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DYNAMICS OF WATER IN THE HYDRATION LAYER OF A SELF-ASSEMBLED SYSTEM AND IN BIO-MOLECULES

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Water molecules in the hydration layer of a bio-molecule (proteins and DNA) and of a self-assembled system (micelles, lipids) show many anomalous behaviours. In this article, we attempt to present a coherent picture of dynamics of water in the hydration layer of protein molecules. We will discuss a simple theoretical model (N Nandi and B Bagchi *J Phys Chem* **101** (1997) 10954) that assumes a dynamic equilibrium between the bound and the free states of the water molecules in the hydration layer. It is shown here that both the observed sub-diffusive translational diffusion and the non-exponential orientational relaxation may arise from a distribution of binding energies of the surface water molecules. The dynamic equilibrium model (DEM) predicts a slow component (about 100 ps) in the orientational relaxation of water molecules in the hydration shell. The rate of the slow relaxation is determined by the rate of transition from the bound to the free state. DEM allows us to establish a relation between the residence time of the water molecules in the hydration layer and the observed slow component in solvation dynamics. It is further shown that the hydration shell of the protein molecule gives rise to enhanced rotational friction which results from the slow relaxation of water in the hydration shell and thus, may be correlated with the residence time. Finally, we argue that the ultra-slow component (of a few ns) might arise from the exchange of the probe (rather than the solvent) between different heterogeneous domains in a self-organized assembly.

Key Words: Hydration Layer; Slow Dynamics; Bio-molecules; Organized Assembly

1 Introduction

Recent experiments have shown that the dynamical response of water molecules in the immediate vicinity of many bio-molecules (proteins and DNA) and complex systems (micelles, lipids) is much slower than that in the bulk[1-4]. This water, which is sometimes called biological water, plays an extremely important role in the stability, structure and function of these systems. The structure and dynamics of water around proteins in particular, have been subject of many recent investigations[1-16]. Notable dynamical features are (a) sub-diffusive mean-square displacement, (b) markedly non-exponential orientational relaxation, (c) several branches of well-separated dielectric relaxation and (d) slow solvation dynamics. Here, we discuss a theory that attempts to explain and correlate many aspects of the dynamics of water at the protein surface.

Biological water experiences a surface that is heterogeneous, even on a molecular length scale. Strong interactions with the adjacent surface lead to partial disruption of the hydrogen bond network of water. Different experimental studies probe such systems on different scales of length and time and, as a result, different studies have often led to conflicting results. For example, while NMR studies seem to predict time scales less than (but around) 300 ps from NOE measurements[8,9], dielectric relaxation experiments have given time scales of the order of 10 ns[1]. While NOE is sensitive to very short length scales (but with limited time resolution), dielectric relaxation has no sensitivity to length scales at all! Both inelastic neutron scattering and polar solvation dynamics are sensitive to both time and length scales and can, therefore, be good probes of the dynamics of hydration layer.

In this article, we discuss a simple theory that is based on the assumption of a dynamic equilibrium between the bound and the free states of water molecules in the hydration layer of a protein molecule (Fig. 1). A simple relation between the solvation correlation time and the residence time of the water molecules in the protein hydration layer is presented[17]. This relation facilitates an understanding of results from two entirely

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different groups of experiments. We also establish a correlation between the enhanced friction on a protein due to the slow water molecules in its hydration layer and the residence time of water in the layer[18]. This relation is approximate but catches the essential aspects of water-surface interactions.

Computer simulations on water molecules at protein surfaces show two entirely different behaviour-one for the bound state and the other for the moving (free) state. Rapid exchange between the two states is observed suggesting the *existence of a dynamic equilibrium between the two states.* Simulations find less number of tightly bound water molecules than observed in diffraction measurements. The residence time (τ_{res}) of water molecules in the hydration layer of myoglobin is found to have a distribution between somewhat less than 30 ps to more than 80 ps which was the longest run time of the simulation[11,12], The water molecules with much longer residence times are those which are either buried inside protein cavities or in the clefts or have multiple interactions with the protein and have higher (than average) binding energies. The binding energy distribution has values ranging from 0.5 to 9 kcal/mole. In a recent simulation of the dynamics of water at a micellar surface[15,16], it was observed that for the water molecules which remain within the hydration layer all through the simulation, the orientational correlation function decays very slowly. In fact, the correlation function does not decay to zero, even after 300 ps. This observation indicates that the rotational motion is restricted which is a signature of a bound state.

