Hydrogen Bond Kinetics in the Solvation Shell of a Polypeptide

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Analysis of a series of molecular dynamics simulations reveals that the kinetics of breaking and forming water-water hydrogen bonds is slower in the first solvation shell of a 16-residue polypeptide than in bulk water. The correlation time of hydrogen bonds persists significantly longer near hydrophobic groups than in bulk water. Hydrogen bonds are found to be stronger in the solvation shell of non-polar groups. We show that the difference in hydrogen bond kinetics in the different environments can be understood in the light of the energetics and the concerted forming and breaking of hydrogen bonds.

Hydrogen bonds are responsible for many of water's peculiar properties. [1,2] The process of forming and breaking of the water-water hydrogen bonds plays a significant role in the dynamical behavior of liquid water. [3] Considerable effort has been invested in the understanding of hydrogen bond kinetics in liquid water. [4–9] Evidently the structure and dynamics of water-water hydrogen bonds also determine many aspects of the thermodynamic and dynamic properties of biomolecules in aqueous solutions. The hydrogen bonds in the first solvation shell are of special importance. Probing these "interfacial" hydrogen bonds presents an enormous challenge to experiments. Direct measurements of hydrogen bond relaxation in the solvation shell only emerged very recently. [10] Computer simulations, on the other hand, provide a powerful tool for the study of water-water hydrogen bond kinetics near the solvated biomolecules. Molecular dynamics simulations have provided tremendous insight into the structural features of hydrogen bonds in the solvation shells. [11,12] In this Letter, we use molecular dynamics simulations to study the dynamic processes of the formation and breaking of water-water hydrogen bonds in the first solvation shell of a polypeptide. We observed significant differences in the dynamic behavior of the hydrogen bonds in bulk water and in the proximity of various groups of the polypeptide.

The polypeptide chain of the last 16 residues (GEWTY-DDATKTFTVTE) to the C-terminus of the immunoglobulin binding protein G (PDB ID 2gb1) is chosen for this study. The 16-mer polypeptide has been shown to form a β-hairpin structure in aqueous solutions. [13] The residues Trp43, Tyr45, Phe52 and Val54 form an extended, flat hydrophobic surface exposed to the solvent. Hydrogen bond behavior near such a surface is of particular interests because of its relevancy to the understanding of hydrophobic hydration. The 16-mer chain is cut from the NMR structure of the entire protein, and the resulting chain is acetylated and amidated at the N-terminus and C-terminus, respectively. The 3 negative charges on the peptide are balanced by 3 Na^+ ions. A water box of $38\text{Å} \times 38\text{Å} \times 38\text{Å}$ is used to solvate the polypeptide, and the water molecules that overlap with the solute atoms are removed, resulting in 1574 water molecules in the box. The SPC [14] model is used for water and the OPLS/AA force field [15] is used to model the polypeptide. The structure of the fully solvated polypeptide chain is first locally minimized in potential energy using the conjugate gradient method. The backbone of the peptide is subsequently fixed in space in the following simulations, but the side chains are free to move. The fixed backbone of the polypeptide helps to better understand the dependence of hydrogen bond behavior on the surface topography. The mobility of the polypeptide backbone, except for the indirect effect of creating a fluctuating environment, should have negligible effects on the observed hydrogen bond dynamics, because the latter moves on a much faster time scale. The recently developed P3M Ewald/rRespa algorithm [16] is used to compute the electrostatic interactions and integrate the equations of motion. An outer time step of 4 femtoseconds(fs) is used to guarantee stable trajectories. RATTLE is used to keep water rigid and bond length fixed. Periodic boundary condition is applied. [17] The system is equilibrated for 500 picoseconds(ps) at 298.15K and 1atm by an isothermal-isobaric MD simulation, using Berendsen thermostat and barostat. [17] Fifteen uncorrelated phase points, evenly spaced in time, are selected from a subsequent 160-ps isothermal-isobaric MD simulation. Fifteen 100-ps microcanonical MD simulations are carried out starting from these phase points. Every 20fs a configuration is used for the analysis below.

