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Molecular Dynamics Study of Hydration Water Behavior in Blue Copper Protein

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Abstract. We carry out the molecular dynamics(MD) simulation of type 1 blue copper protein azurin in room and some lower temperatures to investigate the behavior of hydration water molecules in the protein surface. In this study, we find the anomalous behavior of the water molecules , which depend on the system temperatures. These water molecules have hydrogen bond to the protein surface residues. We specify the residues type, being classified as the hydration donor and the hydration acceptor of water molecules. We analyze the residue type, and the bond length and bond strength between solvent water molecules in each temperature. Moreover, we estimate the B-factor of these residues which indicates the fluctuation of hydration residues in each temperature. B-factor values depend on the system temperatue althought the number of hydration residue do not depend on the temperature.

Keywords: blue copper protein, azurin, MD simulation, hydration water
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INTRODUCTION

In general, protein exists in water or lipid with folding conformation and has a particular function in such a situation . Solvent water molecules have a great influence on the geometrical formation, stability, and functionality of solute protein. In this study, we focus on protein - solvent water interaction and analyze a behavior and physical properties of water molecules surrounding protein. In the last decade, diverse behavior of hydration water has been investigated. Inter-molecular hydrogen bond between water molecules, which is observed in hydration water surrounding protein, has been investigated[1]. Especially, Nakasako et. al. have indicated that the number of hydration water molecules and the adsorption point of hydration water molecules surrounding the protein surface depend on the system temperature in bovine β-trypsin by cryogenic X-ray structure [2]. In theoretical study, glasslike behavior of water molecules near the protein surface has been also observed by the MD simulation of azurin and plastcianin [3, 4]. The boson peak, which is observed in amorphous state , has been observed in this simulation. Odagaki et. al. have also observed a similar glasslike behavior by trapping diffusion approach in supercooled fluids[5].

In this study, we focus on and investigate one of the blue copper protein, azurin. Blue copper proteins are categorized into three types (type1, type2, and type3) by their structures and functions. Azurin is one of the type 1 blue copper protein[6]. The structure of azurin consists of eight β strands and one α helix with 128 residues. The active site of azurin consists of a copper ion and five ligand residues(His46, Cys112, His117, Gly45, and Met121). The functionality of azurin is an electron transfer, which is due to their prominent reactivity. In the active site, electron transfer is with the redox reaction caused by charge transfer of S(Cys112)(π) $\rightarrow$ Cu($d_{x^2-y^2}$), which has been investigated by resonance Raman (RR) spectra[7, 8]. In our previous studies, we investigated physical and chemical properties of azurin concerning with the active site and electron transfer by using molecular dynamics (MD) simulation and density functional theory (DFT) calculations[9, 10, 11]. In these studies, we found the geometrical structure of azurin in solution and studied these electronic structures[11]. The dependence of solvent dielectric constance on the atomic charge and spin density was also calculated[10].

In this study, we carried out the MD simulation of type 1 blue copper protein azurin in room and some lower temperatures to investigate the behavior of hydration water molecules in the protein surface. We estimated the diffusibility of the hydration water molecules in each temperature. We specified the hydrogen bonding atom from azurin surface residues and calculated atomic positional fluctuations of each atoms. we analyzed the residue type, and the bond length, bond angle, and bond strength between solvent water molecules and discussed the temperature dependence of system.

METHOD

In this section, we explain the detail of MD simulation method and analysis method of hydration water and hydrogen bonding.

Computational Detail

In this study, we carry out MD simulation using AMBER99[12] force field in room and some lower temperatures(140K, 220K, and 300K). The total run times of each simulation are 500 ps (1MDsteps=2fs). We adopt the crystal structure of oxidized *Pseudomonas aeruginosa* azurin at pH5.5 from the Protein Data Bank (PDBID:4AZU[13]) as an initial coordinate. For equilibrium of the system, we carried out the 2000 steps energy minimization with the steepest descent method and 50 ps MD calculation with 25 kJ/mol/Å harmonic position restrain force. After that, for the decreasing the harmonic position restrain, energy minimization with the steepest descent method are also carried out 5 times. In each minimization, restrain force is decreased 5 kJ/mol/Å for each step. Finally, MD calculation with no restrain force during 500ps are carried out. The system achieve the equilibrium. Figure 1 shows a snapshot of the equilibrium system of azurin. The cutoff radii for nonbond interactions are 8 Å. The charge distribution around the active site is determined by the semiempirical method (AM1) with the MOPAC program package[14]. The SHAKE algorithm[15] is performed to the bonds involving hydrogen. 3351 TIP3P[16] balk waters are applied to the solvent model (Fig. 1). All simulations are carried out under NPT ensemble without the periodic boundary condition.

