J|A|C|S ARTICLES

Published on Web 03/13/2009

Collective Dynamics of Protein Hydration Water by Brillouin Neutron Spectroscopy

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Abstract: By a detailed experimental study of THz dynamics in the ribonuclease protein, we could detect the propagation of coherent collective density fluctuations within the protein hydration shell. The emerging picture indicates the presence of both a dispersing mode, traveling with a speed greater than 3000 m/s, and a nondispersing one, characterized by an almost constant energy of 6−7 meV. In agreement with molecular dynamics simulations [*Phys. Rev. Lett.* **2002**, *89*, 275501], the features of the dispersion curves closely resemble those observed in pure liquid water [*Phys. Rev. E: Stat. Phys., Plasmas, Fluids, Relat. Interdiscip. Top.* **2004**, *69*, 061203]. On the contrary, the observed damping factors are much larger than in bulk water, with the dispersing mode becoming overdamped at $Q = 0.6$ Å^{-1} already. Such novel experimental findings are discussed as a dynamic signature of the disordering effect induced by the protein surface on the local structure of water.

Introduction

A crucial challenge of modern biophysics is to understand the interplay among structure, dynamics and functionality of proteins. This debated issue can be unraveled only if a clear picture of the intimate coupling between the biomolecule and the surrounding solvent is provided. In fact, the solvent either confers the protein the conformational flexibility required for biological function,[1−5] or is necessary to increase the protein activity.[6] It has been shown that at least a minimum amount of water, namely about $0.2h$ (h = grams of water/grams of protein), is essential for the biological activity of several enzymes.[1,7] At the same threshold value, protein internal stochastic motions on the picosecond time scale, progressively turned on by hydration, attain their full activation.[8] As a consequence, motions over the picosecond scale, characterized by thermal energies (few tens of meV) and THz frequencies, are considered of primary importance for the fulfillment of dynamically driven biological functions.[9] It is clear that the dynamic behavior of hydration water plays a key role for these vital mechanisms. How and to which extent hydration water determines protein functional movements is still a vast subject of investigation.

A huge number of studies about *single-particle* motions of hydration water have shown that its dynamic features are quite different from those of bulk water,[10−16] due to the topological and energetic disorder induced by the interaction with the fluctuating protein surface. The resulting water dynamics shows several similarities with glassy systems,[11] such as slowed-down diffusional motions [10,15,16] or the presence, in the incoherent dynamic structure factor of hydration water, of the vibrational anomaly known as boson peak [12,13,17]

Much less numerous are instead the existing studies about *coherent collective* dynamics of hydration water at THz frequencies. Recently, coherent density fluctuations in the hydration shell of the ribonuclease protein were investigated by MD simulations,[17] which predicted that such kind of collective excitations exist, propagate with thermal energies and exhibit two modes very similar to those previously observed in

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 10.1021/ja807957p CCC: $40.75

liquid water.[18−20] The existence of such propagating modes could be of strong biological relevance, especially under the hypothesis that they are coupled to, and possibly strongly affect, the collective internal protein dynamics. Unfortunately, in spite of the cited MD simulation work, no experimental investigation about collective density fluctuations propagating at thermal energies in hydration water exists. Indeed the subject of collective excitations in biological systems has been approached by thermal Brillouin spectroscopy in a restricted number of cases, where the behavior of the whole biomolecule-water system, rather than of the hydration shell only, was actually investigated.[21−24] In the case of hydrated proteins a rough indication that propagation velocities range from 2000 to 4000 m/s was found, but more precise quantitative information could not be extracted from the data.[21]

Such a lack of studies is mainly due to objective experimental difficulties, which basically arise from the intrinsic nature of thermal Brillouin scattering. Coherent collective excitations at thermal energies usually propagate in disordered materials in the wavevector range $0.1 \lesssim Q \lesssim 1.5$ Å^{-1}, where the low-Q drop of the structure factor of noncrystalline systems in general makes the inelastic scattering intensity intrinsically weak. Moreover, inelastic measurements at such small wavevectors require access to small scattering angles, with consequently tight collimations, which further reduce the useful beam intensity. Finally, a nontrivial problem is the separation of hydration water contributions from those due to the protein, which is possible in the present case thanks to the contrast methods provided by neutron spectroscopy and, as explained later, only under certain experimental conditions.

