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Study of the dynamical properties of water in disaccharide solutions

S. Magazù · F. Migliardo · M. T. F. Telling

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Abstract This work presents quasi-elastic neutron scattering (QENS) and neutron spin echo (NSE) findings on homologous disaccharide (i.e. trehalose, maltose and sucrose)/water solutions as a function of temperature. The dynamical properties of these systems are investigated by QENS, which, on the picosecond scale, allows for the characterisation of the diffusion of both solutes and solvent. On the other hand, NSE investigates the dynamics on the nanosecond scale, allowing for the relaxation times of the disaccharide/water systems to be evaluated. The experimental data highlight a strong slowing down of water in the presence of disaccharides. The whole set of findings indicates, therefore, a noticeable disaccharide–water interaction, which is more intense in the case of trehalose. This feature can justify its higher bioprotective effectiveness.

Introduction

The bioprotective properties of trehalose have been recently subject of attention not only under the aspect of pure physical, biological and chemical research but also for the promising applicative implications (Crowe and Crowe 1984; Storey and Storey 1992; Wright et al. 1992; Chen et al. 2002; Guo et al. 2000; Zhang et al. 2003). Such a research moves from the observation that many organisms, such as, for example, locusts (*Schistocerca gregaria*), artemie saline (Brine shrimps), cryptobiontes (Phylum Tardigrada), bacteria (*Escherichia coli*) and plants (*Myrothamnus flabellifolia*), are able to synthesise trehalose for surviving under environmental stress conditions, such as dehydration and freezing. The disaccharide allows them to undergo in a cryptobiotic (''hidden life'') state and to re-activate the vital functions when the external conditions come back favourable to the life (Crowe and Crowe 1984; Storey and Storey 1992; Wright et al. 1992; Chen et al. 2002).

Trehalose has very important applications in different industrial fields, such as food, pharmaceutical and cosmetic industry. Trehalose can be used as a new multi-functional ingredient with considerable potential for the industry. It can be used by product developers either to improve existing products or to create innovative new products. The mild sweetness, low cariogenicity, low hygroscopicity, high freezing-point depression, high glass transition temperature and protein protection properties are all of immense interest to food technologists (Murray et al. 2000; Oku and Nakamura 2000; Gleeson and Bishop 2000a, b). Trehalose is fully caloric, has no laxative effects and after ingestion is broken down in the body to glucose, but with an even blood glucose response, making it ideally suited for products formulated to provide sustained energy (Murray et al. 2000; Oku and Nakamura 2000; Gleeson and Bishop 2000a, b; Ivy 2001). Unlike other disaccharides, it will not readily hydrolyse to its component parts and subsequently take a part in Maillard reactions with amino acids and proteins. In

S. Magazù (✉) · F. Migliardo
Dipartimento di Fisica, Università di Messina,
P.O. Box 55, S.ta Sperone C.da Papardo,
98166 Messina, Italy
e-mail: smagazu@unime.it

M. T. F. Telling
ISIS Facility, Rutherford Appleton Laboratory,
Chilton, Didcot, UK

food and beverage products, the high stability of trehalose enables the original product characteristics to be retained even after heat processing and prolonged storage (Hiashiyama 2002).

Many and promising are the biomedical and pharmaceutical applications of trehalose. In vitro screening studies have shown that various disaccharides can inhibit polyglutamine-mediated protein aggregation. Oral administration of trehalose decreased polyglutamine aggregates in cerebrum and liver, improved motor dysfunction and extended lifespan in a transgenic mouse model of Huntington disease (Tanaka et al. 2004). These beneficial effects are the result of trehalose binding to expanded polyglutamines and stabilising the partially unfolded polyglutamine-containing protein. A lack of toxicity and high solubility make trehalose promising as a therapeutic drug or lead compound for the treatment of polyglutamine diseases. The saccharide–polyglutamine interaction thus provides a new therapeutic strategy for polyglutamine diseases (Tanaka et al. 2004).

Research from many laboratories over the past several decades indicates that invertebrate oocytes and eggs are extraordinarily difficult to freeze. Since starfish oocytes, eggs and embryos are important cells and developmental biology model systems, there is a great interest to cryopreserve these cells. Previous starfish oocyte cryopreservation studies using slow cooling protocols revealed that these cells are highly sensitive to osmotic stress and form intracellular ice at very high sub-zero temperatures, suggesting that common freezing methodologies may not prove useful. It has been shown that a short exposure to trehalose in hypotonic salt solution followed by ultra-rapid cooling to cryogenic temperatures allows starfish oocytes to be cryopreserved with the average survival rate of 34% (Hamaratoglu et al. 2005).