Because of chemical heterogeneity on the protein surface, there is always a distribution of energies of binding of water molecules to the protein surface. Distribution of residence time is a direct consequence of the distribution of binding energy. We shall denote this distribution by P(ε) which we expect to be bimodal with a sharp peak at small values of ε (due to the free water molecules which are near the hydrophobic residues) and a broad maximum at larger ε values due to the hydrophilic groups. The distribution is shown schematically in Fig. 1. For simplicity, we can assume the following distribution of P(ε), given by

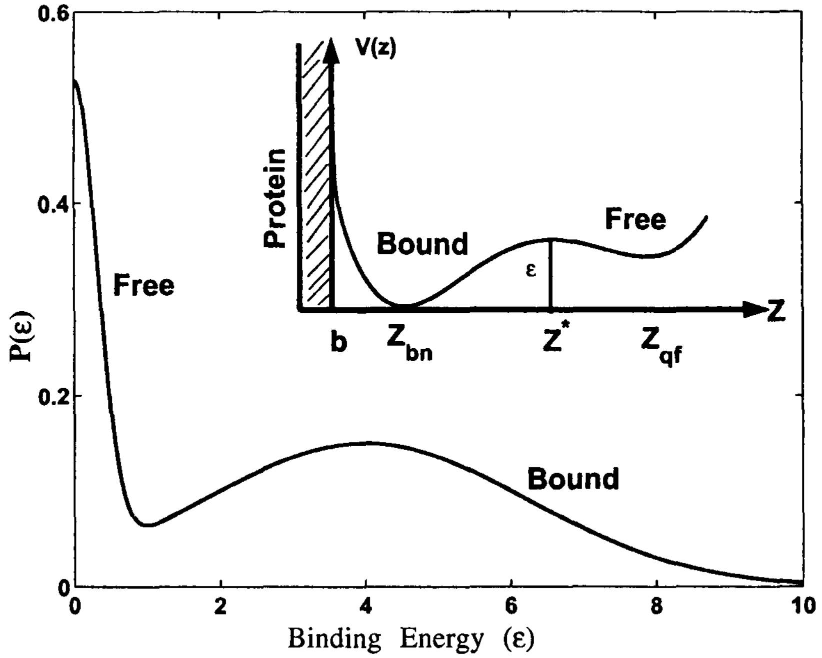

Fig. 1 A schematic illustration of the probability distribution P(ε) of the binding energy, ε, of water molecules on a protein surface. The first peak at zero binding energy corresponds to the quasi-free water molecules while the broad maximum at larger values corresponds to the transiently bound water molecules to sites of large binding energy (like arginine). The inset figure provides a schematic illustration of the potential energy surface V(z) that a water molecule experiences near the protein surface. The points on the Z-axis has the following significance. Z=b denotes a position at the surface where the potential energy becomes much larger than the thermal energy, k_BT. We place the reflective barrier at this position in our calculation of the mean first passage time by the method of images. $Z=Z_{bn}$ denotes the minimum of the potential energy surface.

$$P(\varepsilon) = \rho_b(2\ \pi\sigma\varepsilon)^{-0.5}\exp(-(\varepsilon - <\varepsilon>)^2/2\ \sigma\varepsilon^2) + \rho_f\delta(\varepsilon), \qquad ...(1)$$

where ρ_b and ρ_f are the densities of the free and bound water molecules at the surface, respectively, and $<\varepsilon>$ is the average binding energy at the protein surface.

This article is organized as follows. In the next section we discuss the theoretical formulation and results on orientational relaxation, self-diffusion and solvation dynamics. Section 3 contains theoretical discussion of the additional friction on the protein molecule due to the hydration layer. Section 4 contains discussions on relation with NOE experiments. Section 5 concludes with a brief discussion of the results and of the scope of future studies.

2 Dynamics in the Hydration Layer: Theoretical Formulation

The basic model of the following discussion has been presented earlier. It is assumed that the water molecules in the hydration shell can be divided into two categories, bound and free. The bound molecules are hydrogen bonded to the polar or charged amino acid groups on the protein surface (Fig. 1). These species are transient, as there is a dynamic equilibrium between them, described by

$$\textit{bound} \underset{k_{fb}}{\overset{k_{bf}}{\rightleftharpoons}} \textit{free}$$

The number of bound molecules in the layer is larger than that of quasi-free molecules. The water molecules in the bulk are not considered in this model, although can be included. The rates of inter-conversion from bound to free and free to bound water molecules are denoted by k_{bf} and k_{fb}, respectively. The bound water molecules are assumed to be totally immobile – they can neither rotate nor translate. The free molecules are free to do both, with D_R and D_T as the rotational and translational diffusion coefficients. The rates of transition between the bound and the quasi-free states can be calculated from a simple model, as discussed by Nandi and Bagchi[1,4].

