Water Hydrogen-Bond Dynamics around Amino Acids: The Key Role of Hydrophilic Hydrogen-Bond Acceptor Groups

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Water hydrogen-bond (HB) dynamics around amino acids in dilute aqueous solution is investigated through molecular dynamics simulations and analytic modeling. We especially highlight the critical role played by hydrophilic HB acceptors: the strength of the HB formed with water has a pronounced effect on the HB dynamics, in accord with several experimental observations. In contrast, we evidence that hydrophilic HB donors induce a moderate slowdown in the water HB exchange dynamics due to an excluded volume effect, similar to that of hydrophobic groups. We present an analytic model which rationalizes the effect of all examined amino acid sites on the HB dynamics and whose predictions are in excellent agreement with the numerical simulations. This model provides the acceleration or retardation in the HB exchange time with respect to the bulk through the combination of the solute excluded volume factor with the solute−water HB strength factor, both referring to the HB exchange transition state.

I. Introduction

The dynamics of water molecules within the hydration layer of proteins plays a critical role in their biochemical properties. This includes protein folding, whose rate-limiting step is the core water expulsion,[1,2] and also enzyme catalysis where water facilitates the conformational transitions,[3] favors substrate binding,[4] and is directly involved in the chemical process for hydrolysis reactions. Further examples include proton transport along water chains in proton pump proteins,[5] protein freeze tolerance,[6] thermostability,[7] and the formation of protein aggregates, such as those involved in Alzheimer's or Parkinson's diseases, where the hydration layer lability is a key factor.[8] In return, the protein's presence modifies the water dynamics with respect to the bulk, and the protein and its hydration shell can be dynamically coupled.[3]

The peculiar properties of the hydration layer have been studied via a number of techniques, including NMR,[9,10] neutron scattering,[11−13] time-resolved fluorescence,[14,15] THz spectroscopy,[16,17] and simulations.[18−27] While most experimental techniques only access the average effect of a solute over its entire hydration layer, molecular dynamics is a valuable tool to provide a site-specific picture of hydration dynamics. Because of the great heterogeneity and thus complexity of a protein's solvent exposed surface, our present work is an intermediate step focusing on the hydration dynamics around the protein building blocks, amino acids, in dilute aqueous solution. Through the combined use of molecular dynamics simulations and analytic modeling, we determine and rationalize the role of each chemical group on the surrounding water dynamics.[28]

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The lability of the water hydrogen-bond (HB) network can be measured through the rate at which a water molecule trades HB acceptors. Several of us recently showed that this acceptor exchange mechanism is the main reorientation pathway for water in the bulk[29] and around a range of solutes.[30,31] Accordingly, we calculate via simulations to what extent the HB exchange rate is retarded or accelerated by each type of amino acid site with respect to the bulk and develop an analytic model to elucidate the key factors which affect the water dynamics.

Of the 20 natural amino acids, we have studied all the polar and uncharged (Ser, Thr, Cys, Asn, Gln), positively charged (Lys, Arg, His), and negatively charged (Asp, Glu) amino acids, together with an aromatic one (Tyr) (Figure 1). The hydration dynamics around the nonpolar amino acids whose side chains are hydrophobic is described by the ideas several of us developed in a previous study on hydrophobic hydration dynamics[31] and which are incorporated in the present model. It was shown that in dilute solution water HB exchange around a hydrophobic group is retarded by a moderate factor (approximately 1.5) with respect to the bulk, in agreement with experimental observation (see, e.g., ref 32). Our present work therefore focuses on the hydrophilic side chain sites. Hydrophilic solutes such as ions[30,33,34] or sugars[35−37] have been shown to have a strong effect on the water dynamics, which typically is much more pronounced than that induced by hydrophobic groups.

