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Relationship Between The Excited State Relaxation Paths Of Rhodopsin And Isorhodopsin

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Abstract: The pigment Isorhodopsin, an analogue of the visual pigment Rhodopsin, is investigated via quantum-mechanics/molecular-mechanics computations based on an ab initio multiconfigurational quantum chemical treatment. The limited $\sim$5 kcal mol$^{-1}$ error found for the spectral parameters allows for a nearly quantitative analysis of the excited-state structure and reactivity of its 9-cis-retinal chromophore. We demonstrate that, similar to Rhodopsin, Isorhodopsin features a shallow photoisomerization path. However, the structure of the reaction coordinate appears to be reversed. In fact, while the coordinate still corresponds to an asynchronous crankshaft motion, the dominant isomerization component involves a counterclockwise, rather than clockwise, twisting of the 9-cis bond. Similarly, the minor component involves a clockwise, rather than counterclockwise, twisting of the 11-trans bond. Ultimately, these results indicate that Rhodopsin and Isorhodopsin relax along a common excited-state potential energy valley starting from opposite ends. The fact that the central and lowest energy region of such valley runs along a segment of the intersection space between the ground and excited states of the protein explains why the pigments decay at distinctive conical intersection structures.

1. Introduction

The visual pigment Rhodopsin$^{1,2}$ (Rh) is a G-protein-coupled receptor containing an 11-cis-retinal chromophore (PSB11) bound to a lysine residue (Lys296) via a protonated Schiff base linkage (see green substructure in Figure 1). While the biological activity of Rh is triggered by the light-induced 11-cis $\rightarrow$ all-trans isomerization of PSB11, this reaction owes its efficiency (e.g., short time scale and high quantum yield) to the protein cavity.$^3$ Indeed, computational and experimental studies$^{3-5}$ have shown that, in the apoprotein (opsin), PSB11 isomerizes to a distorted all-trans-retinal (PSBT) identified as the chromophore of the first isolable ground state photocycle intermediate Bathorhodopsin (bathoRh).

Isorhodopsin (isoRh) is a Rh analogue featuring a 9-cis-retinal chromophore (PSB9) embedded in the same opsin environment. It has been shown that isoRh can activate a weaker visual response$^6$ triggered by a light-induced 9-cis $\rightarrow$ all-trans isomerization of PSB9 featuring a longer time-scale$^7$ and a reduced quantum yield.$^8$ Remarkably, as first reported by Yoshizawa and Wald,$^9$ the photoisomerization of isoRh results in the production of the same bathoRh intermediate obtained from Rh.

Figure 1. Schematic structure of the chromophores (in green) of the visual receptor (A) Isorhodopsin and (B) Rhodopsin. The curved arrows indicate the reactive double bonds.
Indeed, it has been shown that Rh, bathoRh and isoRh form, upon irradiation, a three-component photoequilibrium.\textsuperscript{10,11} Below, we show that the fact that Rh and isoRh can be photochemically converted to the same ground state intermediate provides the opportunity to expand our understanding of the structure of the excited-state potential energy surface of visual pigments. Indeed, it is apparent that the distinct photochemical reaction paths of Rh and isoRh (i.e., an approximate minimum energy path—see Section 2 for details—connecting Franck–Condon points on the excited-state energy surface to product valleys on the ground state energy surface) must describe converging excited-state relaxations ultimately leading to the same ground state energy minimum. Furthermore, the comparison of the computed PSB11 and PSB9 reaction paths must provide information on the apoprotein control of the geometrical deformation of the chromophore.

