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Trehalose solution viscosity at low temperatures measured by dynamic light scattering method: trehalose depresses molecular transportation for ice crystal growth

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TITLE RUNNING HEAD: Viscosity of trehalose solution via DLS method (46 characters)

Section/Category: Solution growth; industrial, biological, and molecular crystallization

ABSTRACT:

The inhibitory effects of trehalose on ice crystal growth were discussed on the basis of the viscosity measurements of aqueous solutions via the dynamic light-scattering method. The temperature and concentration conditions of the solution were ranged between 268 and 343K and up to 50 wt%, respectively, which were feasible for applying this novel technique and were useful in the indirect measurement of the macroscopic dynamic properties of the trehalose solutions. A comparison of the viscosity data with those reported in the literatures indicated the validity of this method for measuring the viscosity.

The nonlinearity of the temperature and concentration dependences of the trehalose solutions suggested that two different hydrogen-bonding networks exist in the solutions within the investigated range. Dilute solutions of less than 10 wt% of trehalose exhibited properties very similar to those of pure water. Higher concentration solutions had large viscosities with large temperature and concentration dependences. This was caused by the decrease in the free water in the solution and the development of hydrogen-bonding networks with hydrated trehalose clusters. Sucrose and maltose solutions had the same properties, so this would be the dominant inhibitory process of disaccharides on ice crystal growth. (193 words)

KEYWORDS: A1. Biomaterials, A1. Fluid flows, B1. Organic compounds, B1. Sucrose

PACS-2003 codes: 61.66.Hg Organic compounds, 66.20.+d Viscosity of liquids; diffusive momentum transport, 82.30.Rs Hydrogen bonding, hydrophilic effects, 83.85.Jn Viscosity measurements

Introduction

Trehalose and sucrose in aqueous solutions protect living species from damage caused by freezing [1]. Trehalose is used as a cryoprotectant mainly for animals capable of enduring cold temperatures, whereas sucrose exists in plants [2]. Although maltose has the same chemical composition as trehalose and sucrose ($C_{12}H_{22}O_{11}$), it is not used as a cryoprotecting material in nature. These natural cryoprotecting materials (disaccharides) are also used in medical and engineering applications, such as the cryopreservation of living cells [3].

Sei et al. [4] and Gonda and Sei [5] measured the changes in ice single-crystal shapes and the crystal growth rates for ice in trehalose and sucrose solutions. They suggested that the cryopreservation mechanism of disaccharide solutions appears to be a local increase in the viscosity near a growing ice interface, which inhibited the ice-crystal growth. For a given temperature and concentration, they found that trehalose decreased the growth rate more than sucrose solutions.

In addition to single-crystal observations, the freeze-fractured surfaces of trehalose solution droplets were observed using a field-emission gun type transmission electron microscope (FEG-TEM) [6]. FEG-TEM observations indicated that the size of the ice crystals decreased exponentially with an increase in the trehalose concentration, whereas the number of ice crystals increased exponentially. These results suggest that the size of the ice crystal is suppressed by an increase in nucleation sites in the trehalose solution; therefore, trehalose may act as a nucleation promoter. Thus, the mechanism by which trehalose obstructs crystal growth processes should be investigated by considering the dynamic properties of its aqueous solution.

Both microscopic and macroscopic properties of aqueous trehalose solutions have been studied. For example, infrared [7, 8] and Raman [9-12] spectroscopy studies suggested microscopic conformations of trehalose molecules in solution. These studies also demonstrated that trehalose destroyed water's hydrogen-bonding structure. Such local water structure was also observed via neutron-scattering [13] and laser-light-scattering [14, 15] techniques. These studies revealed the construction of a hydration shell structure around a trehalose molecule. Using nuclear magnetic resonance (NMR) spectroscopy, the

self-diffusion coefficients of both trehalose and water molecules in the aqueous phase were measured [16, 17]. At high trehalose concentrations, even water molecule mobility decreased, possibly due to the high glass transition temperature of trehalose solution [18].

According to the Stokes-Einstein relation, the diffusion constant D varies inversely to the viscosity η :

$$D = k_B T / A \eta R, \quad (1)$$

where T is the absolute temperature, k_B is Boltzmann's constant, and A is a constant that depends on the type of friction between a material with radius R and its surrounding liquid [19]. This relation can be applied to disaccharide solutions when the conditions of the liquid are such that T_g/T is less than about 0.6 [19]. The viscosity of sucrose has been thoroughly studied, but that of trehalose has seldom been investigated. Recently, Longinotti and Corti [20] summarized the experimental data for sucrose and trehalose solutions over a wide range of temperatures and concentrations. Several conventional measurements with mechanical viscometers have been reported [15, 20-26].

