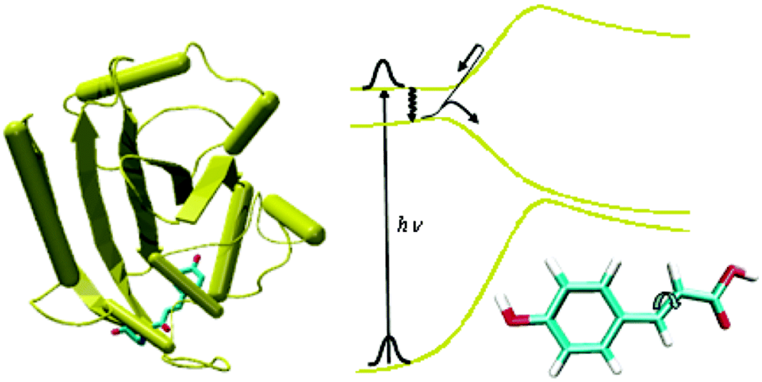
JACS JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Communication

Ab Initio Excited-State Dynamics of the Photoactive Yellow Protein Chromophore

Chaehyuk Ko, Benjamin Levine, A. Toniolo, Leslie Manohar,
Seth Olsen, Hans-Joachim Werner, and Todd J. Martnez

J. Am. Chem. Soc., **2003**, 125 (42), 12710-12711• DOI: 10.1021/ja0365025 • Publication Date (Web): 30 September 2003



Journal of the American Chemical Society is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

J|A|C|S
COMMUNICATIONS

Ab Initio Excited-State Dynamics of the Photoactive Yellow Protein Chromophore

Chaehyuk Ko,[†] Benjamin Levine,[†] A. Toniolo,[†] Leslie Manohar,[†] Seth Olsen,[†]
Hans-Joachim Werner,[‡] and Todd J. Martínez*,[†]

Department of Chemistry and The Beckman Institute, University of Illinois at Urbana-Champaign,
Urbana, Illinois 61801, and Institut für Theoretische Chemie, Universität Stuttgart, Stuttgart, Germany

Received June 4, 2003; E-mail: tjm@spawn.scs.uiuc.edu

Photon absorption by the *trans-p*-coumaric acid chromophore (pCA) of photoactive yellow protein (PYP) leads to trans−cis isomerization, initiating a signaling cascade which ultimately results in negative phototaxis by the purple bacterium *Halorhodospira halophila*.[1] Thus, an optical signal is converted to mechanical motion on a length scale extending from angstroms to micrometers. A detailed account of the initial events in this process requires investigation of pCA chromophore photodynamics. Ideally, such studies should span environments ranging from the gas to solution and protein phases, to elucidate how the protein environment controls and directs the photodynamics, and possibly use this knowledge to design molecular scale optomechanical devices. In this work, we use ab initio quantum chemistry and ab initio multiple spawning (AIMS) dynamics[2] to elucidate the excited-state behavior of isolated pCA, providing a clear explanation of puzzling findings from recent experiments.[3]

Although the active form of pCA in PYP is the anion, we study the neutral form here to connect with recent high-resolution gas-phase spectroscopic experiments.[3] These data provide a special opportunity to compare theory and experiment without introducing uncertainty from a relatively crude treatment of the solution or protein environment. The fluorescence excitation experiments found three distinct energetic regions.[3] Spectral lines are sharp at low excitation energy. With a sharp onset, the spectral lines become broadened as excitation energy is increased. This broadening continues in the highest energy regime, but the form of the spectrum changes. In addition, wavelength-dependent photochemistry was observed. Isomerized *cis*-pCA products were detected only in the intermediate and high energy regimes, with decreasing isomerized product at increasing excitation energy.