Human platelets have extremely important medical applications, including uses in trauma situations, surgery, chemotherapy, bone marrow transplants, and treating immune system diseases including AIDS. Platelets, which are extremely sensitive to cold, are usually stored in blood banks at 22°C for only 5 days. An effective cryopreservation protocol would enable longer term storage and reduce the chronic shortage of platelets. Currently, 6% dimethyl sulfoxide (DMSO) is considered to be the most effective cryoprotectant for platelets, but DMSO must be washed away from thawed cells before transfusion due to cytotoxicity concerns. Trehalose has significant promise as a possible nontoxic replacement for DMSO, by evaluating the effectiveness of a trehalose–phosphate formulation in protecting platelet structure and function following cryopreservation. Finally, platelets protected by the trehalose–phosphate formulation exhibited a virtually normal aggregation response upon thrombin addition similar to fresh platelets (Nie et al. 2005).

Some theories have been formulated to explain how sugars exert their protective action. Green and Angell (1989) have concluded that the bioprotection mechanisms are related to the high glass transition temperature of trehalose. In fact, the higher T_g values of the trehalose/H_2O mixtures, in respect to those of the other disaccharides/H_2O mixtures, imply that at a given temperature the glass transition for trehalose mixtures always occurs at a higher water content. Crowe et al. (1998), however, have noted that vitrification is not sufficient for preservation. Vitrification alone does not explain, for example, why another carbohydrate, dextran, which has a significantly higher glass transition temperature than trehalose, is a much less effective cryoprotectant than trehalose. The water replacement hypothesis, proposed by Crowe, asserting the existence of direct hydrogen bonding of trehalose with the polar head groups of the lipids that constitute biomembranes, accounts for how the nonreducing trehalose preserves the integrity of biological structures. As the systems are dried or frozen, these interactions replace those of the hydration water at the membrane–fluid interface. In such a way, in the opinions of these authors, it prevents the phase transition and the accompanying leakage upon rehydration (Crowe et al. 1998).

Very recently Mason et al. (2005) investigated glucose aqueous solutions by neutron diffraction with isotopic substitution (NDIS) experiments and molecular dynamics (MD) simulations. The results emphasise that glucose molecules have no tendency to aggregate. Solute pairing was found to take place through hydroxyl–hydroxyl hydrogen bonds, in competition with water molecules for the same hydrogen-bonding sites. The authors conclude that the structure of bulk water is not significantly perturbed by the addition of a single sugar, even at fairly high concentrations (1:10 sugar:water), indicating that sugar has a weak influence on water.

The characterisation of molecular mechanisms underlying the numerous functions of trehalose is very important for the understanding and exploitation of the potentialities of the disaccharide.

Recently some experimental findings obtained by several spectroscopic techniques indicate that the structural and the dynamical properties of water result perturbed by disaccharides, and in particular by trehalose (Branca et al. 1999a, b, 2001, 2002; Magazù et al. 2004). The obtained findings are consistent with the

picture of a disaccharide-perturbing effect on the H_2O tetrabonded network of water molecules (Branca et al. 1999a, b, 2001, 2002). Furthermore, elastic neutron scattering and viscosity measurements allowed for the ''strongest'' character in Angell's classification scheme of trehalose in comparison with the other disaccharides to be pointed out.

A confirmation to these results can be found by the systematic computational work performed on a series of 13 disaccharides by Choi et al. (2006) to provide an atomic-level insight of unique biochemical role of the α,α-(1 → 1)-linked glucopyranoside dimer over the other glycosidically linked sugars. Analyses of the hydration number and radial distribution function of solvent water molecules showed that there was very little hydration adjacent to the glycosidic oxygen of trehalose and that the dynamic conformation of trehalose was less flexible than any of the other sugars due to this anisotropic hydration. The trehalose stable hydrogen-bond network is derived from the formation of long-lived water bridges at the expense of decreasing the dynamics of the water molecules, which was evident by both the lowest translational diffusion coefficients and the lowest intermolecular coulomb energy of the water molecules around trehalose.

In the present work, the results of a study performed by means of the integrated employment of complementary techniques such as quasi-elastic neutron scattering (QENS) and neutron spin echo (NSE) on homologues disaccharides (trehalose, maltose and sucrose)/H_2O mixtures as a function of temperature are presented. QENS measurements, performed both on partially deuterated disaccharides in D_2O and hydrogenated disaccharides in H_2O, allow for separation of the solute dynamics from that of the solvent, while NSE measurements on partially deuterated and hydrogenated samples make possible to characterise the dynamics of the entire solute-solvent. All the findings show a marked slowing down of the water dynamics.

Experimental

Samples

Trehalose, maltose and sucrose are homologues disaccharides having the same chemical formula ($C_{12}H_{22}O_{11}$) (molecular weight M_w = 342.3), but different structures. More precisely (Branca et al. 1999a, b), α,α-trehalose (α-D-glucopyranosil α-D-glucopyranoside) is constituted by two pyranose rings, linked by a glycosidic bond between the chiral carbon atoms C1 of the two rings. Maltose (4-*O*-α-D-glucopyranosil-D-glucose) is also constituted by two pyranose rings, but the oxygen bridge links the two carbon atoms C1 and C4 of the two rings. Sucrose (α-D-glucopyranosil *β*-D-fructofuranoside) is constituted by a glucose ring and a fructose ring and it is not a reducing sugar. Molecular structures of (a) trehalose, (b) maltose and (c) sucrose are shown in Fig. 1.

