Nature of biological water: a femtosecond study

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The quasi-bound *biological or structured* water molecules in a protein play a key role in many biological processes. The dynamics of the biological water has been studied by femtosecond spectroscopy and large-scale computer simulations. Solvation dynamics of biological water displays an almost bulk-water like ultrafast component ($\sim$1 ps) and a surprising slow component at the 100–1000 ps time scale. In this article, we discuss several examples of the ultraslow component, its possible origin and implications in biology. We show that the ultrafast ($\sim$1 ps) component arises from an extended hydrogen bond network while the ultraslow component originates from binding of a water molecule to a biological macromolecule.

Introduction

Water is often hailed as the *lubricant of life* for its central role in biology.[1] The hydrophilic and hydrophobic interactions involving water control the structure of a protein and formation of a self-organized assembly.[2,3] The water molecules present at the surface of a protein or DNA guide the incoming substrates and thus act as a molecular usher. Water-mediated interactions are fundamental in DNA replication and repair,[4] protein–protein and protein–DNA interaction,[5] molecular recognition by a DNA[6] or a protein,[7] electron transfer[8] and proton transfer[9] in a protein.

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There is a longstanding interest to understand the nature of *biological water*[10] *i.e.* the water molecules near a biological system. The biological water differs from bulk water in a number of ways. First, clustering of the water molecules at the surface of a protein increases the local density by as much as 25% compared to bulk water.[11] Second, disruption of the water–water hydrogen bonds and replacement by the water–protein hydrogen bonds prevent freezing of water (*i.e.* formation of ice). The hydration layer of many proteins does not freeze even at sub-zero temperatures and thus sustains life at low temperatures.[12–14] Third, in bulk water, mutual polarization of the hydrogen bonded water molecules increases the dipole moment and dielectric constant.[15] Such polarization is absent for a water molecule hydrogen bonded to a biological system. As a result, biological water is less polar than bulk water.

While the static aspects of biological water have been studied for a long time, its dynamics has been explored only recently. In this feature article, we focus on the recent studies on biological water using femtosecond spectroscopy. As will be illustrated later in this article, the biological water differs markedly from bulk water both in the ultrafast (a few ps) and ultraslow time scale. Perhaps, the most significant discovery is the ultraslow component of biological water at the 100–1000 ps time scale. This is 2–3 orders of magnitude slower compared to bulk water. The early works on the origin and the implications of this ultraslow component has been reviewed before.[16–18] Subsequent application of femtosecond spectroscopy and large-scale computer simulations on a wide variety of systems have significantly improved our understanding of biological water and its effect on ultrafast chemical processes.[19–21] In the present feature, we summarize the latest developments in this area.

In this article, we will discuss the results of femtosecond dynamic solvent shift (DSS) studies of biological water. DSS has certain advantages over other techniques. First, the time resolution of the DSS down to femtosecond is vastly superior to other techniques *e.g.* NMR,[22,23] dielectric relaxation

(DR)[16,24] or quasi-elastic neutron scattering (QENS).[24] Second, about 85% of the DSS arises from the first solvation shell of a fluorescent probe.[25] Thus DSS has excellent spatial resolution and reports dynamics in the immediate vicinity of the fluorescent probe. QENS, NMRD and DR on the contrary, capture signals from everywhere and thus, give only an average picture. Since in a dilute solution of a protein, >90% signal (in QENS, NMRD or DR) is due to bulk water it is difficult to study selectively the biological water, using these techniques.

We will begin this feature article with a brief introduction to DSS. We will then illustrate DSS in many systems *e.g.* cyclodextrin, protein, micelles, reverse micelles, lipid vesicles, polymer and DNA.

Solvation dynamics and dynamic solvent shift (DSS)

Solvation dynamics refers to the dynamics or time scale of solute–solvent interaction. If a charge or a dipole is suddenly created in a polar solvent, the solvent dipoles reorganize about the charge (or the solute dipole). The main aim of a solvation dynamics study is to determine the time constant of the solvation process. For this purpose, one chooses a fluorescent probe whose dipole moment is very small in the ground state but is very large in the excited state. When such a solute is in the ground state, the solvent dipoles remain randomly arranged around the solute in its ground state. On excitation of the solute (probe) by an ultrafast laser, a dipole is created suddenly. Immediately after creation of the solute dipole, the solvent dipoles are randomly oriented and the energy of the system is high. With increase in time, as the solvent dipoles reorient the energy of the solute dipole decreases (Fig. 1(A)). As a result, the fluorescence maximum gradually shifts to lower energy *i.e.* towards longer wavelength (Fig. 1(B)). This is known as dynamic solvent shift (DSS).

Evidently, at a short wavelength, the fluorescence corresponds to the unsolvated solute and exhibits a decay. At a long wavelength, the fluorescence originates from the solvated species and a rise precedes the decay. The rise at a long wavelength is a clear signature of solvation. Thus solvation dynamics leads to a wavelength dependence of fluorescence decays with rise at long emission wavelength and decay at short wavelength (Fig. 1(C)). The time-resolved emission spectra (TRES, Fig. 1(B)) may be constructed using the steady-state emission spectra and the fluorescence decays.[25]

Solvation dynamics is monitored by the decay of the time-correlation function $C(t)$ which is defined in terms of the emission energies as,[25]

$$C(t) = \frac{\nu(t) - \nu(\infty)}{\nu(0) - \nu(\infty)} \quad (1)$$

where, $\nu(0)$, $\nu(t)$ and $\nu(\infty)$ are the observed emission frequencies at time zero, t and infinity, respectively. In many cases, a portion of the solvation dynamics is too fast and goes undetected even in a femtosecond set up. Fee and Maroncelli developed a simple method to calculate the amount of solvation missed.[26]

