Quantitative Characterization of Hydration State and Destructuring Effect of Monosaccharides and Disaccharides on Water Hydrogen Bond Network

K. Shiraga¹, T. Suzuki¹, N. Kondo¹, J. De Baerdemaeker² and Y. Ogawa^{1*}

¹ Graduate School of Agriculture, Kyoto University, Kitashirakawa-Oiwakecho, Kyoto 606-8502, Japan

² Division of Mechatronics, Biostatistics and Sensors (MeBioS), Department of Biosystems, K. U. Leuven, Kasteelpark Arenberg 30, B-3001 Leuven, Belgium

* Corresponding author

(Phone) +81-75-753-6169

(E-mail) ogawayu@kais.kyoto-u.ac.jp

(Adress) Kitashirakawa-Oiwakecho, Kyoto 606-8502, Japan

ABSTRACT. Terahertz time-domain attenuated total reflection measurements of monosaccharide (glucose and fructose) and disaccharide (sucrose and trehalose) solutions from 0.146 M to 1.462 M were performed to evaluate (1) the hydration state and (2) the destructuring effect of saccharide solutes on the hydrogen bond (HB) network. Firstly, the extent of hydration water was determined by the decreased amount of bulk water with picosecond relaxation time that was replaced by that

with much longer orientational relaxation time. As a result, we found glucose and trehalose exhibits stronger hydration capacity than fructose and sucrose, respectively, despite of the same number of the hydroxyl groups. For each saccharide, the hydration number tended to decrease with solute concentration. Secondly, the destructuring effect of these saccharide solutes on the HB network of the surrounding bulk water was discussed from the perspective of the fraction of non-hydrogen-bonded (NHB) water isolated from the HB network. We found the fraction of NHB water molecules that are not engaged in the HB network monotonously increased with saccharide concentration, indicating saccharide solutes promote the disruption of the water HB network. However, no noticeable differences were confirmed in the fraction of NHB water between glucose and fructose or between sucrose and trehalose. In contrast to hydration number, the number of NHB water produced by a single saccharide solute was less dependent on solute concentration, and three monosaccharide/disaccharide solutes were found to produce one/two NHB water molecules.

KEYWORDS. Terahertz time-domain attenuated total reflection spectroscopy, complex dielectric constant, saccharides, hydration, hydrogen bond, destructuring effect

1. Introduction

Saccharide molecules have long been known for their peculiar bioprotective characteristics, as seen in the response toward severe external stresses, such as temperature change and dehydration. However, the underlying molecular mechanisms for this are still poorly understood in detail. To explain the bioprotective capacity of saccharides, several hypotheses have been proposed; the “water-replacement” hypothesis, which assumes saccharides directly form hydrogen bonds (HBs) with biomolecules by substituting for hydrated water, is a scenario to interpret the preserved biomolecular three-dimensional structure.^{1,2} However, some recent studies concluded saccharides were preferentially excluded from the biomolecular surface,^{3,4} as opposed to the “water-replacement” hypothesis.

Alternatively, significant slowing down of biomolecular motion is thought to partially contribute to the bioprotective effect, since viscous saccharide solutions decrease the flexibility of proteins (thereby reducing its conformational entropy) and thus stabilize their tertiary structure of proteins over a wide range of temperatures.^{5,6} The origin of the dynamical slowdown in saccharide aqueous solutions is supposed to be the large hydration capacity of saccharides⁷⁻⁹ and/or a aggregation of saccharides at high concentrations.¹⁰ Especially, the abundant hydrophilic groups exhibit a noticeable effect to trap hydrated water molecules in the vicinity of the saccharide surface, increasing the HB activation energy and decreasing the rotational/translational diffusion coefficient.⁹ Thus, this strong hydration effect of saccharides results in thermodynamically stabilizing the entire system, behaving as a buffer toward external stresses. On the other hand, recent computational^{7,8,11-14} and experimental¹⁵⁻²² results have suggested that the peculiar bioprotective effect of saccharides may related to a “destructuring effect” on the water HB network. This concept was originally developed by Branca et al., based on various approaches,¹⁵⁻¹⁷ who pointed out that saccharides with a different strengths, promote distortion of the native tetrahedral HB network of water (destructuring effect), impeding the crystallization process to reduce the freezable water. Accordingly, these facts denote another probable hypothesis for the bioprotective

capacity of saccharides: the strong hydration ability of saccharides stabilizes the conformation of proteins, while the water HB network is “destructured” to prevent freezing upon cooling.

In addition to molecular dynamics (MD) simulations^{7,8,11-14}, some experiments such as Raman spectroscopy,^{15,17-19} infrared spectroscopy,¹⁷ inelastic neutron scattering,¹⁷ dielectric relaxation spectroscopy,^{20,21} nuclear magnetic resonance²² and viscosity measurements¹⁶, have shed some light on the hydration capacity and destructuring effect of saccharides in aqueous solutions. However, details of the saccharide – water interactions remain obscure due to insufficient experimental evidences. For example, only a few experimental methods can quantitatively determine the hydration state (i.e. hydration number), and inconveniently, the definition of hydrated water is dependent on the adopted experimental method and analytical model used, confusing understanding of overall hydration mechanisms. Moreover, the HB destructuring effect has been investigated mainly in terms of distortion of the water tetrahedrality.¹⁵ On another front, in the framework of an “intermittent” HB scheme (the HB pair can break and switch/rejoin after a time), non-hydrogen-bonded (NHB) water temporally appears at sub-picosecond timescales.²³ Since the population of NHB water is supposed to increase as the HBs are destructured, NHB water may be used to infer the HB destructuring effect in aqueous solutions. However, the existence of NHB water around saccharide solutes have yet to be experimentally measured, due to the absence of effective experimental measurement methods to probe the sub-picosecond water dynamics.

Considering that the fundamental characteristics of water dynamics originate from the strong dipole fluctuations between water molecules at a sub-picosecond (10^{-13} s) or picosecond (10^{-12} s) timescale,^{24,25} IR active (dipole allowed transitions) spectroscopic measurements that are sensitive to sub-picosecond and picosecond timescales will provide structural and dynamical characteristics of the HB network of bulk water. Recently, it has been proposed that the complex dielectric constant ($\tilde{\epsilon} = \text{Re}[\epsilon] - i\text{Im}[\epsilon]$) in the terahertz (THz) region is expected to be able to directly characterize changes in the HB network.^{26,27} This is because THz waves, defined here as 0.1 ~ 10 THz, correspond to dynamics at sub-picosecond and picosecond timescales (i.e. $\omega = 1$ THz

corresponds to $\tau=0.16$ ps), and thus, the dielectric responses in the THz region will sensitively reflect dynamical motions such as relaxation and vibration of bulk water molecules engaged in the HB network. In contrast, previous experiments^{20,21} and simulations^{7,13,14} have revealed that the relaxational behaviors of saccharide and hydrated water are retarded compared to that in bulk water. In particular, SPC or SPC/E potential MD simulations^{7,13,14} suggested massive saccharides take longer time (i.e. tens or hundreds of picoseconds) to reorient, and hence, the molecular reorientation of hydrated water concerted with saccharide's dynamics is also slowed down by a factor of 2~3 relative to bulk. Consequently, the relaxation modes of saccharide solutes and hydrated water molecules lie in the low GHz frequencies, causing no responses in the THz region. Additionally, the intermolecular vibration modes of crystallized saccharides (evident above 1 THz)^{30,31} are absent in aqueous solution, since dispersed saccharides in water cannot form saccharide – saccharide HBs.³² Therefore, dielectric responses of dissolved saccharide solutes and hydrated water are negligibly small in the THz frequencies,³³⁻³⁶ and the complex dielectric constant in the THz region solely reflects the dynamics of only bulk water engaged in the HB network, allowing us to directly look into the HB destructuring state of water. At the same time, hydration state can also be evaluated from the reduction in the response of bulk water, which is simply replaced by the corresponding amount of hydrated water.³³ However, despite the recent application of THz spectroscopy to various fields,³⁷⁻³⁹ characterization of the water HB network has been restricted due to the strong absorption of water in the THz region. To overcome this problem, attenuated total reflection (ATR) spectroscopy will be a strong tool to precisely determine the complex dielectric constant of aqueous samples, reducing the strong absorption of water.³²

