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Nature of the water specific relaxation in hydrated proteins and aqueous mixtures

K.L. Ngai [a,*], S. Capaccioli [a,b,1], A. Paciaroni [c,2]

[a] Dipartimento di Fisica, Università di Pisa, Largo B. Pontecorvo 3, I-56127 Pisa, Italy
[b] CNR-IPCF, Institute for Physical and Chemical Processes, Largo B. Pontecorvo 3, I-56127 Pisa, Italy
[c] Dipartimento di Fisica, Università di Perugia, Via A. Pascoli 1, 06123 Perugia, Italy

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ABSTRACT

The dynamic transition found by Mössbauer spectroscopy and neutron scattering in hydrated and solvated proteins has been an active research area for the past three decades. By now a consensus among some researchers has been reached that it originates exclusively from relaxation of the hydration water (HW) coupled to the protein. The dynamic transition temperature T_d depends on energy resolution of the spectrometer and is higher than the glass transition temperature T_g. Recently demonstrated is the presence of yet another transition at T_g, which is independent of the resolution of the spectrometer and coexists with the dynamic transition at a higher temperature T_d. The transition at T_g is similar to that found in various kinds of glass-formers by neutron and dynamic light scattering at short times when molecules are mutually caged via the intermolecular potential. Like in the case of conventional glass-formers, the transition at T_g of hydrated proteins has been explained by the sensitivity of the extent of the caged dynamics to change of specific volume and entropy on crossing T_g. The caged dynamics are terminated by the onset of relaxation of HW, which in turn gives rise to the dynamic transition at $T_d > T_g$. Despite these important roles played by the caged dynamics and the HW relaxation in the observed dual transitions of the hydrated proteins, their exact nature is still unclear. In this paper we clarify their nature in hydrated proteins by use of various experimental data, with the assist of the results from studies of mixtures of water with hydrophilic solutes, taking advantage of the fact that the properties are similar in both systems.

1. Introduction

The dynamic transition in hydrated proteins (*i.e.*, the onset of excess atomic mean square displacements) at temperature T_d, first observed by Mössbauer spectroscopy [1,2] and later by neutron scattering [3–25], has occupied the attention of researchers over the last three decades. The physical origin of the dynamic transition (DT) has become clearer from the properties observed by neutron scattering experiments using spectrometers with widely different resolution on samples where the hydration water (HW) is either H_2O or D_2O and the protein is deuterated. The DT occurs at the same temperature T_d irrespective of the molecular displacements measured are coming from the HW or the protein, indicating that the dynamics of the protein and the HW are strongly coupled together.

As evident from the recent publications of different research groups, seemingly now there is a consensus that the DT is triggered by a fast relaxation of the HW on raising temperature. At or near T_d, its relaxation time spectrum starts to overlap the time-scale of 140 ns of Mössbauer spectroscopy or the time window of the neutron scattering spectrometer, resulting in the observed change. The properties of the fast relaxation of the HW have been explored over a broad range of temperature and relaxation times by broadband dielectric spectroscopy, nuclear magnetic resonance, and adiabatic calorimetry. From these properties, satisfactory explanation has been given on the dependence of dynamic transition temperature T_d on the energy resolution of the spectrometer used and the solvent of the hydrated protein [26–28]. The study of the relaxation and diffusion dynamics of hydrated proteins by neutron scattering is enriched by these other experimental techniques. In principle, the dynamics of both the fast relaxation of the HW and the structural relaxation responsible for glass transition hydrated proteins can be probed over immense time range of 15 orders of magnitude from ps to 10^3 s.

Recently demonstrated is the presence of yet another transition of dynamics at the glass transition temperature T_g of the hydrated

* Corresponding author. Tel.: +39 0502214322; fax: +39 502214333.
E-mail addresses: ngai@df.unipi.it (K.L. Ngai), capacci@df.unipi.it (S. Capaccioli), alessandro.paciaroni@fisica.unipg.it (A. Paciaroni).
[1] Tel.: +39 0502214537.
[2] Tel.: +39 0755852785.

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[23,26] and solvated protein [27]. It is further shown that this transition is observed always at T_g, independent of the resolution of the neutron scattering spectrometer used. The transition at T_g of hydrated and solvated proteins is similar to that found in inorganic, molecular, and polymeric glass-formers by neutron scattering [28,29]. At the short time scales of neutron scattering experiments and at temperature below or in the vicinity of T_g, the molecules are mutually caged by the intermolecular potential, and all true relaxations and diffusion processes, including the fast HW relaxation, are much slower and cannot be responsible for the transition at T_g. Hence the transition at T_g of the hydrated protein comes from caged motion of the coupled HW and the protein in the hydration shell. Notwithstanding, if temperature above T_g is further increased, the thermally activated fast HW relaxation starts to enter the time window of the spectrometer, causing the DT as well as terminating the caged dynamics regime. This relation between the caged dynamics and the HW relaxation basically explains the transition at T_g to be followed by the DT at T_d. Like in the case of conventional glass-formers, the transition at T_g of hydrated proteins has been explained by the sensitivity of the extent of the caged dynamics to change of specific volume and entropy on crossing T_g. Interestingly the fast water relaxation is also sensitive to vitrification, as evidenced by its relaxation time and relaxation strength changing temperature dependence on crossing T_g [30–35]. Thus this property is shared by both the caged dynamics and the fast water relaxation (the Johari–Goldstein β-relaxation in conventional glass-formers [28]), and the latter has been used to explain the former as a consequence of the change of dynamics of the latter [27,36].