According to the continuum theory, the solvation time (τ_s) is given by,[18]

$$\tau_s^{-1} = 2D_R[1 + (D_T k^2/2D_R)] \quad (2)$$

In bulk water, the translational diffusion coefficient, $D_T = 2.5 \times 10^{-9}$ m^2 s^{-1}, rotational diffusion coefficient, $D_R = 2.2 \times 10^{11}$ s^{-1} and $k \sim 2\pi/1.5\sigma$, with diameter of water molecule, $\sigma \sim 2.8$ Å. Using these parameters, $\tau_s \sim 1$ ps in bulk water. According to recent experiments,[27,28] solvation dynamics in bulk water is extremely fast with a major component at the 0.1 ps (100 fs) time scale and a minor component of 1 ps. While the 1 ps component is consistent with the continuum model, the major sub-100 fs component of solvation dynamics can not be explained by the simple continuum model. The ultrafast sub-100 fs component has been ascribed to intermolecular vibration and libration of the extended hydrogen-bond network in bulk water.[29]

Solvation dynamics in biological assemblies

In this section, we discuss solvation dynamics in several organized assemblies. Fig. 2 shows the structure of a few organized assemblies and of several well known solvation probes. Solvation dynamics in many biological and organized assemblies exhibit an ultraslow component in 100–1000 ps time scale whose contribution varies from system to system. We will discuss a simple analytical model which explains the ultraslow dielectric response of biological water. This model seems to be applicable to a wide variety of systems such as proteins, micelles and cyclodextrins. Most biological assemblies are heterogeneous on the molecular length scale. We will discuss that even within one assembly the solvation dynamics may vary markedly from one site to another.

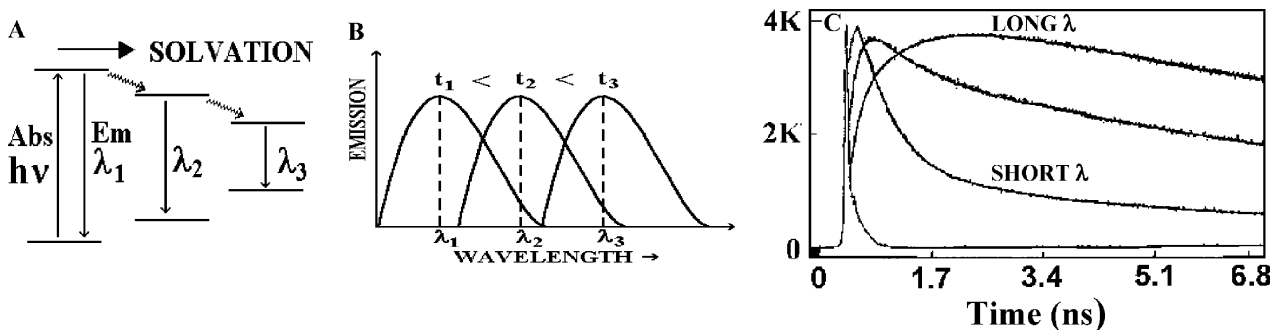

Fig. 1 Solvation dynamics: (A) decrease in the energy of the excited state; (B) time-resolved emission spectra (TRES); (C) wavelength dependent fluorescence decays.

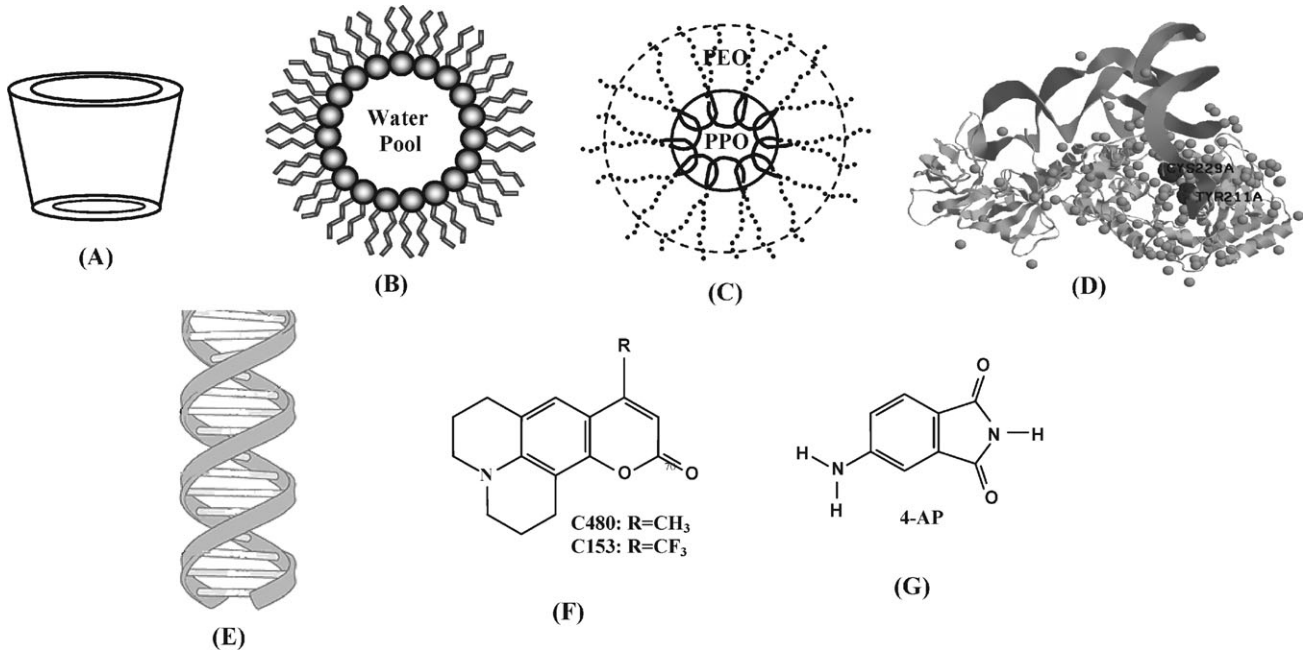

Fig. 2 Structure of: (A) cyclodextrin; (B) reverse micelle; (C) P123 micelle; (D) protein (GlnRS); (E) DNA; (F) Coumarin dyes; (G) 4-aminophthalimide (4-AP).