Here we report the results of an extended Brillouin neutron scattering investigation on hydrated powders of ribonuclease A (RNase), a single-domain enzyme, which is a model system for protein science and the subject of several ongoing investigations.[25] It also fits our specific purposes, because RNase is the subject of the only existing MD simulation study about collective modes in protein hydration water.[17] We studied three RNase powder samples, hydrated at 0.68h, 1.00h and 1.00h respectively, along with the corresponding three dry samples. The first two couples of wet and dry samples were measured by the BRISP spectrometer and the last one by the IN1 spectrometer, at the Institut Laue-Langevin (Grenoble, France).

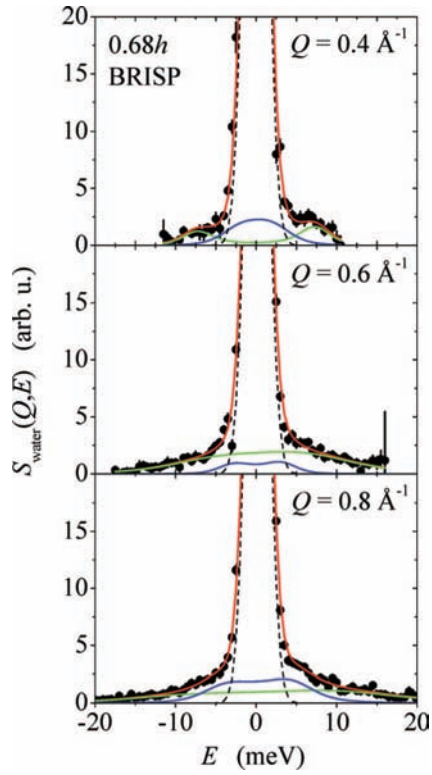

Figure 1. (color online) Fit of the dynamic structure factor of hydration water, for the sample 0.68h measured on BRISP. The Q values are 0.4, 0.6 and 0.8 Å^{-1} from top to bottom. Full red line: global fit function. Full green line: high-frequency DHO. Full blue line: low-frequency DHO. Dashed line: energy resolution function.

Full details about sample preparation and neutron experiments are reported as Supporting Information.

Data Analysis

As discussed in the Experimental Methods section of the Supporting Information, a sample composed of hydrogenated protein and deuterated water is the most suited one for observing possible collective density fluctuations propagating within the protein hydration shell. Nevertheless, the possibility that the strong, albeit incoherent, signal from the protein molecule overwhelms the smaller coherent signal from hydration water must be considered to a deeper extent. The vast literature on neutron scattering studies of hydrogenated proteins shows that their incoherent dynamic structure factor is characterized, in the meV energy scale, by essentially two features: a quasi-elastic diffusional peak and an inelastic vibrational bump, the so-called boson peak, centered at energies of ∼3 meV.[26,27] At the low wavevector transfers of the present experiments, the quasi-elastic peak is known to have a width of ∼0.2−0.3 meV,[28] which is 1 order of magnitude smaller than the narrowest energy resolution window here employed. As to the boson peak, the well-known Q^2-dependence of its scattering intensity[26] makes its contribution negligible at the low-Q values where Brillouin modes are searched for. These arguments hold for both dry and hydrated

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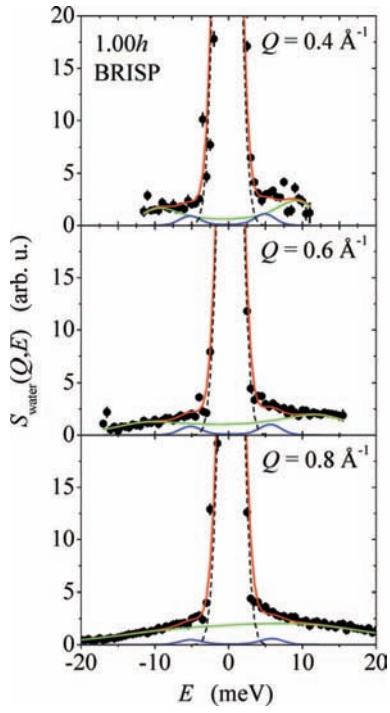

Figure 2. (color online) Fit of the dynamic structure factor of hydration water, for the sample 1.00*h* measured on BRISP. The *Q* values are 0.4, 0.6 and 0.8 Å^{-1} from top to bottom. Full red line: global fit function. Full green line: high-frequency DHO. Full blue line: low-frequency DHO. Dashed line: energy resolution function.

