

Note

Molecular Origin of Thermal Stability of Rhamsan Gum in Aqueous Media†

Masakuni TAKO

Department of Bioscience and Biotechnology, University of the Ryukyus, Nishihara, Okinawa 903-01, Japan

Received September 9, 1992

Rhamsan gum is a commercial polysaccharide produced by *Alcaligenes* ATCC 31961, and is non-gelforming but gives a thermostable, highly viscous solution even at high temperatures (108°C).¹⁾ 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.²⁾ The primary structure of rhamsan gum is similar to that of gellan^{3,4)} and welan,⁵⁾ which also consists of the same tetrasaccharide repeating units without side chains for the former and with regular branching chain either at an α-L-rhamnosyl or α-L-mannosyl residue in the ratio of 2:1 for the latter molecule.

We have proposed a possible mode of a gelation mechanism of gellan gum⁶⁾ and intramolecular associations of welan gum⁷⁾ molecules in aqueous solution. These polysaccharides, gellan, welan, and rhamsan gum, provide an interesting opportunity to investigate polysaccharide structure-function relationships. We report herein the flow behavior and dynamic viscoelasticity of rhamsan gum, and its rheological properties are analyzed with respect to its association characteristics, in comparison with those of gellan⁶⁾ and welan⁷⁾ gum.

Rhamsan gum was kindly supplied by Kelco Division & Co. and was dissolved in hot water (70°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 it reached pH 6.5). In the presence of 0.05% KCl, ethanol (2 vols.) was added to the filtrate, and the precipitate was dried *in vacuo*. Purified 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 50 mM 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 used was identical to that our preceding study and purified as previously described.⁶⁾ The welan gum used was also identical to that in our previous study,⁷⁾ and purified with similar methods as those for rhamsan gum. Viscosity at Various shear rates (1.19-95.03 s⁻¹) and dynamic viscoelasticity at a fixed frequency (3.77 rad/sec) were measured with a rheogoniometer (IR-103, Iwamoto Seisakusho, Co., Ltd.) and shear rate, shear stress, apparent viscosity, dynamic viscosity, and dynamic modulus were calculated as described in our preceding studies.⁶⁻¹¹⁾

The flow curves of rhamsan gum approximated to plastic behavior even at 0.1%, and the yield value was estimated to be 0.4, 1.0, 2.0, 3.2, 5.5, and 7.8 Pa at 0.1, 0.2, 0.3, 0.5, 0.8, and 1.0%, respectively. The flow curves of rhamsan gum solutions shifted to the high shear-stress side in proportion to the concentrations up to 0.8%. However, in 1.0% solution, it shifted to only a little higher shear-stress than that in 0.8%.

Though viscosity of gellan gum was large at low temperature (0°C), it decreased rapidly with increasing temperature.⁶⁾ The branched welan⁷⁾ and rhamsan gum were quite different viscosity properties. The viscosity of welan gum was very large at low temperatures, as with that of gellan gum, and decreased a little with increasing temperature up to 85°C. However, in the case of rhamsan gum, it stayed at the low value and increased gradually with increasing temperature up to 85°C. The increase of viscosity was also observed in a solution of native xanthan,⁸⁾ and deacetylated⁹⁾ and depyruvated¹⁰⁾ xanthan. This might be attributed not only to its formation of intramolecular associations to which the methyl group of acetyl residue contributed, but also to an increase of kinetic energy of the trisaccharide side-chains of xanthan.¹¹⁾

Despite showing low viscosity as mentioned above, the dynamic modulus of rhamsan gum showed very large values at various concentrations, as shown in Fig. 1. The dynamic modulus was constant during increasing temperatures at concentrations below 0.8%, but it increased gradually a little and showed a weak sigmoid curve in 1.0% solution. The phenomenon, showing sigmoid curve, was also observed in a solution of native⁸⁾ and deacetylated⁹⁾ xanthan. Though the tan δ value of the rhamsan gum decreased from 0.3 to 0.21 with increase in concentration from 0.1 to 0.5%, it increased to 0.23 and 0.25 with further increase of the concentration from 0.8 and 1.0% at low temperature (0°C).