Although viscosity measurements via conventional viscometers are very accurate, non-contact methods have been expected to be used to measure viscosity in more realistic systems, such as in a solution containing biomaterials or living cells. Several indirect methods have been proposed, such as the fluorescence recovery after photobleaching (FRAP) [19, 27] and the laser-light-scattering method [15]. However, these indirect methods have been developed very recently, and their accuracies remain relatively low.

In this study, we use dynamic light-scattering (DLS) spectroscopy to measure viscosity under various temperature and composition conditions. This method is applicable to solutions at high pressures [28] and is suggested for measuring viscosity more easily than with conventional mechanical viscometers, especially in dilute solutions. We measure the viscosities of trehalose solutions (η_{treha}) at temperatures in a liquid state and at concentrations less than 50wt% to investigate the validity of the DLS method, and compare these viscosities with those of other disaccharide solutions. We find that the viscosity of trehalose solution is almost the same as that of pure water when the concentration is below 10wt%. At higher concentration, the viscosity depends on both the solute concentration and the

temperature. These tendencies are also observed in sucrose and maltose solutions. We then investigate the inhibitory mechanism of disaccharides for ice-crystal growth for their application as cryoprotectants.

Experimental Methods

Three disaccharides were used for the measurements: research-grade trehalose dihydrate crystal, maltose monohydrate crystal (from Hayashibara Biochemical Lab), and special-quality grade sucrose (from Sigma-Aldrich Japan). Disaccharide solutions with appropriate concentrations (less than 50 wt%) were prepared by mixing measured disaccharide powder with distilled and deionized pure water (resistivity of approximately 18 M Ω cm). The pure water was filtered three times through a 0.2 μ m filter before use. A small amount (mass fraction below 5×10^{-6}) of uniformly sized (204 nm diameter) polystyrene latex particles (Seradyn) was then added to the solution as a light-scattering material.

The details of the viscosity measurements are the same as those in [28], so we give only a brief explanation here. Twenty cubic millimeters of the solution was put in the sample cell of the DLS system, and the temperature of the system was controlled by circulating silicone oil through a cooler (Haake, F8-C40). The temperature was controlled between 268 and 343 ± 0.2 K, which meant that T_g/T ranged from 0.4 to 0.6. Light from an Ar-ion laser (20 mW, $\lambda = 488$ nm) was focused onto the center of the sample cell. The light scattered by the latex particles in the solution was collected by an objective lens, and the time-dependent intensity of the mixing of scattered light through two pinholes was measured with the photomultiplier of the DLS spectrophotometer (Photal, DLS-7000). Since the Stokes-Einstein law was obeyed for the disaccharide solutions in the present study, we could compute the autocorrelation function $q_e(\tau)$ of the temporal fluctuations in the photocurrent output and the translational diffusion coefficient of the solution D_τ from

$$q_e(\tau) = \exp(-D_\tau q^2 \tau), \quad (2)$$

where τ is the relative time and q is the scattering vector. The apparent particle diameter d , the viscosity η , and the diffusion coefficient D_τ are related by the Stokes-Einstein law (Eq. 1). Substituting D_τ

obtained by the DLS measurement and $d = 204$ nm, we obtained the viscosity of the solution around the light-scattering material, which equals the absolute viscosity of the solution.

The systematic uncertainties in the viscosities of the disaccharide solutions were attributed to both the temperature and the refractive index, which are related to the scattering vector. The temperature control uncertainty is discussed above. The refractive index of a disaccharide solution varies with both the solute concentration and the temperature. The refractive index of a sucrose solution depends on its concentration and is related to the Brix value (e.g., International Commission for Uniform Methods of Sugar Analysis (ICUMSA) table [29]). We used these refractive indices to analyze the viscosities of sucrose solutions through DLS measurements at room temperature. However, very little data have been reported for the refractive indices of trehalose and maltose solutions. Lammert et al. [30] found that the refractive index of a trehalose solution nearly equaled that of sucrose at 318 K (the maximum discrepancy in their experimental conditions was 0.015%). Therefore, we assumed that the refractive indices of all the disaccharide solutions were equal to the refractive index of sucrose. Shu et al. [31] demonstrated that the temperature dependence of the refractive index of a sucrose solution (concentration of 50 wt%) was approximately $1.6 \times 10^{-4} \text{ K}^{-1}$, which slightly exceeded that of pure water (approximately $1 \times 10^{-4} \text{ K}^{-1}$.) Since the solution temperature in our study was changed from 268 to 343 K, the variation in the refractive index with temperature was less than 0.015. The maximum uncertainty in the refractive indices of the disaccharide solutions was ± 0.03 , which was equivalent to an uncertainty of 4.4% for viscosity estimations. Due to the variation in the refractive indices, fewer uncertainties occurred than with the measurement variations for the DLS method ($\pm 6\%$) [28].