Ultra-pure powdered trehalose, maltose and sucrose, D_2O and H_2O, purchased by Aldrich-Chemie, were used for both the experiments. Measurements were performed in a temperature range of 273–353 K on hydrogenated trehalose, maltose and sucrose ($C_{12}H_{22}O_{11}$) in H_2O and on partially deuterated trehalose, maltose and sucrose ($C_{12}H_{14}D_8O_{11}$) in D_2O at a weight fraction, values corresponding to 19 water (H_2O and D_2O) molecules for each disaccharide molecule. All the three disaccharides possess hydrogen atoms, belonging to the OH groups, which exchange easily with the deuterium atoms of heavy water. To focus our

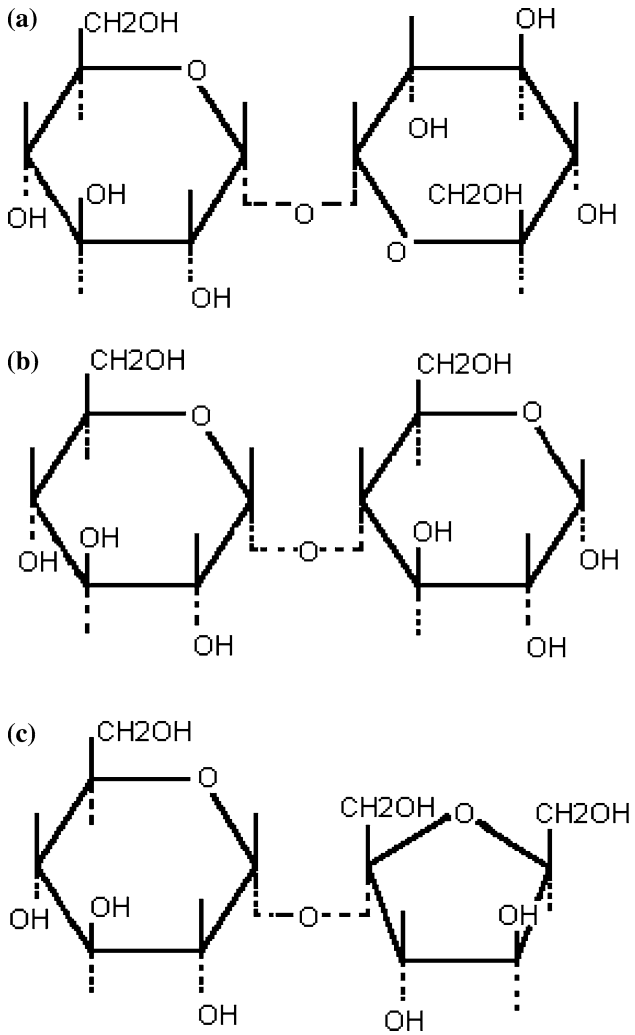

Fig. 1 Molecular structure of **a** trehalose, **b** maltose and **c** sucrose

attention on the disaccharide dynamics in D_2O, the exchangeable atoms were substituted with deuterium before preparing the solutions. We estimated that in the deuterated solutions (at the investigated concentration) the coherent contribution to the total scattering cross section is ~5%. In order to obtain partially deuterated samples, the disaccharides were first dissolved in pure D_2O to exchange the eight labile hydrogen atoms of the disaccharides and subsequently the solutions were lyophilised.

In the case of QENS in protonated samples, we focus the attention on the incoherent scattering arising from the self-correlation function which involves the motions of protons, the ratio between the incoherent cross-section σ_i and the scattering cross-section σ_s being $\sigma_I/\sigma_s = 0.94$, whereas in the case of NSE in partially deuterated disaccharides in D_2O the attention is addressed to the dynamics of the entire solute/solvent system.

It is important to specify that, as pointed out by ultrasonic and viscosity measurements (Branca et al. 1999b, 2001), disaccharides in water solution are strongly bonded to more than ~22 water molecules at room temperature and that this hydration number abruptly increases by lowering temperature. Therefore, at the investigated concentration values, no bulk water is present.

Instruments

The QENS experiment was carried out using the IRIS high-resolution spectrometer at ISIS, the world's leading pulsed neutron and muon source located at the Rutherford Appleton Laboratory (RAL, UK). IRIS is an inverted geometry spectrometer such that neutrons scattered by the sample are energy analysed by means of Bragg scattering from the large-area crystal-analyser array (Adams et al. 2001). We measured the sets of QENS spectra covering a Q,ω-domain extending from $\hbar\omega = -0.3$ to 0.6 mev (energy transfer) and $Q = 0.3$ to 1.8 Å^{-1} (momentum transfer) with a mean energy resolution of $\Gamma = 8$ μeV (HWHM). The raw spectra were corrected and normalised using the standard GENIE procedures and the IRIS data analysis package (Telling and Howells 2003). The estimated error in the measured spectra is 4%, while for the calculated parameters it is 6%.