Given the above observations, Ryan et al. suggested the presence of a barrier to isomerization on S$_1$, estimated to be 3.4 kcal/mol. The change in spectral form in the high energy region was attributed to the existence of a second electronic excited state, suggested to be of n$\rightarrow\pi^*$ character in analogy with previous studies of urocanic acid.[4] The wavelength dependence of the photoisomerization quantum yield is harder to explain. Given a barrier to isomerization on S$_1$, one would expect higher excess energy to lead to more efficient isomerization. The different electronic character of S$_2$ might lead to a state-specific and distinct (relative to S$_1$) relaxation pathway which does not involve torsion. This would be in violation of Kasha's rule[5] and similar to the state of affairs in azobenzene.[6] We show here that Kasha's rule is in fact perfectly obeyed in *trans*-pCA and that the origin of the observed behavior lies instead in nonadiabatic recrossing effects and multiple decay channels. The vertical excitation energies of *trans*-pCA were computed using coupled cluster (EOM-CCSD), state-averaged complete active space self-consistent field (SA-CAS), and multireference perturbation (CASPT2) methods (Table 1). As expected, dynamic electron correlation has a large effect on the vertical excitation energies. However, all methods agree on the character of the lowest excited states: S$_1$ and S$_2$ are $\pi\rightarrow\pi^*$ states and S$_3$ is an n$\rightarrow\pi^*$ state. As in styrene,[7] the lowest two of these excited states arise from combinations of a $\pi\rightarrow\pi^*$ state localized on the alkyl tail and the L$_b$ state[8] of the aromatic ring. These results confirm that a low-lying second excited state exists in the neutral pCA chromophore. Although the original assignment[3] to an n$\rightarrow\pi^*$ state must be questioned, there is also a close-lying but higher n$\rightarrow\pi^*$ state, as found in the pCA anion.[9]

Table 1. Vertical Excitation Energies (in eV) for the Neutral Form of the PYP Chromophore[a]

	S$_1$ $\pi\rightarrow\pi^*$	S$_2$ $\pi\rightarrow\pi^*$	S$_3$ n$\rightarrow\pi^*$
SA-5-CAS(6/5)	5.67	6.07	7.09
SA-5-CAS(6/5)-PT2	4.93	5.17	5.33
SA-3-CAS(6/5)	5.60	6.07	n/a
SA-3-CAS(6/5)-PT2	4.96	5.26	n/a
EOM-CCSD	4.92	5.14	5.55

[a] All methods agree on the character of the three lowest excited states. The 6-31G* basis set is used unless otherwise noted. The S$_0$ geometry was optimized with B3LYP-DFT/6-31G**.

To address the wavelength-dependent excited-state photochemistry, we have computed potential energy curves for torsion about the isomerizable double bond (dihedral angle denoted by φ). The energy was minimized on S$_1$ with respect to all other coordinates for fixed φ, and the results are shown in Figure 1. An isomerization barrier exists, as in *trans*-stilbene.[10] We find this barrier to be 4.4 kcal/mol (2.1 kcal/mol with CASPT2; Table S9 of Supporting Information), in good agreement with the experimental onset of line broadening. This appears to confirm barrier-crossing as the origin of line broadening, if this barrier-crossing can also be connected with excited-state decay. We have carried out a search for S$_0$/S$_1$ minimal energy conical intersections (MECIs), which might form important S$_1$ decay pathways. As expected for a conjugated hydrocarbon,[11] there are low-lying intersections involving isomerization about the alkyl $C{=}C$ bond (the corresponding MECI is described in Table S7 of Supporting Information). Indeed, the entirety of the coordinate driving path from $\varphi = 142°$ to $\varphi = 90°$ exhibits a small S$_0$/S$_1$ energy gap and is likely very close to a seam of conical intersections. We also located a MECI (see Figure S5 of Supporting Information) involving a kinked benzene ring which is only 4.2 kcal/mol above the S$_1$ minimum. This MECI geometry is similar to the previously reported prefulvene S$_1$/S$_0$ MECI.[12] Thus, with ~4 kcal/mol of excess energy, two decay pathways are accessible − one involving a kinked distortion of the benzene ring and a second involving isomerization about the alkyl $C{=}C$ double bond.

[†] University of Illinois at Urbana-Champaign.
[‡] Universität Stuttgart.

