

Retrogradation Mechanism of Rice Starch¹

M. Tako² and S. Hizukuri³

ABSTRACT

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The non-Newtonian behavior and dynamic viscoelasticity of rice starch (Akihikari, 18.8% amylose content) solutions after storage at 25 and 4°C for 24 hr were measured with a rheogoniometer. The flow curves, at 25°C, of Akihikari starch showed plastic behavior >3.0% (w/v) after heating at 100°C for 30 min. The dynamic viscoelasticity of the starch increased after storage at 25 and 4°C for 24 hr and stayed at a constant value with

increasing temperature. A small dynamic modulus of rice starch was observed upon addition of urea (4.0M) at low temperature (0°C), but it produced a sigmoid curve when plotted against increasing temperature. A small dynamic modulus was also observed in 0.05M NaOH solution. However, it increased rapidly after the temperature reached 70°C. Possible models of retrogradation mechanism of rice starch were proposed.

We have proposed a possible model of the gelation mechanism of amylose molecules in aqueous solution (Tako and Hizukuri 1995). Intramolecular hydrogen bonding may take place between OH-6 and the adjacent hemiacetal oxygen atom of the D-glucosyl residues. This bonding is likely due to the flexibility of the α -(1→4)-linkage and extended conformations at high temperatures. In addition, intermolecular hydrogen bonding may take place between OH-2 and an adjacent O-6 of the D-glucosyl residues on different molecules. A part of the intermolecular hydrogen bonding, side-by-side association, breaks down above a transition temperature of 25–35°C. Residual intermolecular, together with intramolecular, hydrogen bonding is lost above another transition temperature at 80–90°C.

We have discussed the molecular origin for the thermal stability of rice amylopectin in aqueous solution and concluded that the molecules are involved in intramolecular hydrogen bonding and van der Waals forces of attraction (Tako 1996; Tako and Hizukuri 1997, unpublished). Intramolecular hydrogen bonding, together with van der Waals forces of attraction, may play a dominant role in the thermal stability of viscosity and dynamic viscoelasticity of rice amylopectin in aqueous solution. Long chains (B3–4) of rice amylopectin may be involved in intramolecular associations.

We have previously proposed a gelatinization mechanism for rice starch (Nihonbare) in aqueous suspension (Tako and Hizukuri 1999). An intermolecular hydrogen bonding of Nihonbare rice starch may take place between O-6 of the amylose and OH-2 of the amylopectin molecules as illustrated in Fig. 1. The short amylopectin chains (A and B1), which are not involved in intramolecular associations, may take part in the intermolecular associations. Intermolecular hydrogen bonding between amylose and amylopectin molecules is thermally stable in the presence of water. This bonding is liable to dissociate with increasing temperature under shearing-flow but is stable under angular flow. However, intermolecular hydrogen bonding, together with intramolecular association within long chains (B3–4) of amylopectin molecules, dissociates above the transition temperature (>50°C) in solutions of 4.0M urea and 0.05M NaOH.

In this study, we have analyzed the rheological behavior of a rice starch (Akihikari) solution after storage at 25 and 4°C for 24 hr with respect to its association characteristics in comparison with solutions of potato amylose and rice amylopectin. We propose a possible mechanism of retrogradation that may offer a new concept of the retrogradation mechanism of rice starch in aqueous solution.

MATERIALS AND METHODS

Materials

Rice starch (Akihikari) harvested in Aomori Prefecture, Japan, was prepared using the alkaline leaching method from polished flour. The extract was fractionated into amylose and amylopectin following the method of Takeda et al (1986). The yield of amylose and amylopectin from 10 g (dry weight) of starch was 1.5 and 7.2 g, respectively.

Methods

Total carbohydrate was determined by the phenol-sulfuric acid method (Dubois et al 1956). Reducing residue was assayed colorimetrically following the method of Somogyi (1952) using Nelson's reagent (Nelson 1944). The heating time was extended to 30 min to give the same reducing power regardless of chain length. Iodine affinity was determined at 25°C by modified amperometric titration (Larson et al 1953). Blue value was determined by a described procedure (Takeda et al 1983). The nonreducing residue was determined by rapid Smith degradation with photometric or fluorometric assay of glycerol (Hizukuri et al 1981). The number-average degree of polymerization of the amylose and amylopectin was determined by the modified Park-Johnson method (Hizukuri et al 1981).

