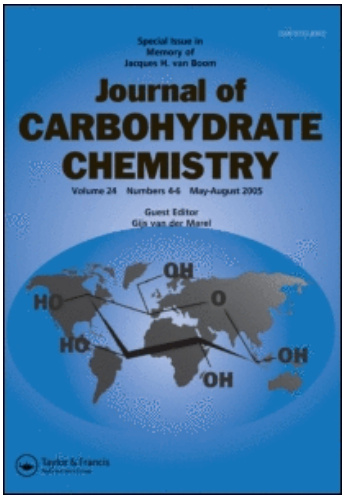

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GELATINIZATION MECHANISM OF RICE STARCH [1]

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ABSTRACT

The non-Newtonian behavior and dynamic viscoelasticity of rice starch (Nihonbare; amylose content, 15.8%) solutions were measured with a rheogoniometer. A gelatinization of Nihonbare starch occurred above 3.0% after heating at 100 °C for 30 min. The Nihonbare starch showed shear-thinning behavior at a concentration of 2.0%, but plastic behavior above 3.0% at 25 °C. The viscosity of Nihonbare starch at a concentration of 2.0% solution decreased gradually with increase in temperature from 10 to 55 °C, then it stayed at a constant value with further increase in the temperature. However, for 4.0% solution, rapid decrease in the viscosity was observed after the temperature reached 25 °C up to 50 °C, then it stayed at a constant value. The dynamic modulus of Nihonbare starch stayed at a constant value during increase in the temperature at 4%. The tan δ of the starch showed low values, 0.28, at low temperature range and stayed at a constant up to 30 °C, then it increased a little with increasing temperature. A little decrease of dynamic modulus of Nihonbare starch was observed at low temperature range upon addition of urea (4.0 M). The dynamic modulus, however, decreased rapidly when the temperature reached 50 °C, which was estimated to be a transition temperature. The dynamic modulus also decreased rapidly in 0.10 M NaOH solution above 50 °C. A possible mode of intermolecular hydrogen bonding between amylose and amylopectin molecules of Nihonbare starch is

proposed. The short chains (A and B1) of the amylopectin molecules may take part in the intermolecular association in aqueous solution.

INTRODUCTION

We have proposed a possible model of the gelation mechanism of amylose molecules in aqueous solution.[2] An intramolecular hydrogen bonding may take place between OH-6 and the adjacent hemiacetal oxygen atom of the D-glucosyl residues. This bonding is likely owing to the flexibility of the α-(1→4)-linkage and extended conformations at high temperature. In addition, intermolecular hydrogen bonding may take place between the OH-2 and 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, 25-35 °C, during increase in temperature. Residual intermolecular, together with intramolecular hydrogen bonding, is lost above another transition temperature, 80-90 °C.

We have previously discussed the molecular origin for the thermal stability of rice amylopectin in aqueous solution and concluded that the molecules are involved in an intramolecular hydrogen bonding.[3] The 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. The intramolecular associations might be involved in long chains (B2-4)[4] of rice amylopectin molecules.[5]

In this study, we analyze the rheological behavior of a solution of rice starch with respect to its association characteristics in comparison with that of amylose and rice amylopectin, and propose a possible mode of intermolecular association between amylose and amylopectin molecules in aqueous solution. This work may offer a new concept for a gelatinization mechanism of rice starch in aqueous solution.

RESULTS AND DISCUSSION

Some characteristics of the starch (Nihonbare) and its components are summarized in Table 1. The amylose content and iodine affinity of the starch was estimated to be 15.8% and 3.60. The iodine affinity and blue value of amylose was estimated to be 21.6 and 1.51. The $\overline{d.p.n.}$ and $\overline{d.p.w.}$ of the amylose was calculated to be 850 and 3620.