In the present study, a comparative study of monosaccharides (glucose and fructose) and disaccharides (sucrose and trehalose) in aqueous solution is performed by THz spectroscopy. Based on the analysis of their dielectric responses in the THz region, the extent of hydration and the destructuring effect on the HB network is characterized. The hydration state is defined as all water molecules whose reorientational relaxation time is longer than those in bulk water. In this

definition, the existence of weakly perturbed hydrated water in the second hydration shell, as well as strongly hydrated water in the first hydration shell, is observed. The destructuring effect is quantified as the fraction of NHB water that is released from the HB network. Our results are compared with previous experiments and MD simulations.

2. Experimental

Powder samples of monosaccharides (glucose and fructose) and disaccharides (sucrose and trehalose) from Wako Pure Chemical Industries, Ltd. were dissolved in distilled water. The solutions were prepared at various mol concentrations, ranging from 0.146 to 1.462 M for both the monosaccharides and disaccharides.

In order to precisely determine the complex dielectric constants of saccharide solutions and distilled water, THz time-domain attenuated total reflection (THz TD-ATR) spectroscopy was employed.³² In this measurement scheme, when a temporal THz pulse was focused on and totally reflected from the ATR prism – sample interface, the THz electric field penetrated into the sample as an evanescent field, as shown in the inset in Figure 1. As a result of the interaction between the evanescent field and the sample, both the attenuated total reflectance $ATR(\omega)$ and phase shift spectrum $\varphi(\omega)$ in the frequency domain can be simultaneously determined after Fourier transform of the time-domain THz pulses with and without a sample. Then, the complex dielectric constant $\tilde{\epsilon}(\omega)$ of the sample was mathematically calculated based on the Fresnel's equation.³² In the present study, all the measurements were performed on a commercial THz TD-ATR spectrometer, TAS7500 (ADVANTSET Co.). The ATR prism, made of silicon crystal, equipped with a temperature controller to maintain sample temperature at 27 °C, was placed at the focal position of the THz radiation. The incident angle of the THz pulse on the prism surface was 57°. The complex dielectric constant was measured from 0.15 to 4.0 THz, with the frequency resolution set at 7.6

GHz. In our measurement, typical standard errors in the real and imaginary parts of distilled water were 0.007 and 0.012 (corresponding to 0.14 % and 0.32 %) at 0.5 THz, 0.019 and 0.009 (0.45 % and 0.37 %) at 1.0 THz and 0.033 and 0.014 (0.86 % and 0.80 %) at 2.0 THz, respectively. These small errors allowed us to proceed to the further discussions in the next section.

3. Results and Discussion

3.1. Principle of Analysis. Figure 1 shows the attenuated total reflectance $ATR(\omega)$ and phase shift $\varphi(\omega)$ for distilled water and the glucose solutions (as representative samples among four saccharide solutions) between 0.15 and 4.0 THz. While $ATR(\omega)$ of the glucose solutions uniformly became higher over the entire frequency range as concentration increased, $\varphi(\omega)$ of distilled water and the glucose solution almost completely was overlapped above 2.0 THz. This is because absorptive bulk water was replaced by solutes and hydrated water molecules, which do not couple with the THz waves. The same tendency was also observed in the previous THz TD-ATR measurement.³²

The concentration dependence of the complex dielectric constant at 27 °C for distilled water and the glucose solutions, calculated from $ATR(\omega)$ and $\varphi(\omega)$, are shown in Figure 2. Although there was only a slight decrease in the real part ($\text{Re}[\epsilon]$: polarizability) of the saccharide solution, a significant attenuation in the imaginary part was observed ($\text{Im}[\epsilon]$: dielectric loss due to absorption). The absorption peaks assigned to glucose – glucose intermolecular forces were absent in the aqueous solution due to the mediation of water.³⁰⁻³² On the other hand, a strong concentration dependence was observed in the imaginary part, especially in the lower frequencies.

Figure 3 compares the complex dielectric constants of examined saccharide solutions at the highest concentration (1.462 M). As with Figure 2, the imaginary part differed more clearly than for

the real part, and no sharp peaks inherent in the saccharides themselves could be observed in the saccharide solutions. The difference between distilled water and disaccharide (sucrose and trehalose) solutions was roughly twice larger than that between distilled water and monosaccharide (glucose and fructose) solutions. Furthermore, slight differences were confirmed between glucose and fructose, as well as between sucrose and trehalose, in the low frequency region (inset in Figure 3), although their error bars were partly overlapped. This result implied different monosaccharides (or disaccharides) exhibited different interaction with water, even though they have the same chemical formula and number of hydrophilic groups. To understand the mechanisms of this in more detail, further discussions are provided in section 3.2. ,

3.2. Decomposition of Raw Data. As mentioned in Introduction, the dielectric properties in the THz region result from picosecond or sub-picosecond dynamics of bulk water, thus the relaxation modes of saccharide solute molecules and their hydrated water molecules can be ignored in the THz region, due to their much long relaxation times relative to bulk water.^{33,40} Previous studies have confirmed this: Debye type relaxation of disaccharides, monosaccharides and hydrated water molecules peaks around a few GHz, and therefore they have almost zero contributions above 0.15 THz.⁴⁰⁻⁴²

On this basis, the complex dielectric constants of saccharide solutions, as well as distilled water, were decomposed into four complex susceptibilities: the Debye-type slow relaxation mode of bulk water ($\tilde{\chi}_{\text{slow}}$), the Debye-type fast relaxation mode of bulk water ($\tilde{\chi}_{\text{fast}}$), the Lorentz-type intermolecular stretching vibration mode ($\tilde{\chi}_{\text{S}}$) of water, and the higher frequency limit (ϵ_{∞}).^{26,27} According to dielectric spectroscopy in the microwave region, the slow relaxation mode is known to be a collective orientation mode of water molecules engaged in the water HB network.⁴³ Since the slow relaxation mode of bulk water (~20 GHz) is clearly distinct from the orientational relaxation of hydrated water (peaked around or below GHz frequencies), the decrease in the slow

relaxation strength is interpreted as the increase in corresponding amount of hydrated water.^{33,34} On the other hand, the fast relaxation mode is an individual relaxation mode of NHB water molecules that are isolated from HB network.^{26,43} Thus, the slow relaxation mode is indicative of the HB bulk water molecules unperturbed by the saccharide solutes, while the fast relaxation mode reflects those water molecules neither bound up in the HB network, nor perturbed in the hydration layer. Previous *ab initio* MD simulations⁴⁴ and low frequency Raman spectroscopy⁴⁵ proved that the intermolecular stretching vibration mode around 5.0 THz is associated with the tetrahedral configuration of the HB network. The final term, the higher frequency limit originates from the polarization of higher frequency resonances, and thus shows no frequency dispersions in the THz region.³³

