

## LETTERS

### 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 nonpolar groups. We show that the difference in hydrogen-bond kinetics in the different environments can be understood in terms of the energetics and the concerted forming and breaking of hydrogen bonds.

Hydrogen bonds are responsible for many of water's peculiar properties.<sup>1,2</sup> Because the process of forming and breaking water–water hydrogen bonds plays a significant role in the dynamical behavior of liquid water,<sup>3</sup> considerable effort has been made to understand hydrogen-bond kinetics in neat liquid water.<sup>4–9</sup> The structure and dynamics of water–water hydrogen bonds also play an important role in determining 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 experiment. Only recently has it become possible to measure hydrogen-bond relaxation in the solvation shell.<sup>10</sup> Computer simulations, on the other hand, provide a powerful tool for the study of water–water hydrogen-bond kinetics near the solvated biomolecules.<sup>11,12</sup> 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  $\beta$ -hairpin 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 (GEWTYD-DATKTFTVTE) 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  $\beta$ -hairpin structure

in aqueous solutions.<sup>13</sup> 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 particularly interesting 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 three negative charges on the peptide are balanced by three Na<sup>+</sup> ions. A water box of 38 Å × 38 Å × 38 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 simple point charge (SPC)<sup>14</sup> model is used for water, and the OPLS/AA force field<sup>15</sup> 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 us 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<sup>16</sup> is used to

compute the electrostatic interactions and integrate the equations of motion. An outer time step of 4 fs is used to guarantee stable trajectories. RATTLE is used to keep the water rigid and the bond length fixed. Periodic boundary condition is applied.<sup>17</sup> The system is equilibrated for 500 ps at 298.15 K and 1 atm by an isothermal–isobaric molecular dynamics (MD) simulation, using a Berendsen thermostat and barostat.<sup>17</sup> 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 20 fs, 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 4.0 Å<sup>12,18</sup> (3.25 Å in the case of Na<sup>+</sup>).<sup>19</sup> Of particular interest are the water molecules in the following 4 environments: **(0)** the water molecule is in bulk, i.e., it's farther than 4.0 Å away from any solute atoms; **(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<sup>+</sup> 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<sup>+</sup>).

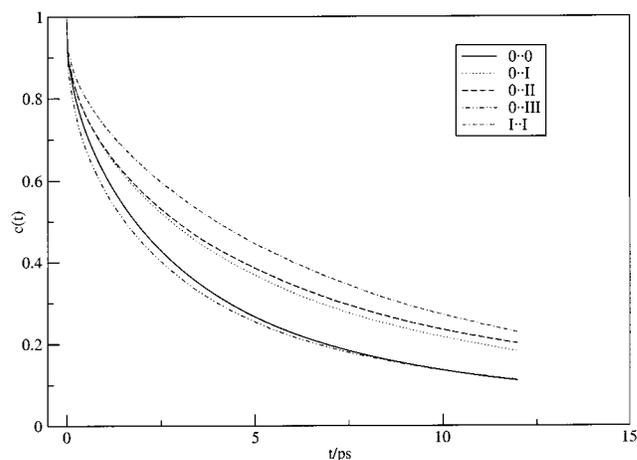
The structural relaxation of hydrogen bonds can be characterized by the hydrogen bond autocorrelation function,

