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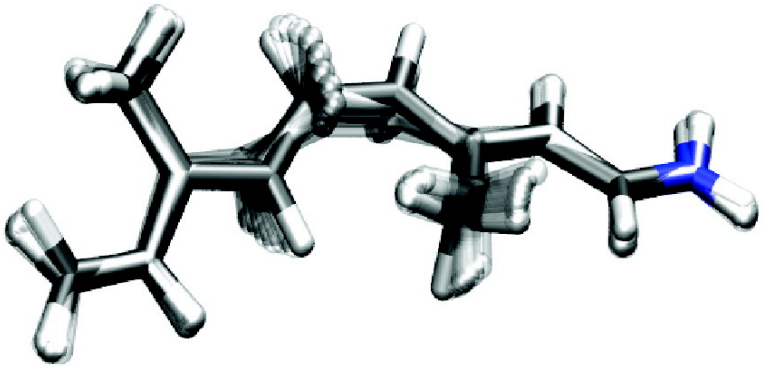

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The Twisted C11═C12 Bond of the Rhodopsin Chromophore—A Photochemical Hot Spot

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Rhodopsin, a G-protein coupled receptor (GPCR), belongs to a super-family of proteins which are instrumental for signal transduction through cell membranes.[1] In the vertebrate eye it mediates scotopic vision: excitation by 500 nm photons drives the highly efficient isomerization of the rhodopsin chromophore, 11-*cis* retinal protonated Schiff base (pSb), to the all-*trans* form in bathorhodopsin which starts the visual cycle.[2]

Recently, the bathorhodopsin structure has been resolved by X-ray analysis of rhodopsin crystals under illumination.[3] According to the diffraction data and their quantum-mechanical refinement,[4] the C11═C12 bond at this very early stage of the photocycle has already adopted a twisted *trans* configuration (Figure 1), with smaller changes of the other torsion angles distributed over the length of the conjugated chain.

How does this reaction come about within the confined space of the protein pocket? The simplest possibility, the classical "one-bond-flip" with complete rotation of one-half of the molecule, is ruled out on the basis of the bathorhodopsin crystal structure. However, neither the "bicycle-pedal" mechanism proposed by Warshel,[5] nor Liu's so-called "hula-twist" model,[6] both volume-conserving, can account for the formation of the all-*trans* photoproduct. In the former, the *cis*-C11═C12 bond would be transferred to one of the neighboring double bonds and in the latter to one of the single bonds; neither is observed in bathorhodopsin.

Cembran et al. have characterized the static evolution of 11-*cis* retinal in S_1 by calculating the minimal energy path (MEP).[7] We have simulated the photoreaction of rhodopsin on the basis of a five-double-bond model of the 11-*cis* retinal pSb chromophore using ab initio molecular dynamics (AIMD).[8] Our findings show that the distortion of the chromophore induced by steric interaction with the protein pocket is sufficient to make the photoreaction super fast, highly efficient, and stereoselective. The analysis of 18 trajectories reveals that the initial photochemical event which culminates in the isomerization of the *cis* double bond involves the three central bonds only, making this twisted region of the chromophore what might be called an inherently hot photochemical spot.

The five-double-bond model was obtained by DFTB/CHarMM[9] optimization of the complete 11-*cis*-retinal pSb within the binding pocket of rhodopsin[10] and cutting off the β-ionone ring and the bond to Lys296 (Figure 1). A molecular ensemble was created by randomly activating the vibrational modes at 0 K (zero point energy sampling, ZPE) using the RHF routine in Gaussian 98.[11] The resulting geometries and velocities were used as starting vectors for the individual trajectories.

In AIMD quantum-mechanical forces derived from a CASSCF wave function[12] are used to integrate the equations of motion and generate the reaction coordinate "on the fly", first on the S_1, then on the S_0 potential energy surface. To locate the hopping point, the vector rotation method[13] was employed which monitors the CI coefficients of the two electronic states involved. The stereochemical outcome of all trajectories was determined by following the reaction on the S_0 surface until the configuration could be unambiguously assigned. Details of the computational setup may be found in Supporting Information.