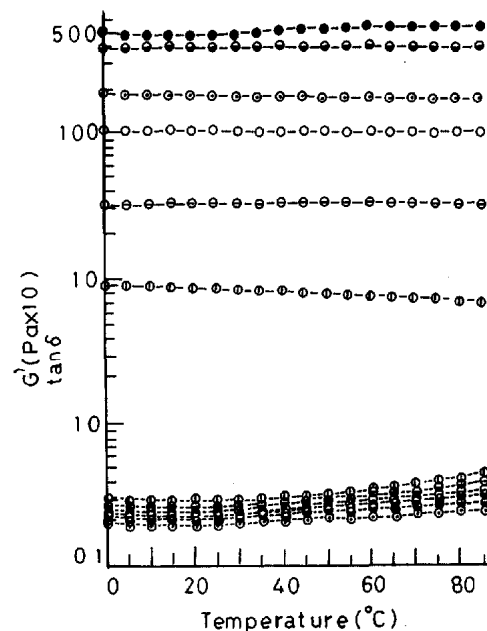


Fig. 1. Effects of Temperature on the Dynamic Modulus of Rhamsan Gum at Various Concentrations and 3.77 rad/s.

The solid lines refer to the dynamic modulus and broken lines to the tan δ. Concentrations: ○, 0.1%; □, 0.2%; △, 0.3%; ●, 0.5%; ⊙, 0.8%; ⊚, 1.0%.

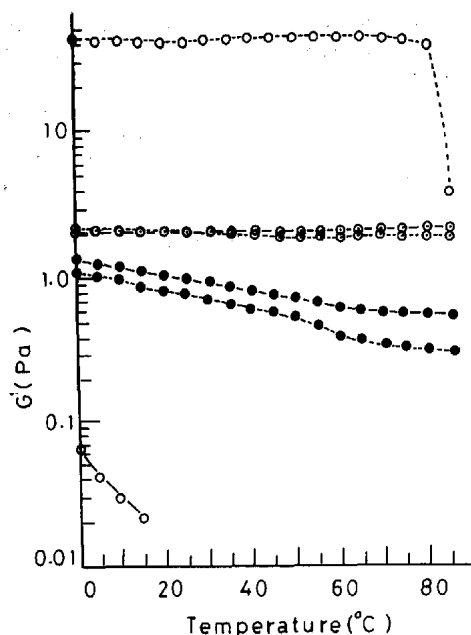


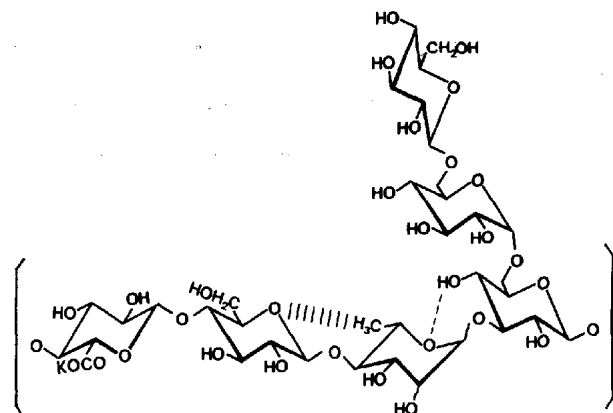
Fig. 2. Effects of Temperature on the Dynamic Modulus, at 3.77 rad/s, for Aqueous Solution of Rhamsan, Welan, and Gellan Gum at a Concentration of 0.2% after Addition of CaCl_2 (6.8 mM).

The solid lines refer to polysaccharide alone and broken lines to that with Addition of CaCl_2 (6.8 mM). \odot , Rhamsan; \bullet , Welan; \circ , Gellan Gum.

A very large dynamic modulus was observed on addition of CaCl_2 (6.8 mM) to a 0.2% solution of gellan gum.⁶⁾ The solution increased with the increase of temperature up to 80°C, and then it decreased rapidly, as shown in Fig. 2. This fact indicates that the gellan gum molecules associate tightly with Ca^{2+} where the carboxyl groups of D-glucuronosyl residues may contribute to cation-bridges on the different molecules with ionic bonding.⁶⁾ On the contrary, the dynamic modulus of welan gum solution (0.2%) decreased a little on addition of CaCl_2 ,⁷⁾ indicating that the side-chains of welan gum molecules⁵⁾ prevent the formation of Ca^{2+} bridges on different molecules. The dynamic modulus of rhamsan gum had almost the same values on addition of CaCl_2 during increase of temperature in comparison with polysaccharide alone. This also indicates that the side-chains of rhamsan gum prevent the formation of Ca^{2+} bridges between different molecules.