Among the amino acid hydrophilic sites, we distinguish those donating an HB to a water molecule (group A) and those receiving an HB from a water (group B) (see Figure 2). Group A comprises the NH, NH_2, and NH_3 amino groups found in Asn, Gln, Lys^+, Arg^+, and His, the OH hydroxyl found in Ser, Thr, and Tyr, and the SH thiol from Cys. Group B includes the CO carbonyl of Asn and Gln, the COO^- carboxylate of Asp^- and Glu^-, the OH hydroxyl of Ser, Thr, and Tyr, and the N lone pair of His. The hydroxyl OH groups belong to both groups

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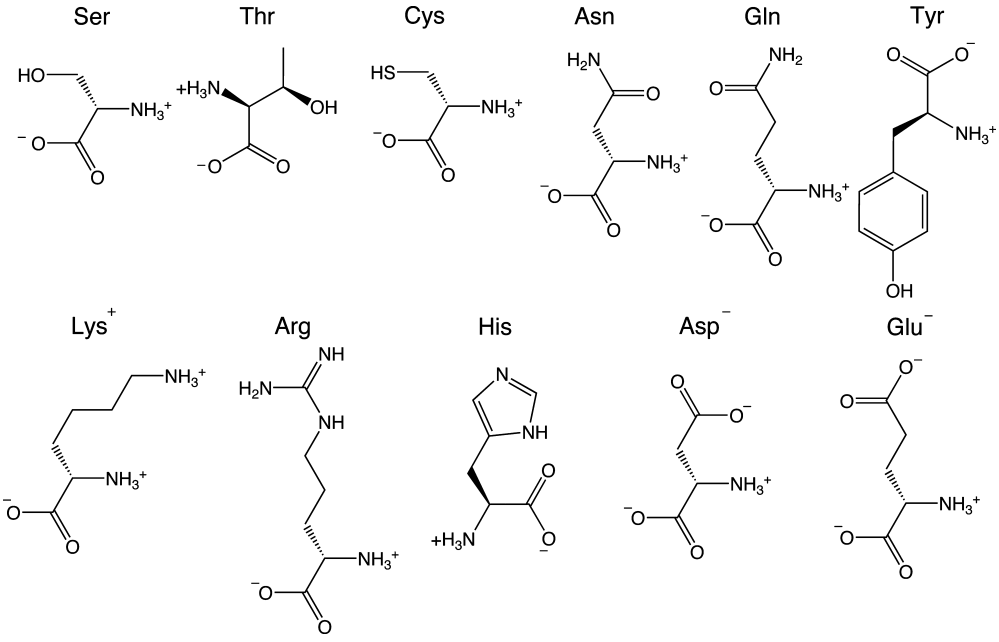

Figure 1. Structures of the 11 amino acids studied in the present work.

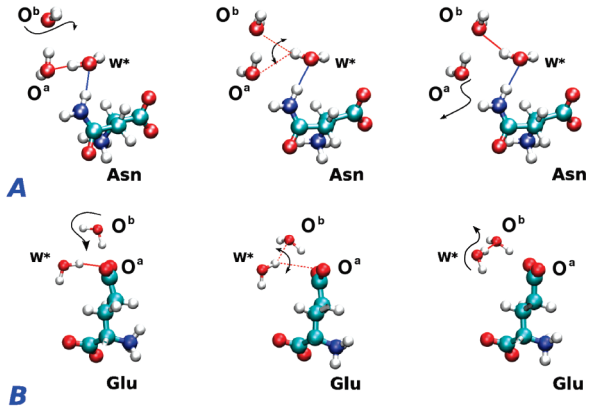

Figure 2. Typical HB acceptor exchange mechanisms. (A) Acceptor exchange mechanism for a water receiving an HB from an amino acid HB donor group, the Asn NH_2 amino group. The rotating water W*, which initially donates an HB to a water oxygen O^a and accepts an HB from the NH_2 group, is found to go through a symmetric transition state configuration where it forms a bifurcated HB with the initial and final HB acceptors, before forming a stable HB with the final acceptor O^b. (B) Acceptor exchange mechanism for a water initially donating an HB to an amino acid HB acceptor group, the Glu^- COO^- carboxylate. The rotating water W* is found to go through an asymmetric transition state configuration when replacing its initial amino acid HB acceptor O^a by a water oxygen O^b.

because they donate and receive HBs, and their two roles will be considered separately.

