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Effects of Dimerization of *Serratia marcescens* Endonuclease on Water Dynamics

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Abstract

The dynamics and structure of *Serratia marcescens* endonuclease and its neighboring solvent are investigated by molecular dynamics (MD). Comparisons are made with structural and biochemical experiments. The dimer form is physiologic and functions more processively than the monomer. We previously found a channel formed by connected clusters of waters from the active site to the dimer interface. Here, we show that dimerization clearly changes correlations in the water structure and dynamics in the active site not seen in the monomer. Our results indicate that water at the active sites of the dimer is less affected compared with bulk solvent than in the monomer where it has much slower characteristic relaxation times. Given that water is a required participant in the reaction, this gives a clear advantage to dimerization in the absence of an apparent ability to use both active sites simultaneously.

Keywords

molecular dynamics; computer simulation; protein–DNA recognition

INTRODUCTION

The biological activity of a protein is uniquely related to its native structure obtained in water. It is often the case that water participates in the biological activity of a protein through molecular recognition and ligand binding. The properties of water molecules appear distinct from bulk water when in proximity to proteins. Experiments and theoretical studies^{1–6} have provided considerable evidence that the properties of water, such as diffusion constant, residence time, dipole moment, and hydrogen bond dynamics, etc., are different when around a protein than those of bulk water. Generally, the diffusion rates of water molecules within 4–5 Å of a protein surface have been found to be significantly different from that in bulk water. Bulk water that has residence times ranging from 0.5 to 50 ps, while water molecules in the grooves, clefts, or buried within a protein have residence times that can be considerably longer, on the order of hundreds of picoseconds or more. In addition, studies of water hydrogen bond kinetics have showed that a substantial relaxation of the hydrogen bond network is necessary

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for structural relaxation of the protein.^{7,8} The dynamics of breaking hydrogen bonds is also slower in the first solvation shell of a polypeptide,⁹ or at vapor-water and hard surface-water interfaces^{10,11} than in bulk water. As a result unique insights into catalytic mechanism can often be obtained through a study of water dynamics near the surface of enzymes.

Serratia marcescens endonuclease (SMnase) is a magnesium-dependent extracellular endonuclease, which is sugar nonspecific, cleaving either DNA or RNA. Under physiological conditions, the protein is a homodimer, each with 245 amino acids. The structure consists of a central six-stranded antiparallel β -sheet flanked by α -helices on both sides (Figure 1). From detailed mutational and structural analyses,¹²⁻¹⁶ residues including Arg57, Arg87, His89, Asn119, Glu127, and Arg131 are proposed to play a central role in catalysis. The active site is located at the base of a cleft formed by a long helix and a long loop, and is open to the solvent. This cleft contains a conserved water cluster that is felt to play a significant role in catalysis.¹⁶⁻¹⁹

In our previous dynamics study of a monomer of SMnase and its complex with DNA,²⁰ one water cluster around the active site in both the monomer and the monomer-DNA complex was identified as having a potential role in catalysis. Water not only participates in the cleavage of DNA but can also stabilize the transition state and the leaving groups in this model.

The monomer and dimer are each catalytically active in SMnase. The two subunits of the dimer function independently of each other with no processivity increase because of dimerization. Yet, this system functions biologically as a homodimer with a relatively flat dimer interface far from the active sites.^{15,21-23} It is not clear why nature selects the dimeric state and how protein dimerization affects the enzyme's biological activity.

In this work, we performed molecular dynamics (MD) simulations of a dimer of SMnase and compared the results with a simulation of a SMnase monomer to understand the role of water in the dimerization and effects of dimerization on the activity of the protein. Specifically, we calculated the local hydration sites and analyzed the interactions between protein residues, particularly, within the dimeric interface and the active site. We then evaluated the residence times of waters and the dynamics of water-water and water-protein hydrogen bonds at the hydration sites within 4 Å of the various biomolecular surfaces. Using these results, we are now able to hypothesize that dimerization is coupled to and influences the structure and dynamics of water, especially those at the active site, which in turn affects the protein's activity.

