Primary Photoprocess in Vision: Minimal Motion to Reach the Photo- and Bathorhodopsin Intermediates

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According to time-resolved spectroscopic measurements, the initial step of the photoreaction of rhodopsin occurs with a time constant of approximately 200 fs. The whole or a part of the retinal molecule cannot move any significant distance in such a short time. In this paper, we propose instead a minimal motion that accomplishes the important task of guiding the molecule to a configuration where it can decay to the ground-state surface, with a minimal loss of strain energy. This motion is proposed to involve a −90° twisting of the C11═C12 double bond and a simultaneous twisting around two other double bonds in retinal to minimize the geometrical changes along the reaction path. The ONIOM method (complete active space self-consistent field for retinal and AMBER for the peptides) is used in a chromophore−cavity model to elucidate and confirm important features of the mechanism. The potential energy surface (PES) obtained according to the proposed mechanism show all of the characteristics of a fast photoreaction, meaning a downhill reaction path from the Franck−Condon point to an avoided crossing between S_1 and S_0. In this motion, only a few carbon and hydrogen atoms move more than 0.3 Å, and the retinal structure is conserved in the protein cavity. We propose that the photorhodopsin intermediate is a retinal molecule formed on the excited-state PES. Bathorhodopsin, however, is a ground-state intermediate, still located inside the protein cavity.

I. Introduction

Wald and co-workers originally showed that the retinal chromophore (Figure 1) in rhodopsin, the visual pigment of the vertebrate eye, performs a cis−trans isomerization around the C11═C12 bond (Figure 1; twist angle β_3) after it has been excited by light[1]. Theoretically, such a reaction may be thought of as passing through an avoided crossing (AC) between the first excited-state (S_1) potential energy surface (PES), where β_3 is twisted 90°, and the ground-state (S_0) PES. The full cis−trans isomerization is completed on the ground-state PES.[2−4]

Time-resolved spectroscopy was used early on to study the primary photoprocess.[5] The first intermediate is called photorhodopsin.[6] Callender et al. found that this intermediate is formed in about 200 fs and assigned it as an excited state of retinal with a 90° twist around the C11═C12 bond.[7] The 200 fs process is then the downhill pathway on the excited PES from the Franck−Condon (FC) region to the AC region. Better time-resolved data were obtained later by Mathies et al.[8,9] The latter authors stressed, however, that their data supported a different model where the first intermediate is the fully cis−trans-isomerized molecule on the ground-state PES.

The measured time constant for the primary reaction (200 fs)[8,9] is surprisingly short in view of the rather recently determined crystallographic structure,[10] which shows that the retinal molecule is embedded in the protein. Although there is no great surprise in the way the retinal molecule is embedded, there is an ambiguity in the understanding of the precise mechanism for cis−trans isomerization. The original suggestion of Wald did not specify any time scales. In theoretical discussions following the Wald paper, it was assumed that the cis−trans isomerization is around one unique double bond, particularly since such a motion is known to return the system to the ground-state potential surface. During times when the time scale for photophysical measurements improved from nanoseconds and picoseconds to femtoseconds, it was taken for granted that the primary process associated with the first spectral change was due to the same cis−trans isomerization. This led to a paradoxical conclusion (that did not cause widespread concern) that the retinal molecule undergoes full cis−trans

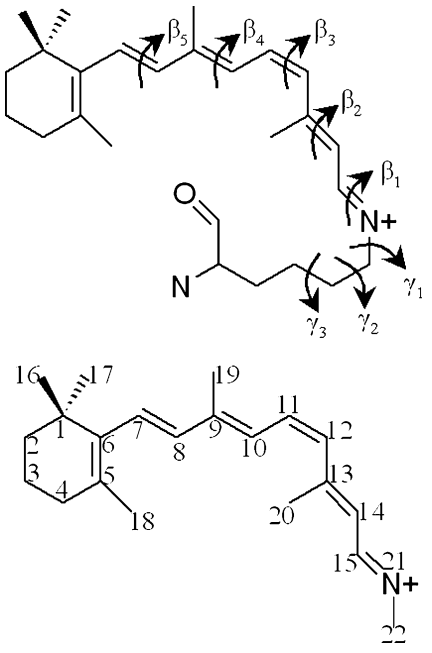

Figure 1. Atom numbering of the retinal chromophore. Torsion angles are represented by β and γ.

