Study of solvent–protein coupling effects by neutron scattering

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Abstract The present work aims to characterize the dynamical behavior of proteins immersed in bio-preserving liquids and glasses. For this purpose, the protein dUTPase was chosen, while the selected solvents were glycerol, a triol, and some homologous disaccharides, i.e., trehalose, maltose, and sucrose, which are known to be very effective bio-preserving agents. The results highlight that the disaccharides show a slowing down effect on the water dynamics, which is stronger for trehalose than in the case of the other disaccharides. Furthermore, a characterization of the medium which hosts the protein is performed by using an operative definition of fragility based on the mean square displacement extracted by elastic incoherent neutron scattering, which is directly connected to Angell's kinetic fragility based on the viscosity. Finally, a study of the dynamics of the protein sequestered within the solvents is performed. The result shows that the protein dynamics is coupled with that of the surrounding matrix.

Keywords Quasi-elastic neutron scattering · Elastic neutron scattering · Protein · Bioprotection

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1 Introduction

The clarification of the molecular mechanisms underlying protein stability and denaturation constitutes a central problem in biological physics [1–3]. From the applicative point of view this understanding is important for the development of various applications such as protein-based drugs stabilized by bioprotective host-matrices. In fact, the denaturation rate of biological agents is a determining factor for the shelf life of protein/enzyme-based pharmaceuticals, and, in turn, the dynamics of the proteins has a direct influence on their shelf life.

Recent experimental findings suggest that the characterization at different length and time scales of the protein–host medium system is essential not only for the understanding of the molecular mechanisms determining stability but also for the comprehension of protein and enzyme reactions at a molecular level [4–6].

The coupling between the dynamics of the host medium with that of the protein may explain the bioprotectant function. More specifically, proteins are complex systems which are not to be considered static but as dynamical systems which perform motions to execute their functions [7]. These motions actually involve the atoms not just of the biological structure itself but also of the surrounding medium with which a coupling exists. Therefore, depending on the circumstances, the protein can be considered “slaved” or “sequestered” by the host medium which can be able to suppress its dynamics, so resulting in a retardation of denaturation processes or a slowing down of biological function [4–12].

The employment of a disaccharide glassy matrix for the preservation of biomolecules is well documented, even if the underlying molecular mechanisms that pilot protection effectiveness are not fully understood yet [13, 14]. Previous analyses of the mean square displacement obtained on the three bioprotectant homologous disaccharides suggest that trehalose is characterized in respect to maltose and sucrose by a lower fragility, namely by a higher structural resistance to temperature changes in approaching the glass transition temperature [15]. These findings indicate a correlation between fragility degree and bioprotection effectiveness and could give also an explanation for the better preservation provided by several bioprotectants.

The fragility concept, introduced by Angell to address the problem of viscous slowdown [16, 17] describes, in its kinetic version, how fast the structural relaxation time (τ_α) increases with decreasing temperature on approaching T_g , defined as the temperature where τ_α becomes equal to 10^2 s. In this case the evaluated quantity refers to a limit value at a given temperature and not to a global behavior and, hence, has a local character. “Strong” systems show a T dependence of $\tau_\alpha(T)$, that can be described by an Arrhenius law $\tau_\alpha(T) = \tau_\infty \exp(\Delta/k_B T)$, whereas “fragile” systems show, close to T_g , a much faster T dependence of the relaxation time, which is also markedly non-Arrhenius, and hence a T dependence of the activation energy Δ [16, 17]. In Angell’s strong-fragile liquid classification an extreme is therefore represented by conformity to the Arrhenius equation:

$$\eta = \eta_0 \exp\left(\frac{E_a}{k_B T}\right) \quad (1)$$

with E_a is the activation energy and k_B is the Boltzmann constant, while the other extreme is represented by a marked departure from Arrhenius behavior which at the limit becomes

a first-order transition from fluid to glass, unless a first-order fragile-liquid-to-strong-liquid transition occurs [16, 17].

