetylation and carbonate was also of salting-out effect in addition to facilitating the deacetylation of KGM chains. So far, it seems to be clear gradually that hydrophobic interaction plays an important role in the gelation process of KGM.

However, whether or not deacetylation is the necessary prerequisite in the gelation of KGM? What is the relationship between hydrophobic interaction and deacetylation? It has been reported that 7 wt% and 8 wt% KGM could gel without the addition of alkali (Dave et al., 1998; Nishinari & Williams, 1992). Hence, it is interesting to consider one possibility that KGM gelation depend on the amount of acetyl groups of the polymer backbone and will be more easier with decreasing the amount of acetyl groups. Further investigation is required to confirm this hypothesis.

The primary objective of this research was to gain further insight into types of molecular bonding and interaction presented in KGM gels and effect of the amount of acetyl groups on the gelation behavior of KGM via investigating the rheological behavior. Furthermore, we aim to develop a better understanding of the effect of lyotropic series of salts on gelation behavior of KGM from a molecular origin.

2. Materials and methods

2.1. Materials

The native KGM which was a gift from Hubei Jian Shi Nong Tai Industry Co. Ltd. (Hubei, China) and further purified by ethanol extraction followed by vacuum-drying, was treated with different quantities of alkali (Na_2CO_3) in aqueous ethanol under the same reaction temperature and time, and then a series of deacetylated products with different degree of deacetylation coded as Da0, Da1, Da2, Da3, Da4, Da5 and Da6 were obtained.

Their weight average molecular weight (M_w) were determined in 1 M KSCN aqueous solution at 30 °C by gel permeation chromatography coupled with multi angle laser light scattering (GPC-MALLS), as shown in Table 1 (since deacetylation did not lead to a remarkable difference in molecular weight, in the following discussions, the difference in molecular weight between KGM samples was not taken into account).

All chemicals used in this study were of analytical grade reagents (Sinopharm Chemical Reagent Co. Ltd., Shanghai, China) and were used without further purification.

2.2. Determination of degree of deacetylation (DD)

Degree of deacetylation (DD) defined as the ratio of removed acetyl groups to total acetyl groups in the KGM backbone was examined by a modified Eberstadt method (Tanghe, Genung, & Mench, 1963) including saponification and successive titration. This method is based on ASTM volumetric method and usually used to determine the acetyl content in cellulose acetate. All reported data are averages of experiments performed three times at least.

2.3. Determination of solubility

0.10 g of KGM samples were dispersed in 24.90 g of crushed ice under an ice-water bath at 0 °C with vigorous stirring for approximate 1 h until crushed ice thawed thoroughly. Then sample solutions were centrifuged at 4000 rpm for 20 min in order to separate soluble and insoluble section. 10.00 g of the upper solutions were dried in an oven at 105 °C until constant (recorded as m) was obtained. The solubility was calculated by equation:

$$\text{Solubility}\,(\%) = \frac{m \times 2.5}{W} \times 100$$

where m is the weight of soluble section in 10 g upper solution, W is the total weight of KGM sample. Experiment was carried out three times and draw average number.

2.4. Fourier transform IR spectra measurements

Fourier transform infrared (FT-IR) measurements of KGM samples were carried out using a FT-IR Spectrometer (Nexus 470, Nicolet, USA) at a resolution of 4 cm^{-1} in the range 400–4000 cm^{-1}. KGM samples were prepared as KBr slices and were scanned against air background.

2.5. Rheological measurements

Powders of native and deacetylated KGM samples were dispersed in deionized water and salt solutions both which have been precooled to below 0 °C. The samples were completely dissolved within 1 h by mechanical stirring at an ambient temperature of below 0 °C. The samples solutions were centrifuged to degas at 4500 rpm for 10 min. Da3 was used as the main sample for most experiments unless as particularly described. The samples aqueous solutions were equilibrated at refrigeratory of 4 °C for the same time before dynamic viscoelastic measurements.

