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Properties of hydration water and its role in protein dynamics

Jan Swenson[1], **Helén Jansson, Johan Hedström and Rikard Bergman**

Department of Applied Physics, Chalmers University of Technology, SE-412 96 Göteborg, Sweden

E-mail: f5xjs@fy.chalmers.se (J Swenson)

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Abstract

The low-temperature properties of confined water and the relation between protein and solvent dynamics have been studied by broadband dielectric spectroscopy with the aim to understand the role of hydration water for protein dynamics. At low temperatures (below approximately 200 K) confined water generally exhibits two relaxation processes: one process that is due to the local β-relaxation, and a faster and even more local process that is interpreted to arise from the motion of Bjerrum-type defects. These relaxation processes, showing Arrhenius temperature dependences, are also observed in glycerol–water solvents of myoglobin containing $\geqslant$50 wt% water. In the temperature regime below 200 K, only a local protein process is observed. The activation energies of this protein process and the β-relaxation in the solvent are similar, suggesting that this local protein process is determined by the β-relaxation in the solvent. At about 200 K the nature of the dynamics changes dramatically and an onset of cooperative and large-scale dynamics is observed for both the water-rich solvent and the protein. We believe that the reason for this crossover is that the β-relaxation in the solvent merges with the non-observable α-relaxation at this temperature, giving rise to a merged α–β-relaxation in the solvent at higher temperatures. Also above this temperature the fastest observed protein processes seem to be determined by the solvent dynamics, as suggested for 'solvent-slaved' protein motions.

(Some figures in this article are in colour only in the electronic version)

1. Introduction

It is well known that water associated with proteins, nucleic acids, polysaccharides and assemblies of smaller molecules that make up living organisms is essential for their structure

[1] Author to whom any correspondence should be addressed.

and function. It determines their mobility, allows them to associate and dissociate, enables proton transfer, and facilitates a large number of biochemical processes [1–4]. Proteins are, for instance, known to be inactive in the dehydrated state up to about 0.2 (g of water)/(g of protein), and full activity requires roughly an equal fraction of water and protein [5, 6]. Experiments [7, 8] and molecular dynamics (MD) simulations [9, 10] have indicated that the protein motions are mainly determined by the water dynamics, i.e. the protein motions are 'slaved' (or 'driven') by the water motions. Furthermore it has been suggested [8] that the most local protein motions are slaved by the β-relaxation in the hydration shell whereas the global conformational changes of a protein are slaved by the α-relaxation in the bulk solvent. This 'slaving' does not mean that a solvent relaxation and its associated protein motions occur on the same timescale (i.e. having the same relaxation rate), but that the relaxation times of the processes show similar temperature dependences, i.e. similar activation energies at a given temperature. The reason for the protein motions being slower than their related solvent motions is that a relaxation process in the protein generally requires a large number of elementary steps, which can only take place if the solvent moves. In this paper we further elucidate the relation between protein and solvent motions by studying myoglobin in a water–glycerol mixture.

Due to the coupling between the dynamics of a protein and its surrounding water, it is important to investigate not only the protein but also the properties of its water to fully understand the protein dynamics. It is known that both the structure and the dynamics of the solvating water molecules are affected by the presence of a protein [11] due to surface interactions and confinement effects, but it is less known what the nature of the water dynamics is in the deeply supercooled regime. In fact, the dynamical properties of supercooled water in confined geometries and around biomolecules is currently intensively debated [12–20]. It has been suggested that such water exhibits a liquid–liquid transition at a temperature of approximately 225 K [12–16]. A liquid–liquid transition is thought to exist in supercooled water since glassy water is known to exist in two forms with different density [21]. Associated with this proposed transition is a dynamical crossover, where the fragile (i.e. a highly non-Arrhenius temperature dependence of the viscosity and its related α-relaxation time) high-temperature behaviour would change to a strong (i.e. an Arrhenius temperature dependence) low-temperature behaviour [22]. Furthermore, it has been suggested [14] that this fragile-to-strong transition in the hydration water around proteins causes them to undergo a similar transition. In a recent letter [19] we argued that no true fragile-to-strong transition is present for such hydration water, but that an apparent fragile-to-strong transition occurs where the merged high-temperature α–β-relaxation splits to a local β-relaxation and a non-observable α-relaxation. However, here we show that the low-temperature (i.e. below about 225 K) data reported in [12–16] is neither due to the α-relaxation nor the β-relaxation of the interfacial water, but to an even more local process that is also observed in ice and has been interpreted to arise from the motion of Bjerrum-type defects [23] (due to a non-perfect hydrogen-bonded network of water molecules). On the other hand, the present study indicates that protein motions are slaved by the solvent motions and that a protein therefore undergoes a similar dynamic crossover as its solvent does when the low-temperature β-relaxation transforms to a merged α–β-relaxation at high temperatures ($\approx$200 K).

