Hydrogen Bonding Controls Excited-State Decay of the Photoactive Yellow Protein Chromophore

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Photoactive Yellow Protein (PYP) is believed to be the primary photoreceptor for the photoavoidance response of the salt-tolerant bacterium Halorhodospira halophila. PYP contains a deprotonated 4-hydroxy-cinnamic acid (or p-coumaric acid, pca) chromophore linked covalently to the γ-sulfur of Cys69 via a thioester bond. Upon absorbing a blue-light photon, PYP enters a fully reversible photocycle involving several intermediates on a time scale spanning from a few hundred femtoseconds to seconds. In previous works we have used mixed quantum/classical (QM/MM) simulations to reveal the detailed sequence of structural changes that follows photon absorption in both wild-type PYP and the Arg52Gln mutant. The first step is a photoisomerization of the chromophore of the double (wt-PYP) or single bond (Arg52Gln). In the protein radiationless decay from the excited state is very efficient because the intersection seam between the ground- (S0) and excited-state (S1) surfaces lies near the excited-state minima. Time-resolved fluorescence decay measurements of chromophore analogues in water indicate that also in solution excited-state decay is very efficient.5 In contrast, calculations on the isolated chromophore by Gromov et al. demonstrate that in vacuum the intersection seam very close to these minima. Thus, radiationless decay is enhanced via electrostatic stabilization of the chromophore’s excited state by H-bond interactions.

The QM/MM MD simulations were carried out at the CASSCF(6,6)/3-21G level of theory, with diabatic surface hopping between the excited- and ground-state potential energy surfaces. In total, 91 excited-state simulations were initiated from different frames of a 50 ns ground-state trajectory (see Supporting Information (SI) for details). In these simulations, an excited-state lifetime between 117 and 1951 fs was observed (SI, Table S1). A simple exponential fit yields an excited-state decay time of ~350 fs, which is in reasonable agreement with the ca. 1 ps time constant that was found in the experiment.5 The predominant decay channel involves rotation around the SB (80 cases) rather than the DB (11 cases), reflecting the lower barrier to SB isomerization (SI, Figure S1), which was also observed in recent simulations.7

Inspection of the hopping geometries in the SB isomerization trajectories reveals that the water configuration near the chromophore is very similar in each of these trajectories. The same is true for the DB isomerization trajectories. However, when comparing the water configurations between SB and DB hopping geometries, we observe differences. In the case of SB isomerization, the carbonyl oxygen atom is H-bonded to three nearby water molecules, whereas the phenolate oxygen is involved in a single weak H-bond interaction (Figure 1a). In the case of DB isomerization, there are at least two water molecules near the ring of the chromophore that donate strong hydrogen bonds to the phenolate oxygen (Figure 1b), whereas the carbonyl oxygen accepts weak hydrogen bonds from one or two water molecules (see SI for animations).

The difference in the H-bonding patterns reflects the different charge distributions in the two S1 minima. In the SB twisted structure, the net negative charge is on the alkene moiety of the chromophore in S1, whereas it is mainly localized on the phenolate ring in the DB twisted structure. Dynamic adaptation to these charge distributions by the highly mobile water molecules stabilizes both S1 minima with respect to S0, bringing the seam closer to the reaction path sampled in the trajectory.

Thus, the rate-limiting step in the decay process is the reorganization of the solvent. To test this, we have repeated the simulations in D2O, which has a slightly higher viscosity. In D2O the solvent reorganization is slower, leading to an increase of the S1 lifetime by ~75 fs (SI, Table S2).

To quantify the stabilizing effect of the H-bonds, we have performed CASSCF(6,6)/3-21G8 geometry optimizations of the chromophore with and without water molecules. In these optimizations, the complete π system of the chromophore was included in the active space, which thus consisted of 12 electrons in 11 π orbitals. In addition to optimizing the local minima on the S1 potential energy surface, we also searched for conical intersections in the vicinity of these minima.

Figure 1. Twisted S1 minima for rotation around the single and double bond and S1 charge distributions.