In order to precisely characterize the solute-dependence and concentration dependence of each complex susceptibility, the measured complex dielectric constants $\tilde{\epsilon}(\omega)$ were decomposed into these four terms by a least-square method,^{26,27}

$$\begin{aligned}\tilde{\epsilon}(\omega) &= \tilde{\chi}_{\text{slow}}(\omega) + \tilde{\chi}_{\text{fast}}(\omega) + \tilde{\chi}_{\text{S}}(\omega) + \epsilon_{\infty} \\ &= \frac{\Delta\epsilon_{\text{slow}}}{1 + i\omega\tau_{\text{slow}}} + \frac{\Delta\epsilon_{\text{fast}}}{1 + i\omega\tau_{\text{fast}}} + \frac{\Delta V_{\text{S}}}{1 - (\omega/\omega_{\text{S}})^2 + i\omega\gamma_{\text{S}}/\omega_{\text{S}}^2} + \epsilon_{\infty}\end{aligned}\quad (1)$$

where, the first and second Debye relaxation components consist of the relaxation strength $\Delta\epsilon$ and relaxation times τ . The third term represents resonance dispersion with a vibrational strength ΔV_{S} , a resonant frequency ω_{S} and a damping constant γ_{S} . Using this equation, Yada et al. have successfully revealed the dynamics of the HB network in distilled water.^{26,27} In this study, we will extend this analysis into saccharide – water mixture solutions, in order to discuss the hydration state and destructuring effect of saccharides.

To successfully fit the results into Eq. (1), τ_{slow} was fixed to be 7.93 ps (corresponding to a relaxation frequency of 0.02 THz) in the present study, based on an empirical formula.^{26,46} This empirical formula originally hold for distilled water, but is supposed to be also valid for aqueous solutions, since precise measurements in the microwave region found that the slow relaxation

frequency of bulk water in aqueous solution are unchanged from that of distilled water, independent of solute concentration.⁴¹ As a result, we obtained a static dielectric constant, $\epsilon_S = \Delta\epsilon_{\text{slow}} + \Delta\epsilon_{\text{fast}} + \Delta V_S + \epsilon_\infty = 77.78$ for distilled water, which is quite in good accordance with the empirical value (77.68) at 27 °C.⁴⁶ Furthermore, the fitted parameters for distilled water at 27 °C ($\Delta\epsilon_{\text{slow}} = 72.02$, $\Delta\epsilon_{\text{fast}} = 2.10$, $\tau_{\text{fast}} = 0.26$ ps, $\Delta V_S = 1.27$, $\omega_S = 5.22$ THz/ 2π , $\gamma_S = 5.68$ THz/ 2π , $\epsilon_\infty = 2.39$) are in good agreement with previous data for water at 20 °C²⁶ and 23 °C.²⁷ Although the intermolecular stretching vibration mode (~ 5.0 THz) has to be taken into account in the fitting procedure,^{26,27} the detailed discussion about this vibration mode is to be avoided here; the measured dielectric constant up 4.0 THz sometimes tends to lead to uncertain fitted parameters for ω_S and γ_S . However, we confirmed that the uncertain fluctuations in ω_S and γ_S are so small that the fitted parameters of the slow relaxation ($\Delta\epsilon_{\text{slow}}$) and fast relaxation ($\Delta\epsilon_{\text{fast}}, \tau_{\text{fast}}$) were not seriously affected.

In Figure 4, the original complex dielectric constant and the decomposed results of distilled water are displayed (the inset is an overview of the imaginary part in the microwave to far-infrared region). The residuals according to the fitting procedure, $\delta\epsilon(\omega) = \tilde{\epsilon}_{\text{exp.}}(\omega) - \tilde{\epsilon}_{\text{fit.}}(\omega)$ where $\tilde{\epsilon}_{\text{exp.}}(\omega)$ and $\tilde{\epsilon}_{\text{fit.}}(\omega)$ are the complex dielectric constant of the experiment (left-hand side in Eq. (1)) and fitting (right-hand side), respectively, are shown in the bottom of Figure 4. Over the entire frequency range measured, $\delta\epsilon(\omega)$ was within ± 0.2 , which was small enough to assure the accuracy of our fitting procedure. Furthermore, the imaginary part of $\tilde{\chi}_{\text{slow}}(\omega)$ and $\tilde{\chi}_{\text{fast}}(\omega)$ for the distilled water and glucose solution (0.731M and 1.462 M) is shown in Figure 5 for comparison. Since dielectric responses in the THz region is selectively sensitive to bulk water dynamics, these deviations of $\tilde{\chi}_{\text{slow}}(\omega)$ and $\tilde{\chi}_{\text{fast}}(\omega)$ in Figure 5 directly reflects the hydration state and HB destructuring effect imposed by glucose molecules. Compared with a relatively smaller concentration dependence in $\tilde{\chi}_{\text{fast}}(\omega)$, we found a significant (about 35 % at 0.15 THz) attenuation in $\tilde{\chi}_{\text{slow}}(\omega)$. The reason for this is that the slow relaxation is a sensitive representation of the reduced volume of bulk water replaced by solutes and hydrated water. Thus, the strong attenuation

in the imaginary part, especially in the lower frequencies as shown in Figure 2 and 3, was due to the decrease in the slow relaxation mode. On the other hand, for both distilled water and the saccharide aqueous solutions, the contribution of $\tilde{\chi}_{\text{slow}}(\omega)$ in the real part is quite small, even in the lower frequency region. As seen in Figure 4, the real parts of the examined samples were dominated by $\tilde{\chi}_s(\omega)$ and ϵ_∞ in the whole region. In our analysis, there were only slight differences in ϵ_∞ between distilled water (2.39) and 1.462 M glucose solution (2.55). According to the Bruggeman effective medium approximation,⁴⁷ which describes the high frequency dielectric constant of aqueous solutions, even the real part of a 1.462 M glucose solution at around 10 THz varies from that of distilled water by only about 6 percent. This may explain the reason why only a slight variation was found in the real part between distilled water and the glucose solutions.

3.3. Hydration States. For saccharide solutions, we can simply consider the solution to be made up of a mixture of three different dielectric substances: saccharide solutes, hydrated water molecules and bulk water molecules. As determined by force-field MD simulations,^{7,13,14} dynamically retarded saccharide solutes and hydrated water reorient 2~3 times slower than bulk water, exhibiting negligible responses in the THz spectrum. Hence, the presence of saccharide solutes and the hydrated water are represented as a decrease in the volume of bulk water in the THz frequencies. As discussed above, the slow relaxation mode results from the collective motion of HB bulk water and therefore $\Delta\epsilon_{\text{slow}}$ is about 30 times larger than $\Delta\epsilon_{\text{fast}}$ (the relaxation strength of NHB bulk water molecules) for distilled water.²⁶ Thus, we can make the approximation that $\Delta\epsilon_{\text{slow}}$ sensitively represents the volume fraction of bulk water. Taking these into consideration, $\Delta\epsilon_{\text{slow}}$ is an effective index of the extent of hydration in these conditions if the quantity of saccharide solutes is known. Thus, hydrated water is defined as the water molecules whose relaxation times are distinctively longer than those of bulk water molecules.