Previous computational studies\textsuperscript{12} on Rh indicate that the level of theory required for a quantitative description of the geometrical and electronic structure of its chromophore must include the treatment of electron dynamic correlation. In particular, the use of a quantum-mechanics/molecular-mechanics (QM/MM) model based on a QM \textit{ab initio} multiconfigurational second-order perturbation theory, allows for a comparison with the observed spectra. Indeed, we have shown: (i) that for a Rh model (S\textsubscript{0}-Rh) derived from monomer A of the X-ray structure of bovine Rh, the CASPT2/CASSCF/6-31G*/AMBER protocol\textsuperscript{13} yields a PSB11 conformation consistent with the experiment;\textsuperscript{14,15} (ii) that the protocol also yields a bathoRh model (S\textsubscript{0}-I)\textsuperscript{16} with a PSB structure close to the one recently resolved via femtosecond Raman spectroscopy\textsuperscript{16} (this is comparable with the one recently reported by Okada and co-workers\textsuperscript{17,18}); and (iii) that, again, the same protocol has been shown to reproduce the \textit{λ}_{\text{max}} change for a small set of modified rhodopsins.\textsuperscript{19} The S\textsubscript{0}-Rh model also features S\textsubscript{0} → S\textsubscript{1} and S\textsubscript{1} → S\textsubscript{2} \textit{λ}_{\text{max}} values (478 and 327 nm) 3 kcal mol\textsuperscript{-1} off the experimental values (498 and 340 nm),\textsuperscript{8} a computed 14.6 D change in dipole moment (∆D) that falls within the observed 13–15 D range\textsuperscript{20} and a S\textsubscript{1} → S\textsubscript{2} oscillator strength value (0.8) that compares well with the experimental quantity (1.0).\textsuperscript{8} Similarly, the computed \textit{λ}_{\text{max}} and photon energy storage of bathoRh are 5 kcal mol\textsuperscript{-1} off the observed values. The method has also been used to evaluate the \textit{λ}_{\text{max}} of PSB11 in methanol yielding an opsin-shift 2 kcal mol\textsuperscript{-1} off the experimental value.\textsuperscript{13} We found that the S\textsubscript{0} → S\textsubscript{1} \textit{λ}_{\text{max}} computed with the ANO-S (C,N[4s3p1d]/H[2s]) correlated basis set yields a reduced 10 nm red-shifted error.\textsuperscript{19}

However, due to its excessive computational cost, such a basis could not be adopted here.

Recently, we have reported the CASPT2/CASSCF/6-31G*/AMBER excited state (S\textsubscript{1}) reaction path that connects the Franck–Condon structure S\textsubscript{0}-Rh to an excited state/ground state conical intersection structure Rh–CI featuring a highly twisted (∼80°) reactive C\textsubscript{11}–C\textsubscript{12} bond.\textsuperscript{12,13} Such conical intersection provides the mechanistic entity allowing for fully efficient decay of the photoexcited chromophore to the ground state (S\textsubscript{0}) and, ultimately, for bathoRh production. We have also shown that trajectory computations with a scaled-CASSCF/6-31G*/AMBER potential that reproduces the S\textsubscript{0}-Rh → Rh–CI reaction path, indicate that S\textsubscript{1} → S\textsubscript{0} decay must take place on a ∼120 fs time scale in line with the experiment.\textsuperscript{21} Comparison of the reaction coordinate associated with the S\textsubscript{0}-Rh → Rh–CI reaction path with the trajectory reveals that the molecular motion associated with the space-saving excited-state isomerization of PSB11, an asynchronous crankshaft (or bicycle pedal) deformation of the C\textsubscript{11}–C\textsubscript{12}–moiety (see Figure 1 for the atom numbering), is also present along the S\textsubscript{0}-Rh → Rh–CI reaction coordinate and is thus driven by the shape of the S\textsubscript{1} potential energy surface.\textsuperscript{21}

In the present work, we compute, using the same CASPT2/CASSCF/6-31G*/AMBER protocol, the S\textsubscript{1} reaction path driving the isomerization of isoRh. Accordingly, we build and validate a suitable S\textsubscript{0}-isoRh model, compute and analyze the reaction coordinate associated to the photoisomerization process, and compare it with the S\textsubscript{0}-Rh → Rh–CI of Rh. We show that the structural differences of the isoRh and Rh paths provide information on the role of the protein cavity in the S\textsubscript{1} and (indirectly) S\textsubscript{0} relaxation of PSB11 and PSB9.