Dynamic viscoelastic measurements were carried out on a stress-controlled rheometer, Rheostress 600 (ThermHaake Co. Ltd., Germany). A parallel plate geometry with 35 mm in diameter and 1.0 mm gap was used to measure dynamic viscoelastic parameters. In order to avoid the destruction of the structure being formed, the stress in all measurements of the present work was set as 0.2 Pa, which is within a linear viscoelastic regime. For each measurement, the sample was poured directly onto the lower parallel plate, which had been kept at each measurement temperature without preshearing or oscillating. Temperature control was established by connection with Haake Universal Temperature Controller kept within ±0.01 °C over an extended time. The dynamic temperature sweep measurements were conducted from 15 to 65 °C at a frequency of 1 rad s^{-1} and with heating or cooling rates of 1 °C min^{-1}. The gelation kinetics was studied at constant temperature as a function of time at a frequency of 1 rad s^{-1}.

3. Results and discussion

3.1. Preparation and physicochemical properties of KGM samples with different degree of deacetylation

In the present work, a heterogeneous method for deacetylation of KGM was employed in aqueous ethanol in the presence of alkali. Powders of KGM samples with different degree of deacetylation (DD) were obtained. The values of degree of deacetylation (DD) of KGM samples are listed in Table 1. Under the same ethanol concentration, temperature and reaction time, the addition of higher amount of alkali is favorable for higher DD.

Moreover, it is equally of interest to observe that, in Table 1, the solubility of KGM decreased with increasing DD. To give straight evidence, solution behavior of KGM samples with different DD was examined by visual observation (as shown in Fig. 1). Da0, Da1 and Da2 presented transparent liquid yet Da3 and Da4 presented opaque state. The simplest interpretation here is that since steric hindrance is removed with decreasing the amount of acetyl groups,

Table 1
Physicochemical properties of KGM with different degree of deacetylation.

	Sample						
	Da0	Da1	Da2	Da3	Da4	Da5	Da6
DD (%)[a]	0.00 ± 0.12	16.87 ± 0.32	32.58 ± 0.23	51.90 ± 0.33	67.32 ± 0.29	80.51 ± 0.30	98.28 ± 0.16
Solubility (%)[a]	82.90 ± 1.85	81.65 ± 1.32	81.56 ± 3.27	81.12 ± 3.23	75.32 ± 3.05	70.61 ± 2.98	31.68 ± 1.56
$M_w \times 10^5$ (g mol^{-1})[b]	7.47	–	–	–	–	5.89	5.58

DD, degree of deacetylation; M_w, weight average molecular weight; R_g, weight average radius of gyration; polydispersity, M_w/M_n.
"–" means no available data.
[a] Data are mean ± SD of 3 replicates.
[b] Measurement by gel permeation chromatography coupled with multi angle laser light scattering (GPC-MALLS) in 1 M NaSCN aqueous solutions at 30 °C.

the polymers are more free to associate to form more numerous junction zones. However, turbidity of Da4 was smaller than Da3 and showed a heterogeneous state owing to effective concentration decreases with reducing of water solubility of Da4. With increasing DD, Da5 and Da6 presented slightly swelling conditions. This result implies that acetyl groups control the water solubility of KGM, confirming that the presence of acetyl groups confers solubility on KGM in aqueous solution although the precise role of the acetyl groups in promoting solubility is still a matter of controversy.

3.2. Effect of degree of deacetylation (DD) on gelation properties of KGM

As above mentioned, the water solubility of KGM samples reduced with increasing DD. So, KGM with the higher DD aqueous solution cannot be obtained at ambient temperature leading to the limitation to the amount of usable samples in present work. Maekaji (1978) proposed that the peptization of KGM gels (deacetylated KGM) was easier at the lower temperature and cyanate had the stronger ability to peptize KGM gels than other reagents such as urea, since the addition of cyanate (salt-in salt) facilitate molecules to hydrate and make molecular chains of KGM more extent in water. Similar phenomenon has also been shown in methylcellulose (Xu, Wang, Tam, & Li, 2004) and curdlan (Funami & Nishinari, 2007) aqueous solutions in the presence of cyanate. Xu et al. (2004) concluded that it was caused by the weakening or destroying of hydrophobic interaction upon addition of salt-in salt and the addition of salt did not change the patterns of molecular forces for gelation. Hence, we attempted to solubilize KGM samples using above method and succeeded ultimately.

