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What determines water-bridge lifetimes at the surface of DNA? Insight from systematic molecular dynamics analysis of water kinetics for various DNA sequences

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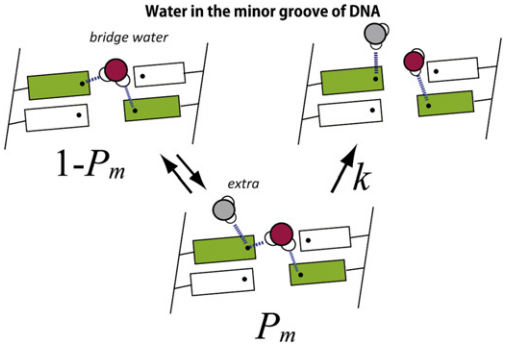
HIGHLIGHTS

- Lifetime of water molecules in the DNA minor groove widely varies for DNA sequences.
- Lifetime is associated with a specific H-bond and structural fluctuations of DNA.
- The water kinetics is consistent with the H-bond switching model of Laage and Hynes.

GRAPHICAL ABSTRACT



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ABSTRACT

The lifetime during which a water molecule resides at the surface of a biomolecule varies according to the hydration site. What determines this variety of lifetimes? Despite many previous studies, there is still no uniform picture quantitatively explaining this phenomenon. Here we calculate the lifetime for a particular hydration pattern in the DNA minor groove, the water bridge, for various DNA sequences to show that the water-bridge lifetime varies from 1 to ~300 ps in a sequence-dependent manner. We find that it follows $1/k(V_{step})P_m$, where P_m and V_{step} are two crucial factors, namely the probability of forming a specific hydrogen bond in which more than one donor atom participates, and the structural fluctuation of DNA, respectively. This relationship provides a picture of the water kinetics with atomistic detail and shows that water dissociation occurs when a particular hydrogen-bonding pattern appears. The rate constant of water dissociation k can be described as a function of the structural fluctuations of DNA. This picture is consistent with the model of Laage and Hynes proposing that hydrogen-bond switching occurs when an unusual number of hydrogen bonds are formed. The two new factors suggested here are discussed in the context of the surface's geometry and electrostatic nature, which were previously proposed as the determinants of water lifetimes.

1. Introduction

Water molecules play crucial roles in the stability of biomolecules and their associations with each other. In order for proteins or nucleic acids to associate, many ordered water molecules surrounding them must be released from their interaction surfaces; this entropically affects the molecular association [1,2]. In addition to such structural

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and thermodynamic effects, the kinetics of water molecules is also of importance in the hydration of biomolecules [3–5]. Experimental measurements using nuclear magnetic resonance (NMR) [6–9], time-resolved fluorescence [10,11], and terahertz spectroscopy [12], and the computational approach of molecular dynamics (MD) simulations [13–21] have been performed to reveal the time-dependent features of the hydration water. Of particular interest is the slow kinetics of water molecules near the biomolecule's surface. Most water molecules reside at the surface sites of biomolecules for a few picoseconds, but some reside at a particular surface site for a long period of over ~100 ps [7,8,14,18,19,22]. This timescale is substantially longer than the timescale of a few picoseconds for hydrogen-bond switching of bulk water (we use the term *lifetime* throughout this paper to denote our particular definition for this time, although it can also be referred to as the *residence time*; see Section 2.2). In this way, the water lifetime varies according to the site at which it resides on the heterogeneous surface of a biomolecule.

What determines water lifetimes at biomolecular surfaces? This has been recognized as one of the important questions in the area of hydration water kinetics [15–18,20–29]. A useful approach to address this question is MD simulation, which allows us to directly examine the time-dependent phenomena at an atomic scale. Previous MD simulations have indicated that the electrostatic nature [15,20,21,23,24] and geometry [17,18,22,28,29] of the surface are major determinants of water lifetimes. Nonetheless, several uncertainties remain. The suggested contributions of the electrostatic effect contradict each other. García and Stiller [23] and Rocchi et al. [20] showed that the lifetime τ varies with the electrostatic character of the hydration site (which is indicated by the subscript of τ) in the order $\tau_{\mathrm{charged}} > \tau_{\mathrm{polar}} > \tau_{\mathrm{nonpolar}} \approx \tau_{\mathrm{bulk}}$, whereas Brunne et al. [15] listed them in a different order, $\tau_{\mathrm{polar}} > \tau_{\mathrm{nonpolar}} > \tau_{\mathrm{charged}}$. On the other hand, Kovacs et al. [16] suggested that the electrostatic condition produces no clear difference in the lifetime. Recently, Schröder et al. [21] provided more detailed information by evaluating different types of amino acids individually, with the result that $\tau_{\mathrm{negatively\ charged}}$ (~30 ps) $> \tau_{\mathrm{positively\ charged}} \approx \tau_{\mathrm{polar}}$ (~10 ps). Compared to the electrostatic nature of the surface, the surface geometry is likely to have a somewhat larger effect on the lifetimes. For example, Makarov et al. [18] showed by MD simulations that water molecules with a lifetime longer than ~80 ps could be found only in less exposed regions such as protein cavities and clefts. Luise et al. [17] and Henchman and McCammon [22] also suggested the importance of surface geometry in studies where the surface geometry was evaluated in terms of solvent accessibility. In spite of these investigations, a uniform, comprehensive picture quantitatively explaining the relationships between these factors and water lifetimes has not yet been provided. This demonstrates the complexity of the problem, and much effort is still devoted to such investigations [30].