Origin of ultraslow dynamics of biological water

On the basis of NOE studies, Wuthrich and co-workers first qualitatively proposed that the water molecules near a protein may be classified as, "bound" and "free".[23] Nandi and Bagchi extended this idea to develop a phenomenological theory for the ultraslow dielectric response.[30] In this model, "bound" water molecules refer to those which are hydrogen bonded to a biological macromolecule and are largely immobilized. In contrast, the "free" water molecules which are not hydrogen bonded to the biological system retain bulk water-like high mobility (Fig. 3(A)). This model envisages a dynamic exchange between bound and free water,[30] the rate determining step in solvation dynamics is the interconversion of bound-to-free water (k_2, Fig. 3(A)),

$$k_2 = \left(\frac{k_B T}{h}\right) \exp\left(\frac{-(\Delta G^0 + \Delta G^*)}{RT}\right) \qquad (3)$$

where, ΔG^* is the free energy of activation for the conversion of free-to-bound water molecules. The activation energy and entropy may be obtained from temperature variation of solvation dynamics. We will discuss this later in this article.

According to the Nandi–Bagchi model the major contribution to the solvation dynamics originates from the water molecules which vastly outnumber the polar residues of a protein or the polar head groups of a micelle. Several recent experiments and simulations considered the contribution of the protein (polar residues and side chains) and water, separately to the solvation dynamics. We will begin with a few systems (cyclodextrin and micelles) which do not have a back bone or side chain typical of a protein. Still, these systems exhibit an ultraslow component of solvation dynamics. The observation of slow solvation dynamics in these non-protein systems suggests that confinement of water and dynamic exchange of bound and free water are the main sources of

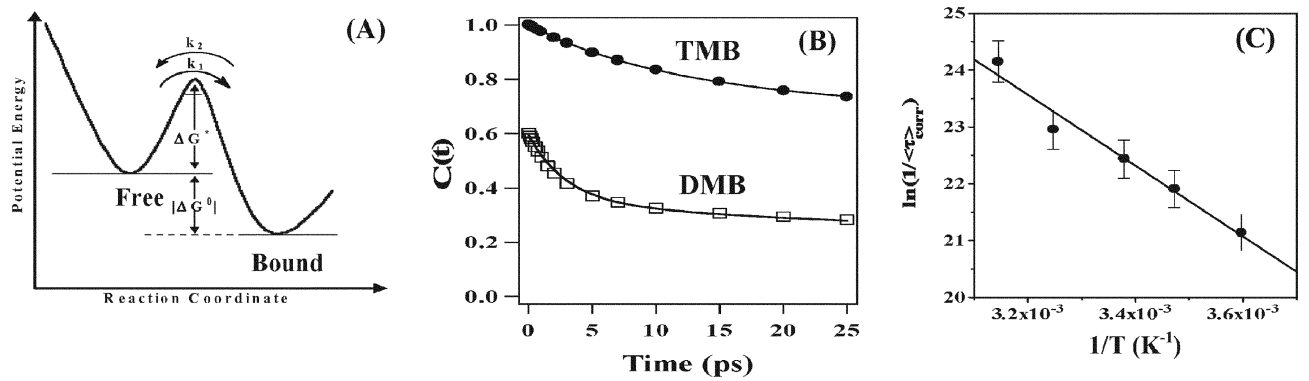

Fig. 3 (A) Bound-free exchange model.[30] (B) Solvation dynamics in dimethyl- (DMB) and trimethyl β-cyclodextrin (TMB).[35] (C) Temperature dependence of salvation dynamics in γ-CD aggregate.[36] Reprinted with permission. Copyright, American Chemical Society.

the ultraslow component. This, however, does not rule out the role of the protein matrix. We will come back to the issue of protein-coupled hydration dynamics later in this article.

Cyclodextrin

A cyclodextrin (CD) is a cyclic polymer of glucose containing 6 (α-), 7 (β-) or 8 (γ-) glucose units. It provides a hydrophobic nanocavity with a height ~8 Å and a largest diameter of ~4.5 Å (for α-), ~6.5 Å (for β-) and ~8 Å (for γ-). An organic guest molecule with a few water molecules may be encapsulated inside the CD cavity. Such a host–guest complex represents, perhaps, the simplest example of a nano-confined liquid (Fig. 2(A)). Cyclodextrin has versatile applications in solubilizing insoluble drugs in water, in targeted drug delivery and as enzyme models.[31,32] Compared to an unsubstituted cyclodextrin, an *O*-methyl or an *O*-hydroxypropyl derivative of a cyclodextrin are more soluble in water and hence, are more useful in drug delivery.[31]

Fleming and co-workers first reported that the solvation dynamics of Coumarin 480 (C480) in γ-CD exhibits an ultraslow component (1200 ps) which constitutes about 10% of the total response.[33] They ascribed the slow components of solvation dynamics to the restricted motion of the confined water molecules, motion of the guest probe molecule in and out of the cavity, and the fluctuations of the γ-cyclodextrin ring. Nandi and Bagchi attributed the slow dynamics inside the CD cavity to the complete suppression of the translational motion of the constrained water molecules within the cavity.[34]

Most recently, Sen *et al*. investigated the role of hydrogen bonding in solvation dynamics in a CD cavity.[35] They chose two derivatives of β-CD, trimethyl β-CD (TMB) and dimethyl β-CD (DMB). In TMB, all the three OH groups of the β-CD cavity are replaced by OMe groups. In DMB, one OH group on each of the seven glucose units, is left unsubstituted. According to a simple MM2 calculation, there is no hydrogen bond involving the water molecules inside the TMB cavity with those outside (minimum O···O distance between a water inside and that outside being ~7.7 Å). The short (~2.4 Å) O···O distance between the water molecules inside the cavity suggests strong hydrogen bonding. For DMB, the hydrogen bonding network involving the seven OH groups connect the water molecules inside the cavity with those outside. Note, for both TMB and DMB almost all (>99%) the probe molecules (C153) are inside the CD cavity and negligibly few probe molecules remain free in bulk water.[35] Hence, in this case the probes report almost exclusively the dynamics inside the CD cavity. It is observed that compared to unsubstituted γ-CD,[33] solvation dynamics in trimethyl β-CD is significantly slower. For DMB, having seven OH groups and an extended hydrogen bond network, there is a major (64%) contribution of the ultrafast bulk water like component (≤1 ps) with a 36% contribution of the slow components—50 ps (18%) and 1450 ps (18%) (Fig. 3(B)).[35] In contrast, TMB which has no OH group, does not display the ultrafast component. For TMB, the fastest component is ~10 ps (24%) and there are two ultraslow components—240 ps (45%) and 2450 ps (31%) (Fig. 3(B)).[35] This suggests that hydrogen bonding between water molecules inside the cavity and those outside (as in γ-CD or dimethyl-β-CD) is essential for ultrafast bulk water like response.