We used the V5-SPAN spectrometer at the Berliner Neutron Scattering Centre (BENSC, HMI, Berlin, Germany) to collect NSE data on trehalose and sucrose water solutions as a function of temperature. In the V5 configuration used for the experiment the incident wavelength was $\lambda_o = 6.5$ Å, scanning a Q range of 0.4–1.5 Å^{-1}.

Results

Quasi-elastic neutron scattering

In Fig. 2, we report the best fit of hydrogenated aqueous solutions of trehalose for three temperature values and of the three disaccharides according to the fitting function:

$$S_{inc}(Q,\omega) = A(Q)\left\{ f_{Disaccharide}\left[F(Q)\frac{1}{\pi}\frac{\Gamma_1(Q)}{\Gamma_1^2(Q)+\omega^2} + (1-F(Q))\frac{1}{\pi}\frac{\Gamma_2(Q)}{\Gamma_2^2(Q)+\omega^2}\right] + f_{hydr}\frac{1}{\pi}\frac{\Gamma_3(Q)}{\Gamma_3^2(Q)+\omega^2}\right\}. \quad (1)$$

In Eq. 1 the first two terms refer to the dynamical structure factors of the hydrated disaccharides, where the translational scattering law is a Lorentzian whose half width at half maximum (HWHM) is Γ_1 and the rotational scattering law is a Lorentzian with HWHM Γ_2. The third term refers to the dynamical structure factor of water, where only the translational Lorentzian with HWHM Γ_3 is present, because the rotational contribution is so broad to be considered as flat. The terms $f_{Disaccharide}$ and f_{hydr} represent the fraction factors of the total scattering from disaccharide and from the strongly bonded water molecules and they fulfil the relation $f_{Disaccharide} + f_{hydr} = 1$. The numerical values of the parameters obtained by the present analysis for the three disaccharides at $T = 320$ K are reported in Table 1, whereas Table 2 shows the values obtained for trehalose mixtures for different temperature values. As it is evident, the present model results in a very good agreement with the experimental data.

The random jump diffusion (RJD) model (Bee 1988) well describes the behaviour of the translational linewidth for the investigated solutions (see Figs. 3, 4):

$$\Gamma_1(Q) = D_s Q^2/(1 + D_s Q^2 \tau) \quad (2)$$

furnishing the values of the self-diffusion coefficient D_s of the molecule and the residence time τ. For trehalose as a function of temperature the RJD model furnishes for the diffusion coefficient D_s and the residence time τ the values of $D_s = 2.83 \times 10^{-7}$ cm^2/s and $\tau = 24.7$ ps, $D_s = 3.82 \times 10^{-7}$ cm^2/s and $\tau = 20.6$ ps, $D_s = 5.35 \times 10^{-7}$ cm^2/s and $\tau = 19.1$ ps and $D_s = 8.50 \times 10^{-7}$ cm^2/s and $\tau = 18.3$ ps for $T = 283$, 295, 308, 320 K, respectively. From the relation $\langle l^2\rangle = 6D\tau$, we obtain the values $\langle l^2\rangle^{1/2} = 0.64$ Å, 0.68 Å, 0.78 Å and 1.00 Å, $T = 283$, 295, 308, 320 K, respectively.

