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LETTER

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Understanding the Mechanisms of Bioprotection: A Comparative Study of Aqueous Solutions of Trehalose and Maltose upon Supercooling

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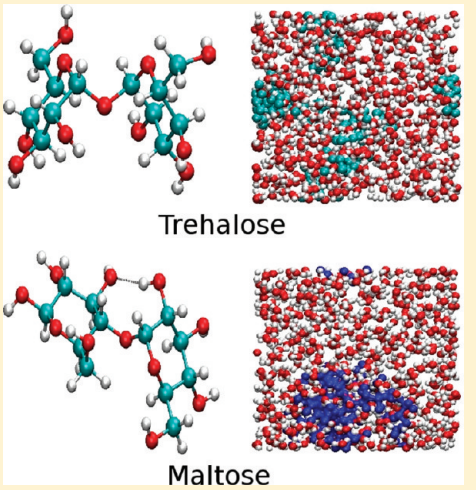
ABSTRACT: We present evidence from molecular dynamics (MD) simulations that dynamics of water in the presence of trehalose and maltose shows two well distinct relaxations. In particular, the fraction of water molecules interacting with the sugar has a completely different dynamical behavior with respect to that of bulk-like water molecules. The analysis of the dynamic of hydration water molecules allows us to comprehend the differences between trehalose and maltose aqueous solutions and the microscopic mechanisms that cause a greater capability of trehalose in preserving biomolecules upon cooling.

SECTION: Statistical Mechanics, Thermodynamics, Medium Effects



Over the past 20 years, aqueous solutions of carbohydrates have increasingly attracted the attention of scientists. The observation that in several anydrobiotic organisms such as tardigrades[1] or certain desert plants[2] the ability to survive severe dehydration conditions at elevated temperatures is accompanied by the synthesis of a large amount of saccharides has stimulated a series of studies that have pointed out the extraordinary biopreservation ability of sugars, particularly of trehalose.[3,4] In fact, this common disaccharide of glucose has been found to be especially effective in preserving membranes structure[5] and in preventing proteins from denaturation at very low water content.[6] It has also been demonstrated that trehalose stabilizes living cells subjected to freezing stresses.[7,8] This evidence has paved the way to several diversified industrial applications of trehalose, which is currently used in pharmaceutical formulations as an eccipient, in the food industry to preserve the flavor of dried products, in many cosmetic lines and importantly in cryogenic technology.

Despite this great number of uses, the microscopic mechanism responsible for the ability of trehalose, and sugar in general, as bioprotectant is still unclear. Green and Angell[9] have shown that trehalose increases the glass-transition temperature of the aqueous solutions with respect to pure water more than other polysaccharides, like maltose. This is particularly true for dilute solutions. According to Crowe et al.,[10] the source of the biopreserving power of carbohydrates has to be attributed to the ability of saccharides in establishing strong hydrogen-bond-based interactions with the polar groups of biomaterials. Because of these interactions, sugars, especially trehalose, would be able to replace water near biological surfaces, which in this way would preserve the hydrogen bond network even if water is partially or totally absent. Raman scattering results about protein−trehalose interactions led Belton and Gil[11] to propose a different mechanism: trehalose would be particularly capable of creating around the protected biostructure a cage containing sluggish water molecules, which would serve both for maintaining a high level of hydration and for smoothing molecular motions, which would lead to denaturation of proteins upon cooling. Recently, Magazù et al. have pointed out that at low concentrations aqueous solutions of trehalose owe their protective attitude to the specific interactions established with water molecules.[12]

Bulk supercooled water occupies an important place in the field of metastable liquids[13] because many of its anomalous properties are enhanced in the supercooled region.[14] In contact with biomolecules a different microscopic dynamics than that of bulk is expected for water.[15,16]

In this Letter, we analyze the dynamics of water upon cooling of trehalose and maltose aqueous solutions to study the microscopic behavior responsible for their properties as crioprotectants and the possible differences in their effectiveness.

