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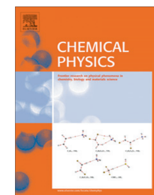

Protein dynamics by neutron scattering: The protein dynamical transition and the fragile-to-strong dynamical crossover in hydrated lysozyme

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ABSTRACT

In this work Elastic Incoherent Neutron Scattering (EINS) results on lysozyme water mixtures in absence and in presence of bioprotectant systems are presented. The EINS data have been collected by using the IN13 and the IN10 spectrometers at the Institut Laue-Langevin (ILL, Grenoble, France) allowing to evaluate the temperature behaviour of the mean square displacement and of the relaxation time for the investigated systems. The obtained experimental findings together with theoretical calculations allow to put into evidence the role played by the spectrometer resolution and to clarify the connexion between the registered protein dynamical transition, the system relaxation time, and the instrumental energy resolution.

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1. Introduction

During the last years considerable efforts have been addressed, through experimental, theoretical and computational studies, to clarify the microscopic nature of the dynamics of biological macromolecules. In this frame, the so-called dynamical transition in protein systems, which, in literature, is referred to as a sharp rise in the Mean Square Displacement (MSD) of hydrated proteins with respect to the dry sample, usually registered in the temperature range $T = 200 \div 240$ K [1–5], is largely debated. In neutron scattering, MSDs are deduced from the measured elastic scattering intensity $S_R(Q,\omega = 0,\Delta\omega)$. The interest in the dynamical transition was stimulated by the fact that the measurable biochemical activity of proteins usually appears around the same temperature range [2,6]. For example, Pieper et al., in the case of hydrated Photosystem (PS) II, have demonstrated that the dynamical transition occurs at the same temperature value at which the electron transport efficiency highly increases [7]; however, several exceptions exist [8–10]: e.g. in Ref. [10] the Authors shown that biological activity is also present in nearly dehydrated conditions.

Since the dynamical transition is absent in dehydrated systems, it has been related to the dynamics of the hydration shell or at least coupled to it. In contrast to bulk water, protein-hydration water can be supercooled down to a glass transition at $T_g = 175$ K [11–13]. Near T_g translational degrees of freedom arrest, this inducing discontinuities in the specific heat and in the thermal expansion coefficient of the hydration water. Because of the dynamic nature of the glass transition, freezing of microscopic degrees of freedom can be observed far above T_g.

Various models have been proposed to explain the mechanisms underlying the observed dynamical transition. Such a transition had been ascribed to a sudden change in an effective elasticity of the protein [3], to motions of specific side groups [6], to a specific fragile-to-strong crossover in dynamics of hydration water [14], to the microscopic manifestation of the glass transition in the hydration shell [15] and to a resolution effects due to a relaxation process that enters the experimentally accessible frequency window [16]. Furthermore, in Ref. [17] the Authors proposed a model in which two different transitions take place at *Tg* and *Td*.

More specifically, performing different quasi-elastic neutron scattering (QENS) experiments, Chen and coworkers in Ref. [14] have evaluated the temperature behaviour of the mean characteristic time of the hydration water in a lysozyme sample (hydrated with $h = 0.3$ g water/g protein); this latter shows a transition from an Arrhenius to a non-Arrhenius behaviour at a temperature value of $T = 220$ K, which the authors put in correspondence with the kink in the extracted temperature MSD behaviour, i.e. with the so-called dynamical transition; this circumstance suggested the

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authors that the kink represents a time-scale independent transition that really occurs in the system due to hydration water. Furthermore, with reference to confined water the authors have shown that a transition from an Arrhenius to a non-Arrhenius behaviour in the mean characteristic time takes place at $T = 224$ K at ambient pressure for confined water in silica nanopores [18,19], at $T = 220$ K in DNA [20] and at $T = 220$ K in RNA [21].

On the other hand, performing different QENS experiments, Doster et al. in Ref. [15] highlighted that the protein dynamical transition is the microscopic manifestation of the glass transition in the hydration shell that occurs at $T = 175$ K [11–13]. Since QENS probes structural relaxation at a time scale of 100 ps, while the calorimetric T_g refers to a time scale of 100 s, it is expected that the dynamical transition is located far above T_g. Therefore the authors conclude that the dynamical transition is a time-scale dependent transition due to the finite spectrometer resolution. These authors also highlighted that in Ref. [14] the employment of a different analysis protocol with respect to the one employed by Chen et al. allows to translate the crossover registered at $T = 220$ K at $T = 175$ K. The origin of the crossover in protein's hydration water at $T = 220$ K was also ruled out in Ref. [22] in which Vogel showed that water performs thermally activated jumps at $T < 200$ K.

Finally investigating the same system of Chen et al. [14] (with $h = 0.4$) also by dielectric spectroscopy technique, Sokolov and coworkers in Refs. [16,23,24] have found that at $T = 220$ K no change in the system mean characteristic time occurs but a secondary fast process, related to water molecules, appears at temperatures lower than $T = 220$ K, and concluded that the dynamical transition is a mere finite instrumental resolution effects. The mean characteristic time of Chen et al. coincides with the main process for temperature values higher than $T = 220$ K and with the secondary fast relaxation for lower temperature values. Finally, Swenson et al. in Refs. [25,26] showed that in confined water the cooperative α relaxation vanishes at about $T = 200$ K implying that, above it, a merging of the α and β relaxations takes places.