Thus, in this paper we focus on two currently debated issues: whether a true fragile-to-strong transition occurs for interfacial water and whether protein motions are solvent slaved. The present investigation is aimed to complement our previous studies of supercooled water confined in a wide range of model systems [17–19, 24–29] and biological materials [17, 19, 30–34] by new differential scanning calorimetry (DSC) measurements on fully hydrated Na-vermiculite clay and nanoporous silica of type MCM-41, and dielectric measurements of myoglobin in a water–glycerol mixture.

2. Experimental details

Two nanoporous silica samples of type MCM-41, with pore diameters $d = 21$ and 36 Å, respectively, were prepared by the modified Beck method [35]. The samples were exposed to 100% humidity after being evacuated at 573 K for 24 h to remove impurities. Similarly, a fully hydrated Na-vermiculite clay (provided by Askania, Sweden) was produced by exposing the sample to 100% humidity for 24 h.

The myoglobin used in this study was a horse heart myoglobin from Sigma. The protein was in form of freeze-dried powder, and as dry protein, powder from the bottle was used. The solvents in this study were different water–glycerol mixtures of various water contents. In this paper the results from the dielectric measurements obtained for the sample with 50 wt% water (equal fraction of water and glycerol) at the solvent level $h = 2.0$ (i.e. 2 g solvent per gram protein) is shown. The results from other solvent levels, as well as other water–glycerol compositions will be shown and discussed elsewhere [36].

For the DSC measurements we used a Q1000 calorimeter from TA Instruments. All measurements were performed with cooling and heating rates of $|\mathrm{d}T/\mathrm{d}t| = 10$ K min^{-1}. The samples were measured in hermetically sealed aluminium pans.

The dielectric measurements on myoglobin in water–glycerol were performed on a broadband dielectric spectrometer from Novocontrol. The sample was investigated in the frequency and temperature ranges 10^{-2}–10^{9} Hz and 120–350 K, respectively. For the lower frequencies (10^{-2}–10^{7} Hz) an Alfa-S high-resolution dielectric analyser was used, and the measurements in the higher frequency range (10^{6}–10^{9} Hz) were carried out on an Agilent 4291B RF impedance analyser. The sample was placed between two gold-plated electrodes and a 'Teflon hat' (a film of thickness 100 μm), placed between the sample and the upper electrode, was used in order to reduce the large contribution of electrode polarization to the spectra at low frequencies. The sample thickness was 0.1 mm (determined by silica spacers) for all measurements, and the sample diameter for the low-frequency part was 20 mm, while electrodes of diameter 10 mm were used for the higher frequencies. After preparation, the sample was placed in a sample holder and cooled down to 120 K and then reheated to 350 K while isothermal (±0.2 K) scans were made at every fifth degree. The imaginary part of the dielectric response, $\varepsilon^*(f) = \varepsilon'(f) - \varepsilon''(f)$, was then analysed. The dielectric loss peaks obtained for the protein sample were fitted to several Havriliak–Negami functions:

$$\varepsilon''(\omega) = \sum \mathrm{Im}\left(\frac{\varepsilon_\mathrm{s} - \varepsilon_\infty}{(1 + (\mathrm{i}\omega\tau)^\alpha)^\beta}\right) \tag{1}$$

where $\omega = 2\pi f$ is the angular frequency, τ the relaxation time, and ε_s and ε_∞ are the static dielectric constant and the limiting value of the dielectric constant at high frequencies, respectively. α and β are shape parameters that determine the symmetric and asymmetric broadening of the relaxation peak, respectively.

