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Structure and slow dynamics of protein hydration water

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ABSTRACT

We report results on the structure, local order and dynamics of water surrounding a lysozyme protein. The local order of water molecules is as much tetrahedral as in bulk water already at close vicinity of the protein but the number of hydrogen bonds depends more on the distance from the protein and gradually recovers bulk value upon moving outer. The dynamics of water seems in general to be more affected than its structure by the presence of the protein. An extremely long-relaxation detected in hydration water appears in the first monolayer around the protein, and the slow down is enhanced at low temperature. The dynamics of water within a layer of thickness 6 Å is sub-diffusive up to about ~1 ns, above 1 ns we observe a crossover toward a hopping regime over a length-scale larger than that of nearest neighbors molecules. This hopping seems connected to transient trapping of water molecules on some specific protein domains.

1. Introduction

The first layers of water surrounding proteins play a crucial role for their biological activity [1]. For this reason a huge amount of theoretical and experimental work [2-10] have been done to elucidate the physical properties of this kind of hydration water. These properties pertain the structure, the hydrogen (H) bonds and the dynamics of hydration water.

The three dimensional H-bond pattern of water is disrupted at the protein surface [11]. Hydration water can in fact bind or not to the protein, and if it does, depending on the hydrophilicity of the surface patches of the protein, it does via H-bonds. The number of water molecules engaged in protein-water H-bonds is linearly dependent on the temperature and experiences an abrupt increase of the slope in the supercooled regime [5]. It is worth mentioning that upon supercooling the protein undergoes the so-called protein dynamical transition [12,13], connected to the behavior of the mean square fluctuations of the protein itself [14]. Concerning water-water H-bonds, it has been shown that hydration water is able to form a two dimensional H-bond network around the protein and the stability of this network depends on the specific protein residue [15].

Hydration water experiences perturbations also from the dynamical point of view. Evidences of two distinct long relaxation times have been found experimentally [16-20]. The translational motion is slowed down with respect to bulk water already at ambient conditions and whereas bulk water shows a single structural α-relaxation, hydration water shows also in simulations two distinct structural relaxations [21-23]. One is the α-relaxation typical of many glass formers [12,24]. The other, the long-relaxation, is characterized by a longer relaxation time and an higher stretching character with respect to the α-relaxation [21-23]. The two relaxations show different temperature behavior. The α-relaxation of hydration water shows upon cooling a fragile-to-strong crossover like in bulk water [12,24]. while the dynamics of the long-relaxation, coupled to protein internal dynamics, shows a strong-to-strong crossover upon cooling and this occurs at the protein dynamical transition [14], therefore at higher temperature than the fragile-to-strong transition of the α-relaxation [23].

In this work we analyze the structure of hydration water in different layers around the protein and characterize its hydrogen bonding behavior. We also show the translational dynamics in a particular layer of thickness 6 Å which corresponds to a double molecular layer of water around the lysozyme. We analyze the dynamics by means of density self correlation functions in the ($\mathbf{r}$,t) and in the ($\mathbf{q}$,t) space to access to complementary information. These functions are respectively called the Self van Hove Function and the Self Intermediate Scattering Function.

The work is organized as follows: we describe in Section 2 the simulation methods and in Section 3 the investigated protein system. In Section 4 we present the results obtained probing the structure of hydration water and its hydrogen bond network. Section 5 is devoted to results on the dynamics, where we first present Self Intermediate Scattering Function and then Self van Hove Functions of hydration

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and bulk water. In Section 6 we draw our conclusion and discuss future directions.

2. Methods

A lysozyme protein immersed in water was simulated through classical Molecular dynamics (MD) all-atoms simulations. The cubic simulation box contains 1 lysozyme protein, 13982 water molecules and 8 Cl^- ions to maintain the total charge neutrality.

Protein interactions were described by the CHARMM force field [25,26] and water was modeled by the SPC/E potential [27]. The cutoff radius for the non-bonded van der Waals interactions was set to 10 Å. The Particle Mesh Ewald algorithm was used for the electrostatic interactions. Equations of motion were integrated with a time step of 1 fs. Equilibration runs were performed in the NPT ensamble for 30 ns at $T = 300$ K and 40 ns at $T = 250$ K. Both pressure and temperature were controlled using weak coupling algorithms [28]. Pressure is fixed at 1 bar. Then we execute production runs of 10 ns for analyzing the structure and further 20 ns for the dynamics. The frequency of coordinates storage is 500 fs and 1 fs respectively. Molecular dynamics simulations were also performed using the SPC/E water model on a box composed of 500 molecules in similar conditions. The Gromacs 4.5.5 [29] simulation package was used for all trajectories. Details on the protocol used to construct the simulation box can be found in Ref. [22].

3. Lysozyme and its hydration water

The lysozyme is an antimicrobial enzyme consisting of 129 amino acid residues having a molecular weight of 14.4 kDa. It folds into a compact globular structure having an ellipsoidal shape with dimensions $a \times c \times c = 2.25 \times 1.3 \times 1.3$ nm. The active site of lysozyme consists of a deep crevice, which divides the protein into two domains linked by an alpha helix. One domain consists almost entirely of beta-sheet structures, while the other domain is predominantly alpha-helical [30]. Our lysozyme is shown in Fig. 1, where we colored it according to the hydrophobicity/hydrophilicity of its residues. Note how hydrophobic sites are in general located more in the internal part of the protein, and this is very common for small globular proteins.