Results

(a) Viscosity of trehalose solution measured by the DLS method

The η_{treha} with various concentrations was measured at room temperature to investigate the validity of this novel technique. **Figure 1** indicates η_{treha} (dots) with different trehalose concentrations C [wt%] measured at 300.3 ± 2.9 K. By fitting the data, we found that η_{treha} increases with C according to

$$\eta_{\text{treha}} = 2.08 \times 10^{-4} C^3 - 8.68 \times 10^{-3} C^2 + 0.141 C + 0.467, \quad (3)$$

with a correlation of 0.99. The cubic η - C relationship was proposed by Elias and Elias [24]. Recently, Longinotti and Corti [20] summarized the concentration dependence of viscosity for an aqueous sucrose solution, and they demonstrated that the specific viscosity (η/η_w , where η_w is the viscosity of pure water at the same temperature [32]) related exponentially to molar concentration c [mol/kg]. The data obtained for the present trehalose solutions also obey this relation with a similar fitting correlation (data not shown):

$$\ln \eta_{\text{treha}}/\eta_w = 0.900 c - 0.0283. \quad (4)$$

The data of η_{treha} were compared with values in the literature for the bulk η_{treha} obtained by several conventional methods. In Fig. 1, several viscosities obtained using these conventional techniques are presented simultaneously. Most of the bulk viscosities of disaccharide solutions were measured by mechanical viscometers: the Ubbelohde viscometer [23-25], the Marion-Krieger Sisco capillary viscometer [26], or the rotational viscometer [15]. The selected literature data plotted in Fig. 1 were measured in a temperature range of 293.2 to 313.2 K. Taking into account this temperature variability, we concluded that the DLS method was feasible and convenient for measuring the viscosities of disaccharide solutions (concentrations of less than 50 wt%).

Viscosities of other disaccharide solutions, sucrose (η_{suc}) and maltose (η_{mal}), were found to be similar to that of η_{treha} at a given concentration within the experimental uncertainties (Fig. 1). Since sucrose solution is one of the most thoroughly investigated solutions, we compared η_{suc} values obtained in the present study with the values found in the literature [20]. A quantitative agreement within the experimental uncertainties suggests the validity of applying the DLS method to viscosity measurements. Furthermore, these three disaccharide solutions had very similar viscosity dependence on concentration relationships at room temperature, particularly in dilute solutions (less than 30 wt%).

(b) Temperature dependence of viscosity of trehalose solutions

We measured the temperature dependence of viscosity to determine how viscosity affects the inhibition of ice crystal growth. The DLS method used in the present study is convenient for this purpose. The temperature dependence of η_{treha} was measured at concentrations ranging from 4.7 to 44 wt%. The highest temperature was set below the structural transition point of trehalose molecules, at approximately 350 K [33]. The lowest temperature, however, was just below the freezing point of the solutions [5]. Since we could measure the DLS signals at all temperatures, we assumed that all the samples were in the liquid state in this temperature range.

We found that the viscosity of a trehalose solution increases with decreasing temperature under all concentration conditions. **Figure 2** depicts an Arrhenius plot of the viscosity of trehalose solutions (all solid marks, crosses, and Xs). As a reference, the plot for pure water is denoted with a thick dashed line. This figure also indicates that η_{treha} follows an Arrhenius temperature dependence ($\eta_{treha} = \eta_0 \exp [Q_{treha}/kT]$, where η_0 is constant and Q_{treha} is the temperature dependence parameter for trehalose solution) under the present study conditions (see Appendix A). The temperature dependence fit an Arrhenius curve even below the melting point for solutions with any concentration. Therefore, below the melting point, the viscosity of a supercooled trehalose solution can be predicted by extrapolating the temperature dependence of the viscosity in the liquid state, at least within our experimental validity (i.e, the temperature is not below $T_g/0.6$ [19]).

The variation of the temperature dependence parameter of trehalose solutions (Q_{treha}) with the solute concentration C indicates interesting properties: Q_{treha} for dilute solutions with concentrations of less than 10 wt% is almost concentration-independent, which is the same as that of pure water ($Q_w = 17.3 \pm 1.4 \text{ kJ mol}^{-1}$). That is, the dynamic properties of dilute solutions are similar to those of pure water. This interesting result is very important when considering the cryoprotection effect of trehalose used in biological and engineering applications because trehalose is usually applied in dilute conditions of less than 10 wt%.

However, Q_{treha} increases linearly with an increase in C above 10 wt%. This result indicates that trehalose solutions have two types of structures in the liquid phase: a dilute solution ($C < 10 \text{ wt\%}$) and a

solution with a concentration higher than 10 wt%. Since the values of Q_{treha} in higher-concentration solutions increased linearly with C , the macroscopic properties of solutions were assumed to be continuously changing with trehalose concentration.