Figure 6 shows the variation in $\Delta\epsilon_{\text{slow}}(C)$, the slow relaxation strength at concentration C , as a function of glucose concentration and “ideal” bulk water relaxation strength. This assumes that all water molecules in the solution (bulk water & hydrated water) contribute to the slow relaxation process. Therefore, the deviation between experimentally determined $\Delta\epsilon_{\text{slow}}(C)$ and “ideal” bulk water relaxation intensity originates from the replacement of bulk water molecules by hydrated water molecules. The apparent bulk water concentration $C_{\text{bulk}}(C)$ is yielded by,

$$C_{\text{bulk}}(C) = \frac{\Delta\epsilon_{\text{slow}}(C)}{\Delta\epsilon_{\text{slow}}(0)} \cdot \frac{\rho_{\text{water}}}{M_{\text{water}}} \quad (2)$$

where, $\Delta\epsilon_{\text{slow}}(0)$, ρ_{water} and M_{water} is the slow relaxation strength of distilled water, density and molecular weight of water, respectively.⁴¹ Then, we can evaluate the effective hydration number N_{hyd} (the number of hydrated water per saccharide solute molecule):^{41,48}

$$N_{\text{hyd}}(C) = \frac{C_{\text{water}}(C) - C_{\text{bulk}}(C)}{C} \quad (3)$$

where, $C_{\text{water}}(C)$ is the mol concentration of water in the solution. The N_{hyd} for monosaccharide and disaccharide solutions are summarized in Figure 7. In our analysis, the hydrated water molecules are defined as those water molecules with reorientational times that are much longer than those of bulk water ($\tau_{\text{slow}} = 7.93$ ps).

Hydration numbers have been quantified by various experimental procedures, such as density⁴⁹, acoustic^{50,51}, viscosity¹⁶ and calorimetry^{52,53}; however, the definition of hydrated water varies between experiments. In density⁴⁹, acoustic^{50,51} and viscosity¹⁶ measurements, water molecules with smaller partial specific volume and compressibility than bulk are regarded as hydrated water. On the other hand, calorimetric measurements defined unfreezable water as hydrated water, since the hydrated water remains unfrozen below subzero temperatures.^{52,53} All these measurements describe a “static” characteristics of hydration phenomena, where ice-like and unfreezable water directly bonded (or immobilized) to the saccharides are probed. However, this

“static” hydration images does not fully describe the hydration phenomena of saccharides, since water molecules in vicinity of solutes are perturbed into various dynamic states, depending on the distance from the saccharide surface. From a “dynamical” point of view, water molecules in the first hydration layer exhibit much slower orientational/translational dynamics than bulk (hereafter referred to as strongly hydrated water).⁷ In the second hydration shell (about 5 Å from the saccharide surface), water molecules are unable to directly form HBs with the saccharide solutes, but their dynamical properties are still indirectly perturbed (weakly hydrated water, as contrasted with strongly hydrated water). While the “static” hydrated water closely corresponds to the strongly hydrated water, our experiments and analysis take both strongly and weakly hydrated water into account, allowing us to look into the overall hydration state of saccharides. This is because, in our analysis, all the water molecules with longer relaxation times (= more retarded reorientational dynamics) than bulk water are regarded as hydrated water. Indeed, N_{hyd} in glucose solution (17.8) at the lowest concentration in the present study was significantly larger than previously reported results, such as from acoustic or calorimetric methods (from 3 to 8 hydrated water molecules depending on the experiments).^{50,51,53} A similar tendency was observed for the other examined saccharide solutions. If we take glucose solution as an example, various force-field and *ab initio* MD simulations have shown that, on average, roughly 10 water molecules forms HBs with glucose solutes,⁵⁴⁻⁵⁷ indicating that glucose molecules equip ~10 strongly hydrated water molecules in their first hydration shell. Since we observed a larger hydration number ($N_{\text{hyd}} = 17.8$ for glucose), the remaining hydrated water molecules (i.e. $17.8 - 10 = 7.8$) were assumed to be weakly hydrated water in the second hydration shell. Our experimental result is in line with the previous MD simulation, which found the rotational correlation time of water in the second hydration shell is 20~50 % longer than that for bulk water.⁷ Recently, THz vibrational spectroscopy combined with MD simulation by Havenith et al. found the long-range hydration state of saccharides,^{36,58} to be approximately 50 water molecules in total that are perturbed by a glucose molecule. Assuming 10 water molecules are strongly hydrated to the glucose solute in the first hydration shell, the rest of

the 40 water molecules exist as weakly hydrated water, which is much larger than our result. The reason for the apparent difference in the number of weakly hydrated water is to that measured by Havenith et al. is they probed very minor dynamical perturbations of hydrated water in the sub-picosecond timescales, whereas we focused attention on the picosecond relaxation motions of water.^{36,58} Therefore, a larger N_{hyd} for glucose (i.e. $40 - 7.8 = 32.2$) observed in their study could arise from the fact that the picosecond dipole dynamics might less sensitively reflect minor differences between bulk and weakly hydrated water than sub-picosecond dipole fluctuations. From this result, we can speculate that only a part of the weakly hydrated water has a relaxation time distinctive from that of bulk water. In other words, most of the weakly hydrated water shows so similar relaxation dynamics to bulk ($\tau_{\text{slow}} = 7.93$ ps) that common Debye relaxation model with very broad frequency dispersion (see the inset in Figure 4) cannot rigorously distinguish them.

Figure 7 also shows a systematic reduction in N_{hyd} , which is associated with concentration in all the investigated saccharides. The decrease in N_{hyd} with concentration increase can be explained by the overlap of the hydration shells and/or solute – solute dipole interactions. Firstly, the overlap effect of the hydration shells is that a hydrated water molecule is occupied by the different two solutes in the concentrated environment, consequently reducing the average hydration number per a solute molecule. In this case, N_{hyd} should exhibit no concentration dependence at low concentrations when the hydration layers are not overlapped. To deduce this overlap effect, we approximated the average solute – solute distance (d_{solute}) in an arithmetical manner: $d_{\text{solute}} = 2\sqrt[3]{3V_{\text{solute}}/4\pi} - 2r_{\text{solute}}$, where V_{solute} represents the effective volume assigned to one solute molecule in the solution (total volume of solution divided by the number of solutes), assuming a spherical shape, and r_{solute} is the effective radius of the solute (see the illustration inserted in Figure 8). For glucose with $r_{\text{solute}} = 3.6$ Å,⁵⁹ $d_{\text{solute}}(0.585 \text{ M})$ is estimated to be 10.4 Å. According to MD simulation studies,^{7,36} the average rotational motions of water within 5 Å (corresponding to the second water layer) of the carbohydrate atoms of the glucose are retarded, but this effect vanishes

beyond 5 Å. This means that at $d_{\text{solute}} = 10$ Å each of the hydration shells are starting to overlap with each other. For this reason, we predict that the moderate decreases in N_{hyd} at higher concentrations (the shaded area in Figure 7) are the result from the overlapping of the hydration layers between neighboring solutes. However, the overlap effect, by itself, is not sufficient to explain the rapid decrease in N_{hyd} at lower concentrations (below 0.585 M and 0.292 M for monosaccharides and disaccharides, respectively). Secondly, in turn, the solute – solute dipole interactions may help to reasonably understand the decreased N_{hyd} below these concentrations. Even if the solute concentration is low, increasing solute – solute intermolecular dipole correlation as solute concentration weakens the solute – water interactions.^{11,60} This leads the reduction in N_{hyd} at low concentration range, before the hydration shells start to overlap.

