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Gelation mechanism of deacetylated rhamsan gum[☆]

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Abstract

Non-Newtonian behaviour and dynamic viscoelasticity of deacetylated rhamsan gum in aqueous solution were measured with a rheogoniometer. The flow curves, at 25 °C, of deacetylated rhamsan gum showed Newtonian behaviour at concentrations below 0.2%, but plastic behaviour above 0.3%. The yield value of the last named solutions was estimated to be 8.0 and 11.0 Pa at 0.3 and 0.5%, respectively. The elastic modulus increased with an increase in concentration and gelation occurred at a concentration above 0.3%. The elastic modulus for a solution of 0.3% decreased a little with increase in temperature up to 30 °C, then it showed a constant value with increase in temperature. However, when the temperature reached 50 °C, which was estimated to be a transition temperature, the elastic modulus decreased rapidly with further increase in temperature. The elastic modulus of the deacetylated rhamsan (0.2%) increased with addition of $CaCl_2$ (6.8 mM) and gelation occurred at low temperature (0 °C). The elastic modulus showed a little high value in the presence of urea (4.0 M). Possible gelation mechanism of the deacetylated rhamsan was proposed.

Keywords: Deacetylated rhamsan gum; Dynamic viscoelasticity; Gelation mechanism

Rhamsan gum is a bacterial polysaccharide produced by *Alcaligenes* ATCC 31961, and is non-gel forming but gives a thermostable, highly viscous solution even at high temperatures > 100 °C (Kang, Veeder, & Cottrel, 1983; Moorhouse, 1987). The main chain of the polysaccharide consists of tetrasaccharide repeating units, 3)-β-D-Glcp-(1 → 4)-β-D-GlcpA-(1 → 4)-β-D-Glcp-(1 → 4)-α-L-Rhap-(1, and every D-glucosyl residue next to the L-rhamnosyl residue is substituted at O-6 by α-D-glucosyl-(1 → 6)-β-D-glucosyl disaccharide side-chains (Jansson et al, 1986). The polysaccharide further contains approximately one *O*-acetyl group per repeating unit, substituting position of which is not known yet. The primary structure of rhamsan gum is similar to that of gellan gum and welan gum which also consists of the same tetrasaccharide repeating units without side chains for the former (Jansson, Lindberg, & Sandford, 1983; O'Neill, Selvendran & Morris, 1983) and with regular branching chain either at an α-L-rhamnosyl or β-L-mannosyl residue in the ratio of 2:1 for the latter (Jansson, Lindberg, Wildmalm, & Sandford 1985) molecule.

Lee and Chandrasekaran (1991) reported that rhamsan gum molecules formed half-staggered double helices stabilized by carboxylate–hydroxymethyl interaction with hydrogen bond in solid state, as in gellan gum molecules (Chandrasekaran, Pulgjaner, Joyce, & Arnott, 1988). The side chain–main chain interaction within the double helix prevent the carboxylate-mediated aggregation of double helices that is necessary for the gelation of rhamsan molecules.

We have proposed a possible mode of intra- and intermolecular associations of gellan gum in aqueous solution, as illustrated in Scheme 1 (Tako, Sakae, & Nakamura, 1989). The intramolecular hydrogen bonding may take place between the OH-4 group of the D-glucosyl residue and the adjacent hemiacetal oxygen atom of the L-rhamnosyl residue, and between OH-3 of the D-glucosyl residue and the adjacent hemiacetal oxygen atom of the D-glucuronosyl residue, to make gellan gum molecule rigid. The intermolecular association may take place between the methyl group and the hemiacetal oxygen atom or the methyl group of the L-rhamnosyl residues on different molecules with van der Waals forces of attraction by cage effect (Tako & Nakamura, 1988; Tako et al., 1989). In the presence of $CaCl_2$, an intermolecular Ca^{2+} bridge, as in ι-carrageenan (Tako, Nakamura, & Kohda, 1987) and alginic acid (Tako & Kohda, 1997), may also take place with ionic bonding

