Coupled relaxations at the protein–water interface in the picosecond time scale

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The spectral behaviour of a protein and its hydration water has been investigated through neutron scattering. The availability of both hydrogenated and perdeuterated samples of maltose-binding protein (MBP) allowed us to directly measure with great accuracy the signal from the protein and the hydration water alone. Both the spectra of the MBP and its hydration water show two distinct relaxations, a behaviour that is reminiscent of glassy systems. The two components have been described using a phenomenological model that includes two Cole–Davidson functions. In MBP and its hydration water, the two relaxations take place with similar average characteristic times of approximately 10 and 0.2 ps. The common time scales of these relaxations suggest that they may be a preferential route to couple the dynamics of the water hydrogen-bond network around the protein surface with that of protein fluctuations.

Keywords: neutron scattering; maltose-binding protein; anomalous diffusion; hydration water

1. INTRODUCTION

The protein–water interface is one of the most intriguing and, at the same time, complex systems in the biophysics and life science fields (Ball 2008). In fact, water plays a major role in affecting both the structure and the dynamics of biomolecules. Water molecules around hydrophobic and hydrophilic sites are important to settle the activity of enzyme proteins and are part of the recognition process by other molecules or proteins (Gregory 1995; Ball 2008). The structural and dynamical features of protein hydration water (water within few Å from the protein surface) are sensibly altered with respect to the bulk, as they are affected by local topography and specific interactions with the protein (Bellissent-Funel *et al.* 1992; Settles & Doster 1996; Mattea *et al.* 2008; Paciaroni *et al.* 2008; Orecchini *et al.* 2009). On the other hand, protein motions also intimately depend on the presence of the solvent surrounding the protein surface as shown by experimental (Paciaroni *et al.* 2002; Cornicchi *et al.* 2005; Weik *et al.* 2005) and theoretical (Vitkup *et al.* 2000; Tarek & Tobias 2002) studies. Despite the huge body of data, there is no exhaustive picture of the dynamical coupling between the protein and its hydration

water. In particular, the nature of this coupling in the *picosecond time region* is not completely unravelled yet, and deserves special interest. It is indeed in this time scale that the relaxation processes of the water hydrogen-bond network are mostly coupled with the protein-surface groups.

In the present work, we investigate the behaviour of picosecond thermal fluctuations of the maltose-binding protein (MBP), a model system often used for biotechnological purposes (Medintz & Deschamps 2006), and of its hydration water. By measuring both the natural-abundance hydrogenated and the perdeuterated protein samples, we gained access to the protein–water interface dynamics with unprecedented accuracy. We found that in MBP and its hydration water, there exist two classes of relaxation motions, with the same characteristic times in the region from tenths to tens of picoseconds.

2. MATERIAL AND METHODS

2.1. Sample preparation

MBP is a two-domain protein, responsible for maltose uptake, that plays a major role in the signal transduction cascade that leads to chemotaxis. The two domains of the protein are of roughly equal size and are connected via a short helix and a two-stranded sheet (Spurlino *et al.* 1991). MBP in its hydrogenated

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(MBP(H)) and perdeuterated (MBP(D)) form was provided by the ILL-EMBL Deuteration Laboratory (D-Lab) in Grenoble. In the adopted protocol, MBP was produced as a histidine-tagged fusion protein, which allowed its purification by immobilized metal-ions affinity chromatography in a one-step procedure. Both the hydrogenated and the perdeuterated forms of this protein were expressed in high-cell density cultures and purified. Thanks to such a procedure a noticeable amount (approx. 200 mg) of both MBP(H) and MBP(D) was made available. The samples were left for 3 days in solution of, respectively, D₂O and H₂O to exchange most of the labile hydrogen/deuterium atoms. The solutions were dialyzed twice against the buffer, lyophilized and then dried under vacuum in the presence of P₂O₅. The MBP(H) and MBP(D) powder samples were finally hydrated by D₂O and H₂O vapour pressure up to a hydration degree of 0.42*h* and 0.37*h*, respectively (*h* = gm water/gm dry protein), in order to have the same number of water molecules per protein.