The number-average chain length was determined also by assaying reducing power after isoamylolysis, which was done in a 0.5% solution at 45°C, pH 3.5 (50 mM acetate buffer), for 12 hr with *Pseudomonas* isoamylase (0.3 U/mg; Hayashibara Biochemical Lab., Japan).

The weight-average chain length of amylopectin was determined by HPLC combined with low-angle laser-light-scattering photometry

TABLE I
Properties of Akihikari Starch

Properties	Starch	Amylose	Amylopectin
Iodine affinity (g/100 g)	4.20	20.7	0.38
Blue value (680 nm)	...	1.57	0.077
β -amylolysis limit (%)	...	88.0	59.0
Number-average degree of polymerization	...	980	...
Weight-average distribution	...	3,800	...
Number-average chain length	19
Amylose content	18.8	100	0

TABLE II
Distribution of Chain Lengths of Akihikari Amylopectin

Fraction	Whole	B4	B3	B2	B1+A1+A2
Chain length					
Number-average	19.0
Weight-average	...	620	83	40	16
Weight (%)	100	1.6	3.8	19.6	75
Mole (%)	100	0.05	0.87	9.38	89.7

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² Associate professor, Department of Bioscience and Biotechnology, University of the Ryukyus, Nishihara, Okinawa 903-0123, Japan.

³ Professor emeritus, Department of Biological Science and Technology, Kagoshima University, Kagoshima 890-0065, Japan.

as described (Hizukuri and Takagi 1984). The chain-length distribution of the amylopectin was examined by gel-permeation HPLC using connected columns (Tosoh, TSK gel G3000SW and G2000SWX2, each 7.5 mm × 60 cm) with a differential refractometer (Tosoh RI-8000) and a low-angle laser-light-scattering photometer (Tosoh LS-8) as detectors. The weight-average distribution was determined also by gel-permeation HPLC.

Viscosity and Dynamic Viscoelasticity Measurements

Viscosity at various shear rates (1.19–95.03/sec) and dynamic viscoelasticity at a fixed angular velocity (3.77/rads) were determined with a rheogoniometer (Iwamoto Seisakusho Co., Ltd, Japan) 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-cooling instrument (LCH-130F, Toyo Co., Ltd.) at 0–90°C and raised at a stepwise rate of 1°C/min. The shear rate ($\dot{\gamma}$) shear stress (τ), and viscosity (η) were calculated with the equation of Margules (Harris 1977). The dynamic viscosity (η') and elasticity (G') were calculated from the modified equation of Markovitz (1952). The loss tangent was calculated from the relationship, $\tan \delta = G''/G'$, where $G'' =$

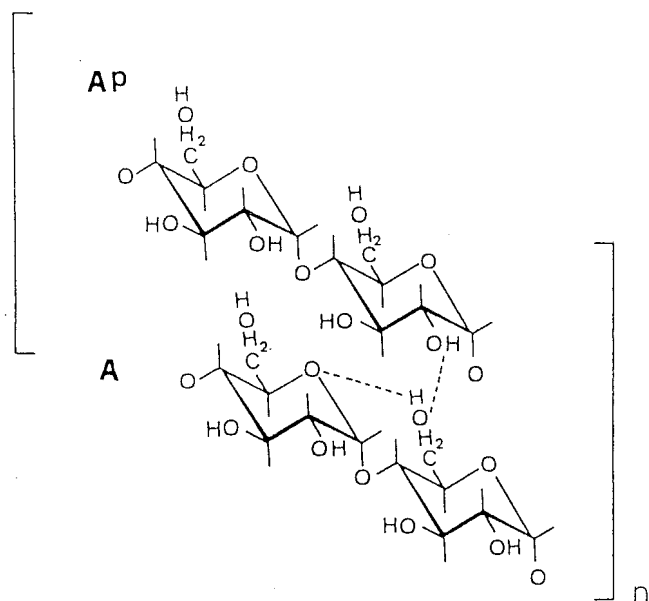


Fig. 1. Possible intermolecular hydrogen bonding of rice (Nihonbare) starch in aqueous solution. Dotted lines = hydrogen bonding. A, amylose; AP, short chain (A and B1) of amylopectin molecules.