The temperature dependence parameter Q of other disaccharide solutions was also observed. Here, η_{suc} with a concentration of 9.1 wt% and η_{mal} at 8.6 wt% exhibited temperature-dependent viscosities similar to that of pure water. We then compared the temperature-dependence parameters of three disaccharide solutions at concentrations exceeding 30 wt%. Figure 2 indicates the temperature dependences of the viscosities of both the sucrose solution ($C = 38.5$ wt%; open squares) and maltose solution ($C = 35.8$ wt%; open triangles). The results indicate that the temperature dependence is nearly the same for all three disaccharide solutions at the same concentration.

Discussions

(a) Solute-solution model of trehalose solution estimated from viscosity measurements

As mentioned in the previous sections, η_{treha} was similar to the viscosity of pure water when C was less than 10 wt%, but increased when C exceeded 10 wt% at room temperature. The temperature dependence of η_{treha} was empirically described by the Arrhenius equation to estimate the temperature dependence parameter Q_{treha} . This approach is reasonable in the present study conditions, at $0.4 < T_g/T < 0.6$. In addition, Q_{treha} increased when C exceeded 10 wt%. Therefore, we assumed that the macroscopic properties of solutions, which control viscosity, would change at approximately 10 wt%. We then considered the solute-solution model of trehalose discussed below.

In dilute solutions ($C < 10$ wt%), the major matrix of the solutions is pure water (or free water) and the trehalose molecules exist as minor solute materials. Since the viscosity in these solutions is nearly the same as pure water viscosity, we conclude that the trehalose molecules make only a small contribution to the macroscopic solution properties, or to the hydrogen-bonding network of the free-water molecules, even though the trehalose molecules tend to break the hydrogen-bond network of water [10-11, 13-15].

In high-concentration solutions ($C > 10$ wt%), free-water molecules are consumed by trehalose molecules due to their high hydration abilities (the hydration number of trehalose is 11 ± 4 [34]). The result is the change in the dynamic structure of the solution from a hydrogen-bond network of free water to a network structure related to hydrated-trehalose molecules, which contributes to the viscosity of the solution. Thus, we define the free-water ratio of the solution, $W = (\text{free water})/(\text{total water})$, to consider the phase change in the network structure of the solution by the following equation:

$$W = (1 - C/C_s)/(1 - C/100), \quad (5)$$

where C_s is the saturation concentration. Based on the trehalose hydration number mentioned above, C_s is 63 ± 7 wt%. Using this equation, the dilute condition ($C < 10$ wt%) corresponds to W exceeding approximately 93%.

As expected, Q_{treha} increases linearly with a decrease in W for $W < 90\%$ (Fig. 3). The error bars for W were estimated from the variation of C_s , and those of Q_{treha} were estimated from the standard deviations of Q_{treha} in Fig. 2. Since Q_{treha} is an energy term, the increase in Q_{treha} for higher-concentration solutions is a reflection of the higher energy barriers in the translational motion of bound molecules, or an increase in the activation energy of the self-diffusion coefficients of both water and trehalose molecules. The self-diffusion coefficients of either solute or solution molecules in disaccharide solutions were previously measured by NMR spectroscopy [16, 17]. From these reported values, the activation energies for both molecules were found to increase gradually with a decrease in W . Therefore, the energy barrier for the translational movement of the matrix is caused mainly by the lack of free water.

A solute-solution model of low- W (or high- C) solutions has been proposed by molecular dynamics (MD) simulation [34]. Researchers suggested that the hydrated clusters of disaccharide molecules began to interact, forming compartment-like hydrogen-bonding networks. These clusters were predicted to be giant hydrated saccharide clusters [5, 34], suggested from FEG-TEM image observations [6]. Therefore, a network structure with a high-energy barrier for the translational motion of the matrix was assumed to be built with these giant hydrated saccharide clusters. Since Q_{treha} does not change step-wise, the phase

change of the network structure from a free-water matrix to a disaccharide-cluster matrix would progress partially in the solution.

In addition, we compare Q_{suc} and Q_{mal} at low W conditions. Figure 3 indicates that the W dependences of Q_{suc} (open squares) and Q_{mal} (open triangles) coincided well with the Q_{treha} vs. W relation within the experimental uncertainties. Here, the hydration numbers used for the C_s estimate in sucrose solution was assumed to be 10.5 ± 4.0 , and that in maltose solution was assumed to be 10.1 ± 3.8 [34]. Therefore, it is assumed that all three disaccharides play similar roles in the structurization of solutions.

(b) Inhibitory effects of trehalose on ice-crystal growth

The volume ratio of free water W decreases with an increase in C . The remaining region is the trehalose-binding (or hydrated) water, which forms clusters under low C conditions, and the macroscopic cluster-binding network in high C solutions. We assumed that this network forms a compartment-like structure [34] in the solution, which gradually develops with an increase in C . Since this network develops all over the solution, its bulk viscosity increases.