Another aspect that makes this problem complicated is the difficulty of determining the lifetime from knowledge about high-water-occupancy sites deduced from X-ray crystallography or MD data, because the lifetime does not correlate with static properties such as the time-averaged water occupancy [18,25,31]. A high-occupancy site is not necessarily one where water molecules reside for a long time: the lifetime can vary from a few to several hundred picoseconds [18]. In other words, distinct kinetics can produce similar time-averaged pictures. It should be noted, however, that the lack of correlation between the time-averaged occupancy and the water lifetime is not unreasonable, because they arise from different physical sources [4]. The water occupancy is determined by the energetic stability of the state in which a water molecule is hydrating the biomolecule, whereas the lifetime is mainly attributed to features of the transition states, which can be characterized by the energy profile along the water dissociation pathway.

In this paper, we revisit the long-standing question of what determines the various water lifetimes among different sites on a biomolecular surface. Here we focus on a typical hydration pattern, the water bridge, in the DNA minor groove, where the two hydrogen atoms of the water are bonded to two different acceptors in the DNA. We calculated the water-bridge lifetimes for various DNA sequences using MD simulations and found that the appearance of a particular hydrogen-bonding pattern and the structural fluctuation of the DNA are crucial factors determining the water-bridge lifetimes. The narrow floor of the minor groove has been known to be favorable for long residence times of water molecules, giving lifetimes of over ~100 ps [6–8,13,14,19], but the length of the lifetime depends on the DNA sequence [14,19]. Consistent with these data, the present MD results showed that the water-bridge lifetime is in the range of ~1 to 300 ps and varies depending on the DNA sequence. Using the sequence-dependent data, we propose that the lifetime τ can be described as $\tau = 1/k(V_{step})P_m$, where V_{step} is the structural fluctuation of DNA and P_m denotes the probability of forming a particular hydrogen-bonding pattern involving more than one donor atom. Furthermore, we provide an atomistic picture explaining how the water dissociates from the DNA minor groove. The associated hydrogen bond breaks when a particular hydrogen-bonding pattern appears. We also find that the breaking rate, k, can be described as a function of the structural fluctuation of DNA.

In this paper, the results are discussed particularly with regard to the following three points. In Section 3.6, the two factors suggested here are discussed in the context of the two previously proposed factors, the surface geometry [17,18,22,28,29] and electrostatic nature of the surface [15,20,21,23,24]. In Section 3.7, the consistency of our atomistic picture with the model proposed by Laage and Hynes [32,33] is discussed. In Section 3.8, our calculated water-bridge lifetimes are compared with associated data obtained from previous MD simulations and experiments.

2. MD Systems and calculations

2.1. MD calculations

The MD trajectories used in the present study are the same as those used in previous work [34]. We briefly describe the calculations. The double-stranded B-form DNA considered here was composed of a 12-mer, $CGCGN_1N_2N_3N_4CGCG$ (Fig. 1), where for $N_1N_2N_3N_4$ all possible arrangements of A, T, G, and C were considered, resulting in 136

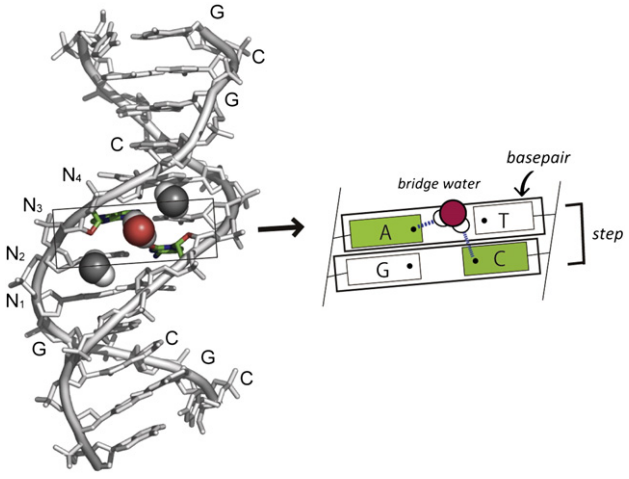

Fig. 1. Hydration of 12-mer DNA observed in the minor groove (a snapshot from the MD trajectories). The lifetime was calculated for the water molecule (shown by red and white spheres) that appears at the site of the N_2N_3 basepair step. This water molecule forms a bridge through hydrogen bonding between the bases of distinct strands [see the schematic view showing a GA step (N_2 = G and N_3 = A) on the right-hand side].

systems with different tetrameric patterns. The force fields for DNA and water were of AMBER *ff*99 [35,36] and TIP3P [37], respectively. About 5000 water molecules were placed around each DNA, and a truncated octahedral periodic boundary condition was imposed. Ions, 39 K^+ and 17 Cl^-, were added to neutralize the system and realize the physiological salt concentration of 0.15 M. For each of the DNA sequences, a 10-ns-long trajectory was generated using the AMBER sander module [38] with the particle mesh Ewald electrostatic calculation [39]. The equations of motion were numerically integrated with a 1-fs time-step. Covalent bonds involving hydrogen were constrained by the SHAKE algorithm [40]. Of the 10-ns-long trajectories, the last 8 ns, which were equilibrated at 1 atm and 300 K, were analyzed. In addition, we carried out a 100-ns-long simulation for AGCC and analyzed the trajectory every 10 ns to check the effects of simulation time length.