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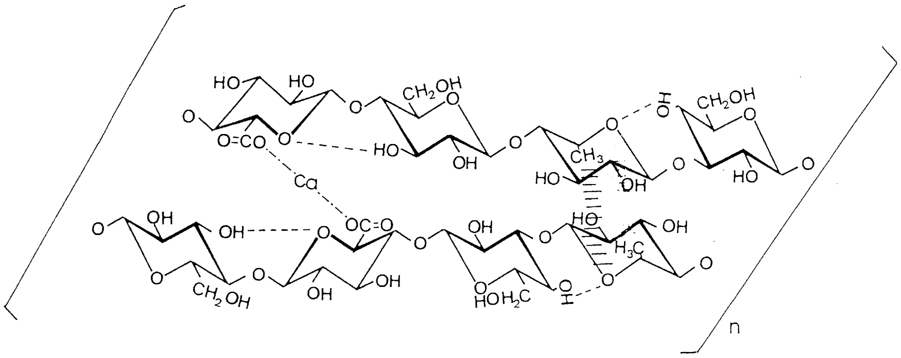


Scheme 1. Possible mode of intra-and intermolecular associations of gellan gum in aqueous solution. Association: - - - -hydrogen bonding; ·——· ionic bonding; / / / / van der Waals forces of attraction.

between the carboxyl oxygen atom of the D-glucuronosyl residues on different molecules.

We have also proposed a possible intramolecular associations of rhamsan gum (native) in aqueous solution, as illustrated in Scheme 2 (Tako, 1993a,b). The intramolecular associations may take place between the OH-4 of the D-glucosyl residue and the adjacent hemiacetal oxygen atom of the L-rhamnosyl residue with hydrogen bonding, and between the methyl group of L-rhamnosyl residue and the adjacent hemiacetal oxygen atom of the D-glucosyl residue with van der Waals forces of attraction where the L-rhamnosyl residues may adopt $^1C_4$ conformation in part (Tako, 1993b). The intramolecular associations may be dominant in thermal stable characteristics for viscosity and dynamic viscoelasticity of rhamsan gum molecules in aqueous solution. Such mechanism of the intramolecular associations have also been proposed in welan and S-657 gum molecules (Tako, 1994; Tako & Kiriaki, 1990). The main chain of the latter also consists of tetrasaccharide-repeating units, as in gellan, welan and rhamsan gum, but every D-glucosyl residue next to the D-glucuronosyl residue is substituted at O-3 by-L-rhamnosyl disaccharide side chains (Chowdhury, Lindberg, Lindberg, & Baird, 1987).

We report herein the flow behaviour and dynamic viscoelasticity of deacetylated rhamsan gum, and its rheological properties are analysed with respect to its association characteristics, in comparison with those of gellan (Tako et al., 1989) and native rhamsan gum (Tako, 1993a,b).

1. Materials and methods

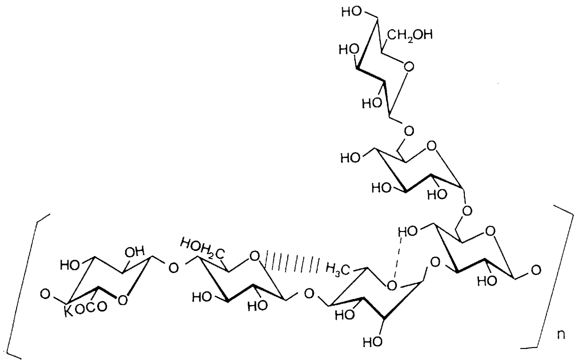
1.1. Materials

Native rhamsan gum was kindly supplied by CP-Kelco, California, USA and was dissolved in hot water (80 °C) as a 0.1% solution. The solution was heated at 90 °C for 20 min, and then cooled at room temperature, centrifuged at 46,000*g* for 1 h, and filtered through Celite 545 (which had been treated with boiling 3 M HCl for 30 min and washed with distilled water until pH 6.0). In the presence of 0.05% KCl, ethanol (2 vols.) was added to the filtrate and the precipitate was dried in vacuo. Purified native rhamsan gum was redissolved in hot water and the solution was deionized by passage through a column of Amberlite IR-120 (H^+), and neutralized with 0.05 M KOH. The solution was filtered through Celite 545 again. Ethanol (2 vols.) was added to the filtrate in the presence of 0.05% KCl, and precipitate was dried in vacuo.