METHODS

Molecular Dynamics Simulation

The systems were simulated using the program ESP²⁴ and the all-atom CHARMM 27 parameter set.²⁵ Equations of motion were integrated using a 0.002-ps time step in a microcanonical ensemble (NVE) with periodic boundary conditions. Electrostatic interactions were treated with an Ewald sum²⁶ using a fast linked-list algorithm. For the simulation of the monomer system, the coordinates of the B subunit of *Serratia marcescens* endonuclease (SMnase) (1QAE) were used as the original configuration. Residues 1–5, not given by the diffraction results, were model built and found to be quite flexible during the simulation. They were not used directly in subsequent analysis. For the simulation of the dimer, all the coordinates from 1QAE were likewise used as the original configuration. The proteins were put into pre-equilibrated boxes of water using standard setup procedures.²⁰ The system set-up of the monomer and dimer is summarized in Table I. After several steepest descent energy minimization steps, the system was equilibrated at 300 K for 2.2 ns (monomer) and 3.0 ns (dimer) to provide stable backbone geometries for the proteins. The trajectory was then

continued to 5.2 ns (monomer) and 6.1 ns (dimer) for analysis. The trajectory coordinates were saved for further analysis at an interval of 0.1 ps.

Solvent Density and Hydration Sites

Hydration sites may be heuristically defined as any local maximum in the water (oxygen) density map that satisfies certain protein proximity conditions. Obtaining the average solvent density map and localizing the hydration sites from the molecular dynamics (MD) trajectory has been described in detail elsewhere.^{20,27} Briefly, for each step of the MD trajectory, the protein was superimposed over a reference frame with the same operation applied to the solvent molecules, taking the periodic boundary conditions into account. Other definitions have been used for different purposes such as highlighting individual side chains.²⁸ The positions of water oxygen atoms were mapped onto a three-dimensional rectangular grid with a 0.5 Å spacing. By collocating the number of water molecules that appeared in each cube, a time averaged, normalized solvent density was constructed.

A density threshold, ρ_{cut} , and a minimum distance separating each site, r_{cut} , is set to determine which sites are to be deemed significant. Assuming the density of normal water is 1.0 g/cm³ and the average volume of a water molecule is around 30 Å³, the average number density of water molecules per grid point is 0.0042. After excluding grid cells with insufficient statistics, a number density threshold, ρ_{cut} , was selected to be 1.4, which is above the average thermal fluctuations in each cell. Next, the position of a site is determined by averaging the position of the cube and its six closest neighbors weighted by their densities. This definition is similar to what we used in previous studies.⁵ Finally, for comparison with the crystal results, we require nonoverlapping sites and no cases of double occupancy. To achieve this, r_{cut} was set to 2.8 Å. For convenience, the sites were sorted and numbered in decreasing order of their density values.

Analysis of Interactions

Protein–protein interactions may be phenomenologically decomposed into H-bond, electrostatic, hydrophobic, and water-mediated interactions. Here, a strong electrostatic interaction was defined between a pair of charged nonhydrogen atoms when the distance was less than 2.8 Å, and a hydrophobic interaction was defined when the distance between a pair of low charged carbon atoms (e.g. not carbonyl) was less than 4.0 Å.³⁰ These are useful but clearly not unique definitions for energy decomposition. For hydrogen bond interaction, we define a donor–acceptor pair, AΩH–D, where A is acceptor, D the donor atom, and H the hydrogen atom, with a geometric criterion of a hydrogen bond: (i) that the distance between H and A should be smaller than 2.3 Å, (ii) that the distance between A and D should be smaller than 3.2 Å, and (iii) that the angle between A, H and D should be between 130° and 180°. These criteria are taken from recent literature,³¹ tightening the AD distance criteria somewhat. A water-mediated hydrogen bond interaction was claimed when a site occupied by water molecules was in proximity to either other waters or protein.

RESULTS AND DISCUSSIONS

Analysis of Interactions

Interface of the Dimer—Crystallographic studies^{22,32} indicated that the SMnase dimer structure is very symmetric with an almost perfect 2-fold rotation axis that relates monomer A of the dimer to monomer B. Inter-subunit noncovalent interactions include 4 salt links, 8 direct hydrogen bonds centering around residues 180–184, 17 water-mediated hydrogen bonds, and several hydrophobic interactions. A primary water molecule is located at the center core of the dimer interface, bridging His184A and His184B. This water molecule also interacts with two

other water molecules that bridge Tyr185 and Val236 (Figure 2A). Another water cluster bridges the side chains of Asn183 from both chains.

