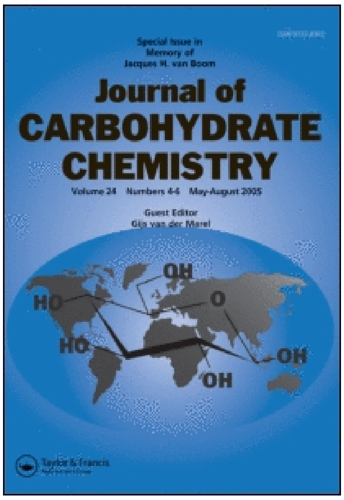

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MOLECULAR ORIGIN FOR THE THERMAL STABILITY OF RICE AMYLOPECTIN[1]

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ABSTRACT

The non-Newtonian behavior and dynamic viscoelasticity of Takanari and Reimei amylopectin solutions were measured with a rheogoniometer. The Takanari and Reimei amylopectin showed plastic behavior at a concentration above 2.0% at 25 °C. The viscosity of Takanari amylopectin decreased a little with increasing temperature at 2.0%. However, a little increase in the viscosity was observed with increasing temperature from 0 to 15 °C, then it stayed at a constant value with further increase in the temperature up to 80 °C at a concentration above 4.0%. An increase in the viscosity was also observed in Reimei amylopectin solution at various concentrations. The dynamic viscoelasticity of Takanari amylopectin increased with increasing concentration at low temperature (0 °C) and it stayed at a constant value with increasing temperature up to 80 °C. On the other hand, dynamic viscoelasticity for Reimei amylopectin showed a weak sigmoid curve. The tan δ of both amylopectins showed low values, 0.32-0.38, at low temperature range and kept constant with increasing temperature up to 80 °C. A little decrease of dynamic modulus of Takanari and Reimei amylopectin was observed upon addition of urea (4.0 M). The dynamic modulus of Takanari and Reimei amylopectin solution decreased rapidly when the temperature reached 45 and 60 °C, which was estimated to be a transition temperature, in 0.10 N NaOH solution. The molecular origin for the thermal stability of rice amylopectin (Takanari and Reimei) was essentially attributed to intramolecular associations in aqueous solution. Possible mode of intramolecular hydrogen bonding and van der Waals forces of attraction of amylopectin molecules are proposed.

INTRODUCTION

Amylopectin is a branched macromolecule composed of (1→4)-α-D-glucan chains which are joined together through α-(1→6) branched-points. On average, there is one branched point for every 18-19 main-chain residues for rice amylopectin molecule.[2-4] The most widely accepted model is a cluster model[5-8] in which short chains, which may be multiply branched, are arranged in a cluster on longer main-chains that are themselves linked together. Molecular weights of amylopectin[8] isolated from many plants are estimated to be in a range of of 5×10^6.

We have discussed the molecular origin for rheological characteristics and gelation mechanism of κ-carrageenan,[9,10] ι-carrageenan,[11] agarose,[12] gellan gum,[13-15] and curdlan[16] in aqueous solutions. We concluded that the rheological characteristics of these polysaccharide molecules might be essentially attributed to intra- and intermolecular associations in which the hemiacetal and ring oxygen atoms of sugar residues might play dominant roles.

Recently, we proposed a possible model of intra- and intermolecular hydrogen bonding of amylose molecules in aqueous solution.[17] Intramolecular hydrogen bonding might take place between OH-6 and the adjacent hemiacetal oxygen atom of the D-glucosyl residues within an amylose molecule. In addition, intermolecular hydrogen bonding might take place between the OH-2 and the adjacent O-6 of the D-glucosyl residues on different molecules to make a gel. Amylose molecules, however, in aqueous solution are notoriously unstable and retrogradation results in an increase in turbidity and eventually precipitation.[18] Accordingly, the retrogradation seems to occur by shrinkage of the amylose molecules which was caused by a decrease of the kinetic energy and Brownian motion of the polymer and water molecules. Much more intense intra- and intermoleculr hydrogen bonding might result in precipitation of the amylose molecules in aqueous solution.

