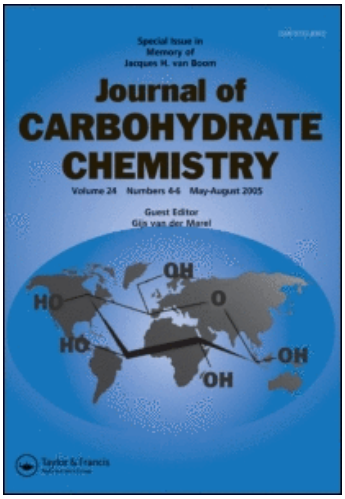

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Evidence for Conformational Transitions in Amylose[1]

Masakuni Tako[a]; Susumu Hizukuri[b]
[a] Department of Bioscience and Biotechnology, University of the Ryukyus, Okinawa, Japan [b] Department of Applied Biochemistry and Technology, Kagoshima University, Kagoshima, Japan

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EVIDENCE FOR CONFORMATIONAL TRANSITIONS IN AMYLOSE[1]

Masakuni Tako* and Susumu Hizukuri[a]

Department of Bioscience and Biotechnology, University of the Ryukyus, Nishihara, Okinawa 903-01, Japan
[a]Department of Applied Biochemistry and Technology, Kagoshima University, Kagoshima 890, Japan

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ABSTRACT

The flow behavior, dynamic viscoelasticity, and optical rotation of an aqueous solution of amylose were measured using a rheogoniometer and a polarimeter, respectively. The amylose solutions showed shear-thinning behavior at a concentration of 1.2%, but plastic behavior above 1.4% at 25 °C. With increasing amylose concentrations the viscosity decreased rapidly with increasing temperature from 20 to 25, 30, and 35 °C. These latter temperatures are estimated to be first transition temperatures at the respective concentrations. Viscosities were scarcely changed until temperatures reached 70, 90, and 90 °C, which were estimated to be second transition temperatures, for 1.2, 1.4, and 1.6% solutions, respectively. Gelation occurred at a concentration of 1.2% at room temperature (25 °C). The dynamic modulus of amylose increased gradually with increasing temperature from 20 to 30 °C and kept a constant value until the temperature reached 65, 75, and 80 °C for 1.0, 1.2 and 1.4% solutions, respectively, which were estimated to be transition temperatures, then dynamic modulus decreased rapidly. The dynamic modulus of amylose stayed at a very low value with addition of urea (4.0 M). The optical rotation of amylose solution (1.0%) increased a little with deceasing temperature up to 25 °C, then it increased rapidly with further decrease of the temperature. Possible mode of intra- and intermolecular hydrogen bonding within and between amylose molecules were proposed.

INTRODUCTION

Amylose is an linear polysaccharide composed of 1,4-linked α-D-glucose residues by definition, but the actual specimens, which are isolated and purified from starch, include slightly branched molecules.[2,3] The physical[4,5] and physico-chemical[6-9] properties of amylose have been extensively investigated, but the gelling mechanism is still not understood at the molecular level.

On the other hand, in the course of the rheological studies of polysaccharides, we have discussed the molecular origin for their rheological characteristics and proposed gelation mechanism for κ-carrageenan,[10,11] ι-carrageenan,[12] agarose,[13] gellan gum[14-18] and curdlan[19] where the hemiacetal oxygen atom of sugar residues might have a dominant role in both intra- and intermolecular associations in aqueous solution.

In the present study, we analyze the rheological behavior of a solution of amylose with respect to its association characteristics, and propose possible modes of intra- and intermolecular hydrogen bonding in aqueous solution. This work may offer a new concept for a gelation and retrogradation mechanism of amylose molecules in aqueous solutions.

RESULTS

The number-average degree of polymerization ($\overline{d.p.n}$) of purified potato amylose was estimated to be 300, with a molecular weight of 49,000 based on the number of glucosyl units per a reducing residue.