The remainder of the paper is organized as follows. We first describe the simulation methodology in section II. We then focus successively on the water dynamics next to HB donor sites (group A) in section III and next to HB acceptor sites (group B) in section IV. We then finish with some concluding remarks in section V.

II. Methodology

A dilute (≃0.07 M) aqueous solution of each amino acid in its zwitterionic form (NH_3^+ and COO^- terminal groups) and in its protonation state corresponding to pH = 7 was simulated at 300 K. Each simulation box contains a single amino acid surrounded by approximately 800 water molecules. The amino acids are described by the CHARMM22[38] force field and the water molecules by the SPC/E[39] model. While the SPC/E model is very simple and much more sophisticated models are now appearing (see, e.g., ref 40), this force field has been shown to provide a very satisfactory description of the water dynamics (see, e.g., ref 41), and its low computational cost allows the long simulations which are necessary in the present case and for future application to large biomolecules. Molecular dynamics simulations have been performed using NAMD,[42] with periodic boundary conditions and a PME[43] treatment of long-range electrostatic interactions. The systems were first equilibrated for 1 ns in the NPT ensemble at 300 K and 1 atm, followed by a 1.5 ns simulation in the NVT ensemble at 300 K with a 1 fs time step. We employ a Langevin thermostat with a friction $\mu = 1$ ps^{-1}: this value was checked to reproduce the NVE dynamical properties of bulk water, and we have verified for several amino acids that the NVT results are identical to the NVE ones.

For each site, the HB acceptor exchange time was calculated following the procedure described in ref 44 as the time to replace a stable HB with the initial acceptor by a stable HB with the final acceptor.

III. H-Bond Donor Sites

We first focus on group A, i.e., on water molecules whose oxygen receives an HB from the amino acid and whose OH replaces an initial HB with a water oxygen O^a by an HB with a different water oxygen O^b (an illustration is given in Figure 2A around the NH_2 group of Asn).

This situation is analogous to the HB exchange in the vicinity of a hydrophobic group that several of us have already characterized:[31] the switching water replaces its initial water acceptor with another water acceptor, in the presence of a solute—a hydrophobic group or here an amino acid HB donor group—which does not directly participate in the exchange process. Just as in the hydrophobic solute case,[31] we find that the HB exchange mechanism is identical to the one determined in bulk water.[29,44] In this exchange process, which can be viewed as a chemical reaction, the first step is the elongation of the

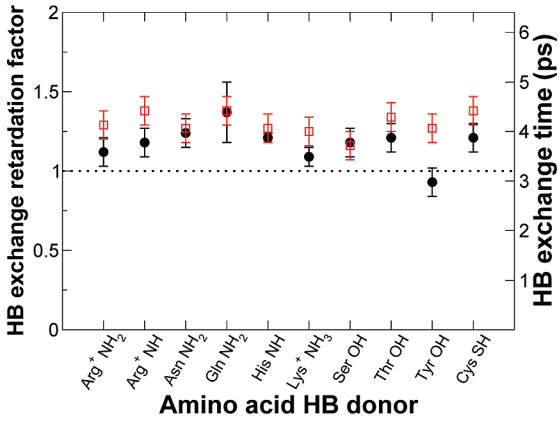

Figure 3. Water HB exchange time and retardation factor with respect to the bulk, determined from the simulations (full black circles) and from the TSEV model prediction ρ_V eq 1 (open red squares) for a water accepting an HB from a series of amino acid HB donor sites (group A). The dashed line indicates the reference bulk case.

initial HB, while a new water's oxygen acceptor arrives from the second shell. Once the initial and final partners' oxygens are equidistant from the rotating water's oxygen, the water OH can suddenly execute a large-amplitude angular jump from one acceptor to another, and at the transition state for this HB exchange, the rotating water forms a symmetric bifurcated HB with its initial and final water acceptors. The HB with the new partner eventually stabilizes, while the initial partner leaves.[29,31,44]