The ice-crystal growth excludes trehalose molecules from solid phase. Since the self-diffusion coefficients of trehalose molecules were one-order of magnitude smaller than that of water molecules [16], the excluded trehalose molecules build up at the freezing interface. This process increases the local viscosity of solution in front of ice crystal, which further increases the local concentration. The freezing temperature at the interface then decrease (constitutional supercooling), reducing the driving force of ice-crystal growth. A crystal usually has facets under low driving force condition. However, the ice crystal in a high-trehalose-concentration solution is round [4, 5]. This surface roughening indicates that the inhibitory effects of trehalose on ice crystal growth not only lower the mass transport but also kinetically interrupt the structurization of ice as a result of the high viscosity of trehalose solution at the freezing interface.

Based on above discussions, we can determine the cryoprotection process of trehalose. The viscosity of a solution, including dissociated living cells (approximately 0.1 mm in diameter [3]) that contains

trehalose as a cryoprotectant increases when it is cooled below the melting point. If ice crystals nucleate in the solution, their growth rates are decreased by the accumulation of the excluded trehalose in front of the ice crystal, which reduces the mass transport of water molecules from the surrounding solution to the ice crystal. The excluded molecules also play roles in decreasing free-water molecules, increasing the viscosity of the solution and the decreasing freezing temperature (constitutional supercooling). The trehalose-binding water network keeps the ice crystal size small and further reduces the ice crystal growth rate, thus promoting the expulsion of water molecules from neighboring cells due to an osmotic pressure difference. The crystal also becomes rounder due to kinetic roughening at the surface. These phenomena ensure the safety of the dispersed living cells by minimizing the danger of mechanical damages from ice crystals. Therefore, the inhibitory mechanisms of trehalose differ from those of anti-freeze protein solutions, which inhibit the kinetics of ice-crystal growth by direct contact with the crystal surface [35, 36].

Therefore, we conclude that disaccharide molecules protect a dispersed living cell from mechanical damage of the surrounding ice crystals by decreasing the size of ice crystals and rounding their shapes. Both resulting from their high hydration properties and from the viscosity increase due to their network formation in the solution.

Conclusions

We applied a novel technique, dynamic light-scattering (DLS) spectrometry, to measure the viscosity of trehalose solutions and other disaccharides, sucrose and maltose. Based on the viscosity data, we determined the role of trehalose on the growth-inhibitory properties of ice crystals. The viscosities of the trehalose solutions increased with concentration at room temperature, with almost similar dependences seen in the three disaccharides. However, the viscosity of the trehalose solution increased with temperature drop. To consider the macroscopic property change related to viscosity under the present experimental conditions, we approximated the temperature dependence using an Arrhenius plot for each concentration solution. The temperature dependence parameters of dilute solutions (less than

10 wt%) were concentration-independent and the same as those of pure water. However, at higher concentrations, they increased with a decrease in the free-water ratio. These properties can be attributed to a change in the water state, from a state where free-water molecules dominate the solution properties to one where most of the water molecules are strongly bound to the trehalose molecules. When the free-water ratio in the solution decreased due to consumption from ice crystal formation or hydration to trehalose molecules, the ice-crystal growth rate was decreased by decreases in the rates of both the water supply from the surrounding environment and the trehalose removal from the ice-solution boundary. These processes are basically similar for all three disaccharides, based on the result of similar viscosities in a given condition. The cryoprotection of a dispersed living cell in disaccharide solutions is therefore caused by the inhibition of extracellular ice growth.

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SUPPORTING INFORMATION PARAGRAPH:

Appendix A: Temperature dependence of viscosity of trehalose solution; Angell plot

Several researchers [11, 20] suggested that the temperature dependence of the viscosity of the trehalose-water system did not obey the Arrhenius law, particularly in the case of a solution with the highest concentration. To verify the consistency of the viscosity data obtained in the present study with those reported in the literature, we created an Angell plot ($\ln \eta$ vs. T_g/T , where T_g is the glass transition

temperature estimated by Green and Angell [18]), which is recommended instead of the Arrhenius plot.

We confirmed that all the data obtained in the present study also fit a universal curve:

$$\eta_{treha} = 6 \times 10^{-5} \exp [21.5 T_g/T] \quad (\text{a1})$$

with a correlation of 0.935 ($n = 77$), (Fig. A). Therefore, we assumed that the obtained data were consistent with the previously obtained data, and that it was feasible to obtain viscosity data with the DLS method under the present study conditions. In the present study, we sought to determine the difference in temperature dependence at various concentrations. We subsequently confirmed that the temperature dependence of the viscosities of the disaccharide solutions obeyed the Arrhenius relation under the conditions of this study.