2.1 *Models of the Rate Process*

Let us assume that $V_i(z)$ denotes the reduced energy of interaction of a water molecule with the site i on the protein surface. Here, z is the direction perpendicular to the surface. $V_i(z)$ will have a minimum at $z \approx 3$-3.5 Å. At smaller distances from the surface, the energy should rise sharply. The potential is shown schematically in Fig. 3 where all the coordinates are also shown. Fortunately, we can obtain a closed form of expression for the escape time by calculating the mean first passage time which is obtained by using the method of images[19]. The physical picture behind this derivation is simple and as follows. The diffusing molecule is modelled as a random walker. The boundary on the bulk side is an absorbing wall (or barrier) while the protein surface is modelled as a reflecting barrier. The random walker (the water molecule) executes a random walk under the influence of a potential surface which is given by $V_i(z)$. The equation of motion is given by a Smoluchowski equation. The first passage time can be obtained from the adjoint of this equation. An elegant description of the method is given in ref. [20]. The final expression for k_{fb} is given by

$$k_{fb} = D[\int_{z_0}^{z^*} dy\, \exp(\beta V(y) \int_{b}^{y} dx\, \exp(-\beta V(x))]^{-1}$$

Note that now the upper limit of integration in the first integral is z* because the bond is supposed to break when the particle reaches z*. The expression for k_{bf} is obtained by reversing the initial and the final states.

2.2 *Non-exponential Orientational Relaxation in the Hydration Shell*

First note that the orientational relaxation of the water molecules in the bulk is largely single exponential, with a time constant of about 4.5 ps. The orientational relaxation of *free* water molecules in the hydration shell of a protein is not expected to slow down appreciably. The existence of the observed slow component should be attributed to those water molecules which are trapped in the hydration shell for a long time. This slow component, which could last for hundreds of picosecond, is a clear signature of bound water molecules.

We can derive an expression for this slow relaxation by using the same dynamic exchange model, described earlier. The model is to consider rotation of the molecules within the hydration layer. The details of the model have been discussed elsewhere[17]- here we present a somewhat simpler version. The starting point is the coupled reaction-diffusion equations of Nandi and Bagchi[4] for the orientation dependent densities $\rho_b(\Omega,t)$ and $\rho_f(\Omega,t)$. The bound molecules undergo only reaction while free molecules undergo both rotational diffusion and reaction. These equations can be solved to obtain the two rate constants, $k_\pm$, given by[17]

$$k_\pm = 0.5\ [-B\pm\sqrt{(B^2-4D_R k_{fb})}], \qquad \ldots(3)$$

with $B = 2D_R + k_{bf} + k_{fb}$. Typically, the rate of free to bound reaction, $k_{fb,}$ will be larger than that for the reverse process, k_{bf}. In the limit when the rate of conversion from bound to free becomes very small, the above expression further simplifies and the two rates are given by $2D_R$ and k_{fb}. Thus, while one time constant remains fast, of the order of 4-5 ps, the other one can slow down appreciably, even to the extent of hundreds of picoseconds. The rate k_{fb} is of course determined by the binding energy. For majority of sites, the time constant may range between 20 to 300 ps or so. The theory thus predicts a bi-exponential decay for a given binding energy. It is important to note that there is a distribution of binding energy. This can lead to highly non-exponential orientational dynamics and can explain the stretched exponential decay of orientational relaxation observed in simulations recently. The general forms of the orientational relaxation function is

$$C\_\{R\}(t) = \int d\varepsilon\ P(\varepsilon)\ [A\ \exp(-k_+\ t) + B\ \exp(-k_-\ t)] \qquad \ldots(4)$$

The constants A and B can be determined as follows. In the limit of large binding energy, the two rate constants are widely separated, one determined by rotational diffusion coefficient of water while the other by the rate of bound to free transition. Since the correlation function is for the quasi-free molecules only, the time derivative at time t=0 should be determined by the concentration of the free molecules. In addition, k_- remains close to the rotational diffusion rate $((2D_R)^{-1}$ as the free molecules will relax according to their rotational relaxation time. We thus have the following expression for the rotational correlation time

$$C\_\{R\}(t) = \rho_b(2\ \pi\sigma\varepsilon)^{0.5}\int d\varepsilon\ \exp(-(\varepsilon - <\varepsilon>)^2/ 2\sigma\varepsilon^2)\ \exp(-k_+t) + \rho_f\exp(-2D_Rt) \quad \ldots(5)$$

where ρ_b and ρ_f are the equilibrium densities of the bound and free water molecules at the surface. The above equation gives a bimodal decay of the orientational correlation function, with an initial fast decay, which, for proteins, may be between 20-30%. That is, the slower component should have the larger amplitude because of preponderance of polar and charged groups on the surface. Another prediction of the above expression is that it is the slow component, which will be markedly non-exponential because the slow one will be mostly affected by the binding energy distribution.

Therefore, the general prediction is that orientational relaxation of water molecules in the hydration layer should be given by a sum of two stretched exponentials. The faster term will have a time constant close to $(2D_R)$ and the slower second term will have a time constant that will be average of k_{bf}.