The flow curves, at 25 °C, of potato amylose aqueous solution at various concentrations are shown in Fig.1. At a concentration of 1.2%, the flow curve approximated shear-thinning behavior, and for 1.4 and 1.6% concentrations, plastic behavior. The yield value for the last-named concentrations were estimated to be 1.2 and 4.1 Pa, respectively. A curious flow behavior was observed in a solution at 1.6%; the shear stress decreased rapidly with an increase of shear rate up to 9.5 s^{-1}, then it increased gradually with increasing shear rate. The phenomenon, showing a decrease of shear stress with increasing shear rate up 9.5s^{-1}, may be caused by a rapid breakdown of an intermolecular association of amylose molecules. This suggests that an intermolecular association of amylose molecules is liable to dissociate under shearing force.

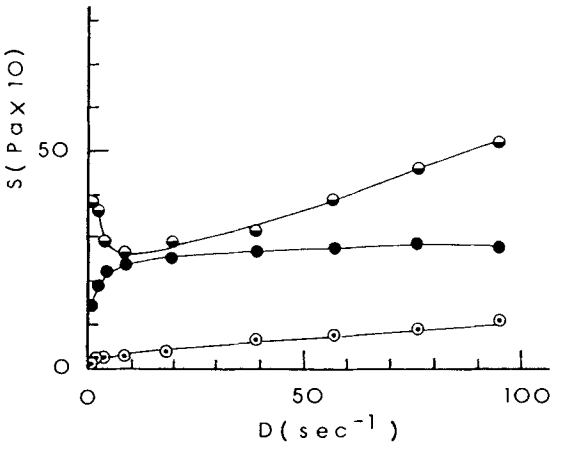


Fig. 1. Flow curves of potato amylose at various concentrations and 25 °C. Concentration: ⊙, 1.2%; ●, 1.4%; ◒, 1.6%.

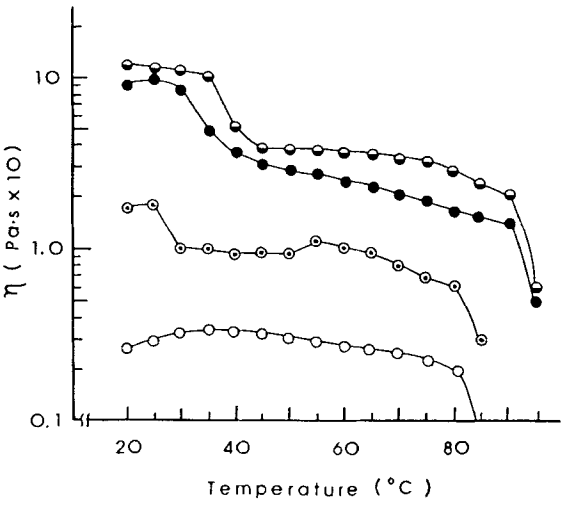


Fig. 2. Effects of temperature on the viscosity of potato amylose at various concentrations. Concentration: ○, 1.0%; ⊙, 1.2%; ●, 1.4%; ◒, 1.6%.

As shown in Fig.2, the viscosity of amylose solution at a concentration of 1.0% stayed at a constant value with increasing temperature up to 80 °C, which was estimated to be a transition temperature, then it decreased rapidly. On the other hand, for 1.2, 1.4, and 1.6% solutions, the viscosity decreased rapidly when the temperature reached 25, 30, and 35 °C, respectively, these temperatures estimated to be first transition temperatures,

at the respective concentrations. However, after reaching the first transition temperature, the amylose solutions essentially maintained a constant viscosity up to 80, 90, and 90 °C, respectively, which were also estimated to be second transition temperatures. The viscosities then decreased rapidly, as observed in a solution of 1.0%. These results indicate that there are two stepwise conformational transitions in amylose molecules under shearing force over a temperature range of 25-35 °C and of 80-90 °C, respectively. This phenomenon showing transition temperatures, was also observed in κ-carrageenan,[10] ι-carrageenan,[12] agarose,[13] gellan gum[14] and curdlan,[19] and suggests that amylose molecules are involved in both intra- and intermolecular association in aqueous solution. For curdlan, particularly, the primary structure of which consists of β-1,3-linked glucoses,[20] the dynamic modulus was very large and stayed constant with increasing temperature up to 40 °C, then it decreased rapidly. However, when the temperature reached 55 °C, in contrast, the dynamic modulus increased gradually with further increase in temperature. This result might be caused by a breakdown of intermolecular hydrogen bonding between OH-6 groups of the D-glucosyl residues on different molecules during increase in the temperature from 40 to 55 °C, and by formation of hydrophobic interactions between the methylene groups at C-6 of the D-glucosyl residues over a high temperature range≥55 °C.[19]

