Landscape Excitation Profiles and Excess Thermodynamic Properties of Disaccharide Aqueous Solutions

S. MAGAZÙ¹, C. MONDELLI² and G. ROMEO¹

¹*Dipartimento di Fisica and INFN, Università di Messina, Papardo Sperone 31, 98166 S. Agata, PO Box 55 Messina;* ²*INFN- Operative Group Grenoble CRG IN13 and Institut Laue-Langevin, 38042 Grenoble Cedex 9, France*

Abstract. We will consider the use of the bond model in characterizing, by the direct calculation route, the full excitation profile of aqueous solutions of disaccharides. The present work sets the temperature behaviour of the excessive value of some thermodynamic quantities, such as configurational entropy and heat capacity of two homologous disaccharide aqueous solutions. The findings represent important data for understanding the better lyoprotectant effectiveness of trehalose in comparison with sucrose.

Key words: disaccharides, neutron scattering, excitation model

Introduction

It is common knowledge that stabilization during drying is widespread in nature: many anhydrobiotic organisms, including fungal spores, yeast cells, and certain soil-dwelling organisms, may persist without water for decades and, in some cases, centuries [1]. When rehydrated, they rapidly resume active metabolism, frequently within minutes. A common feature in these organisms is the synthesis induced by heat shock of a large amounts of trehalose [2]; its analogue in higher plants appears to be sucrose, which may in some cases make up as much as 50% of their dry weight [3]. The synthesis of some kind of sugars is employed, not only in the stabilization of membranes in the dry state by lyophilization, but also in preserving living cells from the damage caused by freezing [4]. It is well known in fact, that the phase transition from liquid water to ice, being connected to the damage of the cellular microarchitecture, is equated with death [5]. Many are the options, not mutually exclusive, that organisms have for dealing with sub-zero temperatures: undercooling, vitrification and sugar accumulation have proven to be useful mechanisms to protect cells to a substantial degree against osmotic injury [6].

The complexity of the energy landscape [7, 8], explored by the system, can be connected with the density of minima of the hypersurface (degeneracy), and with the distribution of the barrier heights between them. These features determine the

structural sensitivity of a system to temperature changes, namely its fragility. In this frame, Angell's classification [9] of glass-forming systems in as “strong” or “fragile” takes particular relevance.

This strong-fragile liquid pattern has been used as the basis for a classification of liquids, to indicate the sensitivity of the liquid structure to temperature change. Strong liquids have a built-in resistance to structural change, and their vibrational spectra and radial distribution show little reorganization despite wide variations of temperature, whereas fragile liquids exhibit little resistance, and much reorganization. Between these two limit cases, intermediate behaviours are interpreted in terms of different kinetic and thermodynamic contributions: thermodynamically strong and kinetically fragile systems are characterized by a low configurational degeneracy, and small barrier heights. In contrast, thermodynamically fragile and kinetically strong systems have a high number of minima on the energy hypersurface and large barrier heights. Strong liquids can be converted to more fragile behaviour by changing their densities; typically, they show very small jumps in ΔC_P at the glass transition temperature T_g , whereas fragile liquids show large jumps.

In an incoherent neutron scattering experiment, waves scattered by different nuclei do not interfere [10]. The observed scattering intensity is the sum of intensities scattered by individual nuclei. It is analyzed by a neutron spectrometer, in terms of energy (frequency, ω) and momentum transfer (scattering vector, $\mathbf{Q}$), to provide $S(\mathbf{Q}, \omega)$. The scattering is elastic for $\omega = 0$, i.e., when, within the energy resolution of the spectrometer, there is no energy transfer between the incident and scattered neutrons. Elastic incoherent neutron scattering (EINS) reflects the displacements of individual atoms in the picosecond-to-nanosecond time range, depending on the energy resolution of the spectrometer. An energy broadening of the elastic peak corresponds to quasielastic scattering. It results from relaxation processes such as non-vibrational, diffusive motions or jumps between conformational substates. Vibrational motions give rise to inelastic scattering with peaks at distinct energy transfer values.