We employ the widely used definition of solvation shells. A water is considered to be proximal to a solute atom and belong to its first solvation shell if the water's oxygen is closer to that atom than to any other solute atoms and the distance is no greater than 4.0Å [12,18] (3.25Å in the case of Na^+ [19]). Of particular interests are the water molecules in the following 4 environments:

0   the water molecule is in bulk, *i.e.* it's farther away from any solute atoms than 4.0Å.

I   the water molecule is in the solvation shell of the carbon atoms of the above mentioned hydrophobic surface formed by Trp43, Tyr45, Phe52 and Val54.

II   the water molecule is in the solvation shell of an oxygen or nitrogen atom.

III   the water molecule is in the solvation shell of a Na^+ ion.

The environment of a hydrogen bond can then be categorized according to the two water molecules forming the bond. *e.g.* **0··III** will signify a hydrogen bond with one water in the bulk and another in the solvation shell of a Na^+.

The structural relaxation of hydrogen bonds can be characterized by the hydrogen bond autocorrelation function,

$$c(t) = \langle h(0)h(t)\rangle/\langle h\rangle \tag{1}$$

where $h(t) = 1$ if the tagged water pair is hydrogen bonded at time t, and $h(t) = 0$ otherwise. [20,9] We adopt a geometric definition of hydrogen bonds, according to which a water pair is hydrogen bonded if the oxygen-oxygen distance is no greater than 3.5Å (the first minimum in the oxygen-oxygen radial distribution function of liquid water) and simultaneously the bonded O—H$\cdots$O angles is no greater than $30°$ (the magnitude of the librational motion that breaks the hydrogen bonds between water). $c(t)$ is the probability that a pair of hydrogen bonded water molecules at time 0 is also hydrogen bonded at time t. We calculated $c(t)$ for hydrogen bonds in various environments (Fig. 1). Compared with hydrogen bonds between bulk water molecules, $c(t)$ decays slightly faster for hydrogen bonds around Na^+, but significantly slower for hydrogen bonds in the vicinity of other solute groups. The relaxation time of hydrogen bonds, τ_{rlx}, can be defined as $c(\tau_{rlx}) = e^{-1}$. [21] $\tau_{rlx}^{I··I} = 6.8$ps and $\tau_{rlx}^{0··0} = 3.2$ps (Table. I). The hydrogen bonds between two water molecules both proximal to the extended hydrophobic surface persist more than twice as long as the hydrogen bonds in bulk. In contrast, the hydrogen bonds around Na^+ ions persist for a shorter time ($\tau_{rlx}^{0··III} = 2.9$ps).

The kinetics of hydrogen bonds for times longer than 1ps is related to the translational pair diffusion of water. [9] It is known that the translational diffusion of water is slower in the solvation shell, [22] therefore the slowdown of hydrogen bond relaxation in the solvation shell in the long time region is partly due to the slowness of water pair's mutual diffusion. To eliminate the contributions from mutual diffusion, we calculated

$$O(t) = \langle h(0)(1-h(t))H(t)\rangle/\langle h(0)H(t)\rangle \tag{2}$$

where $H(t) = 1$ if the pair of water molecules are closer than 3.5Å at time t and $H(t) = 0$ otherwise. $O(t)$ is the conditional probability that a hydrogen bond is broken at time t, given that the involved pair of water molecules haven't diffused away. $O(t)$ describes the time-dependent probability of breaking the hydrogen bond due to the reorientation between the water pair. In Fig. 2, we see that $O(t)$ exhibits significant differences for different environments. Around neutral atoms, hydrogen bonds break more slowly than in bulk water, while around the positively charged Na^+ ions, hydrogen bonds break more rapidly. Therefore diffusion alone cannot account for the slowdown in the long time behavior of hydrogen bonds near neutral atoms.