The structural organization of water around a protein is intimately related to the kind of protein sites that it is close to. A powerful tool to probe the local organization of water molecules around selected protein sites is the Radial Distribution Function (RDF). We calculated the RDF for the oxygen atoms of water with respect to a heavy atom of the protein. In Fig. 2 the RDFs calculated for the two extreme cases observed in our system are presented, namely curves that show two peaks within 6 Å from the protein heavy atom and curves that don't. In the example of the hydrated site we chose a surface site, therefore the protein atom is exposed to water. In this case water molecules organize around the site, creating correlation shells resembling the order of bulk water. Differently, when we selected a buried site the RDF shows a depletion region which extends outer up to about 6 Å, and where the probability of finding water molecules is very low.

From these RDFs we can see that considering water within 4 Å and 6 Å from the protein corresponds to analyze the behavior of the first layer and the first plus the second layers around the protein respectively. We will refer to this water as hydration water.

In Table 1 we report the number of water molecules that reside on average in the various investigated layers for two temperatures of our system, $T = 300$ K and $T = 250$ K. We consider a water molecule inside the layer $(r_1 - r_2)$ when the oxygen atom is located at a distance d such that $r_1 \leq d \leq r_2$ from the closest lysozyme atom.

4. Tetrahedrality and hydrogen bonds in hydration water

We studied the properties of the H-bond network of hydration water in the different layers around the lysozyme reported in Table 1. We start this analysis by calculating the probability distribution $P(\cos\gamma)$ of the angle γ between the two vectors joining the oxygen atom of a central water molecule with the oxygen atoms of two neighbor water molecules at a distance $d < 3.5$ Å, this distance corresponds to the first minimum of the water oxygen-oxygen RDF. The geometry of the angle γ for three molecules is shown in the top part of Fig. 3. This angle is a good probe of the local environment of a water molecule. If the water molecule is surrounded by four water molecules in a perfect tetrahedral structure the angle is $\gamma = 109.5^\circ$ ($\cos\gamma = -0.334$).

Fig. 3 shows the distributions of $\cos\gamma$ of hydration water in the different layers around the lysozyme and in bulk water at $T = 300$ K (upper panel) and $T = 250$ K (lower panel). The distributions are normalized to unit area.

At $T = 300$ K the distribution $P(\cos\gamma)$ of bulk water shows a broad peak with the maximum located at $\gamma \sim 104.5^\circ$ ($\cos\gamma = -0.250$), this peak is the signature of the nearly tetrahedral order present in liquid water at short range distance. At $T = 250$ K this peak becomes sharper, as the population of water molecules assuming tetrahedral structure is enhanced upon decreasing the temperature. Interestingly this angle corresponds to the experimental intermolecular angle HÔH of water, even though the SPC/E model has a fixed geometry with $\hat{O} = 109.47^\circ$. A secondary sharp peak is found at $\gamma \sim 53^\circ$ ($\cos\gamma = 0.6$) at both the temperatures, this peak corresponds to interstitial neighbor molecules. These findings are in agreement with previous results on SPC/E water at ambient conditions [31].

The behavior of the distributions $P(\cos\gamma)$ calculated for hydration water is shown in the same figure. We start by looking at the curve relative to the layer 0–4 Å, which shows at both the temperature the largest deviation with respect to bulk water. At $T = 300$ K, this distribution shows a broad peak centered at $\gamma \sim 109.5^\circ$, this is the tetrahedral peak at the correct tetrahedral angle. It is also evident by comparison with bulk water that there is a larger probability to find neighbor molecules at higher angles (look the shoulder at $\gamma \sim 140^\circ$) and correspondingly a depletion of the population assuming angle values $\gamma = 75$–90°. This effect is more evident at low temperature. The secondary sharp peak due to interstitial water is located at the bulk angle.

By looking at the layer 0–6 Å, we see that as soon as we include the second molecular layer of water in the analysis the tetrahedral peak moves closer to the value of bulk water, but at lower angle. This "jump" is plausibly due to the larger population of hydration water which assumes $\gamma \sim 75$–90° respect to the bulk. This population decreases as we move toward outer layers and correspondingly the tetrahedral peak increases and shifts toward the bulk value. The analysis on the water molecules belonging to outer shell shows that the more we move out from the protein the more hydration water recovers bulk-behavior, in fact the curves approach the bulk distribution progressively. The angle of interstitial water is completely unaffected by neither the environment (protein) nor the temperature.

Globally we can say that the distortions induced by the protein in the water H-bonds network are not much pronounced for hydration water. This is very similar to what happens to for water in hydrophobic confinement [32], in opposition to water in hydrophilic confinement, that completely suppresses the tetrahedral peak of water in the first 4 Å from the wall [31]. The largest deviation found for the shell 0–4 Å is related to the fact that this correspond the the first layer around the protein, therefore water molecules perturbed the most by the interaction with protein. We note in fact that water molecules belonging to this monolayer completely miss water neighbors on the protein side of the shell. On the other hand being the