The elastic intensity I_{el} has been analysed with a simplified model that describes the transitions between different conformations of the disaccharide in terms of proton jumps within an asymmetric double-minimum potential. Next, we have applied the “the bond lattice” model [11] to our data. This model is attractive because the system is viewed as an ensemble of bonds rather than as an ensemble of molecules or ions [11] and, indeed, the existence of intact and broken bonds, whose ratio is temperature-dependent, allows us to explain the existence of configurational and/or diffusive relaxations without specifying the scatterers. We show that the parameters, probabilities of finding a hydrogen in the ground or excited state, obtained by neutron scattering data allow us to explain the temperature behaviour of the excessive value of some thermodynamic quantities, such as C_P and S_C . All the observations outline a picture in which the trehalose/water system, at the investigated concentration, will play the role, in comparison with the sucrose/water mixture, of

the fragile system. We can therefore argue that the extended polymorphism leads to a molecular rigid phase able to adapt physically to the irregular biostructure surfaces.

Experimental Setup

Ultrapure powered trehalose and sucrose, and D₂O, purchased from Aldrich-Chemie, were used to prepare solutions at a weight fraction corresponding to 19 water molecules for each disaccharide molecule. At such a concentration value, the glass transition temperature, as determined by differential scanning calorimetry, is 253 and 250 K for trehalose and sucrose aqueous mixtures, respectively [12].

The experiments were performed on the IN13 backscattering spectrometer at the Institut Laue-Langevin (ILL; Grenoble, France). IN13 is a spectrometer combining a high energy resolution and a wide Q range. The beam scattered from the sample was reflected in almost perfect backscattering geometry by CaF₂ analysers to be finally collected by 32 He [3] detectors. The detectors on the spectrometer cover a large Q -range, from 0.28 to 4.27 Å⁻¹; the incident wavelength was 2.23 Å and the elastic energy resolution was 8 μeV. We performed the elastic neutron scattering experiment and collected data between 18.27 and 309.00 K. The raw data were corrected, at each Q -value, for scattering by the empty sample container, detector response (by using a standard vanadium sample), and for transmission of the sample, and normalized to unity at $Q = 0$ Å⁻¹.

Theoretical Background

Elastic scans make evident a dynamical transition at T_g for trehalose and sucrose aqueous solutions [13], which marks a cross-over in molecular fluctuations between harmonic and anharmonic dynamical regimes which permits each system to access multiple minima of its configuration space. In this frame, the anharmonicity can be seen as an index of polymorphism and therefore of the fragility of the system.

The elastic intensity I_{el} has been analysed with a simplified model [15] that describes the transitions between different conformations of the disaccharide in terms of proton jumps within an asymmetric double-minimum potential. In this model, the hydrogens can fluctuate between two sites of different free energy ΔG and separated by a distance d . In this context, the elastic scattering intensity is given by:

$$S(Q, \omega = 0) = \exp(-Q^2 \langle \Delta u^2 \rangle_G) \left[1 - 2p_1 p_2 \left(1 - \frac{\sin(Qd)}{Qd} \right) \right]$$

in which the first term gives the Gaussian contribution to the proton mean square displacement (normalised to 18.27 K) and where, below the dynamical transition,

Figure 1. Normalised incoherent elastic neutron scattering intensity at different temperatures, for (a) trehalose/water mixture and (b) sucrose/water mixture.

temperature, $\langle \Delta u^2 \rangle_G = \langle \Delta u^2 \rangle_{\text{vib}}$. The second term in brackets takes into account the elastic incoherent structure factor due to the two states. The variables p_1 and p_2 are the probabilities of finding a hydrogen in the ground (site 1) or excited state (site 2) respectively, with $\frac{p_1}{p_2} = \exp(-\Delta G^0/RT)$. As it can be seen from Figure 1, the two-state model fits the data reasonably well. From the fitting procedure, we find that values obtained for p_1 are always higher for trehalose/water than sucrose/water solutions; $d_{\text{trehalose}} = 0.9 \text{ \AA}$ and $d_{\text{sucrose}} = 1.1 \text{ \AA}$ [16]. Such a value indicates that the observed anharmonic dynamics may involve the activation of torsional degrees of freedom within the glucose monomer of the disaccharides. The observation that $d_{\text{trehalose}} < d_{\text{sucrose}}$ at all the investigated temperature values indicates that, in the case of the trehalose/ D_2O mixture, we are in the presence of more hindered dynamical processes than in the case of the sucrose/ D_2O mixture, and hence of a more rigid structure on a nanoscopic scale.

