Untangling complex dynamics of biological water at protein–water interface

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Water becomes profoundly modified near large biomolecules like proteins and DNA to give rise to a range of interesting dynamical properties that are yet to be understood fully. Due to the intimate connection between the function of these bimolecular systems with the structure and dynamics of the hydration water, there has been increasing attention on the properties of this so-called “biological water” (1–5). Divergent views, however, have recently emerged on the details of the dynamical properties, especially regarding the dominant relaxation timescales of relevant time correlation functions (4, 5). Estimates of relaxation times range from a few picoseconds to a few nanoseconds. These have been derived from experiments that use different techniques, different probes, and different protein molecules (3–5). The paper by Qin et al. (6) discusses results of detailed experimental (and simulation) studies of water at the surface of a specific protein, DNA polymerase IV (Protein Data Bank ID code DPo4). The experimental method used is time-dependent fluorescence Stokes shift (TDFSS), which is a popular tool in the study of dipolar solvation dynamics (1). Qin et al. make a valuable contribution toward understanding the myriad anomalous dynamical properties of the protein hydration layer, in particular on the role of amino acid side-chain motions in determining the relaxation behavior of the hydration layer.

The complexity of a protein–water interface, with its narrow width (only a few nanometers thick), makes a quantitative study, and understanding of its structure and dynamics a challenging task (6–11). In addition, a protein surface is highly heterogeneous, with a mixture of seemingly randomly placed amino acid residues of diverse polar character. Water molecules close to the protein therefore experience different environments from point to point as we travel along the protein surface. As mentioned, Qin et al. used TDFSS, using the amino acid residue tryptophan (Trp) as a natural probe (6). By using mutation of the wild variety, the authors placed the probe Trp at different locations on the protein. This allowed the authors to obtain detailed information about the region-specific dynamics of water surrounding the protein. As expected, Qin et al. found that dynamics are heterogeneous with timescales ranging from a few hundred femtoseconds to a few hundred picoseconds. The authors provide a molecular picture in terms of coupling between amino acid side chains and water molecules.

The problem at hand may be understood better if we briefly summarize the results of solvation dynamics of a dipolar solute in neat water. In this case, solvation dynamics are found to proceed in three distinct stages.
(12). The main component is ultrafast with a time constant of about 50 fs. The intermediate component proceeds with a time-scale of about 250 fs. The last, slowest component has a time constant of about 1 ps. These three components are known to have definite dynamical origin. The ultrafast 50–fs component is assigned to the underdamped collective polarization relaxation aided in speed by the large dielectric constant of water (which provides a large force constant for relaxation back to equilibrium) and by the intermolecular vibrational modes located at frequency 190–200 cm$^{-1}$ and the librational mode at 600 cm$^{-1}$. The intermediate component with time constant of $\sim$250 fs is attributed to a mixture of rotation and translation motions of water molecules. The slowest time ($\sim$1 ps) is attributed to the damped polarization relaxation that is slowed down by intermolecular correlations. In this context, it is useful to remember that the longitudinal solvation time of dipolar solute predicted by the continuum model is given by the following (12):

$$t_0^L = \frac{2(\varepsilon_0 + \varepsilon_c)}{2\varepsilon_0 + \varepsilon_c} \tau_0,$$  \[1\]

where $\varepsilon_0$ and $\varepsilon_c$ are the static and infinite-frequency dielectric constants, respectively; $\tau_0$ is the Debye relaxation time of the dipolar solvent; and $\varepsilon_c$ is the dielectric constant of the molecular cavity. For liquid water at ambient conditions, this expression provides an estimate of about 500 fs for the solvation time. As mentioned above, the solvation dynamics proceed quite differently from what is envisaged in the continuum model picture. However, one important insight to be carried forward is that the solvation response time gets shortened by a factor of about 20 from the dielectric relaxation time of water (which is 8.3 ps).

The importance of the local character of protein–water interactions in the hydration dynamics at protein surface is nicely demonstrated in figure 1C of the paper by Qin et al. where probe is placed at three different locations with fairly different results for the solvation time correlation function. The differing dynamics experienced by the probe are then a consequence of differing local solvent dynamics originating from different local structure at the protein surface. These results clearly prove, perhaps for the first time, the heterogeneous nature of water dynamics near a protein surface. Several additional important results have emerged from the studies reported in ref. 6, which deserve further discussion and scrutiny. First, dynamics probed are sensitive not only to the exposure of the Trp residue but also to the polar character of the surrounding medium. Dynamics are slow when the probe is buried inside the protein and fastest when exposed completely to water. The former shows that the electric field of the probe gets screened by the protein and hydration water. Dynamics are also slow when the probe is placed close to a charge on a protein side chain. The latter is due to the presence of slow water molecules that are hydrogen bonded to the charge on the amino acid side chain.