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isomerization around one double bond in 200 fs within the protein cavity. Such an isomerization would make some atoms of the molecule move several angstroms in a very short time. We believe that the atomic movements are much less. The theoretical problem is that it has never been shown in an accurate calculation that there is a properly constrained pathway that allows the system to reach the intermediates that are known to exist. All accurate calculations have concerned the simpler pathway where the isomerization is around one double bond.

A cis−trans isomerization of the ordinary type (around one double bond alone) is unlikely to occur rapidly. Even a half cis−trans isomerization ($\beta_3 = 90°$) around one double bond alone cannot be expected to be finished in 200 fs due to the inertia of the atoms in the retinal molecule itself and the protein constraint that blocks large geometrical changes.

A model based on a minimal motion principle (MMP) for the primary reaction path was proposed where retinal remains in the cavity.[11] In preliminary ab initio calculations, which did not include the protein, we found a downhill reaction path to the so-called "funnel" (a funnel may be defined as the region of smallest energy gap between two PESs, i.e., the AC region). In the present paper, the protein pocket will also be included in the study. In addition, we will calculate the energy gap at the avoided crossing. The results hint that the first intermediate in the visual transduction path, photorhodopsin, is the 90° twisted excited state, likely with bathorhodopsin as the ≥90° twisted ground state. The two intermediates are still located in the original cavity, in a strained and energy-rich conformation.

The interplay between retinal and the surrounding protein is a key factor in the photoreaction of rhodopsin. By comparing femtosecond dynamics of retinal in protein and in solution, Kandori et al. concluded that the presence of the protein enhances the S_1 relaxation rate and the cis−trans isomerization quantum yield compared to solution.[12] The unconstrained motion in a solvent leads to a steep downhill reaction path. However, the motion is continuously slowed by collisions with the solvent molecules. Thereby a great amount of the stored energy is released to the solvent, and less remains to complete the cis−trans isomerization in the ground state. The constraint of the protein therefore acts as a "guide" for the retinal molecule on the S_1 PES, saving energy and preventing the system from engaging in an extensive, energy-wasting motion. Borhan et al.[13] have also investigated the effect of the protein on the motion of retinal and concluded by cross-linking experiments that the β-ionone ring does not begin to move out of its original ground-state position until the transition from bathorhodopsin to the next intermediate lumirhodopsin. The mentioned experiments support the idea that retinal undergoes a minimal motion during the S_1 relaxation.

Earlier models that express constrained motion are the bicycle pedal[14] and Hula twist[15] models. In the present paper, we will show, using accurate quantum mechanical methods, that the MMP pathway chosen by us is energetically downhill and ends up at an avoided crossing. Other important works should be mentioned. Hayashi et al.[16] carried out a direct molecular dynamics study with calculated ab initio gradients pertaining to the excited state. Unfortunately, this calculation could only be performed with severe truncation of the part of the retinal molecule that is treated by ab initio methods. Other important ab initio calculations have appeared recently; Bifone et al. also looked for a minimal motion.[17] Two hydrogen atoms were moved into a sort of trans position, after which "cooling" of the system was performed using a molecular dynamics simulation based on the Car−Parinello method. The final, highly distorted trans form was assumed to be the bathorhodopsin intermediate, theoretically storing 22 kcal/mol compared to the experimental value 32 kcal/mol.[18,19] Olivucci et al. introduced general ab initio methods that are used also in our work.[20,21] Sugihara et al. performed accurate calculations on the ground state, with retinal embedded in the protein.[22] On the basis of the above-mentioned Car−Parinello model of Bifone et al.,[17] Schreiber and Buss interpreted the bathochromic shift.[23] Also in the latter case, the retinal molecule had to be truncated. The present study presents accurate ab initio calculations of a reaction path where complete active space self-consistent field (CASSCF)[24] is used to calculate the wave function of the entire retinal chromophore.