From the observation of an unusually slow (>20 ns) component of anisotropy decay, Roy *et al*. inferred formation of nano-tube aggregates involving Coumarin 153 (C153) and >50 units of γ-CD.[36] The temperature dependence of solvation dynamics in this aggregate (Fig. 3(C)) indicates that the activation energy for bound-to-free interconversion is ~12 kcal mol^{-1} and the entropy of activation is 28 cal mol^{-1} K^{-1}.

Cyclodextrins are polymers of a sugar (glucose). The sugar molecules play a crucial role in molecular recognition at the cell surface. Thus study of water molecules near cyclodextrins and other sugars have biological implications.[37] Heugen *et al*. studied the water molecules near a sugar (lactose) using terahertz spectroscopy and computer simulation.[38] They detected marked slowing down of water molecules in the hydration layer. According to them, the hydration layer extends up to 5.13 Å from the surface of lactose and contains 123 water molecules.[38]

Micelles

Solvation dynamics in the nearly spherical micellar aggregate of several surfactants has been studied by many groups. Most micelles exhibit an ultraslow component at the 100–1000 ps time scale.[39] The dynamics at different regions of the micelle and of different kinds of bound water (singly or doubly hydrogen-bonded to the micelle) have been studied in considerable detail using computer simulations.[40–43] One interesting result is, solvation dynamics in a cationic micelle is slower than that in an anionic or a neutral micelle. This is attributed to the binding of the heavier end of water (oxygen) to the cationic head group of a micelle.[40] Sen *et al*. studied the temperature dependence of solvation dynamics of 4-aminophthalimide (4-AP) in Triton X-100 (TX-100) micelles.[44] They reported a 8-fold decrease of average solvation time from 800 ps at 283 K to 100 ps at 323 K. The activation energy of solvation dynamics is found to be 9 ± 1 kcal mol^{-1} with a positive entropy factor of 14 cal mol^{-1}. It is interesting to note that according to a computer simulation[41] the difference between the water–water and micelle–water hydrogen bond energy is ~8 kcal $mol.^{-1}$ This is very close to the activation energy obtained experimentally. Most recently, Pal and co-workers reported a ~2-fold decrease in solvation time of a hydrophobic probe, 4-(dicyanomethylene)-2-methyl-6-(*p*-dimethylaminostyryl)-4*H*-pyran (DCM) in a micelle with increase in temperature from 298 to 348 K.[45] The weaker temperature dependence may arise from the buried location of DCM and exposed location for 4-AP.

A micelle or in general, any organized assembly is highly heterogeneous over the molecular length scale. Most recently, there have been several attempts to study solvation dynamics in different regions (*i.e*. to spatially resolve) in a micelle. The absorption and emission maxima of a solvation probe are sensitive to polarity and hence, are markedly different in different regions of such an assembly (Fig. 4(A)). Different regions of an organized assembly may be selectively probed by varying the excitation wavelength (λ_{ex}). At a short excitation

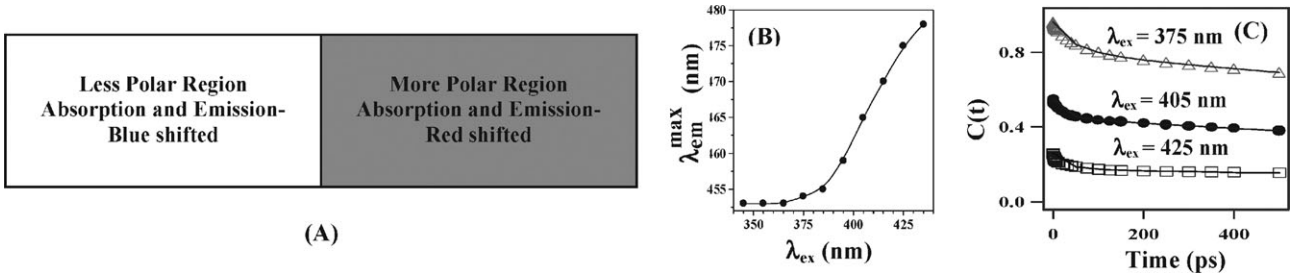

Fig. 4 (A) Spectra in different regions (polarity); (B) REES of C480 in P123 micelle;[49] (C) λ_{ex} dependence of solvation dynamics in P123 micelle.[49] Reused with permission, ref. 49. Copyright 2006, American Institute of Physics.

wavelength (''blue edge'') the probe molecules residing in a non-polar (buried) region are preferentially excited and this gives rise to a blue shifted emission spectrum (Fig. 4(A)). Excitation at a longer wavelength (''red edge'') selects the probe in a polar (exposed) region of the assembly and results in a red-shifted emission spectrum. Such a shift of the emission maximum with variation of excitation wavelength (λ_{ex}) is known as red edge excitation shift (REES).[46–48]

A tri-block co-polymer micelle (*e.g.* PEO_{20}–PPO_{70}–PEO_{20}, pluronic P123) consists of a hydrophobic and nonpolar PPO core and a polar, fast bulk water-like hydrophilic corona containing the PEO block (Fig. 2(C)). Sen *et al.* studied solvation dynamics in different regions of a P123 micelle by varying λ_{ex}.[49] With increase in λ_{ex} from 345 to 435 nm, the emission maximum of C480 exhibits a large red edge excitation shift (REES) by 25 nm (Fig. 4(B)).[49] Solvation dynamics in P123 micelles has been interpreted in terms of three regions—the fast peripheral PEO–water interface with solvation time $\leq$2 ps, a very slow (4500 ps) buried core (PPO) and chain region (60 ps).[49] With increase in λ_{ex}, contribution of the bulk-like ultrafast dynamics ($\leq$2 ps) increases from 7% at λ_{ex} = 375 nm to 78% at λ_{ex} = 425 nm (Fig. 4(C)).[49] There is a concomitant decrease in the contribution of the core-like slow component (4500 ps) from 79% at λ_{ex} = 375 nm to 17% at 425 nm.