As seen in Figure 7, the “hydration ability” of each saccharide was characterized as: for monosaccharides glucose (17.8) > fructose (9.6) for monosaccharides, and trehalose (39.8) > sucrose (23.6) for disaccharides, in spite of the same total number of hydroxyl groups. Interestingly, this order completely matches the number of equatorial hydroxyl groups: ($N_{e\text{-OH}}$) glucose (4.6) > fructose (3.0) for monosaccharides, and trehalose (8.0) > sucrose (6.3) for the disaccharides. From a stereochemical aspect, the equatorial hydroxyl group, $e\text{-OH}$, is the hydroxyl group orientating in the plane of the six-membered ring for the monosaccharide unit (see the inset in Figure 6). As frequently mentioned in previous NMR experiments, saccharide solutes with larger $N_{e\text{-OH}}$ can accommodate more water molecules in their vicinity.⁶¹⁻⁶³ This is because the neighboring O ... O distance between $e\text{-OHs}$ (2.86 Å) is very close to that between water molecules of ice (2.80 Å), forming hexagonal ring structure similar to ice. Thus, $e\text{-OH}$ can “fit” into the water structure with saccharide – water HBs replacing the water – water HBs.⁶¹ Consequently, since the HB lifetime correlation time of $e\text{-OH}$ group – water is much longer than that of water – water, $e\text{-OH}$ allows neighboring hydrated water molecules to be dynamically stabilized and thus $N_{e\text{-OH}}$ is an important factor determining the hydration ability of saccharide molecules.⁶¹ Additionally, the higher hydration ability of trehalose than that of sucrose was highlighted by more symmetric

conformation.⁶⁴ Nearly symmetric topology of trehalose efficiently prevents from forming the intramolecular HBs between the two glucose six-membered ring.⁹ At variance, other disaccharides such as sucrose and maltose are likely to form more internal HBs that reduce their ability for intermolecular forces, decreasing the hydration capacity.

In Figure 7, whereas we could observe noticeable solute-dependent variances in N_{hyd} in the low concentration regime, N_{hyd} of glucose and fructose and N_{hyd} of sucrose and trehalose was undistinguished above 0.585 M. To obtain further insight about this, we calculated the hydration number normalized per single –OH group (normalized N_{hyd}) for each saccharide. Figure 8 shows that the normalized N_{hyd} of glucose and trehalose, representative of the examined monosaccharides and disaccharides. These results indicate the hydration number per hydroxyl group for monosaccharides is almost identical to that of the disaccharides, in line with the previously mentioned depolarized scattering experiment.⁶⁵ Put all the above together, at concentrations above 0.585 M, the examined saccharides (glucose, fructose, sucrose and trehalose) exhibited similar hydration capacity, independent of their chemical structure and the number of (equatorial) hydroxyl groups. In particular, normalized $N_{\text{hyd}} = 1.5\sim 2.0$ above 0.585 M (Figure 8) is very close to the number of average HBs (≈ 2) formed between water and saccharides' hydroxyl groups, as calculated by previous force-field^{54,55} and *ab initio*^{56,57,66} MD simulations. Therefore, we assume that above 0.585 M only strongly hydrated water molecules are present around saccharide molecules due to the overlapping of the second hydration shells, making the fraction of weakly hydrated water negligibly small. To summarize, N_{hyd} above 0.585 M (Figure 7) is virtually independent of solute concentration because only strongly hydrated water directly bonded to the saccharides' hydroxyl groups will be measured. In contrast, below 0.585 M where the hydration shells are still separated, both strongly and weakly hydrated water are measured as hydrated water, and thus, the intrinsic hydration ability (presumably depending on $N_{e-\text{OH}}$) of the saccharide solute is likely to be manifested.

3.4. Destructuring effect on the HB network.

In contrast to the slow relaxation process attributable to the collective reorganization of the HB network, the fast relaxation mode is assigned to single NHB water molecule relaxation. These molecules transiently appear amongst the dynamical HB network.²⁶ In the HB network, where a water molecule preferably forms four HBs with its neighbors, at least two of these bonds must break for a significant rotation (slow relaxation: $\tau_{\text{slow}} = 7.93$ ps) to take place. Although most of these water molecules switch or reform HBs with surrounding water molecules, some of them are assumed to be transiently isolated from the HB within picoseconds.²³ Therefore, the following scenario for the fast relaxation mode is suggested: some water molecules are fleetingly isolated from the HB network, which then individually relax (due to their own permanent dipole moments and/or molecular collision), typically to a sub-picosecond timescale. The existence of such NHB water in the HB network has been predicted from first-principles MD simulation of liquid water.^{67,68} Thus, the fast relaxation process sensitively reflects information about the “destructured” HB network of water. Figure 9 shows the concentration dependence of the relaxation time τ_{fast} and relaxation strength $\Delta\epsilon_{\text{fast}}$ of glucose. Though no significant variations were found in $\Delta\epsilon_{\text{fast}}$, the relaxation time τ_{fast} tended to decrease as concentration became higher. Indeed, the negative linear correlation, as seen in Figure 9, is in line with the tendency of pure water τ_{fast} when the temperature is elevated, where water HB is destructured.

In order to quantify the destructuring effect on the HB network, the fraction of NHB water molecules (f_{NHB}) was determined by calculating the ratio of fast relaxation strength to total relaxation strength ($\Delta\epsilon_{\text{total}} = \Delta\epsilon_{\text{slow}} + \Delta\epsilon_{\text{fast}}$), that is, $f_{\text{NHB}} = \Delta\epsilon_{\text{fast}}/\Delta\epsilon_{\text{total}}$.²⁶ For distilled water, we calculated f_{NHB} to be 0.028, which means 2.8 % of the water molecules were isolated from the HB network, while the remaining ≈ 97 % were engaged in the tetrahedral HB network. Since the analytical errors were quite small for $\Delta\epsilon_{\text{slow}}$ and $\Delta\epsilon_{\text{fast}}$ (0.03 % and 0.85 % in slow and fast

relaxation, respectively), f_{NHB} is presumed to be accurate to the third decimal place. Figure 10 shows the concentration dependence of the fraction of NHB water (f_{NHB}) for each monosaccharide and disaccharide solution. There was a near linear increase as concentration increased from 0.146 to 1.462 M, clearly implying a concentration dependent “destructuring effect” of saccharide molecules on the HB network. The fractions of NHB water in the highest concentration solutions were found to be 4.1 % (glucose), 4.0 % (fructose), 5.6 % (sucrose) and 5.8 % (trehalose).