It is well known that the behavioral properties of a glass-forming system can be described in terms of the $(3N + 1)$ -dimensional potential energy landscape (PEL) in the configurational space [18, 19]. It is important to stress that, while the PEL does not depend on T , the exploration of the PEL (i.e., which parts of the surface are explored) is strongly T dependent. The system explores an energy hyper-surface, whose complexity can be correlated with the density of the minima of the hyper-surface, characterized by a degeneracy $\propto \Delta C_p(T_g)$ and a distribution of the barrier heights between minima $\Delta\mu$. The number and energy depth of the local minima are indeed the basic ingredients of the PEL thermodynamic formalism [18–20]. Stillinger and Weber [20] provided a formally exact partitioning of the configurational space as a sum of distinct basins, associating with each local minimum of the potential energy surface (called an inherent structure) all points in configuration space connected to the minimum by the steepest descent path. This set of points is called a basin. The landscapes of strong liquids are supposed to have a “uniform” roughness, while a multi-length-scale arrangement of the minima, with the introduction of *meta basins*, are expected to characterize the PEL of fragile liquids. Fragile liquids would have high density of minima per unit energy and surfaces with few minima generate strong liquids. In other words, the fragility of a system $\left(\propto \frac{\Delta C_p(T_g)}{\Delta\mu}\right)$, i.e., its structural sensitivity to temperature changes in approaching the glass transition, is determined by these topological features of an energy hyper-surface [18–20].

As a matter of fact, as shown by Scopigno et al. [21] the knowledge of number, energy depth, and shape of the basins of the potential energy landscape may not be sufficient for predicting fragility. It is possible, and actually most likely, that fragility could be derived from other features of the PEL as, for example the minimum-to-minimum barrier heights. Another important property of the dynamics of supercooled liquids is the development of a separation of timescales. The short microscopic dynamics is not much affected by supercooling, while the structural relaxation time (the so-called α relaxation, describing the long-time decay of correlation functions) is significantly changed by T (according to the fragile/strong character of the system). Goldstein [22] interpreted this separation of timescales, suggesting that molecular motions in supercooled liquids consist of vibrations about deep potential energy minima and of visitations of different such minima, stimulating an interest in the study of the properties of the potential energy landscape sampled in supercooled states [22].

An Arrhenius behavior of viscosity in the T_g -scaled plot and a small heat capacity variation $\Delta C_p(T_g)$ characterize the strongest systems, whereas a large departure from the Arrhenius law and a large heat capacity variation $\Delta C_p(T_g)$ characterize the most fragile ones. Between these two limiting cases, intermediate behaviors can be interpreted in terms of different kinetic (η) and thermodynamic contributions ($\Delta C_p(T_g)$): thermodynamically strong (small $\Delta C_p(T_g)$) and kinetically fragile systems (non-Arrhenius η behavior) are characterized by a low minima density of the potential energy hyper-surface and low energy barriers. Conversely, thermodynamically fragile (large $\Delta C_p(T_g)$) and kinetically strong systems (Arrhenius η behavior) are characterized by a high hyper-surface configurational degeneracy and high barrier heights [18, 19].

Hydrogen-bonded systems, such as trehalose, maltose, sucrose, and glycerol, are considered as anomalous due to the large change in heat capacity which they experience at the glass transition, and they occupy an intermediate position in the Angell classification.

On the other hand, generally speaking, proteins in contrast to glass forming liquids have a quite different behavior:

1. Liquids exhibit short-range order (i.e., long-range disorder) and long-range (isotropic) translational diffusion; significant motions occur if the molecules rearrange their positions in a concerted and cooperative way
2. Proteins, instead, are long-range ordered and show short-range diffusion

Water acts as a plasticizer and lubricant to protein motions, expanding the accessible conformational space and decreasing both barrier heights and friction. When the protein is nominally dry, the simplified three-dimensional potential energy funnel, hierarchically organized in tiers with well-separated minima, may trap the protein in inactive conformations on the folding pathway; localized motions, which involve smaller volume changes (than translation), still may occur. In hydrated conditions, the energy landscape is considerably smoothed with lowered energy barriers and lubricated movements which allow the protein to attain the active minimum energy conformation in a straightforward and rapid manner.

In the present work, quasi-elastic neutron scattering (QENS) data on aqueous solutions of disaccharides as a function of temperature are presented. Then, the fragility of some glass forming liquids which show a bioprotective effectiveness is evaluated by following two procedures which make use of the mean square displacement evaluated by elastic incoherent neutron scattering (EINS). Finally, some experimental results highlighting protein–host solvent dynamics are presented.