### 2. Methodology

Our QM/MM scheme is fully described in ref 22. Briefly, the method is based on a hydrogen link-atom\textsuperscript{23} and electrostatic embedding\textsuperscript{24} schemes with the frontier placed at the C\textsubscript{9}–C\textsubscript{10} bond of the Lys296 side chain (see Figure 1). The \textit{ab initio} QM calculations are based on a CASPT2/CASSCF/6-31G* methodology. The active space comprises the full π-system of PSB11 (12 electrons in 12 π-orbitals). The MM (we use the AMBER force field) and QM segments interact in the following way: (i) all QM atoms feel the electrostatic potential of the MM point charges; (ii) stretching, bending, and torsional potentials involving at least one MM atom are described by the MM potential; and (iii) QM and MM atom pairs separated by more than two bonds interact via either standard or re-parametrized\textsuperscript{24,25} van der Waals potentials. CASSCF/6-31G*/AMBER geometry optimization is carried out with the GAUSSIAN03\textsuperscript{26} and TINKER\textsuperscript{27} programs. The construction of the S\textsubscript{0}-Rh, S\textsubscript{0}-isoRh, and S\textsubscript{0}-I featuring PSB11, PSB9, and PSBT chromophores, respectively, has been documented.\textsuperscript{19,22} All protein models used in the computation are derived from monomer A deposited in the PDB archive as file 1HZX.\textsuperscript{15} (For isoRh, no archived crystallographic structure is currently available. Thus, we assume

\textsuperscript{17}Nakanishi, H.; Okada, T. Angew. Chem., Int. Ed. 2006, 45, 4270–4273.

\textsuperscript{22}Ferré, N.; Olivucci, M. J. Am. Chem. Soc. 2003, 125, 6868–6869.
that its average protein cavity is similar to that of Rh.) With the exception of the Glu113 counterion (forming a salt-bridge with NH(+)i), the opsins cavity is set neutral consistently with the experiment.28 While the protein is kept frozen during the optimizations (given the subpicosecond excited-state evolution of the chromophore, a fixed-opsin approximation seems to be adequate), the Lyso296 side chain, the position/orientation of two TIP3P water molecules (W1 and W2 in Figure 1) and the chromophore (PSB11 and PSB9 for Rh and isoRh, respectively) are relaxed. The optimizations were stopped when the maximum force is <0.0015 u.a./bohr and the rms force is <0.0010 u.a./bohr. Due to the excessive computational cost, no second derivative computations could be performed to rigorously determine the nature of the stationary points. At the equilibrium geometries, a single point three-root CASPT2 calculation with a three-root state averaged CASSCF reference wavefunction is carried out using the MOLCAS-6.429 program to evaluate the ground (S0) and second excited state (S2) states. The AMBER charges account for S0 polarization effects in a mean-field way.30 The same charges are used for the excited-state computations with no ad hoc dielectric constant added. Recently, two better resolved crystallographic structures of Rh (1L9H13 at 2.6 Å and 1U1935 at 2.2 Å in the PDB archive) have become available. The new structures display a position of W2 that differs significantly from that defined in our original model. Thus, in order to test the sensitivity of the spectral parameters to water re-location, we also reconstruct and test the S0-Rh, S0-isoRh, and S0-I models using the 1U19 crystallographic structure (see also ref 33). In all cases, the excitation energies and wavelengths are evaluated in terms of the energy gap between the corresponding electronic states. The S1 reaction coordinates defining the two reaction paths discussed below are computed starting from the S0-Rh and S0-isoRh models as follows. First, a chromophore unconstrained geometry optimization is carried out in the excited-state generating two excited-state relaxed structures. Second, starting at these relaxed structures, a relaxed scan along the reactive C11=C12 (for Rh) and C8=C10 (for isoRh) bond torsional coordinates (i.e., C10−C11−C12−C13 and C8−C9−C10−C11 dihedral angles) is computed with a step of −10°. Such a protocol, based on a relaxed scan rather than on a steepest-descent path computation, has been used to overcome convergence problems related with the flatness of the S1 energy surface along the dominating C10−C11−C12−C13 and C8−C9−C10−C11 dihedral angles. The resulting reaction paths are considered mechanistically valid approximations of S1 minimum energy paths. In fact, for Rh, the validity of the computed paths has been assessed by comparison with a recently reported unconstrained S1 trajectory21 computed using the same Rh model and level of theory. The comparison shows that the structural evolution described by the trajectory and by the reaction path is substantially the same.