From the discussion given above, it is clear that the fast HW relaxation plays pivotal roles in the coexisting transitions of dynamics at T_g and T_d. In spite of the experimental data over a broad spectral range available in some archetypal hydrated proteins, its nature is still controversial. Different authors made different conclusions, some of which are incompatible and even conflicting with each other.

The purpose of this paper is to critically examine the different views on the nature of the fast HW relaxation by confrontation with experimental facts. In this way, its true nature is revealed. The fast HW relaxation in hydrated proteins is similar in every respect to that of water in aqueous mixtures with hydrophilic molecules and polymers. We take advantage of the similarity and use the experimental data of aqueous mixtures to strengthen our conclusions. In the next section, some general and key experimental dynamic properties of hydrated proteins are shown by examples. The different conclusions made by different authors on the nature of the water specific relaxation are brought out with the help of the experimental data. Also additional experimental facts will be provided to critically examine the various views on the nature of the fast HW relaxation in hydrated proteins and aqueous mixtures. Finally, we propose our alternative point of view that is consistent with all known experimental facts.

2. Collecting experimental data in hydrated myoglobin

To better understand the nature of the water specific relaxation in hydrated proteins, all pertinent properties obtained from Mössbauer spectroscopy and neutron scattering have to be examined altogether with those from dielectric, NMR, and calorimetric experimental studies. We use the example of hydrated myoglobin for which measurements had been made using all these spectroscopic tools [22]. The relaxation times of all processes seen experimentally by various techniques are shown as a function of reciprocal temperature in Fig. 1. The HW relaxation times τ_W shown were determined by neutron scattering in ($h = 0.4\, g_{water}$/$g_{protein}$) hydrated myoglobin powder (black closed circles) [22]. The single and large closed black square is from Mössbauer spectroscopy [37]. The two open diamonds at the top are taken from dynamic heat capacity data of hydrated myoglobin determining the glass transition temperature T_g to be 170 K [38,39]. Large size red closed triangles are the full set of dielectric data from Refs. [40–42]. The multiplication signs stand for NMR data from Refs. [43,44].

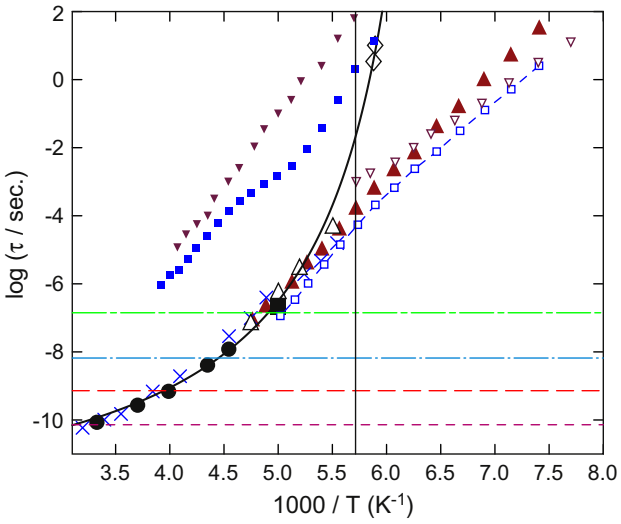

Fig. 1. Part of the data in figure are from the Arrhenius plot of data called the α-relaxation rate $k_S = 1/\tau_\alpha$ for hydrated myoglobin at $h = 0.4$ reproduced in Fig. 3 of Ref. [23]. The large black closed circles are from neutron scattering, large open black triangles are dielectric relaxation data taken from Ref. [40], the lone large closed square is from Mössbauer spectroscopy [37], the two open diamonds at the top are from specific heat spectroscopy [38,39]. The fit to a VFT-equation provided in Ref. [22] is also shown (full line). The same symbols as in Fig. 3 of Ref. [22] are used to represent the relaxation time data of HW. All of them are black in color and large in size to distinguish them from additional data from other sources to be described next. Large size red closed triangles are the full set of dielectric data of τ_W obtained by Swenson et al. [40], only part of which were used by Doster (large open black triangles) and considered by him as the τ_α of the myoglobin HW. The purple inverted open and closed triangles are respectively the dielectric τ_W and τ_P of hydrated myoglobin [41]. Open and closed blue squares are dielectric τ_W and τ_α of hydrated myoglobin at hydration level of $h = 0.33$ [42]. The NMR relaxation times of hydrated myoglobin are represented by the multiplication signs [43,44].

3. Interpretations of the nature of the water specific relaxation

3.1. The interpretation in terms of the structural α-relaxation

The data so far described and shown in Fig. 1 are the data considered by Doster in his interpretation of the nature and property of the water specific relaxation [22]. The symbols used in Fig. 1 are the same as in Fig. 3 of Ref. [22]. The only difference is that Doster took only the high temperature (short time) part of the dielectric HW relaxation times τ_W represented by the large red closed triangles in our Fig. 1, and left out the lower temperature (longer time) part of τ_W. To make this point clear, the high temperature part of the dielectric τ_W he took are shown by the black open triangles of the same size as the closed red triangles, but displaced slightly to make the selected τ_W visible.

Doster identified the relaxation time data from neutron scattering (black closed circles), Mössbauer spectroscopy (closed black square), and the high temperature part of the dielectric τ_W (open black triangles) all with the structural α-relaxation time τ_α of the myoglobin HW. Apparently the τ_W data of hydrated myoglobin (closed red triangles) at lower temperatures with Arrhenius temperature dependence neglected by Doster were considered by him as the secondary β-relaxation of HW, which he assumed to