Dielectric Modulation of Biological Water

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We show that water constrained by vicinal hydrophobes undergoes a librational dynamics that lowers the dielectric susceptibility and induces a "redshift" of the relaxation frequency in the hydration shell. The results shed light on the way proteins enhance their intramolecular interactions as they fold or associate.

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Protein folding involves backbone hydrogen bonds (H bonds) which, due to the high dipole moment and orientational versatility of surrounding water, can occur efficiently only if this water is immobilized, structured, or ultimately removed [1]. It has been appreciated for some time [2,3] that hydrophobes structure water. Hence, it was recognized that the hydrophobic amino acids (a.a.) of a protein play the determining role in protecting backbone H bonds; e.g., they provide an efficient wrapping of exposed hydrogen bonds [4]. We show that hydrophobic caging of surrounding water also enhances Coulombic interactions between charged groups of a protein during folding. The present model incorporates explicitly in the hydration forces the complementary aspects of strength and dynamics. This seems especially appealing because it shows an alternative possibility [5] to correlate the solvent effects to the effective a.a./a.a. interactions. Although we do not discuss here the relative importance of the two possible roles of the protection of the hydrophobic effect, our results suggest that, besides the stabilization of structures, most notably the native structures, hydrophobes can be important in the guidance of folding paths by providing downhill steps along the way.

Hydrophobic hydration was studied from various perspectives, and an extensive review, including long standing experimental observations, was recently published [6]. Although the previous theoretical results are suggestive, they do not provide a molecular description connecting the dielectric properties of the hydration layer to the widely accepted intuitive pictures of the hydrophobic effect, such as local inhibition of H-bond exchange and water structuring. This is precisely the aim of this Letter, in which the reduction of H-bond exchange possibilities of water molecules at a hydrophobic interface is assessed in terms of a self-consistent expression for the dielectric susceptibility.

Hydrophobicity is an indirect effect resulting from a peculiarity of the water structure [6–8]. Water molecules exchange H bonds with neighbors at a fast rate ($\nu_b \cong 1.7 \times 10^{13}\ \mathrm{s}^{-1}$ at room temperature [9]). This trade is associated with a fast and random reorientation of the individual dipoles ($\vec{d}$) of the water molecules under the influence of the thermal energy ($k_B T = \beta^{-1}$). At the interface between water and a non-H-bonding group such as CH_3, a water molecule has fewer opportunities for the H-bond exchange, leading to extended lag times for reorientation of its dipole. This delay enhances the probability for an adjacent dipole to join the slowly fluctuating dipole and create a water dipole pair. These dipole pairs are long-lived states and give rise to a structured water shell around the hydrophobic unit. The spacing r_{ij} of the dipoles in a pair is random and ranges between the typical interspace between bulk water molecules a_0 [$a_0 = (\frac{3}{4}\pi n)^{1/3}$, where n is the density of bulk water] and a critical distance r_c; r_c is determined from equilibrium energy considerations by equating the free energy of a dipole pair at a critical spacing (r_c) with that corresponding to a bulk pair. This gives

$$r_c = a_0\left(1 - \frac{3\varepsilon_0}{d^2 n} T\Delta s\right)^{-1/3} \cong a_0\left(1 + \frac{\alpha}{3}\right), \qquad \left(\alpha = 3\varepsilon_0 \frac{T\Delta s}{d^2 n} \ll 1\right), \tag{1}$$

where d is the dipole moment of a single water molecule and ε_0 stands for the dielectric constant of the vacuum. Δs represents the entropy penalty [10] of a water molecule due to the reduction (f) of H-bond exchange possibilities from m possibilities in bulk water to $m - f$ possibilities at the hydrophobic interface and can be written as $\Delta s = k_B \ln\frac{m}{m-f}$. Here, $f < m$, strictly because only the sides of the water molecule facing the hydrophobe are precluded from H-bond exchange. For temperatures of biologic interest, $T\Delta s$ is much smaller than the Lorentz field of a dipole pair $E_L d = \frac{d^2 n}{3\varepsilon_0}$. This yields $\alpha(f) = \frac{T\Delta s}{E_L d} = \frac{1}{\beta E_L d} \times \ln(1 - \frac{f}{m})^{-1} \ll 1$, which is the limit of the present theory. Because the equilibrium condition (1) yields $a_0 \leq r_c$, it is easy to infer that the hydrophobic effect leads to a depletion of water molecules at the interface. $a_0 \leq r_c$ sets also the limits for the thickness of the hydration layer.