While the presence of the amino acid HB donor group does not modify the exchange *mechanism* with respect to the bulk, it affects the HB exchange time, which is retarded by a factor between 1.1 and 1.4 with respect to the bulk (Figure 3). This slowdown is analogous to the one induced by hydrophobic groups[31] and stems from an excluded volume effect at the HB exchange transition state. The presence of the amino acid hinders the approach of some new water HB acceptors, thus slowing the exchange rate. The decrease in transition state entropy induced by the solute yields the retardation factor ρ_V.[31] Within the transition state excluded volume (TSEV) model, the excluded fraction f of the transition state locations for the new acceptor yields the slowdown $\rho_V = 1/(1 - f)$.[31] The resulting HB jump exchange time τ_{jp} is[31]

$$\tau_{jp} = \rho_V \tau_{jp}^{bulk} = \frac{1}{1-f}\tau_{jp}^{bulk} \qquad (1)$$

where τ_{jp}^{bulk} is the bulk jump exchange time. We have calculated the slowdown ρ_V for each amino acid HB donor, using the procedure detailed in ref 31. Figure 3 shows that the overall agreement with the retardation factors computed directly from the simulations is very good.[45] All retardation factors fall within a narrow range, independently of the amino acid donor group; this is analogous to what has been shown for hydrophobic solutes in dilute solutions[31] and originates from the similar TSEV fractions f (see eq 1) throughout the amino acid groups, independently of their chemical nature.

IV. H-Bond Acceptor Sites

We now turn to water molecules that initially donate an HB to an amino acid hydrophilic site (group B). We only consider exchanges which replace this acceptor by a water oxygen (see the illustration in Figure 2B around the Glu carboxylate group) since in dilute solution exchanges involving a final acceptor other than water are very unlikely. Exchanges involving a different solute are improbable because of the very low concentration. Further, we have computed that (except in one case discussed below) intramolecular exchanges to another acceptor site within the same amino acid are negligible (such exchanges are expected to be more important next to a protein surface with a large density of HB acceptor sites).

