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Changes in vibrational modes of water and bioprotectants in solution

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Abstract

Inelastic neutron scattering (INS) measurements have been performed on trehalose and sucrose/H_2O mixtures at very low temperature as a function of concentration by using the TOSCA spectrometer at the ISIS Facility (DRAL, UK). The aim of this work is to investigate by INS the vibrational behaviour of water in presence of trehalose and sucrose in order to characterize the changes induced by these disaccharides on the H_2O hydrogen-bonded network. In particular, we obtained information about the effects of the two disaccharides in the translational, librational and bending spectral regions of ice. The disaccharide bioprotective effectiveness can be linked by the high destructuring effect emphasised by the analysis of the librational modes region. On the other hand, the analysis of the vibrational region corresponding to the ice bending modes show a high "crystallinity" degree which can justify the *crypto*biotic action of disaccharides.

Keywords: Disaccharides; Water mixtures; Bioprotection; Vibrational properties; Neutron scattering

1. Introduction

Cryobiology studies the effects of very low temperature on life [1]. Living organisms all share basic building blocks. These blocks include the genetic library held by the DNA double helix that is transferred to RNA in order for macromolecules like proteins, polysaccharides and lipids, to be manufactured. Despite their importance, it is H_2O the most fundamental building block to all life. Water is the key to life because it is the primary solvent in all living creatures. In cryobiology, the nature of water transformation from a liquid solvent to a solid structure during freezing provides for the ability to preserve or destroy. Understanding how to manipulate the freezing process is the cryobiologist's ultimate goal [1–3].

Through evolution, certain organisms have adapted to survive at low temperatures well below the freezing point of water (0 °C). These organisms have the ability to create biomolecules, called "cryoprotectants", which act as anti-freeze, lowering the temperature at which intracellular and, to some extent, extracellular ice forms [1–4]. Among cryoprotectants, trehalose and sucrose are very effective in avoiding cellular damage by low temperatures [4].

Many experimental studies [5–10] have been addressed to understand from a molecular point of view the bioprotective mechanisms of disaccharides. Raman spectroscopy [5] and neutron diffraction results [6], together with ultrasonic findings [6], showed that the water hydrogen bonded tetrahedral network is destroyed by trehalose by means of a strong interaction with water molecules, as indicated by the high values of the solute–solvent interaction strength and of hydration number. This feature has as an effect a strong slowing down of water dynamics, as emphasised by quasi elastic neutron scattering (QENS) [10]. Furthermore, elastic neutron scattering (ENS) [8] and viscosity measurements [6] allowed also to point out the "stronger" character in the Angell's classification scheme of trehalose. All these results contribute to clarify the physical processes underlying the biological action of disaccharides and in particular by trehalose, which evidently give rise to a strong interaction with water and not directly with the biostructures to be protected, as hypothesised by Crowe and Crowe [11].

Recent experimental and simulation studies have been addressed to the investigation of the ternary lysozyme/disaccharides/H_2O system. Caliskan et al. [12] have investigated the influence of glycerol and trehalose on lysozyme by

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Raman scattering, focusing particularly the attention on the low-frequency spectra. They conclude that protein is strongly coupled dynamically to trehalose and to glycerol and that glycerol provides superior suppression of protein dynamics than trehalose does at low temperature, while trehalose appears to be more effective at higher temperatures. This conclusion agrees with time-dependent geminate CO recombination measurements for myoglobin Mb in glycerol [13] and trehalose [14,15]. Near 200 K, the ligand escape and conformational rearrangements are faster in the Mb dissolved in trehalose glass as compared to Mb in glycerol [13–15]. Using a model-dependent analysis, it has been found [14] that activation energy for conformational rearrangements of Mb dissolved in glycerol is approximately 3 times higher than those in Mb dissolved in trehalose.