In Fig. 2, we show a typical orientational time correlation function and also a stretched exponential fit to the calculated function. The stretched exponential exponent is found to be about 0.4, signifying considerable non-exponentiality.

2.3 Translation Diffusion: Sub-diffusive Behaviour

Our model also provides specific predictions about translational diffusion of water molecules at the surface. One again expects two different kinds of behaviour. The initially bound molecules will have a inertial component at very short times followed by a plateau whose duration is determined approximately by the inverse of the average rate of bound to free transition. This plateau will be followed by linear growth of the mean square displacement with time. The free water molecules will show, after the initial inertial regime, a linear growth, which can be followed by a plateau if they bind to the surface. As larger fraction of water molecules in the hydration layer of a protein is in the bound state at any given time. The above consideration leads to the following expression for the mean-square displacement (MSD), averaged over all the water molecules in the layer

$$<(\Delta r)^2(t)> = at^2,\ t< t_1\ , \qquad \ldots(6a)$$

$$<(\Delta r)^2(t)> = \int d\varepsilon P(\varepsilon)H(t-k_{bf}{}^{-1})D_R(t-k_{bf}{}^{-1}) + \rho_{qf}6D_Rt,\ t>t_1 \qquad \ldots(6b)$$

The integration is over all the bound states of the water molecules. This formulation gives mean square displacement, which is slower than that of the free molecules.

A numerical solution of the reaction diffusion equation has been carried out[20]. This gives rise to

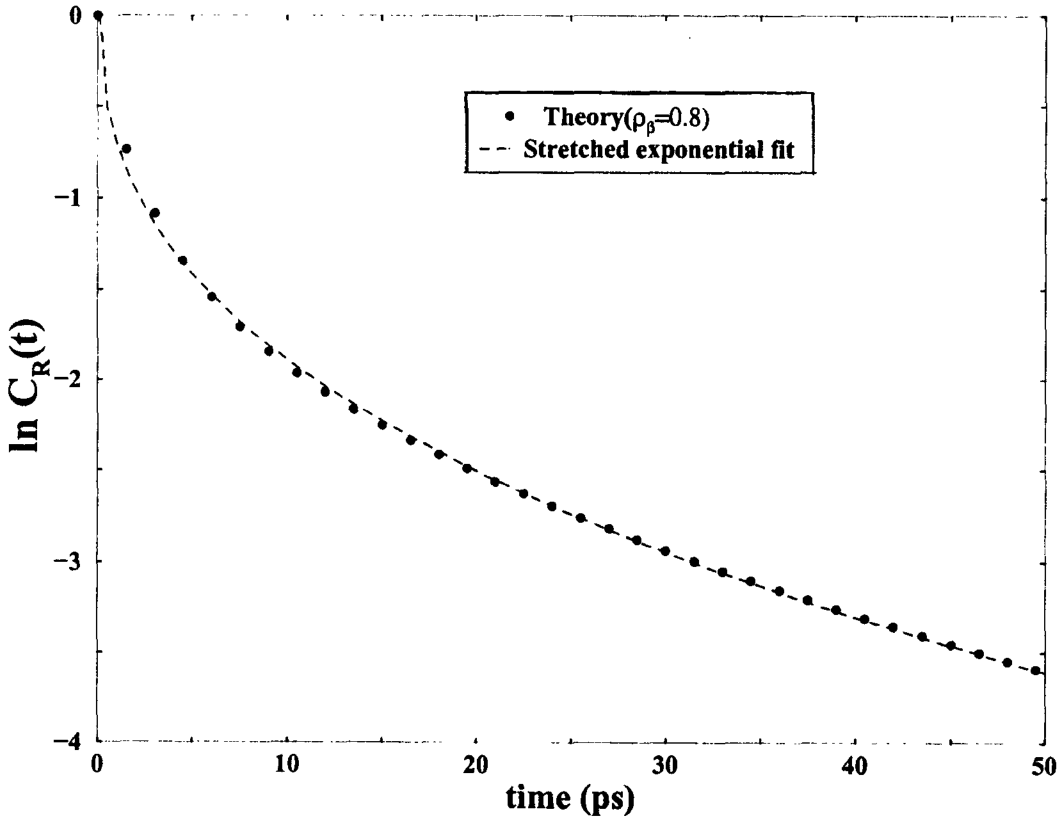

Fig. 2 The calculated orientational correlation time plotted against time. The dashed line shows a stretched exponential fit with exponent equal to 0.38.

a sub-linear growth of MSD at short times, followed by a super-linear dependence.The calculated MSD agrees rather well with the ones computed numerically.