10.1021/ja0365025 CCC: $25.00

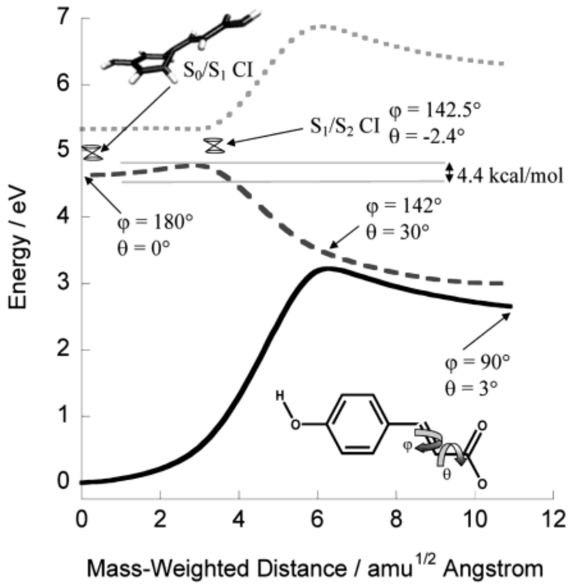


Figure 1. Potential energy curve for photoisomerization in the neutral form of the PYP chromophore determined using a SA-5-CAS(6/5) electronic wave function and the 6-31G* basis set. The torsional coordinate φ was varied from 180° to 90°, and the energy was minimized on S_1 with respect to all other coordinates for the fixed value of φ. See Figure S6 and Table S8 in the Supporting Information for CASPT2 results.

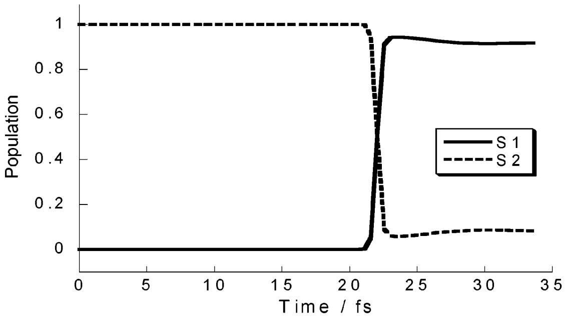


Figure 2. Electronic quenching from S_2 to S_1 determined using AIMS with a SA-5-CAS(6/5) electronic wave function. Almost all of the population excited to S_2 has quenched to S_1 within 30 fs, and the molecule remains completely planar for the period shown in this graph.

The experimentally observed increase in isomerization quantum yield with increasing excitation energy on S_1 is predicted by the presence of the S_1 isomerization barrier. However, the experiments also find a decrease in the isomerization quantum yield when S_2 is excited directly. It is tempting to attribute this to state-specific chemistry on S_2, that is, a violation of Kasha's rule. However, Figure 1 suggests that state-specific chemistry on S_2 would lead to a higher propensity for twisting because there is no S_2 isomerization barrier. Thus, if pCA violated Kasha's rule, one would expect a wavelength dependence opposite to that found experimentally. To resolve this puzzle, we turn to dynamical simulations.

Using a recently developed synthesis of the AIMS[2] and MolPro[13] codes, we modeled direct excitation to S_2, sampling initial conditions from the Wigner distribution for the harmonic approximation to the ground state $v = 0$ vibrational wave function and starting with a single trajectory basis function in each of five independently sampled runs. The electronic structure is solved simultaneously with the nuclear wave packet dynamics using the SA-5-CAS(6/5) electronic wave function and the 6-31G* basis set. The population decay (averaged over all initial conditions) is essentially complete in less than 50 fs (Figure 2). This is insufficient time for much more than bond stretching dynamics, and we observe no twisting at all (not shown). Thus, excitation of S_2 is equivalent to exciting S_1 with the appropriate amount of excess vibrational energy. In other words, Kasha's rule holds perfectly. Why then is there a decrease in isomerization quantum yield? A search for S_2/S_1 MECIs clarifies this point. As expected from the fast decay seen in Figure 2, there are low-lying S_2/S_1 MECIs involving only bond alternation. However, searching for MECIs in the S_1 isomerization barrier region (constraining φ to 142.5°) leads to the location of an intersection which is almost indistinguishable from the barrier geometry (see Figure S4 of Supporting Information) and only 3.2 kcal/mol above the barrier energy. Hence, the decrease in isomerization quantum yield is at least partially a consequence of nonadiabatic trapping by S_2 when the excess energy over the isomerization barrier exceeds 3.2 kcal/mol. In other words, molecules which attempt to twist will have a strong tendency to "up-funnel" to S_2, be turned around to return to *trans*-pCA, and then "funnel" back down to S_1.[14] Similar behavior has been shown to influence product branching ratios in unimolecular decomposition of small molecules.[15]