In this MD study salt bridges, direct hydrogen bonds, water-mediated hydrogen bonds, and hydrophobic interactions in the interface region have also been considered, and the interaction frequencies are listed in Table II. It was found that the residues participating in intersubunit interactions frequently differed between our MD simulations and the crystallographic studies. Experimentally, it was found that Asn245 (A/B) interacted with Lys233 (B/A) through a salt bridge²²; however, in our simulation it is Glu239 (A/B) that is salt linked to Lys233 (B/A). Although the interaction between Asp225 (A/B) and Arg136 (B/A) remains, the salt link in the simulation is asymmetric. Direct hydrogen bond interactions happen not only in the core interface, but also across the peripheral interface region. For example, the water molecule that bridges two asparagine residues in the peripheral interface region is similar in placement to the equivalent water molecule in the experiment. However, in the core region, one direct hydrogen bond interaction between His184A and His184B appears with a frequency of 85%, as shown in Figure 2B. The water-mediated interaction of Tyr185A and Val236A has a similar frequency of 84%. The other water-mediated interactions of Tyr185B and Val236B are less prominent with only 17% frequency by additional water-mediated interactions with His184A and Val236B. Not surprisingly, the instantaneous symmetry in the dimer structure shown in the experiment is destroyed. There were no experimental restraints between the dimers in the refinement procedure. Each monomer was treated as completely independent of the other monomer. That our time averaged structures are not yet as symmetric as the experiment is just a measure of incomplete conformational sampling.

The Active Site—The interactions at the active site have been analyzed and are listed in Table III. It was found that the monomer and each subunit in the dimer, on average, have the same salt bridges and direct hydrogen bond interactions between amino acids that are involved in the activity. In all the systems, Mg^{2+} keeps five water molecules and Asn119 as its ligands. In the monomer and subunit A, only four magnesium-coordinated water molecules have hydrogen bond interactions with the residues nearby, such as Asp86, Arg87, Gln114, and Asn119 (Figure 3A). However, in the subunit B, the fifth magnesium-coordinated water also has interactions with both OE1 and OE2 of Glu127 with strength of 32.0% and 55.5%, respectively (Figure 3B). Glu127's participation in the interactions with the magnesium water cluster is further exemplified by interactions between OE1 of Glu127 and HH12/HH21 of Arg131, whose frequency drops from 90% in the monomer and subunit A to 20% in the subunit B. In addition, a direct hydrogen bond interaction between NE2 of His89B and HH12 of Arg57B with frequency of 49% appears in the subunit B, which reduces the mobility of the side chain of Arg57B. Thus, we expect that such differences might change the distribution and dynamics of water molecules at the active site.

By analyzing the site–site interactions, a water cluster around the magnesium ion was identified at the active site in all the systems similar to the crystallographically resolved one. Some differences in the dimer halves were evident. The monomer and subunit A have similar positions of the hydration sites of the magnesium water cluster (Figures 4A and 4B). The main part is located in the deep cleft of the active site, close to Glu127, His89, and Mg^{2+} . The water cluster seems to extend to the region between Arg57, Asp86, Arg87, and Mg^{2+} , and there are relatively strong interactions among those hydration sites. However, in the subunit B, the water cluster is shifted somewhat and centers around the deep cleft of the active site (Figure 4C). This difference might be an effect of the flexibility of the side chain of Arg57.

Hydration of the Protein-Site Occupancy and Water Residence Times

The average site occupancy is defined as the fraction of time the site is occupied by any water. A site is considered occupied if a water molecule (oxygen nucleus) is found in a spherical volume with radius of 1.5 Å. The occupancy distributions of the hydration sites were estimated by calculating site occupancy in three different 1 ns time blocks. They are illustrated in Figure 5 with error bars computed from block averaging. The uncertainty in the probability of the hydration sites in the monomer system is larger than in the dimer system, especially around occupancies of 0.4 and 0.5. The fluctuations of side chains in the monomer system are greater than either dimer subunit. Those fluctuations then disturb the occupation of water at some sites in our time averaged protein centered calculation. To see how water tracks with the side chain, others have used a side chain centered definition of hydration site, which resolves local features but has varying metrics of normalization for comparison with our system.^{28,29} We find, within a range of error of the occupancy and probability, both monomer and dimer systems have a similar probability distribution with the most probable occupancy around 0.5. Such a deviation from unity is expected given the definition of hydration sites used here; the minimum separation of the sites is 2.8 Å. Thus, the most probable water–water distance, and thus some hydration sites will be sterically governed by the protein side chains. Nearly, 95% of hydration sites in both systems have relative occupancies larger than that of bulk water, which is 0.32 (blue straight line). Occupancies of the bulk sites were calculated using the same procedure as the hydration sites. Bulk sites were chosen such that they have the density of bulk water and are located at least 9 Å away from the protein surface.