To place the results of this work in long-term perspective, we need to consider the understanding that already exists in this problem. Earlier dielectric relaxation studies by Grant, Pethig, Mashimo, and others have observed the presence of a slow timescale of the order of 50–100 ps (1). Nuclear magnetic relaxation (NMR) experiments have provided different results. Initial studies of Wüthrich predicted the presence of a slow component, of the order of 300 ps (7). Later studies contradict this result and assert that water molecules in the protein hydration layer can slow down by no more than a factor of 2–5 from that in bulk water (5). Several computer simulation studies of single-particle orientational relaxation also find that only a few water molecules on the surface experience substantial slow down while most of the water molecules retain the mobility of free water molecules as in the bulk (13).

Because NMR experiments and most molecular dynamics simulations measure and monitor single-particle relaxation dynamics, the values obtained are bound to be different from those measured by dielectric relaxation and solvation dynamics experiments that probe collective response. As is well known, the last two also give vastly different results (12). This salient point is often missed by researchers in the area. Dielectric relaxation time for water (8.3 ps) is slower by a factor 4–5 from the time obtained from NMR where relaxation time is about 2 ps.

Similarly, solvation dynamics provide times that are usually shorter than $\tau_0$, as exemplified in Eq. 1 given above. However, most of these predictions hold only for neat water. Dielectric response of the first hydration layer can be quite different from neat water, as discussed in ref. 13.

In the work of Qin et al., the slow dynamics are found to originate partly from the coupling of water molecules with the amino acid side chains that contains the polar, often charged groups (figure 2 of ref. 6). This coupling may arise partly from hydrogen bond-breaking dynamics of those water molecules that are strongly hydrogen bonded to the polar groups in amino acid side chains (like arginine, methionine). Second, for the exposed probes, an ultrafast component naturally arises from bulk water. Third, we now begin to understand why NMR may miss the slow component that is routinely picked up by TDFSS.

A stated objective of the present work is to establish a connection with the “solvent slaving protein fluctuations” model of Frauenfelder et al. (8, 9). In Frauenfelder’s picture (derived largely from decades-old experimental studies on myoglobin using Mossbauer spectroscopy and dielectric relaxation experiments), protein’s conformational fluctuations, key to the activity of protein, are determined by motions of water. One identifies two different contributions to the energy fluctuations of protein: a $\beta$-relaxation from coupling between side-chain motions with the hydration water and a second contribution (called $\alpha$-relaxation) from the bulk water. The present results seem to agree with the picture of Frauenfelder in terms of separation of timescales and magnitude of the two interactions. Qin et al. also deftly demonstrate the coupling between hydration dynamics and side-chain motion as envisaged by Frauenfelder.

An effective way to describe different solvent response in different layers as we move away from the protein surface is described in Fig. 1, which complements figure 4 of Qin et al. Water dynamics in the first layer are influenced by the side-chain motion. The longer side chains can make transitions between different conformations states that are characterized by different orientations of the dihedral angles that the side-chain bonds subtend in a chosen coordinate system. When the side chains are polar (like arginine), water molecules are relatively strongly hydrogen bonded to the polar atoms. Therefore, transitions of the side chain between different rotomeric states are associated with rotation of the water...
molecules that are hydrogen bonded to the polar residue. Hydrogen bonds may naturally break and reform during such a transition. These motions naturally couple to the solvation dynamics of the excited Trp residue. This whole process may thus introduce two timescales (one due to hydrogen bond equilibrium and the other due to side-chain conformational transitions) which may range from a few tens to a few hundred picoseconds. Recently, attempts have been made to account for the conformational degrees of freedom of side chains through enumeration of side-chain entropy (11). We note that the relation between dynamics and entropy is a well-addressed topic in condensed matter physics, particular in the area of glass transition of a supercooled liquid (14). One can thus make connection with the protein–glass transition where side-chain fluctuations are assumed to play an important role (8–10).

Thus, the scientific discourse of dynamical interaction between water and protein is laden with many possibilities and the work by Qin et al. touches on a few aspects of the problem. Several issues remain to be discussed like the distance into the bulk up to which the effect of the protein is felt and influences water’s structure and dynamics. This is a problem poorly understood. Because different proteins have different side chains, dynamics of hydration layer must vary from protein to protein. We already mentioned the possible relationship of the dynamics with side-chain entropy. Because of the immense complexity of protein–water interfaces and also because of the huge number of proteins that are involved in biological processes, it makes sense to develop an energy landscape picture that can rationalize the general behavior in some semiquantitative fashion. Qin et al. also summarize their results in their figure 4 in terms of an energy landscape (6).

The relationship between amino acid side chain motion and water dynamics has never been addressed adequately in theory or simulation. It clearly depends on feedback amongst the two, leading to a cooperativity that is vital to many of the enzymatic activity of proteins performed through the side chains. For example, the three arginine residues with their positive charge help adenylate kinase (ADK) capture ATP and bring it towards the opening called LID, so that reaction with the AMP can occur to give two ADP (15). Interactions between water and arginine residues give a rugged energy landscape and can alter the catalytic pathway, as discussed in ref. 15. However, the detailed dynamic role of water has not been investigated.

Acknowledgments
I thank Sir J. C. Bose Fellowship and the Department of Science and Technology (India) for partial support of this work.