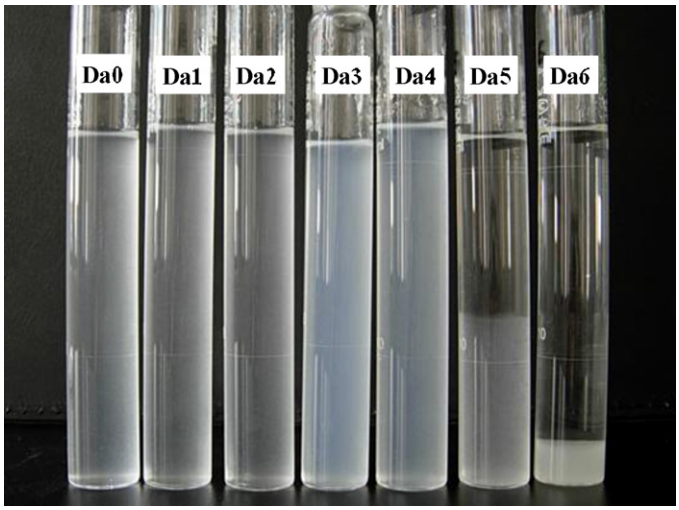

Fig. 1. Photos of 1% KGM samples with different DD solution at ambient temperature.

Temperature dependence of G' in a heating and cooling process for 1.5 wt% KGM with different DD in 0.2 M NaSCN aqueous solutions is shown in Fig. 2. In the case of KGM with lower DD (≤51.90%), G' decreased slightly with raising temperature up to about 50 °C then increased slightly in the heating process. In the cooling process from 65 °C, the rheological curves were not so different from the heating curves, indicating that no gel was formed in the heating process. As for Da4 and Da5, G' decreased slightly with raising temperature before 45 °C and then showed a steep increase until the end of the heating. And in the cooling process, G' almost remained as a constant, suggesting that a thermoirreversible gel was formed. It is obvious that the gelation behavior of KGM shows strong dependence on DD, implying that the presence of acetyl groups indeed plays an important role in the gelation behavior of KGM. It seems that there is a critical DD below which the gelation cannot form under the present experimental conditions. It is reasonable to presume that the difference between the samples will be enlarged, if all samples can be thoroughly dissolved in water other than 0.2 M NaSCN aqueous solutions, and the law still exists.

Rheological response of KGM with lower DD (≤51.90%) in 0.2 M NaSCN aqueous solutions is similar with that of the high methoxy pectin gel (Case et al., 1992) which is representative of stabilization of the gel through a combination of hydrogen bonding and

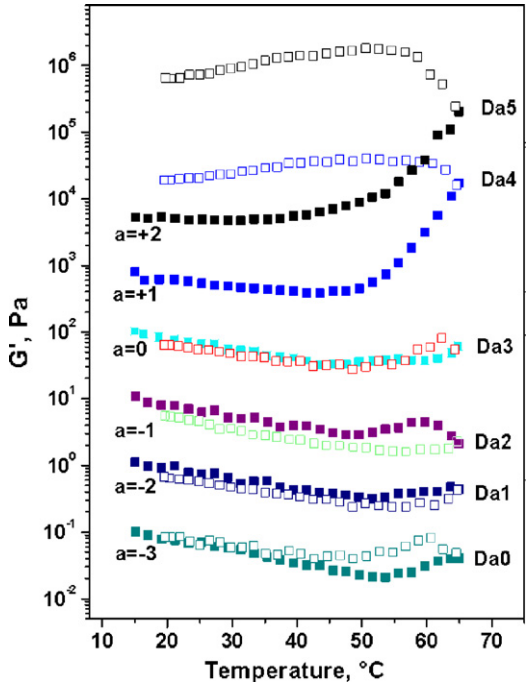

Fig. 2. Temperature dependence of G' in a heating (closed) and cooling (open) process for 1.5% KGM with different DD in 0.2 M NaSCN aqueous solutions at a scanning rate of 1 °C min^{-1}. The data are shifted along vertical axes by 10^a to avoid overlapping.

