

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/H₂O 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 H₂O 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 *cryptobiotic* action of disaccharides.

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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 H₂O 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/H₂O 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/H₂O, lysozyme/maltose/H₂O and lysozyme/sucrose/H₂O 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 $\Delta E/E \approx 1.5\text{--}2\%$ 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 H₂O, purchased by Aldrich-Chemie, were used for the experiment. Measurements were performed at a temperature value of 27 K on hydrogenated trehalose and sucrose (C₁₂H₂₂O₁₁) in H₂O at different weight fraction values corresponding to 2, 7, 10 and 14 H₂O 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 $\sim 5\%$. The multiple scattering contribution has been

minimised by using a thin sample in order to obtain a scattering transmission from the sample $\geq 90\%$. 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 H₂O (the dehydrate form is the natural state of trehalose) and (b) sucrose/2 H₂O are shown as reference.

Let us analyse in detail the different spectral regions.

In the ice $0\text{--}400\text{ cm}^{-1}$ spectrum two bands are observed at low energy: the first one, having a sharp peak at $\sim 56\text{ cm}^{-1}$, is dominant on the second one centred at $\sim 148\text{ cm}^{-1}$. They can be assigned to acoustic modes. It is known, in fact, that in the ice spectrum the peak at $\sim 56\text{ cm}^{-1}$ denotes the first Van Hove singularity in the dynamics of acoustic phonons. The two peaks present at $\sim 224\text{ cm}^{-1}$ and at $\sim 304\text{ cm}^{-1}$ 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

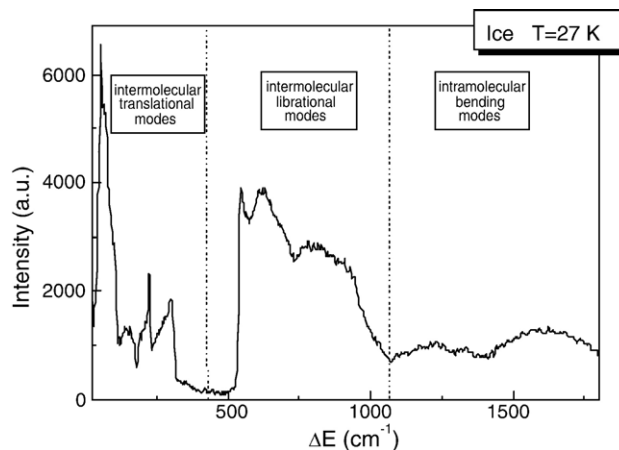


Fig. 1. INS spectrum of ice.

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