2.4 *Polarization Relaxation in the Protein Hydration Layer*

In the presence of the dynamic equilibrium, the theoretical description requires solution of two coupled reaction-diffusion equations. Two rate constants, k_{fb} and k_{bf}, are introduced to describe the rate of transition from bound to free and the reverse, respectively. The resulting equations can be solved to find two wavenumber dependent polarization relaxation times given by

$$\tau_\pm = 2[-A\pm\sqrt{(A^2-4k_{fb}/\tau_s^{bulk}(k))}]^{-1}, \quad ...(7)$$

with $A = [\tau_s^{bulk}(k)]^{-1} + k_{bf} + k_{fb}$. Here $\tau_s^{bulk}(k)$ is the wavenumber dependent bulk solvation time. These rates depend on the binding energy and will vary from site to site.

The above expression of the rate gives simple results in the limit of large activation barrier between the bound and free states. This is because $t_s^{bulk}(k)$ is close to 10^{12} sec^{-1} and the transition rates are expected to be much smaller. The limiting time constants are given by[17]

$$\tau_{fast} \equiv \tau_- \approx \tau_s^{bulk}, \quad ...(8a)$$

$$\tau_{slow} \equiv \tau_+ \approx k_{fb}^{-1}. \quad ...(8b)$$

In the same limit of large activation energy separating the bound state from the free one, the residence time of the bound water molecules is given essentially by k_{fb}^{-1}. The expressions of the rates are given later.

2.5 *Solvation Dynamics of a Static Probe in the Hydration Layer*

A simple way to address the dynamics of polar solvation is to start with the following expression for the solvation energy, $E_{solv}(t)$[21],

$$E_{solv}(t) = -(1/2)\int dr\, \mathbf{E_0(r)}.\, \mathbf{P(r,t)}, \quad ...(9)$$

where $\mathbf{E_0(r)}$ is the instantaneously created, position dependent electric field from the ion or the dipole and $\mathbf{P(r, t)}$ is the position and time dependent polarization. The latter is defined by the following expression

$$P(\mathbf{r}, t) = \int d\Omega\, \mu(\Omega)\, \rho\, (\mathbf{r}, \Omega, t), \qquad \ldots(10)$$

where $\mu(\Omega)$ is the dipole moment vector of a molecule at position **r** and ρ ($\mathbf{r},\Omega$,t) is the position, orientation and time dependent density. Therefore, the time dependence of the solvation energy is determined by the time dependence of polarization that is in turn determined by the time dependence of the density. If the perturbation due to the probe on dynamics of bulk water is negligible, then the time dependence of polarization is dictated by the natural dynamics of the liquid.

A simple but fairly accurate way to describe the slower part of solvation dynamics (that involves re-arrangement of the solvent molecules which are the nearest neighbours of the probe) is to use a wave-vector dependent relaxation time (as is routinely used in the description of the neutron scattering experiments). In the slow relaxation regime we are interested here, one can use a Smoluchowski-Vlasov type kinetic equation of motion to describe the rotational and translational motion of water molecules. This translational-rotational diffusion equation can be solved to obtain the following expression for the k-dependent relaxation time of longitudinal polarization relaxation[21]

$$\tau^{bulk}_{L}(k) = (1/2\, D_R)\, f(k)[1+(D_T k^2/2D_R)]^{-1}, \qquad \ldots(11)$$

with D_R and D_T as the rotational and translational diffusion coefficients, respectively, and f(k) is a force constant which describes orientational correlation among the water molecules at wave-vector k. At the wavenumbers that correspond to the distance of nearest-neighbour separation, f(k) $\cong$ 1.0-1.5. At small wavenumbers ($k \approx 0$), the relaxation time goes over to the standard form of the longitudinal relaxation time.

The above expression indeed reproduces the observed slow decay with time constant about 1 ps. Actually, the above excellent agreement is a bit fortuitous because one expects some perturbation of the values of the transport properties near the solute. The slowest time constant is about 1 ps, which is determined by the individual rotational and translational motions of the molecules which are close to the probe.

In the hydration layer, the situation changes because now the long time component of the solvation dynamics is coupled to slow component of orientational correlation time. This slow component of orientational relaxation is given by eq.(8b). Since the long time component in solvation dynamics comes from the single particle orientation (as discussed above), we deduce that approximately solvation dynamics, in the long time limit, is controlled by the slow component of orientational relaxation. However, translational diffusion continues to play a role, as discussed in the next section where a relation between solvation dynamics and residence time is derived.

2.6 Residence Time of Water Molecules in the Hydration Layer, the Transition Rates, k_{bf} and k_{fb} and the Solvation Time

One often obtains an approximate estimate of the residence time of a water molecule in a layer of width (or, thickness) L_H by using the following expression

$$D_{perp}=L_H^{2}/6\tau_{res}, \qquad \ldots(12)$$

where D_{perp} is the diffusion in the perpendicular (to the protein surface) direction and τ_{res} is the time taken to cross the layer. Note the factor 6 here – not 2, because we are still in 3 dimensions. L_H is typically 4 Å and D_{perp} is to be calculated as follows. In the bulk, D_{perp} is (1/3) D_{bulk}. Typical values of D_{bulk} is 2.5. 10^{-5} cm^2/sec. However, in the hydration shell, both the parallel and perpendicular components of diffusion coefficient decrease – the perpendicular one gets more affected. Thus, it is reasonable to assume that D_{perp} is approximately (1/4)th -(1/5)th of the bulk value. Combining all these considerations, we get an estimate of t_{res} in the 40- 50 ps range. Note that this is much shorter than the estimate (300 ps) sometimes quoted in the literature of NOE experiments.