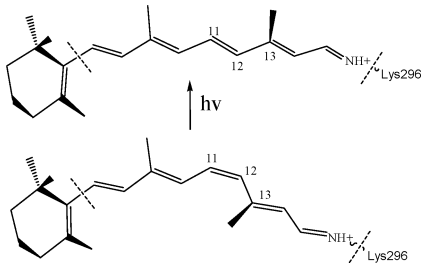

Figure 1. Photoisomerization of the 11-*cis*-retinal chromophore in rhodopsin to the all-*trans* form in bathorhodopsin. The graphs reflect the shapes of the chromophores after optimization within their respective protein pockets. Broken lines denote the limits of the five-double-bond model of this study.

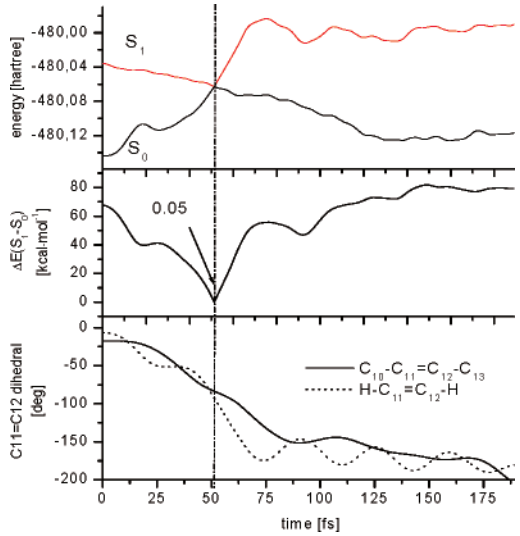

Figure 2. MD run of reference trajectory with panels showing the evolution of (from top) the S_1 and S_0 potential energy, the S_1-S_0 energy difference, and two torsion angles about the C11═C12 bond.

The panels in Figure 2 trace the change of several key parameters along the reference trajectory which was calculated without initial kinetic energy starting with Franck—Condon excitation at $t = 0$. As a consequence of the extended π-system the S_1 potential is initially rather flat, while the S_0 energy increases as the nuclear framework adjusts to the inverted π-density alternation. About 15 fs into the simulation the molecule starts to rotate around the C11═C12 bond which leads to a sharp drop in the S_1-S_0 energy difference. After 51 fs the energy difference is down to 0.05 kcal·mol^{-1}, and the molecule hops to the ground state. The C11═C12 dihedral angle at the point of crossing is −75° in this particular run. Note the activation of the strong H—C11═C12—H out-of-plane (HOOP) vibration as the system continues its trajectory on the ground-state potential surface.

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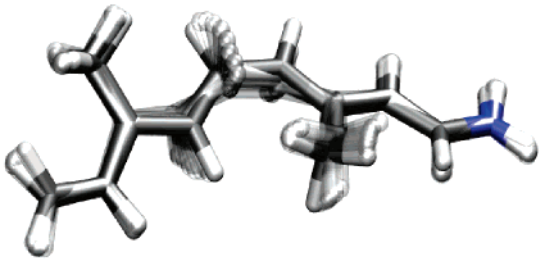

Figure 3. Overlay of snapshots of the model chromophore covering a time span of 100 fs from excitation. The hopping event occurred after 51 fs.

Hopping times and geometries vary in the different runs due to the randomly sampled starting geometries and velocities, but the over-all picture is the same. The initial C11═C12 twist angle varies between −6 and −36°; for the C12−C13 angle the spread is similar. The average of both angles, however, corresponds closely to the values of the energy-minimized chromophore (−18° and 167°, respectively) as it should.