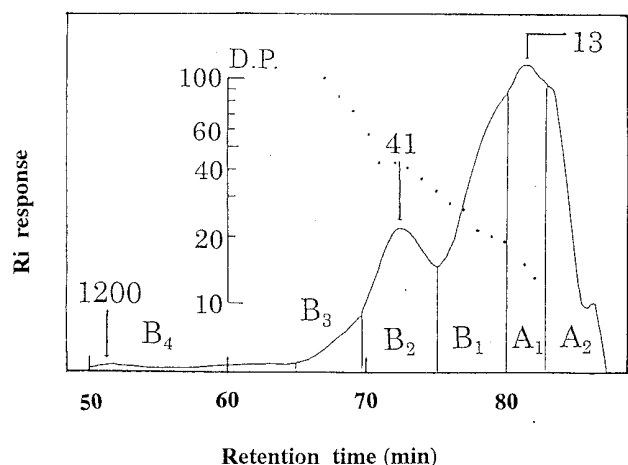


Fig. 2. Gel-filtration HPLC of Akihikari amylopectin debranched with isoamylase.

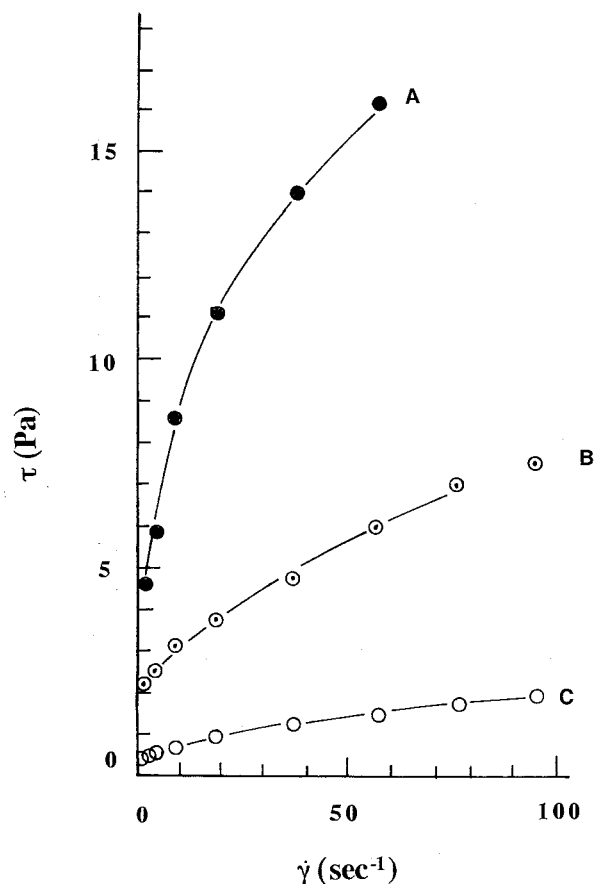


Fig. 3. Flow curves of Akihikari starch at 25°C. Concentrations: 4.0% (A), 3.0% (B), 2.0% (C).