Table 1. Properties of Nihonbare starch

Properties	Starch	Amylose	Amylopectin
Iodine affinity (mg/100mg)	3.60	21.60	0.20
Blue value (680 nm)		1.51	0.076
$\overline{D.p.n.}$		850	
$\overline{D.p.w.}$		3620	
Amylose content	15.8	100	0

The number-average chain length of the amylopectin was found to be 18.0 by reducing power after isoamylolysis. This value was a little shorter than that of waxy-rice amylopectin (Reimei, 18.8; Takanari, 19.0), as reported previously.[3]

The chain length distribution as revealed by an HPLC-RI system is shown in Fig. 1 and data are summarized in Table 2. The distribution pattern was largely bimodal with peaks at d.p. 16.0 and 40.0, 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 (fraction A+B1) was predominant (87.81%) by mole.

A gelatinization occurred in an aqueous solution of Nihonbare starch at a concentration $> 3.0\%$ after heating at 100 °C for 30 min. The flow curves, at 25 °C, of Nihonbare starch at various concentrations are shown in Fig. 2. Though flow curve showed shear-thinning behavior at a percentage of 2.0%, it approximated to plastic behavior above 3.0%, the yield value of the last named solution was estimated to be 1.0 and 9.8 Pa at 3.0 and 4.0%, respectively. This indicates that Nihonbare starch molecules are involved in a secondary association because yield values were estimated above 3.0%.[2,3,6-10]

Figure 3 shows the effect of temperature on viscosity of Nihonbare starch at various concentrations. The viscosity at 2.0% decreased gradually with increasing temperature up to 55 °C, then it stayed at a constant value with further increase in the temperature. On the other hand, for 4.0% solution, the viscosity decreased a little with increasing temperature up to 25 °C, then it decreased rapidly. However, when the

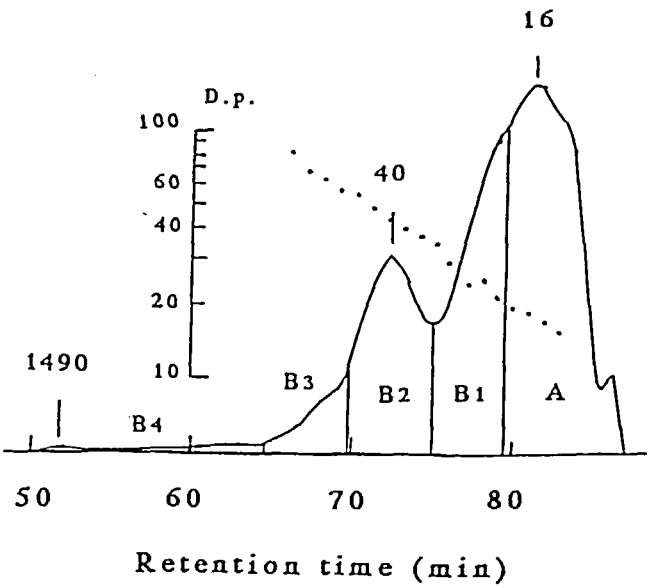


Fig. 1. Gel-filtration HPLC of Nihonbare amylopectin debranched with isoamylase.

Table 2. Distribution of the chain lengths of Nihonbare amylopectin

Fraction	Whole	B4	B3	B2	B1+A
$\overline{C.l.n.}$	18.0				
$\overline{C.l.w.}$		390	63	42	18
Weight (%)	100	1.5	4.0	21.0	73.5
Mole (%)	100	0.08	1.36	10.71	87.81

temperature reached 50 °C, the starch solution essentially maintained a constant viscosity with further increase in the temperature. The result differs from that of amylose[2] and rice amylopectin.[3] The viscosity of amylose solution at 1.2, 1.4, and 1.6% decreased rapidly when the temperature reached 25, 30, and 35 °C, respectively, the temperatures estimated to be first transition temperature, at the respective concentrations. However, after reaching the first transition temperature, the amylose solutions stayed at a constant