The water residence time at a hydration site was evaluated from an occupation survival time correlation function $C_\alpha(t)$ that represents the average number of water molecules that remain in the hydration site for a duration of time.²⁷ The occupation survival function is defined as

$$C_\alpha(t) = \sum_{j=1}^{N_w} \frac{1}{T_{\text{run}} - t + 1} \sum_{t_0}^{T_{\text{run}}-t} p_{\alpha,j}(t_0, t_0+t) \quad (1)$$

where $p_{\alpha,j}(t_0, t_0+t)$ is a binary function that takes the value of 1 if the j th water molecule has occupied the α th site from t_0 to t_0+t ; otherwise, the function is 0. Here, N_w is the total number of water molecules and T_{run} is the length of simulation. From the survival function, in many cases, it is possible to approximate a relaxation time using double exponential fitting,

$$C_\alpha(t) = W_0 [(1-x) \exp(-t/\tau_f) + x \exp(-t/\tau_s)] \quad (2)$$

where W_0 is the occupancy of the α th site, τ_f and τ_s are the fast and slow residence time, respectively, and x will give the weight of the slow relaxation. Nonlinear least-squares fits to the data utilize the Levenberg-Marquardt algorithm as coded in *Numerical Recipes in Fortran77*.³³ Here, we focus on the slow part of the decay. Figure 6 illustrates the probability distributions of water residence times at all hydration sites ranging from 1 to 30 ps. Distributions from both systems are similar. More than half of the hydration sites have water residence times close to 1 ps, which is similar to that of the bulk water. The hydration sites having water residence times longer than 30 ps are mostly located deep in the cleft of the active site, between turns and loops as well as in the dimeric interface of the structure. For example, Site 14_d and Site 2_d (d denotes the dimer system; Figure 2B) are located around the core of the dimeric interface and have residence times of 61 ps and 115 ps, respectively, which indicates that water is considerably less mobile than bulk in this region.

The relaxation of the occupation survival time correlation function depends on the details of the interactions between the protein and the hydration water. A few studies have demonstrated that in proximity to the protein surface or other hydrophobic surfaces, the decay of the survival function can be better described by a stretched exponential^{11,34,35} or a Kohlraush-Williams-Watts model:

$$C_{\alpha}(t) = W_0 \left[(1-x) \exp(-t/\tau_f) + x \exp(-(t/\tau_s)^{\beta}) \right] \quad (3)$$

where β is a stretching parameter between 0 and 1, which reflects the extent to which the relaxation process deviates from exponential behavior. The β value decreases when water closer to the protein surface is taken into account. We found the best fit using the single exponential at short times and the stretched one for the long time decay. Such nonexponential behavior is commonly found in amorphous and glassy condensed phase systems as well.^{36,37}

In this work, we expect that the β value at the active site can provide additional information about heterogeneity in the water dynamics. It was found that the survival functions at some hydration sites at the active site are better described by KWW formula, as illustrated in Figure 7, in which only Site 11, 18, 52, and 58 in the subunit B are shown as an example. The point wise differences in the regression are less than $\sim 10^{-2}$, an order of magnitude smaller than those in a double exponential fit. For comparison, the decay of the survival function for a site in the bulk region is also shown in Figure 7(cyan). The β value in the bulk region was estimated to be 0.961 ± 0.026 . For the hydration sites in the deep cleft of the active site, the averaged β value was 0.599 ± 0.015 , 0.693 ± 0.006 , and 0.697 ± 0.006 in the monomer, subunit A, and subunit B, respectively. The deviation of the relaxation of water residence times from the bulk value indicates a temporal disordered character of hydration water at the active site, particularly in the monomer system. β in the monomer is significantly smaller than that in each subunit of the dimer. Given that the dimer values are statistically indistinguishable from each other and well separated from the monomer value, dimerization clearly induces a strong correlation on the water dynamics in the active site not seen in the monomer.

Hydrogen Bonds Dynamics

Water residence times can indicate how water around the protein differs from bulk water, but it can not explain how the dynamics of water is related to the protein's function. In addition, although water residence times could be dependent on the hydrogen bond network, a simple correlation between water residence time and hydrogen bond lifetime in the absence of strong geometric restrictions has not been found.^{38,39} So, it is necessary to investigate the dynamics of hydrogen bonds within water as well as between water and the protein.

The dynamics of water–water and water–protein hydrogen bonds at the hydration sites and in bulk regions were investigated by calculating the average life time of the hydrogen bonds. The decay was evaluated by calculation of a hydrogen bond time correlation function,¹⁰

$$S_{\alpha}(t) = \frac{\langle h(t_0) H(t_0+t) p_{\alpha}(t_0) \rangle}{\langle h \rangle} \quad (4)$$

where function $p_{\alpha}(t_0)$ is unity when a particular water molecule occupies the α th hydration site at original time t_0 , otherwise it is 0; $h(t)$ is a hydrogen bond indicator, which is unity when the water molecules inside the α th hydration site forms a pair with another molecule by hydrogen bonding at time t , otherwise it is 0; $H(t_0+t)$ is 1 if the tagged pair remains continuously hydrogen bonded during time t_0+t , otherwise it is 0; $\langle \cdot \rangle$ denotes an average over all hydrogen bonds that are present at t_0 . With this construct, $S_{\alpha}(t)$ describes the probability that a water pair, which occupies the α th hydration site, is continuously hydrogen bonded during time t_0 and t .

