Low-Temperature Decoupling of Water and Protein Dynamics Measured by Neutron Scattering

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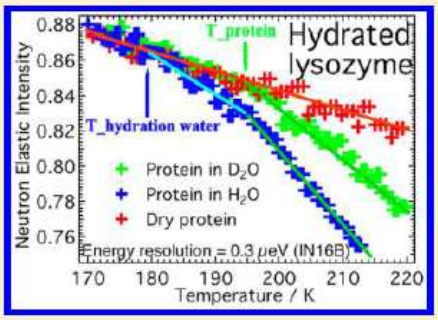
ABSTRACT: Water plays a major role in biosystems, greatly contributing to determine their structure, stability, and function. It is well known, for instance, that proteins require a minimum amount of water to be fully functional. Despite many years of intensive research, however, the detailed nature of protein–hydration water interactions is still partly unknown. The widely accepted "protein dynamical transition" scenario is based on perfect coupling between the dynamics of proteins and that of their hydration water, which has never been probed in depth experimentally. I present here high-resolution elastic neutron scattering measurements of the atomistic dynamics of lysozyme in water. The results show for the first time that the dynamics of proteins and of their hydration water are actually decoupled at low temperatures. This important result challenges the "protein dynamical transition" scenario and requires a new model to link protein dynamics to the dynamics of its hydration water.



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Summary

This paper suffers from several inconsistencies:

(1) the presented elastic intensities are Q-averaged, from 0,2 to 1.9 A^{-1}, while mean square displacements, presented in fig. 5, require a Q-dependent analysis, this is not shown.

(2) the effect of methyl group rotation on the elastic intensities is ignored.

(3) The exact water content of the samples, which determines the onset temperatures, is not given, it is only vaguely fixed between 0,3 and 0,4 g/g.

(4) The protein dynamical transition (PDT) is incorrectly explained as an intrinsic dynamic change of the system at the onset temperature at 200 K, independent of the instrumental resolution. It is thus strange that the instrumental resolution is varied as the essential parameter of the experiment.

(5) Increasing the resolution leads to a downshift of the onset temperature (178 K) for H_2O as expected. By contrast, the protein T_{on} (D_2O) = 200 K does not vary with the resolution. These results are incompatible, since at the lower resolution T_{on} is the same for both, protein and water at 200 K. It certainly does not indicate "decoupling".

(6) There is no good or "worse" resolution, each experiment requires an optimal resolution, which could be high or low, depending on the time scale of the process to be investigated. The H_2O/D_2O exchange requires a detailed cross-section discussion.

Detailed review

This is one of the above mentioned dynamic investigations based on „elastic scattering only“, in the spirit of G. Zaccai, with sensational conclusions, “protein-water decoupling°. Typical results are presented in the figure above. The method of qualitative analysis is the anharmonic onset in the decline of the elastic intensity above a particular temperature T_{0n}. That protein structural relaxation times and those of hydration water overlap is known since 1989. This leads to similar T_{on} values of both systems, highlighted either by D_2O or H_2O, while T_{on} goes to infinity, when water is removed. The author uses H_2O or D_2O hydrated lysozyme to study elastic back-scattering at two different resolutions, 1 and 0,3 μeV. While the respective elastic intensities overlap at 1 μeV with similar $T_{on} \cong$ 200 K (coupling), T_{0n} shifts to 180 K at the higher resolution for the H_2O sample (blue) , while T_{on} for the D_2O-hydrated sample does not change from the original value of 200 K (green), dry (red) no onset, as shown in the figure above. This is the central result, on which the ”decoupling” hypothesis is based. Prof. Colmenero complained at the respective ECNS session, that the term “decoupling” in this context is ill-defined. Winfried Petry pointed out, that the presented elastic intensity data were all Q-averaged. Such averages are problematic, since T_{on} generally depends on Q, the magnitude of the intensity decline increases with Q. In fact an alternative interpretation of this shift in T_{on} could be an effect of **differing magnitude**: Water has a much larger displacement amplitude within a given time than the spatially constrained protein residues. This would shift the noticeable deviation from a straight line at T_{on} to lower temperatures, see figure above.