The gellan gum sample was also kindly supplied by CP-Kelco, California, USA and purified as previously described (Tako et al., 1989). For ^1H NMR analysis, the samples, native rhamsan and gellan gum (deacetylated), was prepared same procedure as described above except final stage by lyophilization, respectively.

1.2. Deacetylation of rhamsan gum

The rhamsan gum was dissolved in 0.2 M KOH and stirred at room temperature (25 °C) for 12 h under atmosphere of N_2 to make deacetylation (Tako & Nakamura, 1984; Tako, Yoza, & Tohma, 2000). The solution was neutralized with 0.1 M HCl. The solution was filtered through Celite 545. In the presence of 0.05% KCl, ethanol (2 vols.) was added to the filtrate, and the precipitate was dried in vacuo. For ^1H NMR analysis, the deacetylated



Scheme 2. Possible mode of intramolecular associations of native rhamsan gum in aqueous solution. Association: - - - -; hydrogen bonding; / / / / / van der Waals forces of attraction.

rhamsan sample was also prepared at final stage by lyophilization.

1.3. ^{1}H Nucelar magnetic resonance (NMR) spectroscopy

The ^{1}H NMR spectra were recorded on a FT-NMR spectrometer (JNM-α500, Nihondenshi, Co., Ltd, Japan) at 500.00 MHz (Tako et al., 1999; Tako et al., 2000). The native and deacetylated rhamsan gum (1.0%, W/V), and gellan gum was dissolved in 4 ml of D_2O, respectively, and recorded at 70 °C. The ^{1}H NMR chemical shifts were expressed in parts per million (ppm) relative to internal sodium 3-(trimethylsilyl) propionic-2,2,3,3-d4(TSP, 0.00 ppm), respectively.

1.4. Viscosity and dynamic viscoelasticity measurements

To compare the rheological behaviour of deacetylated rhamsan gum with that of native rhamsan and deacetylated gellan gum, the viscosity and dynamic viscoelasticity were measured under the same conditions as those of previous studies (Tako, 1993a,b; Tako et al., 1989).

Viscosity at various shear rates (1.19–95.05 s^{-1}) and dynamic viscoelasticity at a steady angular velocity (3.77 rad/s) were measured with a rheogoniometer equipped with a coaxial cylinder (18 mm diam.) and rotating outer cylinder (22 mm diam.), 60 mm long (IR-103, Iwamoto Seisakusho, Co., Ltd, Japan). Circulating oil from a Thermo-cool (LCH-130F, Toyo Co., Ltd, Japan) controlled the temperature of the sample over the temperature range from 0 to 85 °C, and raised it in steps at a rate of 1 °C/min. Shear rate ($\dot{\gamma}$), shear stress (τ), and apparent viscosity (η) were calculated using the equation of Margules (Harris, 1977). Dynamic viscosity (η') and elastic modulus (G') were calculated by a modification of Markovitz's equation (Markovitz, 1952). The mechanical loss tangent was calculated from the relationship tan $\delta = G'', /G'$ where G″ is the loss modulus ($\omega\,\eta'$) and ω is the angular velocity of the outer cylinder. The values reported are the means of two determinants.

2. Results and discussion

2.1. Identity of deacetylated rhamsan gum

The identity of the polysaccharide as deacetylated rhamsan gum was established by ^{1}H NMR spectroscopy, as illustrated in Fig. 1. The chemical shift for native rhamsan gum (A) of major signal at 1.916 ppm was attributed to the methyl proton of the acetyl groups (Tako et al., 2000). This acetyl group was removed by treatment in 0.2 M NaOH solution (B). The signals at 1.430 and 1.329, and 1.202 and 1.189 for the native rhamsan gum correspond to the methyl protons of the L-rhamnosyl residues (Jansson et al., 1986; Jansson et al., 1983; Jansson et al., 1985). The signals mean that there are two kinds of conformation on the L-rhamnosyl residues involved. These signals, however, converged as a major signal at 1.323 ppm by treatment of 0.2 M NaOH solution, the chemical shift of which was in agreement with that of the methyl protons of L-rhamnosyl residues of deacetylated gellan gum (not shown in the figure). This indicates that the conformations of L-rhamnosyl residues of deacetylated rhamsan gum have been converged to a single conformation by treatment of 0.2 M NaOH solution. The result means that the conformation of the L-rhamnosyl residues of the deacetylated rhamsan gum is identical with that of gellan gum molecule which is also deacetylated polymer.