2.2. Calculation of water-bridge lifetimes

As shown in Fig. 1, our analysis was focused on the minor groove of the central dimeric basepair step N_2N_3 (= TA, CG, CA, GA, GG, AT, AC, AA, GC, or AG), but use of the 136 tetrameric patterns allowed us to examine the effects of the flanking bases N_1 and N_4 on the N_2N_3 site. Fig. 1 shows the hydration pattern most frequently observed in the minor groove, which we previously called the *1-water bridge* [34]. This water molecule corresponds to the first layer of the *hydration spine* [41], which forms a hydrogen-bonding bridge between the two bases of the different strands (see Fig. 1). Note that any base A, T, G, or C can participate in this type of hydration, because each base has an acceptor atom, N or O, in the minor groove; however, the probability of bridge formation differs among basepair steps [34,42]. Here, we characterize the lifetimes of water molecules that appear at the bridge-forming site. The time correlation function $C(t)$ was calculated in a similar way to that in a previous work [43],

$$C(t) = \frac{\langle h(0)h(t)\rangle}{\langle h\rangle}. \quad (1)$$

Then, we obtained the lifetime,

$$\tau = \int_0^\infty C(t)dt. \quad (2)$$

This quantity corresponds to the time when $C(t)$, under the assumption of exponential decay, decreases to e^{-1}. The same single-exponential treatment was also employed in previous studies [15,16,20,23], although the mathematical expression used in some of these [15,20] is described in a different form. Here, $h(t)$ indicates whether a water molecule resides at the bridge-forming site or not, that is, $h(t)$ is 1 when the two associated hydrogen bonds (see Fig. 1) are formed, but it becomes 0 when either hydrogen bond breaks. Note that our definition of $h(t)$ is slightly different from the previous one [43], where only a single hydrogen bond was considered. MD snapshots were saved every 1 ps, and, for each of the snapshots, we judged whether a water bridge is formed by checking the formation of the two associated hydrogen bonds (see Fig. 1). For the formation of hydrogen bonds, the criterion of $d_{A\cdots H}<$ 2.5 Å and $\theta_{A\cdots H—O}>135°$ was used, where A denotes the acceptor atom of DNA on the minor groove. If a water bridge was formed at the N_2N_3 site, the index of the water molecule was recorded for the calculation of the time correlation function $C(t)$ (see Eq. (1)). From this record, we picked all pairs of snapshots with a time interval t, and incremented the count for $h(0)h(t)$ by one if the same water molecule was found. We then calculated $C(t)$ using Eq. (1) and finally obtained the lifetime τ with Eq. (2).

We use the term *lifetime* rather than *residence time* for the following reason. Even when a water bridge breaks, the water can still reside in the same region. The current definition for τ does not count such residence, so it should be referred to as the *water-bridge lifetime*. To check whether our conclusion depends on the definition of τ, we also performed calculations using another criterion, whether or not a water molecule resides within 3 Å of the minor groove acceptors (in this case, τ is the *residence time*). The values of τ themselves changed, but the change was almost uniform for all the DNA sequences. Therefore, the choice of the definition of τ does not change our conclusion (data not shown).

2.3. Evaluation of DNA structural fluctuations

In this study, structural fluctuations of DNA are discussed in the context of water kinetics. The structural fluctuations were evaluated using the relative positioning of two adjacent basepairs (i.e., basepair step; see Fig. 1). The fluctuation V_{step} was obtained for each of the dimeric basepair steps N_2N_3 using principal component analysis of the distributions of the step conformations (for the details, see Refs. [34,44], where V_{step} is called the basepair step deformability). In the analysis of the MD trajectories, each basepair was assumed to be a rigid plane, and the relative configuration between the two adjacent planes of N_2 and N_3 was described with six step parameters (i.e., shift, slide, rise, tilt, roll, and twist [45]) using the 3DNA software [46]. The distributions of these parameters can be characterized with six eigenvalues λ_i (i = 1–6), and the structural fluctuation V_{step} was calculated as

$$V_{step} = \prod_{i=1}^{6} \sqrt{\lambda_i}. \quad (3)$$

3. Results and discussion

3.1. Water-bridge lifetimes for various sequences

The water-bridge lifetimes τ calculated for all 136 cases are plotted in Fig. 2 (the probability of forming multiple hydrogen bonds P_m is explained later). The value of τ varies widely from 1 to 300 ps, depending not only on the central basepair step N_2N_3, but also on the flanking bases N_1 and N_4. These τ values were obtained from the time correlation functions of the water bridges, $C(t)$. Fig. 3 shows three typical cases with N_2N_3 = GC, demonstrating that different decays of $C(t)$ are well characterized by the τ values.

We first believed that DNA basepair step fluctuations caused the differences in τ , expecting that basepair steps with large fluctuations would have smaller τ values and vice versa. However, no simple relation was found. For example, the AT and GC steps, extracted from AATT and AGCT, respectively, showed comparable fluctuations, V_{step} = 2.7 $Å^3\,deg^3$ and 4.3 $Å^3\,deg^3$, respectively; however, the lifetimes τ were quite different: τ_{AT} = 40 ps and τ_{GC} = 281 ps. These results indicate that the water-bridge lifetime cannot be described simply in terms of DNA structural fluctuations, unlike the particular hydration patterns we studied previously [34]. However, a quantitative description of the water-bridge lifetime is possible using another quantity, P_m, which is introduced in the following section.