In this study, we analyze the rheological behavior of a solution of amylopectin with respect to its association characteristics in comparison with that of amylose, and propose modes of intramolecular associations in aqueous solution. This work may offer a new concept for a thermal stability and retrogradation mechanism of amylopectin molecules in aqueous solution.

RESULTS AND DISCUSSION

Some characteristics of Takanari and Reimei amylopectin are summarized in Table I. Though the average chain length of Takanari and Reimei amylopectin showed almost the same values (18.8 and 19.0), number average degree of polymerization of the former had a much larger value (32,000), about double, than that of the latter (16,000). The blue value, λ_{max}, and limiting viscosity for the Takanari and Reimei amylopectin were estimated to be 0.071 and 0.075; 531 and 522nm; 153 and 154, respectively.

The flow curves, at 25 °C, of Takanari and Reimei amylopectin solution at various concentrations are shown in Fig. 1. The flow curve for Takanari amylopectin was approximated to plastic behavior and the yield value was estimated to be 0.3, 2.3, and 3.5 Pa at percentages of 2.0, 4.0, and 6.0%. The flow curves of Takanari amylopectin solution shift linearly with the glucan concentrations over high shear-stress. Similar plastic behavior was observed and yield value was estimated to be 0.2, 3.0, and 5.0 Pa at 2.0, 4.0, and 6.0% in Reimei amylopectin solution, respectively. This indicates that both Takanari and Reimei amylopectin molecues are involved in a secondary association in aqueous solution because yield value was estimated at various concentrations.[9-15]

Figure 2 shows the effect of temperature on viscosity of Reimei and Takanari amylopectin at various concentrations. The viscosity for Reimei amylopectin solution at 2.0% stayed at a constant value with increasing temperature up to 80 °C. However, a little increase in the viscosity was observed with increasing temperature up to 15 and 10 °C, then it stayed at a constant value with further increase in the temperature at 4.0 and 6.0% concentration, respectively. On the other hand, for Takanari amylopectin, the viscosity decreased a little with increase in temperature at a concentration of 2.0%. However, the viscosity increased a little with increase in the temperature up 5 and 15 °C, then it stayed at a constant value with further increase in the temperature at concentration of 4 and 6%, respectively, as in the case of Reimei amylopectin. The

Table 1. Characteristics of rice amylopectins

Amylopectin	$\overline{\text{D.P.n.}}$	$\overline{\text{C.L.n.}}$	$\overline{\text{C.L.w.}}$	Blue value	λ max	[η]
Takanari	32,000	18.8	22.0	0.071	531	153
Reimei	16,000	19.0	24.5	0.075	522	154

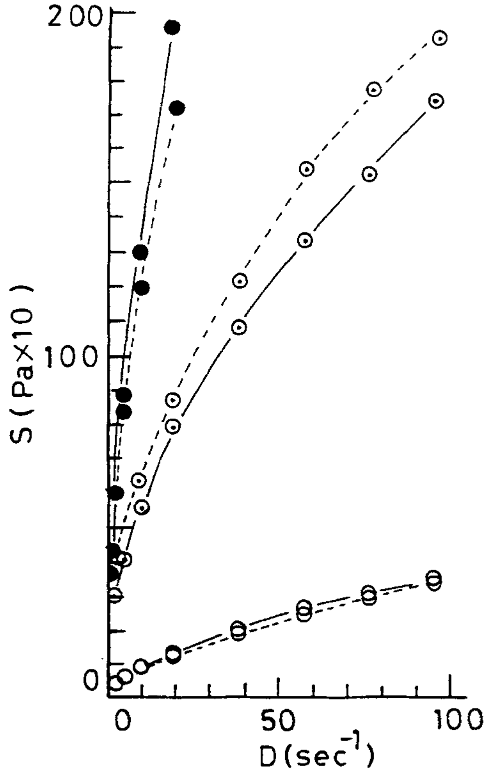


Figure 1. Flow curves of rice amylopectin at various concentrations and 25°C. The dotted lines represent Takanari amylopectin and solid lines Reimei amylopectin. Concentration: ○, 2.0%; ⊙, 4.0%; ●, 6.0%.