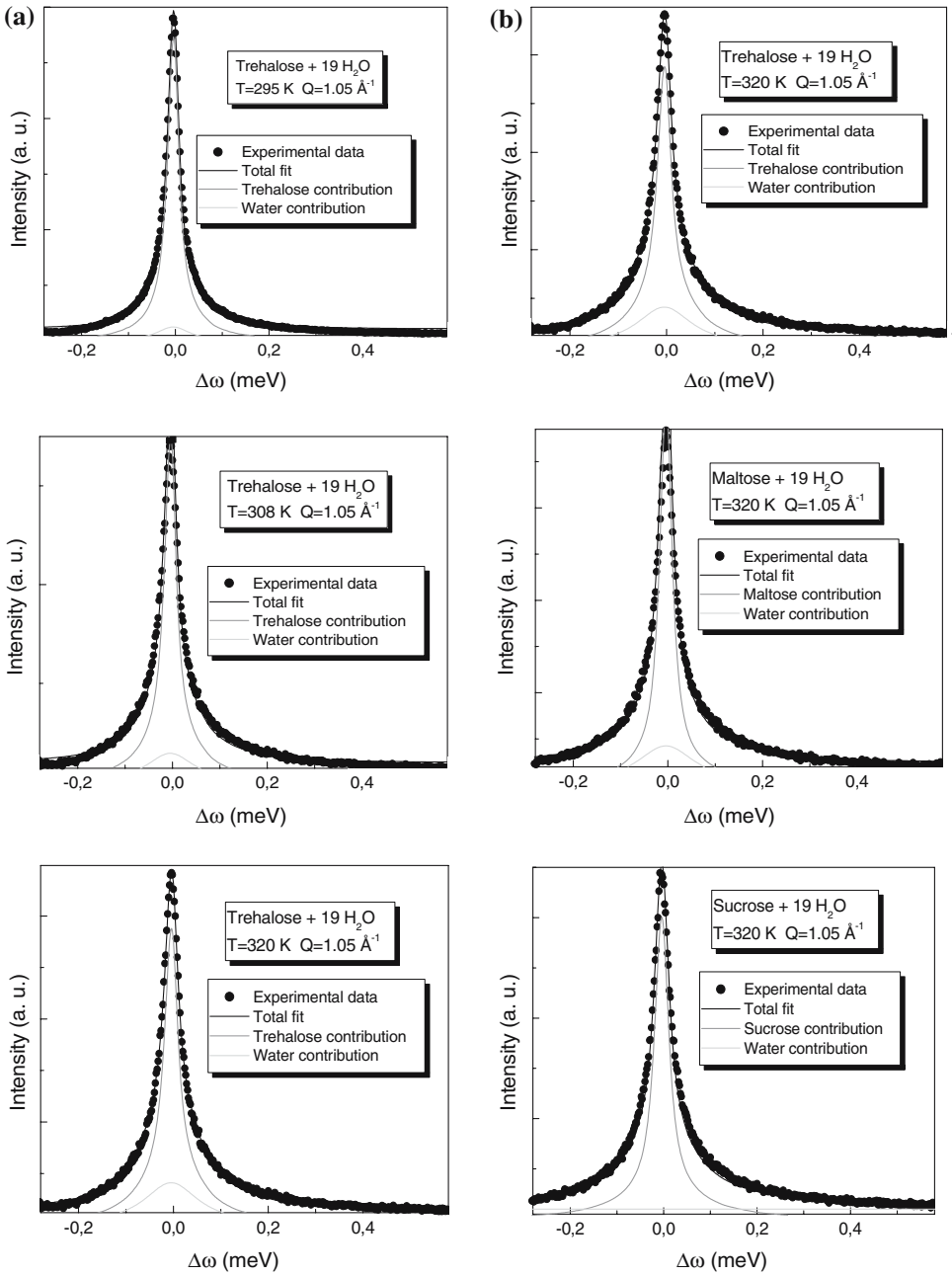

Fig. 2 Best fit of hydrogenated aqueous solutions of **a** trehalose for three temperature values and **b** of trehalose, maltose and sucrose at $T = 320$ K. The total fit (*black line*) is composed by the disaccharide contribution (*grey line*), resulting by the fit of disaccharide/D_2O spectra, and the water contribution (*light grey line*), where only the translational part is present

Table 1 Parameter values for trehalose, maltose and sucrose mixtures at $T = 320$ K as obtained by QENS and NSE data fitting

	f_{hydr}	D (cm^2/s)	τ (ps)	$\langle l^2\rangle^{1/2}$ (Å)	Γ_2 (μeV)	τ_w (ns)	β
Trehalose	0.328	8.50×10^{-7}	18.3	1.00	59	0.16	0.72
Maltose	0.348	1.00×10^{-6}	14.3	1.00	75		
Sucrose	0.378	1.23×10^{-6}	13.8	1.00	89	0.14	0.70
Water in trehalose		8.31×10^{-6}	3.7	1.35		0.11	0.80
Water in maltose		8.46×10^{-6}	3.4	1.31			
Water in sucrose		8.60×10^{-6}	3.0	1.24		0.10	0.78

The linewidth Γ_2 of the rotational contribution of disaccharides results nearly constant as a function of Q. For trehalose aqueous solutions, we obtain the values of $\Gamma_2 \sim 30$, $\Gamma_2 \sim 41$, $\Gamma_2 \sim 53$ and $\Gamma_2 \sim 59$ μeV for $T = 283$, 295, 308, 320 K, occurring on a time scale of 21.9, 16.1, 12.4 and 11.2 ps, respectively.

By the behaviour of the translational linewidth of water, we conclude that the water dynamics in

Table 2 Parameter values for trehalose and water in trehalose mixtures for different temperature values as obtained by QENS data fitting

T (K)	f_{hyd}	D (cm^2/s)	τ (ps)	$\langle l^2\rangle^{1/2}$ (Å)	Γ_2 (μeV)	D (cm^2/s), water in trehalose	τ (ps), water in trehalose
283	0.032	2.83×10^{-7}	24.7	0.64	30	2.04×10^{-6}	5.5
295	0.108	3.82×10^{-7}	20.6	0.68	41	3.47×10^{-6}	4.3
308	0.223	5.35×10^{-7}	19.1	0.78	53	5.46×10^{-6}	4.1
320	0.328	8.50×10^{-7}	18.3	1.00	59	8.31×10^{-6}	3.7