In fact, the above method gives only a rough estimate of the residence time. In reality, the residence time in the biological layer will certainly have a broad distribution. The molecules that are bound to the hydration sites of the protein will have much longer residence time. The residence time of these molecules are expected to be a function of the binding energy. In fact, the expression derived above for the mean first passage time can be used to obtain a reliable estimate of the residence time. The final expression of the mean first passage time is given by

$$T(x_0) = (1/D) \int dy\, \exp(\beta V_i(y))\, \beta \int dz\, \exp(-\beta\, V_i(z)). \qquad \ldots(13)$$

This $T(x_0)$ has a dependence on the initial position x_0 and also on the hydrogen bond strength, through $V_i(z)$. Thus, this T(x_0) will also have a distribution. The average residence time will be given by double averaging

$$\tau_{res} = \langle\langle T(x_0) \rangle\rangle = \int dx_0 P(x_0) \int d\varepsilon P(\varepsilon) T(x_0). \qquad \ldots(14)$$

Here $P(x_0)$ is the initial population distribution in the potential well and $P(\varepsilon)$ is the distribution of the hydrogen bond energy. $P(\varepsilon)$ can have a wide distribution. One can even include the effects of hydrophobic interaction by including a repulsive surface. Note that the above expression for the residence time is perfectly general. Actually, the residence time of bound water should be a sum of time to get free and time to travel across the layer. The above expression can be used to find out the distribution of residence times in the hydration shell. The main idea is to translate the distribution of binding energy to the distribution of residence time. This is trivial because we can calculate the residence time at each energy.

One intuitively expects a relation between the solvation dynamics and the residence time of a water molecule in the hydration layer because the residence time is a measure of the mobility of the water molecules. It turns out that there can exist two such relations, one for the fast, 1 ps component and another for the slow, 20-40 ps component of solvation. We have already discussed that the time constant for the fast part of solvation is given by eq. (8a) This equation allows us to make a simple connection with the residence time of the free molecules for whom the residence time can be defined as $\tau_{res} = L_H^2/(6\, D_{perp})$. We now use this relation to eliminate D_T from the above equation to obtain the following expression

$$\tau_{solv}/\tau_{rot} = [1 + AL^2\, \tau_{rot}/\tau_{res}]^{-1}, \qquad \ldots(15)$$

where τ_{solv} is the solvation time from the hydration shell. L is the length of the shell. $A/\sigma^2 = 4\pi^2/4 \approx 10$. We have also used $D_{perp} = D_T/3$. We have also used the relation $\tau_{rot} = (2D_R)^{-1}$. This relation shows that in the limit of long residence time, solvation is determined by rotational diffusion.

For strongly bound water molecules, on the other hand, the residence time is given by the inverse of the rate of transition from the bound to the free state[17]

$$\tau_{res} = [k_{fb}]^{-1}. \qquad \ldots(16)$$

This is an interesting result that shows that the long time component of polar solvation dynamics is equal to the residence time of the water molecules.

Because of the existence of binding sites of different energies on the protein surface, it is convenient to discuss dynamics in terms of a distribution of residence time. Once an expression of the residence time is available, it is easy to translate the distribution of binding energy to the distribution of residence time. This is trivial because we can calculate the residence time at each energy.

3 Connection with NOE Experiments

NOE provides us with an approximate estimate of the residence time from the sign of the NOE signal. When the sign of the signal is positive, there is significant dynamics at the Larmor frequency, ω_0. NOE signal is found to be negative for the protons inside the protein, implying that for those protons there are no dynamics, which populate at 600 MHz. The functional relation between the NOE cross-section and the density of states is given by[8,9]

$$\sigma^{NOE} = 6J(2\omega_0) - J(0), \qquad \ldots(17)$$

where ω_0 is the Larmor frequency of the protons. A simple exponential approximation for the decay of J(t) with correlation time τ_{NOE} shows that σ^{NOE} changes sign when the product $\omega_0\tau_{NOE}$ is greater than 1.12. At ω_0 = 600 MHz, one finds the signal to be positive which in turn implies that τ_{NOE} is less than 300 ps. Since NOE is sensitive to the spatial resolution, it is safe to assume that the stochastic modulation populating $J(\omega)$ is due to exchange of water between the hydration layer and the bulk. Note however that NOE can only give an upper estimate of the residence time.