The reaction paths defined above are computed in the field of the fixed crystallographic structure of the protein (with the exception of Lyso296). Such a field affects the computed paths through the van der Waals interactions between protein and chromophore centers and the electrostatic interaction between protein centers and the chromophore electron density (see point i above). This approximation seems to be adequate if one considers that the S1 evolution is completed on a subpicosecond time-scale.

3. Results and Discussion

3.1. Energy Profiles. In Figure 2, we report the energy profile of the S1 reaction paths computed starting from S0-Rh (left) and S0-isoRh (right) energy minima. In the same figure, we
also report the $S_0$ and $S_2$ energy profiles computed along the $S_1$ paths. In both cases, the initial relaxation (from $-10^\circ$ to $-20^\circ$), leading to a rapid $\sim 15$ kcal mol$^{-1}$ energy decrease, is largely dominated by a stretching mode corresponding to the inversion of the bond order along the chromophore backbone (double-bond expansion and single-bond contraction). This initial change has been previously reported, and it is connected with the positive charge translocation from the $-C_14=\text{N}$ region toward the $\beta$-ionone ring. The stretching relaxation is completed at the $-20^\circ$ structures and remains unchanged after these points. It is apparent from inspection of Figure 2, that, in both cases, the force field driving the subsequent isomerization is flat and connects the Franck–Condon points to a $S_0/S_1$ conical intersection (a real crossing between electronic states of the same spin multiplicity). Since the intersections provide a fully efficient decay channel, the energy surface is consistent, for both pigments, with the observed subpicosecond excited-state lifetime. However, a closer look reveals that, while in Rh the conical intersection (i.e., the Rh–CI structure) is reached when the isomerizing $C_{11}=C_{12}$ double bond is between $-70^\circ$ and $-80^\circ$ twisted, in isoRh the energy degeneracy is only intercepted when the reactive double bond is $-90^\circ$ twisted (isoRh–CI). Furthermore, while the Rh energy profile features a flat $-30^\circ$ energy minimum, the corresponding isoRh intermediate is shifted to the $-50^\circ$ region and features a higher ($\sim 6$ kcal mol$^{-1}$) barrier. The origin of these minima (and of the related barriers) may be associated to a change in character of the reaction coordinates that display a rapid change in the $C_{9}=C_{10}$ and $C_{11}=C_{12}$, $C_{13}=C_{14}$ torsions for Rh and IsoRh, respectively. However, an analysis of the $S_1$ energy profile of isoRh, in terms of protein–chromophore van der Waals and electrostatic interactions, indicates that the latter determine the depth of the $-50^\circ$ minimum (see Sections 8 and 9 in the Supporting Information).

One remarkable feature documented in Figure 2 is related to the intersection points. In fact, the $S_0$, $S_1$, and $S_2$ energy profiles developing from the very different $S_0$-Rh and $S_2$-isoRh Franck–Condon structures, seem to merge after the intersection regions are reached (i.e., at $-80^\circ$–$90^\circ$ twisting of the corresponding reactive bonds). This indicates that the $S_1$ potential energy valleys controlling the isomerization of Rh and isoRh join, from different directions, a common energy surface region characterized by the degeneracy of the $S_0$ and $S_1$ states. This region belongs to the $S_0/S_1$ intersection space, that is the $n$-dimensional space ($n$ being the number of vibrational degrees of freedom of the system) of electronic energy degeneracy. This conclusion is reinforced by inspection of the distinct Rh–CI and isoRh–CI chromophore structures (see Figure 2, top). As detailed in Figure 3, a superposition of these structures demonstrates that they are similar, with the largest differences corresponding to the orientation of a nearly planar $-C_{9}=C_{10}=C_{11}=C_{12}$ segment (on $S_1$ $-C_{10}=C_{11}$)–($-C_{12}$) containing the double bond character) of the carbon backbone. Such a segment is found to be substantially planar also at the common $S_0$–I intermediate. Of course, the $C_{10}$ and $C_{11}$ centers do not lie on such plane. In fact, the values of the $C_8-C_9-C_{10}=C_{11}$ and $C_{10}=C_{11}=C_{12}-C_{13}$ dihedral angles are $-90^\circ$ and $-155^\circ$ and $-161^\circ$ and $-80^\circ$ for isoRh–CI and Rh–CI respectively. Consistently, the $C_9-C_{10}$