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Trehalose[17] and maltose[18] are two naturally occurring disaccharides of glucose. They are both composed of two glucose monomers, and, in fact, they have the same molecular formula $C_{12}H_{22}O_{11}$. The molecular conformations of both disaccharides have been determined by X-ray (for trehalose) and neutron (for maltose) diffraction. The difference between them resides in the glucosidic linkage: whereas in maltose it connects the carbon atoms 1 and 4 of the two monomers, in trehalose, it binds the carbon atoms 1 and 1. Because of this difference, trehalose and maltose have different chemical reactivity. Trehalose is a nonreducing sugar, whereas maltose can be easily oxidated under alcalin conditions and it is more reactive.

We have simulated with molecular dynamics (MD) three systems: bulk water, an aqueous solution of trehalose, and an aqueous solution of maltose. Water is described with SPC/E site model potential.[19] The bulk is composed of 500 water molecules. The aqueous solutions of trehalose and maltose are both composed of 484 water molecules and 5 sugar molecules; the concentration of both solutions is ∼0.5 M, which is a typical concentration used in biopreservation.[21] The intramolecular and intermolecular interactions of both sugars have been modeled with the use of the CHARMM[22,23] type force field developed by Ha et al.[24] We have used the DL_POLY package.[25] Cubic periodic boundary conditions were applied. The cutoff radius of interactions was set equal to 9.0 Å. Electrostatic long-range interactions were treated with Ewald summation technique. All simulations were performed in the NPT ensemble at atmospheric pressure by using the Berendsen algorithm.[26] The investigated temperatures range between T = 300 and 230 K. For each temperature, the systems were equilibrated for a time varying from 1 ns at high T to 30 ns at low T. After each equilibration, production runs were conducted. The total time required for the simulations presented in this work is ∼30 000 h, corresponding to roughly 3.5 years on a single processor.

In Figure 1, we show the snapshots of two equilibrated configurations at T = 300 K of the aqueous solutions of trehalose and maltose. We note that in the aqueous solutions of trehalose the five molecules of the sugar are homogeneously distributed in the simulation box. In the aqueous solutions of maltose, the five molecules of sugar are strongly interacting with each other instead. This result is also supported by computational[27] and experimental[28] studies that proved that sugar−sugar interactions in maltose are stronger than in trehalose. Therefore, a more destructuring effect on the surrounding water hydrogen bond network is to be expected for trehalose.[28] In fact, it has been demonstrated[29] from the analysis of the orientational order q that the presence of trehalose in aqueous solutions also at dilute concentrations has an effect on the tetrahedrality of the surrounding water similar to that caused by a temperature increase. According to ref 30, the balance between sugar−sugar and sugar−water interactions is directly related to the topology of trehalose, whose nearly symmetric conformation prevents extensive internal H-bonding between the two glucose rings, whereas sucrose and, to a lower extent, maltose are able to form more frequently internal HBs that reduce their abilities for intermolecular H-bonding.

To study the dynamical behavior of water in the sugar solutions, we calculated for water oxygens the self-intermediate scattering function (SISF) $F_S(q,t)$, which is the Fourier transform of the tag-particle density autocorrelation function. The dynamical behavior of SPC/E bulk water simulated upon supercooling[31,32] follows the predictions of the idealized version of mode coupling theory (MCT).[33] When a liquid approaches the crossover temperature T_C according to MCT, the dynamics is dominated by the cage effect. After an initial ballistic motion, the particle is trapped in the transient cage formed by its nearest neighbors. After the cage relaxes, the particle enters the Brownian diffusive regime. Below T_C, according to the idealized version of MCT, the system becomes nonergodic. In real structural glasses, hopping processes restore