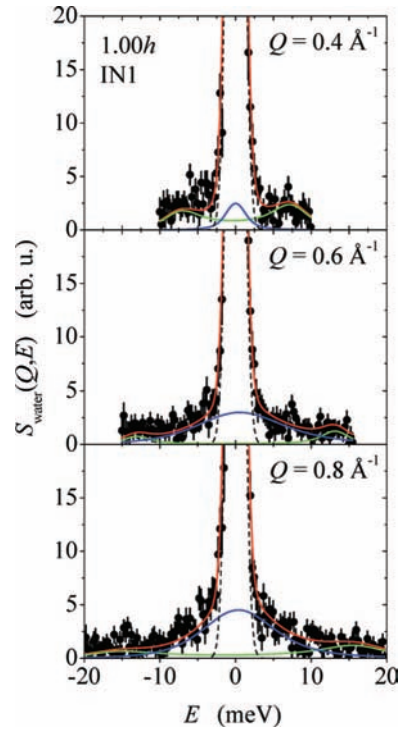

Figure 3. (color online) Fit of the dynamic structure factor of hydration water, for the sample 1.00*h* measured on IN1. The *Q* values are 0.4, 0.6 and 0.8 Å^{-1} from top to bottom. Full red line: global fit function. Full green line: high-frequency DHO. Full blue line: low-frequency DHO. Dashed line: energy resolution function.

samples. Indeed, despite hydration has the effect of broadening the quasi-elastic peak, the resulting line width remains well within the employed energy resolution.[28] Similarly, hydration is known to shift the boson peak energy up to ~4 meV, but does not affect the Q^2-drop of the peak scattering intensity, so that the boson peak contribution results negligible even for the wet protein spectra.

For these reasons, hydration-induced variations of the strong incoherent signal from the protein hydrogen atoms are not visible by the present experiments, and the incoherent contribution can thus be removed by direct subtraction of the normalized spectrum of the dry sample from the normalized spectrum of the corresponding wet sample:

$$S_{\mathrm{water}}(Q,E) = S_{\mathrm{wet}}(Q,E) - S_{\mathrm{dry}}(Q,E) \qquad (1)$$

The resulting dynamic structure factors are displayed at selected values of the exchanged wavevector in Figures 1, 2 and 3. They are denoted by the symbol $S_{\mathrm{water}}(Q,E)$ because, as argued in the following, they are dominated by the coherent signal of the sole hydration water.

In principle, the result of eq 1 still contains the water incoherent contribution, along with three coherent terms due to water−water correlations, water−protein correlations and possibly residual protein−protein correlations (in case the protein coherent scattering changes upon hydration). Incoherent scattering from deuterated water is small compared with coherent scattering and can thus be neglected. In addition, an estimate of the three coherent terms suggests that, in the investigated *Q* range, the water−water correlations produce by far the most important contribution to the coherent signal (for further details see Supporting Information). We can therefore conclude that the spectrum after eq 1 is a good approximation of the hydration water dynamic structure factor.

The model adopted to interpret the dynamic structure factor of the RNase hydration shell is based on the present-day picture of bulk water dynamics. To-date experimental evidence show that in the THz domain two vibrational modes propagate in bulk water:[19,20] a high-frequency dispersive mode and a low-frequency mode of almost constant energy. The response function of a damped harmonic oscillator (DHO) is commonly employed for describing such density fluctuations.[18−20,29−31] This model presents the peculiarity that the parameter describing the characteristic oscillation energy of the DHO corresponds to the energy position of the maximum in the longitudinal current spectrum ($C_{\mathrm{L}}(Q,E) = (E^2/Q^2)S(Q,E)$), rather than in the dynamic structure factor. This makes the model particularly well suited for a comparison of the present experimental data with the existing MD simulation results,[17] where the dispersion curves of the RNase hydration shell were in fact determined from the longitudinal current spectra. The experimental $S_{\mathrm{water}}(Q,E)$ was thus fitted to the convolution of the instrumental energy resolution with the DHO-based model function