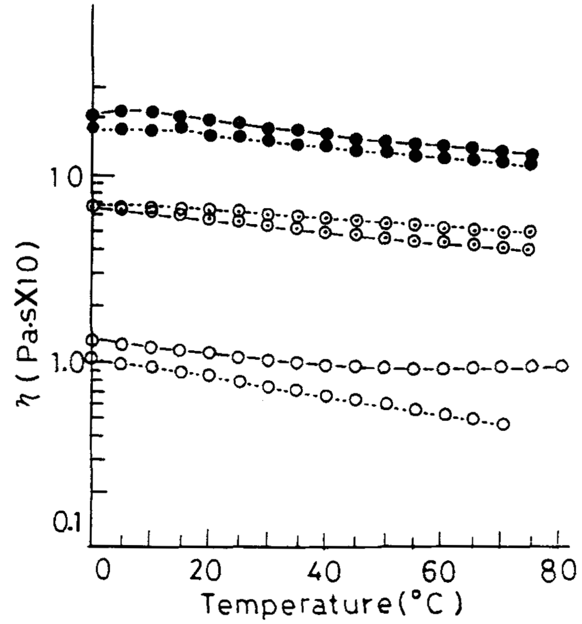


Figure 2. Effects of temperature on the viscosity of rice amylopectin at various Concentrations. The dotted lines represent Takanari amylopectin and solid lines Reimei amylopectin. Concentration: ○, 2.0%; ⊙, 4.0%; ●, 6.0%.

phenomenon, showing increase in the viscosity, was also observed in a solution of native xanthan,[19] deacetylated,[20] depyruvated,[21] and deacylated[22] xanthan. This might be attributed not only to its formation of intramolecular associations (to which the methyl group of the acetyl residues and the OH-3 of the D-glucosyl residues contributed), but also to an increase of kinetic energy of the trisaccharide side chains of xanthan. The results suggest that both Reimei and Takanari amylopectin molecules are involved in a secondary association in aqueous solution.

As shown in Fig. 3(A), dynamic viscoelasticity of Reimei amylopectin increased with increase in concentration and showed very large values at a concentration of 6.0%. The dynamic modulus decreased a little with increasing temperature up to 35 °C, then it increased gradually with further increase in the temperature at a concentration of 2.0%. For 4.0% solution, the dynamic modulus showed a constant value up to 50 °C, then it decreased a little. However, when the temperature reached 60 °C, the dynamic modulus increased again. A constant dynamic modulus was observed in a solution of 6.0% during

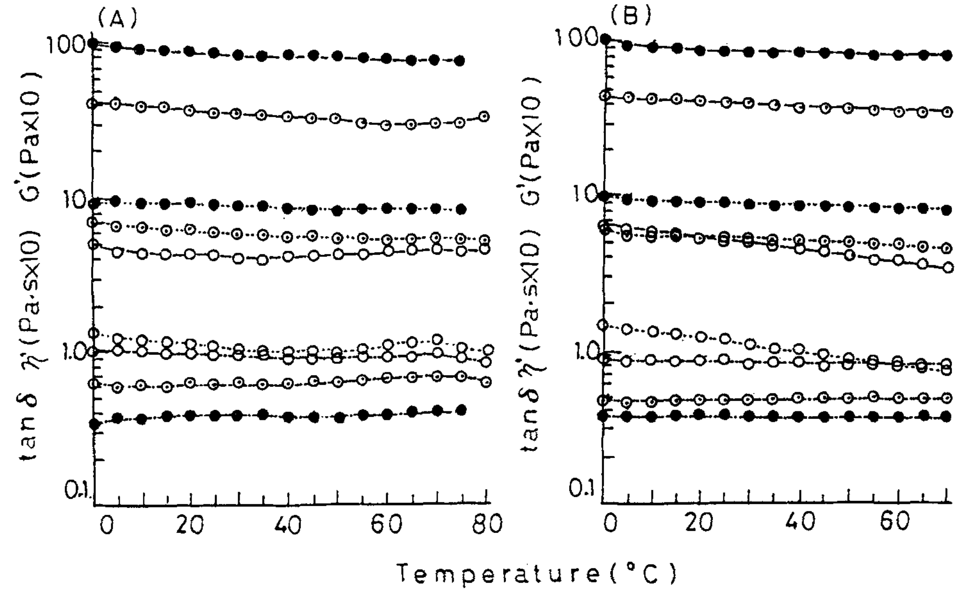


Figure 3. Effects of temperature on the dynamic viscoelasticity of Reimei (A) and Takanari (B) amylopectin at various concentrations. (----), Dynamic viscosity; (——), dynamic modulus; (·—·—), tan δ. Concentration: O, 2.0%; ⊙, 4.0%; ●, 6.0%.