Because the spacing r_{ij} between the dipoles i and j in a pair is a random variable, the vector dipole field $\vec{E}$ at each site in the system is also random [11]. To derive the average thermodynamic properties of the system, we need the probability distribution of these internal fields. For a system of N dipoles in thermal equilibrium interacting through the field $\nu_{ij} = \frac{d}{4\pi\varepsilon_0 r_{ij}^3}$, with their thermal averages denoted by $\langle\vec{d}\rangle = d\langle\vec{\mu}\rangle$, and independently distributed in the volume V with a probability V^{-1}, the probability distribution of their random dipole fields $\vec{E}$ is given by the integral equation [11]

$$P(\vec{E}) = (2\pi)^{-3}\int d\vec{\rho}e^{i\vec{\rho}\vec{E}}\left[V^{-1}\int d\vec{E}P(\vec{E})\int d\vec{r}e^{i\nu(r)\vec{\rho}\langle\vec{\mu}\rangle}\right]^N. \tag{2}$$

Our integration over r is taken between a_0 and r_c, which is the linear dimension of the correlated region. We look for a solution of (2) in this domain. First, we compute the quantity

$$\begin{aligned} V' &\equiv V - \int d\vec{E}P(\vec{E})\int d\vec{r}e^{-i\nu(r)\vec{\rho}\langle\vec{\mu}\rangle} \\ &= \frac{\rho d}{3\varepsilon_0}\int d\vec{E}P(\vec{E})|\langle\vec{\mu}\rangle\cos\theta_\mu|\int_{z_c}^{z_0} dz\left[\frac{1-\cos z}{z^2}\right. \\ &\quad \left. + i\,\mathrm{sgn}(\langle\vec{\mu}\rangle\cos\theta_\mu)\frac{\sin z}{z^2}\right], \end{aligned} \tag{3}$$

where $z = \frac{\rho d}{4\pi\varepsilon_0 r^3}|\langle\vec{\mu}\rangle\cos\theta_\mu|$ and θ_μ is the angle between $\vec{\rho}$ and $\langle\vec{\mu}\rangle$. $\mathrm{Re}(V')$ describes the scatter of local fields in a disordered system of dipoles, which prevents the occurrence of a preferred orientation of the dipoles. $\mathrm{Im}(V')$ determines the most probable value of the local field which tends to order the dipoles coherently. Note the V' is independent of $\vec{E}$ and θ_μ. By integrating over all possible field orientations, we make the direction immaterial. Averaging over θ_μ may slightly modify the effective dipole d, which, anyway, enters our calculation as a parameter. If $\alpha(f) \ll 1$, the domain of integration is small and $\mathrm{Re}V' \cong \frac{\rho d}{3\varepsilon_0}\int d\vec{E}P(\vec{E})|\langle\vec{\mu}(\vec{E})\rangle\cos\theta_\mu|(z_0 - z_c)z_c^{-2}(1-\cos z_c)$. Next, we perform a power-series expansion of $\cos z_c$ and keep only terms linear in α. This gives $\mathrm{Re}V' \cong \frac{\rho^2 E_L^2}{18n}\alpha(f)\overline{|\langle\vec{\mu}\rangle|^2}$, where $\overline{|\langle\vec{\mu}\rangle|^2} = \int d\vec{E}P(\vec{E})\times|\langle\vec{\mu}(\vec{E})\rangle\cos\theta_\mu|^2$. The equation for the dipole field distribution (2) now can be integrated, yielding

