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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.[1] 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.[2] 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[3] and the Arg52Gln mutant.[4] 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- (S_0) and excited-state (S_1) 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 lies rather far away from the accessible S_1 minima.[6] These findings suggest that specific interactions that are present in the protein as well as in water control the ultrafast decay of the chromophore.

To identify interactions that bring the S_1/S_0 seam closer to the S_1 minima, we have performed a series of excited-state simulations of a chromophore analogue in water. The results of the simulations demonstrate that in water excited-state decay occurs near either the single-bond (SB) twisted S_1 minimum, in which the bond adjacent to the phenol ring is rotated by 90°, or the double-bond (DB) twisted S_1 minimum, in which the ethylenic bond is twisted at 90° (Figure 1). Although computations at a higher level of theory indicate that, in vacuum, the S_1/S_0 intersection seam is not readily accessible from these minima, we found that including few water molecules at key positions around the chromophore can bring the 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]

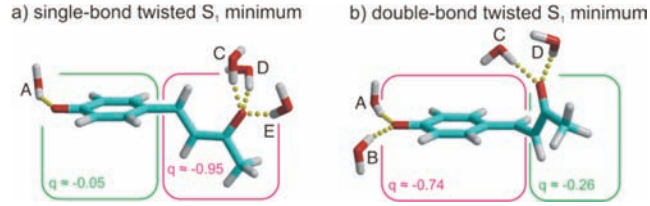

Figure 1. Twisted S_1 minima for rotation around the single and double bond and S_1 charge distributions.

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 S_1 minima. In the SB twisted structure, the net negative charge is on the alkene moiety of the chromophore in S_1, 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 S_1 minima with respect to S_0, 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 D_2O, which has a slightly higher viscosity. In D_2O the solvent reorganization is slower, leading to an increase of the S_1 lifetime by ~75 fs (SI, Table S2).

To quantify the stabilizing effect of the H-bonds, we have performed CASSCF/6-31G* 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 S_1 potential energy surface, we also searched for conical intersections in the vicinity of these mimima.

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Table 1. S_0-S_1 Energy Gap (kJ/mol) at the Single- (Top) and Double-Bond Twisted (Bottom) Minima for Different Water Configurations, and Barrier to S_1/S_0 Seam

waters	$\Delta E(S_1,S_0)$	ΔE(seam)
none	171.1	–
A, B	222.2	–
C	132.0	97.1
A, B, C, D	143.2	–
A, C, D, E	51.5	8.1
C, D, E	25.6	4.4

waters	$\Delta E(S_1,S_0)$	ΔE(seam)
none	91.2	29.3
A, B	32.2	5.2
C	102.3	–
A, B, C	46.9	9.3
A, B, C, D	64.6	–

Table 1 lists the energy gap between S_1 and S_0 at the SB and DB twisted S_1 minima. Table 1 also includes the relative energy position of the corresponding S_1/S_0 conical intersection with respect to each mimimum. Without water, there is a significant S_0-S_1 energy gap at the S_1 minima. In the DB twisted structure, the seam lies ~29 kJ/mol above the S_1 minimum, while no conical intersection was found near the SB twisted S_1 minimum. Including two water molecules near the ring of the chromophore leads to a substantial reduction of the S_0-S_1 gap at the DB twisted S_1 minimum. In this case, a DB twisted S_1/S_0 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 S_1 minimum: the S_0-S_1 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 S_0-S_1 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.[1] These H-bonding interactions facilitate DB photoisomerization by enhancing radiationless decay from the DB twisted minimum.[3] In simulations of the Arg52Gln mutant, the predominant decay channel involves photoisomerization around the SB.[4] 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 S_1, 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 S_1 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. This is possible because, by replacing a rather bulky arginine side chain by a much smaller glutamine side chain, the chromophore pocket has become more exposed to the solvent. With three strong H-bonds at the carbonyl and the H-bond between the phenolate oxygen and the Glu46 side chain transiently broken, the system finally decays to the ground state.

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

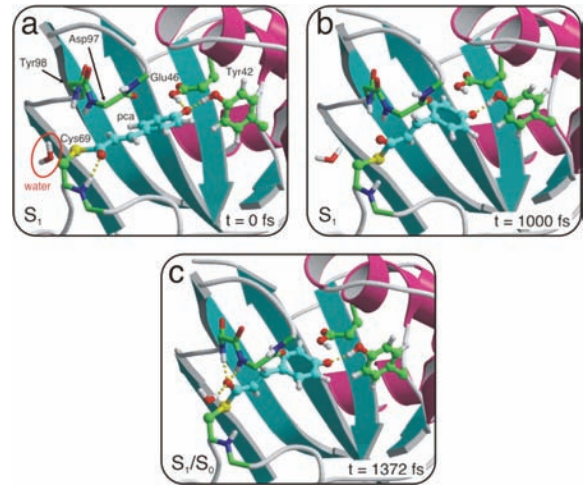

Figure 2. Snapshots from an excited-state trajectory of the Arg52Gln mutant:[4] (a) at photoexcitation, (b) twisted configuration without H-bonds to the carbonyl oxygen, and (c) at conical intersection seam. Image (c) shows how two backbone amino groups and a bulk water molecule donate the three H-bonds required for excited-state decay from the single-bond twisted structure.

minimum and the barrier to reach that minimum.[3,4,7,8] 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.[9]

In summary, ultrafast solvent reorganization dynamics enhances photoisomerization of the PYP chromophore in water. By H-bond interactions, the water molecules selectively stabilize both S_1 minima and displace the S_1/S_0 seam very close to these minima. Similar effects have been observed in recent simulations of the GFP chromophore in water,[7,10] 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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