3. Results and discussion

Figure 1 shows results from DSC measurements on a fully hydrated Na-vermiculite clay and two fully hydrated MCM-41 samples with pore diameters of 21 and 36 Å, respectively. As is evident from the figure, only the MCM-41 sample with a pore diameter of 36 Å shows a strong endothermic peak due to melting of ice in the pores (approximately 30% of the water crystallized in this sample). For the other two samples the confinement is severe enough to prevent the supercooled water from crystallizing. However, despite no significant crystallization occurring, it is not possible to observe any glass transition. One may argue that

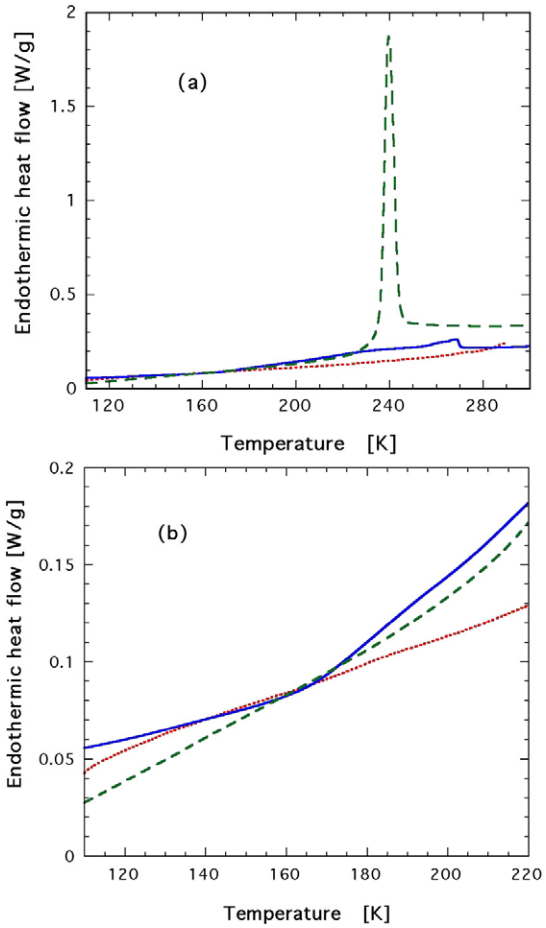

Figure 1. DSC data (heating rate 10 K min^{-1}) of fully hydrated Na-vermiculite clay (dotted line) and MCM-41 with pore diameters of 21 Å (solid line) and 36 Å (dashed line). In (b) the figure is plotted on an expanded scale to verify that no calorimetric T_g can be observed for any of the samples.

this is a natural result due to the severe confinement of the water, but the fact is that for all other liquids we have confined in the same or similar host materials there is no difficulty in observing their calorimetric glass transitions, even for more severe confinements than in the present cases [37]. Thus, the absence of any calorimetric glass transition temperature T_g for the confined supercooled water is an anomalous result in comparison to other liquids, and since the molecular motions that are responsible for the glass transition are given by the viscosity-related α-relaxation it is likely that also a dielectric α-relaxation is absent in the temperature range of an expected T_g. The idea that no α-relaxation is observed in the deeply supercooled regime of such severely confined water is also consistent with recent detailed analysis of the dielectric main relaxation process of confined supercooled water [19]. In [19] it was shown that the 'universal' main relaxation process in systems of severely confined deeply supercooled water exhibits all the typical features of a local β-relaxation. Thus, in this low-temperature regime the more cooperative α-relaxation cannot be observed. We believe that the reason for this is that deeply supercooled water requires an exceptionally extended three-dimensional hydrogen-bonded network in order to show the α-relaxation (and thereby a calorimetric T_g), in