2.2. Incoherent neutron scattering

Thermal neutrons are a powerful and widely used probe to directly obtain information on the fast (nano- and picosecond) motions of biological samples. Neutrons exchange with the sample nuclei a momentum $\hbar\mathbf{Q}$ and an amount of energy $E = \hbar\omega$ with a probability given by the dynamical structure factor $S(\mathbf{Q}, \omega)$ (Lovesey 1986). In isotropic samples like the present ones, the dynamical structure factor depends only on the wavevector modulus Q .

Within the incoherent approximation, the signal of the MBP(H)+D₂O sample comes mainly from the non-exchangeable protein hydrogen atoms, while the spectrum of the MBP(D)+H₂O is composed, respectively, of the contribution from the hydration water (approx. 72%) and the exchangeable protein hydrogens (approx. 28%).

In order to extract the contribution coming solely from the protein hydration water, $S_{\text{H}_2\text{O}}(Q, \omega)$, we made the assumption that, in the meV region, the protein exchangeable hydrogen atoms and the non-exchangeable ones have the same vibrational behaviour. On these bases, we may write

$$S_{\text{H}_2\text{O}}(Q, \omega) = S_{\text{DH}}(Q, \omega) - 0.26 \cdot S_{\text{HD}}(Q, \omega), \quad (2.1)$$

where $S_{\text{DH}}(Q, \omega)$ and $S_{\text{HD}}(Q, \omega)$ are the dynamical structure factors of, respectively, MBP(D)+H₂O and MBP(H)+D₂O, and 0.26 is the ratio between the number of exchangeable and non-exchangeable protons.

2.3. Neutron scattering experiment

The incoherent neutron scattering measurements were performed on the high-flux time-of-flight spectrometer IN5 at ILL (Grenoble). The incident wavelengths $\lambda = 5.2$ and $8.5 \text{ \AA}$ were employed, achieving, respectively, the elastic wavevector ranges $0.4 \text{ \AA}^{-1} < Q < 2.2 \text{ \AA}^{-1}$ and $0.3 \text{ \AA}^{-1} < Q < 1.3 \text{ \AA}^{-1}$, and the energy resolutions of 0.11 and 0.2 meV. The samples were placed in a slab aluminium cell, at an angle of 135°

with respect to the incident neutron beam, and measured at 100, 200 and 300 K. The collected data were treated with a standard correction procedure, taking into account empty cell contribution, transmission and non-uniform detector efficiency as a function of the scattering angle. The values of the transmission coefficients were 0.95 and 0.93 for, respectively, MBP(D)-H₂O and MBP(H)-D₂O. Multiple scattering was neglected.

3. RESULTS AND DISCUSSION

A rather concise view of the spectral features shown by the MBP and its hydration water is reported in figure 1. Both systems exhibit a pretty harmonic behaviour at 100 K, with the elastic peak sharply emerging from an about three orders of magnitude smaller inelastic signal, where a rather large bump in the range of few meVs appears. In more detail, this broad inelastic peak is centred at around 4 and 6.5 meV for, respectively, the MBP and its hydration water. The former represents an excess of inelastic scattering over the expected flat Debye level, which should occur if the protein vibrational density of states followed a simple harmonic ω^2 trend. This peak, already revealed in several protein powder samples (Doster *et al.* 1990), is reminiscent of the so-called boson peak found in glasses and amorphous materials (Elliott 1992; Frick & Richter 1993). The similarity between proteins and glasses in the inelastic spectral behaviour should not be surprising because the dynamical behaviour of native proteins shows more general features that can be related to so-called ‘glassy’ dynamics (Iben *et al.* 1989; Angell 1995). The protein–glass analogy, by which the dynamics of a single protein macromolecule is interpreted in terms of that of a many-particle glass-forming system, resides mainly in the existence of a huge number of nearly conformational substates, regulating the kinetic response of the protein (Iben *et al.* 1989) and constituting a potential energy hypersurface similar to that of a many-particle glass-forming system (Angell 1995). The exact dynamics and topology that give rise to this bump in proteins are still under investigation, even though it now seems clear that it arises from harmonic vibrations distributed throughout the system (Tarek & Tobias 2001).