In the present paper we report the results of experiments and of theoretical-numerical calculations performed on lysozyme samples in different environments by using the IN13 and the IN10 spectrometers at the Institut Laue-Langevin (ILL, Grenoble, France); in particular we collected EINS data on the dry and hydrated protein in absence and in presence of a disaccharide, i.e. sucrose, at different instrumental energy resolutions, i.e. of 8 μeV for IN13 and 1 μeV for IN10.

The effect of bioprotectant systems [27–31] on the protein dynamics, by evaluating the behaviour of the elastic scattering intensity and of the extracted MSD as a function of temperature and of the exchanged wavevector Q will be discussed; on this score, due to the dynamics–function relation, the changes induced by the presence of the disaccharide could affect the biological functions of the protein. Furthermore, starting from the connexions between both the measured elastic scattering intensity and the extracted MSD with the employed instrumental energy resolution (on this regard, several contributions are reported in literature during the last years [32–39]), it is shown that the observed protein's dynamical transition does not require necessarily any discontinuous change in the temperature behaviour of the system relaxation time, and hence it is not necessarily connected to a real transition of the system. In particular, the kink in the MSD temperature behaviour appears when the system relaxation time crosses the instrumental resolution characteristic time. Finally, the time-scale independent temperature behaviour of the system relaxation time is evaluated from different neutron spectra obtained at different instrumental energy resolutions and the change from Arrhenius to super-Arrhenius behaviour registered at $T = 220$ K has been related to protein–water coupled motions.

2. Experimental section

Elastic Incoherent Neutron Scattering (EINS) data were collected on lysozyme samples in different environments by using the IN13 and the IN10 spectrometers at the Institut Laue-Langevin (ILL). These spectrometers worked at two different instrumental energy resolutions, i.e. of 8 μeV for IN13 and 1 μeV for IN10. The experimental set up for IN13 was: incident wavelength 2.23 Å; Q-range $0.28 \div 4.27$ Å^{-1}; elastic energy resolution (FWHM) 8 μeV, corresponding to a time of about 270 ps [40,41]. The experimental set up for IN10 was: incident wavelength 6.27 Å; Q-range $0.30 \div 2.00$ Å^{-1}; elastic energy resolution (FWHM) 1 μeV, corresponding to a time of about 2190 ps [40,41]. The relationship between the elastic energy resolution and the corresponding resolution time are extensively reported in Refs. [40,41]. Raw data were corrected for empty cell and normalised by vanadium sample. Measurements were performed for 12 h in the $20\ \mathrm{K} \div 320$ K temperature range on dry and D_2O hydrated lysozyme by IN10 and IN13 spectrometers and on lysozyme/H_2O/sucrose mixture by IN10. More specifically, lysozyme in D_2O at a hydration value of $h = 0.4$, and lysozyme in sucrose/H_2O at a hydration value of $h = 0.4$, h = (g of water + disaccharide)/(g of protein) have been employed. It is believed, in fact, that 0.4 g of water/g of lysozyme is sufficient to cover the protein surface with a single layer of water molecules and to fully activate the protein functionality [42–44].

3. Results and discussion

It is well known that, due to the finite energy instrumental resolution, the experimentally accessible quantity, i.e. the measured scattering law $S_R(Q,\omega,\Delta\omega)$, is the convolution of the scattering law with the instrumental resolution function $R(\omega,\Delta\omega)$ [45,46]. In the case of elastic contribution it corresponds to a time integral of the intermediate scattering function weighted in time by the instrumental resolution function:

$$S_R(Q,\omega=0;\Delta\omega) = S(Q,\omega)\otimes R(\omega;\Delta\omega)\big|_{\omega=0} = \int_{-\infty}^{\infty} I(Q,t)R(t)e^{-i\omega t}dt\bigg|_{\omega=0} = \int_{-\infty}^{\infty} I(Q,t)R(t)dt \qquad (1)$$

In the ideal case in which the resolution function is a Dirac's delta in the ω-space, one obtains that the elastic measured scattering law is the scattering law evaluated at $\omega = 0$.

Fig. 1 shows the comparison between the normalised time behaviour of an intermediate scattering function $I(t;\tau)$ (black dashed line) at a fixed τ value ($\tau = 1.5 \cdot 10^3$ ps) and the resolution function $R(t;\tau_{RES})$ (in red) taken at three different τ_{RES} values ($\tau = 10^5$ ps for simulating the ideal case, $2 \cdot 10^3$ ps and $3 \cdot 10^2$ ps which correspond to the characteristic times of the energy resolution spectrometers IN10 and the IN13 respectively) together with the associated measured elastic scattering functions $S_R(\omega = 0,\Delta\omega)$ (in blue) obtained by Eq. (1). More specifically, in Fig. 1a it is shown how, due to the fact that $\tau \ll \tau_{RES}$, there is no effect of the resolution function on the measured elastic scattering intensity, i.e. $S_R(Q,\omega = 0,\Delta\omega) = S(Q,\omega)$; in such a case the blue area that represents the $S_R(\omega = 0,\Delta\omega)$ coincides with the area under the intermediate scattering function, i.e. $S(\omega = 0)$. In Fig. 1b the case of the IN10 spectrometer is considered; it is shown how, in the measured elastic scattering intensity, the resolution function gives rise to a weighted value of the intermediate scattering function; in this case the measured elastic scattering law $S_R(\omega = 0,\Delta\omega)$ (the blue area) is very close to the area under the intermediate scattering function (black dashed line) and the effects of a finite resolution are quite small. In Fig. 1c the case of the IN13 spectrometer is taken into account; it clearly emerges that the resolution function originates the exclusion of a portion of the intermediate scattering function

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