The electronic structure of polyenes can be partly understood on the basis of the simple Hückel model,[25] which predicts the shape and the energy order of the π-orbitals quite correctly. From the orbital coefficients of the occupied orbitals, one derives the bond orders that are proportional to the respective bond lengths. This model may be improved by letting the coupling matrix element depend on the bond length.[25] From such a simple model, it follows that the HOMO → LUMO excited state has reversed short and long bonds compared to the ground state in the central part of the molecule. Rotation barriers are therefore smaller around the bonds that are double bonds in the ground state. This qualitatively correct result explains the physics of the system, but for accurate energy surfaces and spectra the Hückel method is of course inadequate. In symmetric polyenes, there is a lower excited state called the 2^1A_g state that is important in the photophysics. For retinal, only the HOMO → LUMO excited state is present at a low energy.

The main challenge of the MMP model is to find a downhill path from the FC point to the AC region. To account for the blocking effect of the protein, the steric repulsion energy imposed on the chromophore by the protein must be included in the S_1 energy. In this paper, we report rigorous quantum chemical calculations on the whole retinal chromophore along an S_1 reaction path in which protein blocking effects are considered using the ONIOM method.[26,27] The assumption is made that the amino acids of the protein do not move during the very fast S_1 relaxation.

II. Theory and Calculations

A. Computational Details. The ONIOM[26,27] method has been used both to optimize the geometry of the ground state and in reaction path calculations. In an ONIOM calculation, different parts of the system, called layers, are modeled by different methods. Ab initio methods are usually used in the high layer whereas methods based on molecular mechanics are used in the low layer. The entire retinal chromophore was included in the high layer whereas the low layer was chosen to include Lys296 (attached to retinal) and the amino acids closest to the β-ionone ring: Gly121, Glu122, Met207, Phe212, Phe261, Trp265, and Ala269. CASSCF was used for the high layer, and the AMBER force field[28] for the low layer.

The geometry of retinal in the ground state was optimized using an active space corresponding to 6 electrons in 6 π-orbitals. During the geometry optimization, the amino acids were kept frozen in their crystallographic positions. The optimized bond lengths and dihedral angles are shown in Table 1.

For the reaction path calculations, state average (SA) CASSCF was used with an active space corresponding to 10 electrons in 10 π-orbitals. The amino acids were kept frozen in every step of the reaction path.

TABLE 1: Calculated Bond Lengths and Torsion Angles for the Ground (Franck−Condon) State Geometry and the AC Geometry of the S_1 State[a]

	bond length (Å)			torsion angle (deg)	
bond	ground state[b]	AC[c]	angle	ground state [b]	AC[d]
C6−C7	1.4930	1.4930	C5−C6−C7−C8	−60.6	−50.6
C7═C8	1.3496	1.3422	C6−C7═C8−C9	179.7	179.7
C8−C9	1.4712	1.4324	C7−C8−C9−C10	−179.4	−179.4
C9═C10	1.3681	1.4570	C8−C9═C10−C11	176.6	−127.3
C10−C11	1.4468	1.3480	C9−C10−C11−C12	176.9	176.9
C11═C12	1.3680	1.4691	C10−C11═C12−C13	−7.8	−90.0
C12−C13	1.4535	1.3852	C11−C12−C13−C14	164.0	164.0
C13═C14	1.3795	1.4172	C12−C13═C14−C15	177.9	−164.1
C14−C15	1.3965	1.3922	C13−C14−C15−N21	173.5	173.5
C15═N21	1.3098	1.3160	C14−C15═N21−C22	179.6	179.6

[a] For reasons of convenience, tosion angles are referred to in the text by the two central atoms. For example, C10−C11═C12−C13 is referred to as the C11═C12 twist angle. [b] Optimized using the ONIOM method. CASSCF was used for the chromophore with 6 electrons in 6 π-orbitals in the active space. [c] Optimized using the algorithm for locating ACs[29] with 6 electrons in 6 π-orbitals in the active space. [d] Obtained as described in section II.B.