In the case of the quasi-solid cubic gel phase of P123, it is observed that there is a bulk water like ultrafast component ($\leq$2 ps).[50] This is attributed to the nearly free water molecules in the voids or pores of the gel. The PPO core in a gel gives rise to a very long component of 4500 ps. There is a third component of 500 ps which is ascribed to chain–chain entanglement. With rise in λ_{ex}, contribution of the bulk-like ultrafast component increases and that of the core region decreases.[50]

Reverse micelles and lipid vesicles

In a reverse micelle, a nanosized water droplet surrounded by a layer of surfactant is dispersed in a non-polar solvent (Fig. 2(B)). Relaxation dynamics of water in different regions of the water nano-droplet has been studied by many groups. The water molecules close to the head group of the surfactant is more restricted compared to those in the core of the water pool.[19,51–56] λ_{ex} dependence of solvation dynamics of Coumarin 343 (C343) in the water pool of an AOT (aerosol-OT, sodium dioctyl sulfosuccinate) reverse micelle, suggests existence of two such environments.[57] Recent, ultrafast vibrational spectroscopic studies of the nanoscopic water in an AOT reverse micelle suggest presence of bulk-like water in the core of the water pool and confined water in hydration layer of the surfactants.[58–60] Most recently, excitation wavelength dependence and hence, heterogeneity has been observed in a microemulsion containing an ionic liquid and water and even, in a neat ionic liquid.[61]

There are many recent computer simulations on water nano-droplets in reverse micelles[43,62] and in other nano-confined systems.[63,64] It is proposed that after excitation, the solute with increased dipole moment migrates to a more polar region.[56,62,63] This may give rise to a ns (1000 ps) component of relaxation.

In a lipid vesicle, a bilayer membrane of surfactants encloses a polar water pool. Solvation dynamics in a lipid displays an ultrafast component ($<$0.3 and 1.5 ps) arising from the ''water pool'' with two slow components—250 ps and 2000 ps arising from the bilayer membrane. With increase in λ_{ex}, the relative contribution of the ultrafast components ($<$0.3 ps and 1.5 ps) increases from 48% at λ_{ex} = 390 nm to 100% at λ_{ex} = 430 nm.[65] The 250 ps component may be assigned to the structured water molecules.[66] The 2000 ps component may arise from the chain dynamics of the surfactants or migration of the probe molecule.

Proteins

Among all kinds of the *structured* water molecules perhaps the most important are those near a protein. Recent femtosecond studies have addressed many new issues regarding biological water. First, the *structured or biological* water near a protein are shown to have anomalously slow solvation dynamics compared to bulk water. Secondly, even within the same protein the water molecules in different regions (buried and exposed) are found to display different dynamic behaviour. Thirdly, the dynamics of the water molecules are observed to change markedly when a substrate binds to a protein.

If one uses an extrinsic fluorescence probe, one has to worry whether the probe impairs the biological activity of the protein. Therefore, many groups prefer to use a fluorescent amino acid as an intrinsic probe.[18,67,68] In bulk water, solvation dynamics of tryptophan occurs in $\sim$1 ps. However, in a protein the solvation dynamics is found to be 10–20 times slower.[18,67] It is also interesting to note that dynamics in a denatured protein is slower than that in bulk water.[18] Zhong and co-workers studied solvation dynamics of melittin, in a random-coiled primary structure and detected two

components—0.62 ps and 14.7 ps.[67] They assigned the faster component to bulk water and the slower to surface-type hydration dynamics of the protein. At a membrane–water interface, melittin folds into a secondary α-helical structure, and the relaxation time of interfacial water was found to be as long as 114 ps. This indicates a well ordered water structure along the membrane surface.[67]

Though use of tryptophan (and other intrinsic probes) is an attractive option, the complicated photo-physics of tryptophan poses difficulty in interpretation of the results. Excited state photo-physics of tryptophan involves inter-conversion between multiple conformers,[69] internal Stark effect[70] and multiple excited states[71] apart from solvation dynamics. Thus the fluorescence decay of tryptophan does not necessarily represent only solvation dynamics and it is non-trivial to separate the contributions of the different processes.

A better strategy is to use well characterized fluorescent probes which bind to a protein non-covalently. Location of the non-covalent probe inside a protein may be inferred by measuring its distance from a tryptophan residue using fluorescence resonance energy transfer (FRET). Fleming and coworkers studied solvation dynamics of a non-covalent probe, eosin, in the hydration layer of lysozyme using three pulse echo peak shift (3PEPS).[72] They detected a minor (8%) slow component of 530 ps.[72] The slow component is absent for free eosin in bulk water and hence, is assigned to protein bound water. The dynamic solvent shift (DSS) of DCM non-covalently attached to a protein (human serum albumin, HSA) exhibits a slow component (600 ps) and very long component (10 ns *i.e.* 10 000 ps).[73] The 600 ps component is assigned to bound water. The ultraslow 10 ns component may arise from overall tumbling of the protein.