A combined Raman and MD simulation study performed by Descamps and coworkers (A. Lerbret, Etude de l'action bioprotectrice des sucres: une investigation par dynamique moléculaire et spectroscopie Raman, PhD thesis, University of Lille, 2005) on lysozyme/trehalose/H_2O, lysozyme/maltose/H_2O and lysozyme/sucrose/H_2O solutions has been focused on the hydration properties of the protein in presence of disaccharides and their dependence on concentration, pointing out that trehalose hydrates preferentially lysozyme more than maltose and sucrose and confirming the highest destructuring effect.

This work is aimed to describe the effects of trehalose and sucrose in water mixtures on the vibrational properties of water in order to clarify the physical mechanisms of their bioprotective action. The dependence of the induced changes on concentration is pointed out. The present findings furnish useful information on the trehalose and sucrose amount which produces a destructuring effect on the water tetrahedral network, giving confirmation and explanation to previous neutron scattering results [5–10].

2. Experimental

INS measurements have been performed by using the indirect geometry time-of-flight spectrometer TOSCA at the ISIS Pulse Neutron Facility (DRAL, UK) [16]. The energy resolution of TOSCA is $\Delta E/E \approx 1.5$–2% for energy transfers up to several hundred meV. This high resolution coupled with the high intensity of the ISIS source makes TOSCA ideal for studying the dynamics of water and water mixtures below 2000 cm^{-1} (250 meV) [16].

Ultra pure powdered trehalose and sucrose and H_2O, purchased by Aldrich-Chemie, were used for the experiment. Measurements were performed at a temperature value of 27 K on hydrogenated trehalose and sucrose ($C_{12}H_{22}O_{11}$) in H_2O at different weight fraction values corresponding to 2, 7, 10 and 14 H_2O molecules for each disaccharide molecule.

The samples, contained in thin walled aluminium cells, were cooled to 27 K by a liquid helium cryostat. For all the investigated hydrogenated samples, the measurement time was 12 h for each run.

For the data treatment, the standard GENIE programme has been used [16]. For all the plots, the error bars to be considered are ~5%. The multiple scattering contribution has been minimised by using a thin sample in order to obtain a scattering transmission from the sample ≥90%. The multiphonon neutron scattering contribution (MPNS), which can be significant at high temperature and large momentum transfer, has been calculated directly from the measured spectra by using a method of sequential iterations. Since measurements were performed at low temperature, the MPNS contribution is not large at the translational modes region (i.e. at low Q region) [17–19].

3. Results and discussion

Previous INS results on trehalose and its homologous, i.e. maltose and sucrose [9], emphasised the differences among disaccharide vibrational properties starting by a comparison with the ice spectrum obtained at the same temperature value. In the previous paper [9], the effects on the tetrahedral network of water have been discussed pointing out the strongest interaction of trehalose with water which justifies its superior bioprotective effectiveness. In order to analyse the modifications induced by trehalose on water, we subdivided the ice spectrum, shown in Fig. 1, in different regions [17–19] which will be selectively discussed. Furthermore, due to the spectral features observed by increasing the water content in the mixtures, in Fig. 2, the INS spectra of (a) trehalose/2 H_2O (the dehydrate form is the natural state of trehalose) and (b) sucrose/2 H_2O are shown as reference.

Let us analyse in detail the different spectral regions.

In the ice 0–400 cm^{-1} spectrum two bands are observed at low energy: the first one, having a sharp peak at ~56 cm^{-1}, is dominant on the second one centred at ~148 cm^{-1}. They can be assigned to acoustic modes. It is known, in fact, that in the ice spectrum the peak at ~56 cm^{-1} denotes the first Van Hove singularity in the dynamics of acoustic phonons. The two peaks present at ~224 cm^{-1} and at ~304 cm^{-1} with cutoffs on their right-hand sides are due to molecular optical modes [17–19].

