Molecular Origin of Thermal Stability of Rhamsan Gum in Aqueous Media

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Rhamsan gum is a commercial polysaccharide produced by Alcaligenes ATCC 31961, and is non-gelling 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-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 gellan34 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 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 gellan8) and welan9) 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 in our preceding study and purified as previously described.9) 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 welan9) 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).

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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%.
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$_2$, indicating that the 
side-chains of welan gum molecules$^7$ 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 
than 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.$^{79}$ 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 Ca(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.

Though the primary structure of rhamsan gum is similar to 
that of gellan and welan gum, its rheological characteristics differ 
from those of the former but essentially agree with those of the 
latter. Thus, the rhamsan gum molecules seem to be involved 
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-rhamnopyranosyl 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.$^{10}$ However, 
for rhamsan gum, when the temperature reached 108°C, which 
was estimated to be a transition temperature, the viscosity 
decreased rapidly.$^{10}$ 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 
ergy 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 
especially 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.

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