$$S_{\mathrm{mod}}(Q,E) = a(Q)\delta(E) + [n(E)+1]\left\{\frac{a_{\mathrm{H}}(Q)\Gamma_H(Q)E}{(E^2-\Omega_{\mathrm{H}}^2(Q))^2+(\Gamma_{\mathrm{H}}(Q)E)^2} + \frac{a_{\mathrm{L}}(Q)\Gamma_{\mathrm{L}}(Q)E}{(E^2-\Omega_{\mathrm{L}}^2(Q))^2+(\Gamma_{\mathrm{L}}(Q)E)^2}\right\} \qquad (2)$$

where $n(E)$ is the Bose thermal factor. The first term in eq 2 describes the elastic and quasi-elastic response of the system: since the quasi-elastic line width of hydrated proteins is much narrower than the instrumental resolution functions into play, it was simply modeled by a Dirac delta function with intensity

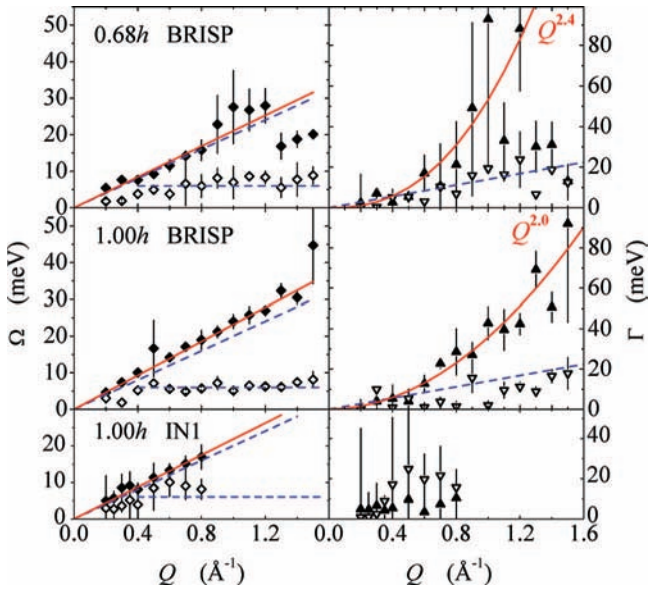

Figure 4. (color online) Left panels: dispersion curves of the high-frequency (full diamonds) and low-frequency (empty diamonds) mode in RNase hydration water. The red continuous lines represent the linear fits yielding the propagation speed of the high-frequency mode (see text). The blue dashed lines report the dispersion relation in pure liquid water, composed of a high-frequency mode propagating at 3040 m/s and a low-frequency mode localized at 6 meV.[19] Right panels: damping factors of the high-frequency (full triangles) and low-frequency (empty triangles) mode in RNase hydration water. The power laws describing the Q-dependence of the high-frequency damping factor at the two hydration levels measured on BRISP are compared with the behavior observed in bulk water (straight line in dashed blue).[19]