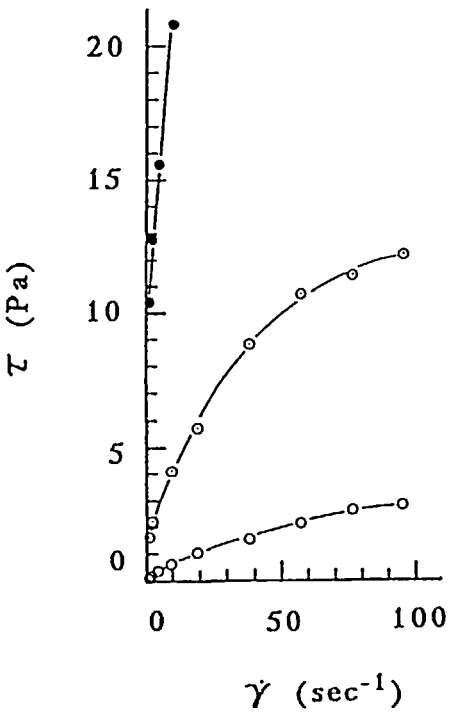


Fig. 2. Flow curves of Nihonbare starch at various concentrations and 25 °C. Concentration: ○2.0%; ◉, 3.0%; ●, 4.0%.

value with increasing temperature up to 80, 90, and 90 °C, respectively, which were also estimated to be second transition temperatures. The viscosities then decreased rapidly. In the case of rice amylopectin in 4.0 and 6.0% solution, the viscosity increased a little with increasing temperature up to 5 and 15 °C, respectively, then it stayed at a constant value with further increase in the temperature. The results indicated that the secondary associations of amylose and rice amylopectin molecules had thermal-stable characteristics in aqueous solution. Therefore rapid decrease in vicosity of Nihonbare starch indicates that a secondary association of the molecules is liable to dissociate with increasing temperature under shearing-force. This suggests that a secondary association of Nihonbare starch seems to take place between amylose and amylopectin molecules.

As shown in Fig. 4, the dynamic modulus of Nihonbare starch increased with increase in concentration and showed very large values at a concentration of 4.0%. For the starch solution at 2.0%, the dynamic modulus decreased rapidly from 10 °C to 15 °C,

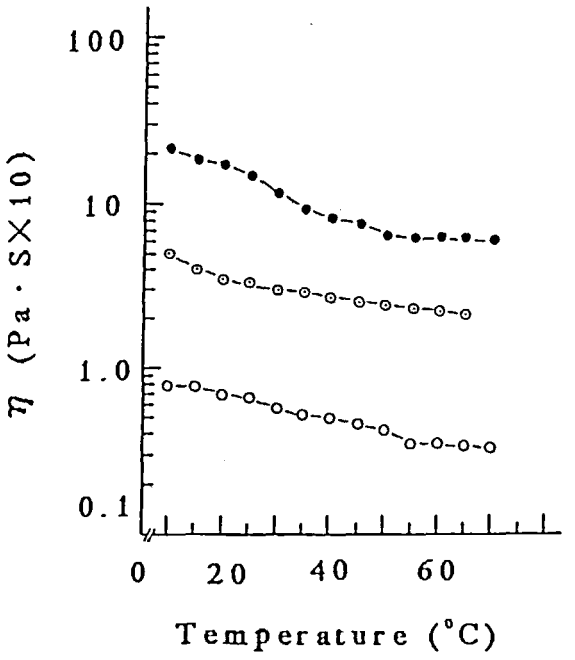


Fig. 3. Effects of temperature on the viscosity of Nihonbare starch at various concentrations. Concentration: ○, 2.0%; ◉, 3.0%; ●, 4.0%.