How difficult it can be to localize T_{on} was demonstrated by Wood et al. (JACS 2008, 130, 4586): “Coincidence of Dynamical Transitions in a soluble protein and its hydration water, direct measurement by neutron scattering and MD simulation”. This is one of the papers, where elastic neutron scattering has the purpose to complement the time domain simulations. In their study a per-deuterated protein is decorated with H_2O, while the protonated protein is hydrated with D_2O, which yields a better discrimination between protein and water displacements. At 1 μeV they derive “identical” T_{on} values near 190 K for both systems. The magnitude effect of water takes over above 220 K, while there could be a second protein onset above 230 K (see figure)

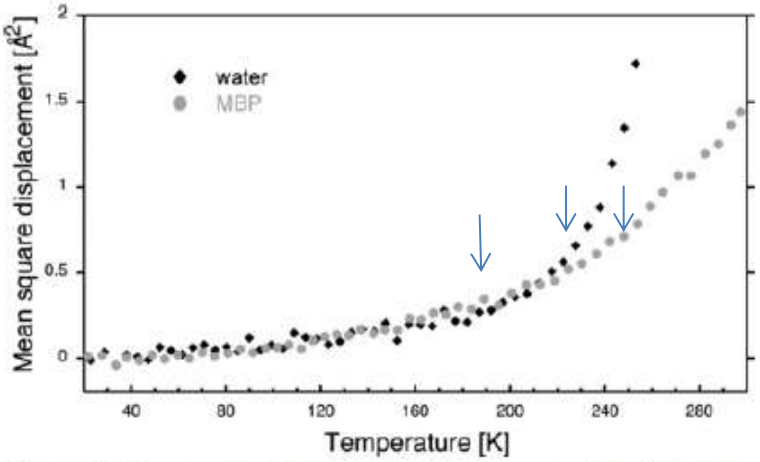

Figure 1. Mean square displacements of ns − ps motions in maltose binding protein (H−MBP−D_2O sample; gray circles) and in its hydration water (D−MBP−H_2O sample; black diamonds). Dynamical transitions (changes in slope of temperature-dependent mean square displacements) in the protein and in its hydration water take place at similar temperatures (~220 K).

The physics of the dynamical transition and T_{on}

The most problematic aspect of the Benedetto paper is the incorrect discussion of the mechanism of the "protein dynamical transition". It is presented as a structural change of water, the "fragile-strong transition", which leads to a dynamic change. Few people still believe in this effect at 220 K. In our studies we find no evidence of it (Doster et al. PRL 2010). Since our paper on "Elastic Resolution Spectroscopy" in 2001. Phys. B., it is well established, that the PDT reflects the overlap of structural relaxation times with the respective resolution time of the instrument. In 1999 it has been shown by us with hydrated lysozyme, that the anharmonic onset of displacements varies with the instrumental resolution (Doster/Settles, in Hydration Processes in Biology, IOS Press 1999). This has been discussed in detail by Doster in EBJ 2008, Concepts and misconception of the PDT. In this paper a phenomenological concept of T_{on} was presented. It was shown, that for an exponential process and a Gaussian resolution function the harmonic onset occurs when the ratio of the instrumental resolution time τ_{res} and the structural relaxation time τ_c has reached a level of 0,2 :

$$\tau_{res}/\ \tau_c(T_{on})\ \sim 0{,}2 \qquad (1)$$

In this experiment τ_{res} is enhanced by a factor of three, while T_{on} decreases be 20 K. Is this result plausible?

To proceed further, we assume an activated process for both water and protein motions, defined by an Arrhenius law:

$$\tau_c(T) = A \exp(\ H/RT) \qquad (2)$$

We are interested in the temperature shift Δ from the initial temperature T_1^{on}, when the resolution is enhanced by a factor 'f', which would be three in the present case. Thus $T_1^{on} = T_2^{on} + \Delta$.

It is then straightforward to derive the follow equation for Δ:

$$\Delta = \ln(f) \ (T_1^{on})^2 \ / (H/R + \ln(f) \ T_1^{on}) \qquad (3)$$

if we now assume plausible values for T_1^{0n} = 200 K and an activation enthalpy of H = 20 kJ/mol, which applies to hydration water, then a temperature down shift of

$\Delta \cong$ 17° K results. This is close to the experimental value for water of 20° K.

Water thus behaves quantitatively as expected. **The sensational effect is, that the onset of protein anharmonicity does not shift within experimental accuracy in spite of enhanced instrumental resolution, $\Delta \cong$ 0° K.** There is no good explanation for this discrepancy. The activation enthalpies, which appear to be similar at 200 K, will not change for the protein in response to a modified instrumental resolution. Therefore the magnitude effect, which can modify the onset temperatures, needs to be reinvestigated, in particular the Q-dependent elastic intensities have to be studied. This paper reflects in part the current state of the art, which in my opinion is not exciting. Even if the conclusions of this paper were correct, what does it mean for biology or biophysics if there is a "decoupling" of protein and water at 180 K. I am afraid, very little.