2.2. Flow characteristic

As reported previously, the flow curves of native rhamsan gum showed plastic behaviour even at a concentration of 0.1% (W/V) and shifted over high shear-stress values in proportion to the solution concentrations up to 0.8% at 25 °C. However, a 1.0% native rhamsan solution shifted only slightly at higher shear-stress values than that at

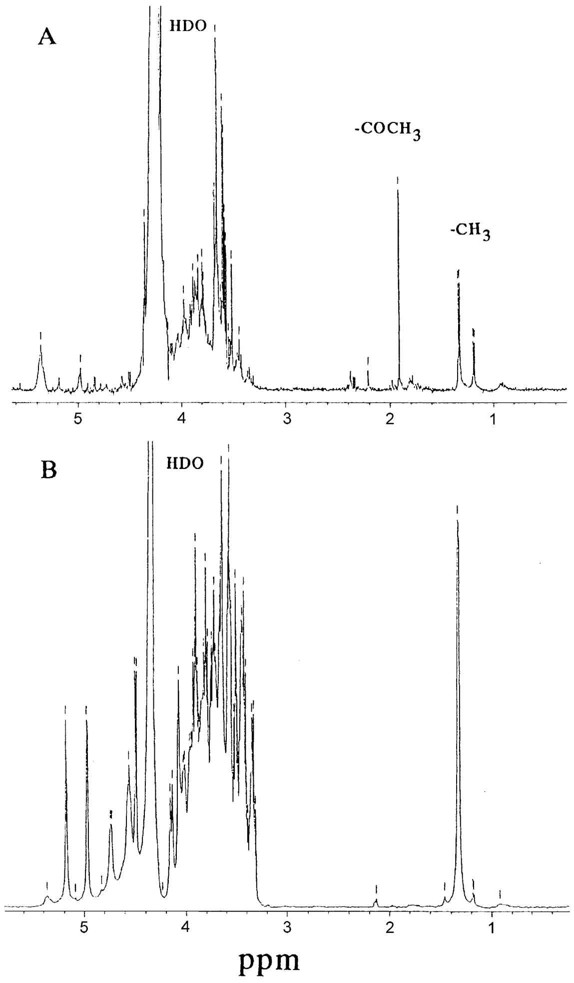


Fig. 1. ^{1}H-NMR spectra of native (A) and deaccetylated (B) rhamsan gum in D_2O at 70 °C.

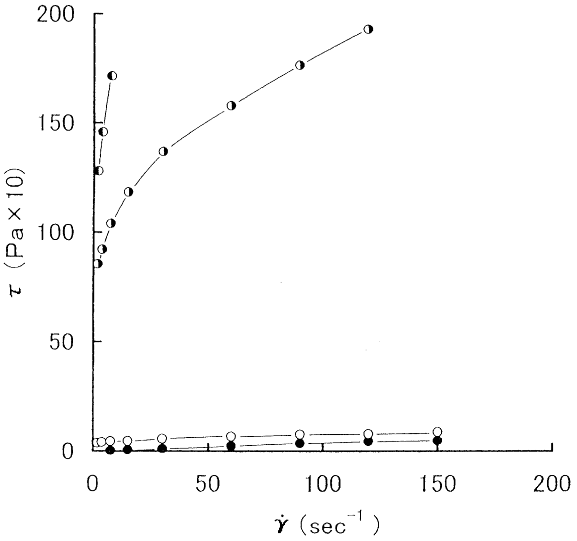


Fig. 2. Flow curves of deacetylated rhamsan gum in aqueous solution at 25 °C. Concentration: ●, 0.1%;○, 0.2%; ◑ 0.3%; ◐ 0.5%.

0.8% concentration. This might be due to the breakdown of the inter- or intramolecular associations under shear force (Tako, 1993,b). Consequently, the molecular chains of native rhamsan gum in aqueous solution are susceptible to orientation along shear force at high shear-rates. This might be caused by involving intramolecular associations, as mentioned above.

