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Neutron scattering evidence of a boson peak in protein hydration water

Alessandro Paciaroni,[1] Anna Rita Bizzarri,[1,2] and Salvatore Cannistraro[1,2,*]
[1]*Unitá INFM, Dipartimento di Fisica dell'Universitá, I-06100 Perugia, Italy*
[2]*Dipartimento di Scienze Ambientali, Universitá della Tuscia, I-01100 Viterbo, Italy*
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Measurement of the low temperature neutron excess of scattering of H_2O-hydrated plastocyanin relative to D_2O-hydrated protein allowed us to reveal the presence of an inelastic peak at about 3.5 meV. This excess of vibrational modes, elsewhere termed ''boson peak,'' is due to the dynamical behavior of the water molecules belonging to the H_2O-hydration shell surrounding the protein. The relevance of the boson peak to the dynamical coupling between the solvent and the protein, and hence to the protein functionality is addressed. [S1063-651X(99)50809-X]

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Recently, a molecular dynamics (MD) simulation approach has allowed us to show the existence of an excess of low frequency vibrational modes over the estimated Debye level in the density of states of hydration water around plastocyanin (PC) [1], a copper containing protein involved in the photosynthetic process. Such a vibrational anomaly was traced back to the so-called boson peak, a sort of signature of the disordered, amorphous state, whose origin, still amply debated [2–8], might be connected to the low temperature thermal anomalies in glasses. The presence of such a low frequency peak, generally located in the 0.1–5 meV range, has been detected by neutron scattering and Raman spectroscopy in a large variety of glassy systems [2,9], such as polymers, glass-forming liquids, and protein macromolecules [10,11]. However, no experimental evidence of this type has been provided for the protein hydration water shell whose thermodynamical and dynamical properties have been suggested to be consistent with those of an amorphous state [12–14]. Indeed, the knowledge of the structural and dynamical properties of hydration water is of the utmost relevance to the understanding of the protein functionality which, on the other hand, crucially depends on the presence of at least a minimum amount of solvent water [15,16]. In this respect, it is believed that about 0.38 g of H_2O per g of protein are sufficient to cover the entire protein surface and to fully activate the protein functionality [15,16]. Actually, the peculiar dynamical behavior of the hydrogen bond network of water at the protein interface is able to modulate the protein dynamics probably through the solvent-exposed lateral chains [17] and is believed to provide the required flexibility for the sampling of the protein conformational substrates [12,18]. These are local minima in the protein-solvent system potential energy and appear to be of biological relevance [19,20].

The present paper reports neutron spectroscopic evidence of a low frequency excess of inelastic scattering in the dynamical structure factor of the PC hydration water. The neutron spectra of PC samples hydrated with D_2O and H_2O have been collected at three different temperatures (100, 220, and 300 K) at the cold neutron multichopper time-of-flight spectrometer IN5 (ILL, Grenoble, France). A neutron incident wavelength of $\lambda = 4.5$ Å, corresponding to an elastic resolution of approximately 150 μeV has been used; the dynamical structure factor has been measured in a wide energy range between -2.5 meV (energy loss) and about $2k_BT$ (energy gain), and an exchanged wave-vector range from 0.2 to 2.4 $Å^{-1}$.

An amount of 200–250 mg of the sample has been held in a flat aluminum cell with internal spacing of 1.5 mm, placed at an angle of 135° to the incident beam. Spectra have been accumulated for 10–12 h at each temperature. The initial data reduction has been performed using standard ILL programs that correct for incident flux, cell scattering, and shielding using an angle-dependent slab correction. To compensate for detector efficiency, the data have been normalized with a Vanadium spectrum. An average transmission of 96% has been obtained; therefore, multiple scattering corrections have not been applied. Poplar PC (molecular weight 10 500 Dalton) was obtained by chromatographic purification from E. Coli cells where the PC gene had been cloned, according to procedures reported in Ref. [21]. Controlled hydration of lyophilized PC in both D_2O and H_2O has been carried out in a chamber under vacuum in the presence of a saturated KCl solutions. The degree of hydration has been determined by weighing. We have investigated PC hydrated with H_2O and D_2O at a hydration level at 0.38 g of solvent per g of protein; therefore, a bulk phase is not present, avoiding exchange between phases and ice formation.