The photoreaction proceeds completely regio- and stereoselective: in all trajectories studied only the C11═C12 bond was affected, and in each case the C13 methyl group started to rotate clockwise. Of the 18 trajectories 13 continued, after hopping to the ground state, to yield the 11-*trans* product, which translates into a quantum yield of 72%.[14] With an average excited-state lifetime of 61 fs the reaction is also extremely fast. Both results are in very good agreement with experimental data: a 50 fs time scale for the S_1 to S_0 internal conversion was deduced from resonance Raman data[15] and, more recently, from flourescence quantum yields.[16] The most recent determination of the rhodopsin quantum yield gave a value of 65%.[17]

The mean C11═C12 dihedral angle during surface crossing is −90°, but even at values as low as −70° the angle continued to increase smoothly on the S_0 surface toward the *trans* configuration. Supportive of the high quantum efficiency of the reaction is the strong HOOP coordinate at the isomerizing bond. In all trajectories leading to *trans* this HOOP vibration was found to be *in-phase* with the C11═C12 rotation during surface crossing, its large amplitude motion carrying the system to the 11-*trans* product. The opposite holds true as well, as we have shown in related studies on 3- and 4-double-bond models of retinal: out-of-phase motion of this coordinate will force the system back to the *cis* product.[18,19]

The activity of the HOOP modes has been used in a recent femtosecond-stimulated Raman study to investigate the molecular structures along the photoisomerization coordinate in rhodopsin.[20] For photorhodopsin, the primary transient following S_1 to S_0 conversion, a highly twisted geometry was deduced, with dihedral angles of +45, +25, −110, and +30° along the C9 to C13 carbon chain. In our simulation, where the molecule reaches a plateau on the S_0 surface about 20 fs after hopping (Figure 1), the corresponding angles are +54, −2, −112, and +11°.

A remarkable feature of the reaction is evident from a glance at the snapshots taken from the model chromophore during the reference trajectory (Figure 3). In the short time the system spends on the excited-state surface the nuclear motion leading to double-bond isomerization is completely restricted to the coupled rotation of the three bonds from C10 to C13, as if the nuclei outside this region were fixed in space (which they are of course not). Only very late in the simulation, when the system has crossed back to the ground state, do the two termini start to rotate, thereby adjusting to the changed stereochemistry at the center of the chromophore.

Crucial for the fast and selective isomerization is the inherent torsion of the carbon chain from C11 to C13. As the C12−C13 bond starts to planarize, which is a consequence of the inverted π-density in the excited state, the C13 methyl group starts to push against the C10 hydrogen atom, thereby initiating a bicycle-pedal rotation, with the C11═C12 and the C9═C10 bonds acting as pedals and the C10−C11 bond as wheel. This coupled rotation, which eventually would lead to the 9-*cis* product, is aborted as soon as the molecule returns to the ground state, and the original π-density alternation is restored. However, the nuclear displacements and the momenta developed in this short time span are sufficient to fix the system in the 11-*trans* configuration in more than 70% of the observed trajectories.

Will this model work for the real chromophore in the true protein environment as well? The answer is probably yes. The van der Waals interactions between chromophore and binding pocket are minimal in the isomerizing region. Only the hydrogen at C12 exhibits repulsive interaction with one of the amino acids, which in the protein might be more supportive for successful *cis*−*trans* isomerization. The additional double bond will not significantly affect the selectivity and the rate of the reaction; this one can infer from the reactivity of shorter 3- and 4-double-bond models.[17,18]

The vital central twist of the chromophore is not due to direct interaction by the protein, but is a necessity resulting from bridging the ends of the chromophore which are oriented by respective parts of the binding pocket.[21] Thus, protein participation in the photoreaction is neither likely nor necessary to postulate at this stage. Participation is needed and obvious once the molecule returns to the ground state. Only then forces are beginning to build up against the fixed ends of the chromophore which finally determine the energy and the geometry of the bathorhodopsin intermediate.

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Supporting Information Available: Complete ref 11 and 12, computational details, discussion, Cartesian coordinates, and movie of the reference trajectory. This material is available free of charge via the Internet at http://pubs.acs.org.

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