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Molecular Dynamics of the M intermediate of photoactive yellow protein in solution

Minoru Sakurai,[1]* Mariko Shiozawa,[1] Shohei Arai,[1] Yoshio Inoue,[1]
Narutoshi Kamiya[2] and Junichi Higo[3]

[1]Department of Biomolecular Engineering, Tokyo Institute of Technology, 4259 Nagatsuta-cho, Midori-ku, Yokohama 226-8501, Japan

[2]Biomolecular Engineering Research Institute, 6-2-3, Furuedai, Suita, Osaka 565-0874, Japan

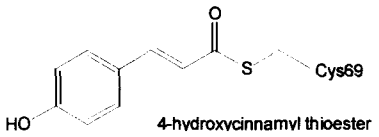
[3]Laboratory of Bioinfomatics, School of Life Science, Tokyo University of Pharmacy and Life Sciences, 1432-1 Horinouchi, Hachioji, Tokyo, 192-0392, Japan

PYP consists of a water-soluble apoprotein and 4-hydroxycinnamyl chromophore bound to Cys69 via thiolester linkage. Upon absorption of a photon, the photocycle is initiated, leading to formation of several photo-intermediates. Among them, M intermediate is important to understand the signal transduction mechanism of PYP, because it is a putative signaling state. As well known, the dynamics of a protein is closely correlated with the occurrence of its function. Here we report the results of 10 ns molecular dynamics (MD) simulation for the M intermediate in aqueous solution and discuss the characteristic feature of this state from a viewpoint of structural fluctuation.

Key words: photoactive yellow protein, molecular dynamics simulation, water, hydrogen bond, intermediate

INTRODUCTION

The photoactive yellow protein (PYP) found in *Ectothiorhodospira halophila* is a small water-soluble photoreceptor in which a 4-hydroxycinnamyl chromophore is covalently linked to Cys 69 through a thioester bond [1]. Upon absorption of a photon, PYP enters a photocycle that generates several spectrally distinct intermediate states.



4-hydroxycinnamyl thioester

The longest lived photobleached intermediate (M) is presumed to be the signaling state of PYP [2]. Thus, the elucidation of its three dimensional structure is essential for understanding the function of PYP. Currently, the interpretation of the structure of M has been much controversial [2-6]. The time-resolved Laue diffraction

* To whom correspondence should be addressed.
E-mail msakurai@bio.titech.ac.jp

study revealed that the structural changes on going from the ground state to M are limited to a small region in the vicinity of the chromophore [2]. However, several experimental data for PYP in the solution-state insist the occurrence of large conformational changes (or fluctuations) with a significant increase in accessible surface area [3-6]. The atomic level of structural analysis by usual experimental techniques may be difficult for M in water because of its partially unfolded character and limited lifetime. Instead, molecular dynamics (MD) simulation may be helpful for this purpose. Our 5 ns MD simulation for the M intermediate demonstrated that the conformational fluctuation of the M intermediate in water is large enough to allow the invasion of water molecules into the interior of the protein [7]. Here we extended the simulation to 10 ns. On the basis of the results, we discuss the molecular dynamics of the M intermediates.

MATERIALS AND METHODS

The MD simulation was carried out according to our previous report [7]. The starting configuration of M was derived from the X-ray coordinates, 2PYP, in Protein Data Bank. The AMBER95 all-atom force field and the TIP3P model were used for the protein part of M and water, respectively. Most of the force field parameters for the chromophore were taken from those of the analogous fragments in amino acids. However, the barrier heights for the single bond rotations of the chromophore backbone were determined so as to fit the results of *ab initio* calculations at the restricted Hartree-Fock (RHF)/6-31G* level. The protein was solvated in a droplet of water generated with a radius of 30 Å around the protein molecule. The net charge of –6.0 was compensated by adding six sodium ions. Consequently, the system studied contained 10965 atoms. After an energy minimization starting from this initial system, a 10 ps heating from 0 to 300 K and a 200 ps equilibration at 300 K were done. Subsequently, a 10 ns simulation was done at 300 K for data sampling. The temperature was controlled using the Nosé-Hoover algorithm. The other simulation conditions were as follows: the time step of the numerical integration was 1 fs; all heavy atom-hydrogen atom bonds were held rigid by the SHAKE algorithm; electrostatic interactions were calculated with a cell multipole method. Coordinate trajectories were stored at a rate of 10 per ps. The simulation was carried out using the MD simulation program PRESTO.

RESULTS AND DISCUSSION

The top of Figure 1 shows the time dependence of the root-mean-square deviation (RMSD) with respect to the starting X-ray structure. The RMSD value tends to increase until about 1 ns. During the period from 1 to 3.7 ns, it oscillates about a mean value of 2.0 Å. After this, it begins to decrease gradually. From 4 to 10 ns, RMSD again becomes stable. Thus, the time evolution of the RMSD up to 10 ns may be divided into three phases. We found that there is good correlation between the time evolution of the RMSD and that of the distance between the phenolic oxygen of the chromophore and the NH_2 –type hydrogen atoms of Arg 52 (the bottom of Figure 1). In the crystalline state, one of the NH_2-type hydrogen atoms of Arg 52 is 2 Å distant from the phenolic oxygen of the chromophore, suggesting the presence of the hydrogen bond