Hydrogen bond energy is found to vary in different environments (Fig. 3 and Table. I), and this difference in energy can partly account for the difference in kinetic behavior. Hydrogen bonds between two water molecules both of which are in the solvation shell of hydrophobic groups are stronger ($\bar{E}_{ww}^{I··I} = -4.42$kcal·mol^{-1}) than hydrogen bonds in bulk ($\bar{E}_{ww}^{0··0} = -4.17$kcal·mol^{-1}). ($RT = 0.59$kcal·mol^{-1} at $T = 298.15$K.) This supports the "iceberg" model for hydrophobic effect, [23] which states that water molecules form orderly clathrate structures around hydrophobic solutes, with strong hydrogen bonds between each other. The increased strength of hydrogen bonds contributes to the slowness of breaking hydrogen bonds. Around the Na^+ ions, on the other hand, hydrogen bonds are weaker. The small Na^+ ions create a strong electric field **E** which align the water dipoles in a radial fashion. Such dipole alignment distorts the hydrogen bond configurations and weakens the hydrogen bonds, leading to more rapid bond breaking. Energetic considerations, however, cannot account for the slower breaking of hydrogen bonds between a bulk water molecule and a water molecule in the solvation shell of the hydrophobic surface, since such hydrogen bonds have almost identical dimer energy distribution as those in bulk.

The forming and breaking of water-water hydrogen bonds is a highly concerted process. When a hydrogen bond breaks, each of the two involved water molecules usually forms a new hydrogen bond with another water molecule in its coordination shell. Conversely, when a hydrogen bond forms, each of the two involved water molecules usually breaks an existing hydrogen bond with another water molecule. The formation and breaking of hydrogen bonds occurs by having water molecules switch bonding alliance with one another, and the formation of a hydrogen bond usually accompanies the breaking of another. [24] Consequently the dynamic behavior of a hydrogen bond will depend on the number of water molecules that are adjacent, but not hydrogen bonded, to the two water molecules forming the hydrogen bond. The more such "replacement" water molecules there are, the higher the probability that the hydrogen bond is traded with a new one. To investigate the effects of such cooperativity, we calculated the number of water molecules that are closer than 3.5Å but are not hydrogen bonded to the water molecule of interest in different environments, n_{adj} (Fig. 4 and Table. II). The direct relationship between the number of "replacement" water molecules and the probability of breaking a hydrogen bond is manifest. For water in the solvation shell of the hydrophobic surface (**I**), oxygen and nitrogen atoms (**II**), the number of "replacement" water molecules, n_{adj}, is smaller than the bulk value (**0**). The smaller n_{adj} slows down the hydrogen bond relaxation around these solvation shells. Around the positively Na^+, large n_{adj}, in addition to high E_{ww}, accelerates the breaking of hydrogen bonds. (We believe that this acceleration of hydrogen bond dynamics in the vicinity of cations contributes predominantly to the observed faster structural relaxation of water-water hydrogen bonds in aqueous NaCl and KCl solutions. [25])

It is worth noting that we are using a nonpolarizable water model for this study. Experimentally water has a nonzero po-

larizability ($\alpha_{xx}, \alpha_{yy}, \alpha_{zz} = (1.47\text{Å}^3, 1.53\text{Å}^3, 1.42\text{Å}^3)$). [26] Water polarizability makes hydrogen bond kinetics more dependent on the local environment, and introduces further cooperativity into hydrogen bond kinetics. The effects of water polarizability on hydrogen bond kinetics is investigated in another work. [27]