3.2. Identification of multiple hydrogen bonds enables explanation of different τ values

To explain the differences in τ, we introduce another quantity P_m, which is the probability that a particular hydrogen-bonding pattern appears. The hydrogen-bonding pattern, often observed during the MD trajectories in this study, is shown in the center panel of Fig. 4; in addition to the bridge-forming water, an extra water molecule appears and forms an additional hydrogen bond to the same acceptor in the DNA, that is, the acceptor has *multiple hydrogen bonds*. We refer to this as the M state and refer to the water-bridge state without multiple hydrogen bonds as the S state. With this in mind, we classified the water-bridge-forming states into M or S states, with and without

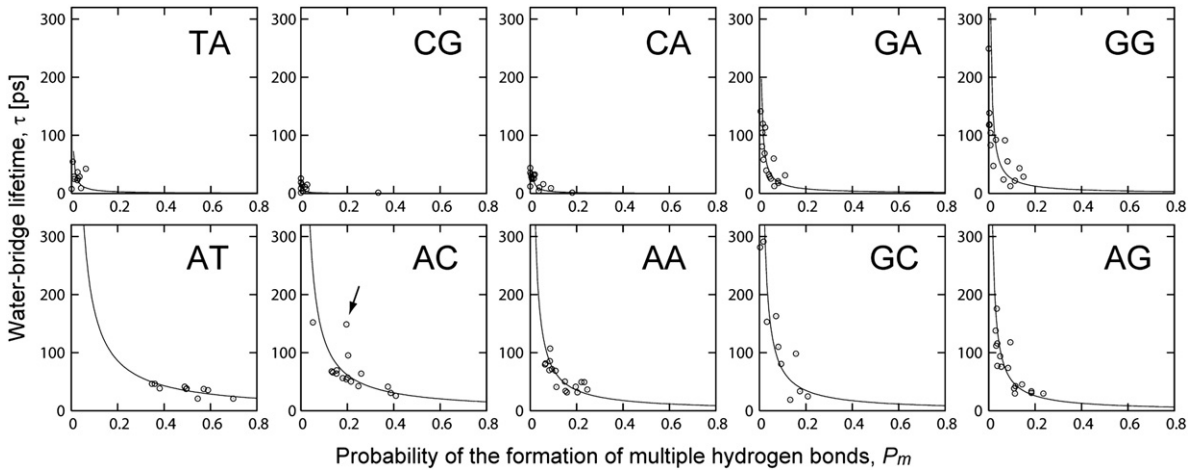

Fig. 2. Water-bridge lifetime τ for the 10 distinct dimeric steps (for the probability of formation of multiple hydrogen bonds P_m, see the explanation in Section 3.2 and Fig. 4). Points in each graph denote different tetrameric patterns with the same dimeric step at the center. The fitted curves $\tau = 1/kP_m$, where the values of k were obtained by least-squares fitting, are drawn. The arrow in the AC step data indicates a point with a large deviation from the fitted curve (see Section 3.4). Values of τ and k are listed in the Supplementary data, Tables S1 and S2, respectively.

multiple hydrogen bonds. These two states correspond to the center and the left panels in Fig. 4, where the relative probabilities are denoted by P_m and $1-P_m$, respectively. To calculate the P_m values, for all MD snapshots in which a water bridge is formed, we judged whether such multiple hydrogen bonds are formed or not. The probability P_m was obtained as a ratio of the number of snapshots with the multiple hydrogen bonds to that of snapshots with any water bridge.

With the probability of the formation of multiple hydrogen bonds P_m, it now becomes possible to explain the differences in τ. Fig. 2 shows that τ and P_m roughly follow $\tau = 1/kP_m$. Here, we note that τ is the lifetime of the water bridges, not that of the M or S states, and can be calculated irrespective of these states. In Fig. 2, the parameter k was adjusted for each of the dimeric basepair steps. The physical meaning of k will be discussed in the following paragraph; k is just considered as a parameter at this point. The relation $\tau = 1/kP_m$ indicates that as P_m becomes lower, the lifetime τ becomes longer. All cases with long lifetimes ~300 ps (see GC steps in Fig. 2) have small P_m values. Note that the relation $\tau = 1/kP_m$ is not seen clearly in three cases, TA, CG, and CA, because both τ and P_m are small for these.

3.3. Interpretation of $\tau = 1/kP_m$

What does the relation $\tau = 1/kP_m$ mean? We consider a possible mechanism by which a water molecule might dissociate from the water-bridge-forming site. The proposed mechanism is shown in Fig. 4, where breaking of the associated hydrogen bond occurs exclusively when multiple hydrogen bonds are formed. Here, k is the rate constant of the hydrogen-bond breaking in the M state. This mechanism is quite reasonable, because it straightforwardly leads to the relation $\tau = 1/kP_m$. We simply consider the first-order reaction, i.e., we assume that the time correlation function $C(t)$ of Eq. (1) follows a single exponential decay. (Indeed, our $C(t)$ satisfied this condition except during the initial short period; see the inset of Fig. 3.) If the hydrogen-bond breaking is allowed only in the state with multiple hydrogen bonds, changes in $C(t)$ with time should be proportional to $C(t)$ multiplied by the probability P_m:

$$-\frac{dC(t)}{dt} = kP_m C(t), \tag{4}$$

where k is the rate at which the breaking occurs in the M state. Then, we obtain under the condition $C(0) = 1$,

$$C(t) = \exp\left(-\frac{t}{\tau}\right), \tag{5}$$

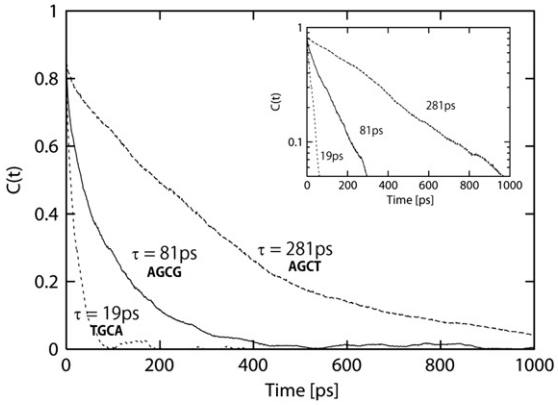

Fig. 3. Time correlation functions, $C(t)$, of water bridges for three typical cases: GC steps of TGCA ($\tau = 19$ ps), AGCG ($\tau = 81$ ps), and AGCT ($\tau = 281$ ps). In the inset, the same data are shown on a logarithmic scale.

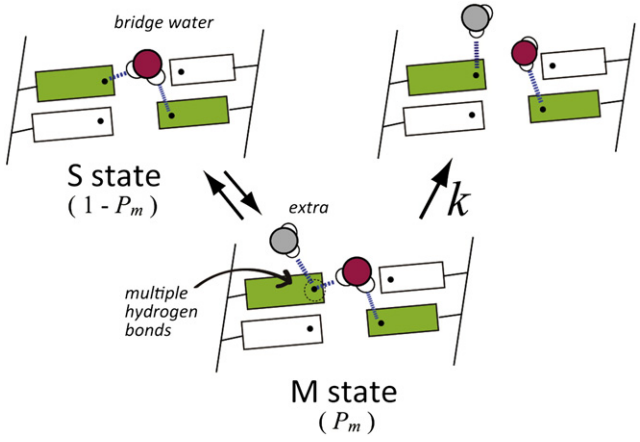

Fig. 4. Multiple hydrogen bonds (center) to an acceptor and the proposed mechanism for water dissociation from the bridge-forming site. The water-bridge-forming states can be classified into two cases, those with and without multiple hydrogen bonds, which are called M and S states, respectively. P_m and $1-P_m$ denote the probabilities of being in the M and S states, respectively (see Section 3.2). The parameter k denotes the rate constant in the M state at which the hydrogen bond between DNA and the bridge water breaks.

where

$$\tau = \frac{1}{kP_m}. \tag{6}$$

In this way, we can derive the relationship $\tau = 1/kP_m$ starting from the mechanism shown in Fig. 4.

As we have seen, the formation of multiple hydrogen bonds makes the lifetime shorter. We have also explained how it assists in the dissociation of water molecules. This picture seems to be reasonable from an energetic point of view as well. If a water bridge breaks without any extra water molecules on the DNA acceptor, the acceptor becomes naked. Such a situation is energetically unfavorable, and thus it is unlikely to occur. However, if an additional hydrogen bond is formed by an extra water molecule, at least one hydrogen bond remains at the acceptor, even when the other hydrogen bond is broken. Thus, the energy barrier for breaking either one of the hydrogen bonds will not be as high, consequently allowing water molecules to exchange frequently in a short time.

3.4. k is related to the structural fluctuations of DNA

In the relation $\tau = 1/kP_m$, k denotes the hydrogen-bond-breaking rate in the M state (see Fig. 4). Fig. 2, where different fitting lines of $\tau = 1/kP_m$ are drawn for each basepair step, shows that k differs according to the type of basepair step. What physical quantity is associated with this difference? We found that the dominant factor is the structural fluctuation V_{step} of the basepair step. This can be seen in Fig. 5, where all 136 points for the different tetrameric DNA sequences are colored according to their V_{step} value. There is a clear correlation between k and V_{step}: rigid basepair steps (blue) appear in the outer region with large k values, whereas flexible steps (red) appear in the inner region with small k values. Regarding k as a function of V_{step}, we can rewrite Eq. (6) as

$$\tau = \frac{1}{k\left(V_{step}\right)P_m}. \tag{7}$$

This equation clearly shows that P_m and V_{step} are the two determinants of the sequence dependence of τ.

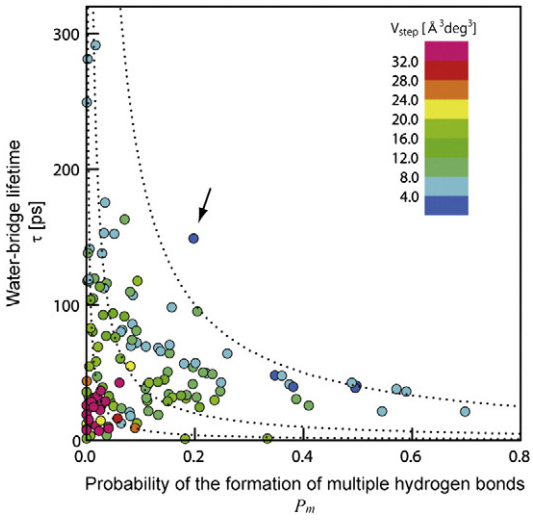

Fig. 5. Water-bridge lifetimes τ for all 136 basepair steps plotted against the probability of formation of multiple hydrogen bonds. Each point is colored according to the basepair step fluctuation V_{step}. For clarity, dotted curves $\tau = 1/kP_m$ with $k = 0.05$, 0.25, and 1.25 are drawn. The arrow corresponds to the point indicated by an arrow in Fig. 2 (see the caption of Fig. 2 and Section 3.4).