There is, however, a misconception that needs to be clarified. In the initial analysis of the NOE data, it was assumed that the translational self-diffusion coefficient of water is only about 1.5×10^{-6} cm^2/sec. This gave an estimate of the residence time in the same range as 300 ps. Thus, it is often stated that NOE provides an estimate of the residence time in the range of 300 ps. This is not correct because the self-diffusion coefficient of water in the hydration layer in general does not decrease by 16 fold as assumed in the above analysis. A more realistic value reduces the estimate significantly.

4 Relation of the Rotational Friction on Protein with the Residence Time

As mentioned in the introduction, despite considerable interest, this has remained an unsolved problem for a long time. We expect the residence time to give a measure of the additional friction on the rotating protein or bio-molecule due to the hydration layer. How to find this additional contribution? Fortunately, we know the answer in the two opposing limits. When the residence time of the hydration water is very long, even longer than the rotational correlation time of the protein (τ_{RP}), then the friction would be larger and this can be approximately incorporated by increasing the size of the protein. However, if the residence time is very short, the effect of the hydration layer will not be felt by the protein. Thus, the ratio of the two times, τ_{res}/τ_{RP} is an important quantity.

One way to proceed is to assume a splitting of the total friction as is common in the dielectric friction theories[22]

$$\xi = \xi_{hyd} + \xi_{bw}\ , \qquad \ldots(18)$$

where ξ_{hyd} is the hydrodynamic contribution, which can be equated to the total on the protein friction in the absence of the biological water and is equal to $8\pi\eta R^3$, where η is the viscosity of bulk water. The biological water contribution now needs to be calculated microscopically which we now describe.

If R is the radius of the protein, then the torque due to this friction can be calculated from the torque-torque time correlation function (TTTCF). This TTTCF will be proportional to the number of water molecules on the surface, which is equal to $4\pi R^2 L\ \rho$ where ρ is the number density of water at the hydration shell. The friction can be calculated by using Kirkwood formula which is given by[22]

$$\xi_{bw} = (\beta/3) \int dt \langle N(0).\ N(t)\rangle, \qquad \ldots(19)$$

where <..> denotes a standard time averaging. We assume that the decay of the torque occurs by orientation of the water molecules, which, as we have already shown, is related to the residence time of the water molecules. Thus, the biological water friction is given by

$$\xi_{bw} = \beta\ (4\pi R^2 L\ \rho) \langle\chi^2\rangle\ \tau_R/3\ , \qquad \ldots(20)$$

where $\langle\chi^2\rangle$ is the mean square torque by a single water molecule on the protein. Note that the rotational correlation time is closely related to the residence time, τ_{res}. $\langle\chi^2\rangle$ can approximately be calculated by assuming that the water molecule faces a polar stationary surface and the mean square torque comes from the different orientations and positions of the water molecule in the layer. That is, $\langle\chi^2\rangle$ can be expressed in terms of ion-water pair correlation function.

There is an interesting aspect of this problem - the additional friction due to the biological water must also contain the dielectric friction due to the interaction of polar charges of the amino acids with the water molecules. The dielectric friction contains a significant part that is local and this part is absorbed in the above expression of ξ_{bw}.

When the size of the protein is very large (R>>L), the hydrodynamic term dominates, as expected and the influence of hydration layer on total rotational friction is not significant. However, when R is not too much larger than L (may be a factor of 5 or so), then the contribution from the biological water can be non-negligible, especially if the residence time is long. On the contrary, if the residence time is short, then the effect of the hydration layer on the rotational motion can be neglected.

In the limit of very long residence time (corresponding to slow hydration water dynamics), an interesting scenario can develop. In this ice–like situation, the mean-square torque also decreases (because even equilibrium fluctuations decrease). Although it is very hard to calculate either $\langle\chi^2\rangle$ or τ_R in this condition, the product $\langle\chi^2\rangle\ \tau_R$ must obey the following relation

$$\langle\chi^2\rangle\ \tau_R = 18 k_B T\ \eta/\rho\ . \qquad \ldots(21)$$

Thus one recovers the correct limiting friction (that R should go over to R+L in the hydrodynamic friction). This relation, valid in the case of immobile hydration layer, can be used to get a measure of $\langle\chi^2\rangle$. While the above is by no means a complete theory, it clarifies the relation between the friction due to the hydration shell and the dynamical quantity like the residence time. Details of this work is available elsewhere[18].