FIGURE CAPTIONS:

Figure 1. Viscosities (η) of disaccharides vs. concentration (C) at room temperature. The dots indicate the relative viscosities of trehalose (η_{treha}), the open triangles indicate the relative viscosities of sucrose, and the open squares indicate the relative viscosities of maltose. The error bars for all the trehalose solution data indicate the maximum variations in η_{treha} observed in the DLS measurements, which are almost the same as the measurement uncertainties. The dotted line indicates the fitting curve for η_{treha} in Eq. (4). This figure also indicates the η_{treha} near room temperature obtained in previous reports with various conventional methods. Open diamonds [26], open inverted triangles [23], Xs [24], and crosses [17] are plotted.

Figure 2. Temperature dependence of viscosities for various concentrations of disaccharide solutions: η_{treha} , η_{suc} , and η_{mal} . Dots represent η_{treha} at a concentration of 4.75 wt%, crosses represent η_{treha} at a concentration of 9.93 wt%, solid diamonds represent η_{treha} at a concentration of 14.7 wt%, solid squares represent η_{treha} at a concentration of 24.5 wt%, solid triangles represent η_{treha} at a concentration of 29.4

wt%, Xs represent η_{treha} at a concentration of 34.7 wt%, and solid inverted-triangles, represent η_{treha} at a concentration of 44.3 wt%. The thin dotted line denotes the Arrhenius curve fit for each concentration. The thick dashed line indicates the temperature dependence of the viscosity of pure water, η_w [32]. The thick short dotted vertical line on each curve is the melting point of the solution [5]. Open squares denote 38.5 wt% concentrations of sucrose solutions (η_{suc}), and triangles denote 35.8 wt% concentrations of maltose solutions (η_{mal}).

Figure 3. Temperature dependence parameter of trehalose solutions (Q_{treha}) (dots) vs. the free-water ratio (W) estimated by Eq. (5). The Q_{suc} estimated from the experimental data presented in Fig. 2 is denoted by open squares, and Q_{mal} estimated from the experimental data presented in Fig. 2 is denoted by open triangles. Error bars for the W estimation were from the uncertainty of the hydration number [34], and those for Q were from the standard deviations of the Q estimate in Fig. 2.

Figure A. Angel plot of viscosities for various concentrations of trehalose solutions ($\ln \eta_{treha}$ vs. T_g/T ; where T_g is the glass transition temperature estimated by Green and Angell [18]). The dotted line is the fitting curve shown in Eq. (a1).