It is helpful to show the actual fluctuations of the basepair step parameters. Fig. 6 shows two typical cases, the AT and TA steps, where V_{step} is very small (2.7 Å^3 deg^3) and very large (84.9 Å^3 deg^3), respectively. The AT step exhibits very small fluctuations, and all six step parameters settle around the equilibrium value. On the other hand, the TA step shows larger fluctuations (~4 Å for shift and slide, and ~40° for twist).

Previous studies [34,44] show that the structural fluctuation V_{step} of a dimeric step N_2N_3 is not constant and somewhat depends on its flanking bases N_1 and N_4. Consequently, k also depends on the flanking bases. The flanking-base dependence of V_{step} may partly explain why several points deviate from a single fitted line (Fig. 2). In AC steps, for example, a large deviation from the fitted line (indicated by an arrow in Fig. 2) may be due to the fluctuation of a small value yielding a small k value (see Fig. 5; the same point is colored dark blue).

3.5. On the reliability of the calculated water-bridge lifetimes

In this study, we tried to provide a general explanation for the water-bridge lifetimes in distinct DNA sequences in terms of the formation of multiple hydrogen bonds and DNA fluctuations. Of course, we admit that our data are somewhat weak from a quantitative point of view. The values of the lifetime provided here can fluctuate on the simulation timescale. The relation $\tau \propto 1/P_m$ naturally causes a large change in τ upon a small change in P_m when P_m takes a relatively small value of P_m~0–0.1, giving a high sensitivity of τ for small P_m values. To see to what extent τ fluctuates, we picked the AGCC sequence, which had a small P_m and large τ, and carried out the simulation extensively up to 100 ns. Fig. 7 shows the results of τ, P_m, and V_{step} evaluated every 10 ns (8 ns only for the first window). The values of τ from the 10 different windows were distributed from 60 to 300 ps, which shows that the lifetime τ is very sensitive when the P_m value is small, as we expect. However, our important finding that the relationship among τ, P_m, and V_{step} is given by Eq. (7) still holds. More precise evaluation of capricious quantities such as τ is not easy, because of the limitations of our current computational resources, but it will become possible when our reachable MD timescales are extended in the near future.

3.6. Factors that affect water lifetimes

So far, from MD simulation studies on protein hydration, the surface geometry [17,18,22,28,29] and electrostatic nature [15,20,21,23,24] have been recognized as the determinants of water lifetimes or residence times. We introduced two new factors, the hydrogen-bonding pattern and the biomolecule's structural fluctuations in this study. All four of these factors are important when hydration of biomolecules is discussed.

The surface geometry is expected to have a close relationship with the effect of the hydrogen-bonding pattern, including the formation of multiple hydrogen bonds. We imagine that a less exposed surface is less likely to form multiple hydrogen bonds, because it is less likely to interact with two water molecules at once. Thus, the effects of both hydrogen bonding and the surface geometry may be due to the same phenomenon, described in different ways. Nonetheless, it is useful to describe the variety of water lifetimes in terms of the hydrogen bonding, because it helps us to understand how such factors affect the water lifetime at an atomic level. The appearance of a particular hydrogen-bonding pattern, multiple hydrogen bonds, assists the water dissociation by lowering the cost of hydrogen-bond breaking.

The electrostatic effect cannot be addressed using the present results, because all hydration sites considered here are polar, consisting of O or N, which have nearly the same atomic charges. However, by considering such similar electrostatic conditions, we were able to clarify the effects of other factors, excluding the electrostatic effect.