Perhaps, the best strategy is to covalently attach a fluorescence probe at a selected site of a protein.[74–77] Guha *et al.* studied solvation dynamics at the active site of an enzyme, glutaminyl-tRNA synthetase (GlnRS).[75] For this purpose, they attached a fluorescence probe, acrylodan, covalently to a cysteine residue C229 near the active site. Solvation dynamics in GlnRs displays two slow components—400 and 2000 ps. When the amino acid glutamine (Gln) binds to the enzyme (GlnRS) the 400 ps component slows down about two-fold to 750 ps while the 2000 ps component remains unchanged. When $tRNA^{gln}$ binds to GlnRS the 400 ps component does not change but the 2000 ps component becomes slower (2500 ps). From this, the 400 ps component is assigned to the water molecules at the Gln binding site and the 2000 ps to the $tRNA^{gln}$ binding site. This suggests water molecules at different sites of a protein are different because of local interactions. A mutant Y211H–GlnRS was constructed in which the glutamine binding site is disrupted. The mutant Y211H–GlnRS labeled at C229 with acrylodan exhibits significantly different solvent relaxation.[75] This demonstrates that the slow dynamics is, indeed, associated with the active site. In summary, water inside a protein relaxes ~100–1000 times slower compared to bulk water and becomes even slower when a substrate binds to a protein.[75] The slow dynamics implies pre-organization at the active site of an enzyme which is consistent with recent computer simulations of enzyme catalysis.[78]

Dynamics in the non-native state of a protein has been the subject of many recent studies. Early NMR studies indicate that there is considerable residual structure present in a partially unfolded protein.[22] Sen *et al.* studied solvation dynamics in the molten globule state of a protein using both a covalent and non-covalent probe.[76] In the native state, the covalent probe resides at the surface and exhibits much shorter solvation time (120 ps) compared to that (1400 ps) of a non-covalent probe (bis-ANS) which goes deep inside the protein. However, in the molten globule state when the protein opens up, solvation times of both the probes becomes similar (~200 ps).[76] Samaddar *et al.* showed that the solvation time is of the order native > molten globule > pre-molten globule.[77] This suggests that the pre-molten globule (of GlnRS) is less compact and more labile compared to the molten globule.

The mitochondrial respiratory membrane protein cytochrome C is cationic in nature and carries a net positive charge (+8) at a neutral pH (~7). Binding of cytochrome C to anionic surfactants (*e.g.* sodium dodecyl sulfate, SDS) or membranes results in partially folded or molten globule-like states. Cytochrome C forms two partially folded intermediates—I_1 (in the presence of SDS) and I_2 (in the presence of SDS and urea).[79] Solvation dynamics in these two partially folded states of cytochrome C are found to be drastically different. The most prominent differences are detected at very early times (<20 ps, Fig. 5(A)).[80] I_1 displays an ultrafast component—0.5 ps (5%) and two slow components—90 ps (85%) and 400 ps (10%). In

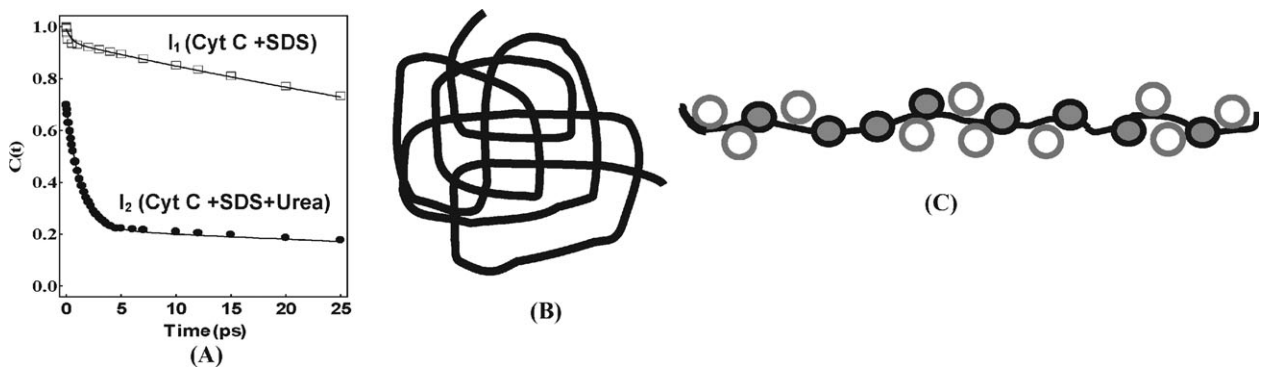

Fig. 5 Solvation dynamics in a protein: (A) Folding intermediates of cytochrome C;[80] (B–C) Schematic pictures of lysozyme (native, B) and lysozyme denatured by SDS and DTT (C). Reprinted with permission, ref. 80. Copyright 2006, American Chemical Society

the case of I_2, a portion (30%) of solvation dynamics is faster than the time resolution of our set up (0.3 ps). This is followed by a major ultrafast component of 1.3 ps (47%) and two slow components—60 ps (12.5%) and 170 ps (10.5%).[80] Thus contribution of the ultrafast bulk water-like response is only 5% in I_1 and 78% in I_2. The faster solvation dynamics suggests that I_2 is more open and labile compared to I_1. It is evident that presence of urea opens up the structure substantially so that 78% of the solvent response is almost bulk water like.

For gel-electrophoresis, a protein needs to be converted into a linear extended polymer so that it can pass through the pores of a poly-acrylamide gel. For this purpose, a surfactant (sodium dodecyl sulfate, SDS), which destroys the noncovalent interactions in the protein, and a mercaptol (dithiothreitol, DTT), which ruptures the disulfide bonds, are added. Dutta *et al.* studied solvation dynamics in a globular protein lysozyme in the native state, in the SDS denatured state and finally, after addition of DTT.[81] The solvation time of C153 in the native state of lysozyme is $\sim$300 ps (Fig. 5(B)). On addition of SDS, the protein expands and becomes decorated by small SDS micelles. Under this condition, the average solvation time increases nearly 20 times to 7250 ps. Subsequent addition DTT and consequent rupture of S–S bonds converts the protein to a linear polymer like structure (Fig. 5(C)). This makes solvation faster and the average solvation time decreases to 1100 ps.[81]