An alternative way to analyze the hydrogen bond dynamics is to calculate the intermittent hydrogen bond correlation function,^{40,41}

$$C_h(t) = \frac{\langle h(t_0) h(t_0+t) \rangle}{\langle h(t) \rangle} \quad (5)$$

which describes the probability that a hydrogen bond is intact after duration of time t , given that it was intact at t_0 . This correlation is expected to generate a longtime tail because it does not depend on the continuous presence of a bond. Since our interest is in the interior water or water at the active site, it is not appropriate to use Eq. (5), because we have an insufficient sample of water hydrogen bonded pairs inside the protein to reasonably eliminate the statistical error.

Similar to the water residence times at the hydration sites, the decay of the $S_\alpha(t)$ is expected to obey a double exponential in many cases,

$$S_\alpha(t) = (1 - x) \exp(-t/\tau_{\text{HB-f}}) + x \exp(-t/\tau_{\text{HB-s}}) \quad (6)$$

Here, we focus on the slow τ_{HB} . Figure 8 illustrates the probability distributions of the lifetimes of water–water hydrogen bonds, $\tau_{\text{HB-WW}}$, at all hydration sites in the monomer (blue) and dimer (magenta) system. For comparison, $\tau_{\text{HB-WW}}$ in the bulky region was also evaluated, which is about 0.46 ± 0.02 ps (cyan straight line). This value is in agreement with the results in the literature on pure water.^{8,41,42} In both systems, less than 2% of hydration sites have $\tau_{\text{HB-WW}}$ smaller than 0.5 ps. About 65% and 55% of the hydration sites in the monomer and dimer, respectively, have $\tau_{\text{HB-WW}}$ in the range of 0.6 and 1.0 ps. The H-bond lifetimes exhibit a strong variability in their values at the different hydration sites as expected from chemistry and geometry.

The hydration sites having $\tau_{\text{HB-WW}}$ less than 0.5 ps are located on the interior of the protein, which indicates that water in these sites are less mobile and might have strong interactions with the protein and/or play structural roles. The hydration sites in the cleft of the active site have $\tau_{\text{HB-WW}}$ longer than 2.0 ps. As one moves away from the protein, $\tau_{\text{HB-WW}}$ is observed to decrease to the bulk value of 0.5 ps. Thus, relaxation of hydrogen bonds between water molecules behaves as expected whereas the relaxation is significantly slower near the protein–water interface than in the bulk water. Such results are consistent with those from other groups^{9,43,44} and with the expectations from other dynamical correlations.⁴⁵

The profiles of the relaxation probability of the hydrogen bonds of water with water are found to be similar in both systems. However, we expect that $\tau_{\text{HB-WW}}$ at the particular sites in the deep cleft of the active site will be different according to the interaction analysis discussed above. The average $\tau_{\text{HB-WW}}$ was calculated to be 2.09 ± 0.02 , 1.96 ± 0.01 , and 1.64 ± 0.01 ps in the monomer, subunit A and subunit B, respectively. In our simulation, we found that $\tau_{\text{HB-WW}}$ in the subunit B is smaller than that of the other two systems. This indicates that this property can have significant fluctuations especially in deep the cleft of the active site. It also demonstrates sampling limits.

To better describe dynamic behavior of hydrogen bonds at the active site, the decays of $S_\alpha(t)$ at the hydration sites in the active site have also been analyzed using a stretched exponential. It was found that like the occupation survival functions, the decays of water–water hydrogen bond correlation functions are better described by KWW formula as illustrated in Figure 9, in which Site 11, 18, 52, and 58 in the subunit B and a site in the bulk region (cyan) are shown. The standard deviations of the regression are mostly in the range of $\sim 10^{-4}$, an order of magnitude smaller than those with the double exponential fit.