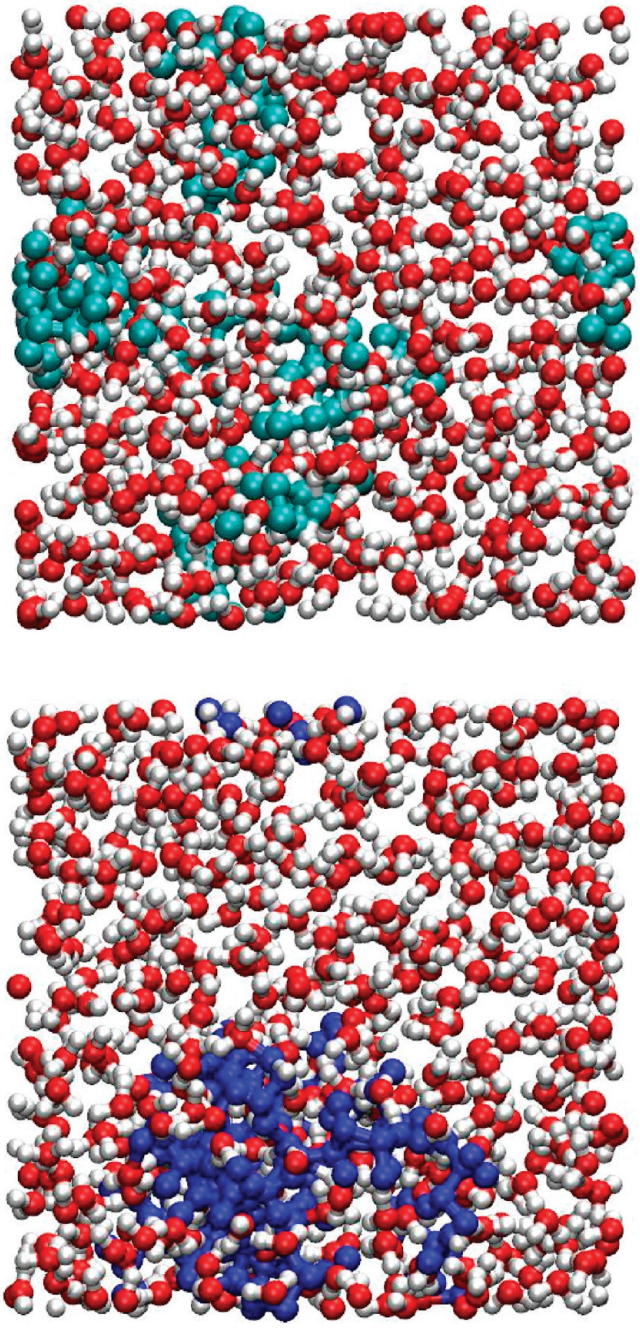

Figure 1. Snapshots of equilibrated configurations at T = 300 K of the aqueous solution of trehalose (top) and maltose (bottom). To better distinguish the sugar molecules from water, we represent trehalose atoms with the PDB color code of carbon (green-blue) and represent maltose atoms with the PDB color code of nitrogen (blue). We can see that trehalose does not show the same tendency to aggregation that is found for maltose.

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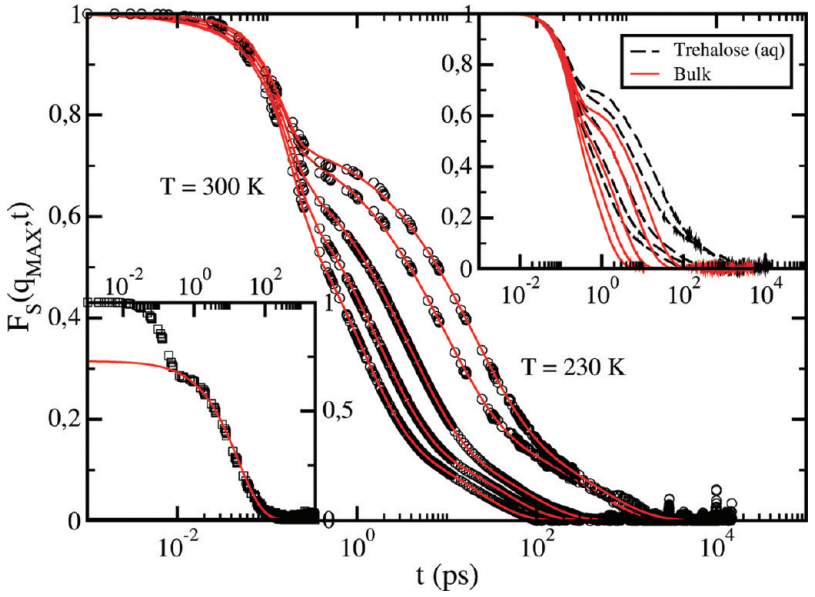