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Fig. 1

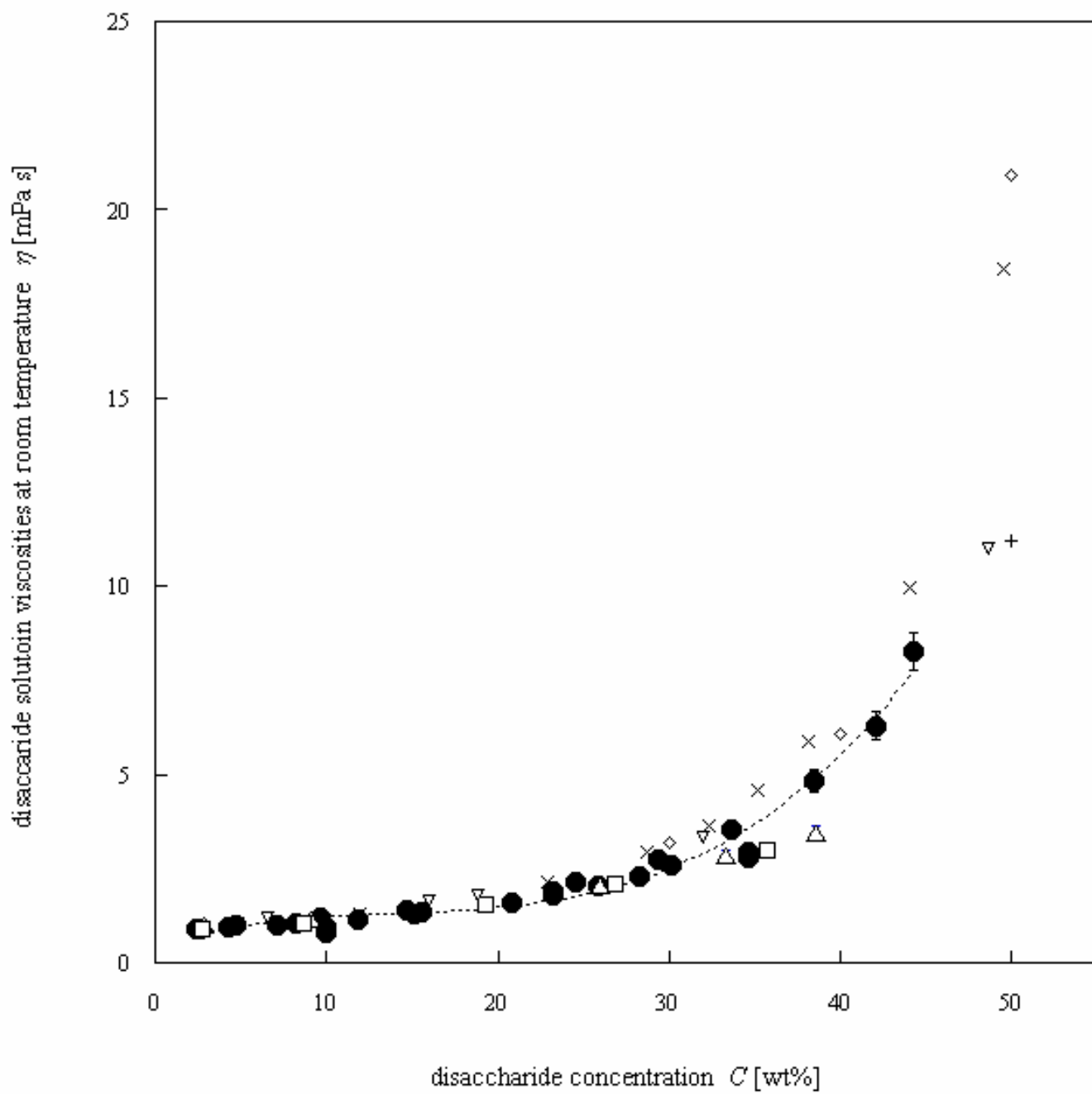