To capture more details of the dynamic process of the hydrogen bonds in different environments, we studied the forming and breaking of hydrogen bonds in the time period shorter than 1ps. Rapid librational and vibrational motions dominate the hydrogen bond dynamics on this time scale. We calculated the first passage time of hydrogen bonds, $\bar{\tau}_{HB}$, defined as the time between the formation of the hydrogen bond and the first breaking of the bond (Table. I). [20,7] The hydrogen bond dynamics on this short time scale is expectedly sensitive to the definition of the hydrogen bond. [21] It is a highly complex many-body problem that makes a quantitative model all but impossible. Therefore, we only give a qualitative rationalization of the results. When the hydrogen bond has only one water molecule in the solvation shell of C, O, O$^-$ and N atoms (**0··I** and **0··II**), the mean first passage time is little different from that in bulk (**0··0**). The influence of these solute atoms on the fast evolution of the nearby hydrogen bonds is a 3-body effect and therefore weak. When the hydrogen bond is between two water molecules in the solvation shell of the hydrophobic surface (**I··I**), $\bar{\tau}_{HB}$ is much longer than the bulk value. We speculate that when both water molecules are in the solvation shell, the geometric confinement from the solute atoms reduces the amplitude of the high frequency rotational and librational motion of water molecules, thereby fixing the hydrogen bond in its bonding conformation. One of the water molecules has to move out of the geometric confinement before it can reorient to break the hydrogen bond. This lengthens the lifetime of the hydrogen bond. When the hydrogen bond is around Na$^+$, on the other hand, $\bar{\tau}_{HB}$ is much shorter than the bulk value. The strong electric field distorts the hydrogen bonds to such extent that they rapidly vacillate between the intact and broken states.

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TABLE I. Hydrogen bond relaxation time τ_{rlx} (defined as $c(\tau_{rlx}) = e^{-1}$), mean first passage time $\bar{\tau}_{HB}$, and average dimer energy between the hydrogen bonded water pairs E_{ww}, in the different environments as defined in the text. The difference between τ_{rlx} and $\bar{\tau}_{HB}$ indicates that there are rapid recrossings of the transition state of breaking hydrogen bonds.

Environment	τ_{rlx}(ps)	$\bar{\tau}_{HB}$(ps)	E_{ww}(kcal·mol^{-1})
0··0	3.2	0.26	-4.17
0··I	5.0	0.27	-4.17
0··II	5.4	0.27	-4.21
0··III	2.9	0.21	-3.98
I··I	6.8	0.31	-4.42

TABLE II. The average number of hydrogen bonds that a water forms with other waters, n_{HB}, and the average number of adjacent waters within 3.5Å that a water is not hydrogen bonded to, n_{adj}. n_{HB} and n_{adj} are collected for water in different environments, as defined in the text. There is a direct relationship between n_{adj} and the dynamic behavior of hydrogen bonds. (*c.f.* Table. I). In contrast, no obvious relationship exists between n_{HB} and the hydrogen bond dynamics.

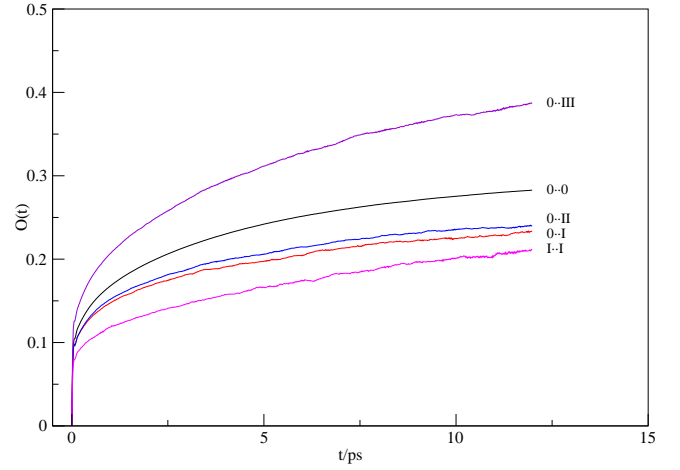| Environment	n_{HB}	n_{adj}
0	3.5	1.7
I	3.2	1.1
II	2.8	1.2
III	2.1	3.2