2 Experimental methods

Ultra-pure powdered trehalose, maltose, sucrose, glycerol, D₂O, and H₂O, purchased from Aldrich-Chemie, were used for the experiments. Hydrogenated mixtures of trehalose, maltose, and sucrose (C₁₂H₂₂O₁₁) in H₂O and of partially deuterated trehalose, maltose, and sucrose (C₁₂H₁₄D₈O₁₁) in D₂O at weight fraction values corresponding to 19 water (H₂O and D₂O) molecules for each disaccharide molecule were prepared by dilution. All the three disaccharides possess hydrogen atoms, belonging to the OH groups, which exchange easily with the deuterium atoms of heavy water. Therefore, to focus our attention on the disaccharide dynamics in D₂O, the exchangeable atoms were substituted with deuterium before preparing the solutions. In order to obtain partially deuterated samples, the disaccharides were first dissolved in pure D₂O at a concentration of ~40% by weight, to exchange the eight labile hydrogen atoms of the, disaccharides and the solutions were, subsequently, lyophilized. The procedure was repeated in order to allow the exchange of all the exchangeable hydrogens. The solution samples were obtained by dissolution of the D₂O-exchanged lyophilized disaccharides in pure D₂O. In the deuterated solutions (at the investigated concentration) the coherent contribution to the total scattering cross-section is ~5%. In the case of QENS in protonated samples, attention is focused 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 $\sigma_i/\sigma_s = 0.94$.

The gene encoding for the dUTPase was amplified by PCR from the genomic DNA of *Mycobacterium tuberculosis* H₃₇Rv strain using the forward primer 5'-GGGAATTCCATATGTCGACCACTCTGGCGATCGTCCGC-3' and the reverse primer 5'-CGCGGATCCTCACAACTCGCATGTCCGCCGAGGA-3', the former also containing

an NdeI restriction site (CATAT), while the latter containing a BamHI restriction site (GGATCC), and both containing digestion helping sequences (GGGAATTC and CGC for the forward and the reverse primers, respectively). The amplified dUTPase gene was digested with NdeI/BamHI. An additional fragment was cut out from the plasmid pET-3a (Novagen) with BamHI/PstI. The digested dUTPase gene sequence then, together with this additional fragment, was ligated into a plasmid pET-3a predigested with NdeI/PstI. The resulting DUT-pET-3a plasmid was digested with NdeI/PstI and was ligated into a plasmid pET-19b predigested with NdeI/PstI. The resulting new plasmid harboring the His-tagged version of *M. tuberculosis* dUTPase was transformed into an *Escherichia coli* BL21(DE3) (pLysS) strain for protein expression. The enzyme was expressed as described by Barabas and coworkers [23] and purified according to the Novagen protocol.

QENS measurements were performed in a temperature range of 273–323 K by 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). The high resolution combined with the most powerful pulsed neutron source in the world make IRIS the best instrument to study diffusive dynamics [24]. We measured 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 $1.8 \text{ \AA}^{-1}$ (momentum transfer) with a mean energy resolution of $\Gamma = 8 \text{ } \mu\text{eV}$ (FWHM). The raw spectra were corrected and normalized using the standard GENIE procedures and the IRIS data analysis package [25].

EINS experiments were performed on pure disaccharides and trehalose, maltose, and sucrose/H₂O mixtures in a temperature range of 20–310 K by using the IN13 spectrometer at the Institute Laue Langevin (ILL) in Grenoble (France) with a Q range of 0.28 – $4.27 \text{ \AA}^{-1}$ and an energy resolution of $\sim 8 \text{ } \mu\text{eV}$ (FWHM).

3 Results and discussion

QENS measurements, performed both in partially deuterated disaccharides in D₂O and in disaccharides in H₂O, allow to separate the solute dynamics from that of the solvent. We performed a fitting procedure on deuterated and non-deuterated aqueous solutions of disaccharides according to the fitting function:

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

where the first two terms refer to the translational and rotational contributions of hydrated disaccharide and the third one refers to hydration water. The random jump diffusion (RJD) model [26], expressed by $\Gamma_1(Q) = D_s Q^2 / (1 + D_s Q^2 \tau)$, well describes the behavior of the translational linewidth for the investigated solutions, as is evident in Fig. 1, furnishing the values of the self-diffusion coefficient D_s of the molecule and for 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 $D_s = 2.83 \times 10^{-7} \text{ cm}^2/\text{s}$ and $\tau = 24.7 \text{ ps}$, $D_s = 3.82 \times 10^{-7} \text{ cm}^2/\text{s}$ and $\tau = 20.6 \text{ ps}$, $D_s = 5.35 \times 10^{-7} \text{ cm}^2/\text{s}$ and $\tau = 19.1 \text{ ps}$, and $D_s = 8.50 \times 10^{-7} \text{ cm}^2/\text{s}$ and $\tau = 18.3 \text{ ps}$ for $T = 283, 295, 308$, and 320 K , respectively. From the relation $\langle l^2 \rangle = 6D\tau$ we obtain the values $\langle l^2 \rangle^{1/2} = 0.64, 0.68, 0.78$, and $1.00 \text{ \AA}$ $T = 283, 295, 308$, and 320 K , respectively.