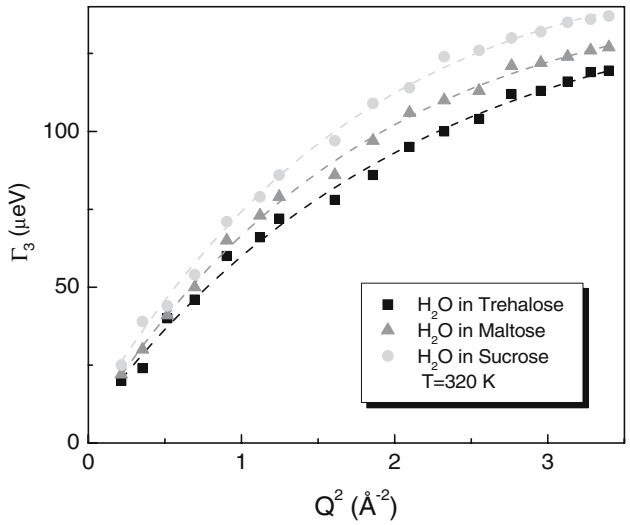

Fig. 3 Linewidth of the translational contribution as a function of Q^2 for water in trehalose, maltose and sucrose aqueous solutions at T = 320 K

trehalose solutions for T = 283, 295, 308, 320 K resembles that of water at ~256, ~261, ~263 and ~268 K, indicating that the water has a diffusive behaviour strongly triggered by the trehalose molecules and suffers of a noticeable frozen effect, whereas it is similar to that of water at ~271 K in the case of maltose solution and at ~277 K in the case of sucrose solution. Analogously to the trehalose aqueous solutions, all the disaccharides show a slowing down effect on the water dynamics, which is stronger for trehalose than the other disaccharides.

Neutron spin echo

In Fig. 5 a comparison between the NSE intensities of hydrogenated aqueous solutions of trehalose for three temperature values and of two disaccharides is shown.

The fitting procedure has been carried out according to the Kohlrausch–Williams–Watts (KWW) function:

$$S_{self}(Q,t) = f(Q)\exp\left[-\left(\frac{t}{\tau_w}\right)^{\beta}\right] + \mathrm{BKG}, \qquad (3)$$

where β is the stretching parameter describing the shape of the relaxation function ($0 < \beta \leq 1$) and τ_w is the

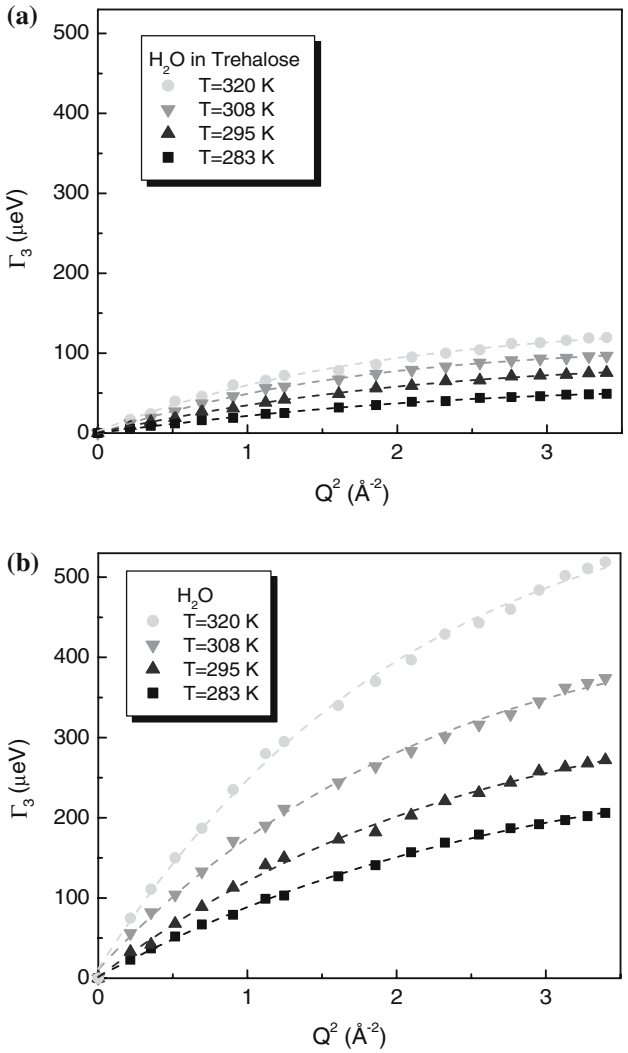

Fig. 4 Linewidth of the translational contribution as a function of Q^2 for **a** water in trehalose aqueous solutions at four temperature values, **b** for pure water at the same temperature values

relaxation time. The fitting lines in Fig. 5 are obtained according to Eq. 3. The fitting procedure furnishes for the relaxation time τ_w the values of τ_w = 0.16 and τ_w = 0.14 ns for trehalose/D_2O and sucrose/D_2O solutions, respectively, and for the stretching parameter β the values of β = 0.72 and β = 0.70 for trehalose/D_2O and sucrose/D_2O solutions, respectively, whereas for trehalose/H_2O solution we obtain the values of