Saccharides are generally known to act overall as “structure makers” in which intermolecular HBs between water molecules are facilitated,⁶⁹ but the evidence of HB destructuring effects were seen in the present study. These effects have also been reported in numerous MD simulations^{7,11,12} and various other experiments.^{15-17,70} In the MD simulations, Lerbret and co-workers^{8,12} speculated that the probability of forming intact HBs between water molecules (p_{HB}) will become smaller in saccharide solutions. Additionally, they also found the average size of the HB cluster had a negative correlation with concentration. These computational results point to the saccharide molecules “disturbing” the native HB network of water. Such destructuring effects on HBs around saccharide solutes have also been manifested in other computational studies that calculate the so-called q distribution (orientational order / tetrahedral distribution factor).⁷¹ The q distribution represents the extent to which a tetrahedral arrangement has been adopted with the four nearest neighboring water molecules.^{7,8} Within 4 Å of the saccharides (approximately corresponding to the first hydration shell), the population of water molecules with a high- q value (around $q = 0.78$) decreased, while on the other hand, the population with an extremely low- q ($q \approx 0$) population increased.⁷ The q distribution of water in the second hydration shell (around 4 ~ 5 Å) and that in bulk water exhibited no significant difference. This suggests that the local HB configuration is disordered, presumably producing more NHB water molecules, in the hydration monolayer. Pangnotta et al.⁷² used neutron scattering to experimentally determine the water – water radial distribution functions, finding the average number of water – water HBs decreased, and inversely, the population of NHB water (= a water molecule forming 0 HBs) increased in the trehalose solutions. This experimental evidence

was supported by classical MD simulation study done by Pomata et al.,¹⁴ who insisted the population of isolated (=NHB) water molecules increased as fructose concentration and such isolated water amounted to up to 85 % of the total population at 5 M aqueous solution. These previous computational and experimental results point to the “destructuring effect” of saccharides. Our results (Figure 10) are good accordance with these results. The increased existence of NHB water supports the hypothesis that saccharides obstruct the crystallization process and thereby reduce the freezable water, namely destroying the water compatible with that of ice.¹⁵ A plausible reason for this destructuring effect is: [1] steric constraints imposed by the stiff saccharide molecules, reducing the free volume in which the surrounding water molecules can adopt a perfectly tetrahedral arrangement with their nearest neighbors,⁷ and/or [2] donated HBs from glucose to water which can affect the orientation of the surrounding water molecules, slightly modifying the water structure.⁷³

To more quantitatively gain insight into the destructuring effect, the number of NHB water released by single solute molecule (N_{NHB}) was quantitatively computed by Eq. (4),⁷⁴

$$N_{\text{NHB}}(C) = \frac{C_{\text{bulk}}(C)}{C} (f_{\text{NHB}}(C) - f_{\text{NHB}}(0)) \quad (4)$$

where, $f_{\text{NHB}}(0)$ and $f_{\text{NHB}}(C)$ denote the fraction of NHB water in distilled water and saccharide solution with concentration C . In Eq. (4), the first term represents the number of NHB water per solute molecule, irrespective of the distance from the solute surface. Whereas, the second term accounts for the NHB number that is not associated with the existence of a solute (i.e. NHB water locating distant from the solute molecule). By subtracting the second from the first term, we can extract the number of NHB water, N_{NHB} , that is “directly” released by the saccharide solute. The calculated N_{NHB} is summarized in the inset in Figure 10. Unlike the hydration number (N_{hyd}) shown in Figure 7, less concentration dependence was observed for N_{NHB} in each saccharide solution. This may be attributed to the production process of the NHB water not being closely associated with solute – solute dipole correlations. Also we found that N_{NHB} of the

monosaccharides and disaccharides is about 0.35 and 0.65, respectively, which indicates roughly three monosaccharide (disaccharide) solutes produce one (two) NHB water molecule. Since the number of hydroxyl groups is more abundant in disaccharides by a factor of 1.6 than in monosaccharides, a larger N_{NHB} in disaccharides is assumed to be correlated with the number of OH groups. This idea is supported by the simulation study of Godec et al., who insisted that some fraction of water molecules around the hydrophilic functional groups are released from the HB network.⁷⁴ Further, slight increase in the hydrophobic portion and more complicated chemical structure may also be reasons for larger NHB number of disaccharides than monosaccharides.

In Figure 10, no significant differences were observed between sucrose and trehalose. This is in apparent conflict with the Raman spectroscopy results of Branca et al.^{15,70} and Lerbret et al.¹⁸, who concluded trehalose exhibits the largest destructuring effect among disaccharides. However, it should be noted that they discussed the HB destructuring effect in terms of “distorted” tetrahedral HB component from the blueshift in the OH stretching band, while our observation was provided from the standpoint of non-hydrogen-bonded water molecules “isolated” from the water HB network. In combination with previous Raman data, the destructuring effect of saccharides was described as follows: both trehalose and sucrose accommodate almost the same fraction of NHB water around them but the tetrahedral HB coordination in trehalose solution is peculiarly distorted in structure relative to sucrose and the other disaccharides, indicating solute-dependence of the destructuring effect is supposed to be quite small.

4. Concluding Remarks

Comparative THz TD-ATR measurements on monosaccharide (glucose and fructose) and disaccharide (sucrose and trehalose) solutions were used to experimentally characterize the hydration state and the HB destructuring effect. Since the dielectric responses in the THz region are

dominated by water dynamics mediated by HBs within a sub-picosecond and picosecond time window, which does not include any relaxational contribution from the saccharide solutes and hydrated water. Thus the complex dielectric constants of saccharide solutions can be decomposed into the slow relaxation of HB bulk water, fast relaxation of NHB bulk water and intermolecular stretching vibration modes of water.

In the present study, we demonstrated that THz spectroscopy could be used to quantify the relatively “global” hydration state (including a full subset of strongly hydrated water and a fraction of weakly hydrated water) of saccharide solutions, since water molecules with exhibited longer relaxation time than $\tau_{\text{slow}} = 7.93$ ps (at 27 °C) are defined as hydrated water. The subsequently determined hydration number was highly correlated with the number of equatorial hydroxyl groups in the saccharide solute, and was inversely dependent on solute concentration due to overlaps of the hydration shells and solute – solute dipole correlation interactions. Furthermore, the destructuring effect on the HB network is discussed in terms of the fraction of NHB water. From our experimental results, it can be seen that saccharide molecules release more water molecules from the HB network; a result in good accordance with MD simulations and neutron diffraction measurement. Focusing on the fraction of NHB water, we also found no noticeable difference in the destructuring effect between glucose and fructose, as well as between sucrose and trehalose. Specifically, three monosaccharide/disaccharide solutes produced approximately one/two additional NHB water molecule. While little difference in the destructuring effect between sucrose and trehalose is seemingly inconsistent with previous Raman measurements,^{15,17-19} this discrepancy may originate from different definitions of the destructuring effect: the population NHB water measured in our study while Raman spectroscopy evaluated the “distorted” HB network of water.

Overall, our results suggest that THz spectroscopy can directly provide a window on the dynamics of water and the HB interactions with saccharide solutes. Further investigation combining THz techniques with other experimental methods and MD simulations will help to organize a

comprehensive picture about water – saccharide interactions, such as the peculiar bioprotective features.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

ACKNOWLEDGMENT

We are grateful to Mr. Motoki Imamura and Mr. Akiyoshi Irisawa (ADVANTEST Corporation, Japan) for their technical supports. We also acknowledge Professor Garry John Piller (Graduate School of Agriculture, Kyoto University, Japan) for his help and useful discussions. Financial support was provided by Industry-Academia Collaborative R&D from Japan Science and Technology Agency (JST).