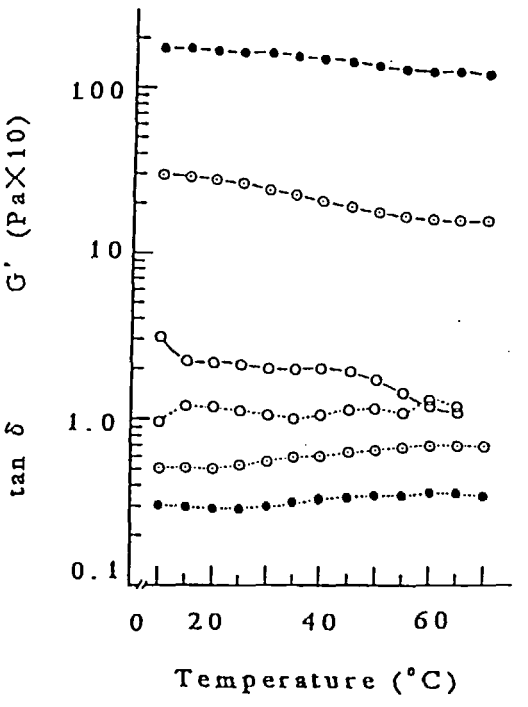


Fig. 4. Effects of temperature on the dynamic modulus of Nihonbare starch at various concentrations. The solid lines represent dynamic modulus and dotted lines represent tan δ . Concentration: ○, 2.0%; ◉, 3.0%; ●, 4.0%.

remained constant with increasing temperature to 50 °C and then decreased rapidly again with increasing temperature to 65 °C. The dynamic modulus in 3.0% solution stayed at a constant up to 35 °C, then decreased a little with further increase in the temperature. For 4.0% solution, the dynamic modulus stayed at a constant value during increase in temperature. On the other hand, the tan δ value of Nihonbare starch solution decreased from 0.96 to 0.28 with an increasing concentration from 2.0 to 4.0% at low temperature (10 °C). For 2.0% solution, the tan δ value increased gradually with increasing temperature. The tan δ value for 3.0 and 4.0% solution stayed at a constant value with increasing temperature up to 20 and 30 °C, then it increased a little with further increase in the temperature. The results essentially are in agreement with those of rice amylopectin, indicating that the secondary association of Nihonbare starch is stable even at a high temperature range.

The dynamic viscoelasticity of Nihonbare starch (4.0%) was measured in the presence of urea (4.0 M), which is known to disrupt hydrogen-bonding. As shown in Fig. 5, a little decrease in the dynamic modulus for Nihonbare starch was observed upon addition of urea and large values were maintained during increase in temperature up to 50 °C, which was estimated to be a transition temperature, then it decreased rapidly with further increase in the temperature. This indicates that urea prevents hydrogen-bonding in starch molecules above the transition temperature > 50 °C, and hydrogen bonding has a dominant role in the secondary association of starch molecules in aqueous solution.

Figure 6 shows the effects of temperature on the dynamic modulus of Nihonbare starch (4.0%) after being dissolved in 0.05 and 0.10 M NaOH, respectively. In spite of a little decrease in the dynamic modulus in 0.05 M NaOH solution during increase in temperature, it stayed at low values and decreased rapidly when the temperature reached 50 °C, which was estimated to be a transition temperature, in 0.10 M NaOH solution of Nihonbare starch. The tan δ values of Nihonbare starch in 0.10 M NaOH solution were higher than those in 0.05 M NaOH solution during increase in the temperature. The result, showing transition temperature, indicates that a secondary association dissociates above the transition point. Such transition temperature was also observed in rice amylopectin alkaline (0.10 M NaOH) solution at 60 °C.[3]

CONCLUSIONS

The rheological characterisics of rice starch (Nihonbare) differ from those of amylose[2] and rice amylopectin[3] in aqueous solution. Gelation did not occur even at a