increase in the temperature. A weak sigmoid curve was observed in solution of 2.0 and 4.0%, respectively. The dynamic viscosity also showed a sigmoid curve during increase in temperature at concentration of 2.0 and 4.0%, respectively. On the other hand, tan δ values decreased from 1.20 to 0.33 with increasing concentration from 2.0 to 6.0% at low temperature (0 °C), then showed a sigmoid curve, as that in dynamic viscoelasticity during increase in the temperature in a solution of 2.0 and 4.0%. The phenomenon, which shows a sigmoid curve, was also observed in a solution of native xanthan,[19] deacetylated xanthan,[20] rhamsan[15,23] and S-657[24] gum. The last-named polysaccharides, rhamsan and S-657 gum, are substituted disaccharide branched chains where intramolecular associations seems to be involved even at high temperature range, as those in xanthan gum.

For Takanari amylopectin dynamic modulus values were very large as were those for Reimei amylopectin at low temperature (0 °C) and various concentrations (Fig.

3(B)).The dynamic modulus decreased as did viscosity (Fig. 2) with increasing temperature at a concentration of 2%. However, it stayed at a constant value during increase in the temperature in 4.0 and 6.0% solution, respectively. The tan δ values for Takanari amylopectin also decreased from 0.88 to 0.36, as did those for Reimei amylopectin, with increase in the concentration from 2 to 6% at low temperature (0 °C), and stayed almost constant during increase in the temperature. In spite of showing large dynamic visco-elasticity, a sigmoid curve was not observed during increase in the temperature in Takanari amylopectin solutions. The results also suggest that Reimei and Takanari amylopectin molecules are involved in a secondary association in aqueous solution.

A little decrease in the dynamic modulus for Reimei and Takanari amylopectin (6.0%) was observed upon addition of urea (4.0 M) and large values were maintained during increase in temperature up to 80 °C (not shown in Figure). This indicates that a secondary association in both amylopectin (Reimei and Takanari) molecules is stable in the presence of urea (4.0 M) even at high temperature range (80 °C).

Figure 4(A) and (B) shows the effect of temperature on the dynamic modulus of Reimei and Takanari amylopectin (6.0%) after being dissolved in 0.05 and 0.10 N NaOH, respectively. In spite of a little decrease in the dynamic modulus in 0.05 N NaOH solution during increase in temperature, it stayed at low values and decreased rapidly when the temperature reached 60 and 45 °C, which was estimated to be a transition temperature in 0.10 N NaOH solution of Reimei (A) and Takanari (B) amylopectin, respectively. The dynamic modulus, however, recovered very large values by cooling. The tan δ values of Reimei and Takanari amylopectin in 0.10 N NaOH solution were higher than those in 0.05 N NaOH solution during increase in the temperature. They increased rapidly above the transition temperature, 60 and 45 °C, respectively. The result, showing transition temperature, indicates that a secondary association dissociates above the transition point.

A little decrease in the dynamic modulus of Reimei and Takanari amylopectin was observed in 85% dimethyl sulfoxide solution and large values were observed during increase in the temperature even at 85 °C (not shown in Figure). This indicates that a secondary association in both amylopectin (Reimei and Takanari) molecules is stable in a solution of 85% dimethyl sulfoxide even at a high temperature range (80 °C).