We have shown that the apparent violation of Kasha's rule in pCA is not a true violation at all. The observed dependence of product yield on excitation wavelength does not come about from an electronic state dependence of the reaction pathway. Instead, there is conical intersection-mediated nonadiabatic recrossing on S_1 which prevents isomerization more efficiently with increasing energy. Population which is trapped in the trans form can then decay to S_0 through an S_0/S_1 intersection involving a kinked benzene ring geometry. Longer time dynamics simulations are in progress to quantify the relative importance of these two effects on the wavelength-dependent quantum yield. It remains to be seen whether these effects also occur in solvated or protein environments, and, if so, whether there is any biological significance to this behavior.

Acknowledgment. This work was supported by the National Science Foundation (CHE-02-311876). T.J.M. is a Packard Fellow and Dreyfus Teacher-Scholar.

Supporting Information Available: CASSCF and CASPT2 energies and Cartesian coordinates of geometries discussed in the text. Active space orbitals and CI vectors (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Hellingwerf, K. J. *Antonie van Leeuwenhoek* **2002**, *81*, 51.
(2) Ben-Nun, M.; Quenneville, J.; Martínez, T. J. *J. Phys. Chem. A* **2000**, *104*, 5161.
(3) Ryan, W. L.; Gordon, D. J.; Levy, D. H. *J. Am. Chem. Soc.* **2002**, *124*, 6194.
(4) Ryan, W. L.; Levy, D. H. *J. Am. Chem. Soc.* **2001**, *123*, 961.
(5) Kasha, M. *Faraday Discuss.* **1950**, *9*, 14.
(6) Rau, H.; Luddecke, E. *J. Am. Chem. Soc.* **1982**, *104*, 1616.
(7) Hemley, R. J.; Leopold, D. G.; Vaida, V.; Roebber, J. L. *J. Phys. Chem.* **1986**, *85*, 134. Molina, V.; Merchan, M.; Roos, B. O.; Malmqvist, P.-A. *Phys. Chem. Chem. Phys.* **2000**, *2*, 2211.
(8) Platt, J. R. *J. Chem. Phys.* **1949**, *17*, 484.
(9) Molina, V.; Merchan, M. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 4299.
(10) Syage, J. A.; Lambert, W. R.; Felker, P. M.; Zewail, A. H.; Hochstrasser, R. M. *Chem. Phys. Lett.* **1982**, *88*, 266.
(11) Klessinger, M.; Michl, J. *Excited States and Photochemistry of Organic Molecules*; VCH Publishers: New York, 1995.
(12) Palmer, I. J.; Ragazos, I. N.; Bernardi, F.; Olivucci, M.; Robb, M. A. *J. Am. Chem. Soc.* **1993**, *115*, 673.
(13) Werner, H.-J.; Knowles, P. J.; Lindh, R.; Schuetz, M.; and others (see http://www.molpro.net). MOLPRO, version 2002.2, a package of ab initio programs.
(14) Martínez, T. J. *Chem. Phys. Lett.* **1997**, *272*, 139. Yarkony, D. R. *J. Chem. Phys.* **2001**, *114*, 2601.
(15) Waschewsky, G. C. G.; Kash, P. W.; Myers, T. L.; Kitchen, D. C.; Butler, L. J. *Faraday Trans.* **1994**, *90*, 1581.

JA0365025