Water–water hydrogen bonds at the buried hydration sites and the bulk sites relax in a similar way for short time periods. However, for the long time decay, the relaxation process at the hydration sites is slower. The stretching parameter, $\beta_{\text{HB-WW}}$, associated with the h-bond dynamics was found to be 0.713 ± 0.007 , 0.729 ± 0.011 , and 0.776 ± 0.007 in the monomer, subunit A, and subunit B, respectively. Those values are significantly different from that in the bulk region, which was estimated to be 0.979 ± 0.035 . Thus, we see the expected geometry

induced change of dynamics because of a local reduction of dimensionality. That $\beta_{\text{HB-WW}}$ in the subunit B is slightly larger than those in the monomer and subunit A is further indication of the range of fluctuations of water properties in the deep cleft of the active site.

The probability distribution of the lifetimes of hydrogen bonds between protein and water hydration sites, $\tau_{\text{HB-WP}}$, was also analyzed with the double exponential as shown in Figure 8. It was found that 55% (monomer) and 52% (dimer) of the interactions have $\tau_{\text{HB-WP}}$ smaller than 0.5 ps. The longest relaxation time reached about 10 ps in the monomer and 21 ps in the dimer system. In particular, the relaxation of interactions between the magnesium ion and water in the hydration sites showed heterogeneity. Site 21_d and Site 6_d (Figure 4C) have $\tau_{\text{HB-WP}}$ with Mg^{2+} of 20.9 and 19.1 ps, respectively, while the similar sites around Mg^{2+} in subunit A have only about 2.1 ps. This correlates well with the magnesium mobility, which because of side chain flexibility obtains some range of excursions or fluctuations.

It is noteworthy that the properties of hydration sites depend on the resolution used to define the hydration sites. When a different r_{cut} or ρ_{cut} is used, the number of hydration sites is changed as expected but the corresponding water residence times and lifetimes of hydrogen bonds between water and other molecules also change. In addition, relatively mobile side chains such as at loops and turns will also influence the properties of hydration. There is the trivial sort of change in hydration occupancy and dynamics due to reference frame such as for the subunit B where the interaction between the side chain of Arg57B and the side chain of His89B causes hydration sites, in our definition, to be more localized in this region. More interesting are examples involving additional interactions, such as the interaction between the side chain of Glu127B and the water molecules that coordinate with Mg^{2+} . This interaction in turn leads to better stabilization of the ion–water cluster.

Table IV lists the lifetimes of hydrogen bonds between water and the main residues involved in the activity of the protein. $\tau_{\text{HB-WP}}$ does not follow any simple trends from the monomer to the dimer system. Each appears to be potentially understandable because of local chemistry and finite averaging. In the subunit B, $\tau_{\text{HB-WP}}$ of HH12 of Arg57 drops from 1.0 ps in the monomer to 0.47 ps, because the interaction of HH12 of Arg57B with NE2 of His89B increases a chance for water–water hydrogen bond. The oxygen from Arg87 interacts twice as strongly with a water molecule coordinated to Mg^{2+} in the dimer than in the monomer; it has a lifetime of 3.41 ps, much larger than 0.63 ps in the monomer. Thus, this measure appears less informative regarding global protein properties.

CONCLUSIONS

MD simulations of SMnase and its subunit have been employed to explore the effect of dimerization on the dynamics of water and the activity of the enzyme. Interactions between finite element sites, sites with water molecules, sites with protein, and between protein moieties were analyzed. At the dimeric interface of the protein, MD simulations and the experiment²² agree on the interactions bridging the two subunits, which include salt links, direct hydrogen bonds, water-mediated hydrogen bonds, and apparent hydrophobic interactions. However, the residues participating in these interactions show more variability in the MD simulations than the experiment. In the core of the dimer interface, a water cluster with a direct hydrogen bond mediating His184A and His184B fluctuates between the side chains of His184A and His184B. Other fluctuations between water mediated and direct hydrogen bond interactions occur not only in the core of the interface, but across the whole interface region. The symmetric structure of the dimer in the experiment is not maintained instantaneously but only as a long time average. The broken symmetry apparently influences the properties of water and protein residues at the active site and the correlations with the

interface. Analysis of the water site interactions at the active site showed that the monomer and the subunits of the dimer differ.

In addition, the solvent densities, occupancies, water residence times, and the lifetimes of hydrogen bonds between waters and between water and protein were evaluated. It was found that the dimerization does not greatly influence the average hydration profile around the protein. Both systems have similar hydration patterns, including cluster locations, occupancy distributions, water residence time distributions, and hydrogen bond lifetime distributions. However, a more detailed investigation of the water residence times and hydrogen bond lifetimes deep in the cleft of the active site showed striking differences. Using a stretched exponential fitting, it was found that the stretching parameter of the residence time, β and h-bond lifetime, $\beta_{\text{HB-WW}}$, are smaller in the monomer than in each subunit of the dimer. The hydrogen bond lifetime between waters is shorter in subunit B than in both the monomer and subunit A system. Both of these results indicate that water at the active sites of the dimer is less affected vs. bulk than for the monomer where it has slower characteristic residence times. This change in water behavior gives a clear advantage to dimerization in the apparent absence of an ability to use both active sites simultaneously; both the entry of substrate and release of products would be facilitated.