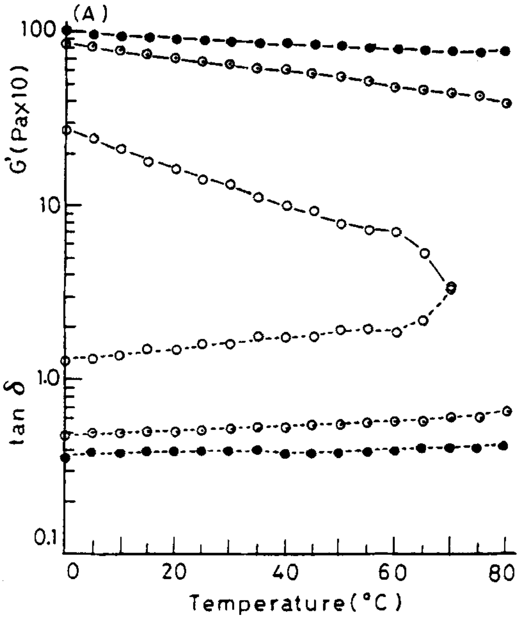


Figure 4(A). Effects of temperature on the dynamic modulus of Reimei amylopectin at a concentration of 6.0% in a NaOH solution. The solid lines represent the dynamic modulus and dotted lines tan δ. (○), In 0.05 N NaOH; (⊙), in 0.10 N NaOH; (●), in aqueous solution.

CONCLUSIONS

The rheological characteristics of amylopectin and amylose are quite different. Gelation did not occur even at a concentration of 6.0% for rice (Reimei and Takanari) amylopectin solutions at low temperature (0 °C) in aqueous solution. Thus, we propose that the rice amylopectin molecules are involved in intramolecular hydrogen bonding, as illustrated in Scheme 1, between the OH-6 and the adjacent hemiacetal oxygen atom of D-glucosyl residues. This bonding is thermally stable even at a high temperature range > 80 °C, as is intermolecular hydrogen bonding in amylose at a high temperature range.[17]

However, a little decrease of the dynamic modulus was observed upon addition of urea (4.0 M) for Reimei and Takanari amylopectin (6.0%) and large values persisted during an increase in temperature up to 80 °C. This suggests that another intramolecular

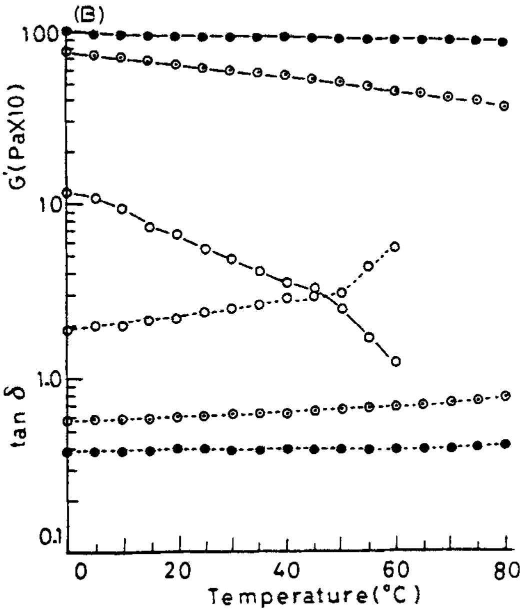
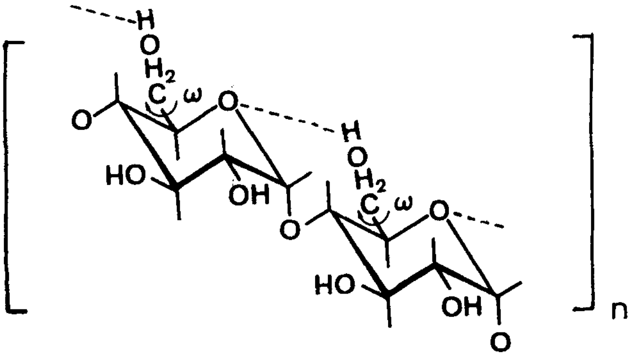


Figure 4(B). Effects of temperature on the dynamic modulus of Takanari amylopectin at a concentration of 6.0% in a NaOH solution. The solid lines represent the dynamic modulus and dotted lines to tan δ. (O), In 0.05 N NaOH; (⊙), in 0.10 N NaOH; (●), in aqueous solution.