It is observed from Fig. 3 that in presence of trehalose and sucrose the sharp peak of the low-energy acoustic modes appears significantly lower and broader and shifted at 72 cm^{-1}. The shift at higher energy is indicative of a strong interaction between disaccharides and water molecules. Important changes are also appreciable for the other bands and the second peak of the optical

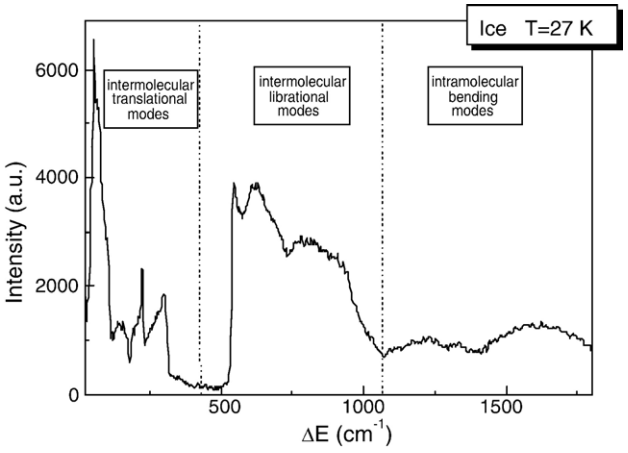

Fig. 1. INS spectrum of ice.

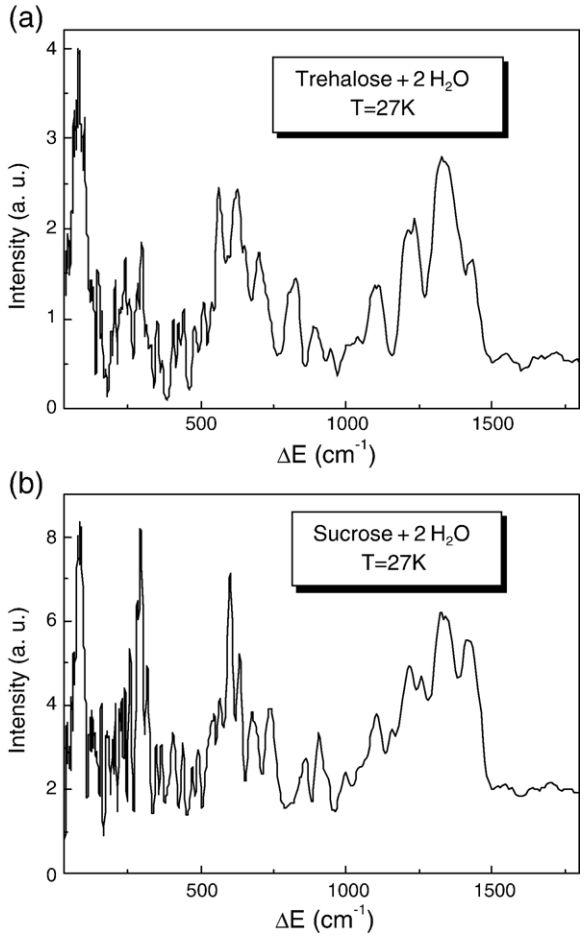

Fig. 2. INS spectra of (a) trehalose/2 H_2O mixture and (b) sucrose/2 H_2O mixture at $T=27$ K.

mode is present but deformed. The water contribution in this region starts to become marked only for the concentration value corresponding to 14 H_2O molecules for each disaccharide molecule, showing a spectrum more similar to that of water. Comparing trehalose and sucrose spectra, it is evident that sucrose is more strongly influenced by water and for sucrose mixtures the spectral features relative to the disaccharide contribution are more rapidly lost than for trehalose mixtures.

In the ice 400–1060 cm^{-1} region, it was found that the characteristic value for the librational band in the INS spectrum for different ice forms is the position of its low-energy cut-off [17–19]. The observed shifts of the cut-off position are proportional to the transverse forces between the water molecules which are of different intensity for the different ice forms [17–19].