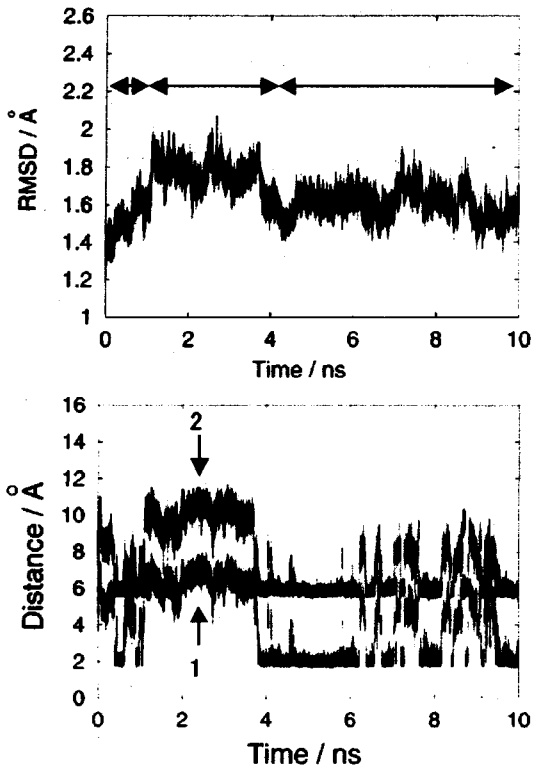


Figure 1. The time dependence of RMSD (top) and the distances between the phenolic oxygen of the chromophore and two of the NH_2-type hydrogen atoms of Arg 52 (bottom). One distance (line 1) was measured from this oxygen to the nearest hydrogen atom to it among the four NH_2-type hydrogen atoms at the start of the data sampling period and the other (line 2) from that to the farthest hydrogen atom.

between these atoms. In the first phase, one can see the repeated formation and collapse of this bond (see line 1 of Figure 1). In the second phase, this bond continues to be broken. The dynamics of the final phase is similar to that of the first phase. These results suggest the possibility that the overall conformational changes of the protein are induced by the formation and collapse of the above hydrogen bond.

In the crystalline structure of M, the 50- and 100-loops cover the chromophore so as to shield it from solvent. However, in the second phase of the simulation two water molecules were found to intervene between the hydroxy group of the chromophore and the side chain of Arg 52. The collapse of the direct hydrogen bond between these groups allows large fluctuations of the 50-loop and the backbone fragment including Cys 69 to which the chromophore is binding. The previous 5 ns simulation indicated that the root-mean-square fluctuation (RMSF) with respect to the average MD structure exhibits a large value at the loop region including Met 100. This tendency was found to be still kept from 5 to 10 ns (data not shown).

The large fluctuation of the protein backbone in the second phase allows rearrangements of hydrogen bonding networks in the protein. In fact, we observed the rearrangement of hydrogen bonds among Glu 46, Tyr 42 and Thr 50 (data not shown). As a result, it was found that a water molecule entered into the protein interior at 4.7 ns. We picked up two water molecules responsible for access to the protein interior. Figure 2 shows the time dependence of the distances between these molecules and the side chain of Glu 46. The molecule indicated by line 1 starts to move toward Glu 46 at about 2 ns and eventually binds to it at 4.7 ns. Such a binding state was kept until 8.5 ns. After that, this molecule is released from the interior, but instead the other water molecule indicated by line 2 entered into it. Namely, the first molecule was exchanged for the second one in the hydrogen bonding with Glu 46.

The present 10 ns MD simulation on the M intermediate in water brings us some important information. Obviously, in water the dynamics of the protein are activated mainly by the collapse of the hydrogen bond between the

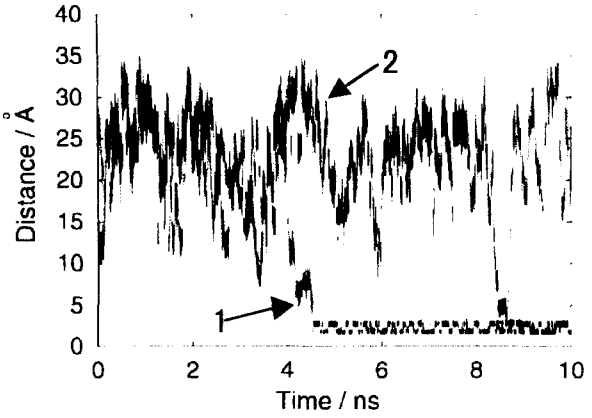


Figure 2. Time dependence of the distances between two water molecules and the side chain of Glu 46.

chromophore and the side chain of Arg 52. The resulting large fluctuations of the 50- and 100-loops causes the invasion of water molecules into the interior, leading to the hydration of Glu 46. In the M state, the side chain carboxyl of Glu 46 is deprotonated. Thus, the M state should be energetically unstable, because the negative charge is embedded in a low dielectric medium of the protein matrix. Therefore, the hydration of Glu 46 is a physically reasonable event. The water molecule exchange shown in Figure 2 clearly indicates that the invasion of water molecules into the protein interior is never an accidental phenomenon. On the basis of these results, it is reasonable to interpret that in the M state a channel is formed to allow water exchange between the exterior and interior of the protein. In conclusion, the M state in solution fluctuates more largely than does that in the crystalline state.

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