REFERENCES

- (1) Clegg, J. S.; Seitz, P.; Seitz, W.; Hazlewood, C. F. *Cryobiology* **1982**, *19*, 306-316.
- (2) Crowe, J. H.; Leslie, S. B.; Crowe, L. M. *Cryobiology* **1994**, *31*, 355-366.
- (3) Timasheff, S. N. *Biochemistry* **2002**, *41*, 13473-13482.
- (4) Cottone, G.; Giuffrida, S.; Ciccotti, G.; Cordone, L.; *Prot. Struct., Funct., Bioinf.* **2005**, *59*, 291-302.
- (5) Hédoux, A.; Ionov, R.; Willart, J.-F.; Lerbret, A.; Affouard, F.; Guinet, Y.; Descamps, M.; Prévost, D.; Paccou, L.; Danéde, F. *J. Chem. Phys.* **2006**, *124*, 014703.
- (6) Lerbret, A.; Affouard, F.; Bordat, P.; Hédoux, A.; Descamps, M. *J. Chem. Phys.* **2009**, *131*, 245103.
- (7) Lee, S. L.; Debenedetti, P. G.; Errington, J. R. *J. Chem. Phys.* **2005**, *122*, 204511.
- (8) Bordat, P.; Lerbret, A.; Demaret, J. -P.; Affouard, F.; Descamps, M. *Europhys. Lett.* **2004**, *65*, 41-47.
- (9) Lerbret, A.; Affouard, F.; Bordat, P.; Hédoux, A.; Guinet, Y.; Descamps, M. *J. Non-Cryst. Solids* **2011**, *357*, 695-699.
- (10) Green, J. L.; Angell, C. A.; *J. Phys. Chem.* **1989**, *93*, 2880-2882.
- (11) Roberts, C. J.; Debenedetti, P. G. *J. Phys. Chem. B* **1999**, *103*, 7308-7318.
- (12) Lerbret, A.; Bordat, P.; Affouard, F.; Descamps, M.; F. Migliardo, F. *J. Phys. Chem. B* **2005**, *109*, 11046-11057.
- (13) Branca, C.; Magazù, S.; Maisano, G.; Migliardo, P. *J. Chem. Phys.* **1999**, *111*, 281-287.
- (14) Branca, C.; Magazù, S.; Maisano, G.; Migliardo, F.; Migliardo, P.; Romeo, G. *J. Phys. Chem. B* **2001**, *105*, 10140-10145.
- (15) Branca, C.; Magazù, S.; Migliardo, F.; Migliardo, P. *Physica A* **2002**, *304*, 314-318.
- (16) Lerbret, A.; Bordat, P.; Affouard, F.; Guinet, Y.; Hédoux, A.; Paccou, L.; Prévost, D.; Descamps, M. *Carbohydr. Res.* **2005**, *340*, 881-887.
- (17) Paolantoni, M.; Sassi, P.; Morresi, A.; Santini, S. *J. Chem. Phys.* **2007**, *127*, 024504.
- (18) Eaves, J. D.; Loparo, J. J.; Fecko, C. J.; Roberts, S. T.; Tokmakoff, A.; Geissler, P. L. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 13019-13022.
- (19) Bagchi, B. *Chem. Rev.* **2005**, *105*, 3197-3219.
- (20) Ball, P., *Chem. Phys. Chem.* **2008**, *9*, 2677-2685
- (21) Yada, H.; Nagai, M.; Tanaka, K. *Chem. Phys. Lett.* **2008**, *464*, 166-170.
- (22) Yada, H.; Nagai, M.; Tanaka, K. *Chem. Phys. Lett.* **2009**, *473*, 179-283.
- (23) Höchtl, P.; Boresch, S.; Steinhauser, O. *J. Chem. Phys.* **2000**, *111*, 9810-9821.
- (24) Weingärtner, H.; Knocks, A.; Boresch, S.; Höchtl, P.; Steinhauser, O. *J. Chem. Phys.* **2001**, *115*, 1463-1472.
- (25) Fuchs, K.; Kaatze, U. *J. Chem. Phys.* **2002**, *116*, 7137-7144.

- (26) Mashimo, S., Kuwabara, S., Yagihara, S., Higashi, K. *J. Phys. Chem.* **1987**, *91*, 6337-6338.
- (27) Sonoda, M. T.; Skaf, M. S. *J. Phys. Chem. B* **2007**, *111*, 11948-11956.
- (28) Pomata, M. H. H.; Sonoda, M. T.; Skaf, M. S.; Elola, M. D. *J. Phys. Chem. B* **2009**, *113*, 12999-13006.
- (29) Upadhyaya, P. C.; Shen Y. C.; Davies A. G.; Linfield, E. H. *J. Biol. Phys.* **2003**, *29*, 117-121.
- (30) Upadhyaya, P. C.; Shen Y. C.; Davies A. G.; Linfield, E. H. *Vib. Spectrosc.* **2004**, *35*, 139-143.
- (31) Nagai, M.; Yada, H.; Arikawa, T.; Tanaka, K. *Int. J. Infrared Millimeter Waves* **2006**, *27*, 505-515.
- (32) Arikawa, T.; Nagai, M.; Tanaka, K. *Chem. Phys. Lett.* **2008**, *457*, 12-17.
- (33) Shiraga, K.; Ogawa, Y.; Kondo, N.; Irisawa, A.; Imamura, M.; *Food Chem.* **2013**, *140*, 315-320.
- (34) Heugen, U.; Schwaab, G.; Bründermann, E.; Heyden, M.; Yu, X.; Leitner, D. M.; Havenith, M. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 12301-12306.
- (35) Heyden, M.; Bründermann, E.; Heugen, U.; Niehues, G.; Leitner, D. M.; Havenith, M. *J. Am. Chem. Soc.* **2008**, *130*, 5773-5779.
- (36) Kawase, K.; Ogawa, Y.; Watanabe, Y.; Inoue, H.; *Opt. Express* **2003**, *11*, 2549-2554.
- (37) Hoshina, H.; Sasaki, Y.; Hayashi, A.; Otani, C.; Kawase, K.; *Appl. Spectrosc.* **2009**, *63*, 81-86.
- (38) Xie, L.-H.; D, H.; Jin, B.-B.; Han, Y.; Tai, Q.; Yi, M.-D.; Yang, T.; Wu, P.-H.; Huang, W.; *Macromol. Chem. Phys.* **2012**, *213*, 1441-1447.
- (39) Perticaroli, S.; Nakanishi, M.; Pashkovski, E.; Sokolov, A. P. K. *Phys. Chem. B* **2013**, *117*, 7729-7736.
- (40) Cametti, C.; Marchetti, S.; Gambi, C. M. C.; Onori, G. *J. Phys. Chem. B* **2011**, *115*, 7144-7153.
- (41) Yokoyama, K.; Kamei, T.; Minami, H.; Suzuki, M. *J. Chem. B* **2001**, *105*, 12622-12627.
- (42) Okada, K.; Yao, M.; Hiejima, Y.; Kohno, H.; Kajihara, Y. *J. Chem. Phys.* **1999**, *110*, 3026-3036.
- (43) Sharma, M.; Resta, R.; Car, R. *Phys. Rev. Lett.* **2005**, *95*, 187401.
- (44) Walrafen, G. E. *J. Chem. Phys.* **1964**, *40*, 3249-3256.
- (45) Vidulich, G. A.; Evans, D. F.; Kay, R. L. *J. Phys. Chem.* **1967**, *71*, 656-662.
- (46) Aspnes, D. E. *Am. J. Phys.* **1982**, *50*, 704-709.
- (47) Schrödle, S.; Hefter, G.; Kunz, W.; Buchner, R.; *Langmuir* **2006**, *22*, 924-932.
- (48) Gharsallaoui, A.; Rogé, B.; Génotelle, J.; Mathlouthi, M. *Food Chem.* **2008**, *106*, 1443-1453.
- (49) Shiio, H.; *J. Am. Chem. Soc.* **1957**, *80*, 70-73.
- (50) Galema, S. A.; Hoeiland, H.; *J. Phys. Chem. B* **1991**, *95*, 5321-5326.
- (51) Bociek, S.; Franks, F. *J. Chem. Soc., Faraday Trans. 1* **1979**, *75*, 262-270.
- (52) Furuki, T.; *Carbohydr. Res.* **2002**, *337*, 441-450.
- (53) Leroux, B.; Bizot, H.; Brady, J. W.; Tran, V. *Chem. Phys.* **1997**, *216*, 349-363.
- (54) Caffarena, E. R.; Grigera, J. R.; *Carbohydr. Res.* **1997**, *300*, 51-57.
- (55) Molteni, C.; Parrinello, M. *J. Am. Chem. Soc.* **1998**, *120*, 2168-2171.
- (56) Suzuki, T. *Phys. Chem. Chem. Phys.* **2008**, *10*, 96-105.
- (57) Leitner, D. M.; Gruebele, M.; Havenith, M. *HFSP J.* **2008**, *2*, 314-323.
- (58) Pappenheimer, J. R.; Renkin, E. M.; Borrero, L. M. *Am. Physiol.* **1951**, *167*, 13-46.
- (59) Chen, C.; Li, W. Z.; Song, Y. C.; Weng, L. D.; Zhang, N.; *Comp. Theor. Chem.* **2012**, *984*, 85-92.
- (60) Uedaira, H.; Uedaira, H. *J. Sol. Chem.* **1985**, *14*, 27-34.
- (61) Uedaira, H.; Ikura, M. *Bull. Chem. Soc. Jpn.* **1989**, *62*, 1-4.
- (62) Sato, Y.; Miyawaki, O. *Food Sc. Technol.* **2000**, *6*, 136-139.
- (63) Taga, T.; Senma, M.; Osaki, K.; *Acta. Cryst.* **1972**, *B28*, 3258-3263.