In order to point out the differences due to the trehalose and sucrose concentration in the librational spectral region, in Fig. 4, the inelastic intensity of trehalose and sucrose/7 H_2O, trehalose and sucrose/10 H_2O and trehalose and sucrose/14 H_2O are shown. A depression of the intensity of the ice librational cut-off is observed and, as it can expected, it is more evident for trehalose and sucrose/7 H_2O, the cut-off becoming more and more sharp by increasing the water content. In order to evaluate the shift of the cut-off position, which for ice at $T=27$ K is at ~ 550 cm^{-1} [17–19], we considered the first peak appearing in the band present starting from ~ 450 cm^{-1}. For all the investigated concentration values in trehalose mixtures, we observe the same shift of ~ 16 cm^{-1}, whereas for sucrose no shift occurs. This result can give a confirmation of previous light and neutron scattering findings [5,6,10], emphasising that trehalose is more capable than sucrose to modify the spectral features of ice, affecting the intermolecular interaction forces and the arrangements of the H_2O molecules.

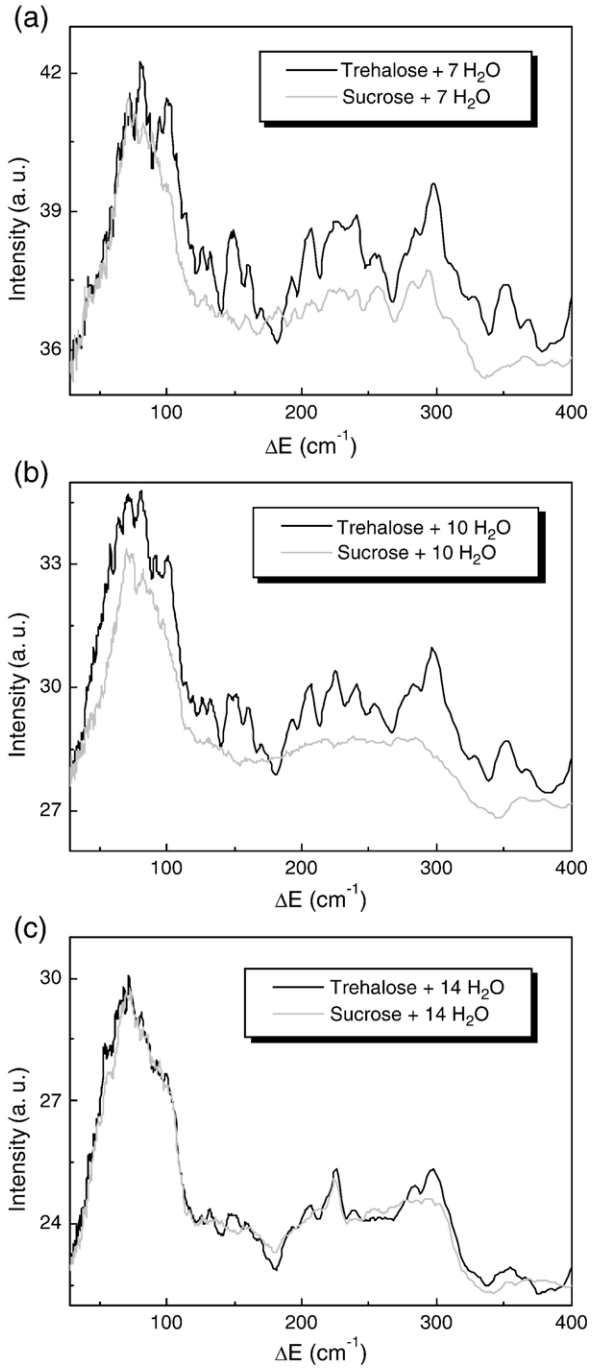

Fig. 3. INS spectra of (a) trehalose and sucrose/7 H_2O mixtures, (b) trehalose and sucrose/10H_2O mixtures and (c) trehalose and sucrose/14 H_2O mixtures in the translational spectral region.