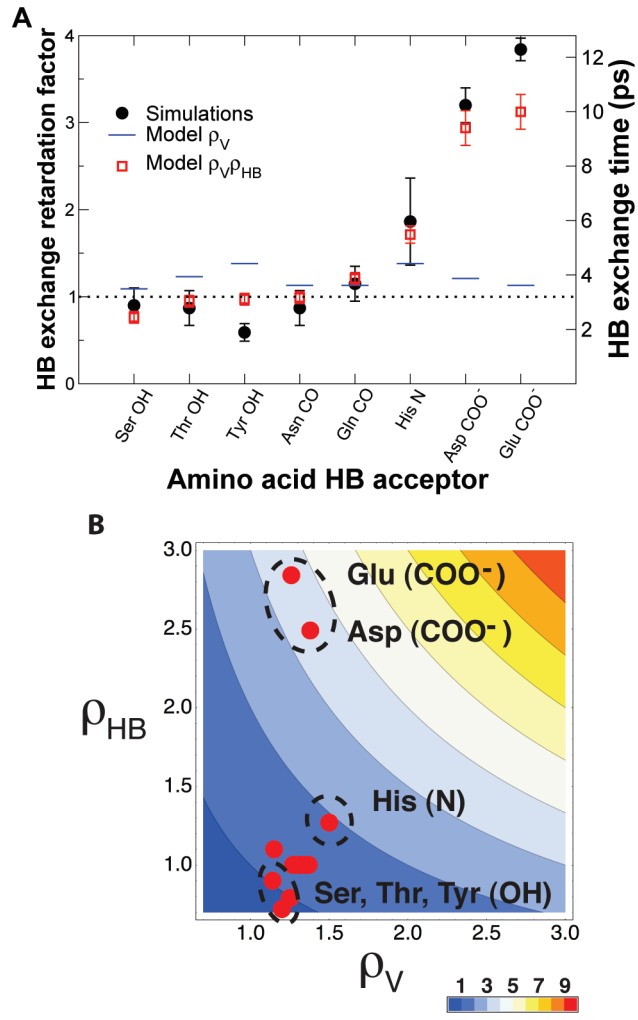

Figure 4. (A) Water HB exchange time and retardation factor with respect to the bulk, determined from the simulations (full black circles), from the TSEV model only eq 1 (blue dashes) and from both the TSEV eq 1 and TSHB eq 3 factors (open red squares) for a water donating an HB to a series of amino acid HB acceptor sites (group B). The dashed line indicates the reference bulk case. (B) Contour plot of the overall retardation factor as a function of the ρ_V TSEV and ρ_{HB} TSHB factors, with a spacing of 1 between successive contours.

As shown in Figure 4A, the amino acid's effect on the exchange time is much more pronounced in this group B case than for group A when the amino acid donates an HB, ranging from a nearly 2-fold acceleration (Tyr hydroxyl) to a 3-fold slowdown (Glu carboxylate). The purely steric factor originating from the TSEV effect eq 1 cannot account for these results since, as shown above, it always leads to a slowdown, which is in addition always moderate (less than 2-fold). A key difference between the present exchange process and the bulk exchange is that of course the initial acceptor is not a water oxygen (although the final acceptor is always the same in the present cases). The strength of the initial HB affects the exchange rate because elongation of this HB is part of the rate-limiting step to reach the transition state in the exchange mechanism:[44] the stronger the initial HB, the more energetically expensive the

TABLE 1: Transition State Geometry for the HB Exchange Process for Different Amino Acid HB Initial Acceptors, Averaged over All the Successful HB Exchange Events Identified in the Simulations[a]

amino acids	$\Delta\theta$ [°]	$R_a^{\ddagger}$ [Å]
Asn CO	71	3.17
Gln CO	72	3.20
His N	74	3.33
$Asp^- COO^-$	63	3.33
$Glu^- COO^-$	60	3.31
Ser OH	73	3.17
Thr OH	73	3.21
Tyr OH	75	3.32

[a] $R_a^{\ddagger}$ is the water oxygen−amino acid acceptor distance in the transition state geometry, and $\Delta\theta$ is the jump angle.[29]

elongation is, and thus the slower the exchange rate. We will term this the transition state HB (TSHB) effect since it is the cost to elongate the initial HB to its transition state length that is determinant. In addition, since the transition state geometry corresponds to equal HB strengths with the initial and final acceptors,[30,44] changing the strength of the initial HB will affect the transition state configuration (see Table 1).

The respective roles of the TSEV effect for the final partner and of the TSHB effect for the initial partner can be understood within a more general model for the exchange activation free energy. In the bulk, this activation free energy was shown to result from three terms:[44] first, a translational term which gives the largest contribution, associated with the elongation of the initial bond and the approach of the final acceptor; second, a term due to fluctuations in the HB coordination of the initial and final acceptors; and third, an angular barrier. The last two terms are small in the bulk situation,[44] and their changes induced by a solute are assumed to be negligible. We therefore focus on the change in the translational activation free energy induced by a solute. This is the free energy difference between the transition state configuration (where the distance from the rotating water to the initial and final acceptors is, respectively, $R_a^{\ddagger}$ and $R_b^{\ddagger}$) and the reactant geometry (with distances R_a^R and R_b^R). We assume that these two changes in distances can be considered independently, while averaging over the other coordinates, i.e.

$$\Delta G_{trsl}^{\ddagger} = G(R_a^{\ddagger}, R_b^{\ddagger}) - G(R_a^R, R_b^R) \simeq [G(R_a^{\ddagger}) - G(R_a^R)] + [G(R_b^{\ddagger}) - G(R_b^R)] = \Delta G_a^{\ddagger} + \Delta G_b^{\ddagger} \quad (2)$$

so that the change in the activation free energy due to the solute compared to the bulk water situation is the sum of two terms $\Delta\Delta G^{\ddagger} = \Delta\Delta G_a^{\ddagger} + \Delta\Delta G_b^{\ddagger}$, associated with the initial and final acceptors, respectively.