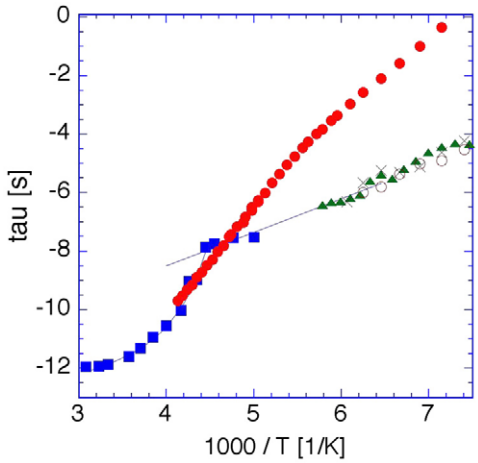

Figure 2. Relaxation times for confined water from dielectric spectroscopy and quasi-elastic neutron scattering (QENS). The data points correspond to the main relaxation process of water confined in approximately 20 Å pores of MCM-41, obtained by QENS [12] (squares) and dielectric spectroscopy [29] (filled circles). In addition, the faster 'universal' dielectric relaxation process, due to the motion of Bjerrum-type defects, of supercooled water in clay [25] (triangles), phospholipids membrane DMPC [44] (open circles) and myoglobin (crosses) is shown for comparison. The lines are fits to the high- and low-temperature QENS data, taken from [12].

contrast to most other liquids where the cooperatively rearranging regions are smaller or can adapt their shape to the confinements such that the α-relaxation can appear [19]. An apparent fragile-to-strong transition occurs around 200 K (depending on the confinement) where the low-temperature β-relaxation merges with the non-observable α-relaxation. As is evident in figure 2, such a crossover in the temperature dependence of the dielectric main relaxation process from a low-temperature β-process to a merged α–β-process at high temperatures is clearly seen at about 180 K for water confined in 21 Å pores of MCM-41. However, as is also shown in figure 2, an even more dramatic transition from a high-temperature non-Arrhenius dependence to a low-temperature Arrhenius behaviour has been observed to occur at about 225 K in quasi-elastic neutron scattering (QENS) and ^{1}H nuclear magnetic resonance (NMR) diffusion measurements on confined and hydration water [12–16]. In figure 2 we show that the low-temperature behaviour observed in the QENS and NMR measurements extrapolates to the second, and faster, 'universal' dielectric process. This dielectric process seems to be present in all systems containing deeply supercooled water, as well as in ordinary ice [25]. It has been interpreted to arise from the motion of so-called Bjerrum-type defects, which are orientationally disordered water molecules that are hydrogen bonded to fewer than four other water molecules [23]. Such water molecules can perform local reorientations, and first-principles calculations have shown [38–41] that this leads to a migration of the defects. Moreover, the first-principles calculations show that long-range proton diffusion is directly coupled to the migration of the defects [38–41]. This explains how the propagation of very local reorientational motions of single water molecules can give rise to the long-range proton diffusivity measured by QENS and NMR. Hence, the long-range motion of the protons, as measured by these techniques, is completely decoupled from the long-range motion of the water molecules. The 'fragile-to-strong transition' observed at about 225 K in [12–16] is therefore only a result of a decoupling between the merged α–β-relaxation and the diffusion of single protons. Since this proton diffusion is not related to the α-relaxation (or viscosity) of the deeply

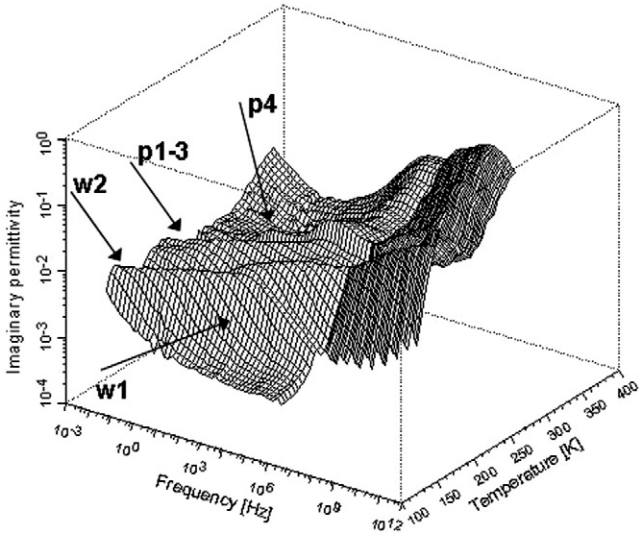

Figure 3. Imaginary part (ε'') of the dielectric spectra as a function of frequency and temperature for a sample containing an equal weight fraction of myoglobin, water and glycerol (i.e. 50 wt% water in the solvent, and a total solvent content $h = 2$). The intensity at the highest frequencies and lowest temperatures was too low to give reliable data points, and consequently these data points have been removed from the measured spectra. At least two solvent processes, denoted w1 and w2, and four protein processes[2], p1–p4, could be observed. See the main text for assignments of the processes.