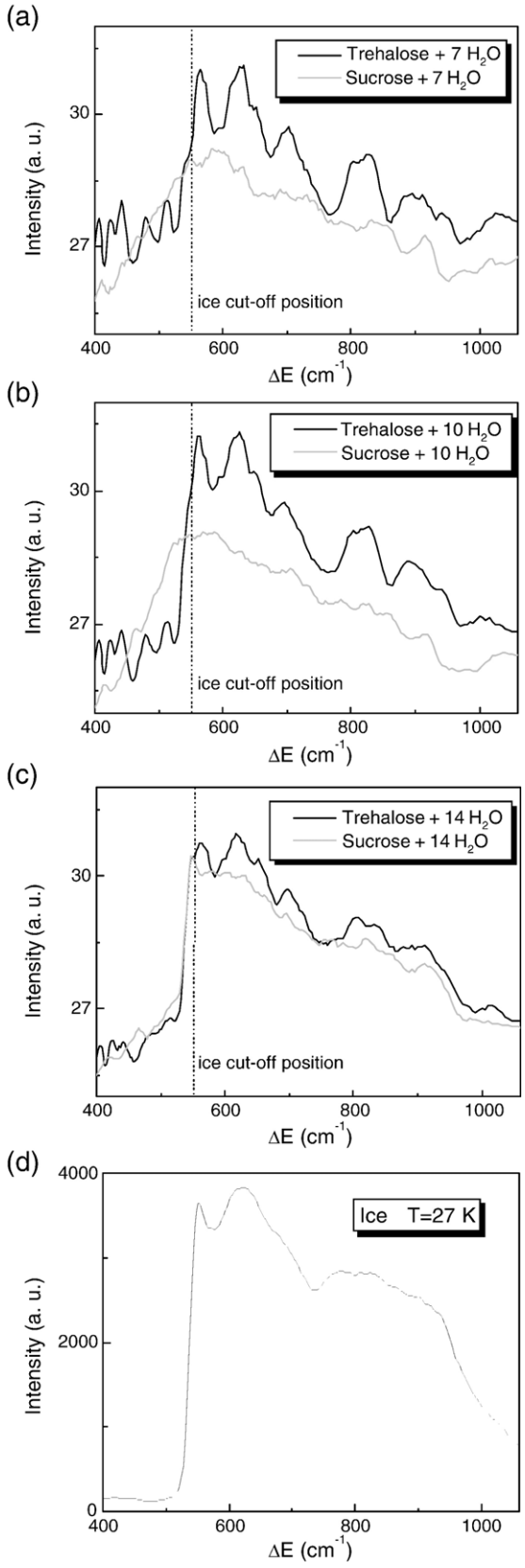

Fig. 4. INS spectra of (a) trehalose and sucrose/7 H_2O mixtures, (b) trehalose and sucrose/10 H_2O mixtures, (c) trehalose and sucrose/14 H_2O mixtures and (d) ice in the librational spectral region.