To obtain the bond lengths at the AC, the method of Bearpark et al.[29] was used. In this method, the S_1 energy is minimized simultaneously with the energy gap between S_1 and S_0 in the process of locating the AC. The calculations were performed using SA-CASSCF with 6 electrons in 6 π-orbitals in the active space.

The basis set 6-31G has been used throughout this paper in conjunction with CASSCF, and in all calculations the Gaussian 03[30] program package was used.

B. Derivation of the MMP structure at the AC. The basic idea is that the MMP structure at the AC has to involve a twist of β_3 by an angle close to 90° (or −90°).[2−4,20,21,31−33] Conservation of the retinal structure in the original protein cavity can be achieved if nearby bonds are twisted simultaneously with β_3. According to the NEER principle[33] and consistent with the simple Hückel model,[25] only double bonds are twisted in S_1. The NEER principle was found to be reliable in our earlier work.[11]

While β_3 is locked at 90° (or −90°), the nearby bonds are twisted from 0° to 360° in steps of 1° to find a structure, consistent with MMP, at the AC. For a set of trial twist angles at the AC, the geometrical change to the Franck−Condon (FC) point (obtained in the ground-state optimization using ONIOM) is defined as

$$\Delta = \sum_i \sqrt{(x_{\mathrm{FC},i} - x_{\mathrm{AC},i})^2 + (y_{\mathrm{FC},i} - y_{\mathrm{AC},i})^2 + (z_{\mathrm{FC},i} - z_{\mathrm{AC},i})^2} \quad (1)$$

The geometry that gives the lowest value of Δ is chosen. In the summation, only the carbon atoms of the bulkiest groups are included, i.e., those of the β-ionone ring and the methyl groups.

In an earlier paper,[11] we reported on a downhill reaction path in which β_2, β_3, and β_4 were twisted. The AC structure showed a large Δ = 13.4 Å, consistent with a large van der Waals energy. Thus, in the present paper, we have modified the calculation of the AC structure in the following way. β_3 is still locked at −90°. β_2 and β_4 were varied as before. In the ground-state structure, the C6−C7 bond is turned −60° because of steric hindrance. This bond seems to lack π-bond character both in the ground state and the excited state. The corresponding torsion angle was therefore also varied along with a variation of −1° and +1° in C−C−C bond angles of the polyene chain. In the lysine residue, bonded to retinal, the torsion angles γ_1, γ_2, and γ_3 were varied from −10° to 10° in steps of 1°. All of the mentioned internal coordinates were varied in all possible combinations during the search of the minimally distorted AC structure. In this search, Δ was finally minimized to 1.2 Å for β_2, β_3, and β_4 equal to −164.1°, −90°, and −127.3°, respectively. Δ corresponding to the β-ionone ring by itself was as low as 0.5 Å.

TABLE 2: Calculated Energy Gaps between S_1 and S_0 for Different Geometries

	energy gap (kcal/mol)	
method	FC	AC
SA-CASSCF (6,6)[a]		2.80[c]
SA-CASSCF (10,10)[b]	75.64[d]	2.65[d]

[a] SA-CASSCF with 6 electrons in 6 π-orbitals. [b] SA-CASSCF with 10 electrons in 10 π-orbitals. [c] Calculated for the model system of retinal with the β-ionone ring replaced by a hydrogen atom. [d] Calculated for the entire retinal chromophore.

To obtain the bond lengths at the provisional AC structure, the method of Bearpark et al.[29] was used. In this calculation, it was necessary to replace the β-ionone ring by a hydrogen atom. Only the bond lengths were optimized. The bond angles and twist angles calculated by minimizing Δ above were kept frozen during this optimization. The optimized bond lengths and the energy gap between S_1 and S_0 are given in Tables 1 and 2, respectively. The calculated structure at AC was overlaid with the ground-state structure (obtained in the ONIOM calculation) and is shown in Figure 2.