A van't Hoff plot of $\ln(p_2/p_1)$ vs. $1/T$ allows us to estimate the evaluation of enthalpy and entropy involved in the processes, unambiguously, once ΔG^0 is assumed to be a positive-definite quantity from its very physical meaning. This is analogous to the “bond-lattice” description [17] of the thermodynamic excitation of a system of bonded particles. The corresponding configurational entropy contains contributions from both the excitation entropy and the entropy of mixing of the unexcited and excited states and is given by $S_c = X \Delta S_0 - X R \ln X - (1 - X) X R \ln(1 - X)$, where X is the fraction of excited states and the contribution of the process to the heat capacity per mole of molecules is in turn given by $C_P = T(dS_c/dT)P$.

Figure 2. The fraction of constraints broken at different temperature. We note that the trehalose/water mixture shows higher fraction of excited states than the sucrose/water mixture.

Results and Discussion

The asymmetric double-minimum potential model allows us to estimate the probabilities of finding a hydrogen in the ground or excited state respectively and by a van't Hoff plot of $\ln(p_1/p_2)$ vs. $1/T$, to estimate the enthalpy and entropy involved in the processes for the disaccharide/water solution. In terms of the bond model, the system is driven to a given state of configurational excitation X . Its form, shown by the plot of X vs. T in Figure 2, can be taken to advantage to simplify the description of liquid properties which are necessarily complex in multidimensional energy landscape [7, 8] terminology. The inherent structure energy [11] is the potential energy of the structure to which the system has been driven by the TS term in the Helmholtz free energy $A = E - TS$ which the system must minimize in order to be in thermal equilibrium at the temperature T , i.e., it is the manner in which the system samples its landscape, not the landscape itself, that changes with temperature.

When the system has sufficient kinetic energy to sample its entire energy landscape, the overwhelming number of minima that it samples are shallow, reflecting the fact that deep minima are very rare. But, as the temperature decreases, the system is unable to surmount the highest energy barriers, and is therefore forced to sample the much rarer deeper minima. At a low enough temperature the system becomes stuck in a single minimum. This corresponds to the glass transition. We note the existence of a plateau, at low temperature, at which the height of the barriers separating sampled inherent structures increases abruptly.

The S_c vs. T curves (Figure 3) from the bond lattice model are sigmoidal in shape, so that S_c approaches zero in a physically plausible asymptotic fashion as $T \sim 0$. The larger the value of ΔC_P (Figure 4), the more rapidly the entropy increases with temperature. In fact, a liquid is classified as fragile (strong) if a large (small)

Figure 3. Comparison of configurational entropy vs. temperature of trehalose/water mixture and sucrose/water mixture.

Figure 4. Excess heat capacities of trehalose/water and sucrose/water solutions as a function of temperature.

variation in heat capacity crossing T_g , occurs. The experimental measurements for the two sugar/water systems show data points with error bars smaller than the symbols into the figures and trehalose/water solution shows an higher fragility with respect to sucrose/water solution.

Concluding Remarks

In the bond lattice approach, the system of strongly bonded particles is transposed to its 'bond lattice' in which the elements of the lattice can justifiably be considered as weakly interacting and can therefore be treated, in the first approximation, as being independently excitable.

Hence we note the higher trehalose/water mixture heat capacity, and higher configurational entropy, than the sucrose/water mixture. In fact, the larger the value of heat capacity the more rapidly the entropy increases with temperature, so the more rapidly the state point will float to the top the landscape. In terms of the bond model, the system is driven to a given state of configurational excitation that can be considered to simplify the manner in which the system samples its landscape, and how this landscape changes with temperature. Thus the trehalose/water solution, with higher heat capacities per mole of heavy atoms, has a steeper excitation profile, and a fragility that is thermodynamic in origin.

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