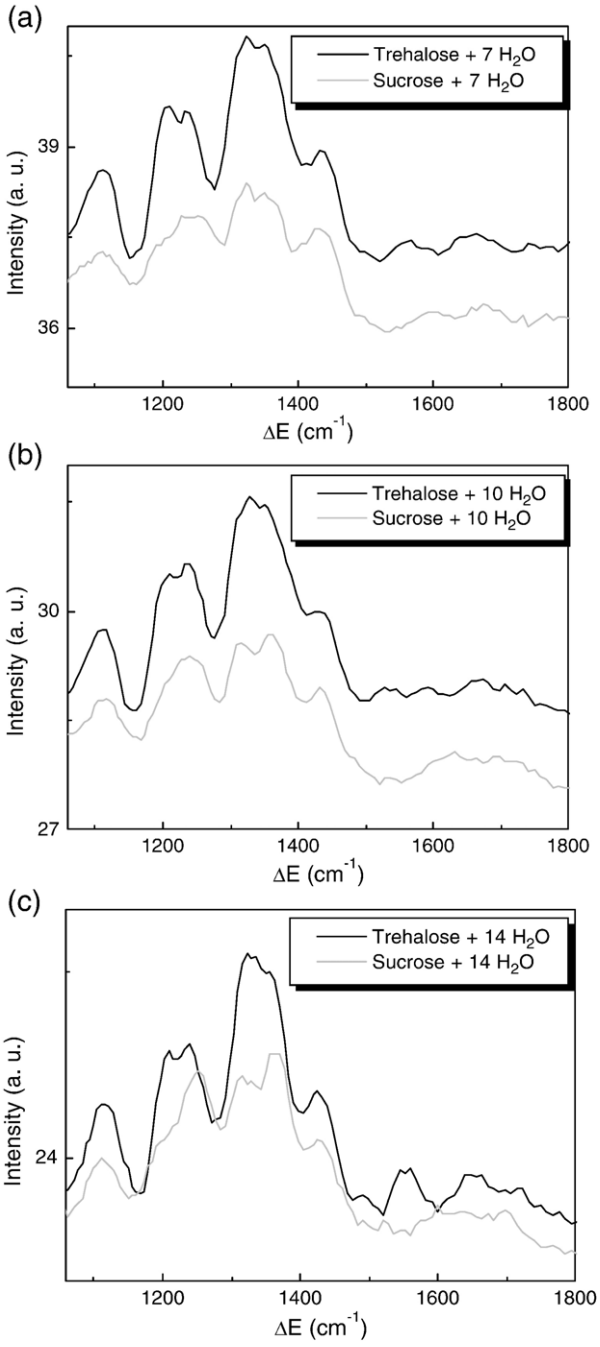

Fig. 5. INS spectra of (a) trehalose and sucrose/7 H_2O mixtures, (b) trehalose and sucrose/10 H_2O mixtures and (c) trehalose and sucrose/14 H_2O mixtures in the bending spectral region.

The 1060–1800 cm^{-1} spectral region corresponds to the range of the bending vibrational modes of ice [17–19]. The ice spectrum is characterised by two distinct bands centred at ~1224 cm^{-1} and ~1608 cm^{-1}, respectively. For the investigated trehalose and sucrose/H_2O mixtures, shown in Fig. 5, these features are totally changed. The trehalose spectra appear more "structured" and show distinctly the three typical peaks of the "crystalline state" of trehalose, i.e. trehalose/2 H_2O, as it is evident by a comparison with Fig. 2. These peaks correspond to the hybridized H–C–H, C–C–H and C–O–H bending modes,

as indicated by simulation [20]. By increasing the water content, the three peaks are still evident even if they are less marked. For sucrose mixtures, the spectra show that these peaks are larger and broader.

This trend finds a correspondence with elastic incoherent neutron scattering (EINS) findings [8], which have shown the increased rigidity of the disaccharide–water system by increasing disaccharide concentration. By the EINS results, we concluded that trehalose, besides modifying significantly the structural and dynamical properties of water, forms with H_2O a more rigid unique entity than sucrose. The present findings help to give an explanation to the previous results, because the locally more ordered structure of trehalose, pointed out by the analysis of the bending region in comparison with sucrose, can justify the higher rigidity of this system [8]. This allows the trehalose–H_2O system to encapsulate biological structures and to protect them in a more rigid environment.

4. Conclusions

In this paper, INS results on trehalose and sucrose/H_2O mixtures as a function of concentration are shown. The experimental data point out that trehalose interacts more strongly with water than sucrose, influencing significantly its vibrational modes and creating optimal conditions for avoiding the formation of ice. Furthermore, trehalose shows a more "cryptocrystalline" character than sucrose, which makes trehalose the most effective as cryo- and cryptoprotectant.

The present findings furnish information to understand the role of disaccharides, and in particular trehalose, in protecting biomolecules. The point to be emphasised is that the interaction with biomolecules is activated by trehalose by means of water. Therefore, in order to better explain the bioprotection mechanisms by disaccharides, it is fundamental to fully characterise the disaccharides/water interaction. As reported in Refs. [12–16], the bioprotective action of trehalose is due to different cofactors linked to the high glass transition temperature value and hence to the superior "strength" and to the capability to deeply modify the structural and dynamical properties of water.

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