On the other hand, the bump appearing in the spectra of hydration water at approximately 6.5 meV could be explained in terms of the O–O–O bending-like modes occurring in the hydrogen-bond network of supercooled water and amorphous ice (Sakamoto *et al.* 1962; Walrafen *et al.* 1996; Schober *et al.* 1998), and giving rise to the TA1 transverse acoustic dispersion curve of ice crystals (Renker 1971). This peak was also observed in hydration water of myoglobin (Settles & Doster 1996) and in simulations of water-hydrating ribonuclease (Tarek & Tobias 2002).

The overlap of the two bands at 4 and 6.5 meV supports the hypothesis that a large portion of the protein low-frequency vibrational motions are effectively coupled with those of the vicinal water. We expect this coupling to relate the collective hydrogen-bond

Figure 1. Dynamical structure factor at $Q = 1.2 \text{ \AA}^{-1}$ for (a) MBP and (b) hydration water at 100, 200 and 300 K. The spectra at 100 and 200 K have been rescaled by the Bose factor to 300 K. The dotted line is the energy resolution. The $S(Q, E)$ at $Q = 1.2 \text{ \AA}^{-1}$ has been calculated by interpolating the neutron scattering data collected as a function of the scattering angle (Paciaroni *et al.* 1999).

network dynamics of water with the harmonic vibrations of the protein-surface polar groups.

The two bands are also well visible at 200 K, where a small quasi-elastic contribution also appears in the lower energy region, for both the protein and hydration water. In figure 1, the spectra have been rescaled by the Bose factor to the common temperature of 100 K, to directly highlight deviations from the pure harmonic behaviour, ideally well represented by the lowest temperature curve (Cusack & Doster 1990; Paciaroni *et al.* 1999). The quasi-elastic contribution at 200 K marks the onset of the anharmonic dynamics corresponding to the well-known protein dynamical transition, which in globular proteins such as MBP was found to take place just at about this temperature (Paciaroni *et al.* 2008; Wood *et al.* 2008).

At 300 K, the quasi-elastic signal is by far larger than the inelastic bump, thus indicating that large amplitude anharmonic motions dominate the dynamics of both the protein and its hydration water. As the reported dynamical structure factors were normalized to the number of (incoherent) scatterers, it is also possible to quantitatively compare the features of the two systems. In particular, the factor of more than two, by which the quasi-elastic signal of the hydration water is higher than that of the protein, should originate from thermal fluctuations whose amplitude is much higher in the former than in the latter system. The superposition of the high-temperature spectrum with the

Figure 2. Dynamical structure factor at $Q = 1.2 \text{ \AA}^{-1}$ for (a) MBP and (b) hydration water at 100, 300 K and quasi-elastic intensity after subtraction of the vibrational contribution. The spectrum at 100 K has been rescaled by the Bose factor to 300 K.

low-temperature curve above the inelastic bands indicates that above 4 and 6.5 meV, for the protein and the hydration water, respectively, the signal comes from harmonic motions.

To give new insight into the character of the stochastic motions at the origin of the quasi-elastic signal, this contribution was estimated at 300 K by subtracting the purely vibrational part from the total spectrum, within the hypothesis that harmonic and anharmonic motions are statistically independent (Cusack & Doster 1990). In figure 2, we show the spectra measured at 100 and 300 K, collected in the high-energy-resolution configuration, which extends the accessible time range, together with the result of the subtraction of the purely vibrational contribution. The good statistics of the data is witnessed by the quite regular behaviour of the curves, which decrease towards zero just in correspondence to the inelastic bands mentioned above. Such trend is excellently described with a Gaussian curve, which is a quite reasonable functional behaviour in the short time scale, i.e. the so-called ballistic regime. This trend has been already observed in hydrated protein powders (Doster *et al.* 1990; Diehl *et al.* 1997), but never for protein hydration water.

In the past, the quasi-elastic spectrum of protein powders was interpreted by following the formalism of the mode coupling theory (Doster *et al.* 1990). In this theory, developed to elucidate the properties related to the glass transition phenomenon (Gotze 1998), the key role is played by the imaginary part of the dynamic susceptibility, which is related to the dynamic structure