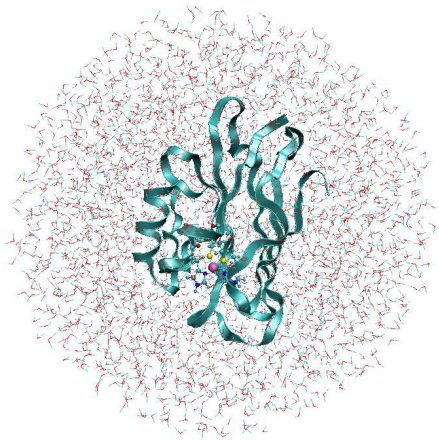

FIGURE 1. Secondary structure of azurin in solution with 3351 TIP3P balk waters.

Analysis method

Intermediate Scattering Correlation Function

From trajectories of water molecules by these simulations, we have analyzed the hydration water. In each temperature, we calculated the intermediate scattering function at q=1.8Å^{-1} defined by,

$$I(q,t) = \frac{1}{3N}\langle \sum_{i=1}^{N} \mathbf{exp}[i\mathbf{q}\cdot(\mathbf{R_i(t)} - \mathbf{R_i(0)})]\rangle. \qquad (1)$$

where N is the number of hydrogen atoms in water and $\mathbf{R_i}$ is the position vector of the *i*-th hydrogen atom. *I(q,t)* is the incoherent part of the total intermediate scattering correlation function and is the measure of the disuffusibility of hydration water.

Hydrogen bonding

Interactions between hydrogen bond "donors" and hydrogen bond "acceptors" are evaluated by using "ptraj" module in AMBER8 program package. From the lists of hydrogen bonding pairs, we estimate and classify the averaged hydrogen bonding distance, residue type, and atom type in each temperature.

B-factor, which is estimated from the root mean square fluctuation (RMSF) of the hydrogen bonding atoms, is also calculated. B-factor is index of measuring the fluctuation of atoms defined by,

$$\text{B-factor} = \frac{8}{3N}\pi^2 \sum_{i}^{N} \langle |R_i(t) - R_i(0)|^2 \rangle. \quad (\text{Å}^2) \qquad (2)$$

We analyze the fluctuation of hydrogen bonding atoms concerning with the side chain type and the bonding distance between side chain atoms to water molecules from B-factor.

RESULTS AND DISCUSSION

Intermediate Scattering Correlation Function

We calculated the intermediate scattering correlation function *I(q,t)* of first hydration shell waters, which is the 3Å thichness layer form the protein surface[17], at the fixed wave vector $q = 1.8\text{Å}^{-1}$ in three different temperature simulations. The *I(q,t)* at low temperature indicates a slow decay shown in Fig. 2. Similar result is obtained by P. A. Paciaroni et. al. in azurin and plastcianin by using GROMOS87 MD force field[3, 4]. In the 140K and 220K systems, two relaxation peaks are observed. It indicates that the hydration waters are trapped by protein surface

TABLE 1. B-factor averaged of the hydration donor and hydration acceptor residues during 50ps MD simulation and the number of hydrogen bonding residues in each temperature ($Å^2$)

	140K	220K	300K		140K	220K	300K
donor				acceptor			
ASP	14.312 (11)	29.158 (11)	44.242 (11)	THR	12.023 (6)	23.386 (8)	36.611 (7)
GLN	18.097 (6)	32.633 (6)	39.360 (6)	ASN	19.867 (6)	34.859 (6)	43.480 (6)
ASN	18.231 (5)	30.486 (5)	42.726 (4)	SER	15.088 (4)	28.755 (5)	36.611 (3)
THR	12.173 (6)	26.576 (7)	35.918 (8)	TYR	13.758 (1)	26.576 (1)	44.326 (2)
SER	15.085 (7)	30.473 (9)	41.510 (8)	ARG	11.818 (1)	26.573 (1)	37.018 (1)
TYR	13.758 (1)	23.850 (1)	44.326 (1)	GLN	18.098 (6)	32.633 (6)	39.360 (6)
(total)	(37)	(39)	(38)	(total)	(24)	(24)	(25)

atoms and slowly decayed. From these simulations and analysis of protein surface, we found that hydration water is less diffusibility than waters far from the protein and the behavior of these waters strongly depends on the system temperature.

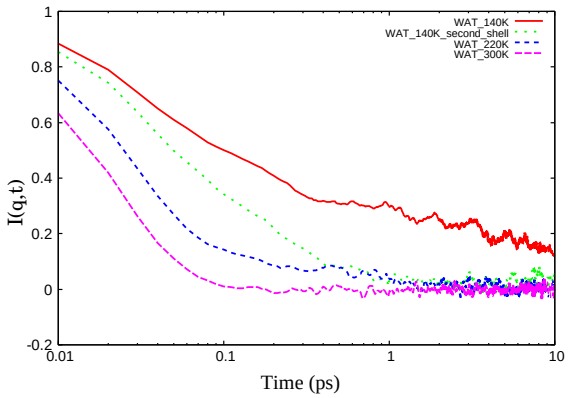

FIGURE 2. Intermediate scattering correlation function at q=1.8$Å^{-1}$ for 140K, 220K, and 300K, respectivly.