As shown in Fig. 2, though the flow curves of deacetylated rhamsan gum were shifted to very low shear stress and approximated to Newtonian behaviour at concentrations of 0.1 and 0.2%, it shifted to high shear stress and approximated to plastic behaviour above 0.3% at 25 °C. The yield value of the last named solution was estimated to be 8.0 and 12.0 Pa at 0.3 and 0.5%, respectively. Thus, the flow behaviour of the deacetylated rhamsan gum differed from that of native rhamsan (Tako, 1993a,b). The result indicates that an intermolecular association may take place above 0.3% solution of deacetylated rhamsan gum because the flow characteristic of native rhamsan gum was attributed to intramolecular hydrogen bonding and van der Waals forces of attraction, as illustrated in Scheme 2 (Tako, 1993a,b).

2.3. Dynamic viscoelasticity

As reported previously (Tako, 1993a,b), the elastic modulus of native rhamsan gum showed very high values at various concentrations (0.1–1.0%) and remained constant with increase in temperature. The tan δ values of native rhamsan decreased from 0.31 to 0.20 with increase in concentration from 0.1 to 0.5%. However, further concentration increases, from 0.8 to 1.0%, produced increases in tan δ values from 0.23 to 0.25. This might suggest that the breakdown of intramolecular associations take place with increasing concentrations under steady frequency.

As shown in Fig. 3, elastic modulus of deacetylated rhamsan gum showed low value at concentration of 0.1% and stayed constant with increase in temperature up to 10 °C, then it decreased gradually with further increase in temperature. The elastic modulus increased with increase in concentration from 0.2 to 0.5%. The elastic modulus decreased a little with increase in temperature up to 50 °C, which was estimated to be a transition temperature, then it decreased rapidly in 0.2% solution. A weak gelation occurred at low temperature and the elastic modulus stayed at a constant value with increase in temperature up to 50 °C, which was also estimated to be a transition temperature, then it decreased rapidly in 0.3% solution. The elastic modulus also showed a constant with increase in temperature up to 30 °C, then it contrary increased with further increase in temperature. However, the elastic modulus decreased rapidly when the temperature reached 50 °C, which was also estimated to be a transition temperature in 0.5% solution. On the other hand, tan δ showed a value of 0.63 in 0.1% solution at low temperature (0 °C) and increased gradually with increase in temperature up to 40 °C, then it increased rapidly with further increase in the temperature. The ***tan*** δ value decreased with increase in concentration 0.2 % (0.27) and stayed constant with increase in temperature up to 50 °C, then it increased rapidly. Though tan δ value also decreased with further

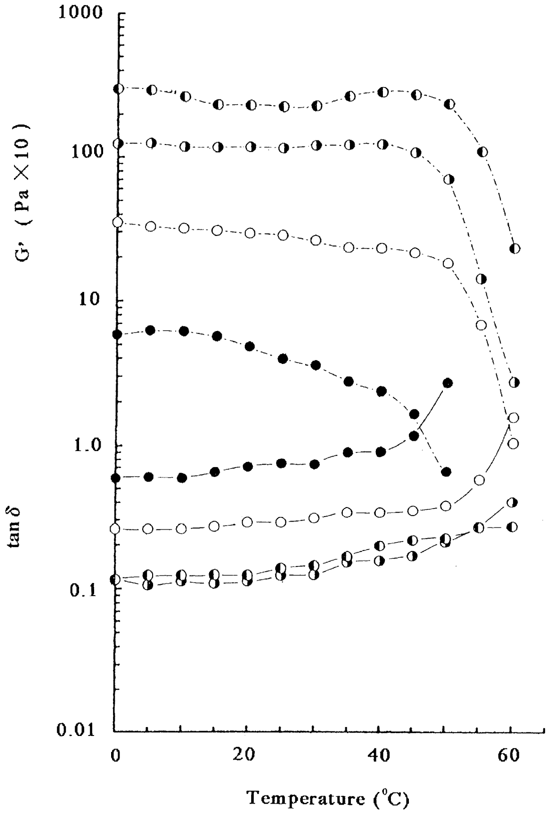


Fig. 3. Effect of temperature on elastic modulus of deacetylated rhamsan gum at various concentrations. Concentration: ●, 0.1%; ○, 0.2%; ◑, 0.3%; ◐, 0.5%.