supercooled water it cannot define the fragility of the liquid. Thus, also this crossover in the temperature dependence of the observed relaxation time should not be considered as a true fragile-to-strong transition, as it is not reflecting any transition in the relaxational dynamics of the water molecules.

Figure 3 shows the imaginary part (ε'') of the dielectric spectra as a function of temperature and frequency for myoglobin in a water–glycerol mixture of 50 wt%. The amount of myoglobin was the same as for each solvent component. As is evident from the figure, the sample exhibits a complicated relaxation behaviour, although two dielectric loss peaks seem to dominate the spectra. However, a closer look at the peak denoted p1–p3 in figure 3 shows that it actually contains three relaxation processes of the protein[2], although not all of them are present in the whole temperature range. In addition to these three protein processes one slower protein process[2] (p4) and two faster solvent processes (w1 and w2) can be observed. In figure 4(a) we present relaxation times obtained from the curve fitting (using equation (1)) of the spectra that are shown in figure 3. The solvent process w1, which is only observed at low temperatures, is due to the motion of Bjerrum-type defects, as discussed above for confined water. The slower solvent process w2 is at low temperatures identical to the 'universal' β-relaxation in deeply supercooled confined water, as also discussed above for confined water. At approximately 200 K this β-process transforms to a merged α–β-relaxation of the water–glycerol mixture, in analogy with the scenario for confined supercooled water where a merging with a non-observable α-relaxation occurs at about the same temperature. At the crossover temperature at 200 K the dynamics changes from a low-temperature Arrhenius behaviour to a non-Arrhenius dependence at high temperatures.

[2] Due to the inhomogeneous nature of the sample a Maxwell–Wagner polarization may arise. If so, one of the processes p2–p4 might not be due to protein motions.