\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure3}
\caption{CASSCF/AMBER structures for the conical intersections of Rh and isoRh. The planes $\alpha$ and $\beta$ show the mutual orientation of the $-C_9=C_{10}-C_{11}=C_{12}$–segment in Rh–CI and isoRh–CI, respectively. The same planes are reported on the structures of Figure 2.}
\end{figure}


of plane α but in the clockwise direction. This inverse behavior is illustrated by the pair of curved arrows on the Rh–CI and isoRh–CI structures of Figure 2. Not only the nature of the −C9=C10/C11=C12− segment rotation appears to be reversed, but also the ∼2:1 (i.e., −40°:+20°) ratio of the C9=C10 and C11=C12 bond deformations of Rh becomes a ∼1:2 (i.e., +15°:−30°) ratio in isoRh. Such a reversed ratio has been previously described.38 In other words, not only the direction of rotation of the plane is reversed, but also the degree of synchronicity of the torsions delimiting it.

While the discussion above has been focused on the last part of the reaction path of both pigments (from −40° to −80° Rh and from −60° to −90° for isoRh) the total changes (from the Franck–Condon point to the intersection) in the value of the dihedral angles associated with the torsion about the −C9=C10− and −C11=C12 reactive bonds, are given in Table 1. From Table 1 and Figure 2, it is also apparent that there are other (lesser) torsional deformations that contribute to the S1 reaction coordinate of Rh and isoRh. For instance, in both pigments, the −C13=C14− double bond undergoes a ∼+20° increase from the Franck–Condon point to the conical intersection.

### 4. Conclusions

The computational investigation of a photochemical reaction in the protein environment is currently a formidable research target. A primary requirement to be fulfilled is the accurate mapping of the force field (i.e., of the potential energy surface) driving the reaction. In the present contribution, we have provided evidence that a CASPT2/CASSCF/6-31G*/AMBER strategy can be used to study the structure of a rather wide region of the excited-state potential energy surface of visual pigments spanning the Rh and isoRh analogues (isoRh can be seen as a diastereoisomer of Rh). These results provide an unprecedented

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**Table 1.** Franck–Condon to Conical Intersection Differences in the Dihedral Angles of the Rh and IsoRh Chromophores

<table>
<thead>
<tr>
<th></th>
<th>Rh</th>
<th>IsoRh</th>
</tr>
</thead>
<tbody>
<tr>
<td>C9−C10</td>
<td>−82°</td>
<td>−72°</td>
</tr>
<tr>
<td>C11−C12</td>
<td>+32°</td>
<td>+30°</td>
</tr>
<tr>
<td>C13−C14</td>
<td>+19°</td>
<td>+16°</td>
</tr>
<tr>
<td>C7−C11</td>
<td>−17°</td>
<td>+30°</td>
</tr>
</tbody>
</table>

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(39) Cooper, A. FEBs Lett. 1979, 100, 382–384.
“wide angle” view of the excited-state potential energy structure that is schematically illustrated in the diagram of Figure 6.

According to the energy profiles of Figure 2, the relaxation of Rh and isoRh from their Franck–Condon point toward the S₁/S₀ intersection is driven by a 15 and 22 kcal mol⁻¹ excess energy, respectively. Thus, remarkably, the longer living (100 fs fluorescence lifetime) isoRh pigment has more vibrational excess energy available than the shorter living Rh (50 fs fluorescence lifetime). The slower excited-state dynamics of isoRh may be associated with the existence along the S₁ relaxation path of a deeper energy minimum (at ~50°). Alternatively, for the case of Rh, trajectory computations indicate that a shallow energy minimum (e.g., at ~30° in Figure 2) does not affect the excited-state dynamics. Thus, the isoRh slower dynamics and, consequently, the slower photoproduction appearance time of isoRh (600 fs) with respect to Rh (200 fs) cannot be explained on the only basis of the structure of the S₁ reaction paths. The observed presence of vibrational excess energy as well as non-stationary vibrational states in the two pigments is also in line with the results of the same Rh trajectory computation that reveals a ~10 kcal mol⁻¹ increase in the S₁–S₀ energy gap in the 30°–40° region. This set of trajectories is required. As suggested by recent trajectory studies on reduced PSB₁¹ models, as well as anticipated by the figure legend, but suggests that can be impulsively populated by continuing the evolution along the S₂-Rh → S₁-Rh direction and after decaying at Rh–Cl. It is also interesting that, consistent with the diagram in Figure 6, in order to populate the bathoRh valley (centered at S₂-Rh) the average direction of that structural evolution must change. In fact, bathoRh could be effectively populated only if the system (i.e., the center of the vibrational wavepacket), after achieving the photoRh configuration, reverts the C₉=C₈=C₁₀=C₁₁ dihedral angle change, moving in an almost orthogonal (i.e., weakly coupled) mode. This change in motion could be the origin of the picosecond time scale required for generation of bathoRh from photoRh.