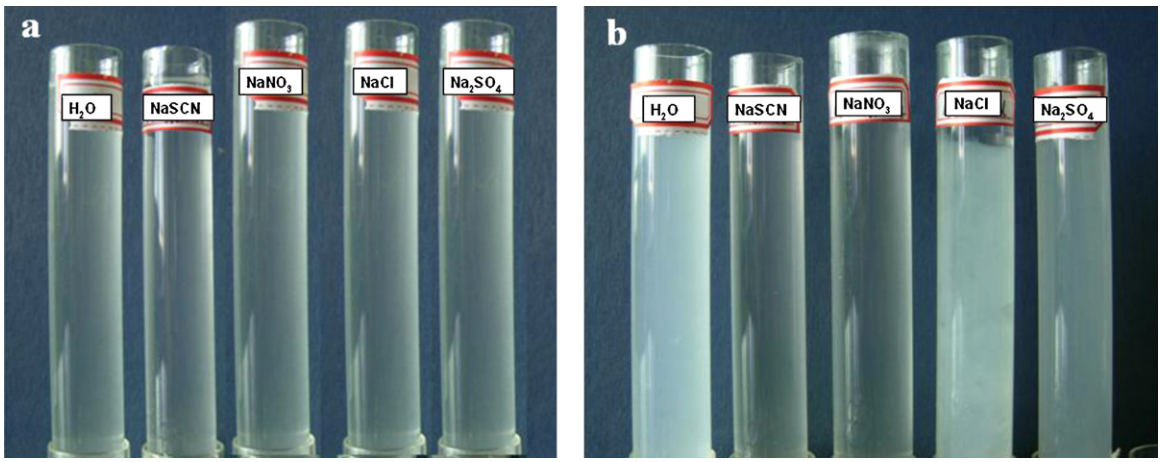

Fig. 3. Photos of 1.5% Da0 (a) and Da3 (b) in water and salts aqueous solutions without heating. The concentrations of NaSCN, $NaNO_3$, Na_2SO_4 and NaCl were 1 M, 1 M, 0.1 M and 1 M, respectively.

hydrophobic interaction. Oakenfull (1984) reported that bonding in a ratio such that the hydrogen bonding had a greater contribution to the stabilization than did the hydrophobic interactions in the high methoxy pectin gel. Whereas, rheological response of KGM with higher DD (>51.90%) in 0.2 M NaSCN aqueous solutions is similar with that of curdlan (Zhang, Nishinari, Williams, Foster, & Norton, 2002). It has been reported that hydrogen bonding and hydrophobic interaction are both responsible for network formation of curdlan and hydrophobic interaction has a more remarkably influence on gelation behavior. Therefore, the present result can be explained by considering both hydrogen bonding and hydrophobic interaction as molecular forces for KGM gelation formation and various DD leading to difference of the ratio of hydrogen bonding to hydrophobic interaction. Although a detailed dynamic molecular level picture of the aggregation process is lacking, it is deduced that while hydrogen bonding is not negligible in the gelation of KGM, this event is governed mainly by hydrophobic interaction with increasing DD.

3.3. *Identification of hydrophobic interaction of gelation of KGM*

There are three methods employed to identify the presence of hydrophobic interactions in polymers (Case et al., 1992): (a) the relative effects of organic solvent; (b) the relative effects of the lyotropic series of salts; (c) the use of fluorescent probe. In many instances, temperature for gelation is greater than the boiling point of the organic solvent leading to the limitation of the use of these organic solvents. In addition, the fluorescent probe used for monitoring the presence of hydrophobic interaction in polymers has become a classical method. However, the signal from fluorescence spectroscopy is strongly influenced by the transparency of sample solution. Basing on the properties of our samples, in this study, we attempted to use lyotropic salts to document the presence of hydrophobic interaction.

The lyotropic series (also called Hofmeister series) of salts has been used extensively with proteins to investigate hydrophobic interaction. Hofmeister (1888) found that salts could affect the solubility of proteins in water to various degrees. In general, salts may either enhance or reduce the hydrophobicity of a solute in water. The so-called "Hofmeister series" is an order of ions ranked in terms of how strongly they affect the hydrophobicity. According to this theory, ions can be classified as either kosmotropes (structure makers) or chaotropes (structure breakers). The structure makers or breakers refer to the ability of an ion to stabilize or weaken (or destroy) the structure of water, respectively. A typical Hofmeister order for ions was reported as $SO_4^{2-} > F^- > Cl^- > Br^- > NO_3^- > ClO^- > I^- > SCN^-$ and $Al^{3+} > Ca^{2+} > Mg^{2+} > H^+ > Na^+ > K^+$ (Zavitsas, 2001). Ions on the left hand, called kosmotropes, can be strongly hydrated, exhibiting strong interactions with water molecules. As a result, they tend to cause "salt-out" or to enhance hydrophobicity of a solute in water. In contrast, ions on the right hand (called chaotropes) can be weakly hydrated, tending to cause "salt-in", which increase the solubility of a nonpolar solute (Dougherty, 2001). And anions were proved to have stronger effects than cations because of the asymmetry of charge in a water molecule (Von Hipple & Wong, 1964; Xu et al., 2004). Lately, this method was also employed to study the nature of polysaccharides gels such as methylcellulose (Xu et al., 2004), curdlan (Funami & Nishinari, 2007) and hydroxypropylmethylcellulose (Liu, Joshi & Lam, 2008). Whereas, there are few works focused on the effects of salts on the sol–gel transition of KGM owing to the absence of significant hydrophobic side groups. Case et al. (1992) reported that Na_2SO_4 facilitated the gelation of KGM, whereas, NaSCN and $NaNO_3$ suppressed it. Yin, Zhang, Huang, and Nishinari (2008b) also found that the influence of the lyotropic salts on the gelation of KGM is dependent on the essential salting-out or salting-in nature of the salt. Here, we expect to develop a better understanding of the effects of lyotropic series of salts on gelation behavior of KGM from a molecular origin.