Figure 2. Main frame: self-intermediate scattering function (SISF) of the oxygen atoms of water from the aqueous solution of water and trehalose at the peak of the oxygen−oxygen structure factor $q = 2.25$ Å^{-1}, from $T = 300$ (bottom) to 230 K (top). The red lines are the fit to eq 2. Top inset: SISF of the oxygen atoms of the water molecules for $q = 2.25$ Å^{-1} for bulk water (continuous red lines) and water in solution with trehalose (long dashed black lines). The tails of the correlators calculated for the solution suggest the presence of an additional and slower relaxation with respect to the bulk. Bottom inset: SISF of the oxygen atoms of the water molecules in the trehalose solution non-hydrogen-bonded to trehalose at $T = 230$ K (square) and fit via a single KWW equation (line). We recover in this way the same α relaxation found in the bulk. Therefore, the long time tails of the total correlator of the solution shown in the top inset come only from water hydrogen bonded to the disaccharides.

ergodicity. T_C is therefore a crossover temperature from a liquid-like to a solid-like regime. These activated processes are not relevant above T_C for most liquids.

The oxygen SISF for bulk water shows therefore a two-step relaxation behavior, a fast one corresponding to the initial ballistic regime leading to the rattling in the cage, and a slow one corresponding to the relaxing cage time region and referred to as α-relaxation region. Therefore, it was proposed and verified with simulations[31] that the SISF for supercooled water can be well-described by the equation

$$F_S(q_{max},t) = [1 - f_{q_{max}}] \exp\left[-\left(\frac{t}{\tau_{short}}\right)^2\right] + f_{q_{max}} \exp\left[-\left(\frac{t}{\tau_\alpha}\right)^\beta\right] \quad (1)$$

where q_{max} is the peak of the structure factor. The Gaussian form takes into account the initial fast relaxation of the particle trapped in the cage. $f_{q_{max}}$ is the Debye−Waller factor related to the cage. The stretched exponential is the functional form connected to the cage relaxation typical of glass formers. For bulk water, the α relaxation times follow a power law in temperature, as predicted by MCT.

We show in Figure 2 the SISFs of the oxygen atom of water molecules of aqueous solution of trehalose at the peak of the oxygen−oxygen static structure factor. In the inset, we compare the SISFs calculated for the aqueous solution of trehalose and the SISFs calculated for bulk water.

It can be observed that whereas the SISFs for bulk water are consistent with the MCT predictions, for water in the presence of trehalose or maltose (not shown) already at ambient temperature, the correlator shows an additional tail at long times, which does not allow to fit the SISFs with the eq 1. Computational and experimental results have found that hydration water, that is, water in direct contact with biological molecules like proteins, shows strong differences in the dynamics with respect to the bulk.[35] On this basis, we hypothesized that the tail appearing in the correlator of the aqueous solutions comes from hydration water. We therefore proposed here a new relation to fit the SISFs that implies two very well distinct dynamic behavior for water close to the sugars and the remaining water

$$F_S(q_{max},t) = [1 - f^A_{q_{max}} - f^B_{q_{max}}] \exp\left[-\left(\frac{t}{\tau_{short}}\right)^2\right] + f^A_{q_{max}} \exp\left[-\left(\frac{t}{\tau_\alpha}\right)^{\beta_\alpha}\right] + f^B_{q_{max}} \exp\left[-\left(\frac{t}{\tau_{long}}\right)^{\beta_{long}}\right] \quad (2)$$