$$P(E;\overline{\langle\vec{\mu}\rangle};f) = [2A(\overline{\langle\vec{\mu}\rangle},f)\sqrt{\pi}]^{-3}e^{-E^2/[4A^2(\overline{\langle\vec{\mu}\rangle},f)]}, \tag{4}$$

where $A^2(\overline{\langle\vec{\mu}\rangle},f) = \alpha(f)\frac{N}{N_0}\frac{E_L^2}{18}\overline{|\langle\vec{\mu}\rangle|^2}$. N_0 is the number of sites in the correlated region, N of which are occupied by molecular dipoles ($N_0 \cong fN$, $f > 1$). Equation (4) gives a self-consistent integral equation for the probability distribution of the internal field of a system of dipoles correlated over a short region of space through an entropic effect. Although the present picture disregards the quantum-mechanical aspect of the H bonds, we can say that it captures the specific and local aspects of interactions of water molecules at the hydrophobic interface.

Now we evaluate the dielectric susceptibility of the system. We recall that the polarization of the whole system can be expressed in terms of the polarization of a single dipole in an effective field averaged over the probability distribution of all fields [11]. The result is then multiplied by the number of dipoles in the correlated region. The input thermodynamic variable is the thermal average of the dipole moment $\langle\vec{d}\rangle = d\langle\vec{\mu}\rangle$ oriented along the random local field acting upon it. This is expressed by the Langevin function $L(x)$, $\langle\vec{\mu}\rangle = L(\beta Ed) = \coth(\beta Ed) - (\beta Ed)^{-1}$. If an external perturbing field $\vec{E}_h$ is applied in the z direction, then the average polarization in the direction of the applied field is $Q_z = Nd\int d\vec{E}'P(\vec{E}';\overline{\langle\vec{\mu}\rangle};f)L(\beta E_z' d)$, where E_z' is the z component of the effective field $\vec{E}' = \vec{E} + \vec{E}_h$ ($\vec{E}$ is the random internal field). The dielectric susceptibility in the z direction in the limit as the externally applied field approaches zero is simply

$$\begin{aligned} \chi_z &= \lim_{\vec{E}_h\to 0}\frac{1}{\varepsilon_0}\frac{\partial Q_z}{\partial E_h} \\ &= \frac{Nd^2\beta}{\varepsilon_0} - \frac{Nd^2\beta}{\varepsilon_0}\int d\vec{E}P(\vec{E};\overline{\langle\vec{\mu}\rangle};f) \\ &\quad \times\left[\coth^2(\beta E_z d) - \frac{1}{(\beta E_z d)^2}\right], \end{aligned} \tag{5}$$

where $P(\vec{E};\overline{\langle\vec{\mu}\rangle};f)$ is given by (4). It must be emphasized that Eq. (5) holds for a spherical symmetry that is initially assumed in the derivation of $P(\vec{E};\overline{\langle\vec{\mu}\rangle};f)$. The specific components of the dielectric susceptibility tensor can be derived once the present treatment for $P(\vec{E};\overline{\langle\vec{\mu}\rangle};f)$ is refined to incorporate the local geometrical aspects of the problem encountered. Here, the average susceptibility χ is approximated by $\chi = \frac{1}{3}(\chi_x + \chi_y + \chi_z)$, and its relation to the dielectric constant ε is $\varepsilon = 1 + \chi$. We denote $\frac{n\beta d^2}{3\varepsilon_0} = \chi_b$ and assume that $\chi_b = 80$ at room temperature (as for bulk water). We notice the departure of (5) from the well-known Langevin-Debye behavior ($\chi \propto T^{-1}$).