Gelation of amylose occurred at a concentration above 1.2% at room temperature (25 °C). Figure 3 shows the effects of temperature on the dynamic modulus of amylose at various concentrations. The dynamic modulus increased with an increase in the temperature up to 30 °C, then it stayed at a constant value. However, at temperatures of 65, 75, 80, and 80 °C, estimated to be transition temperatures at concentrations of 1.0, 1.2, 1.4 and 1.6%, respectively, the dynamic modulus decreased rapidly with further increase of the temperature. On the other hand, the tan δ value of amylose solution decreased with an increase in the concentration. The tan δ had a very low value, 0.08-0.02, during increase of the temperature up to 75, 80, and 80 °C, then it increased rapidly with further increase of the temperature at 1.2, 1.4, and 1.6%, respectively. This suggests that intra- and intermolecular association of amylose molecules dissociate above the transition temperature. Though a transition temperature was observed in a range of 65-80 °C under frequency, it was also observed in a temperature range of 25-35 °C under shearing force (Fig. 2). This indicates that an intermolecular association is stable up to 65-80 °C under frequency.

As the gelling properties of amylose solution are strongly correlated to the molecular weight,[21,22] effects of temperature on the dynamic modulus of two types of amyloses (AS-

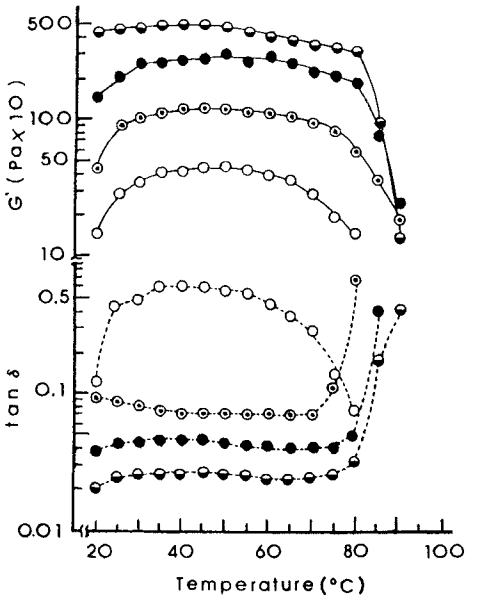


Fig. 3. Effects of temperature on the dynamic modulus of potato amylose at various concentrations. The full lines refer to the dynamic modulus and dotted lines to the tan δ. Concentration: ○, 1.0%; ⊙, 1.2%; ●, 1.4%; ◒, 1.6%.

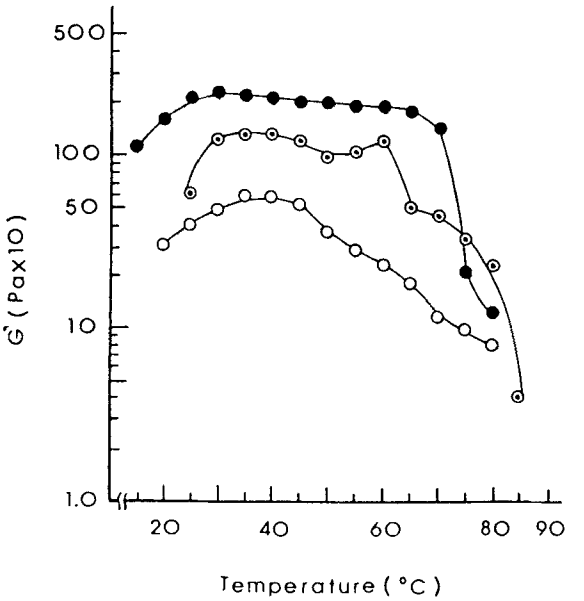


Fig. 4. Effects of temperature on the dynamic modulus of enzymically synthetic amylose. Symbols: ○, AS-110 at 0.8%; ⊙, AS-110 at 1.0%; ●, AS-320 at 1.4%.