Figure 1(a) shows the dynamical structure factor, $S^{PC+D_2O}(2\theta,\nu)$, at a fixed scattering angle ($2\theta = 88°$) of the PC sample partially hydrated with D_2O at three different temperatures. These spectra are generally dominated by the strong incoherent contribution arising from the protein protons which, being uniformly distributed within the whole macromolecule, monitor the overall protein dynamics [22]. Due to the much smaller neutron scattering cross section of deuterium with respect to hydrogen, the contribution to the incoherent scattering of the deuterated solvent is practically negligible [22].

The spectra of Fig. 1(a) (and the following ones) can be generally interpreted in terms of three main components, i.e., the elastic, the quasielastic, and the inelastic ones, whose relative intensity depends on the temperature [22–24]. By restricting our analysis to the inelastic region, we note the

*Author to whom correspondence should be addressed. Electronic address: cannistraro@pg.infn.it

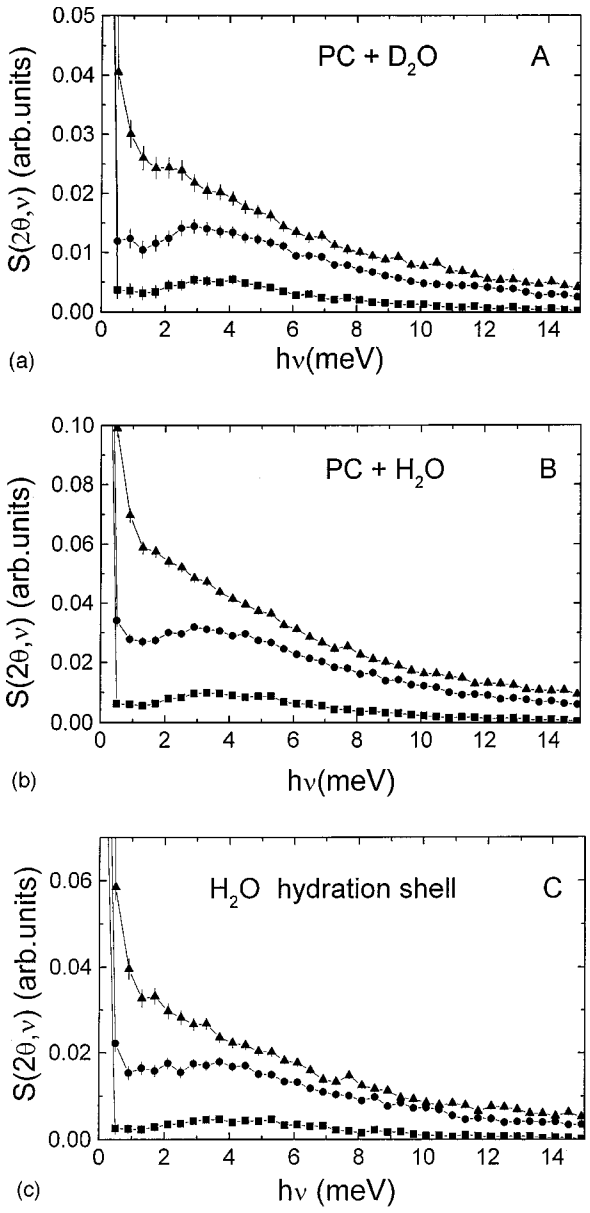

FIG. 1. Experimental dynamical structure factor, $S(2\theta,\nu)$, as a function of energy, $h\nu$, at a fixed angle $2\theta=88°$ of (a) D_2O hydrated PC, (b) H_2O hydrated PC, (c) PC hydration water. In all of the figures, the curves refer to the following temperatures: 100 K (squares), 220 K (circles), and 300 K (triangles). Error bars are indicated and solid lines are a guide to the eye.

presence of a broad peak, centered at about 3 meV, clearly visible at 100 and 220 K temperatures, and possibly merging into the broad quasielastic contribution at 300 K. Generally, the frequency dependence of the dynamical structure factor can be cast in the form $\sim 1/\nu n(\nu,T)g(\nu)$, where $n(\nu,T)$ is the Bose factor and $g(\nu)$ is the density of states. In the Debye approximation and at low frequency, $g(\nu)$ turns out to be proportional to ν^2 and $n(\nu,T)$ is approximated by $k_BT/h\nu$; accordingly, a constant trend as a function of frequency is expected for the dynamical structure factor. Therefore, the observed low frequency excess of modes represents a deviation from the flat Debye level. As previously mentioned, a similar excess of vibrational modes has been observed in many protein systems, including azurin [24], whose structure and functional role are very similar to those of PC, and has been attributed to global, collective, and damped vibrations involving protein domains and secondary structure elements.