It is expected that the more significant influence of salts on KGM samples solutions should be presented with increasing DD due to the enhancing of hydrophobic interaction (basing on our foregoing analysis and guess). So, rheological response of 1.5% Da0 in various salts solutions at 50 °C were compared to that of Da3 (data not shown). Da3 in various salts solutions showed a more drastically difference as compared to Da0 as expected. Visual examination correlated well with rheological response of them, as shown in Fig. 3. It can be seen that, at least in present salts concentrations, there is little difference between the images for Da0, whereas there is evident difference between the images for Da3. Hence, the effects of various salts on gelation behavior of Da3 (partially deacetylated KGM) were studied systematically in detail by rheological measurements as following.

From some preliminary tests, we knew that Da3 could be more thoroughly hydrated to form homogeneous aqueous solutions in NaSCN and $NaNO_3$ aqueous solutions with any salt concentration than in water, yet could not be fully dissolved when Na_2SO_4 and NaCl concentrations were more than 0.2 M and 1.5 M, respectively. But Da3 could not form gels when NaSCN and $NaNO_3$ concentrations were too high. Therefore, the suitable salt concentrations of NaSCN, $NaNO_3$, Na_2SO_4 and NaCl in 1.5% Da3 ranged from 0.1 to 0.5 M, 0.3 to 2.0 M, 0.05 to 0.2 M and 0.25 to 1.5 M were used, respectively.

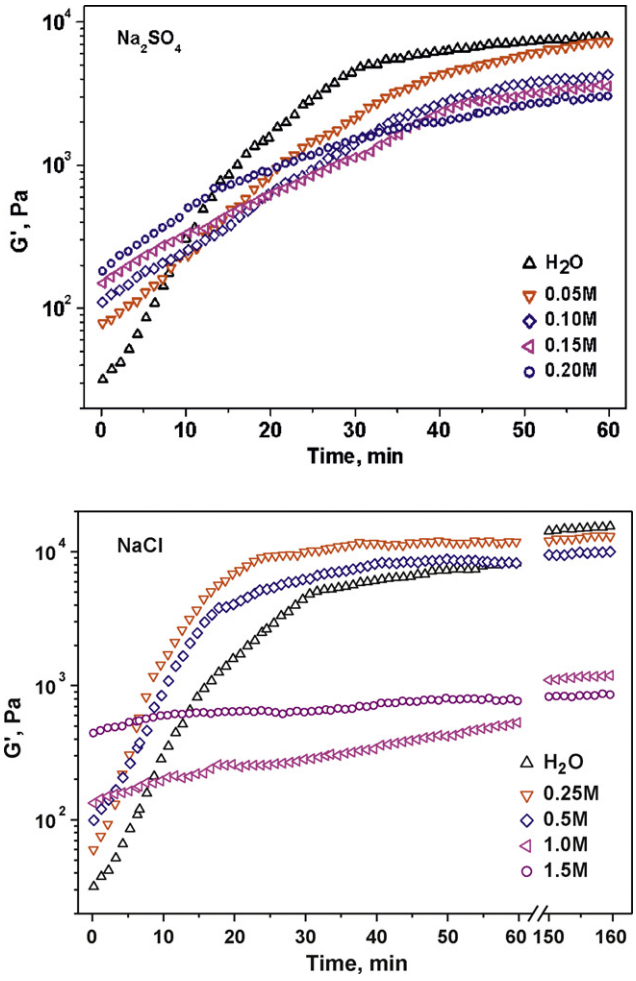

Fig. 4. Time dependence of G' for 1.5% Da3 in Na_2SO_4 and NaCl aqueous solutions with different concentrations at 55 °C.