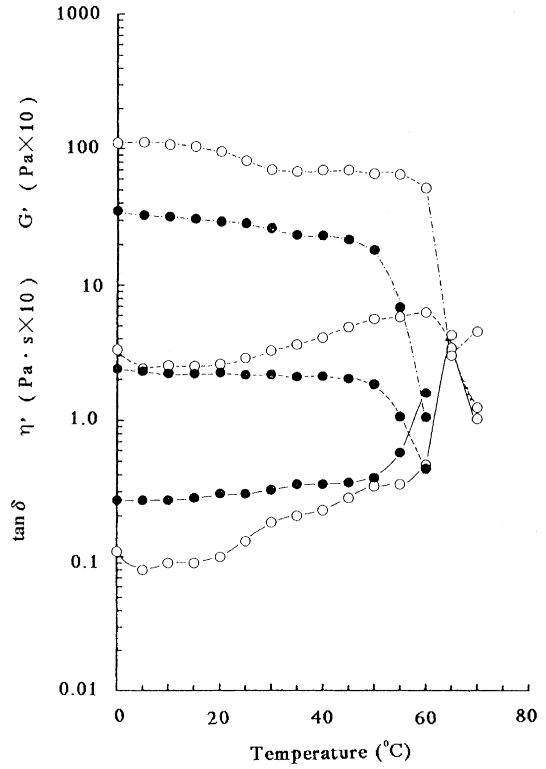


Fig. 4. Effect of temperature on dynamic viscoelasticity of deacetylated rhamsan gum (0.2%) with addition of $CaCl_2$ (6.8 mM). Symbols: ●, in aqueous solution; ○, with addition of $CaCl_2$.

increase in concentration to 0.3% (0.11), no more decrease was observed with increase in concentration (0.5%: 0.11). The tan δ value in the both solutions increased a little with increase in temperature and showed the same value at low temperature in 0.3% (0.13) and 0.5% (0.13) solution. The tan δ value stayed at a constant up to 20 °C, then it increased a little with further increase in temperature, respectively. The transition temperature as estimated in elastic modulus, at 50 °C, indicates that an intermolecular association dissociates above the temperatures.

A very high elastic modulus (10.1 Pa) was observed in 0.2% deacetylated rhamsan gum (3.4 Pa) with addition of $CaCl_2$ (6.8 mM) at low temperature (0 °C) and decreased a little with increase in temperature up to 30 °C, then it stayed constant with further increasing temperature. However, the elastic modulus decreased rapidly when the temperature reached 60 °C, which was estimated to be a transition temperature. On the other hand, dynamic viscosity showed only a little high value (0.3 Pa) than that in aqueous solution (0.23 Pa) and decreased with increase in temperature up to 5 °C, then it stayed at a constant value up to 20 °C. The dynamic viscosity, however, increased gradually with further increase in temperature up to 60 °C (0.64 Pa), which was estimated to be a transition temperature, then it decreased rapidly. The tan δ value decreased with addition of $CaCl_2$ at low temperature and decreased a little with increasing temperature up to 5 °C, it increased gradually with further increase in temperature up to 60 °C, which was estimated to be a transition temperature, then the value increased rapidly (Fig. 4).

As reported previously (Tako, 1993a,b), the dynamic viscoelasticity of native rhamsan gum kept almost the same value with addition of $CaCl_2$ (6.8 mM) in comparison with that of aqueous solution even at low temperature (0 °C). The results indicates that an intermolecular association take place with addition of $CaCl_2$ in deacetylated rhamsan gum solution (0.2%). The intermolecular association may be attributed to ionic bonding through Ca^{2+} cation between carboxyl groups of D-glucuronosyl residues on different molecules.

As shown in Fig. 5, dramatic increase in elastic modulus, which approximated about four times (46 Pa) than that of deacetylated rhamsan gum (10.1 Pa), was observed in 0.2% solution of gellan gum on addition of $CaCl_2$ (6.8 mM) at low temperature (0 °C) and increased a little with increasing temperature up to 80 °C, which was estimated to be a transition temperature, then it decreased rapidly. This behaviour suggests that gellan gum tightly binds Ca^{2+}, with carboxyl groups of the D-glucuronosyl residues contributing to cation-bridges between the different molecules with ionic bonding (Tako et al., 1989). This suggests that side-chains of deacetylated rhamsan gum, as same as native polymer (Tako, 1993a,b) prevent the intermolecular Ca^{2+} bridges on different molecules (Lee & Chandrasekaran, 1991).