The dynamical structure factor, $S^{PC+H_2O}(2\theta,\nu)$, of PC hydrated with normal water, is shown in Fig. 1(b) for the same three temperatures and scattering angle of those of Fig. 1(a). Again, the low temperature curves show, in the inelastic region, a peak located approximately at about 3 meV, over which the quasielastic contribution superimposes at 300 K. It should be kept in mind that, now, both the protein and the hydration water solvent protons contribute to the dynamical structure factor. Therefore, if we carefully normalize $S^{PC+D_2O}(2\theta,\nu)$ and $S^{PC+H_2O}(2\theta,\nu)$ to the number of unitary hydrogen scatterers, the difference

$$S^{H_2O}(2\theta,\nu)=S^{PC+H_2O}(2\theta,\nu)-S^{PC+D_2O}(2\theta,\nu) \qquad (1)$$

provides information about the dynamics of the water molecules belonging to the hydration shell around PC. To properly normalize our data we have followed the procedure reported in Ref. [26]. We have considered that each H_2O-hydrated PC macromolecule contains 558 nonexchangeable protein protons, about 48 solvent-exposed, fast exchanging protein protons, about 100 buried protein protons with a long exchanging time, and 455 H_2O solvent protons. The D_2O-hydrated PC sample contains 558 nonexchangeable protein protons and 50 protein protons with a long exchanging time (under the assumption that about 50% of slow exchangeable protons have actually exchanged with deuterium solvent, according to Ref. [26]). For normalization of the data, we have used the energy integral $\int S(2\theta,\nu)hd\nu$ in the whole investigated energy range. The dynamical structure factor, $S^{H_2O}(2\theta,\nu)$ of Eq. (1), calculated as described, is shown in Fig. 1(c), for the three analyzed temperatures. Surprisingly enough, a broad peak, centered at about 3.5 meV, is well visible at 100 and 220 K, whereas it appears to be masked by the quasielastic contribution at 300 K.

More rigorously, we should take into account that $S^{H_2O}(2\theta,\nu)$ contains contributions from both the hydration shell and the exchangeable PC protons, the latter being about 14% of the total. Therefore, to reliably attribute the boson peak observed in $S^{H_2O}(2\theta,\nu)$ [Fig. 1(c)] to the hydration shell protons, we must check that the intensity of the corresponding peak in the inelastic region is consistent with the number of protons in the H_2O hydration shell. To estimate the number of hydrogen scatterers contributing to such an inelastic peak, we have computed, at the lowest temperature where the quasielastic contribution is practically negligible, the integral $\int S(2\theta,\nu)hd\nu$ from 1.2 to 7.5 meV. We found that this area is consistent with a number of hydrogen scatterers five times higher than that expected if only exchangeable protein protons would contribute to the low frequency vibrational peak (the estimate error on the calculated area being about 10%). Such a finding strongly supports the existence of an excess of low frequency vibrational modes due to the water hydrogen from the hydration shell surrounding PC.

We have, moreover, derived the dynamical structure factor $S^{H_2O}(q,\nu)$ by transforming $S(2\theta,\nu)$ into a constant wave vector q ($q=2.1\,\text{Å}^{-1}$) format, using an interpolation proce-

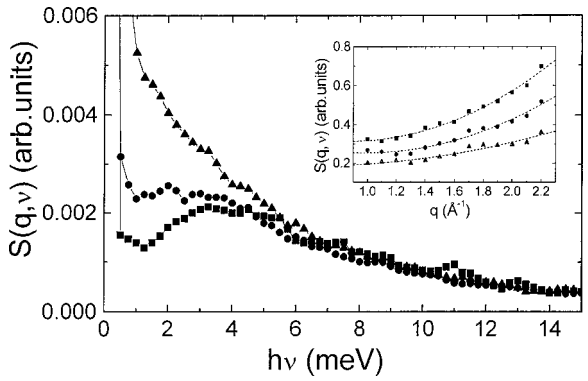

FIG. 2. Experimental dynamical structure factor, $S(q,\nu)$, as a function of energy, $h\nu$, at a fixed wave vector $q=2.1\ \text{Å}^{-1}$, of PC hydration water. The same symbols as in Fig. 1 have been used. All of the spectra have been rescaled by the Bose factor to the common temperature of 100 K. The solid lines are a guide to the eye. Inset: experimental dynamical structure factor, $S(q,\nu)$, as a function of the wave vector q, for three fixed values of the energy (1, 3, and 5 meV from top to bottom) at 220 K. The dashed lines indicate the q^2 trend.