$$c(t) = \langle h(0)h(t) \rangle / \langle h \rangle \quad (1)$$

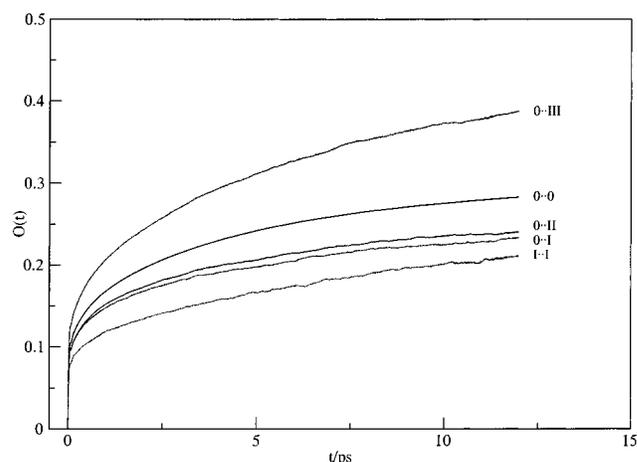
where  $h(t) = 1$  if the tagged water pair is hydrogen-bonded at time  $t$  and  $h(t) = 0$  otherwise.<sup>9,20</sup> We adopt a geometric definition of water–water hydrogen bonds, according to which a water pair is hydrogen-bonded if the oxygen–oxygen distance is no greater than 3.5 Å (the first minimum in the oxygen–oxygen radial distribution function of liquid water) and simultaneously the bonded O–H••O angle 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  $t = 0$  is also hydrogen-bonded at time  $t$ . We calculated  $c(t)$  for hydrogen bonds in various environments (Figure 1). Compared with hydrogen bonds between bulk water molecules,  $c(t)$  decays slightly faster for hydrogen bonds around Na<sup>+</sup> but significantly slower for hydrogen bonds in the vicinity of other solute groups. The relaxation time of hydrogen bonds,  $\tau_{\text{rlx}}$ , can be defined as  $c(\tau_{\text{rlx}}) = e^{-1}$ .<sup>21</sup>  $\tau_{\text{rlx}}^{0\cdots\text{I}} = 6.8$  ps and  $\tau_{\text{rlx}}^{0\cdots\text{0}} = 3.2$  ps (Table 1). 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<sup>+</sup> ions persist for a shorter time ( $\tau_{\text{rlx}}^{0\cdots\text{III}} = 2.9$  ps).

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

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



**Figure 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<sup>+</sup> (**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**).

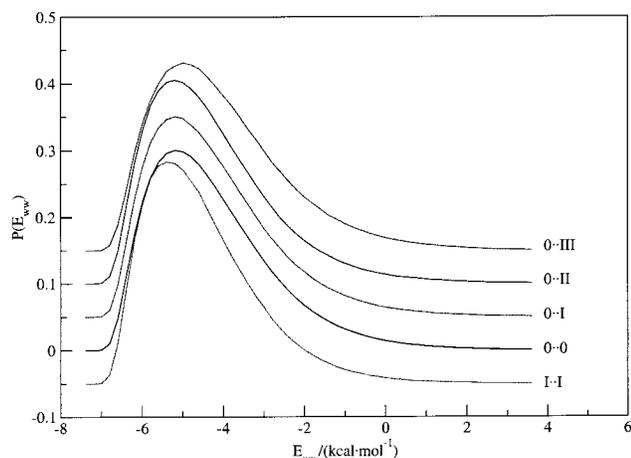


**Figure 2.** The conditional time-dependent probability of breaking hydrogen bonds,  $O(t)$  (see eq 2), in different environments.

where  $H(t) = 1$  if the pair of water molecules are closer than 3.5 Å 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 have not diffused away.  $O(t)$  describes the time-dependent probability of breaking the hydrogen bond due to the reorientation between the water pair. In Figure 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<sup>+</sup> 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.

It is interesting to relate the hydrogen-bond kinetics with rotational dynamics of single water molecules. It has been shown that rotational dynamics of water molecules near solutes differs from that in bulk solution in a similar fashion as hydrogen bond kinetics.<sup>11</sup> We define

$$n(t) = \langle h(0)(1 - h(t))H(t) \rangle / \langle h \rangle \quad (3)$$



**Figure 3.** The distribution of dimer energies between hydrogen-bonded water pairs. The curves have been shifted by different multiples of 0.05 for easy viewing. Hydrogen bonds are stronger when both involved water molecules are proximal to the extended hydrophobic surface and are weaker when around the  $\text{Na}^+$  ions.