Equation (5) describes the dielectric behavior of the hydrophobic hydration shell. This is revealed by the dependence of χ on the depletion parameter f. Figure 1 displays χ against the relative depletion parameter $\frac{f}{m}$. The values are divided by $m + 1 = 5$, which is the number of all water species in the hydration shell. Molecules experiencing high constraints ($\frac{f}{m} \to 1$) exhibit a low susceptibility. Even $\frac{f}{m}$ of 0.25 yields a drastic reduction in χ. Therefore, hydrophobic interfaces drop the dielectric permittivity of the environment. We notice that χ decreases from the value corresponding to bulk water ($f > 1$) towards its value at the hydrophobic interface

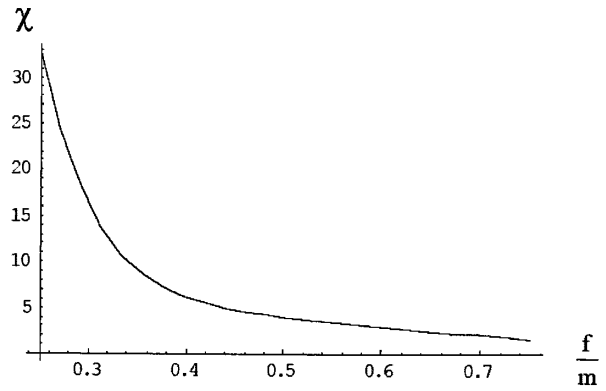

FIG. 1. Average dielectric susceptibility χ against the depletion parameter f/m.

($f \rightarrow m$). Consequently, Coulombic interactions between charged groups are systematically enhanced in the direction of a neighboring hydrophobe up to an order of magnitude. The fact suggests that hydrophobic residues play an active role in mediating intramolecular interactions between the polar side-chain residues of a protein, and intermolecular interactions as well.

Finally, it should be observed that the pair correlation of the water molecular dipoles changes the dispersion properties of the hydration layer. To probe this phenomenon, the complex dielectric constant $\varepsilon^* = \varepsilon' - i\varepsilon''$ has to be examined. Both ε' and ε'' are frequency dependent and usually described by a Debye model with a single characteristic frequency of the dipole reorientation of, e.g., bulk water. Wherever water is structured, the reorientation of every dipole becomes more difficult since it requires surmounting an additional potential barrier due to the local field that tends to orient the dipoles coherently (E_s). The maximum most probable value of E_s is obtained from the imaginary part of Eq. (3) [12], $E_s = \frac{1}{\rho|\langle\vec{\mu}(\vec{E})\rangle|}\mathrm{Im}(N\frac{V'}{V})$, which, for all $\alpha \ll 1$, can be approximated by $E_s \cong E_L\alpha(f)\frac{N}{N_0}$. Besides, we recall that the tendency of reorientation of a molecular dipole is lowered by the configuration entropy penalty Δs. This prompts us to express the relaxation frequency ($\nu_{s,f}$) of the dipole moment in the vicinity of a hydrophobe in terms of free energy,

$$\nu_{s,f} = \nu_0 \exp[-\beta((E_L + E_s)d - T(s_b - \Delta s))]$$
$$\cong \nu_b \exp\left[\left(1 + \frac{N}{N_0}\right)\ln\left(1 - \frac{f}{m}\right)\right], \quad (6)$$

where ν_0 is the frequency of reorientation of an isolated dipole; $E_L d - Ts_b$ is the energy required for the orientation of a molecular dipole in bulk water, with s_b standing for the corresponding entropy. The right-hand side of (6) gives the reduction of the bulk relaxation frequency (ν_b) in the proximity of a hydrophobe. The $f = 0$ term corresponds to bulk water ($\nu_{s,f=0} = \nu_b$) in which all the H bonds per water molecule are fully satisfied. The sudden drop of the relaxation frequency of water in the vicinity of a hydrophobe is a consequence of precluding H bonds, which causes an anisotropic rotation [2]. We notice that the anisotropic reduction in numbers of available partners at the hydrophobic interface induces a shift of the relaxation frequency of the molecular dipole in the hydration shell. Therefore, ε' and ε'' characterizing the hydration shell of a hydrophobe are given by the superposition of f Debye-type contributions. This is an important verifiable result that provides a way to probe the water molecules next to hydrophobes. Clearly, the dielectric loss, which is proportional to ε'', will now be characterized by a convolution of f separate peaks. Their characteristic frequencies will systematically be "redshifted" in comparison with that of the bulk water. The maximum drop of the relaxation frequency occurs next to the interface ($\frac{f}{m} \rightarrow 1$). Assuming that $m = 4$ for a bulk water molecule, using (6) we can compute three characteristic frequencies of water molecules constrained at a hydrophobic interface, $\nu_{s,1}$, $\nu_{s,2}$, and $\nu_{s,3}$. These readily yield $\nu_{s,1} \cong 0.56\nu_b$, $\nu_{s,2} \cong 0.35\nu_b$, and $\nu_{s,3} \cong 0.16\nu_b$, respectively. For water at $T = 293.15$ K, $\nu_b = 1.7 \times 10^{13}$ s^{-1} [9], and the outmost redshifted characteristic frequency in the spectrum is $\nu_{s,3} = 2.72 \times 10^{12}$ s^{-1}. We therefore conclude that water next to a hydrophobe moves almost 1 order of magnitude slower than its bulk counterpart, as predicted by early NMR experiments [3].