dure to take into account the variation of q with ν at fixed scattering angle because of the neutron kinematics. $S^{H_2O}(q,\nu)$ at the three temperatures is shown in Fig. 2 after having been rescaled by the Bose factor to the common temperature of 100 K. This representation allows the three spectra to superimpose into a common curve, at and above the inelastic peak, suggesting that in this region the vibrations are essentially harmonic. The 3.5-meV boson peak is significantly evident at 100 K and its inelastic nature is confirmed by the q^2 dependence shown in the inset of Fig. 2 at three different energies. At 220 K, even if the peak is still well visible, some scattering fills up the low temperature minimum. This additional scattering does not appear to be of a quasielastic nature (the elastic line is not broadened) and is reminiscent of the onset of fast dynamical processes hypothesized to occur in some glasses [2]. At 300 K, the strong increase of the elastic linewidth due to the activation of diffusive motions could probably cover the boson peak. It should be mentioned, however, that such a peak was not observed in the neutron scattering of hydrated phycocyanin (fully deuterated) [25] and hydrated myoglobin [26]. In this respect, it might be argued that the observed low frequency excess of scattering [Fig. 1(c)] might be a peculiarity of the hydration water shell of PC or, alternatively, its presence in phycocyanin and myoglobin systems results in being masked by the large water translational band at 6–8 meV which is also typical of bulk water. At our hydration conditions, indeed, such a band could result in being suppressed due to the lack of nearest neighbors to the hydration water molecules.

The present experimental evidence of a low frequency vibrational anomaly (boson peak) just in the protein hydration water confirms our previous MD simulation results on the same system [1]. Such evidence witnesses, on one hand, the reliability of the force field and the overall simulation procedure employed, and, on the other, strengthens the perspective of assessing the origin of such a peak by exploiting the atomic spatial and temporal resolution of MD [27].

Moreover, the presence of the boson peak supports the glassy character of the water belonging to the PC hydration shell. Indeed, the glassiness of the protein-water system is still an open problem. A wealth of spectroscopic studies have indicated that the internal flexibility of hydrated proteins drastically increases above 180–220 K as compared to temperatures below this transition temperature (which is likely connected to the onset of most of the biological functionality). Owing to the phenomenological analogies of the dynamical behavior between proteins and glassy systems, this temperature has been related to the well-known dynamical glass transition [17,19]. The question concerns whether the measured dynamical transition is an inherent property of the protein or whether the protein dynamics is driven by the solvent properties (slaved to the solvent [18,19,28]). In any case, the present result constitutes additional evidence of the complexity of the protein solvent system. Actually, we have recently shown, by MD simulation of the PC water system, that the protein hydration water shell displays a number of properties that are typical of complex systems: namely, a sublinear trend with time of the water oxygen mean square displacements [14], a stretched exponential relaxation of the hydration layers [29], and a flickering noise in the power spectrum of the solvent potential energy [30]. All of these properties have been traced back to a temporal and a spatial disorder at the protein interface (typical of self-similar systems) [29,30]. Such an aspect could find a correspondence with the current models that invoke the intervention of the topological disorder, or microscopic heterogeneities, in causing the boson peak in glassy systems. It is believed that density fluctuations on domains of medium order, due to the intrinsic heterogeneities of glassy systems, could be responsible for the strong scattering of acoustic localized excitations and then lead to the observed enhancement of low frequency modes [3,7,8]. In this connection, a relationship connecting the domain correlation length, the velocity of the acoustic waves in the medium, and the frequency of the peak has even been proposed. We are not able, at present, to transpose the physical meaning of these models to our system, considering also the uncertainty of the acoustic velocity in the hydration water.

What we find worthy of noting, however, is the simultaneous presence of a boson peak in the protein and in the hydration water, occurring at similar energies. As we have already remarked [1], this could reflect an extensive dynamical coupling between the protein and the hydration solvent. The intimate contact between the solvent exposed protein residues and the water molecules could give rise to a sort of vibrational coupling, possibly at the origin of the peculiar spectral features of both the systems. The density fluctuations of the whole system, appearing of long range and collective character, and of biological relevance, would primarily arise from the fact that solvent water could ''inject'' its dynamics into the protein side chains [17] whose librations have been shown to be strictly dependent on the hydrogen bond network restructuring dynamics occurring at the protein-solvent interface [11]. The mechanisms of the dynamical coupling occurring at the protein-solvent interface, which seems to be responsible for the observed vibrational anomalies, deserves further investigation.

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