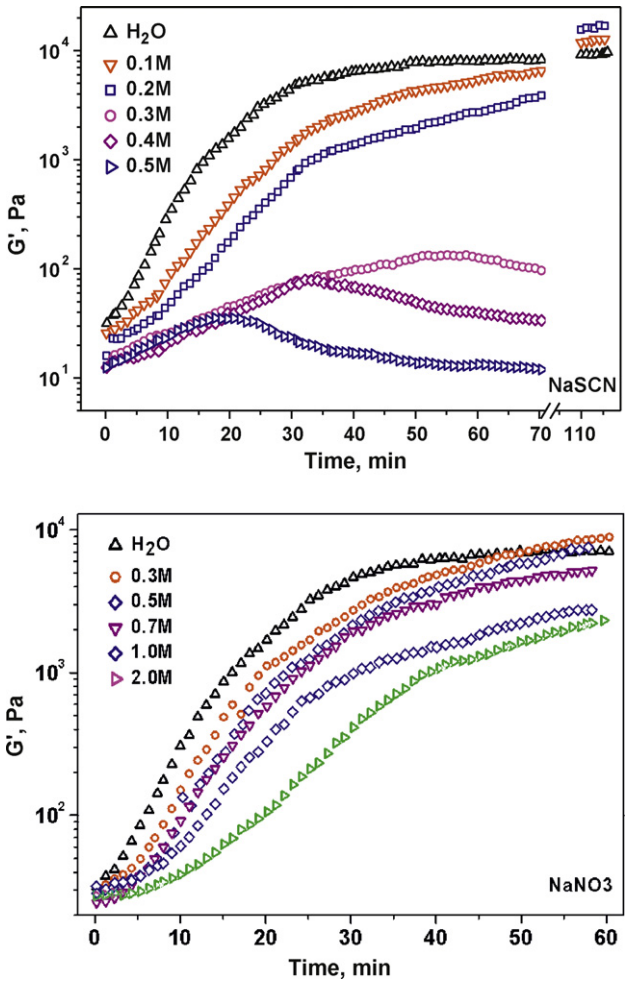

Fig. 5. Time dependence of G' for 1.5% Da3 in NaSCN and $NaNO_3$ aqueous solutions with different concentrations at 55 °C.

Figs. 4 and 5 show that the gelation behavior of Da3 was dramatically changed upon the addition of salts. In the presence of salt-out salts (Na_2SO_4 and NaCl), G' for Da3 salt aqueous solutions at the initial stage was larger than that for Da3 aqueous solutions (control). And the higher salt concentration, the greater the value of G'. However, G' of Da3 salt aqueous solutions were overtaken by that of Da3 aqueous solutions with time elapsing. Furthermore, G' for Da3 salt aqueous solutions was more smaller finally with the increase of salt concentrations, as shown in Fig. 4. Whereas, in the presence of salt-in salts (NaSCN and $NaNO_3$), the values of G' for Da3 aqueous solutions in the beginning of measurements were larger than that for Da3 salt aqueous solutions. However, G' for Da3 salt aqueous solutions tended to be more greater with increasing salt concentrations and exceed the value of G' for Da3 aqueous solutions with time elapsing excluding NaSCN concentration over 0.3 M. It was suggested that the slower the gel formed, the greater the value of G'. It was difficult to observe the exact plateau values of G' for Da3 in higher salts concentrations under the present experimental condition due to the long measuring time. Exceptionally, when NaSCN concentration was higher than 0.3 M, the plateau values of G' could not be observed, as shown in Fig. 5. Yin et al. (2008b) also found that when the concentration of NaSCN is up to 1 M, KGM could not form a gel. These findings correlate well with the previous studies (Case et al., 1992; Yin et al., 2008b) and the theory of salting-out and salting-in. The differences as compared to previous studies are that the amount of acetyl groups removed from KGM backbone is approximately only half of other studies and rheological curve is only a part of the whole gelation process due to the gel occurring in the dissolving process of Da3 (partially deacetylated KGM).