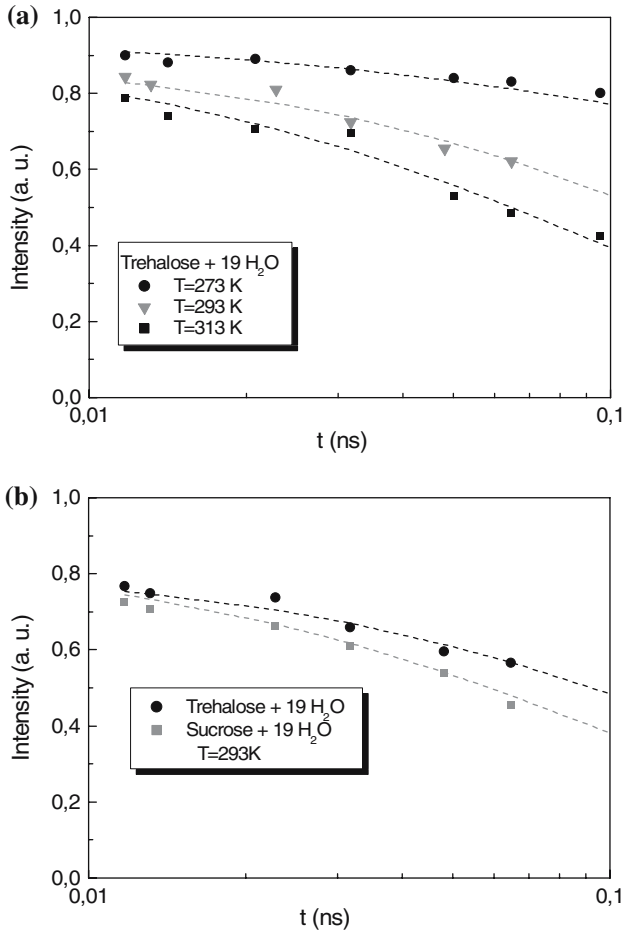

Fig. 5 Comparison among the NSE intensities of hydrogenated aqueous solutions of **a** trehalose for three temperature values (T = 273, 293, 313 K) and of **b** trehalose and sucrose at the same temperature

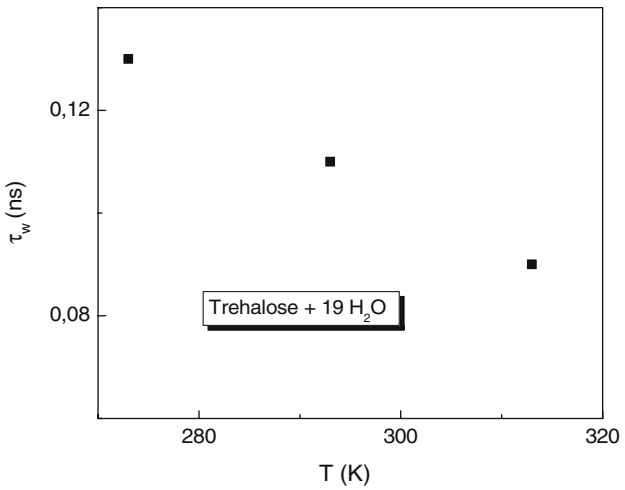

Fig. 6 Temperature dependence of the relaxation time τ_w for trehalose/H_2O solutions

τ_w = 0.11 ns and β = 0.80 and for sucrose/H_2O solution the values of τ_w = 0.10 ns and β = 0.78 at T = 293 K. In Fig. 6, the temperature dependence of τ_w for the trehalose solutions is shown. These results complement those obtained by QENS, confirming a slower dynamics in the trehalose/H_2O solutions.

Discussion and conclusions

In conclusion, the QENS and NSE findings reported in the present paper furnish a clear response about the dynamics slowing down of disaccharide/water solutions. The diffusive dynamics of both disaccharide and water in the solutions has been characterised by QENS measurements, whereas NSE allows for the dynamical properties of the disaccharide/water solution as a whole to be characterised. The diffusion coefficients extracted by the analysis procedure are in good agreement with those obtained by NMR measurements (Branca et al. 1999b) as well as the general trend; i.e. trehalose diffusion was slower than maltose and sucrose. In all the cases, it is clearly pointed out that the dynamics of disaccharide and water is strongly coupled, with a higher coupling strength in the case of trehalose water solution.

It is interesting to notice that DMSO (dimethyl sulfoxide), which is also a known cryoprotectant, has been shown by QENS to have a dynamical behaviour described by a model of jump translational diffusion convoluted with isotropic rotational diffusion and the similar disaccharide capability to slow down the water dynamics (Bordallo et al. 2004).

All the studies performed on disaccharides solutions clearly support the hypothesis of a privileged water–disaccharide interaction. These results, dealing with dynamical properties, complete the structural information obtained by Raman spectroscopy (Branca et al. 1999a), suggesting that trehalose binds more strongly water molecules, so disrupting their tetrahedral configuration arrangements and reducing more effectively the amount of those H-bond configurations, which, decreasing temperature, promote the formation of ice.