- (64) Lupi, L.; Comez, L.; Paolantoni, M.; Perticaroli, S.; Sassi, P.; Morresi, A.; Ladanyi, B. M.; Fioretto, D. *J. Phys. Chem. B* **2012**, *116*, 14760-14767.
- (65) Bonanno, G.; Noto, R.; Fornili, S. L. *J. Chem. Soc., Faraday Trans.* **1998**, *94*, 2755-2762.
- (66) Neumann, M. *J. Chem. Phys.* **1985**, *82*, 5663-5672.
- (67) Ohmine, I. *J. Phys. Chem.* **1995**, *99*, 6767-6776.
- (68) Wu, B.; Zhang, Y.; Wang, H. *J. Chem. Phys. B* **2008**, *112*, 6426-6429.
- (69) Branca, C.; Magazù, S.; Maisano, G.; Bennington, S. M.; Fâk, B. *J. Phys. Chem. B* **2003**, *107*, 1444-1452.
- (70) Errington, J. R.; Debenedetti, P. G. *Nature* **2001**, *409*, 318-321.
- (71) Pagnotta, S. E.; McLain, S. E.; Soper, A. K.; Bruni, F.; Ricci, M. A. *J. Phys. Chem. B* **2010**, *114*, 4904-4908.
- (72) Groot, C. C. M.; Bakker, H. J. *J. Chem. Phys.* **2014**, *140*, 234503.
- (73) Shiraga, K.; Suzuki, T.; Kondo, N.; Ogawa, Y. *J. Chem. Phys.* **2014**, *141*, 235103.
- (74) Godec, A.; Smith, J. C.; Merzel, F. *Phys. Rev. Lett.* **2011**, *107*, 267801.

< Figure captions >

Figure 1. Attenuated total reflectance (ATR) and phase shift spectrum of distilled water and the glucose solutions (0.731 M and 1.462 M). The insets show the schematic illustration of THz TD-ATR spectroscopy.

Figure 2. Real part and imaginary part of the complex dielectric constants of distilled water and the glucose solutions (0.731M and 1.462 M) at 27 °C. The inset shows concentration dependence of the glucose solution, at the selected frequencies (black circle: 0.2 THz and gray diamond: 1.0 THz). The standard errors of the measurements are not depicted since it is much smaller than the symbol size (see the text for details).

Figure 3. Comparison of the complex dielectric constants of distilled water, 1.462 M monosaccharide solutions and 1.462 M disaccharide solutions. The inset displays the real and imaginary part at 0.5 THz with error bars originated from experiments.

Figure 4. (Top) Real part and (middle) imaginary part of the decomposed complex dielectric constant of distilled water. The experimentally determined complex dielectric constant (black solid line) can be expressed as the superposition of the slow relaxation of HB bulk water ($\tilde{\chi}_{\text{slow}}$), the fast relaxation of NHB bulk water ($\tilde{\chi}_{\text{fast}}$), the intermolecular stretching vibration ($\tilde{\chi}_{\text{S}}$) and the higher frequency limit (ϵ_{∞}). The overhead view of the imaginary part between 1 GHz ~10 THz is also displayed in the inset. (Bottom) The residuals, $\delta\tilde{\epsilon} = \tilde{\epsilon}_{\text{exp.}} - \tilde{\epsilon}_{\text{fit.}}$; the real and imaginary part is shown in green and purple, respectively.

Figure 5. Imaginary susceptibilities of (top) slow relaxation and (bottom) fast relaxation. The insets show the real part of each component.

Figure 6. The slow relaxation intensity ($\Delta\epsilon_{\text{slow}}$) as a function of glucose concentration (0.146~1.462 M). The broken line represents the “ideal” bulk water relaxation intensity under assumption that all water molecules dynamically behave as bulk water. Since decrease in $\Delta\epsilon_{\text{slow}}$ is assigned to decrease in the volume of bulk water molecules, the deviations of the fitted $\Delta\epsilon_{\text{slow}}$ from ideal bulk water are attributed to hydration effect. Error bars are negligibly small and not depicted. Inset: the molecular structure of α -glucose (left) and β -glucose (right), and the equatorial hydroxyl groups (*e*-OHs) are shown in red color.

Figure 7. The hydration number of monosaccharides (glucose and fructose) and disaccharides (sucrose and trehalose) from 0.146 M to 1.462 M, calculated by Eq. (3). The broken lines are drawn only to guide the eye, and the shaded area represents the concentration region where the second hydration shells of saccharides are overlapped. The bars are originated from both experimental and fitting errors.

Figure 8. The number of hydration number per a single hydroxyl group (normalized hydration number) of glucose and trehalose from 0.146 to 1.462 M. The inset shows the illustration of the relationship of $2^3\sqrt{3V_{\text{solute}}/4\pi}$, d_{solute} and r_{solute} .

Figure 9. Concentration dependence of the fast relaxation time (τ_{fast}) of glucose solution and the fast relaxation strength of glucose solution ($\Delta\epsilon_{\text{fast}}$). The bars are originated from both experimental and fitting errors.

Figure 10. The fraction of NHB water molecules in the HB network, calculated by $f_{\text{NHB}} = \Delta\epsilon_{\text{fast}}/\Delta\epsilon_{\text{total}}$, versus concentration. The bars are originated from both experimental and fitting errors. The insets show the number of NHB water per a saccharide solute, calculated by Eq. (4). The broken lines are drawn only to guide the eye.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

Figure 8

Figure 9

Figure 10

Decomposition into
Debye and Lorentz
functions