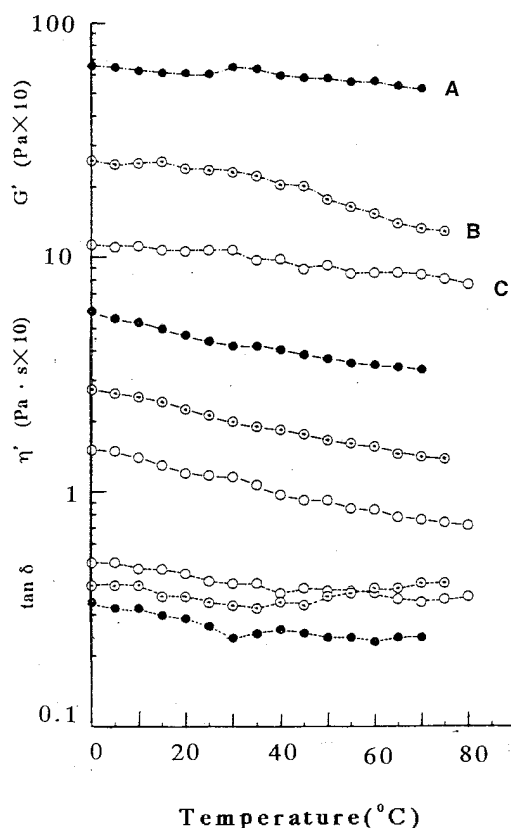


Fig. 4. Effects of temperature on the dynamic viscoelasticity of the Akihikari starch at 3.77 rad/sec. Concentrations: 4.0% (A), 3.0% (B), 2.0% (C).

$\omega\eta'$ is the loss modulus and ω is the angular velocity of the outer cylinder. Values reported are the mean of at least two determinations.

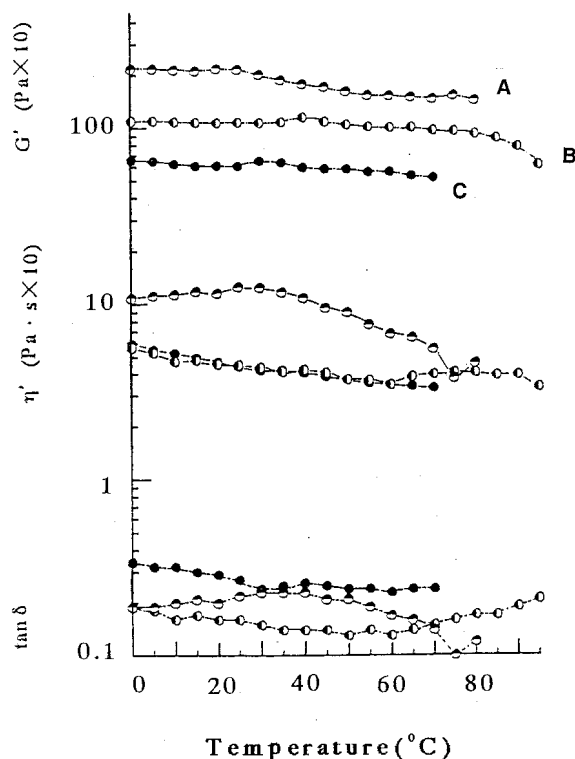


Fig. 5. Effects of temperature on dynamic viscoelasticity of Akihikari starch (4.0%) after storage at 4°C for 24 hr (A), after storage at 25°C for 24 hr (B), after preparation (C).

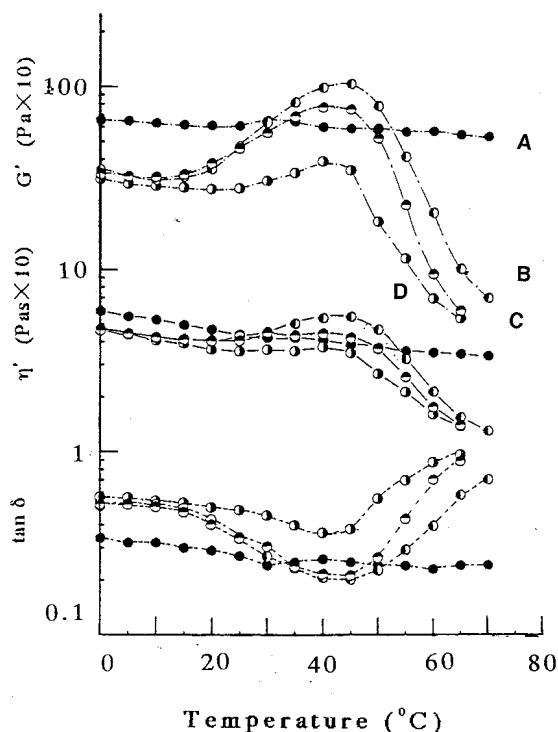


Fig. 6. Effects of temperature on dynamic viscoelasticity of Akihikari starch (4.0%) after preparation in aqueous solution (standard) (A), after storage at 4°C for 24 hr (B), after storage at 25°C for 24 hr (C), after preparation with addition of urea (4.0M) (D).

