Evidence for Large Structural Fluctuations of the Photobleached Intermediate of Photoactive Yellow Protein in Solution

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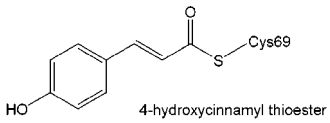
The photoactive yellow protein (PYP) found in *Ectothiorhodospira halophila* is a small water-soluble photoreceptor in which a 4-hydroxycinnamyl chromophore is covalently linked to Cys 69 through a thioester bond.[1] PYP is thought to function as a



4-hydroxycinnamyl thioester

photoreceptor initiating the negative phototaxis of this bacterium.[2] Upon absorption of a photon, PYP enters a photocycle that generates several spectrally distinct intermediate states.[3] The longest-lived photobleached intermediate (pB) is presumed to be the signaling state of PYP.[4] Thus, the elucidation of its three-dimensional structure is essential for understanding the function of PYP. Currently, the interpretation of the structure of pB has been very controversial.[4,5] The time-resolved Laue diffraction study revealed that the structural changes on going from pG to pB are limited to a small region in the vicinity of the chromophore.[4] However, several experimental data for PYP in the solution-state indicate the occurrence of large conformational changes (or fluctuations) with a significant increase in accessible surface area.[5] The atomic level of structural analysis by usual experimental techniques may be difficult for pB 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 demonstrates that the conformational fluctuation of the pB intermediate in water is large enough to allow the invasion of water molecules into the interior of the protein.

The starting configuration of pB was derived from the X-ray coordinates, 2PYP, in the Protein Data Bank. The AMBER95 all-atom force field[6] and the TIP3P model were used for the protein part of pB and for 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 (to allow the temperature to become uniform after the rapid heating) at 300 K were done. Subsequently, a 5 ns simulation was done at 300 K for data sampling. The temperature was controlled using the Nosé−Hoover algorithm.[7] 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;[8] electrostatic interactions were calculated with a cell multipole method.[9] Coordinate trajectories were stored at a rate of 10 per ps. The simulation was carried out using the MD simulation program PRESTO.[10]

The top of Figure 1A 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. Thus, the time evolution of the rmsd up to 5 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 1A). 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 between these atoms. In the first phase, one can see the repeated formation and collapse of this bond (see the red line of Figure 1A). In the second phase, this bond continues to be broken. In the final phase, it is again formed. 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 pB, the 50- and 100-loops cover the chromophore so as to shield it from solvent (blue ribbon in Figure 2A). 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. This is evident from the data for root-mean-square fluctuation (rmsf) with

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(1) Baca, M.; Borgstahl, G. E. O.; Boissinot, M.; Burke, P. M.; Burke, P. M.; Williams, D. R.; Slater, K. A.; Getzoff, E. D. *Biochemistry* **1994**, *33*, 14369−14377.

(2) Sprenger, W. W.; Hoff, W. D.; Armitage, J. P.; Hellingwerf, K. J. *J. Bacteriol.* **1993**, *175*, 3096−3104.

(3) (a) Hoff, W. D.; van Stokkun, I. H. M.; van Ramesdonk, H. J.; van Brederode, M. E.; Brouwer, A. M.; Fitch, J. C.; Meyer, T. E.; van Grondelle, R.; Hellingwerf, K. J. *Biophys. J* **1994**, *67*, 1691−1705. (b) Imamoto, Y.; Kataoka, M.; Tokunaga, F. *Biochemistry* **1996**, *35*, 14047−14053. (c) Ujj, L.; Devanathan, S.; Meyer, T. E.; Cusanovich, M. A.; Tollin, G.; Atkinson, G. H. *Biophys. J*. **1998**, *75*, 406−412. (d) Devanathan, S.; Pacheco, A.; Ujj, L.; Cusanovich, M.; Tollin, G.; Lin, S.; Woodbury, N. *Biophys. J*. **1999**, *77*, 1017−1023.