On the basis of these illustrative arguments, we infer that further experimental investigations of far-infrared dynamics of water around a hydrophobe would be relevant probes of pair correlation. A conceptually simple experiment which can reveal the pair and higher order correlation of hydrophobic caged water is the measurement of the residual polarization. At the initial time all the dipoles are aligned in the external field. After the field has been turned off, most of the molecular dipoles depolarize rapidly in a time $\frac{1}{\nu}$, but those molecular dipoles structured at the hydrophobic interface (dipole pairs plus higher correlated dipoles) will take much longer to depolarize, namely, a time $\frac{1}{\nu_{s,f}}$. It can be shown that, for a long enough time, the residual polarization has an inverse logarithmic time dependence, $Q_r \sim \frac{1}{\ln(\nu t)}$. Nevertheless, the surrounding correlated water might be responsible for setting an interaction between otherwise noninteracting hydrophobes. Under such circumstances, we may conclude that a hydrophobe-hydrophobe interaction is a solvent-induced effect. This particular aspect of the hydrophobic interaction is currently suggested by molecular dynamics simulations [13].

Thus, a self-consistent, classical statistical-mechanical formulation of the hindered rotational motion of water molecules, based on the short-range pair correlation of the molecular dipoles, explains not only the static dielec-

tric behavior of water confined at a hydrophobic interface for which it appears most appropriate but also the dispersion properties. The decrease of the dielectric coefficient of hydrophobic caged water was not addressed previously, although some early speculations on this fact were noted briefly [1,14]. The present approach produces a consistent picture of hydration at hydrophobic interfaces: a thin layer of water molecules adjacent to the hydrophobic core, with low correlation, entropy, dielectric constant, and slow reorientation of their intrinsic molecular dipoles. Averaging the heat capacity of water molecules undergoing librational dynamics over the probability distribution of Eq. (4) reproduces the trend of the excess heat capacity of solvation, a fingerprint for hydrophobic hydration [6]. Finally, we mention implications of these results for the role of the solvent in protein folding and functioning. First, the present work supports the general idea that water structured by hydrophobes enhances the effective forces between charged groups [1]. Further insights come from inspecting (4) and the imaginary part of (3). We see that the internal dipolar field becomes strong, with large variances, at various sites in the hydration layer. This observation implies that the effective mean dipole moment in the hydration layer may exhibit important fluctuations. This appears to be the dominant cause of slaving protein processes by the surrounding solvent, as suggested recently [15]. The hydration shell couples the protein to the surrounding thermal bath, thereby inducing an important modification of the folding time [16]. The typical coupling is described by a damping rate of the solvent [16], and the characteristic frequency ν_s determined above [see Eq. (6)] can be regarded as the upper limit of this rate.

Finally, we put the results presented here in a larger perspective. Immobilized water is also commonly encountered in areas of materials processing, and yet its most basic properties, such as density and dielectric constant, cannot readily be characterized *in situ* [17]. Therefore, some estimate of the confined water properties of the type we presented above will be practical for controlling the formation of nanoparticles, improving the quality of surface catalysts for chemical reactions, and, perhaps for elaborating protocols, for "fixing" broken proteins.

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