The TSEV effect pertains to the hindered approach of the final acceptor and therefore corresponds to an entropic contribution to $\Delta\Delta G_b^{\ddagger}$.[31] In contrast, the TSHB effect enters in the free energy change $\Delta\Delta G_a^{\ddagger}$, and has a significant enthalpic component.

To determine the free energy cost $\Delta G_a^{\ddagger}$ to stretch the initial HB with different amino acid acceptor sites, we first compute the potential of mean force along the r_{HA} distance, where H is the rotating water hydrogen and A is the amino acid acceptor site. This potential of mean force is then renormalized to correct for the volume excluded by the solute presence and thus inaccessible for the water[46] (we stress that this excluded volume effect for the normalization of the potential of mean force should not be confused with the excluded volume effect on the transition state new HB acceptor locations eq 1). This correction has a dramatic effect on the free energy.[46] The free energy cost to stretch the bond is then calculated as the difference between the potential of mean force values at the transition state and reactant r_{HA} distances. $\Delta\Delta G_a^{\ddagger}$ then follows from the difference with the bulk situation. The resulting exchange retardation/acceleration TSHB factor

TABLE 2: Exchange Retardation/Acceleration TSHB Factor ρ_{HB} Equation 3 for the Different Amino Acid HB Acceptor Sites[a]

amino acid site	ρ_{HB}
Asn CO	0.9
Gln CO	1.1
His N	1.3
$Asp^- COO^-$	2.5
$Glu^- COO^-$	2.8
Ser OH	0.7
Thr OH	0.8
Tyr OH	0.7

[a] ρ_{HB} is a measure of the HB strength with water.

$$\rho_{HB} = \exp(\Delta\Delta G_a^{\ddagger}/RT) \quad (3)$$

also provides a useful comparison of the HB strengths for the different amino acid acceptor sites, referenced to the water−water HB (see Table 2). The strongest HBs are accepted by the carboxylate and imidazole N groups, which is in line with their good proton-acceptor character.[47] However, we stress that while $\Delta G_a^{\ddagger}$ is related to the total HB free energy, i.e., the free energy cost to fully break the initial HB, it is a transition state quantity: because the HB acceptor exchange is concerted, the free energy cost to elongate the bond to its transition state length is only a fraction of the total HB free energy. The TSHB factor thus provides a better estimate of the effective HB strength in solution than the full HB free energy.

The ρ_V TSEV and ρ_{HB} TSHB factors are combined within a new model to describe the Activation of Water Hydrogen-bond Acceptor Exchanges (AWHAE) through

$$\tau_{jp} = \exp(\Delta\Delta G^{\ddagger}/RT)\tau_{jp}^{bulk} \simeq \exp(\Delta\Delta G_b^{\ddagger}/RT) \times \exp(\Delta\Delta G_a^{\ddagger}/RT)\tau_{jp}^{bulk} = \rho_V\rho_{HB}\tau_{jp}^{bulk} \quad (4)$$

Figure 4A shows that this AWHAE model leads to an excellent prediction of the exchange times for all the amino acid acceptor sites, despite their very different characters (the exception is Tyr[45]). The TSHB factor gives the dominant contribution, but the TSEV cannot be neglected. Both terms must be considered to properly describe the change in the exchange time. The decomposition of the overall effect into the ρ_V and ρ_{HB} contributions in Figure 4B evidences that, while for all amino acids the ρ_V TSEV factor is very similar due to similar topologies, the ρ_{HB} TSHB term covers a quite broad range of values, due to the very different HB strengths of the different acceptor sites.