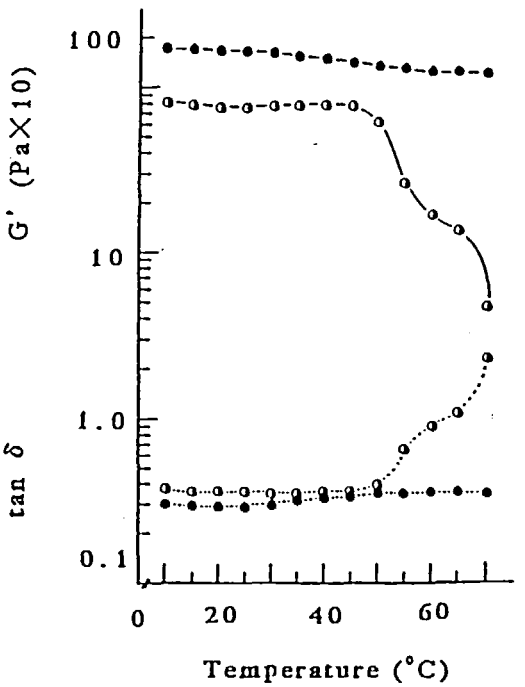


Fig. 5. Effects of temperature on the dynamic modulus of Nihonbare starch at a concentration of 4.0% (●) with addition of urea (◑). The solid lines represent dynamic modulus and dotted lines represent tan δ.

concentration of 4.0%, but gelatinization took place above 3.0%. Though the viscosity decreased rapidly with increasing temperature, little decrease in the dynamic modulus was observed.

Thus, we conclude that gelatinization of Nihonbare starch may occur between O-6 of the amylose and OH-2 of the amylopectin molecules with hydrogen bonding, as illustrated in Scheme 1. The short chains (A and B 1), which are free from intramolecular association, of amylopectin molecules may take part in the intermolecular association.The intermolecular hydrogen bonding between amylose and amylopectin molecules has a thermal stability in aqueous solution. This bonding, however, liable to dissociate with increasing temperature under a shearing-flow, but is stable under angular flow. However, the intermolecular hydrogen bonding, together with intramolecular association within long chains (B2-4) of the amylopectin molecules, dissociate above the transition temperature in 4.0 M urea (50 °C) and 0.10 M NaOH (50 °C).

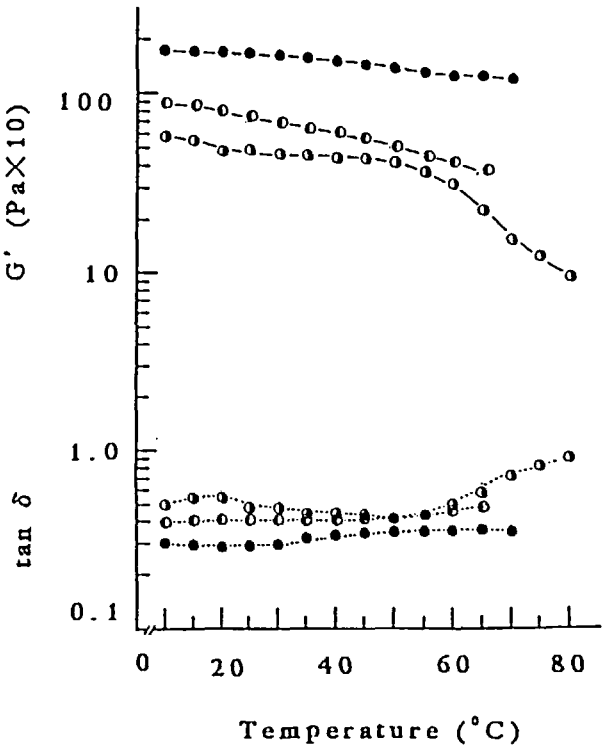
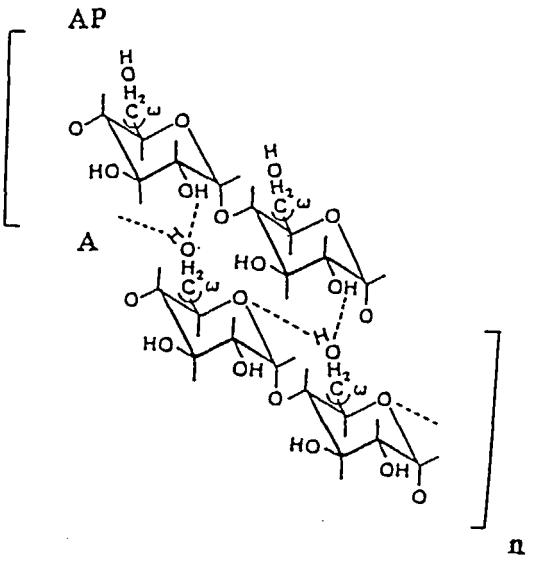