Raman spectra were also confirmed by neutron diffraction results (Branca et al. 2002), showing that the peaks of the partial distribution functions associated with the tetrahedral coordination of water molecules are markedly deformed. On the other hand, ultrasonic findings (Branca et al. 1999b) show that trehalose presents the highest values of the solute–solvent interaction strength and hydration number. Furthermore, the amount of water for each trehalose molecule decreases by increasing temperature

approaching an almost constant value at the highest temperatures. This trend can be ascribed to the enhanced thermal motions at higher temperatures which lead to lower residence times of water molecules in the disaccharide hydration shells. Therefore, the average number of water molecules moving together with trehalose will be lower, decreasing the hydration number. However, it should be also pointed out that, by increasing temperature, owing to the rupture of a certain fraction of hydrogen bonds in water, the number of water molecules available for bonding with trehalose also increases. As a consequence, the hydration number is determined by these two opposing effects. At temperatures higher than about 70°C, these effects compensate and the hydration number tends to a constant value. These results indicate that trehalose strongly binds a greater number of water molecules than the other disaccharides.

Elastic neutron scattering experiments on trehalose, maltose, sucrose/H_2O mixtures as a function of concentration, temperature and exchanged wave vector (Magazù et al. 2004) showed that the decrease in the elastic intensity above the dynamical transition temperature is very less marked in the case of trehalose/water mixture than for the other disaccharide/water mixtures (Magazù et al. 2004). This circumstance indicates that trehalose shows a larger structural resistance to temperature changes and a higher ''rigidity'' in comparison with the sucrose/H_2O mixture which shows the ''softest'' character.

These findings have been confirmed by a combined pulsed-gradient spin-echo NMR and MD simulation study performed by De Pablo and co-workers (Ekdawi et al. 2003), aimed to compare the diffusive behaviour of trehalose and sucrose in aqueous solutions. They have found that sucrose and trehalose exhibit different mobilities when dissolved in water. The higher mobility observed in the sucrose system is attributed to its small hydration number and more compact shape. Interestingly at concentrations below 72 wt%, the diffusion of water appears to be largely independent of the type of sugar. In 80 wt% disaccharide solutions, water diffuses twice as fast in sucrose solutions than in trehalose solutions. In addition, the mechanism of water diffusion changes from a continuous trajectory to a hopping mechanism with increasing disaccharide concentration.

Another confirmation has been furnished by MD simulation studies performed by Descamps and coworkers (Bordat et al. 2004). In this work, several analysis tools such as the size of H-bonded water clusters, the Voronoi tessellation, the orientational order parameter or the dynamical structure factor have been combined to differentiate the actions of trehalose, sucrose and maltose. In particular, the distributions of Voronoi volumes, which provide useful information about the local molecular environment or the local-free volume, emphasise a dilation and a distortion of the hydrogen-bonded network of water from its tetrahedrality. Concerning with water dynamics, the relaxation times of water in the presence of disaccharides result 1.2 to 10 times longer than the ones of pure water, depending on temperature, and the trehalose/water solution shows the longest relaxation times, revealing that the dynamics of trehalose molecules is imposed to a larger number of water molecules.

The physical picture obtained from the studies performed on these systems shows that the highest bioprotectant effectiveness of trehalose in comparison with the other disaccharides is due to the combined effect of different co-factors. What emerges is that the biological action of disaccharides can be explained by the strong interaction with water molecules. This capability implies that disaccharides and in particular trehalose promote an extensive layer of structured water around its neighbourhood, which affects the tetrahedral H-bond network of pure water, providing a structure whose spatial positions and orientations are not compatible with those of ice, as pointed out by Raman and INS findings (Branca et al. 1999a; Magazù et al. 2005).

By the ENS results (Magazù et al. 2004) it has been shown that the glass-forming properties of disaccharides play an important role in the bioprotection mechanisms. We concluded that trehalose, besides modifying significantly the structural and dynamical properties of water, forms with H_2O a less fragile entity able to encapsulate biological structures and to protect them in a more rigid environment. The trehalose–water system shows a macroscopic amorphous conformation in which nanocrystallised domains are mixed with remaining liquid, so revealing a ''cryptocrystalline'' character. The INS findings (Magazù et al. 2005) help to give an explanation to the previous results, because the locally more ordered structure of trehalose, or as it can be conveniently said its ''cryptocrystallinity'', can justify the higher rigidity of this system.

By a biological point of view, the superior cryo- and cryptobiotic action of trehalose can therefore find a full elucidation, since trehalose shows a more marked perturbing effect and a more evident ''cryptocrystalline'' character, which make it so effective as cryo- and cryptoprotectant, respectively. All these results contribute to clarify the physical processes underlying the biological action of trehalose, which evidently gives rise to a strong interaction with water and not directly with the biostructures, as hypothesised by Crowe et al. (1998).

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