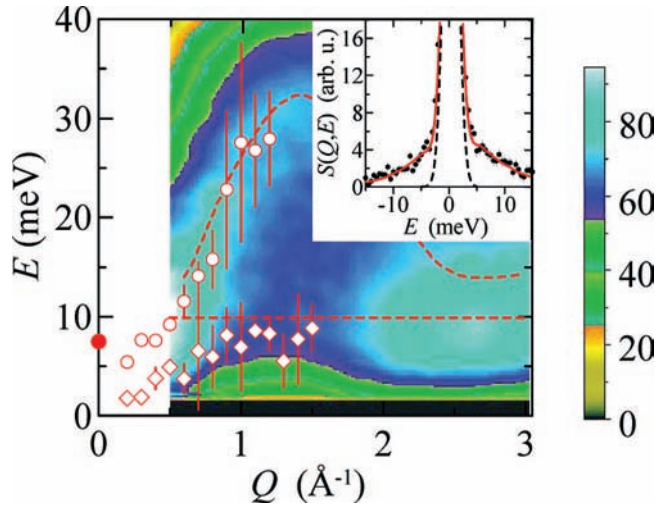

Figure 5. The experimental dispersion curves of hydration water in the 0.68h sample are compared with the contour plots of the longitudinal currents resulting from MD simulations of 0.5h powders of RNase.[17] Red open dots: experimental dispersive mode. Red open diamonds: experimental nondispersive mode. Red dashed lines: dispersive and nondispersive mode from simulations. The full red dot at $Q = 0$ is taken from Raman scattering in pure supercooled water.[42] Inset: Comparison between the experimental (full circles) and simulated (full line) spectra of RNase hydration water from which the corresponding dispersion curves and longitudinal currents were derived. The simulated data are taken from Figure 2 of ref 17. For a meaningful comparison, the simulated data were convoluted with the energy resolution function of BRISP (dashed line) and rescaled to the intensity of the experimental data.

$a(Q)$. The second term between curly brackets describes the response of the two DHOs, which are characterized by their oscillation energies $\Omega_H(Q)$, $\Omega_L(Q)$, damping factors $\Gamma_H(Q)$, $\Gamma_L(Q)$, and weights $a_H(Q)$, $a_L(Q)$. The subscripts H and L stand for high- and low-energy respectively. The seven parameters, characterizing the two DHOs and the elastic peak intensity, were all left free to vary during the fitting procedure, which was independently applied to the spectra at each single value of the wavevector transfer. In this way all the fit parameters were determined as a function of Q, although for $Q < 0.3$ Å^{-1} the available energy range, restricted by both kinematic constraints and energy resolution, was too limited to obtain reliable fits. The same modeling method was applied to all the three available dynamic structure factors of RNase hydration water.

The best-fit functions at some selected Q-values are compared with the experimental data in Figures 1, 2, and 3. The model function reproduces quite well the two spectra measured on BRISP. The IN1 spectra are well reproduced too, although the rather inferior quality of the data would not allow a reliable choice of the most adequate fitting model without the more accurate indication obtained by BRISP.

As a general result, the high-frequency DHO is well defined and easily determined, especially at the intermediate Q-values between 0.4 and 0.8 Å^{-1}. At higher Q's, it becomes strongly overdamped and its energy position is thus less well-defined. The low-frequency DHO is more difficult to determine, because its typical energy results to be systematically close to the tails of the resolution function, and also because the mode appears to be overdamped at all wavevector transfers. Nevertheless, its removal from eq 2 eventually produces an overall worsening of the fit quality. The dispersion curves associated to the energies of the two best-fitting DHOs are plotted in Figure 4, along with the relevant damping factors. As concerns the high-frequency mode, a linear fit of the low-Q portion of the dispersion curve provides the propagation speed of the excitation. The resulting velocities are 3200 ± 150 m/s, 3520 ± 100 m/s and 3320 ± 150 m/s, for the three hydration levels 0.68h measured on BRISP, 1.00h on BRISP and 1.00h on IN1 respectively. On the contrary, the low-frequency mode is characterized by a rather constant energy amounting to ∼6−7 meV.

Discussion

From the above analysis, a complex picture of the THz dynamics in the RNase hydration shell emerges, and presents at once remarkable similarities with bulk water and interesting elements of novelty.

On a purely qualitative ground, coherent collective excitations in hydration water resemble the behavior observed in bulk water, as in both systems they are characterized by a high-frequency mode of dispersive nature and a low-frequency mode of almost constant energy. These findings are also consistent with the picture suggested by MD simulations of hydrated RNase,[17] where the protein hydration shell presents a dispersive mode propagating with a speed of 3800 m/s and a nondispersive mode centered at ∼10 meV. The good agreement of the present experimental results with MD simulations[17] is witnessed by Figure 5, where a direct comparison between experimental and simulated data is presented.