Scheme 1. Possible mode of intramolecular hydrogen bonding of rice amylopectin. The dotted lines refer to hydrogen bonding.

association seems to be involved in amylopectin molecules in aqueous solution. Thus, we also propose that the rice amylopectin molecule is somehow involved in intramolecular association with van der Waals forces of attraction between linked glucosyl units. This interaction has also thermostable characteristic even at high temperature range <80 °C

The intramolecular hydrogen bonding and van der Waals forces of attraction proposed, however, dissociate above a transition temperature (45 and 60 °C) in 0.10 N NaOH solution. This may be due to increase in kinetic energies of the side chains of amylopectin molecules at temperature above 45 and 60 °C. Consequently, these intramolecular associations may have a dominant role in thermal stability of rice amylopectin molecules in aqueous solution.

EXPERIMENTAL

Materials. Waxy rice (Takanari and Reimei) were a gift from Dr. Maruyama (Ministry of Agriculture and Forestry of Japan). Amylopectins were isolated from the samples by the sodium dodecyl benzenesulfonate method.[25] Isolated amylopectins were stored at 4 °C, and then defatted by dissolving in dimethyl sulfoxide and precipitating with alcohol.[26]

Methods. Total carbohydrate was determined by the phenol-sulfuric acid method.[27] The reducing residue was assayed colorimetrically by Somogyi's method[28] using Nelson's[29] reagent, the heating time being extended to 30 min to give the same reducing power regardless of the chain length, or by the modified Park-Johnson method as previously described.[30] The number-average degree of polymerization ($\overline{\text{d.p.n.}}$) was calculated from the reducing residue and total carbohydrate values.

The number-average chain length ($\overline{\text{c.l.n.}}$) of each amylopectin was determined also by assaying reducing power after isoamylolysis, which was carried out with a 0.5%(W/V) solution for 12 h at 45 °C and pH 3.5 (50 mM acetate buffer) with *Pseudomonas* isoamylase (0.3 $U.mg^{-1}$; Hayashibara Biochemical Lab.).

The weight-avearge chain length ($\overline{\text{c.l.w.}}$) of each amylopectin was determined by HPLC combined with low angle laser-light-scattering photometry as described.[31] Blue values and λ_{max} of iodine-stained solutions were determined by the procedures described elsewhere.[32] The limiting viscosity number [η] was determined at 22.5 °C in M KOH with an Ostwald-type viscometer.

High-performance anion-exchange chromatography involved a Dionex BioLC Model 4000i system and a Model II PAD pulsed amperometric detector consisting of an amperometric flow-through cell with a gold working electrode, a silver-silver chloride reference electrode, and a potentiostat. The following pulse potentials and durations were used at range 2 (sampling period, 200ms): E_1 0.10 (t_1 300), E_2 0.60 (t_2 120), E_3 0.80 V (t_3,300 ms). The response time of the detector was set to 1.0 s. A Dionex HPIC-AS6 column (250*4mm i.d.) and an AG6 guard column (50*4 mm i.d.) were used. The eluent A was 150 mM sodium hydroxide prepared from carbonate-free aqueous 50% sodium hydroxide in 18 MΩ cm deionzed water. The eluent B was 150 mM sodium hydroxide containing 500 mM sodium acetate. The gradient programme was; % of eluent B = 40 at 0 min, 50 at 2 min, 60 at 10 min, and 80 at 40 min. A solution of debranched amylopectin (3 mg) in M sodium hydroxide (0.2 mL) was made with deionized water, and aliquots (20-30 µL) were analysed.

Viscosity and dynamic viscoelasticity measurements. Viscosity at various shear rates (1.19-95.03 s^{-1}) and dynamic viscoelasticity at a fixed frequency (3.77 rad s^{-1}) were determined with a rheogoniometer consisting of a coaxial cylinder (1.8 cm diam.) with a rotating outer cylinder (2.2 cm diam.). The temperature of the sample was controlled by circulating oil from a thermo-cool instrument (LCH-130F, Toyo Co., Ltd.), over the temperature range of 0-85 °C and raised at a stepwise rate of 1 °C min^{-1}. Shear rates (D), shear stress (S), and viscosity (η) were calculated with the equation of Margules.[33] Dynamic viscosity (η') and elasticity (G') were calculated by modification of Markovitz's equation[34] The loss tangent was calculated from the relationship, $\tan \delta = G''/G'$, where $G'' = \omega\eta'$ is the loss modulus, and ω is the angular velocity of the outer cylinder.

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