For the carboxylate groups of Glu and Asp, in addition to the intermolecular exchange discussed so far where the final acceptor is a water oxygen, an additional intramolecular exchange can occur, in which the water switches between the two carboxylate oxygens. Such an intramolecular route remains however much slower than the intermolecular exchange, as shown by the jump times (43 ps between carboxylate oxygens

vs 12.3 ps from a carboxylate to a water for Glu; 32 ps vs 10.3 ps for Asp). Such intramolecular transfer has previously been reported around a molecular anion.[48,50]

Experimentally, the pronounced effect of an HB acceptor on the water dynamics is well documented for systems such as ions[33,49,50] and carbohydrates.[35] For halide ions, the strong HB between F^- and water is clearly reflected in a marked slowdown in the water dynamics, while the very weak HB with I^- leads to an acceleration of water dynamics.[33,49] Further evidence is provided by carboxylic acids, where replacing the −COOH moderate HB acceptor group with the $-COO^-$ strong HB acceptor results in a clear slowdown of the surrounding water dynamics.[51] Neutron scattering experiments on oligo-peptide aqueous solutions[12] also measured much slower water dynamics around the hydrophilic backbone than around the hydrophobic side chain and are thus consistent with our model. This collection of experimental observations thus evidences the importance of hydrophilic HB acceptor groups for the water HB dynamics, in perfect agreement with our model.

Table 2 and Figure 4 show that the same HB acceptor group has a similar effect on the water dynamics, which is thus a local property, independent of the rest of the solute (see the CO and OH groups). In the simulated HB exchange times (Figure 4), the only exception is the carboxylate group, whose effect differs in Asp and Glu (while it is similar in the model, see Table 2). Further analysis of the simulations (not shown) shows that for both amino acids the side chain folds to bring the carboxylate anion close to the $-NH_3^+$ end group. However, while this interaction is stable for Glu, the shorter side chain of Asp leads to constraints and large fluctuations in the COO^-−NH_3^+ distance and thus to a larger lability of the carboxylate hydration shell, reducing the exchange slowdown. A calculation with both amino acids in their neutral rather than zwitterionic form leads to the same exchange times for both, thus confirming the role of the unstable side chain folding for Asp.

A quantitative comparison with NMR, neutron scattering, or ultrafast infrared spectroscopy results requires the calculation of the water reorientation time measured by these techniques. It was recently argued[29,44] that water reorientation proceeds along two independent pathways: the first route is via the jump exchange of HB acceptors, and the second contribution is through the slower diffusive tumbling of the intact HB axis (frame) between successive jumps. The analytic extended jump model (EJM) associated with this mechanism successfully describes the reorientation dynamics of water in the bulk[29,44] and around various solutes[30,31] and interfaces.[52,53] The exchange term is determined by the jump exchange time τ_{jp} and by an angular factor α depending on the jump amplitude $\Delta\theta$, which are computed from the simulations. The reorientation time τ_{reor}^{frame} of the intact HB axis is calculated as the reorientation time between HB exchanges.[29,44] This time is similar to the bulk HB tumbling time for HB donors (4.9−7.6 ps range) but increases for HB acceptor sites (8.9−14.1 ps range) where the reorientation of the HB axis is slower because of the bulky amino acid group attached to the HB acceptor (see Table 3). The EJM thus provides the reorientation time[29,44]

$$\tau_{reor} = \left[\frac{\alpha(\Delta\theta)}{\tau_{jp}} + \frac{1}{\tau_{reor}^{frame}}\right]^{-1} = \left[\frac{\alpha(\Delta\theta)}{\rho_V \rho_{HB} \tau_{jp}^{bulk}} + \frac{1}{\tau_{reor}^{frame}}\right]^{-1} \quad (5)$$

with the angular factor provided by the Ivanov model[54] applied to the second-order reorientation time considered here

$$\alpha(\Delta\theta) = 1 - \frac{1}{5}\frac{\sin[(5/2\Delta\theta)]}{\sin[(1/2\Delta\theta)]} \quad (6)$$

The reorientation time calculated from the extended jump model is then compared with the direct calculation of the reorientation time from the simulations. This latter definition is however approximate, since the computed orientational correlation function follows a water molecule which is initially in the amino acid hydration layer but which may leave this layer on a time scale comparable with the reorientation time. The reorientation time is thus estimated from a fit of the simulated orientational correlation function on the 1−5 ps interval. Notwithstanding this approximation, the comparison of the model and simulation reorientation times in Figure 5 evidences that the model provides an excellent description of the water rotational dynamics.