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Further insights can be gained on quantitative grounds. The first experimental observation of a high-frequency mode, usually called *fast sound*, in bulk liquid D_2O was achieved in 1985 by neutron scattering and provided a speed of 3310 ± 250 m/s.[29] Later on, X-ray inelastic scattering measurements on H_2O reported a value of 3200 ± 100 m/s.[30] More recent neutron scattering investigations,[18,19] performed at higher-energy resolution, proposed a global data-analysis strategy to take into account for the large number of neutron and X-ray data become available in the meantime, thus ending up with a high-frequency sound velocity of 3040 ± 80 m/s.[19] These values are consistent with those found herewith for RNase hydration water. The agreement is particularly good at the 0.68*h* hydration level, whereas the results at 1.00*h* from BRISP, where the experimental points are systematically above the dispersion curve of bulk water, might suggest a possible increase of the sound speed with increasing water content.

An interesting element of novelty is represented by the wavevector dependence of the damping factor Γ_H of the high-frequency mode. At GHz and lower frequencies the hydrodynamic theory predicts a Q^2-dependence for the damping factors of sound waves propagating in simple liquids, which is readily confirmed by experimental results. On the contrary, this is not observed at THz frequencies, where the damping factors usually display an almost linear Q-dependence. Similarly, the most recent analyses of bulk water in the THz range pointed out a linearly increasing damping factor.[19] The widths Γ_H of the RNase hydration water determined from the present study show instead a much faster increase with Q. Fits with a power law Q^x of the Γ_H obtained by BRISP in the low-Q region ($Q \leq 1.2$ Å^{-1}) yield best-fit values of the exponent x equal to 2.4 ± 0.8 and 2.0 ± 0.3, for the 0.68*h* and the 1.00*h* samples, respectively. The resulting best-fit curves are plotted in Figure 4 and compared with the behavior of the damping factors in bulk water. The fact that the high-frequency mode becomes rapidly overdamped suggests that the relevant excitations can propagate through a shorter mean free path than in bulk water. The interaction with the protein surface is indeed expected to distort the local structure that liquid water would otherwise assume. Water molecules are known to form a complex hydrogen-bond network, with an almost perfect tetrahedral arrangement and a well-defined second-coordination shell. The hydrogen-bond network was soon hypothesized to sustain the propagation of the high-frequency sound, mainly because the associated wavelength (~20 Å) spans several water molecules.[29] The perturbation due to the interaction with the protein surface might introduce distortions of the water hydrogen-bond network, resulting in a disordering effect which would decrease the mean lifetime of the collective excitations propagating through the network itself.

With this respect, a comparison with the dynamics of glassy systems is particularly interesting. In the THz frequency region, vitreous materials are characterized by large damping factors, which rapidly increase with wavevector transfer and therefore are commonly modeled by a power law Q^x. Reported best-fitting values of the exponent x are often equal to 2, and can in some cases reach values as high as 4.[32−34] The similar exponent values found here for hydration water are well consistent with the hypothesized disordering effect due to the interaction with the protein surface, and can suggest that hydration water has a glass-like behavior as far as collective modes are concerned.

Such analogy with vitreous systems can be further developed if the energy of the Ioffe−Regel limit is considered. The Ioffe−Regel cross-over occurs in glassy systems when the wavelength λ of a propagating mode becomes comparable to its mean free path l, $\lambda_{IR} \approx l$, which can be rewritten in terms of frequency ν and mean lifetime τ of the propagating mode: $\nu_{IR} \approx \tau^{-1}$. As the damping factor Γ is proportional to the inverse lifetime of the mode through the relation $\Gamma \approx 2\hbar\tau^{-1}$, the Ioffe−Regel condition provides a connection between energy and damping factor: $E_{IR} \approx \pi\Gamma$. The Ioffe−Regel energy E_{IR} defines the cross-over between weakly and strongly scattered sound waves by the disorder of the medium,[35] and was conjectured to be the limit above which phonons cease to exist as propagating plane waves and become localized.[36] Based on experimental observations in a number of different systems, some authors hypothesized as a general rule that the Ioffe−Regel energy corresponds to the position of the boson peak in glasses: $E_{IR} \approx E_{BP}$.[34,36,37]

In the present case, the Ioffe−Regel energy of the high-frequency mode of hydration water can be readily deduced by the crossing of the linear dispersion curve with the power-law fit of the damping factor, which provides $E_{IR} = 4.3$ and 4.7 meV for the 0.68*h* and 1.00*h* hydration levels respectively. These values are actually in good agreement with the position of the boson peak in hydration water, which was observed at about 4 meV.[12,13,17]

The whole of these considerations provide new experimental evidence that hydration water presents some of the peculiar behaviors of amorphous systems, which is actually not an unheard of idea. A number of studies on protein hydration water, ranging from structural studies[38] to microscopic investigations of *single-particle* dynamics[11,12,39,40] and measurements of macroscopic thermodynamical properties,[41] have contributed to build up a picture of the hydration shell as a glass-like system. The present study strengthens this interpretation, emphasizing for the first time the effect of the vitreous character of hydration water on *coherent collective* dynamics.