In spite of several recent computational studies on the photoduced isomerization of visual pigments, two key features currently remain to be understood. The first regards the nature of photoRh, an elusive intermediate, that no computational study has been able to model. The second and more fundamental point regards the molecular factor at the basis of the high quantum yield of Rh (67%) with respect to that of isoRh (22%). While it is likely that the understanding of the structure of the energy surfaces of these molecules constitutes a prerequisite for the solution of these problems, this cannot be provided by the present knowledge. In this case, the calculation of a suitable set of trajectories is required. As suggested by recent trajectory studies on reduced PSB₁ models, as well as anticipated by


→ S₀-isoRh direction. It shall be noted that these mechanisms are a generalization of the bicycle pedal mechanism originally proposed by Warshel on the basis of a semiempirical quantum chemical model of Rh and only recently confirmed via scaled-CASSCF/AMBER trajectory computations on the S₂-Rh model. Also notice that, as apparent from Figure 5 and Table 1, more moderate changes of other torsional angles follow the two paths. This includes, in both pigments, the C₁₃=C₁₄ bond that constitutes the reactive bond in microbial rhodopsins (e.g., in bacteriorhodopsin).

It is informative to follow the evolution of the C₁₀-C₁₁, C₁₀-C₁₁, and C₉=C₈=C₁₀ dihedral angles (i.e., of the torsional deformation of the C₁₃=C₁₂ and C₆=C₇=C₁₀ bonds) along the converging paths. As stressed above and shown in Figure 6, the excited-state evolution of both pigments points toward the same intersection space segment. This region, that has been investigated in a previous report, would constitute the bottom of a single S₁ potential energy valley connecting the S₂-Rh and S₂-isoRh Franck–Condon points. Interestingly, the experimentally derived values of the C₁₀-C₁₁, C₁₀-C₁₁, and C₆=C₇=C₁₀ dihedrals for photorhodopsin (photoRh), that is the primary (non-isolable) photoproduct of Rh and precursor of bathoRh, place this critical structure close to the intersection space segment (e.g., to a conical intersection point that located almost halfway between Rh–Cl and isoRh–Cl). Of course, this does not mean that photoRh is a point of the intersection space (most likely, it would differ in the value of the double-bond expansion/single-bond contraction coordinate of the branching space leading far from the S₀/S₁ degeneracy. See also the figure legend) but suggests that can be impulsively populated by continuing the evolution along the S₂-Rh → S₁-Rh direction and after decaying at Rh–Cl. It is also interesting that, consistent with the diagram in Figure 6, in order to populate the bathoRh valley (centered at S₂-Rh) the average direction of that structural evolution must change. In fact, bathoRh could be effectively populated only if the system (i.e., the center of the vibrational wavepacket), after achieving the photoRh configuration, reverts the C₉=C₈=C₁₀=C₁₁ dihedral angle change, moving in an almost orthogonal (i.e., weakly coupled) mode. This change in motion could be the origin of the picosecond time scale required for generation of bathoRh from photoRh.

experimental studies, other modes such as the HOOP vibrational modes accompanying the isomerization motion may play a fundamental role in these processes.

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**Supporting Information Available:** Details of the QM/MM scheme; coordinates of all optimized structures; tables of energies, charge distribution, $\Delta\mu$ and $f$; comparison with experimental data; bare chromophore scan; complete torsional motion; and complete ref 26. This material is available free of charge via the Internet at http://pubs.acs.org.