TABLE 2. Averaged bond distance between solvent water molecules for hydration donor residues (Å)

	140K	220K	300K
hydration donor			
ASP	2.789	2.805	2.873
GLN	2.815	2.918	2.978
ASN	2.864	2.948	2.978
THR	2.864	2.948	3.007
SER	2.943	2.991	3.054
TYR	3.011	3.101	3.093

TABLE 3. Averaged bond distance between solvent water molecules for hydration acceptor residues (Å)

	140K	220K	300K
hydration acceptor			
THR	2.859	2.884	2.954
ASN	3.084	3.126	3.140
SER	2.960	3.044	3.054
GLN	2.960	2.955	2.984
TYR	3.014	3.094	3.056
ARG	3.135	3.137	3.150

Hydrogen bonding

We estimated the number of hydration donor residues and hydration acceptor residues in each temperature. We found that asparatic acid and threonin are rishest amino acids among residue as the hydration donor and the hydration acceptor. The number of asparatic acid in the hydration donor is 11 in every temperatures. On the other hand, in the hydration acceptor residues, the number of threonin is 6, 8, and 7 at 140K, 220K, and 300K, respectively. The total number of hydration donor residues is 37, 39, and 38 at 140K, 220K, and 300K, and number of hydration acceptor residues is 24, 24, and 25 in each temperature. ASP, GLN, ASN THR, SER, TYR residues become a hydration donor and ASP, GLN, THR, SER, TYR residues become a hydration acceptor. The number of hydrogen bonding in each residue is shown in Table 1. These amino acids have acidic or polar acids. The acidic side chain and polar side chain of protein surface have the hydrogen bonding with water molecules, which do not depend on the system temperatures. Moreover, we also calculated B-factor of hydrogen bonding residues to estimate the fluctuation of the residues shown in Table 1. We can find that the B-factor values increase being proportional to the system temperature.

On the other hands, we calculated the trajectories of the water molecules in the first hydration shell. The trajectories of some common water molecules during the 250ps simulation are shown in Fig. 3. In whole, diffusion of water molecule in lower temperature is less weak than one of 300K. Especially, in the 140K system, some water molecules are strongly trapped and hardly move. We also observed similar behavior of water molecules in 220K. However we could not observe in 300K. We found

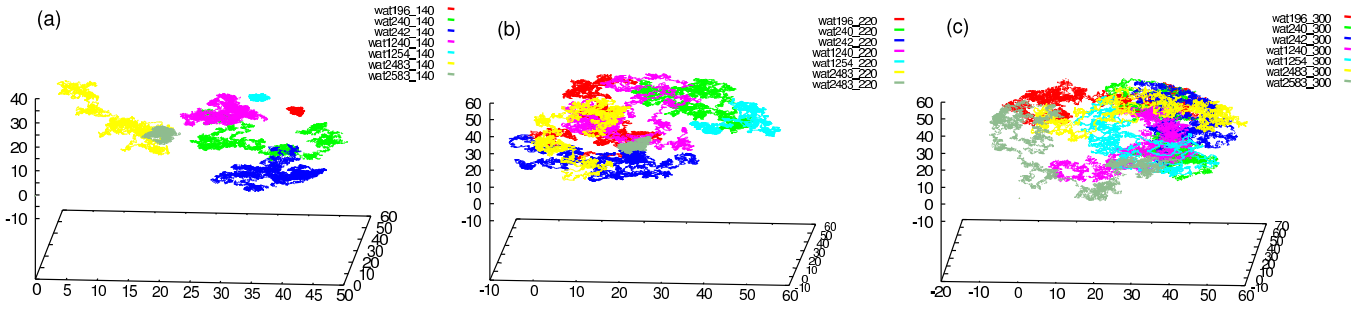

FIGURE 3. The trajectories of water molecules in the first hydration shell at each temperature during 250ps simulations (a): 140K, (b):220K, (c):300K.

that these strongly trapped water molecules have hydrogen bonding with asparatic acid. The averaged hydrogen bonding distance of hydration donor and hydration acceptor are shown in Table 2 and 3, respectively. The averaged bond distance of asparatic acid is the shortest in every temperature.

CONCLUDING REMARKS

In this study, we specified the residues having the hydrogen bond with water molecules and analyzed the residue type, bond length, and bond strength in each temperature.

From the intermediate scattering correlation function *I(q,t)*, we found that the water molecules, which belong to the first hydration shell water, have less diffusibility than water molecules far from protein surface.

Hydrogen bond is generated between water molecule and acidic or polar side chain on the protein surface . Hydrogen bonding residues behave as the hydration donor, acceptor or both together, and do not depend on the system temperatures. B-factor values of hydrogen bonding residues increase being proportional to the system temperature. Especially, aspartic acid has the strong hydrogen bonding with water. We found that water molecules are trapped by aspartic acid in low tempareture.

In this study, we have not remarked to the the effect of protein surface topography. Not only the hydrophobicity and hydrophilicity of protein surface, but also protein surface topography is important to understand the role of the hydration water. Near future, we will present the investigation of the hydration water behavior and hydration network of water molecules surrounding azurin from the view point of protein surface topography.

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