The dynamic modulus of gellan gum (0.8%) was higher than that of the polymer alone upon addition of urea (4.0 M) and gel formation was observed at low temperature (0°C); for welan gum, it was nearly independent of the addition of urea and kept a very large value during increases in temperature as in the polysaccharide alone.⁷⁾ A little decrease of the dynamic modulus, however, for rhamsan gum was observed on addition of urea. This may be due to the substitution of disaccharide side-chains, suggesting that the side-chains of rhamsan gum take part in an intramolecular hydrogen bonding with the backbone, as suggested by Talashek *et al.*¹²⁾

The dynamic viscoelasticity of the rhamsan gum solution (0.2%) was independent of pH change between 2.1 and 11.2 by addition of 10 mM HCl or $\text{Ca}(\text{OH})_2$. The tendency is in agreement with that of welan gum but disagreement with that of gellan gum.^{6,7)} However, the dynamic viscoelasticity of rhamsan gum in a pH 2.1 or 11.2 solution decreased rapidly when the temperature reached 75°C, which was estimated to be a transition temperature. This indicates that a secondary structure dissociates rapidly above the transition temperature (75°C) in pH 2.1 and 11.2 solution, respectively.



Scheme. Possible Mode of Intramolecular Associations of Rhamsan Gum in Aqueous Solution.

Associations; ----, hydrogen bonding; //, van der Waals interaction.

from those of the former but essentially agree with those of the latter. Thus, the rhamsan gum molecules seem to be involved in intramolecular associations as in welan gum molecules.⁷⁾ As shown in Scheme, 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 interaction. The intramolecular associations may be dominant in thermostable characteristics in rhamsan gum molecules in aqueous solution, as in the welan gum molecules.⁷⁾ Welan gum is primarily promoted for excellent stability for retention of viscosity even at a temperature of 140°C.⁴⁾ However, for rhamsan gum, when the temperature reached 108°C, which was estimated to be a transition temperature, the viscosity decreased rapidly.¹⁾ Furthermore, the dynamic modulus of rhamsan gum in pH 2.1 and 11.2 solution decreased rapidly when the temperature reached 75°C. Such decrease of the viscosity and dynamic modulus was not observed in a solution of welan gum.⁷⁾ These might be due to the dissociation of the intramolecular associations (Scheme) which might be caused by too large kinetic energy of the long side-chains of the rhamsan gum molecules at above temperatures (108 and 75°C).

A sigmoid curve of the viscosity and dynamic viscoelasticity of native xanthan during an increase in temperature might be essentially attributed to the involving two alternate intramolecular associations between the methyl group of the acetyl residue and the adjacent hemiacetal oxygen atom of the D-glucosyl residue, and between an alternate hydroxyl group at C-3 and the adjacent hemiacetal oxygen atom of the D-glucosyl residue.^{11,13)} This supports involvement of the intramolecular associations in rhamsan molecules, because a weak sigmoid curve was also observed in a solution of the polysaccharide. Accordingly, the Scheme provided an explanation not only for the rheological characteristics of the rhamsan gum, but also for those of the gellan,⁶⁾ welan,⁷⁾ and xanthan^{11,13)} gums in aqueous media.

Acknowledgments. The author thanks Dr. J. Baird, Kelco Division, Merk & Co., Inc. for providing the polymer samples, rhamsan and welan gum. The author acknowledges support of this investigation by a Grant-in-Aid for Scientific Research (No. 03806023) from the Ministry of Education, Science, and Culture of Japan.

References

- Carbohydr. Res.*, **156**, 157–163 (1986).
- 3) M. A. O'Neill, R. R. Selvendran, and V. J. Morris, *Carbohydr. Res.*, **124**, 123–133 (1983).
 - 4) P.-E. Jansson, B. Lindberg, and P. A. Sandford, *Carbohydr. Res.*, **124**, 135–139 (1983).
 - 5) P.-E. Jansson, B. Lindberg, G. Widmalm, and P. A. Sandform, *Carbohydr. Res.*, **139**, 217–223 (1985).
 - 6) M. Tako, A. Sakae, and S. Nakamura, *Agric. Biol. Chem.*, **53**, 771–776 (1989).
 - 7) M. Tako and M. Kiriaki, *Agric. Biol. Chem.*, **54**, 3079–3084 (1990).
 - 8) M. Tako, T. Nagahama, and D. Nomura, *Nippon Nōgeikagaku Kaishi*, **51**, 513–518 (1977).
 - 9) M. Tako and S. Nakamura, *Agric. Biol. Chem.*, **48**, 2987–2993 (1994).
 - 10) M. Tako and S. Nakamura, *Agric. Biol. Chem.*, **52**, 1585–1586 (1988).
 - 11) M. Tako and S. Nakamura, *Agric. Biol. Chem.*, **53**, 1941–1946 (1989).
 - 12) T. A. Talashek and D. A. Brant, *Carbohydr. Res.*, **160**, 303–316 (1987).
 - 13) M. Tako, *ACS Symp. Ser.*, **489**, 268–281 (1992).