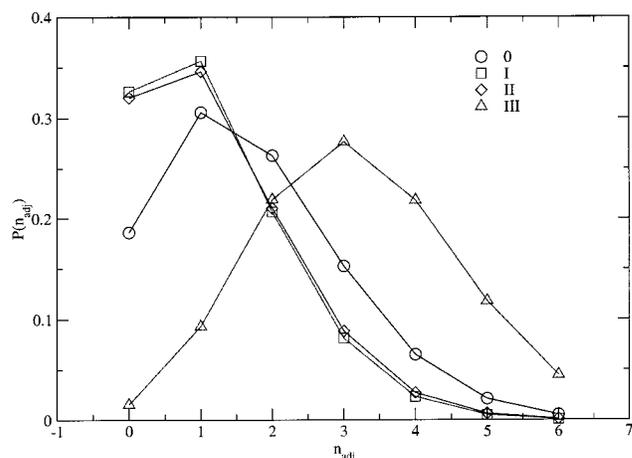
to be the time-dependent probability that a hydrogen bond is broken at time  $t$  but that the pair of water molecules remains in a proximity of  $3.5 \text{ \AA}$ . Intuitively, the relaxation of  $n(t)$  should scale with the rotational time constant  $\tau_2 = \int_0^\infty dt \langle P_2[\hat{\mathbf{e}}(t) \cdot \hat{\mathbf{e}}(0)] \rangle$ , where  $P_2(\cos \theta)$  is the second-order Legendre polynomial, and  $\hat{\mathbf{e}}$  is the unit vector pointing from one hydrogen atom to the other hydrogen atom in the water molecule. In another publication,<sup>23</sup> we show that for different water models M and M'

$$n_M \left( \frac{\tau_2^M}{\tau_2^{M'}} t \right) \approx n_{M'}(t) \quad (4)$$

We also discuss the relationship between  $n(t)$  and  $\tau_2$  in bulk and in the solvation shells of simple hydrophobic and ionic solutes.

Hydrogen-bond energies are found to vary in different environments (Figure 3 and Table 1), 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 ( $-E_{\text{ww}}^{\text{I}\cdots\text{I}} = -4.42 \text{ kcal mol}^{-1}$ ) than hydrogen bonds in bulk ( $-E_{\text{ww}}^{\text{0}\cdots\text{0}} = -4.17 \text{ kcal mol}^{-1}$ ) ( $RT = 0.59 \text{ kcal mol}^{-1}$  at  $T = 298.15 \text{ K}$ ). This supports the "iceberg" model of the hydrophobic effect,<sup>24</sup> 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  $\text{Na}^+$  ions, on the other hand, hydrogen bonds are weaker. The small  $\text{Na}^+$  ions create a strong electric field,  $\mathbf{E}$ , which aligns 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, because such hydrogen bonds have almost identical dimer energy distribution as those in bulk.

The formation and breaking of water–water hydrogen bonds are highly concerted processes. 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



**Figure 4.** The distribution of the 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  $\text{Na}^+$  ions has more such adjacent water molecules.

**TABLE 1: Hydrogen Bond Relaxation Time,  $\tau_{\text{rx}}$  (defined as  $c(\tau_{\text{rx}}) = e^{-1}$ ), Mean First Passage Time,  $\bar{\tau}_{\text{HB}}$ , and Average Dimer Energy Between the Hydrogen-Bonded Water Pairs,  $E_{\text{ww}}$ , in the Different Environments<sup>a</sup>**

| environment <sup>b</sup> | $\tau_{\text{rx}}$ (ps) | $\bar{\tau}_{\text{HB}}$ (ps) | $E_{\text{ww}}$ (kcal mol <sup>-1</sup> ) |
|--------------------------|-------------------------|-------------------------------|---|
| 0 $\cdots$ 0             | 3.2                     | 0.26                          | -4.17                                     |
| 0 $\cdots$ I             | 5.0                     | 0.27                          | -4.17                                     |
| 0 $\cdots$ II            | 5.4                     | 0.27                          | -4.21                                     |
| 0 $\cdots$ III           | 2.9                     | 0.21                          | -3.98                                     |
| I $\cdots$ I             | 6.8                     | 0.31                          | -4.42                                     |