The elastic modulus of deacetylated rhamsan gum solution (0.5%) increased a little with addition of urea (4.0 M) at low temperature (0 °C), as shown in Fig. 6. The elastic modulus, however, kept constant values during increase in temperature and decreased rapidly when the temperature reached 50 °C

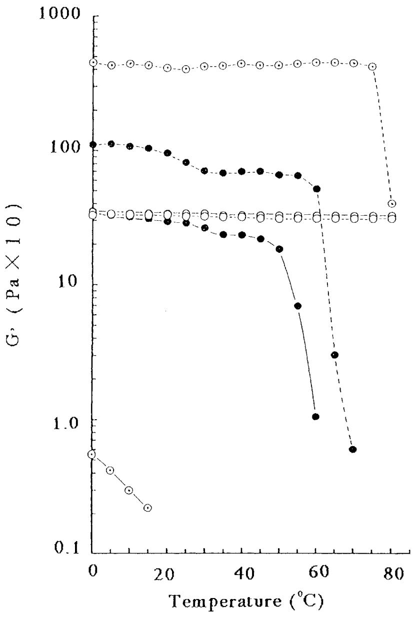


Fig. 5. Effect of temperature on elastic modulus of native rhamsan, deacetylated rhamsan and gellan gum at a concentration of 0.2% with addition of $CaCl_2$ (6.8 mM). The solid lines refer to aqueous solution and dotted lines to addition of $CaCl_2$. Symbols: ○, acetyl rhamsan; ●, deacetylated rhamsan; ◉, gellan gum.

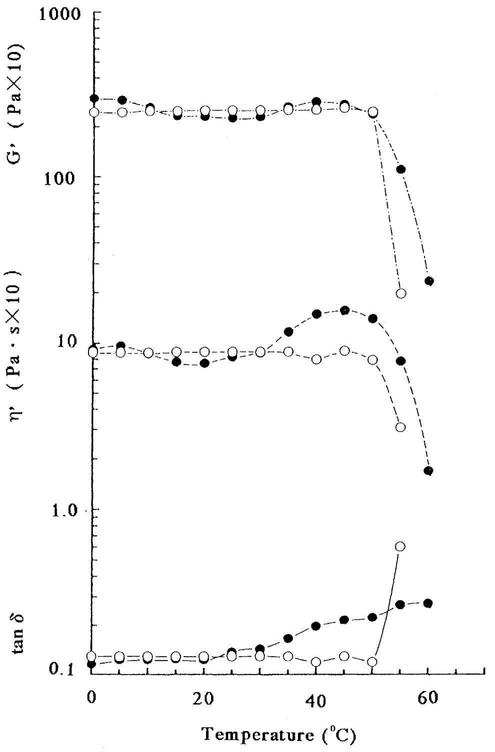


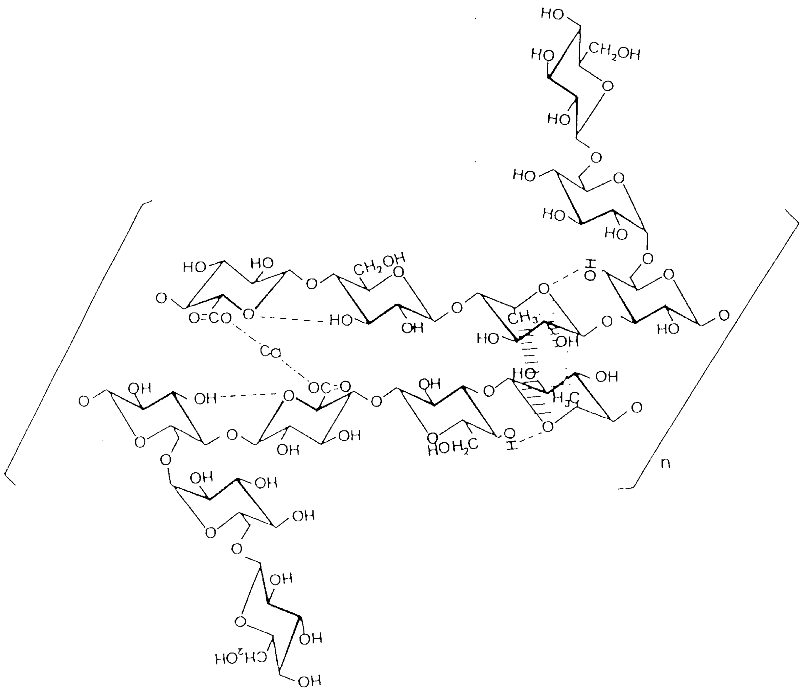
Fig. 6. Effect of temperature of dynamic viscoelasticity of deacetylated rhamsan gum at a concentration of 0.5% with addition of urea (4.0 M). Symbols: ●, aqueous solution; ○, with addition of urea.