When the cation is same, variation of anions allows us to study the effects of anions. A summary of the effects of various anions on G'_0 which is the value of G' at 0 min reflecting the ability for salts to affect gelation formation indirectly is shown in Fig. 6. The approxi-

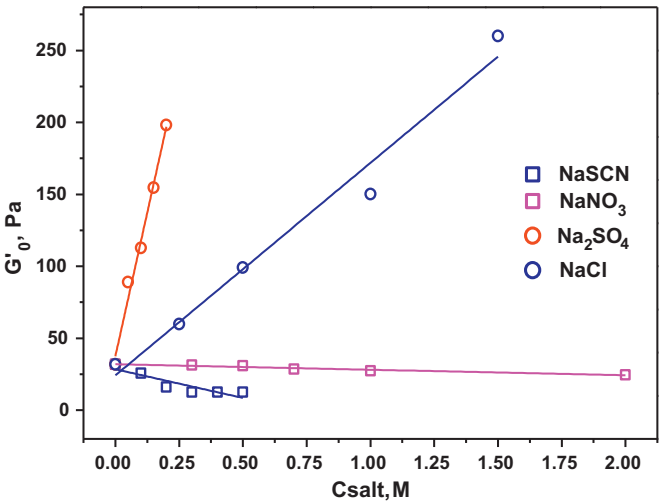

Fig. 6. The G' at 0 min (G'_0) as a function of salt concentration.

mately linear dependences of G'_0 on salt concentration are observed for all the concentration ranges studied. As shown in Fig. 6, the effects of salts on the G'_0 are remarkably different, resulting in the different slope for each line. The greater the salt effect, the steeper the trend line. In addition, a line with a positive slope means that the salt is a salt-out salt, while the down-going line indicates the salting-in trend. It is obvious that Na_2SO_4 had the stronger ability for salting-out than NaCl and NaSCN had the stronger ability for salting-in than $NaNO_3$.

With regard to molecular level mechanisms of the effects of salts on solutions, two viewpoints have been extensively accepted. One was proposed by Muta et al. (Muta, Kawauchi, & Satoh, 2003; Muta, Koji, Emi, & Satoh, 2002; Muta, Miwa, & Satoh, 2001), which considered the electron-pair acceptance/donation (EPA/EPD) ability of water. The hydration of ions changes the charge density on the water hydrogen and oxygen atoms, resulting in the change of EPA/EPD ability in water, and then causes the stabilization or destabilization of the solution system. This explanation is constructive, since for physical hydrogels hydrogen bonding is considered as one of the main reasons for the establishment or destruction of a solubilization balance. Collins (1997), on the other hand, reported that ion effects on water structure were caused by a competition between ion–water interactions and water–water interactions. The former was dominated by the charge density of ions, and the latter by hydrogen bonding. In our systems, the latter would be more useful to explain the rheological results of KGM hydrogels.