factor via the fluctuation dissipation theorem $\chi''(q, \omega) = \pi S(q, \omega) / (n(\omega) + 1)$ (Lovesey 1986), where $n(\omega)$ is the Bose factor. In this representation, the quasi-elastic scattering is composed of two terms: the fast local motions (β relaxation) of particles caged in a heat bath of nearest neighbours and the slow collective motions (α relaxation) arising from the rearrangement of the cages. To correctly describe the slow relaxation, it is critical to carefully estimate the elastic signal, which is much more intense than the relaxation contributions. Then, we first fitted the low-energy side of the spectra with an elastic peak convoluted with a Lorentzian contribution, representing possible molecular roto-translations motions, together with the slow α component, which we approximated with a Cole–Davidson function $\chi''_{\alpha}(\omega) = \text{Im}\{1/[1 + (i\omega\tau_{\alpha})^{d_{\alpha}}]\}$, often used to represent the α process in liquids and glasses. This procedure allowed us to reasonably estimate the elastic peak, which was then subtracted out of the spectra. In the following, $\chi''(q, \omega)$ has been fitted in the energy range from 0.01 to 5 meV, by representing each component by a Cole–Davidson function

$$\chi''(\omega) = A_{\alpha} \text{Im}\left\{\frac{1}{1 + (i\omega\tau_{\alpha})^{d_{\alpha}}}\right\} + A_{\beta} \text{Im}\left\{\frac{1}{1 + (i\omega\tau_{\beta})^{d_{\beta}}}\right\}. \quad (3.1)$$

In figures 3 and 4, where we report the dynamic susceptibility of MBP and its hydration water at 300 K, it can be seen that the fitting procedure with the two relaxation components describes remarkably well the experimental data. It should be noted that both components have been convoluted with the energy resolution function, which is quite an important step to reliably fit the spectra in the very low-energy range. In addition, the fit also takes into account the ballistic behaviour, which gives rise to the fast Gaussian decrease in the high-energy range.

In table 1, we provide the values of the parameters derived from the fitting procedure. Quite interestingly, the τ_{α} values of MBP and hydration water are rather similar. It is quite difficult to provide a precise figure for the error bars of τ_{α} , as these values come from the very low-energy part of the spectrum, which can be affected by a systematic error because of the subtraction of the elastic peak. Nevertheless, the results reasonably show that in both the protein and the hydration water there is a distribution of fluctuations centred at approximately 10 ps. This characteristic time is consistent with the trend of the average translational relaxation time of lysozyme hydration water (Chen *et al.* 2006). MBP and hydration water also show a quite similar fast component characteristic time $\tau_{\beta} = 0.16 \pm 0.05$ ps. In the case of protein powders, the fast β -like motions have been related to rotation/reorientation of protein side-chains, which escape from the cage formed by their neighbours, while slow α -like relaxations correspond to collective motions involving the rearrangement of large protein domains (Doster *et al.* 1990). The present results indicate that, at room

Figure 3. Dynamical susceptibility of MBP at 300 K and at $Q = 1.2 \text{ \AA}^{-1}$. The continuous line is the fit to equation (3.1), the short- and the long-dashed lines are the corresponding fast and the slow Cole–Davidson components, respectively. The dot-dash line is the fit to equation (3.2).

Figure 4. Dynamical susceptibility of protein hydration water at 300 K and at $Q = 1.2 \text{ \AA}^{-1}$. The continuous line is the fit to equation (3.1), the short- and the long-dashed lines are the corresponding fast and the slow Cole–Davidson components, respectively. The dot-dash line is the fit to equation (3.2).

temperature, the dynamics of hydration water molecules can also be described in terms of two relaxations with characteristic times similar to those of the biomolecule. As this double-decay regime has also been observed for supercooled bulk water alone (Sokolov *et al.* 1995; Sciortino *et al.* 1996), we may speculate that the fast and the slow relaxations we detected are the privileged channels through which the solvent injects its effective supercooled dynamics on the biomolecule. The effective supercooled dynamics of the solvent would be a consequence of the disorder and the dynamical heterogeneity induced by the protein surface on the network of water molecules. Actually, this is another evidence of the strong coupling between the protein and the hydration water dynamics (Paciaroni *et al.* 2008; Orecchini *et al.* 2009).

The two relaxations we mentioned above can be represented in an alternative way as the sum of two power-law components, respectively, also called Von Schweidler and critical decays (Gotze 1998):

$$\chi''(q, \omega) \propto A_{\text{slow}} \omega^{-b} + A_{\text{fast}} \omega^{+a} \quad (3.2)$$

Table 1. Results from the fit of experimental data to equation (3.1), as shown in figures 3 and 4.