C. Reaction Path Calculations of the Entire Chromophore Including Nearby Amino Acids. Through the use of the bond-length-optimized AC structure in the S_1 state, a reaction path consisting of 20 steps for the entire chromophore (including the β-ionone ring) was created by means of interpolation between FC and AC. In the first 10 steps, the bond lengths of retinal were relaxed followed by relaxation of the twist angles in the last 10 steps. The bond angles were altered in all 20 steps (Supporting Information). There have been prior investigations of rhodopsin with the ONIOM method.[34−36] However, the present work is, to the best of our knowledge, the first attempt to study an S_1 reaction path of the retinal chromophore inside the protein.

The calculated energy of the first excited PESs obtained by means of the ONIOM method is the sum of the SA-CASSCF energy of the chromophore and the van der Waals interaction energy, modeled by a Lennard-Jones potential, between the chromophore and the surrounding amino acids. For steps 10−20 in the reaction path, the torsion angles γ_1, γ_2, and γ_3 were changed. Since the concomitant energy change of a lysine bonded by a Schiff base cannot be calculated using AMBER, the energy change was calculated using the B3LYP functional[37−39]

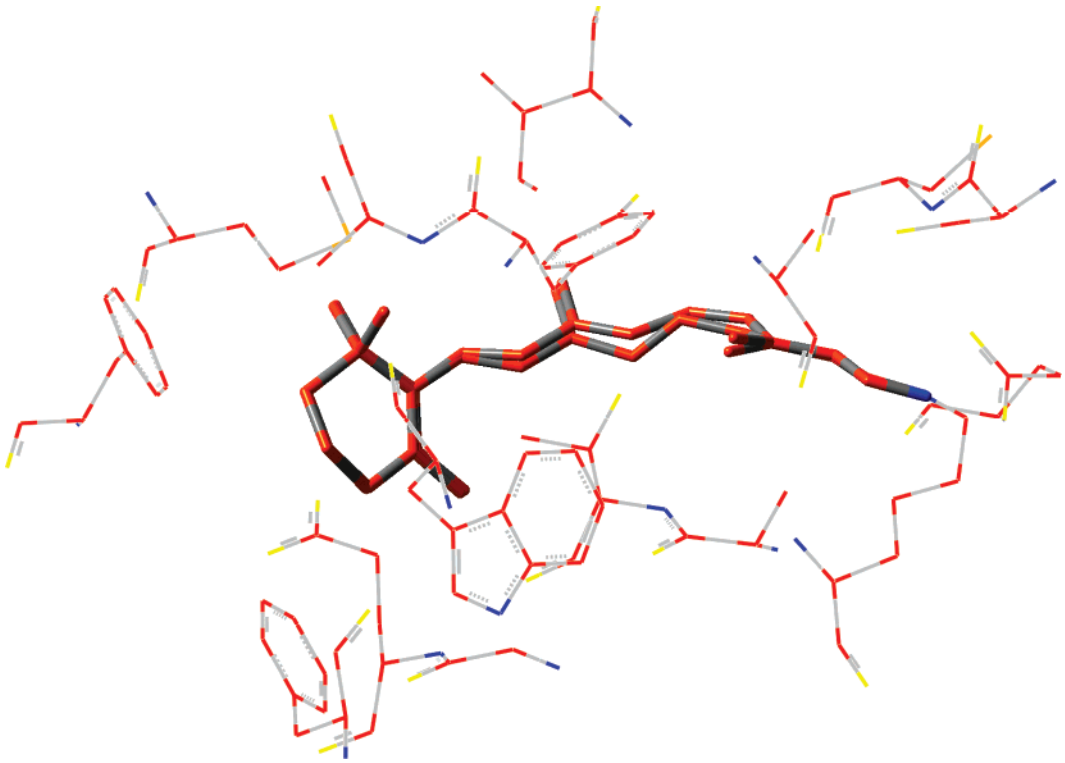

Figure 2. Model of the cavity−chromophore system with the ground-state structure of retinal obtained using ONIOM overlaid with the structure at the AC.