Sen *et al.* studied solvation dynamics in lysozyme in the presence of urea and SDS.[82] Using circular dichroism, they showed that a small amount of SDS (3 mM) causes partial recovery of the protein denatured by 7 M urea. Solvation dynamics in this system (3 mM SDS and 7 M urea) is very close to that in the native protein.[82] However, addition of a large amount of SDS (28 mM) causes complete loss of the tertiary structure of the native protein. Under this condition, the SDS micelles are squeezed inside the polypeptide chain of the protein. This results in a 3.5 times retardation of solvation dynamics compared to that in the native state.[82]

Several groups have studied temperature dependence of solvation dynamics in a protein.[83] Solvation dynamics of ANS (anilinonaphthalene sulfonate) bound to BSA (bovine serum albumin) displays a component (300 ps) which is independent of temperature in the range of 278–318 K and a long component which decreases from 5800 ps at 278 K to 3600 ps at 318 K.[73] The temperature independent part (300 ps component) is ascribed to a dynamic exchange of bound to free water with a low barrier. The temperature variation of the long component of solvation dynamics corresponds to an activation energy of 2.1 kcal mol^{-1}. The activation energy is ascribed to local segmental motion of the protein along with the associated water molecules and polar residues. The entropy of activation is found to be -13 cal K^{-1} mol^{-1}. The observed negative entropy suggests an ordering of the local structure at the transition state of the protein segment during dipolar relaxation.[83]

Recently, many groups have investigated dynamics of water in a protein in unprecedented detail using large scale molecular dynamics simulations.[11,19–21,84–87] In a very early simulation, Levitt and Sharon[11] reported a dramatic accumulation of water molecules at the protein surface. They found that the number of the water molecules at the surface of a protein is nearly double of that expected from the accessible surface area. The density of water molecules at the protein surface is 1.25 g cm^{-3} which is 25% higher than that in bulk water. They classified the water molecules at the protein surface into four regions depending on their distance from the protein surface. The water molecules closest to the protein (region I) were found to be energetically more stable by 1.9 kcal mol^{-1} than bulk water. The diffusion coefficient of the water molecules in region I is found to be smaller than that of bulk water by a factor of 4.[11] The energy and diffusion coefficient of the water molecules increase with distance from the protein. About half of the water molecules reside at a distance >10 Å (region IV) and their properties are almost identical to those of bulk water.[11] More recent simulations suggest that the residence time of biological water at a particular site could be longer than that in bulk water by several orders of magnitude.[19] While the residence time of water in a site in bulk water is $\sim$1 ps, in a protein the residence time may be 10–100 ps.[19–21] According to Makarov and Petit, there is a significant variation in the residence times of water molecules at the 294 hydration sites of myoglobin.[19] The buried sites (*i.e.* cavities, grooves or concave surfaces) display a long residence time (>80 ps). In contrast, the exposed or convex sites are characterized by relatively short residence time (<10 ps).[19] These are markedly longer than the residence times in bulk water (0.34 ps and 4.1 ps). Bagchi and co-workers carried out an atomistic simulation on solvation dynamics in a 36-residue globular protein, HP-36.[20] The secondary structure of this protein contains three short α-helices. The solvation dynamics of the polar amino acid residues in helix-2 ($\langle\tau\rangle$ = 11 ps) is found to be faster than that of the other two helices (the average time constant is smaller by a factor of two). However, the interfacial water molecules around helix-2 exhibit much slower orientational dynamics than that around the other two helices. A careful analysis shows that the origin of such a counterintuitive behaviour lies in the dependence of the solvation time correlation function on the surface exposure of the probes—the more exposed is the probe, the faster the solvation dynamics.[20]

Marchi and co-workers studied folding of a simple alanine-octa-peptide (A_8) confined in a reverse micelle (RM) using MD simulation.[21] In a confined environment (*i.e.* RM), the folded structure of the protein is found to be much more stable than the unfolded structure. For smaller RM, they found a stable helical structure of the polypeptide which quickly assumes an extended structure as the size of the RM increases. MD simulations by Marchi and co-workers suggest multiple time scale of peptide–water interaction (solvation) ranging from femtoseconds to tens of picoseconds.

Evidently, the water molecules in the hydration layer are subject to several different influences from the protein. Prominent among them is the short range hard-sphere type interaction with surface atoms and the longer ranged electrostatic interactions. In specific instances, the latter may be modelled as hydrogen bonds between water and the polar/charged atoms of the protein (as envisaged in the Nandi–Bagchi model[30]). The recent simulations by Rossky and co-workers show that the stability and dynamics of the water network surrounding a protein is strongly influenced by the

electrostatic interactions.[84] Golosov and Karplus suggested that the long (~100 ps) component of polar solvation dynamics arises from coupled hydration and protein conformational dynamics.[85] Singer and co-workers attempted to examine the effect of protein fluctuations and water dynamics separately by freezing the motion of the protein and the water in their simulations.[86] They found that the slow component at the 10–100 ps time scale is recovered only when both the protein and the water molecules are free to move. They however, did not probe dynamics up to several hundred picoseconds. Although the details of hydration dynamics will certainly vary from protein to protein (or even from site to site in a protein) several general features may be identified. The slowness of the hydration layer is due to the formation of a hydrogen-bond network surrounding the protein. For instance, lysozyme is found to form about 30 quasi-stable hydrogen bonds (''pinning sites'') which helps to stabilize the network.[87]

The results discussed in this section illustrates that the ultraslow and ultrafast dynamics of water molecules in a protein is markedly affected by structural change, addition of urea, surfactant and salts. Before closing this section, it is important to note that the presence of ultraslow waters in the hydration layer is extremely beneficial for a protein. In a biological system, a protein is often attacked by small ions (*e.g.* Na^+ or Cl^-) which attract the water molecules very strongly. If the response of the water molecules were fast they would leave as soon as a small ion approaches a protein. This would lead to dehydration and would consequently cause destruction of the structure of a protein. Because of the slow response and long residence time, a water molecule does not leave a protein within the short contact time during which an ion is present near a particular water molecule. The slow water molecules, thus, help to keep the structure of a protein intact.