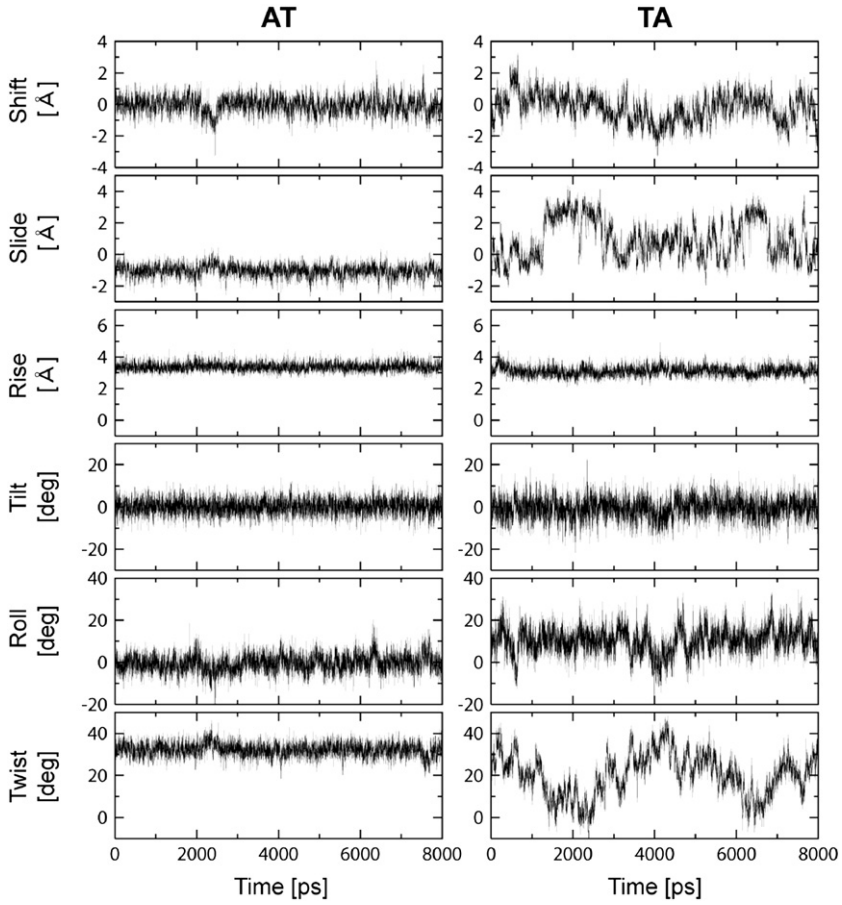

Fig. 6. Fluctuations of six step parameters for the AT step of AATT (left, $V_{step} = 2.7$ Å^3 deg^3) and the TA step of TTAA (right, $V_{step} = 84.9$ Å^3 deg^3), which show very small and very large fluctuations, respectively.

The effect of the structural fluctuation is intuitively reasonable; nonetheless, it has not previously been taken as a factor determining water lifetimes because it was difficult to capture its effect. The present study clarifies this point and shows that structural fluctuations also contribute to the variety of water lifetimes. Rigid sites tend to give longer lifetimes and vice versa. It may be worth noting a study by Pizzitutti et al. [47]. Their MD simulations using two protein models, with and without protein atoms fixed, showed that the water lifetime drastically increased when the protein atoms were fixed. Although the variety of water lifetimes is not the subject of their study (i.e., the lifetime was evaluated for the water molecules in the entire hydration layer), there is a close relationship between the Pizzitutti et al. [47] study and ours with respect to the underlying physical mechanism.

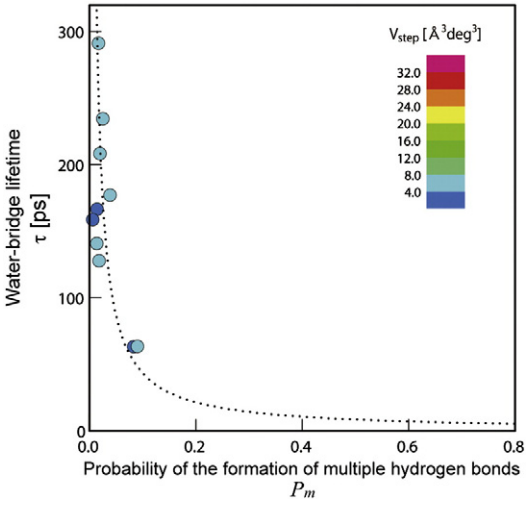

Fig. 7. Simulation time dependence of the water-bridge lifetime. Each point denotes the result from a 10-ns-long window in the 100-ns trajectory for the AGCC sequence, except for the point for the first window, where the length is 8 ns. The dashed line is the fitted curve $\tau = 1/kP_m$, where k is 0.23.

Thus far, we have shown the relationship between structural fluctuations and hydration water motion. However, which is cause and which is consequence? It is still difficult to clearly address this question, as we discussed in previous work [34] (for the details of our test simulation, see p. 1145 in Ref. [34]). Nonetheless, we expect that DNA fluctuations are likely to govern the water motion. This is because previous MD results show that DNA-sequence-dependent fluctuation is strongly correlated with the type of DNA basepair steps [34]; all the pyrimidine–purine steps, TA, CG, and CA (or TG), are very flexible, while the others are not so flexible. We therefore expect that the different base-stacking interactions in distinct basepair steps produce sequence-dependent fluctuations, consequently affecting the motion of the interacting water molecules.

In the present study, we focused on a particular hydration pattern in the DNA minor groove, i.e., the water bridge. Compared with that in the minor groove, hydration in the major groove is rather complicated, because various hydration patterns can exist there due to its wideness. The same situation applies to protein hydration. We believe that a similar description of water lifetimes is possible in these cases, but a longer simulation will be required to provide one. Such a further systematic analysis will reveal a more general relationship between the water lifetimes and the various factors affecting them.

3.7. On the consistency between the resultant atomistic picture and the Laage–Hynes model

As discussed in Section 3.3 and shown in Fig. 4, water dissociation occurs in the state with multiple hydrogen bonds. This picture is consistent with the model proposed by Laage and Hynes [32,33]. Laage and Hynes [32,33] showed that hydrogen-bond switching happened under a particular condition on the hydrogen-bond number. In the case of liquid water [32], for example, the water oxygen usually receives two hydrogen atoms from the neighboring waters, but sometimes receives three or one. When such over- and under-coordinated water molecules encounter each other, one excess hydrogen flips from the former to the latter (see Ref. [32]). Let us again consider Fig. 4. If we focus on the number of hydrogen bonds, the process is consistent with that of Laage and Hynes [32,33]. As in their model, our results show that hydrogen-bond switching occurs when there are an unusual number of hydrogen bonds.