A final comment is devoted to the observation of the constant-energy low-frequency mode in the RNase hydration water. In pure water, the existence of such a nondispersive mode between 5.5 and 6 meV was definitely confirmed by high-resolution neutron and X-ray inelastic scattering experiments.[18−20] An optical mode of ~7.5 meV was also observed at much lower

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wavevector transfers by Raman scattering.[42] The value of ~6−7 meV found herewith for the RNase hydration shell is well comparable with such previous results for pure water. A less quantitative agreement is instead observed with respect to MD simulations, which locate the nondispersive mode at the higher energy of 10 meV.[17]

The global picture emerging from the ensemble of the presented results indicates a close similarity between the collective dynamic behavior of bulk and hydration water. Such analogy is somewhat unexpected, considering that the interaction between water molecules and protein surface has in most cases remarkable effects: the cited slowing down of the single-particle dynamics for instance can amount to 2 orders of magnitude in terms of time.[10,15,16] Nevertheless, the coherent collective dynamics of hydration water on the THz frequency domain appears substantially unaffected by the presence of the biomolecule. The apparently contradictory behavior of coherent and incoherent dynamics might actually be reconciled if one considers the nature of the corresponding motions. Rotational and translational incoherent diffusion requires an equilibrium dynamics involving the continuous breaking and reforming of hydrogen bonds between different water molecules and between water molecules and protein external side chains. The perturbation induced by the protein surface on the hydrogen bond network of water is likely to hinder such equilibrium dynamics, thus resulting in the known slowing-down of diffusional processes. On the other hand, the propagation of short-wavelength excitations requires the vibration of atoms and molecules about their equilibrium positions only, without need for bond breaking. As water−water and water−protein hydrogen bonds have substantially the same strength and nature, the presence of the protein can be expected to produce only minor effects on the propagation speed of collective modes. However, the protein surface strongly affects the damping of these modes, probably because of the more disordered structure and the quasi-2D arrangement of hydration water.

A still debated subject in the dynamics of pure water is the connection of the two THz collective modes with ordinary sound, which propagates at GHz and lower frequencies with the much lower speed of 1320 m/s. In the framework suggested by the dynamical analogies between bulk and hydration water, an interesting extension of the present study would be the experimental determination of the ordinary speed of sound in hydration water, aimed at verifying whether the dynamical similarity with pure water extends to the lower frequency regimes. In the GHz frequency window, the traditional technique of laser-light Brillouin scattering would be well adapted to this end.

Conclusion

In the present study of wet RNase by thermal neutron Brillouin spectroscopy, we could observe the presence of coherent collective excitations in the protein hydration shell. The dispersion relation, determined by a thorough analysis of the experimental spectra, reveals the existence of two modes: a high-frequency mode propagating between 3000 and 3600 m/s and a low-frequency mode localized at 6−7 meV. Such a structure of the dispersion curves bears a strong resemblance with those of bulk liquid water in the same energy range, as if the presence of the protein had negligible effects on water dynamics. On the other hand, the influence of the biomolecule manifests itself in the much larger damping factors than in bulk water, an observation which strengthens the idea that the hydration shell of proteins presents some analogies with glassy systems.

Acknowledgment. We acknowledge the ILL for providing neutron beam time and Dr. A. Ivanov for his help with the experiment on IN1. We thank A. Laloni for his keen assistance during the BRISP experiments. Dr. G. Lorusso and Dr. E. Cornicchi are acknowledged for their help with the data reduction.

Supporting Information Available: Experimental Methods and Data Reduction. This material is available free of charge via the Internet at http://pubs.acs.org/.

JA807957P