Fig. 2

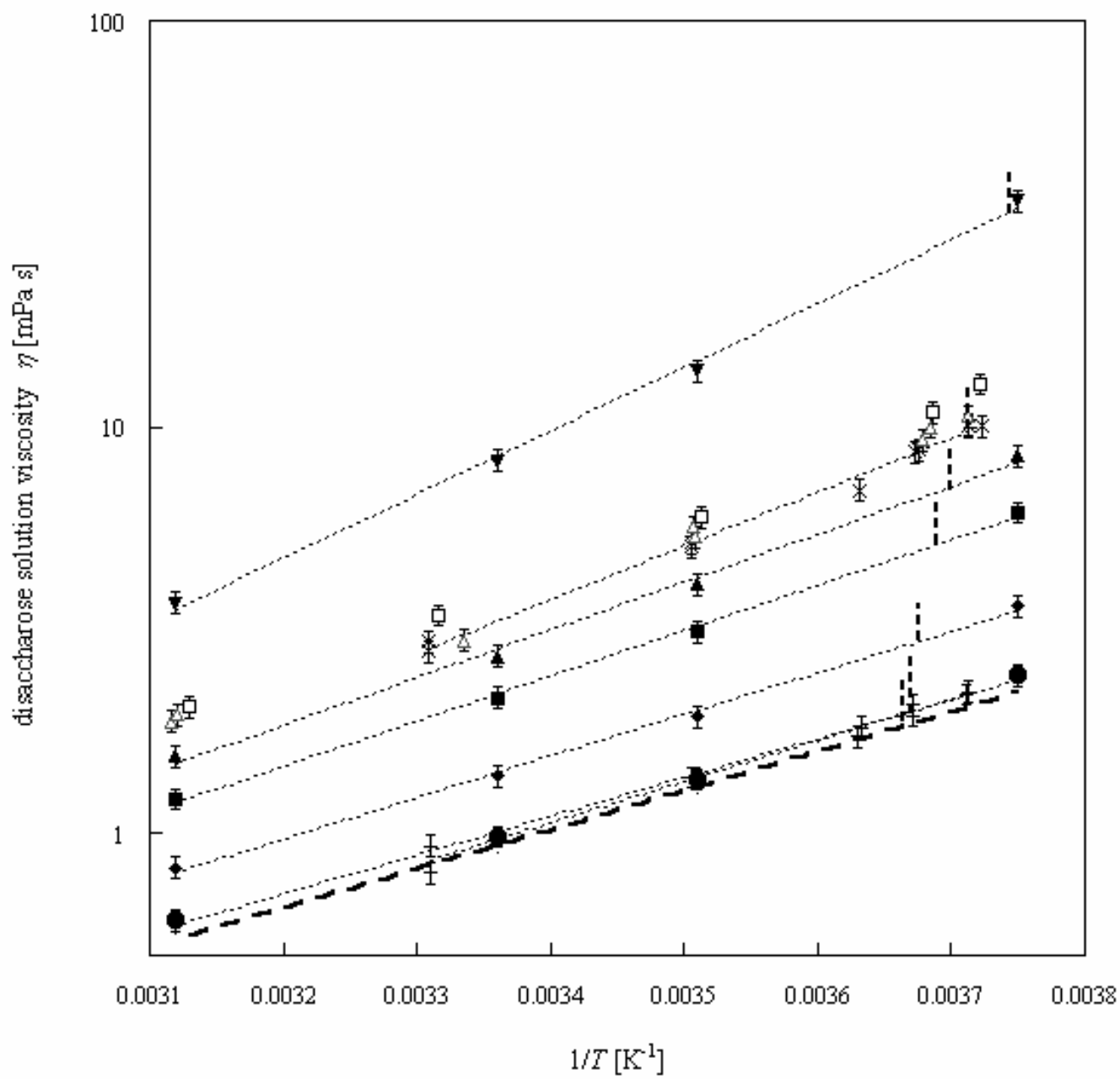


Fig. 3

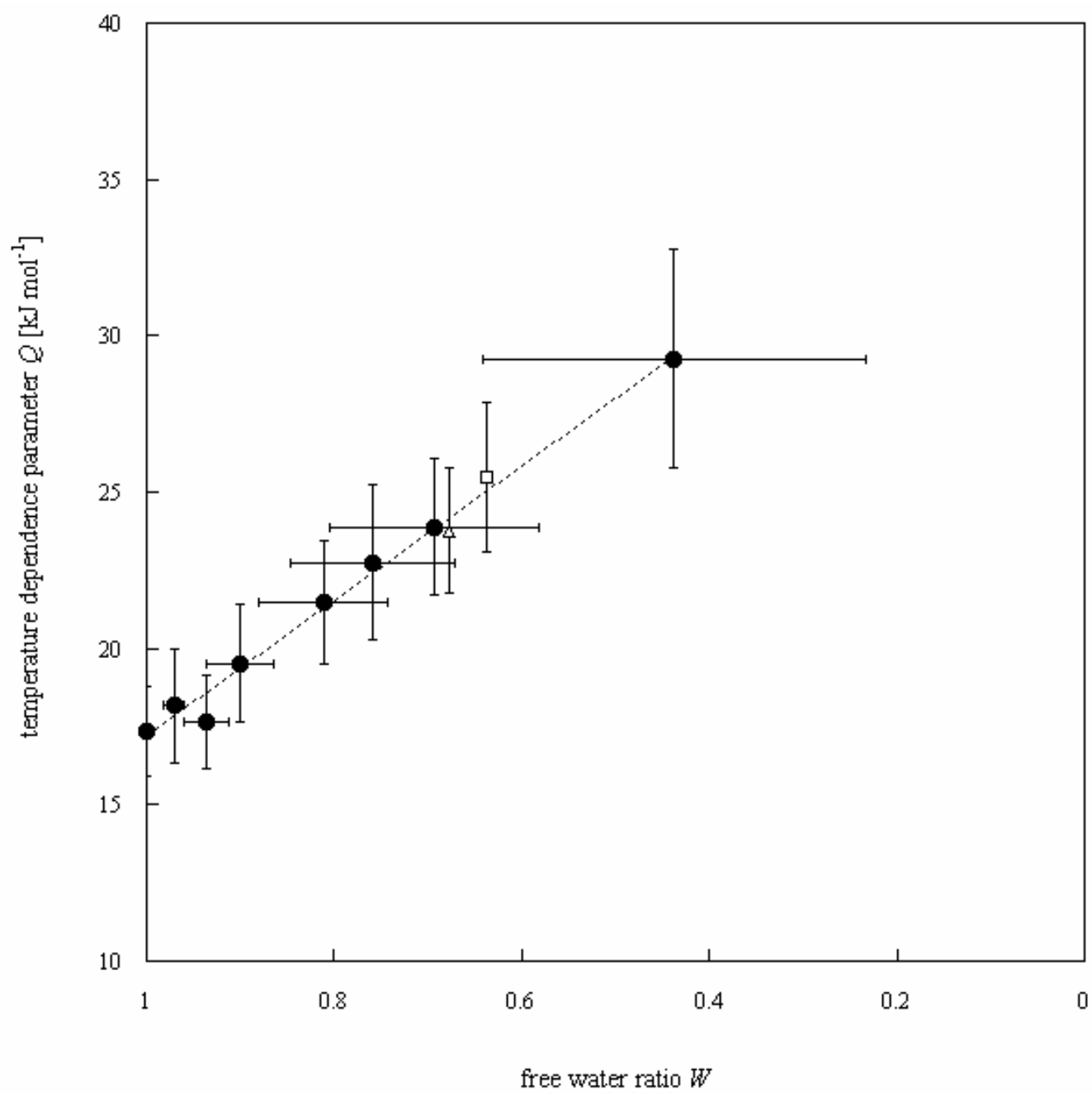


Fig. A