Fig. 1 Linewidth of the translational contribution for H_2O in trehalose aqueous solutions at different temperature values. In the *insert* the linewidth of the translational contribution for pure water is shown. The *dashed lines* are the results of the fitting procedure performed according to the RJD model [26]

By the behavior of the translational linewidth of water, we conclude that the water dynamics in trehalose solutions for $T = 283, 295, 308$, and 320 K resembles that of water at $\sim 256, \sim 261, \sim 263$, and ~ 268 K, respectively, indicating that the water has a diffusive behavior strongly triggered by the trehalose molecules and suffers of a noticeable freezing 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. By the obtained findings, it is evident that all the disaccharides show a slowing down effect on the water dynamics and that this effect is stronger for trehalose than the other disaccharides.

Let us consider the transport properties of the host medium. Figure 2 shows the viscosity of glycerol as a function of temperature. Experimental data have been taken by Martinez and coworkers [16]. In the procedure we are proposing, an important parameter is the Stickel temperature T_s , which marks both a change in the susceptibility spectra of glass

Fig. 2 Viscosity of glycerol as a function of temperature. Experimental data have been taken by Martinez and coworkers [16]. The *dashed lines* are the asymptotes for low and high viscous regions. The Stickel temperature T_s is indicated [29]

formers [27] and departure of the relaxation times from the Vogel–Tamman–Fulcher (VTF) function [28]:

$$\eta(T) = \eta_0 \exp\left(\frac{DT_0}{T - T_0}\right) \quad (3)$$

where η_0 , D , and T_0 are system-dependent parameters. In this equation the D parameter controls how closely the system obeys the Arrhenius law ($D = \infty$) and T_0 , the VTF temperature, is the result of an extrapolation of experimental data.

In the present model the Stickel temperature is evaluated considering the low viscosity region, chosen in the 192–205 K range, and the high viscosity region, chosen in the 307–320 K range. The following step is to linearly fit the two different regions by two asymptotes, whose equations are:

$$\log^{\text{low}} \eta = \log^{\text{low}} \eta_0 + b^{(1)} \cdot (1/T) \quad (4.1)$$

$$\log^{\text{high}} \eta = \log^{\text{high}} \eta_0 + b^{(h)} \cdot (1/T) \quad (4.2)$$

for low and high viscous regions, respectively, which in the case of glycerol have the form:

$$\log^{\text{low}} \eta = -8.68 + 2,547.68 \cdot (1/T) \quad (5.1)$$

$$\log^{\text{high}} \eta = -32.28 + 8,091.40 \cdot (1/T) \quad (5.2)$$

The Stickel temperature is evaluated by identifying the temperature value where the two asymptotes intersect. In the case of glycerol viscosity, the obtained value for the Stickel temperature is $T_s = 234.74$ K.

By the asymptotes (5.1) and (5.2) the following set of straight lines has been created:

$$\log \eta = [\log^{\text{high}} \eta_0 + b^{(h)} \cdot (1/T)] (1 - \psi) + [\log^{\text{low}} \eta_0 + b^{(1)} \cdot (1/T)] \psi, \quad (6)$$

namely

$$\log \eta = [-32.28 + 8,091.40 \cdot (1/T)] \cdot (1 - \psi) + [-8.68 + 2,547.68 \cdot (1/T)] \cdot \psi \quad (7)$$

where ψ fulfils the requirements

$$\psi(T) = \begin{cases} 1 & \text{in high viscous states } (T > T_L) \\ 0 & \text{in low viscous states } (T < T_s) \end{cases} \quad (8)$$

where T_L is the temperature below which the temperature dependence of the density of the supercooled liquid becomes different from linear, typical for the vicinity of the melting point. The ψ parameter is proportional to the number of the degrees of freedom responsible for the formation of highly viscous states and manifested in α relaxation.