RESULTS AND DISCUSSION

Some characteristics of the starch (Akihikari) and its components are summarized in Table I. Estimated values for starch were amylose content 18.8%, iodine affinity 4.20 units. Estimated values for amylose were iodine affinity 20.7 units, blue value 1.57 units. The number-average degree of polymerization and weight-average distribution of amylose were 980 and 3,800, respectively. The number-average chain length of the amylopectin was determined as 19.0 by assaying reducing power after isoamylolysis. This value was almost the same as that of waxy-rice amylopectin of Takinari (19.0) (Tako and Hizukuri 1997) but a little longer than that of Nihonbare amylopectin (18.0) (Tako and Hizukuri 1999).

The chain length distribution as revealed by an HPLC-RI system is shown in Fig. 2 and data are summarized in Table II. The distribution pattern was largely bimodal with peaks at dp 13 and 41, but shoulders were observed at these peaks. Furthermore, small amounts of a super long chain and a long chain (B4) component were present. The shortest chain fraction (A+B1) was predominant (89.7 mol%).

Though flow curves of the starch at 25°C, Akihikari 2.0% solutions showed shear thinning behavior that approximated plastic behavior at >3.0%; yield value was estimated to be 0.2 and 0.4 Pa at concentrations of 3.0 and 4.0%, respectively (Fig. 3). This indicates that an intermolecular association in Akihikari starch molecules takes place at >3.0% (Tako and Hizukuri 1999).

As shown in Fig. 4, the dynamic viscoelasticity of Akihikari starch increased with increasing concentration and showed very large values at 4.0%. For the 3.0% solution, the dynamic modulus was constant with increasing temperature up to 30°C, then it decreased gradually with further increase in temperature. For the 4.0% solution, the dynamic modulus stayed at a constant value during the increase in temperature. On the other hand, the tan δ

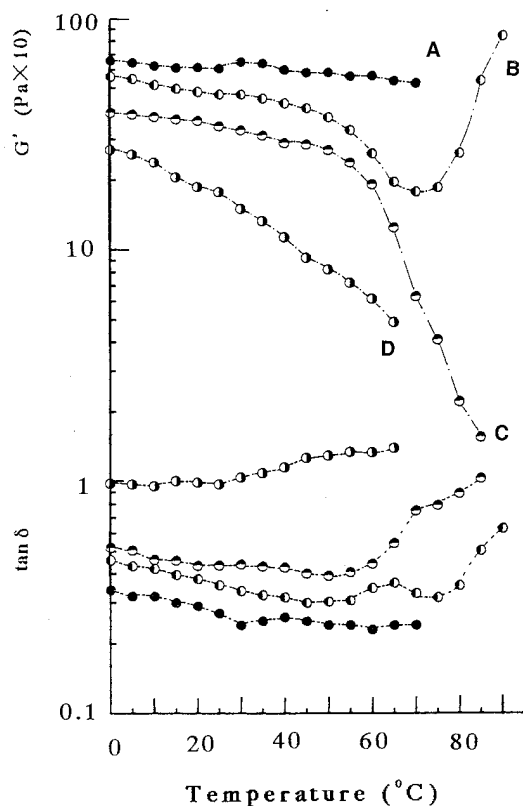


Fig. 7. Effects of temperature on dynamic modulus of Akihikari starch (4.0%) in alkaline solution (0.05M NaOH) after preparation in aqueous solution (standard) (A), after storage at 4°C for 24 hr (B), after storage at 25°C for 24 hr in alkaline solution (C), after preparation in 0.05M NaOH (D).