DNA

Most recently, many groups have studied dynamic solvent shift in DNA. The interior of the DNA double helix does not contain any water. However, there are a lot of water molecules in the exterior of DNA and in particular, in the minor and major grooves. Also, the inherent negative charge of DNA and the counter ions may participate in ionic solvation. Zewail and co-workers used 2-aminopurine as an intrinsic probe and a minor groove binding non-covalent probe, pentamidine.[88] They detected a bi-exponential decay with an ultrafast sub-picosecond bulk-water like component and a relatively long (~10 ps) component. Berg and co-workers studied a series of oligo-nucleotides in which a solvation probe (C480) replaced a native base pair.[89,90] This gives very precise information on the dynamics of water in different regions of DNA. When the probe (C480) is in the centre of the helix, the time scale of relaxation is broadly distributed over six decades of time scale from 40 fs to 40 ns and obeys a power-law, $(1 + \tau/\tau_0)^{-\alpha}$. The very long (~40 ns) component is assigned to the reorganization dynamics of DNA. Since the interior of the double-helix is devoid of water, the observed Stokes shift seems to originate from the electric field of DNA on the probe. The very long component (~40 ns) of solvation may also arise from relaxation of the counter ions (ionic atmosphere).[89,90] When the probe (C480) is attached at the end of the helix an additional very fast component of 5 ps is detected. The 5 ps component and the increased mobility (''fraying'') at the end of the helix is ascribed to increased exposure of the probe to bulk water and lower counter-ion concentration.[90]

Many groups carried out computer simulations to explain the multiple time scale of relaxation times in DNA.[91,92] Bagchi, Hynes and co-workers studied energy–energy time correlation function (TCF) of the four individual bases (A, T, G and C) of a 38-base-pair long DNA duplex using atomistic MD simulations.[91] For each base, they detected a very fast (60–80 fs) component followed by a 1 ps and a slow 20–30 ps component. They further detected a very slow 250 ps component. They showed that the slow decay stems from the interaction of the nucleotides with the dipolar water molecules and the counterions. They however, did not include the relaxation of the ionic atmosphere which may be a possible source of the very slow ns component.[93,94]

Implications of slow biological water in polar reactions

In many chemical reactions, the transition state is more polar than the reactant (*e.g.* proton and electron transfer in neutral reactants). Such a reaction is usually very fast in bulk water because of the stabilization of the transition state and consequent reduction in the energy of activation.[19] In the case of proton migration, an additional fast component arises from rupture and formation of a hydrogen-bond network.[95] For biological water, the slow solvation, reduced polarity and disruption of hydrogen bond network seriously inhibit proton and electron transfer. For instance, in bulk water excited state proton transfer (ESPT) from pyranine to water exhibits two time scales—3 ps and 90 ps.[96] However, when pyranine is encapsulated in a cyclodextrin cavity the ESPT exhibits an additional slow component at the ~1500 ps time scale.[97,98] Similar retardation is also observed inside a P123-CTAC aggregate.[99] Proton transfer involves primarily three steps—initial proton transfer, recombination of the ion pair and dissociation of the ion pair. Recently, several groups examined, in detail, these three steps in many confined systems.[96–99]

Nibbering and co-workers reported that in bulk water ESPT from pyranine to a directly hydrogen bonded acetate ion (proton acceptor) occurs in 0.15 ps.[100] When pyranine and acetate are both encaged inside a cyclodextrin cavity in close proximity ESPT is found to be very slow (90 ps).[101] It is shown that inside the CD cavity the acetate is separated from pyranine by two water molecules as bridges.[101] Thus in this case, there is no direct proton transfer from pyranine to acetate.[101]

Recent MD simulations on proton transfer from phenol to amine in a hydrophobic nanocavity indicate that the reaction free energy of proton transfer depends on the location of the probe.[102] The proton transfer is found to be slower near the hydrophobic wall of the cavity.[102]

In the classic Marcus theory, it is assumed that solvation is complete at each point along the reaction co-ordinate (*i.e.* solvent polarization or solvation).[103] In many cases

electron transfer (ET) is found to be faster than solvation. Yoshihara and co-workers first reported that if an electron acceptor is dissolved in a neat donor (solvent) ultrafast ET occurs in a timescale faster than solvation.[104] Obviously, in this case only a fraction of solvation is complete within the time scale of ET. Bagchi and co-workers showed that about 30–40% solvation is enough for ET to occur.[105] Yoshihara and co-workers observed a Marcus-type bell shaped dependence of the rate of ET on free energy change even when ET is faster than solvation.[104] Photo-induced electron transfer (PET) in an organized assembly is interesting for two reasons. First, close proximity of the donor and acceptor in a confined system is expected to cause very fast electron transfer (ET). Second, the ultraslow component of solvation is much slower than the time scale of ET. Thus PET in an organized assembly corresponds to a situation where ET is faster than solvation. Most recently, Marcus type inversion is detected in a cyclodextrin nano-cavity and in micelles.[106] According to Tachiya and Murata, ET requires coincidence of energy of the reactant (neutral D and A) and the products (D^+ and A^-)[107] and hence, is restricted to those donor–acceptor pairs which have right distance, orientation and re-organization energy. Thus PET in micelles also takes place between these *right* pairs of donor and acceptor and does not exhibit λ_{ex} (*i.e.* location) dependence.[108,109]

Conclusions

The synergistic development of femtosecond dynamics and computer simulations has vastly improved our understanding of biological water. The results described in this feature article demonstrate that solvation dynamics of biological water is dramatically slower than that in bulk water. It is shown that the ultrafast response arises from an extended hydrogen-bond network. Disruption of this network and binding of water to a biological system leads to a dramatically slow component. The response time seems to vary from site to site within an assembly, as demonstrated by the excitation wavelength dependence. The slow response of the biological water may help a protein to retain its structure under adverse conditions. Thus, the dynamics of biological water may be regarded as a new parameter in protein biochemistry. The slow solvation affects dynamics of those reactions whose transition state is more polar than the reactant. The unequivocal separation of the role of the biomolecule and the role of water in the ultrafast dynamics is still elusive though recent simulations have made major advances towards this goal. The ultimate challenge may be the study of dynamics of biological water under *in vivo* conditions *e.g.* inside a biological cell and then, relate it to the biological function.

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