Table Optical rotation of amylose at 589 nm

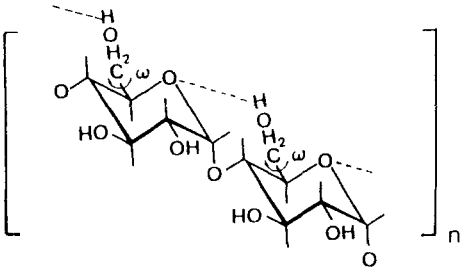
Sample	Temperature °C							
	80	70	60	50	40	30	25	20
Potato	+1.054	+1.065	+1.075	+1.087	+1.098	+1.110	+1.123	+1.165
AS-110	+1.049	+1.058	+1.069	+1.078	+1.085	+1.094	+1.275	
AS-320	+0.954	+0.965	+0.974	+0.983	+0.990	+0.997	+1.002	+1.065

For a solution in water, C 1.0% (W/V).

110 and AS-320; MW 110,000 and 320,000) were measured with a rheogoniometer. Gelation was also observed in AS-110 and AS-320 amylose solutions at a concentration of 1.0% at room temperature (25 °C). An increase of the dynamic modulus was observed with increasing temperature up to 30 °C, then it essentially showed a constant value until the temperature reached 45, 60, and 70 °C, which were estimated to be a transition temperature, for AS-110 (0.8 and 1.0%) and AS-320 (1.4%) amylose solution, respectively, as shown in Fig.4. The dynamic modulus, however, decreased rapidly above the transition temperature. The phenomenon, showing an increase of the dynamic modulus and staying at a constant value with increasing temperature, was in agreement with that of potato amylose (Fig.3). A little increase of the dynamic modulus during increase of the temperature up to 30 °C may be due to formation of an intermolecular association under frequency. The transition temperature indicates that a conformational change of the amylose molecules occurred above the temperature.

The dynamic viscoelasticity of potato amylose in solution (1.6%) was measured in the presence of urea (4.0M), which is known as a hydrogen-bonding breaker. The least dynamic viscoelasticity was observed, indicating that urea prevents hydrogen-bonding in amylose molecules (not cited in Figure). This result indicates that hydrogen bonding has a dominant role in both intra- and intermolecular associations of amylose molecules in aqueous solution.

The optical rotation of a 1.0% solution of potato, AS-110, and AS-320 amylose at various temperatures was determined after dissolving the sample at 145 °C, and then cooling the temperature from 80 to 20 °C (Table). The optical rotation increased gradually with decreasing temperature up to 25 °C, then it increased rapidly. This suggests that an intermolecular hydrogen bonding of amylose molecules may take place at a temperature below 25 °C.



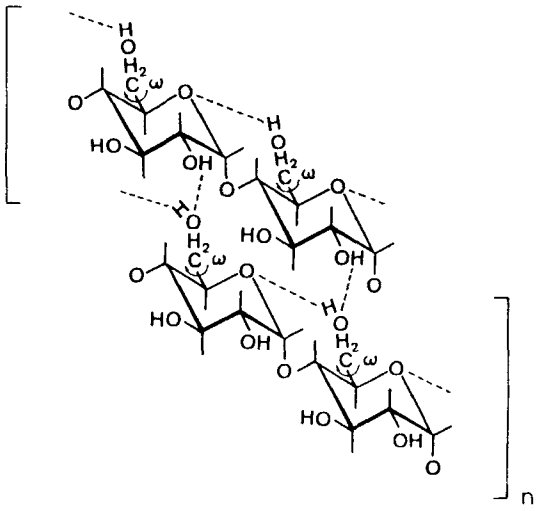
Scheme 1. Possible mode of intramolecular hydrogen bonding of amylose. The dotted lines refer to hydrogen bonding.