By using the following model function for the fitting [29]:

$$\psi(T) = \begin{cases} 1 - \exp\left[-\left(\frac{T - T_\psi}{\Delta_\psi}\right)^n\right] & T > T_\psi \\ 0 & T < T_\psi \end{cases} \quad (9)$$

we obtain the result shown in Fig. 3 and with fit parameters $T_\psi = 165.67$ K and $\Delta_\psi = 82.67$ K. The parameters T_ψ and Δ_ψ define the temperature and the temperature

Fig. 3 Temperature dependence of the ψ function. The ψ parameter is proportional to the number of the degrees of freedom responsible for the formation of highly viscous states and manifested in α relaxation. *White data* indicate the $\psi(T)$ values between T_g and T_m , the *grey data* indicate the $\psi(T)$ values below T_g and the *black data* indicate the $\psi(T)$ values above T_m

interval in which the function $\psi(T)$ undergoes the most significant change, as shown in Fig. 4a and b.

Once the ψ function is determined, it will be possible to express the mean square displacement as a function of ψ , $\langle u^2 \rangle = \langle (u')^2 \rangle (1 - \psi) + \langle (u'')^2 \rangle \psi + \dots$, as well to connect the fragility M_S of the system to the distance $b^{(i)}$ between the Stickel point and the experimental data via the relation $M_S = b^{(i)}$ [29]. This distance, in fact, takes larger and larger values for substances that are more and more “fragile”, according to Angell’s classification [18, 19].

In Fig. 5, a comparison among the EINS spectra of dry trehalose and sucrose powders and their water mixtures is shown. It is evident that a dynamical transition occurs for trehalose and sucrose and for their mixtures. The decrease in the elastic intensity above the dynamical transition temperature can be attributed to the excitation of new degrees of freedom [1], especially at low Q , and is very less marked in the case of trehalose and its mixtures than for sucrose and its mixtures. This circumstance clearly indicates that trehalose shows a higher “rigidity” in comparison with sucrose.

Figure 6 shows the mean square displacement as a function of temperature for glycerol [30] as determined by EINS measurements performed by using the backscattering spectrometer IN13 at the Institute Laue Langevin.

The interpretative model to describe the observed behavior can be visualized as follows for the elementary flow process (the α -relaxation): an atom jumps in the fast processes (β -relaxation motions) with a Gaussian probability distribution characterized by a mean-square amplitude $\langle u^2 \rangle_{\text{loc}}$, defined as the difference between the mean square displacement of the ordered and disordered phase [15]:

$$\langle u^2 \rangle_{\text{loc}} = \langle u^2 \rangle_{\text{anharm}} - \langle u^2 \rangle_{\text{harm}}. \quad (10)$$

If the amplitude of the fast motion exceeds a critical displacement u_0 , a local structural reconfiguration (the α -relaxation) takes place. Assuming the time scale of the fast motion to be independent of temperature, the waiting time for the occurrence of an α -process at a

Fig. 4 Temperature dependence of the parameter ψ **a** as a function of T_ψ and **b** as a function of Δ_ψ

given atom is proportional to the probability of finding the atom outside of the sphere with radius u_0 . In this picture, one obtains for the viscosity [15]:

$$\eta = \eta_0 \exp \left[u_0^2 / \langle u^2 \rangle_{loc} \right]. \quad (11)$$

In Fig. 7 the linear dependence between the logarithm of the viscosity and the local mean square displacement for glycerol is shown.

We can evaluate the “fragility” degree by elastic neutron scattering measurements as follows:

$$M_M = \left. \frac{d(u_0^2 / \langle u^2 \rangle_{loc})}{d(T_g/T)} \right|_{T=T_g^+} \quad (12)$$

It has been demonstrated that a linear relationship between M_M and m exists [15].

On the basis of the relationships introduced, we will try now to get information on the fragility degree of the glassy systems which when host proteins are present show an

Fig. 5 Comparison among the EINS spectra of dry trehalose and sucrose powders and their water mixtures. w and D_w indicate the H_2O and D_2O weight fraction in the disaccharide mixtures, respectively

extraordinary bioprotectant effectiveness. EINS data collected by the IN13 spectrometer allowed us to evaluate the mean square displacement of trehalose, maltose, and sucrose. The mean square displacement has been obtained by employing the approximation:

$$I^{\text{Gaussian}}(Q, t_R) = e^{-\frac{1}{6} Q^2 \langle (\Delta r(t_R))^2 \rangle} \cong 1 - \frac{1}{6} Q^2 \langle (r(0) - r(t_R))^2 \rangle \quad (13)$$

where $\langle (\Delta r(t_R))^2 \rangle$ represents the full amplitude of motion, under the following condition:

$$\langle (\Delta r(t_R))^2 \rangle Q^2 \leq 2. \quad (14)$$

From the temperature behavior of the mean square displacement we have evaluated the fragility parameter following both the procedures, i.e. by the relaxation function approach, which refers to the global behavior of the mean square displacement ($M_S = b^{(i)}$), and by the mean square displacement evaluated locally at the glass transition temperature