Fig. 6. Effects of temperature on the dynamic modulus of Nihonbare starch at a concentration of 4.0% in a NaOH solution. The solid lines represent the dynamic modulus and dotted lines represent tan δ. (◐), In 0.05 M NaOH; (◑), in 0.10 M NaOH; (●), in aqueous solution.

Consequently, the gelatinization of rice starch may take place between amylose and side-chains (A or B1) of amylopectin molecules in aqueous solution. Such mode of intermolecular interaction, involving side-chains, has been proposed in xanthan-galactomannan[11,12] and xanthan-glucomannan[13,14] gelling systems, where trisaccharide side-chains of the former molecules might play a dominant role in the D-mannose-specific interaction.

EXPERIMENTAL

Materials. Rice starch (Nihonbare) which was harvested in Shiga Prefecture, Japan, was prepared by the alkaline leaching method from polished flour. The extract



Scheme 1. Possible gelatinization mechanism of rice (Nihonbare) starch in aqueous solution. The dotted lines refer to hydrogen bonding. AP, Short chain (A or B1) of amylopectin; A, amylose molecules.

was fractionated into amylose and amylopectin by the method of Takeda *et al* .[15] The yield of amylose and amylopectin from 10 g (dry weight) of starch was 1.5 g and that of amylopectin was 7.2 g.

Methods. Total carbohydrate was determined by the phenol-sulfuric acid method.[16] The reducing residue was assayed colorimetrically by Somogyi's method[17] using Nelson's[18] reagent, the heating time being extended to 30 min to give the same reducing power regardless of the chain length. Iodine affinity was determined at 25 °C by modified amperometric titration.[19] Blue value was determined by a described procedure.[20] The non-reducing residue was determined by rapid Smith degradation with photometric or fluorometric assay of glycerol.[21] The number-average degree of polymerization ($\overline{d.p.n.}$) of the amylose and amylopectin were determined by the modified Park-Johnson method.[21]

The number-average chain length ($\overline{c.l.n.}$) 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-average chain length ($\overline{c.l.w.}$) of amylopectin was determined by HPLC combined with low angle laser-light-scattering photometry as described.[22] The chain-length distribution of the amylopectin was examined by gel-permeation HPLC, using connected columns (Tosoh, TSKgel G3000SW and G2000SW×2, 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-avearge distribution (d.p.w.) was determined also by gel-permeation HPLC.

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 as range 2 (sample period, 200 ms):E_1 0.10 (t_1 300), E_2 0.60 (t_2 120), E_3 0.80 V (t_3 300 mn). The response time of the detector was set to 1.0 s. A Dionex HPIL-AS6 column (250*4 mm 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 angular velocity (3.77 $rads^{-1}$) 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 samples was controlled by circulating oil from a thermo-cool instrument (LCH-130F, Toyo Co., Ltd.), over the temperature range of 10-80 °C and raised at a stepwise rate of 1 °C min. The shear rate (γ), shear stress (τ), and viscosity (η) were calculated with the equation of Margules.[23] The dynamic viscosity (η') and elasticity (G') was calculated from the modified equation of Markovitz.[24] 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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