Salting-out nature. The addition of a salt will affect the structure of water, which is mainly due to the interactions between ions and water molecules. NaCl, which is categorized as a salt-out salt, exhibits stronger interaction with water than hydrogen bonding between water molecules. Thus, some of the original hydrogen bonding network (including cagelike structures) formed by water molecules is destroyed by the salt and this effect is similar to increasing temperature. In other words, ions tend to compete with KGM chains for the water molecules, and they succeed in attracting more water molecules surrounding them due to their stronger hydration abilities. This competition causes the decrease of KGM solubility in water. As a result, at the same temperature, there are more hydrophobic aggregates of KGM in a salt KGM solution than in a nonsalt KGM solution. Thus, upon heating it will be easier for a salted KGM solution to meet the requirement for the critical number of hydrophobic aggregates to form a gel, so that the gel is formed easily, or it is better to say that the salt accelerates the formation of a KGM gel. The increased salt content results in fewer free water molecules available around KGM chains and a stronger hydrophobic environment for KGM. Therefore, at higher salt concentration, G'_0 is greater. For the samples with various salt contents, the rheological curves appeared similar, merely amplitude tended to be mild with increasing salt content. In other words, the presence of salts did not affect mechanism of degelation or disassociation of KGM. The same conclusion was also put forward in the study of Methycellulose (Xu et al., 2004). When the anions have different charges, both ionic charge and size have to be taken into account. Our experiments show that the hydration ability is strongly affected by the ion's charge value. During the sample preparation, we noted that each salt used shows an individual concentration limitation with the same content of KGM sample in solution. If the added salt is higher than its concentration limit, the sample could not be fully dissolved. The salt concentration limit was found to be dependent on the anionic charges. In present study, for the single-charged ion (Cl^-), the salt concentration limit is about 1.5 M, while for the double-charged ion (SO_4^{2-}) they falls below 0.2 M. Thus, it is concluded that the higher the anionic charge, the stronger the salt-out effect, which results in lowering the necessary concentration of the salt that brings deacetylated KGM to aggregate. Bockris and Reddy (1998) also reported that the extent of ionic hydration depended upon the valence of the ion. Another possible explanation for salting-out phenomenon is by means of Gibbs equation. In the presence of a salt, more energy is consumed to break not only the hydrogen bonding (including cage structures) between the deacetylated KGM chains and water molecules but also the interactions between ions and water. As a result, the enthalpy change is positive and increases with the addition of a salt. With regard to the entropy change, according to the Gibbs equation ($\Delta G = \Delta H - T\Delta S$), since $\Delta H > 0$, ΔS must be positive to keep a negative value of the free energy change ΔG. The gelation involves the destruction of hydrogen bondings between deacetylated KGM chains and water as well as the interactions between ions and water molecules, which are relatively ordered at low temperatures. As temperature increases, the bonds between ions and water molecules are broken, and thus both ions and water become random and less ordered. So, these "free" ions would contribute more positive entropy change to the gelation process.

Salting-in nature. Although many salts such as Na_2SO_4 and NaCl assist in the fomation of gels, some salts such as NaSCN and $NaNO_3$ on the other hand seems to suppress the gel formation. According to Hofmeister (1888), both SCN^- and NO_3^- are classified as chaotropes or salt-in ions, which are weakly hydrated in water. These big singly charged ions, acting like hydrophobic molecules, exhibit weaker interactions with water than water itself. Song, Ryong, and Mu (1991) demonstrated from an experiment in NMR spectroscopy that SCN^- had a stronger binding affinity with poly(vinylpyrrolidone) in an aqueous solution. From this viewpoint, if adding a salt can create a more stable environment, the solubility of a polymer in water will be increased. Thus the salt-in behavior may be explained. However, considering the cage structure proposed previously for the gelation process of deacetylated KGM solutions, these big weakly hydrated ions are not likely to approach deacetylated KGM chains, let alone have a "binding affinity". More possibly, they can sit comfortably between deacetylated KGM chains, repulsing water molecules to the macromolecules. Alexandridis, Athanassiou, and Hatton (1995) attributed the salt-in behavior to two possible mechanisms: one was indirect where salt-in ions facilitate the hydration of nonpolar solutes; and the other was more direct, whereby they are assumed to have almost no effect on the water structure but replace some of the water molecules in the hydration shell of the solutes. The former explanation seems unsuitable to our system, as it is difficult to use this proposed facilitation to explain the salt-out phenomenon; the latter is recommendable to our study. Adding salt-in ions to a deacetylated KGM aqueous system to some extent is equal to adding another solvent or replacing water, so that deacetylated KGM chains are dispersed in the solution resulting in fewer chances of interconnection. Therefore, on heating it is more difficult for deacetylated KGM to form hydrophobic aggregates at the same temperature.

4. Conclusions

Gelation behaviors of konjac glucomannan (KGM) samples with different degree of deacetylation (DD) obtained using a heterogeneous deacetylation method were studied to probe the effect of degree of deacetylation on hydrophobic association. Compared to polysaccharides whose gelation mechanism is known, we speculate hydrogen bonding and hydrophobic interaction are both presented in KGM gelation and hydrophobic interaction plays an more important role increasing DD. Hydrophobic characteristic of partially deacetylated KGM was confirmed using the lyotropic series of salts. Furthermore, the effects of lyotropic series of salts on gelation behavior of KGM was clarified from a molecular level. Although a qualitative analysis for the types of molecular driving forces in

KGM gel was given, yet the magnitude of hydrophobic interaction with different DD cannot be obtained attributed the limitation of rheological measurements in present work. Fluorescent probe technology as a complementary methodology for quantification of hydrophobic interaction involved in KGM gels have been studied, supporting above results further (data to be submitted).

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