(4) Genick, U. K.; Borgstahl, G. E. O.; Ng, K.; Ren, Z.; Pradervand, C.; Burke, P. M.; Srajer, V.; Teng, T.-Y.; Schildkamp, W.; McRee, D. E.; Moffat, K.; Getzoff, E. D. *Science* **1997**, *275*, 1471−1475.

(5) (a) Rubinstenn, G.; Vuister, G. W.; Mulder, F. A. A.; Dux, P. E.; Boelens, R.; Hellingwerf, K. J.; Kaptein, R. *Nat. Struct. Biol*. **1998**, *5*, 568−570. (b) van Brederode, M. E.; Hoff, W. D.; van Stokkun, I. H. M.; Groot, M.-L.; Kroon, A. R.; Hellingwerf, K. J. *Biophys. J*. **1996**, *71*, 365−380. (c) Hoff, W. D.; Xie, A.; van Stokkum, I. H. M.; Tang, X.-J.; Gural, J.; Kroon, A. R.; Hellingwerf, K. J. *Biochemistry* **1999**, *38*, 1009−1017. (d) Kandori, H.; Iwata, T.; Hendriks, J.; Maeda, A.; Hellingwerf, J. K. *Biochemistry* **2000**, *39*, 7902−7909. (e) Craven, C. J.; Derix, N. M. Hendriks, J.; Boelens, R.; Hellingwerf, K. J.; Kaptein, R. *Biochemistry* **2000**, *39*, 14392−14399.

(6) Cornell, W. D.; Cieplak, P.; Bayly, C. I.; Gould, I. R.; Merz, K. M., Jr.; Ferguson, D. M. Spellmeyer, D. C. Fox, T.; Caldwell, J. W.; Kollman, P. A. *J. Am. Chem. Soc.* **1995**, *117*, 5179−5197.

(7) Hoover, G. W. *Phys. Rev.* A **1985**, *31*, 1695−1697.

(8) Ryckaert, J.-P.; Ciccoti, G.: Berendsen, H. J. C. *J. Comput. Phys*. **1977**, *23*, 327−341.

(9) Ding, H.-Q.; Karasawa, N.; Goddard, W. A., III. *J. Chem. Phys*. **1992**, *97*, 4309−431.

(10) Morikami, K.; Nakai, T.; Kidera, A.; Saito, M.; Nakamura, H. *Comput. Chem*. **1992**, *16*, 243−248.

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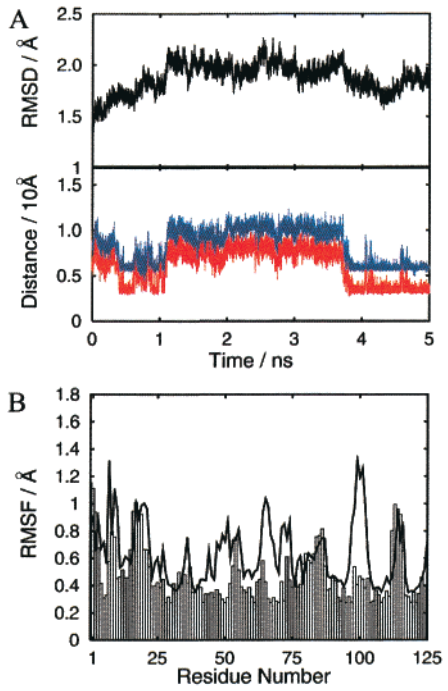

Figure 1. (A) The time dependence of rmsd (top) and the distances between the phenolic oxygen of the chromophore and two of the NH_2-type hydrogen atoms of Arg 52 (bottom). One distance (red line) was measured from this oxygen to the nearest hydrogen atom to it among the four NH_2-type hydrogen atoms at the start of the data sampling period and the other (blue line) from that to the farthest hydrogen atom. (B) The plot of rmsf (solid line) and X-ray B-factor (stick histogram) against the residue number.

respect to the average MD structure (solid line in Figure 1B). In addition, the fluctuation of the 100-loop is much larger than that expected from the X-ray B factor (histogram in Figure 1B). The red ribbon of Figure 2A shows a snapshot at 3.5 ns. Interestingly, the chromophore was reoriented so as to push out the 100-loop (the yellow stick model in Figure 2A). As a result, this loop undergoes a large conformational change. In the second phase, the top of the 100-loop was found to move maximally about 3 Å from the position of the X-ray structure.