In this equation, in addition to the Gaussian relaxation at short times, there are two distinct Kohlrausch−William−Watts (KWW) functions. The first one corresponds to the α relaxation of the systems and has to be referred to the bulk-like water molecules that are not in direct contact with the sugars. We introduced the second stretched exponential function to account for the much slower dynamical behavior of hydration water molecules. We see in Figure 2 that this functional form is able to reproduce perfectly the shape of the SISFs of the water molecules for both trehalose and maltose (not shown) aqueous solutions. From the fit, we extracted the α relaxation time τ_α and the relaxation time of the hydration water molecules τ_{long} and the respective stretching parameters.

To confirm this picture, we have calculated the SISFs only for water molecules not hydrogen-bonded to the sugars, and we have found that the long time tail disappears in the SISFs and that the

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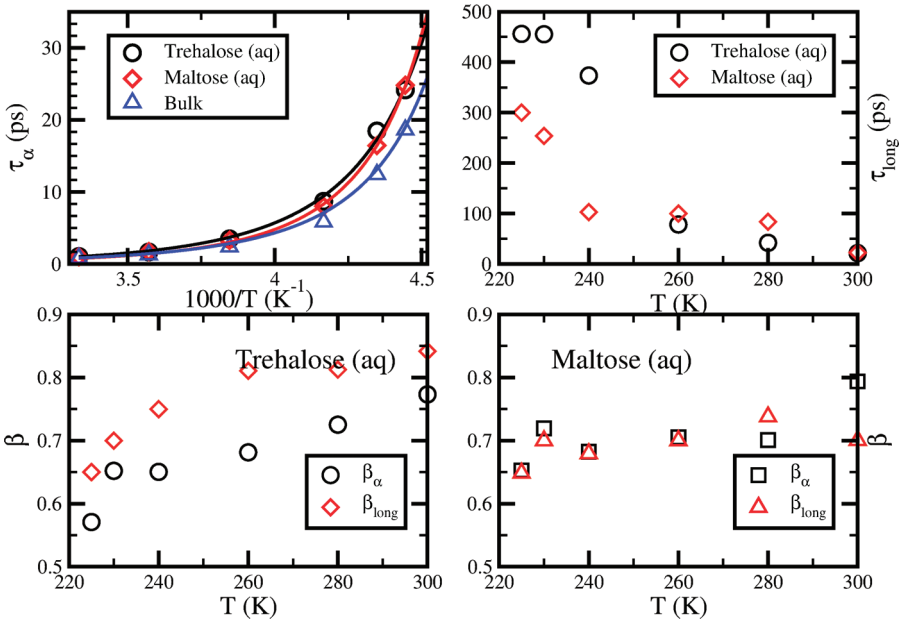

Figure 3. Top left: α-relaxation times for water in trehalose and water in maltose. The continuous line is the fit to eq 3. Top right: long-relaxation times for water in trehalose and water in maltose. The behavior of the long time part of the correlators does not follow the power law predicted by MCT. The relaxation times of the hydration water of trehalose are slower for supercooled temperatures. Bottom left: β_α and β_{long} stretching parameters for water with trehalose. Bottom right: β_α and β_{long} stretching parameters for water with maltose. We observe that in the case of water with trehalose the stretching parameter β_{long} is constant at higher temperatures and below 260 K shows a gradual decrease upon further decreasing temperature by analogy to the same parameter extracted form hydration water[39] and from percolating model systems.[40,41].

slow part of the correlator can be perfectly fitted via a singular KWW function with the same parameters of the α relaxation part of eq 2 from the total correlator, as shown in the bottom inset of Figure 2 for the SISF corresponding to $T = 230$ K. This confirms our hypothesis that α relaxation characterizes the dynamical properties of bulk-like water molecules in solution.