<sup>a</sup>The difference between  $\tau_{\text{rx}}$  and  $\bar{\tau}_{\text{HB}}$  indicates that there are rapid recrossings of the transition state of breaking hydrogen bonds.  
<sup>b</sup> Environments are defined in the text.

shell. Conversely, when a hydrogen bond forms, each of the two involved water molecules usually breaks existing hydrogen bonds with other water molecules. The formation and breaking of hydrogen bonds occur by having water molecules switch bonding alliance with one another, and the formation of a hydrogen bond usually accompanies the breaking of another.<sup>25</sup> 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 is 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  $3.5 \text{ \AA}$  but are not hydrogen-bonded to the water molecule of interest in different environments,  $n_{\text{adj}}$  (Figure 4 and Table 2). 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) or of oxygen and nitrogen atoms (II), the number of "replacement" water molecules,  $n_{\text{adj}}$ , is smaller than the bulk value (0). The smaller  $n_{\text{adj}}$  slows down the hydrogen-bond relaxation around these solvation shells. Around the positively charged  $\text{Na}^+$  ions, large  $n_{\text{adj}}$ , in addition to high  $E_{\text{ww}}$ , 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.<sup>26</sup>)

**TABLE 2: The Average Number of Hydrogen Bonds that a Water Forms with Other Waters,  $n_{\text{HB}}$ , and the Average Number of Adjacent Waters within 3.5 Å to Which a Water Is Not Hydrogen-Bonded,  $n_{\text{adj}}$ <sup>a</sup> for Water in Different Environments**

| environment | $n_{\text{HB}}$ | $n_{\text{adj}}$ |
|-------------|-----------------|------------------|
| <b>0</b>    | 3.5             | 1.7              |
| <b>I</b>    | 3.2             | 1.1              |
| <b>II</b>   | 2.8             | 1.2              |
| <b>III</b>  | 2.1             | 3.2              |

<sup>a</sup> There is a direct relationship between  $n_{\text{adj}}$  and the dynamic behavior of hydrogen bonds (see Table 1). In contrast, no obvious relationship exists between  $n_{\text{HB}}$  and the hydrogen-bond dynamics.

It is worth noting that we are using a nonpolarizable water model for this study. Experimentally, water has a nonzero polarizability ( $\alpha_{xx}$ ,  $\alpha_{yy}$ , and  $\alpha_{zz}$  are 1.47, 1.53, and 1.42 Å<sup>3</sup>, respectively).<sup>27</sup> 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 are investigated in another work.<sup>23</sup>

To capture more details of the kinetics of hydrogen bonding in different environments, we studied the forming and breaking of hydrogen bonds in the time period shorter than 1 ps. Rapid librational and vibrational motions dominate the hydrogen-bond dynamics on this time scale. We calculated the first passage time of hydrogen bonds,  $\bar{\tau}_{\text{HB}}$ , defined as the time between the formation of the hydrogen bond and the first breaking of the bond (Table 1).<sup>7,20</sup> The hydrogen-bond dynamics on this short time scale is expectedly sensitive to the definition of the hydrogen bond.<sup>21</sup> It is a highly complex many-body problem that makes a quantitative model all but impossible. Therefore, we only give a qualitative description of the results. When the hydrogen bond has only one water molecule in the solvation shell of C, O, O<sup>-</sup>, 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 three-body effect and therefore weak. When the hydrogen bond is between two water molecules in the solvation shell of the hydrophobic surface (**I••I**),  $\bar{\tau}_{\text{HB}}$  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

this “geometrically confined” region before it can reorient to break the hydrogen bond. This lengthens the lifetime of the hydrogen bond. When the hydrogen bond is around Na<sup>+</sup>, on the other hand,  $\bar{\tau}_{\text{HB}}$  is much shorter than the bulk value. The strong electric field distorts the hydrogen bonds to such extent that they rapidly fluctuate between the intact and broken states.

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