It should be emphasized that even though the resultant picture agrees with that of Laage and Hynes, the present study provides new insights, because the most important point of this study is that we were able to provide an explanation for the variety of water lifetimes. In this paper, we show that the differences in the probabilities of forming such a particular hydrogen-bonding pattern are responsible for the variety of water lifetimes.

3.8. Comparison of water-bridge lifetimes with the associated values reported from MD simulations and experiments

Water lifetimes that were reported previously, based on MD simulations and experiments, are tabulated in Table 1. Note that different definitions are used for the words *lifetime* or *residence time*, and in some cases direct equality is not assured between the quantities measured using MD simulations and experiments.

Comparison between the present study and the MD simulation study of Pal et al. [19] is straightforward, since both employ the same evaluation based on the relaxation time of the time correlation functions $C(t)$, though there is a slight difference in the treatment of the hydrogen bonding. The water-bridge lifetime averaged over all possible tetramer basepair steps considered here is 58.3 ps, which agrees well with the value of 51.3 ps reported by Pal et al. On the other hand, Auffinger and Westhof [13,14] reported lifetimes of ~1200 or ~700 ps, but these values cannot be directly compared with our lifetimes because these values are the longest time of water residence observed for each site (i.e., maximum residence time). The values corresponding to their lifetimes in this study are the times at the tail ends of the correlation functions $C(t)$ (see Fig. 3), which show good agreement with the Auffinger and Westhof lifetimes. For reference, data for protein hydration water, obtained by Makarov et al. [18], are also given in Table 1. They calculated the residence times using the time correlation function in the same way as in this study and Pal's. Their MD results show that the residence times vary from 1 to 200 ps, depending on the site of hydration. It would be interesting to consider whether their variability can be ascribed to the fluctuations and the hydrogen-bonding patterns of the hydration site, as we show for DNA.

Table 1
Reported values associated with hydration water kinetics.

	Reported values	Reference
MD calc.		
DNA	Water-bridge lifetime ~1–300 ps, average 58.3 ps (minor groove)	This work
DNA	Maximum residence time 700 ps O2# @ T 700 ps N3# @ G 1200 ps O2# @ C	Auffinger, Westhof 2000 [13], 2001 [14]
DNA	Hydrogen-bond lifetime 51.3 ps (minor groove) 71.3 ps (N3# @ A) 39.1 ps (N3# @ G) 72.3 ps (O2# @ T) 20.2 ps (major groove)	Pal et al. 2006 [19]
Myoglobin	Residence time 1–200 ps*	Makarov et al. 2000 [18]
NMR	Residence time	
DNA	>1000 ps (minor groove of AATT)	Liepinsh et al. 1992 [7]
	200 ps (minor groove, 300 K)	Denisov et al. 1997 [6]
	<1 ns (277 K)	Sunnerhagen et al. 1998 [9]
	300 ps (minor groove, 283 K)	Phan et al. 1999 [8]
Fluorescence spectroscopy	Time constants of solvation correlation functions	
DNA	1.4 ps/19 ps (minor groove)	Pal et al. 2003 [10]
Myoglobin mutants	1–8 ps/20–200 ps	Zhang et al. 2007 [11]

* With two exceptions, 456 and 452 ps.
\# Acceptor atoms at the water-bridge-forming sites.

Experimental evaluations of hydration water kinetics have been performed using NMR [6–9] and fluorescence spectroscopy [10,11]. Earlier NMR experiments reported a very long residence time (1 ns) for the minor groove of the AATT sequence [7], but this was updated later to smaller subnanosecond values (see Table 1: Denisov et al. [6], Sunnerhagen et al. [9], and Phan et al. [8]), which are consistent with our result of 1–300 ps. However, we again note that a more definite assessment is needed to establish consistency between the observable quantities in MD simulations and NMR experiments.

Recently, measurements using fluorescence spectroscopy have been performed [10,11]. These studies report two time constants for the measured solvation time correlation functions (e.g., 1.4 and 19 ps for the DNA minor groove [10], and 1–8 ps and 20–200 ps for myoglobin [11]). The MD-derived lifetimes are in the same time range, but the interpretation of the measured quantities is controversial [48–50].

4. Conclusion

What determines the variety of water-bridge lifetimes at the surface of DNA? We suggested two factors, namely the appearance of unusual hydrogen-bonding patterns and DNA structural fluctuations, from comparative MD analysis on distinct DNA sequences. The results showed that the water-bridge lifetime in the DNA minor groove varies from 1 to 300 ps, depending on the sequence. This variety can be described using the two suggested factors as $\tau = 1/k(V_{step})P_m$. From this relation, we provide an atomistic picture for the water kinetics. Water dissociation occurs when a particular hydrogen-bonding pattern appears. The DNA structural fluctuation V_{step} contributes to the rate constant k.

Despite much effort, the problem of explaining the variety of water lifetimes still remains. To obtain a more complete picture of this phenomenon, further factors might be required in addition to the two factors suggested in this study and the previously considered factors, the surface geometry [17,18,22,28,29] and electrostatic nature [15,20,21,23,24].

The resultant water kinetics in the DNA minor groove is consistent with the Laage–Hynes model [32,33] in that the hydrogen-bond switching occurs when an unusual number of hydrogen bonds occur. The present study provides the more profound insight that different probabilities of this particular hydrogen-bonding pattern among different hydration sites are responsible for the variety of water lifetimes.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.bpc.2011.09.006.

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