In the framework of MCT, the behavior of the α-relaxation time has to follow a power law

$$\tau_\alpha = (T - T_C)^{-\gamma} \qquad (3)$$

where T_C is the MCT crossover temperature that in glass formers corresponds to the temperature where all cages are frozen and ergodicity persists only because of hopping.

In Figure 3, we show the temperature dependence of the relaxation times τ_α and τ_{long} and of the stretching parameters β_α and β_{long} for both the disaccharides aqueous solutions and the bulk.

We observe that the α-relaxation times of both solutions are consistent with the MCT power law (eq 3), with a crossover temperature $T_C \approx 208$ K for both trehalose and maltose aqueous solution. Therefore, the crossover temperature is about 20° higher than that calculated for the 1 atm isobar in bulk water (also shown in the figure). We see that the dynamic properties of noninterfacial water are similar to those of the bulk. This is analogous to what is found for water close to hydrophilic surfaces where a bulk-like behavior is recovered once the water closest to the surface is excluded.[36−38]

Importantly, we observe that noninterfacial water molecules are substantially insensitive to the specific kind of sugar present in the solution, trehalose, or maltose, as also demonstrated by the similar trend of the stretching exponent β_α for the two solutions.

When we analyze the dynamics of hydration water molecules, we observe important differences between the aqueous solutions of trehalose and maltose. At low T, the relaxation times of hydration water molecules are much higher in the trehalose solution, highlighting that this disaccharide has a greater attitude to keep water localized close to its surface with respect to maltose. In aqueous solution of maltose, the stretching parameter β_{long} has a temperature dependence roughly equal to that of the stretching parameter β_α. In the presence of trehalose, the stretching parameter β_{long} follows a different trend that is very similar to the trend recently experimentally observed[39] for the stretching parameter of protein hydration water. It is constant at high temperatures, and it shows a monotonic decreasing starting from a crossover temperature possibly connected to the onset of proteins' functionalities.[39] This behavior has been interpreted as that of percolating water molecules throughout the protein surface. We therefore observe for trehalose hydration water a dynamics that is more akin to that of protein hydration water than that of maltose. We also note that the same behavior for the stretching exponent is found in percolating model systems both in real space[40] and in configuration space.[41] Also in these works the stretching exponent is constant down to the percolation transition temperature; then, it shows a gradual decrease upon further decreasing temperature, therefore closely resembling the behavior that we obtained for the β_{long} exponent of trehalose.

These findings confirm the hypothesis that the bioprotecting functions of disaccharides are connected to their ability to maintain a favorable environment for water in contact with biomolecules also despite the lowering temperature or water content.[11,12] Our microscopic analysis of interfacial water dynamics indicates that in this respect the modification induced by trehalose on water dynamics is more compatible than those induced by maltose with those induced by proteins. As we observed in the

snapshots of our simulations, this is due to the fact that trehalose does not form clusters in solutions and therefore offers to water, and indirectly also to biomolecules, a much wider interaction surface. On the contrary, maltose, preferring sugar−sugar interactions, slows down hydration water dynamics essentially for steric reasons, and this results in its minor efficiency as bioprotectant. Stereochemical differences have an important influence also in aqueous solution of monosaccharides.[42]

In summary, we have shown that water slow dynamics in the presence of disaccharides such as trehalose or maltose shows two different well distinct relaxation processes, a faster one that shows bulk-like features due to noninterfacial water and a slower one characterizing the dynamical properties of hydration water molecules. This well-distinct dynamic differentiation has recently been observed through light scattering[43,44] and Fourier transform infrared spectroscopy[45] experiments on trehalose aqueous solutions. We have proposed a useful relation that is able to describe quantitatively both the α relaxation and the long relaxation. Our analysis gives evidence that the main differences between the two disaccharides solutions occur in the dynamic behavior of hydration water molecules, which have for trehalose a better similarity with that of water close to protein surface. Hence, trehalose, with respect to maltose, would be able to slow down more consistently the hydration water dynamics and to create a dynamical environment more similar to that which is needed to maintain functional conformation of proteins.

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