	A_α	d_α	τ_α (ps)	A_β	d_β	τ_β (ps)
MBP	40	0.9 ± 0.1	9.2 ± 1.0	50	0.7 ± 0.2	0.16 ± 0.05
H ₂ O	205	0.82 ± 0.05	14.5 ± 1.0	40	0.7 ± 0.2	0.16 ± 0.05

The two components on the right side describe, respectively, the slow α and the fast β dynamical processes. It should be noticed that in the case of Brownian motion of large particles suspended in a solvent composed of many small particles, the two coefficients are both equal to 1, corresponding to the Lorentzian function describing the diffusion of the large particles and to the white noise resulting from the faster dynamics of the solvent.

We also fitted our data to equation (3.2) to compare the present spectral features with those already reported in literature for other biomolecules (Doster *et al.* 1990; Sokolov *et al.* 1999). In the case of the MBP, we estimate the exponents to be $b = 0.4 \pm 0.07$ and $a = 0.6 \pm 0.1$, in good agreement with those found in the case of myoglobin, i.e. $b = 0.55 \pm 0.1$ and $a = 0.4 \pm 0.1$ (Doster *et al.* 1990), and DNA, i.e. $b = 0.3$ (Sokolov *et al.* 1999). This similarity supports a common description for the dynamics of proteins and DNA when they are in hydrated solid-state phase.

In the case of protein hydration water, we find $b = 0.45 \pm 0.05$ and an anomalously low and ill-defined value of $a = 0.1 \pm 0.1$. The b exponent is very similar to that of a hard-sphere liquid, which is 0.532 (Fuchs *et al.* 1992), and—more interestingly—to that of supercooled water $b = 0.50 \pm 0.05$ (Sokolov *et al.* 1995; Sciortino *et al.* 1996). This agreement is a further analogy between the glassy properties of protein hydration water and supercooled water. A similar Von Schweidler parameter has been found in the case of the hydration water of myoglobin (Settles & Doster 1996), thus suggesting that the slow-relaxation of protein hydration water, on the time scale of several picoseconds (at 300 K), is significantly independent of the secondary and tertiary protein structure.

In the time scale explored by the present experiment, it can be supposed that the Von Schweidler exponent is also able to completely describe the trend of the time-dependent mean square displacements of protein and its hydration water through the power law $\langle u_T^2(t) \rangle \sim t^b$ (Sciortino *et al.* 1996; Gotze 1998). The fractional value of the b exponent, instead of the unity value of Brownian diffusion in bulk water, is the signature of a hindered, anomalous diffusion in this time range. This subdiffusive behaviour can be a consequence of the fact that water molecules move around the rather rough protein surface, characterized by a fractal dimension, resulting in a non-homogeneous spread of the residence times between successive jumps (Bizzarri *et al.* 2000). Actually, from our results, it also turns out that the protein atoms undergo subdiffusive motions. Indeed, we expect the amplitude of the mean square displacements involved in these subdiffusive motions to reach a plateau for longer times, so that the protein structural integrity will not be affected,

at least at room temperature. This is supported by recent molecular dynamics simulations findings, which have shown that oligopeptide chains undergo configurational subdiffusion with a $\sim t^{0.5}$ functional dependence, toward a maximum-amplitude plateau attained in the nanosecond time range (Nesius *et al.* 2008). We may also suppose that the comparable values of the b exponents for the protein and its hydration water are related to a similar subdiffusive behaviour, which is in turn due to the dynamical coupling between the two systems in the time scale of tens of nanoseconds.

4. CONCLUSIONS

The quasi-elastic neutron scattering spectra of both MBP and its hydration water show a two-step power behaviour, which we have described with two different models. In the first one, the data are represented by a phenomenological analytical function with two Cole–Davidson terms. We found that they correspond to relaxations with characteristic times that are similar for the protein and its hydration water. The same data have also been described in terms of an asymptotic law including the Von Schweidler and the critical decays. This approach allowed us to put in relationship the low-frequency relaxation with subdiffusive phenomena occurring in both proteins and hydration water. This common subdiffusive behaviour, together with the similarity of their relaxation characteristic times, are new elements that confirm the dynamical coupling between protein and hydration water in the picosecond time scale.

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