FIG. 2. The conditional time-dependent probability of breaking hydrogen bonds, $O(t)$ (*c.f.* Eqn. 2), in different environments.
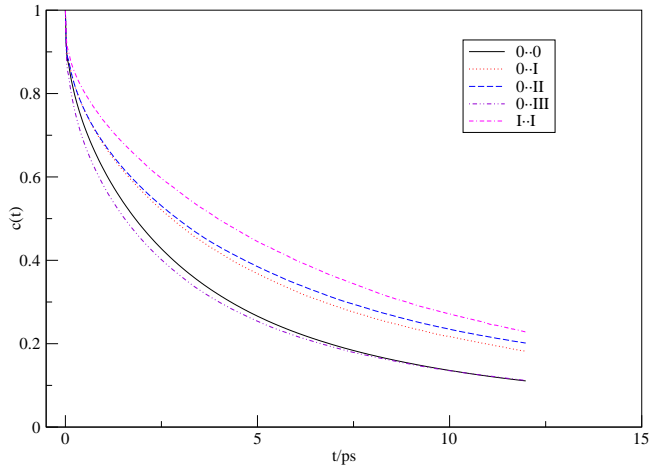

FIG. 1. The hydrogen bond autocorrelation function, $c(t)$, in bulk and in various solvation shells. Different solvation shells are denoted by numerals as defined in the text. Compared with hydrogen bonds in bulk water, $c(t)$ decays slightly faster for hydrogen bonds between a bulk water and another water in the solvation shell of Na$^+$ (0··III). For hydrogen bonds around other solute groups, $c(t)$ decays significantly slower. When both water molecules of a hydrogen bond are in the solvation shell of the extended hydrophobic surface, the decay in $c(t)$ is twice as slow (I··I).
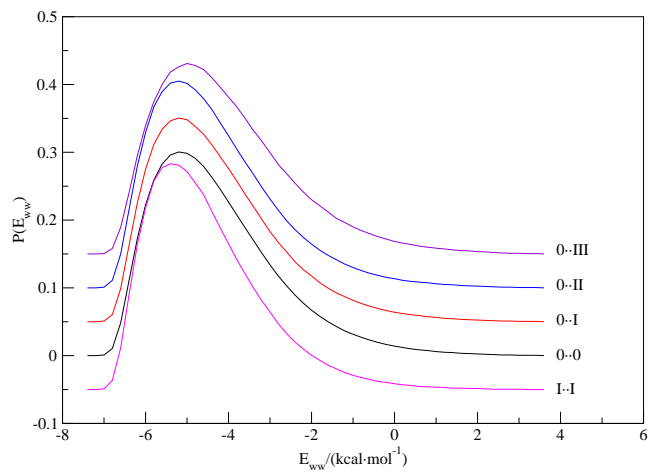
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FIG. 3. The distribution of dimer energies between hydrogen bonded water pairs. Hydrogen bonds are stronger when both involved water molecules are proximal to the extended hydrophobic surface, and are weaker when around the Na^+ ions.
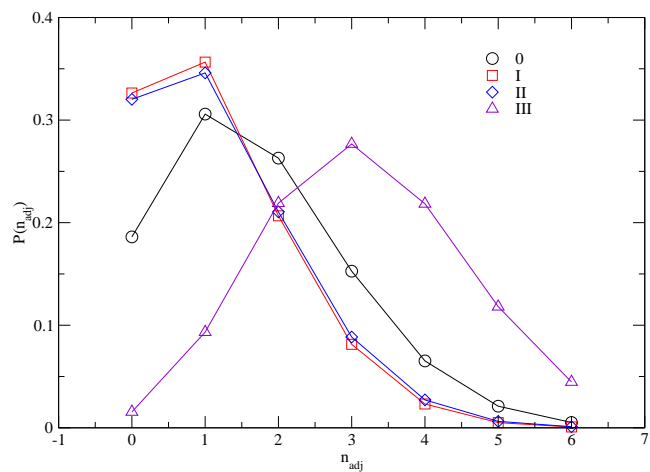

FIG. 4. The distribution of number of adjacent yet non hydrogen bonded water molecules to a water molecule in different environments. The probability to find such adjacent water molecules in the solvation shell of the hydrophobic surface and oxygen or nitrogen atoms (I and II) is smaller than in bulk (0). Water around Na^+ ions has more such adjacent water molecules.