which was estimated to be a transition temperature. As reported previously (Tako, 1993b), elastic modulus of gellan gum solution also increased on addition of urea (4.0 M) at low temperature (0 °C), but it decreased rapidly with increasing temperature. The dynamic viscosity also showed a little high value with addition of urea and kept a constant value during increase in temperature up to 50 °C, then it decreased rapidly. The tan δ also showed a constant value during increase in temperature up to 50 °C, then it increases rapidly. The transition temperature (50 °C) indicates that an intermolecular association dissociates above the temperature even in the presence of urea. The result suggests that a hydrophobic interaction between the methyl groups of the L-rhamnosyl residues on different molecules may participate in the interaction, because urea is a hydrogen bonding breaker.

3. Conclusions

Rheological characteristics of deacetylated rhamsan gum differed from those of native polymer: Gelation occurred in deacetylated rhamsan gum solution at a concentration of 0.3% at low temperature. A very high elastic modulus was observed with addition of $CaCl_2$ (6.8 mM), as in gellan gum (Tako et al., 1989). The elastic modulus showed a little high value in urea (4.0 M) solution as compared with that in aqueous solution. The results, however, essentially agreed with those of deacetylated gellan gum (Tako, 1993b; Tako et al., 1989). Particularly, the chemical shift of ^{1}H NMR spectra at 1.323 ppm for deacetylated rhamsan and gellan gum corresponded to adopting the same single conformation of L-rhamnosyl residues.

Thus, we propose possible intra-and intermolecular associations of deacetylated rhamsan gum molecules in aqueous solution, as illustrated in Scheme 3, which was



Scheme 3. Possible mode of intra- and intermolecular associations of deacetylated rhamsan gum in aqueous solution. Association: - - - - - hydrogen bonding; ·——·—— ionic bonding; / / / / van der Waals forces of attraction.

similar to that of gellan gum (deacetylated) molecules (Tako et al., 1989). The intramolecular hydrogen bonding may take place between OH-4 group of the D-glucosyl residue and adjacent hemiacetal oxygen atom of the L-rhamnosyl residue, and between OH-3 of the D-glucosyl residue and the adjacent hemiacetal oxygen atom of the D-glucuronosyl residue, to make the deacetylated rhamsan gum rigid (Tako et al., 1989). The intermolecular association may take place between the methyl group and the counter hemiacetal oxygen or methyl group of the L-rhamnosyl residues on different molecules with van der Waals interactions by cage effect (Tako, 2000; Tako & Nakamura, 1988; Tako et al., 1989). In the presence of $CaCl_2$, an intermolecular Ca^{2+} bridge, as in ι-carrageenan (Tako et al., 1987), gellan gum (Tako et al., 1989) and alginic acid (Tako & Kohda, 1997), may also take place with ionic bonding between the carboxyl oxygen atom of the D-glucuronosyl residues on different molecules. Although the L-rhamnosyl residues in part of native rhamsan gum may adopt a $^1C_4$ pyranose-ring conformation, they may exist in a $^4C_1$ pyranose-ring conformation in deacetylated rhamsan gum. Such a conformational changes of the L-rhamnosyl residues may contribute to the gel formation of deacetylated rhamsan gum, in which the methyl groups, which oriented at axial, of L-rhamnosyl residues may participate in the intermolecular van der Waals interaction which was derived from increasing elastic modulus in the presence of urea (4.0 M) in the both deacetylated rhamsan and gellan gum solutions.

Thus, the associations depicted in Scheme 3 may provide an explanation not only the rheological characteristics of native deacetylated rhamsan gum, but also for those of gellan gum (deacetylated) in aqueous solution.

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