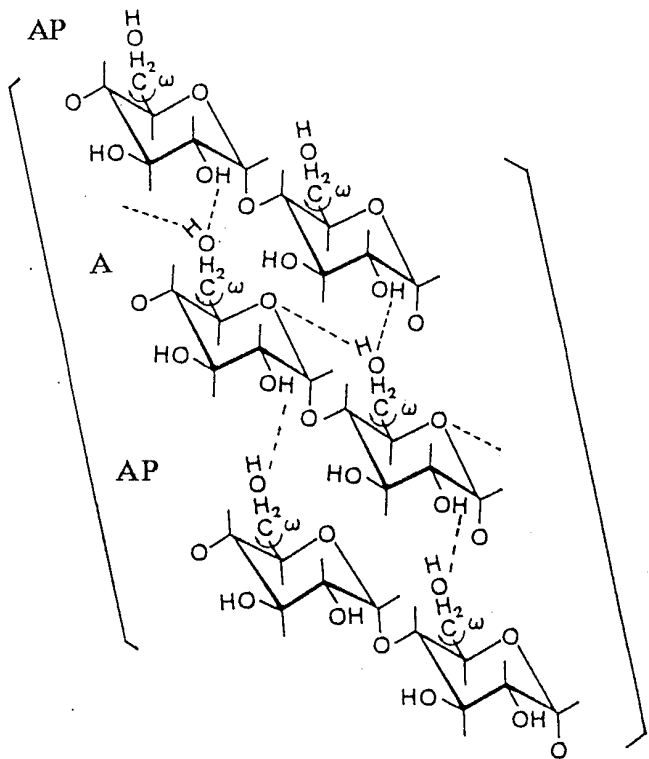


Fig. 8. Possible retrogradation mechanism of Akihikari starch. Dotted lines = hydrogen bonding. A, amylose; AP, short chain (A and B1) of amylopectin molecules.

value of Akihikari starch solution decreased from 0.50 to 0.43 when concentrations were increased from 2.0 to 4.0% at low temperature (0°C). The $\tan \delta$ value for a 4.0% solution decreased slightly with increasing temperature.

The dynamic modulus of an Akihikari 4.0% solution increased when the solution was stored at 25 and 4°C for 24 hr. A larger dynamic modulus was observed when the solution was stored at 4°C for 24 hr. (Fig. 5). Though dynamic viscosity had almost the same value during the increase in temperature after storage at 25°C, it increased greatly after storage at 4°C. On the other hand, the $\tan \delta$ value was low after storage at 25 and 4°C for 24 hr.

The dynamic modulus of an Akihikari 4.0% solution was small and remained constant up to 10°C upon addition of urea (4.0M). At >10°C, the dynamic modulus increased gradually with increased temperature and reached a maximum at 40°C, then decreased rapidly (Fig. 6). A similar curve was also observed with dynamic viscosity. A larger sigmoid curve was observed after storage at 25 and 4°C for 24 hr. A sigmoid curve and increased dynamic modulus as a result of increased temperature was also observed with increasing temperature in native xanthan (Tako et al 1977, 1998; Tako 1992), rhamsan (Tako 1993), and S-657 gum (Tako 1994), where intramolecular van der Waals forces of attraction and hydrogen bonding might be contributors. This suggests that van der Waals forces of attraction, where H-1 and OH-6 of D-glucosyl residues of amylopectin molecules participate in an intramolecular association (Tako 1996; Tako and Hizukuri 1997, unpublished), may contribute to an increase in the dynamic modulus as temperature is increased. This occurs after the addition of urea (4.0M), a reagent known to break hydrogen bonds.

The dynamic modulus of an Akihikari 4.0% solution remained small in alkaline solution (0.05M NaOH) at low temperature (0°C) and decreased gradually with increasing temperature (Fig. 7). After storage at 25°C for 24 hr, the dynamic modulus was large and decreased slightly with increased temperature up to 50°C, then it