V. Concluding Remarks

In conclusion, we have investigated via simulation the water HB dynamics around amino acids and designed a simple analytic AWHAE model that describes very satisfactorily those dynamics. HB donor groups have an effect analogous to hydrophobic groups and retard the water dynamics through a transition state excluded volume effect, which varies very little with the nature of the site. HB acceptor groups induce in addition a transition state HB strength effect which can dramatically accelerate or retard the water dynamics. Strong HB acceptors such as carboxylates thus induce a pronounced slowdown in the water dynamics. This model provides an intuitive but rigorous framework to understand at a molecular level the influence of amino acids on the surrounding waters. The model is expected to benefit numerous studies. These include for example the rationalization of the experimentally measured influence of amino acids on water viscosity[55] and the understanding of the kosmotropic/chaotropic behavior of each amino acid, invoked to explain why some amino acids stabilize the protein structure while others do not.[55] The model can also be easily extended to confinement situations, where

TABLE 3: HB Axis Tumbling Time τ_{reor}^{frame} (Equation 5) Extracted from the Orientational Time Correlation Function $\langle P_2[\mathbf{u}_{OH}(0)\cdot\mathbf{u}_{OH}(t)]\rangle$ Computed between HB Exchanges,[44] with $\mathbf{u}_{OH}(t)$ Being the OH Bond Orientation at Time t

donor	τ_{reor}^{frame} [ps]
Arg^+ NH_2	7.6
Arg^+ NH	5.1
Asn NH_2	5.6
Gln NH_2	6.3
His NH	6.0
Lys^+ NH_3	7.1
Ser OH	6.9
Thr OH	6.6
Tyr OH	4.9
Cys SH	6.8
acceptor	**τ_{reor}^{frame} [ps]**
Asn CO	8.9
Gln CO	10.8
His N	12.2
Asp^- COO^-	10.5
Glu^- COO^-	12.3
Ser OH	10.2
Thr OH	14.1
Tyr OH	9.9

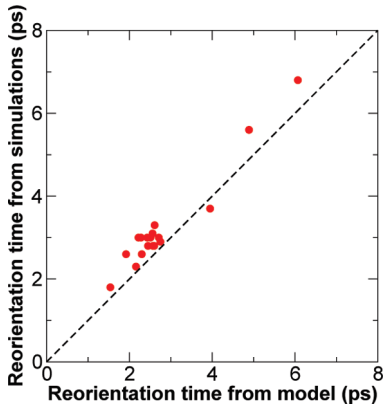

Figure 5. Comparison between the reorientation time calculated from the extended jump model (eq 5) and from the simulations for a water initially HB to the amino acid. The estimated uncertainty on the simulation times is 20%.

the structuring of the liquid is expected to add a contribution to the free energy cost of the final acceptor's approach ($\Delta\Delta G_b^{\ddagger}$ in eq 2) because of the increased free energy barrier along the potential of mean force between the first and second shells. The application of this model to understand the hydration dynamics around proteins and to distinguish the respective roles of each solvent-exposed group is currently underway in the group. This will allow easy discrimination between labile and strongly bound waters, based on the nature of the HB acceptor and the local topology, without performing lengthy simulations. This is critical in a number of phenomena. First, labile, easily displaced waters have been shown to play a fundamental role in enzyme catalysis[56] and drug-screening.[4] In proton pump enzymes, the key role of internal waters has been evidenced,[5] and our model can be used to predict the stability of the HB chain involved in the proton transport. Finally, strongly bound waters on the protein surface extend the influence of the protein electric field, thus playing a central role in long-range molecular recognition.[57]

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Supporting Information Available: Tables with data for Figures 3, 4, and 5. This material is available free of charge via the Internet at http://pubs.acs.org.

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