Kinetic studies have indicated that the rate-limiting step for the reaction is the association of the protein to its substrate.^{13,14,23} Thus, considering that the cleaving reaction of the protein is water-assisted, we expect the change in the dynamics of water at the active site by dimerization may be related to DNA binding with the enzyme and the mechanism of the cleavage of DNA. How dimerization is coupled to the catalytic process of the enzyme will be further investigated in our next work.

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FIGURE 1.

Structure of SMnase: Helices, pink; sheets, yellow; loops, grey; magnesium, green sphere; water, red spheres. The active site is near the magnesium.

FIGURE 2.

A: Interactions in the core of the dimeric interface in the experiment: Red dots, water molecules; blue dash lines, hydrogen bond interactions. B: Interactions in the core of the dimeric interface in MD simulation: Red dots, hydration sites in MD; blue dash lines, hydrogen bond interactions.

FIGURE 3.

The magnesium cluster at the active site. Five water molecules are shown. A: The monomer and the subunit A; B: The dimer. Green sphere: magnesium; blue dash lines: hydrogen bond interactions. The water molecule behaving differently in the subunit B is circled.

FIGURE 4.

A: Site–site interactions at the active site in the monomer: Green sphere, magnesium; red dots, hydration sites; blue dash lines, hydrogen bond interactions. B: Site–site interactions at the active site in the subunit A in the dimer: Green sphere, magnesium; red dots, hydration sites; blue dash lines, hydrogen bond interactions. C: Site–site interactions at the active site in the subunit B in the dimer: Green sphere, magnesium; red dots, hydration sites; blue dash lines, hydrogen bond interactions.

FIGURE 5. Occupancy distributions of the hydration sites within 4 Å of the monomer (black) and the dimer (red). Blue straight line: bulk water.

FIGURE 6. Water residence time distributions around the monomer (black square) and the dimer (red triangle).

FIGURE 7.

The survival time correlation functions for the water molecules at some hydration sites at the active site in the subunit B. *: data points from MD; solid line: fitted curves according to Eq. (3). Red: Site18 ($s_r = 3.04 \times 10^{-3}$); brown: Site58 ($s_r = 9.52 \times 10^{-4}$); blue: Site52 ($s_r = 6.29 \times 10^{-4}$); black: Site11 ($s_r = 4.77 \times 10^{-4}$); cyan: bulk site ($s_r = 3.30 \times 10^{-4}$) s_r is the standard deviation of the regression. Inset: the differences of survival functions between MD and the fitted data.

FIGURE 8.

Distributions of hydrogen bond lifetimes of water–water and water–protein. Blue: water–water in the monomer; magenta: water–water in the dimer; black: water–protein in the monomer; red: water–protein in the dimer; cyan straight line: water–water in the bulk.

FIGURE 9.

The water–water hydrogen bond correlation functions at some hydration sites at the active site in the subunit B. Data points (*) from MD; solid lines are curves fit to the stretched exponential. Red: Site18 ($s_r = 4.08 \times 10^{-4}$); brown: Site58 ($s_r = 1.61 \times 10^{-3}$); blue: Site52 ($s_r = 6.37 \times 10^{-4}$); black: Site11 ($s_r = 4.28 \times 10^{-4}$); cyan: bulk site ($s_r = 8.15 \times 10^{-4}$). s_r is the standard deviation of the regression. Inset: the differences of water–water H-bond correlation functions between MD and the fitted data.

Table I
System Set-Up of the Monomer and Dimer System

	Monomer	Dimer
Box size (Å)	$71.52 \times 64.94 \times 56.10$	$121.24 \times 19.62 \times 119.56$
No. TIP3P	7571	55,498
No. Na ⁺	25	50
No. Cl ⁻	25	50