5 Origin of the Ultra-slow Component Observed in the Solvation Dynamics in Lipids and Micelles

Many experiments have reported observation of a very slow component in the time dependence of the fluorescence Stokes shift of a polar probe in micelles, lipids and membranes[1,5]. This component seems to have a significant weight (estimated to be as high as 50% in some cases). The time constant can be as high as a few ns. Given that solvation dynamics in water are almost within 1 ps and that in the protein surface within less than 100 ps, this large value is indeed surprising. There could be several possibilities, including structural changes in the self-assembled systems. Since many of the probes used are rigid molecules, one can rule out the possibility of structural change in the probe molecules themselves. While the possibility of structural change in these systems cannot be ruled out, there are two reasons why such changes may not play significant role here. These structural changes are expected to occur even slower, may be in the ns scale. Second, the probe molecules, which are expected to be largely in the hydrophobic core, may not experience overall change in the environment. One should also consider the possibility of constrained water molecules contributing to such slow solvation. Simulations and theoretical studies rule out the existence of such very slow water molecules on the surface of these assemblies. There still remains the possibility of water molecules constrained inside the hydrophobic core. For example, in micelles, water molecules could seep into the core up to 3-4 hydrocarbon layers counting from the head group carbon atom. These water molecules will solvate by individual rotations. However, it is unlikely that the individual rotation of these water molecules slows down by three orders of magnitude.

We propose the following scenario. Before excitation, a significant number of probe molecules are in the hydrophobic core. As the probe is excited at time t=0, the probe develops a large dipole moment. The free energy surface of the probe changes as a result, with the outer surface becoming more stable than the hydrophobic core. There will be a net flow of probe molecules from inside the core to the outside. The total flow will be determined by the magnitude of the dipole moment change. This process is slow because it involves transport through the hydrophobic core. If the thickness L_H is 10 A, the self-diffusion of the probe is 10^{-6} cm^2/sec, then the time takes to cross the hydrophobic core to reach the surface is 1.67 ns. This is in the correct range.

We next need to address the amount of energy gained by transporting to the surface. If the change in dipole moment on excitation is $\Delta\mu$ and if the radius of the probe is a, then the gain in energy (ΔE) is given, within the continuum model, by

$$\Delta E = \frac{2(\Delta\mu)^2}{a^3}\left(\frac{\varepsilon_0 - 1}{2\varepsilon_0 + 1} - \frac{\varepsilon_{sa} - 1}{2\varepsilon_{sa} + 1}\right)$$

where ε_0 and ε_{sa} are the static dielectric constant of water and the self-assembly (hydrophobic core), respectively. What is probably involved in ε_0 is the dielectric constant of the hydration layer, so ε_0 is less than 80. However, ε_{sa} is much lower, of the about 3 or so. If the change of dipole moment is 5D and radius is 4 A, then the gain in energy is about 380 cm^{-1}. This is a very crude estimate, as one should also include the increase in energy due to the hydrophobic interactions. The main idea of the above calculation is to demonstrate that one can indeed account for a significant shift in fluorescence Stokes shift within this model. Note that the time constant of this shift is determined by the time taken by the probe to diffuse from the core to the surface.

6 Concluding Remarks

In this article, we have discussed several aspects of protein hydration dynamics. They include solvation dynamics of a natural probe on protein surface, orientational relaxation and residence time of the water molecules, the friction on a protein due to the presence of the hydration layer and the relationship between NOE and solvation dynamics experiments. The emphasis of this article has been on understanding the elementary processes that give rise to the complex dynamics in the hydration layer.

The most notable aspects of hydration dynamics are the recently discovered slow solvation dynamics of a natural probe and non-exponential orientational relaxation of the water molecules in the layer. It is demonstrated here that both these aspects can be understood in terms of a simple model which assumes the existence of a dynamic equilibrium between bound and free water molecules. This dynamic equilibrium introduces a slow time scale in the relaxation of water molecules in the layer and is also responsible for the observed highly non-exponential orientational dynamics. The slow time is equal to the inverse of the rate of transition from the bound to the free state of water at the interface.

Another aspect of this work is the establishment of a relationship between the residence time of water molecules in the layer and the solvation dynamics. It is shown that in the limit of large binding energies, these two become equal to each other. This result is expected on physical grounds. The theory also provides a relationship for the general case.

The effect of the hydration layer on the mobility of the protein is a problem that has remained unsolved for a long time. This is clearly a very difficult problem. In this article, we have presented an expression of this friction. The novel aspect of this expression is the dependence of this friction on the residence time of the water molecules. In the limit of vanishing residence time, the effect of the hydration layer becomes negligible. In the opposite limit of very slow dynamics in the hydration layer (the glassy state), the effect becomes significant. Further work is required in this problem.

We have established that NOE experiments are likely to be sensitive to strongly bound water molecules, with binding energy more than 8-10 kcal/mole. In this case the residence time can be of the order of 300 ps. The existing NOE measurements could give an upper limit of residence time which is in agreement with the present theory.

The present work suggests many future problems. It should be possible now to explore the dynamics of denatured proteins by using TDFSS. In this case we expect a slow component from protein motion. Although a beginning has been made for the hydration friction, further theoretical work will be worthwhile.

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