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Table 1. S0−S1 Energy Gap (kJ/mol) at the Single- (Top) and Double-Bond Twisted (Bottom) Minima for Different Water Configurations, and Barrier to S1/S0 Seam

| waters     | ∆E(S0,S1) | ∆E(seam) |
|------------|-----------|----------|
| none       | 171.1     | —        |
| A, B       | 222.2     | —        |
| C          | 132.0     | 97.1     |
| A, B, C, D | 143.2     | 8.1      |
| A, C, D, E | 51.5      | 8.1      |
| C, D, E    | 25.6      | 4.4      |

Table 1 lists the energy gap between S1 and S0 at the SB and DB twisted S1 minima. Table 1 also includes the relative energy position of the corresponding S0/S1 conical intersection with respect to each minimum. Without water, there is a significant S0−S1 energy gap at the S1 minima. In the DB twisted structure, the seam lies ~29 kJ/mol above the S1 minimum, while no conical intersection was found near the SB twisted S1 minimum. Including two water molecules near the ring of the chromophore leads to a substantial reduction of the S0−S1 energy gap at the S1 minima. In this case, a DB twisted S1/S0 conical intersection was found only 5 kJ/mol higher in energy than this minimum. The two water molecules have the opposite effect at the SB twisted S1 minimum: the S0−S1 gap increases to 222 kJ/mol, and no decay channel can be found near this structure. In contrast, including water molecules near the carbonyl oxygen atom reduces the S0−S1 energy gap at the SB twisted structure. With three H-bonds to the carbonyl oxygen atom, the gap becomes significantly smaller (25.6 kJ/mol) and reaching the seam from the minimum requires only 4.4 kJ/mol.

In the protein environment there are two H-bonds to the phenolate oxygen atom and one to the carbonyl oxygen. These H-bonding interactions facilitate DB photoisomerization by enhancing radiationless decay from the DB twisted minimum. In simulations of the Arg52Gln mutant, the predominant decay channel involves photoisomerization around the SB. Since these observations seem to conflict with our new results, we have performed a more careful analysis of the mutant trajectories.

At the start of the mutant simulations there is no difference in the H-bonding interactions between the wild-type and the mutant. However, shortly after the excitation to S1, when the SB starts twisting, the H-bond between the carbonyl oxygen atom and the Cys69 backbone amino group breaks in the Arg52Gln mutant (Figure 2). After reaching the SB twisted S1 minimum, new H-bonds are formed between the carbonyl oxygen atom and the backbone amino groups of Tyr98 and Asp97. While oscillating around the minimum, a water molecule from outside the protein moves into the chromophore pocket and donates the required third H-bond.

With the new insights we can reinterpret the functional role of the wild-type protein in the photochemical process. In the protein DB isomerization is favored over SB isomerization due to electrostatic interactions between the chromophore and the charged guanidinium group of Arg52, which lower the energy of both the twisted DB minimum and the barrier to reach that minimum. However, the crucial factor for the ultrafast decay from the twisted DB minimum are the H-bonds between the phenolate ring of the chromophore and the side chains of Tyr42 and Glu46 that stabilize the intersection seam. Weakening these H-bond interactions by point mutations will increase the excited-state lifetime, as observed in experiment.

In summary, ultrafast solvent reorganization dynamics enhances photoisomerization of the PYP chromophore in water. By H-bond interactions, the water molecules selectively stabilize both S1 minima and displace the S1/S0 seam very close to these minima. Similar effects have been observed in recent simulations of the GFP chromophore in water, suggesting that solvent stabilization provides a general mechanism for excited-state decay of chromophores in solution.

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Supporting Information Available: Details of the QM/MM scheme, surface hopping algorithm, optimized coordinates and CASPT2 energies. Tables S1 and S2 for QM/MM dynamics results. Figure S1 for barriers to SB and DB isomerizations. Figure S2 for Mulliken charges. Animations of photoisomerization trajectories. This material is available free of charge via the Internet at http://pubs.acs.org.

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