DISCUSSION

Transition temperatures at which viscosity decreased rapidly were observed in the temperature range of 25-35 °C and 80-90 °C in aqueous solutions of potato amylose. Furthermore, a transition temperature at which dynamic modulus decreased rapidly was also observed in temperature ranges of 65-80 °C and 45-70 °C in potato and synthetic (AS-110 and -320) amylose, respectively. These results support the notion that intramolecular hydrogen bonding[10,12-19] occurs in amylose and has a great influence on gelling properties together with intermolecular hydrogen bonding in amylose molecules in aqueous solution. As reported previously,[19] the OH-6 group and hemiacetal oxygen atom of the D-glucosyl residues of curdlan molecules, which consists of (1-3)-linked β-D-glucose residues,[20] might take part in both intra- and intermolecular hydrogen bonding in aqueous solution.

Thus, we conclude that intramolecular hydrogen bonding may take place between OH-6 and the adjacent hemiacetal oxygen atom of the D-glucosyl residues, as illustrated in Scheme 1. This bonding is likely owing to the flexibility of α-(1-4)-linkage and extended conformation at high temperature. Since amylose molecules may exist in several conformations depending on the molecular weight and on the conditions under which the amylose is precipitated from solutions,[20,21] it could also exist in another type of intramolecular hydrogen bonding between OH-3 and the adjacent hemiacetal oxygen atom of the D-glucosyl residues.

In addition, intermolecular hydrogen bonding may take place between the OH-2 and the adjacent O-6 of the D-glucosyl residues on different molecules, as in the solid state[4,5,23](Scheme 2). A part of the intermolecular hydrogen bonding, side-by-side,



Scheme 2. Possible mode of intermolecular hydrogen bonding of amylose. The dotted lines refer to hydrogen bonding.

association,[5] breaks down above a transition temperature, 25-35 °C, during increase in the temperature under shearing force (Fig. 2). Residual intermolecular, together with intramolecular hydrogen bonding is lost above another transition temperature, 80-90 °C, under shearing force. Under frequency (Fig. 3 and 4), however, both intra- and intermolecular hydrogen bonding of amylose molecules are stable until the temperature reached 65-80 °C. This model corresponds to a double-stranded helix. Within the double helix, interstrand stabilization is achieved through the intermolecular hydrogen bonding. A tertiary structure of amylose molecules may consist of two identical, left-handed, 6-fold helices in aqueous solution as in the solid state.[5]

Amylose molecules, however, in aqueous solution are notoriously unstable and retrogradation results in an increase in turbidity and eventually precipitation.[23] 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[10,12,14] and results in new intramolecular hydrogen bonding within the OH-2 and the adjacent OH-3 of the D-glucosyl residues.[24] Much more intense intra- and intermolecular hydrogen bonding may result in precipitation of the amylose molecules in aqueous media.

EXPERIMENTAL

Materials. A potato amylose was supplied by Sigma Chem. Co., Ltd., and dissolved in 1M NaOH at low temperature (4 °C). The solution was neutralized with 1M acetic acid, then distilled water was added (5 vols). After addition of ethanol (4 vols), the suspension was centrifuged at 10,000 g for 15 min. The precipitate was washed with ethanol and ether (3 times). The purified potato amylose was dried *in vacuo* ($CaCl_2$). Enzymically synthesized amyloses (AS-110 and 320; MW , 110,000 and 320,000, Nakano Co., Ltd.) were also used without purification.

Analytical Methods. The number-average degree of polymerization ($\overline{d.p.}_n$) was calculated on the basis of the proportions of reducing and total carbohydrate. The reducing residues were determined by the Somogyi[25] and Nelson[26] methods. Total carbohydrate was determined by the phenol-sulfuric acid method.[27]

Methods. Aqueous solutions were obtained with an autoclave (MC-30321, Ikemoto Rika Kogyo Co., Ltd.) by heating mixtures of amylose and distilled water in sealed flasks at 145 °C for 15 min. Optical rotations were measured at 589 nm with an automatic digital polarimeter DIP-180 (Japan Spectroscopic Co., Ltd.), for a solution of 1.0% (W/V) in water, with cooling system.

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 (1.8cm diam.) with a rotating outer cylinder (2.2cm 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 20-95 °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.[28] Dynamic viscosity (η') and elasticity (G') were calculated by modification of Markovitz's equation.[29] 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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