The second phase corresponds to a kind of preparation period for searching another stable conformation, since the occurrence of backbone's large fluctuations 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. The conformation attained in the final stage of the simulation had a unique feature. Namely, Glu 46 was partially exposed to solvent,[11] although this is completely embedded into the interior in the crystalline state. Surprisingly, a water molecule existed in the interior, forming hydrogen bonds with the side chain of Glu 46 (Figure 2B). This water seems to have entered into the interior by passing through the bottom side of the 50-loop in the view of Figure 2B. It was confirmed that this water molecule continued to stay there during the last 200 ps.

The present 5 ns MD simulation on the pB intermediate in water brings us some important information. Obviously, in water the dynamics of the protein are activated mainly by the collapse

(11) Accessible surface area (ASA) was calculated using the GEPOL93 program written by J. L. Pascual-Ahuir, E. Silla and I. Tunon. We calculated separately the contribution of each residue to the total ASA.

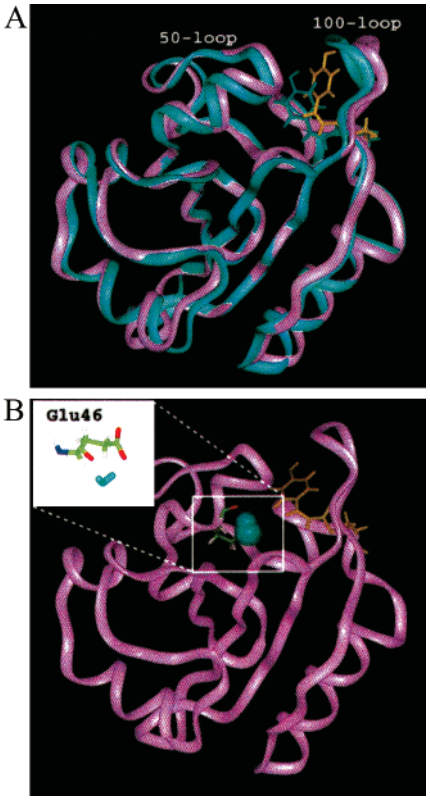

Figure 2. Snapshots of the MD trajectories at 3.5 ns (A) and 5 ns (B). (A) The MD structure (red ribbon) is superimposed on the X-ray structure (blue ribbon). In both cases, the chromophore is represented by a stick model. (B) The water molecule existing in the protein interior is represented by a blue space-filling model.

of the hydrogen bond between the chromophore and the side chain of Arg 52. The resulting large fluctuations of the 50- and 100-loops assist in the invasion of water molecules into the interior, leading to the hydration of Glu 46. In pB the side chain carboxyl of Glu 46 is deprotonated,[12] and its counterion is protonated Arg 52, located near the surface of the protein. In the crystalline structure, the distance between these charges is about 10 Å. Thus, the crystalline pB state is a high-energy one that has the negative charge embedded in a low dielectric medium of the protein matrix. Therefore, the hydration of Glu 46 is a physically reasonable event. On the basis of these considerations, it is evident that the X-ray structure of pB is a local minimum unique to the crystalline environment. In addition, in three-dimensional crystals the so-called crystal packing effect may block the occurrence of large fluctuations of the loop moieties as observed in the present simulation. In conclusion, the X-ray analysis of the photochemical intermediate contains some critical matters.

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Supporting Information Available: The values of the rotation barriers of the chromophore single bonds (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) Imamoto, Y.; Mihara, K.; Hisatomi, O.; Kataoka, M.: Tokunaga, F.; Bojkova, N.; Yoshihara, K. *J. Biol. Chem.* **1997**, *272*, 12905−12908.