Table II
Interactions of Protein–Protein in the Dimeric Interface^a

Subunit A	Subunit B	Frequency (%)
Salt Links		
Arg136A, NH1	Asp225B, OD2	81.1
Arg136A, NH2	Asp225B, OD1	79.4
Asp225A, OD1	Arg136B, NH2	10.2
Asp225A, OD2	Arg136B, NH1	23.3
Lys233A, NZ	Glu239B, OE1	71.9
Lys233A, NZ	Glu239B, OE2	12.0
Glu239A, OE1	Lys233B, NZ	46.8
Glu239A, OE2	Lys233B, NZ	38.1
Direct Hydrogen Bonds		
Asp138A, OD2	Ser179B, HG1	32.5
Val182A, O	Asn183B, HD22	63.6
Asn183A, HD22	Val182B, O	75.4
His184A, HN	Val182B, O	41.2
His184A, NE2	His184B, HD1	84.8
His184A, HD1	Ala181B, O	66.6
Lys233A, HZ1	Lys244B, O	23.3
Lys233A, HZ3	Asn245, OT1	54.7
Ser229A, HG1	Glu239B, OE2	68.4
Glu239A, O	Ser229B, HG1	52.6
Water-Mediated Hydrogen Bonds		
Lys48A, HZ2	Ser19B, N	10.5
Asp138A, OD1	Ser179B, HG1	13.1
Asp138A, OD2	Ser179B, HG1	12.2
Asn177A, HD22	Asn183B, OD1	15.3
Asn177A, HD22	Asn178B, OD1	29.7
Asn178A, OD1	Asn178B, OD1	34.5
Asn178A, OD1	Asn177B, HD22	27.8
Asn178A, OD1	Asn178B, HD21	29.0
Ser179A, HG1	Asp138B, OD2	46.6
Asn183A, HD21	Asn183B, OD1	20.6
Asn183A, HD21	Asn178B, OD1	39.0
His184A, NE2	Val236B, HN	43.0
His184A, NE2	Tyr185B, HN	17.5
Asp225A, OD1	Glu239B, O	28.3
Asp225A, O	Glu239B, OE2	18.1
Gly235A, O	Lys233B, HZ3	11.6
Leu240A, O	Asp225B, OD2	14.3

^aHydrophobic interactions are not included.

Table IIIThe Frequencies of the Interactions at the Active Site^a

		Monomer (%)	Subunit A (%)	Subunit B (%)
Arg87, HN	OD1, Asp86	26.1	26.2	—
Arg87, HN	OD2, Asp86	41.7	40.0	94.0
Arg87, O	Asn119, HD22	10.3	3.4	13.0
Ser95, OG	Arg131, HH12	93.0	96.2	90.1
Glu114, OE1	Asn119, HD22	73.1	91.0	25.2
Glu127, OE1	Arg131, HH11	95.6	98.1	25.4
Glu127, OE2	Arg131, HH21	68.7	47.7	21.0
His89, NE2	Arg57, HH12	—	—	48.6
H ₂ O (Mg)	Asn119, O	51.7	11.0	85.6
	Asp86, OD1	—	11.7	—
	Glu127, OE1	—	—	70.0
H ₂ O (Mg)	Asp86, OD1	18.1	20.1	20.8
	Asp86, OD2	10.0	27.6	—
H ₂ O (Mg)	Asp86, OD1	35.4	31.5	—
	Asp86, OD2	52.9	28.8	50.0
	Arg87, O	24.9	41.5	62.6
H ₂ O (Mg)	Arg87, O	10.0	11.3	—
	Glu114, OE1	18.1	—	68.4
H ₂ O (Mg)	His89, NE2	—	—	8.3
	Glu127, OE1	—	—	32.0
	Glu127, OE2	—	—	55.5

^aH₂O (Mg): the magnesium coordinated water molecule.

Table IVThe Lifetimes of Hydrogen Bond Interactions Between Protein and Water at the Hydration Sites^a

		Monomer (ps)	Subunit A (ps)	Subunit B (ps)
Arg 57	HE	0.72	0.75	0.66
	O	0.85	0.48	0.69
	HH22	0.57	0.79	0.22
	HH12	1.0	0.85	0.47
	HH11	1.98	0.85	0.31
Asp86	HH21	0.32	0.69	0.32
	OD1	4.95	3.04	2.07
	OD2	6.43	2.83	1.65
Arg87	O	0.63	1.5	3.41
	HH12	1.14	1.65	3.61
	HH11	0.62	0.72	1.12
	HH21	1.03	0.87	0.36
His89	O	1.53	1.34	0.24
	NE2	1.45	0.86	0.99
	HD1	2.03	1.08	0.51
Asn119	HD21	0.28	0.55	0.46
	O	0.25	0.79	0.97
Glu127	OE1	1.01	3.53	2.18
	OE2	4.64	1.22	2.41
Arg131	HE	0.54	1.14	2.01
	HH22	0.71	0.49	0.23
	HH21	0.37	0.68	1.29

^a Only residues involving the activity of the protein are included.