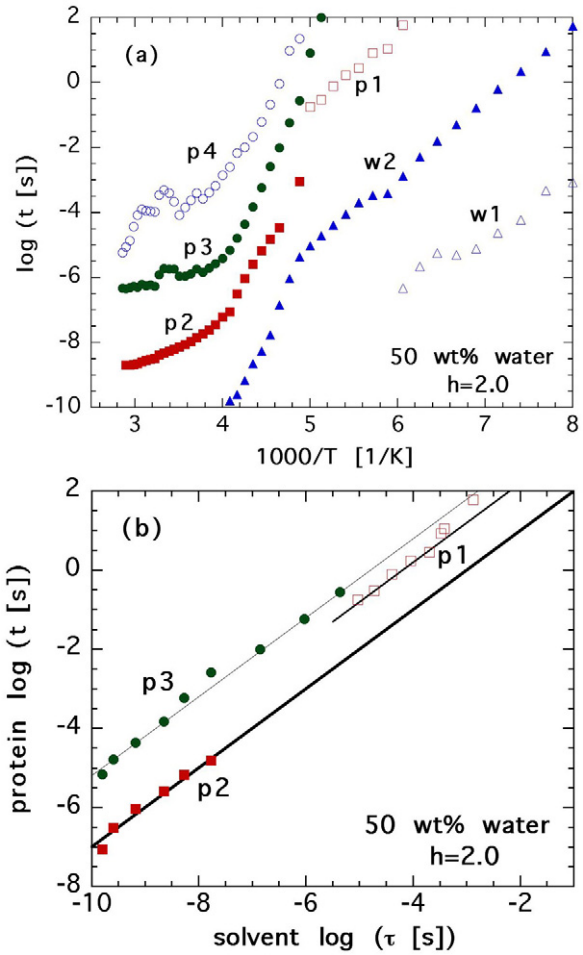

Figure 4. (a) Dielectric relaxation times obtained for the solvent and protein processes shown in figure 3. Process w1 corresponds to the motion of Bjerrum-type defects in supercooled water (open triangles), w2 to the main relaxation process in the solvent (filled triangles), p1 to local motions in the protein (open squares), p2 to motions of polar side groups of the protein (filled squares), and p3 and p4 to conformational protein fluctuations (open and filled circles). (b) The relaxation times of the three fastest protein processes (p1–p3) are shown as a function of the relaxation time for the main process in the solvent (w2). Note the linear dependences between both the local protein process and the low-temperature (β-like) solvent process (below 200 K) as well as between the two fastest protein processes and the main (α) solvent relaxation above 200 K.

In figure 4(a) it is also seen that only one protein process (p1) is present below 200 K. This process also shows an Arrhenius temperature dependence, and is therefore attributed to local non-cooperative protein motions. At about 200 K, where the local β-relaxation in the solvent merges with the non-observable α-relaxation, there is a significant change in the protein dynamics. Several cooperative protein processes appear, suggesting that the onset of more large-scale solvent motions give rise to a glass-transition-like phenomenon in the protein. The origin of the fastest cooperative protein process (p2) has been attributed to motions of polar side groups [30, 42], and the slower processes (p3 and p4) are most likely due to more global conformational fluctuations in the protein [43]. A more complete account of the results from

the analysis of this system as well as other compositions and volume fractions of the water–glycerol solvent will be given elsewhere [36].

In order to investigate the relation between the solvent dynamics and the observed protein processes the relaxation times of the three fastest protein processes (p1–p3) have been plotted as a function of the relaxation time for the main process in the solvent (w2); see figure 4(b). If two relaxation processes have the same temperature dependence there should be a linear relation (i.e. slope of unity in a log–log plot) between them. This is also what is observed in figure 4(b) for the three fastest protein processes. This implies that the local protein process (p1) has the same activation energy as the low-temperature β-relaxation (w2 below 200 K) in the solvent, and that the two cooperative protein processes (p2 and p3) exhibit a similar non-Arrhenius temperature dependence as the solvent does in the temperature range where the α- and β-relaxations are merged (i.e. process w2 above 200 K). Therefore, our findings support a recent study by Fenimore *et al* [8], where it was proposed that the most local protein motions are slaved by the β-relaxation in the solvent whereas the more global protein motions are slaved by the α-relaxation in the solvent. These results further suggest that no global, and biologically most important, protein motions can occur in water-rich solvents at low temperatures where no α-relaxation is present in the solvent. This is of course an important result for cryopreservation of food.

Finally, it should be noted that although solvent dynamics is essential for protein motions and functions there are other characteristics that make proteins unique. In fact, recent studies of other soft biological materials, such as lipid membranes, have indicated that certain types of motions in these materials are also solvent slaved [44]. This suggests that the concept 'solvent slaving' is not unique for proteins.

4. Conclusions

In this paper we show that the earlier proposed [12–16] fragile-to-strong transition of supercooled water in confined geometries and around biomolecules is not a true transition since the low-temperature (below $\approx$225 K) Arrhenius behaviour is most likely due to a proton transport process facilitated by the migration of Bjerrum-type defects that are present in both supercooled water and ice. The Bjerrum-type defects are due to water molecules that are not fully hydrogen bonded, and these water molecules can only perform local reorientations at low temperatures. Therefore, the apparent fragile-to-strong transition at about 225 K is most likely due to a decoupling of the motion of the Bjerrum-type defects (and the associated proton transport process) from the merged high-temperature α–β-relaxation.

The most interesting result of this study is, however, that we found a similar crossover in the protein dynamics as for the solvent around 200 K where there is an onset of global cooperative motions both in the protein and the solvent. Thus, below $\approx$200 K it seems that there are only local protein motions that are slaved by a local (β-like) relaxation in the solvent, whereas at higher temperatures (above 200 K) a merged α–β-relaxation in the solvent determines the more global protein motions. This behaviour is therefore consistent with the findings by Fenimore *et al* [8] that large-scale conformational changes of the protein are slaved by the α-relaxation in the solvent and more local protein motions are slaved by the β-relaxation in the hydration shell.

Acknowledgments

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