Fig. 6 Mean square displacement as a function on temperature for glycerol. The arrow indicates the distance between the experimental data and the Stickel temperature, which is a measure of fragility

Fig. 7 Linear dependence between the logarithm of the viscosity and the local mean square displacement

(Eq. 12). Both the procedures agree in indicating an increasing fragility parameter for trehalose, maltose, and sucrose. Therefore the analysis performed on the three disaccharides clearly shows that trehalose is characterized with respect to maltose and sucrose by a lower fragility, namely by a higher structural resistance to temperature changes as the temperature decreases towards the glass transition values. This correlation between fragility degree and bioprotection also gives an explanation for the better preservation provided by other bioprotectants: to cite an example, sucrose, which, with respect to raffinose, despite its lower T_g , offers a better preservation. Sucrose in fact appears to be less fragile than raffinose.

Now let us analyze the dynamics of dUTPase embedded in the hydrated trehalose matrix. In Fig. 8 we show the linear dependence of $\log \eta$ versus $(\langle u^2 \rangle_{\text{loc}})^{-1}$, where for η values the viscosity of the trehalose/H₂O mixtures were employed and $\langle u^2 \rangle_{\text{loc}}$ is the local mean square displacement of the D₂O-hydrated dUTPase/trehalose (5% by weight of trehalose) system. We can observe that a linear relationship between the solvent, composed of trehalose-H₂O,

Fig. 8 Linear dependence of $\log \eta$ versus $(\langle u^2 \rangle_{\text{loc}})^{-1}$, where η is the viscosity of the trehalose/H₂O mixture, and $\langle u^2 \rangle_{\text{loc}}$ is the local mean square displacement of the hydrated dUTPase/trehalose system

and the mean square displacement of hydrated dUTPase/trehalose system is verified. This result is a signature of a strong coupling between protein and surrounding matrix, showing that a correlation exists between the protein dynamics and the viscosity of surrounding environment.

4 Conclusions

The experimental findings reported in the present paper furnish a clear response about the dynamics of disaccharide/water solutions. The diffusive dynamics of both disaccharide and water in the solutions has been characterized by QENS. It is clearly pointed out that the disaccharides strongly affect the water dynamics, with a higher effect in the case of trehalose-water solutions. Disaccharides, and trehalose to a greater extent, besides imposing an order on the tetrahedral hydrogen bond network of water, significantly slow down the dynamics of water. The increased slowing down effect of the diffusive dynamics observed for trehalose is evidently linked to its extraordinary capability to “switch off” metabolic functions. More specifically, trehalose plays a fundamental role in preserving extremophiles, organisms living in extreme environments [31–33], which have evolved biomolecules (proteins, membranes) and molecular mechanisms that ensure metabolic flux is maintained at conditions that would ordinarily be considered abiological. Life under extreme conditions is limited primarily by the stability of macromolecules and their ability to exert their biochemical functions under nonconventional conditions that normally lead to the collapse of structures or to functional inactivation [34–36]. Trehalose allows to some extremophiles to pass into a state of “suspended life” or “cryptobiosis”, where the metabolic functions are maintained at an undetectable level, and to come back to life when environmental conditions are again favorable.

As far as the host solvent is concerned, the interrelation between the macroscopic and microscopic definitions of the fragility for hydrogen-bonded glass-forming systems such as homologous disaccharides and glycerol is analyzed.

The fragility, as evaluated through both the total temperature behavior of the mean square displacement and the local behavior at the glass transition temperature, for sucrose, maltose, and trehalose confirm that the stronger systems show a better bioprotection effectiveness.

Finally, analysing the dynamics of dUTPase embedded in the hydrated trehalose matrix, a relationship between the solvent, composed by trehalose-H₂O, and the mean square displacement of the hydrated dUTPase/trehalose system is verified. This result indicates a strong coupling between protein and surrounding matrix, showing that a correlation exists between the protein dynamics and the viscosity of surrounding environment.

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