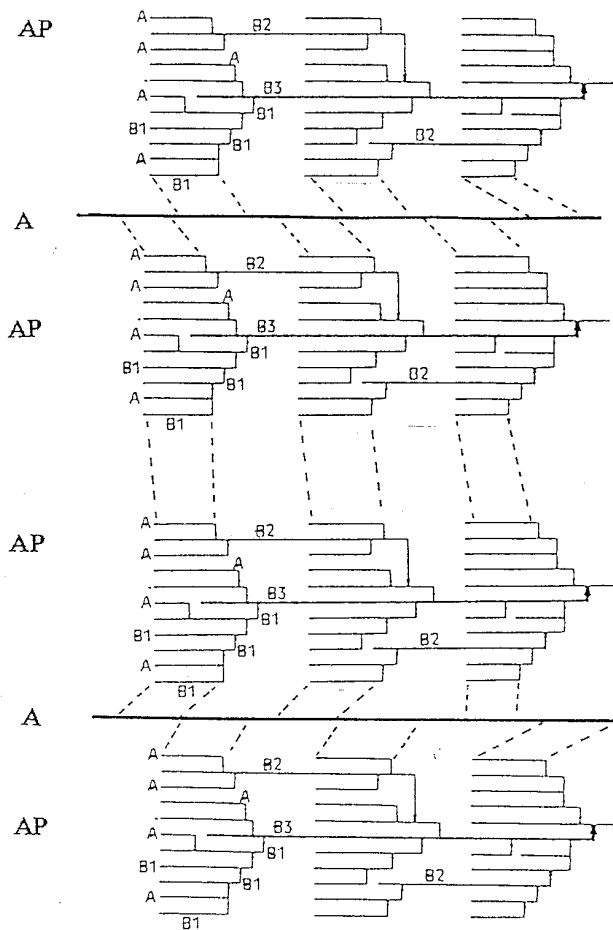


Fig. 9. Possible association sites (dotted lines) between amylose and amylopectin molecules of rice starch. Two or more short side-chains (A and B1) of amylopectin molecules may take part in the interaction with an amylose molecule. After storage at low temperature range and for long times, and after saturation of hydrogen bonding between amylose and amylopectin molecules (Fig. 8), self-association within amylopectin molecules may take place.

decreased rapidly. Thus, 50°C was assumed to be a transition temperature. When the starch solution was stored at 4°C for 24 hr, the dynamic modulus was larger than that of a solution stored at 25°C for 24 hr. The modulus decreased slightly with increased temperature up to 45°C, then decreased rapidly with further temperature increases. At >70°C, however, the dynamic modulus increased rapidly with further temperature increases. Similar increases in the dynamic modulus were also observed for curdlan solutions where the van der Waals forces of attraction between H-6 of D-glucosyl residues on different molecules might contribute to an increase in the dynamic modulus at temperatures >55°C (Tako and Hanashiro 1997). This suggests that van der Waals forces of attraction may be responsible for the increase in dynamic modulus at high temperatures, even in weak alkaline starch solution.

CONCLUSIONS

The rheological characteristics of Akihikari rice starch differ from those of amylose (Tako and Hizukuri 1995) and rice amylopectin (Tako 1996; Tako and Hizukuri 1997) but were essentially comparable with those of Nihonbare rice starch (Tako and Hizukuri 1999). This suggests that intermolecular hydrogen bonding between amylose and amylopectin molecules of Akihikari starch may take place after preparation in aqueous solution (Fig. 1). The dynamic viscoelasticity increased when Akihikari starch solution was stored

at 25 and 4°C for 24 hr. Furthermore, Akihikari starch (4.0%) showed a curious dynamic modulus in solutions containing 4.0M urea and 0.05M NaOH after storage at 4°C for 24 hr.

Thus, we conclude that after formation of intermolecular hydrogen bonding between O-6 of the amylose and OH-2 of the amylopectin molecules (Fig. 1), another intermolecular hydrogen bond may form between OH-2 of a D-glucose residue of the former molecule and O-6 of a D-glucose residue of a short side chain (A and B1) of the latter molecule (Fig. 8). Much more intense intermolecular hydrogen bonding may take place during storage at 25 and 4°C for 24 hr. Two or more short side chains (A and B1) of an amylopectin molecule may associate with an amylose molecule because Akihikari starch consists of ≈19% amylose and 80% amylopectin (Tako et al 1984, 1998; Tako and Nakamura 1986; Tako 1991). After saturation of intermolecular hydrogen bonding between amylose and amylopectin molecules (Fig. 8), an intermolecular association may also take place between amylopectin molecules through hydrogen bonding (Fig. 9). This bonding may be caused by a decrease of Brownian motion and kinetic energy of amylopectin and water molecules during storage at 25 and 4°C for 24 hr. At this stage, side-by-side association between O-3 and OH-3 of D-glucosyl residues on different amylopectin molecules may also take place.

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