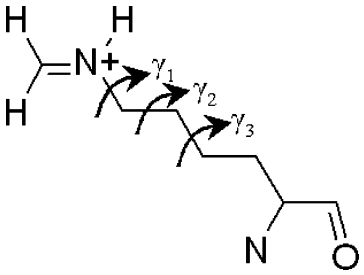

Figure 3. Model system used to calculate the energy for twisting γ_1, γ_2, and γ_3.

with the 6-31G* basis set. For each combination of γ_1, γ_2, and γ_3 along the reaction path, the geometry of the bonded lysine residue model (Figure 3) was reoptimized with the values of γ_1, γ_2, and γ_3 frozen.

The results are displayed in Figure 4. The upper left graph shows the SA-CASSCF energy of the chromophore. The upper right one shows the van der Waals energy whereas the lower left one shows the energy for twisting γ_1, γ_2, and γ_3. Finally, the lower right graph is the sum of the three different types of energy. The amino acids included in the low layer were Gly121, Glu122, Met207, Phe212, Phe261, Trp265, Ala269, and Lys296.

Figure 5 is the same reaction path as in Figure 4 but with additional amino acids close to the retinal molecule included in the low layer of the calculation: Glu113, Ala117, Thr118, Ser186, Cys187, Ile189, Tyr268, and Ala292. The SA-CASSCF energy of the chromophore and the energy for twisting γ_1, γ_2, and γ_3 are the same as in Figure 4 because the chromophore moves in exactly the same way in the two figures, but the van der Waals energy is different due to the increased number of amino acids in the low layer.

D. Relaxation on the Ground-State PES at AC. We finally reoptimized the C−C bond lengths and C−C−C bond angles of the chromophore in the ground state at the torsion angles

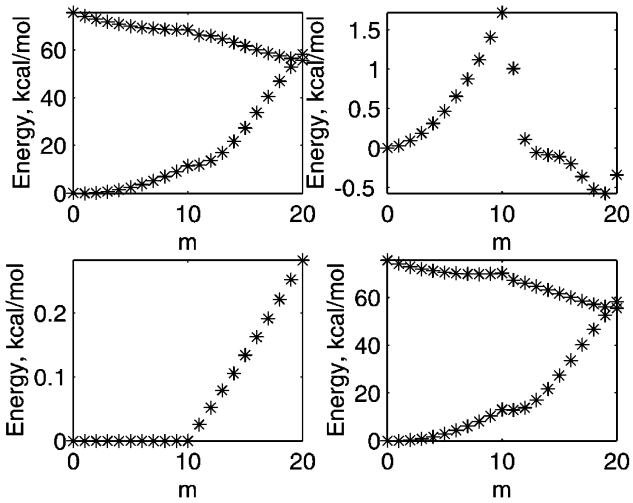

Figure 4. Energy of the reaction path calculated by the ONIOM method including the amino acids Gly121, Glu122, Met207, Phe212, Phe261, Trp265, Ala269, and Lys296. SA-CASSCF energy of the chromophore (upper left), van der Waals energy (upper right), energy for twisting γ_1, γ_2, and γ_3 (lower left), and total energy (lower right). *m* represents steps on the reaction path (Supporting Information).

calculated for the AC. In this constrained geometry, we optimized the ground state of retinal using ONIOM. CASSCF was used for retinal with 6 electrons in 6 π-orbitals. The positions of the amino acids were frozen as before, and the twist angles of the chromophore were also frozen. Hence, only the bond lengths and bond angles of the chromophore were optimized. For the partially optimized structure, a SA-CASSCF (equal weights for S_0 and S_1) single-point calculation was performed with 10 electrons in 10 π-orbitals. The corresponding energy lowering was determined to be 13.7 kcal/mol. For this new geometry, the energy gap between S_0 and S_1 was approximately 19.3 kcal/mol.

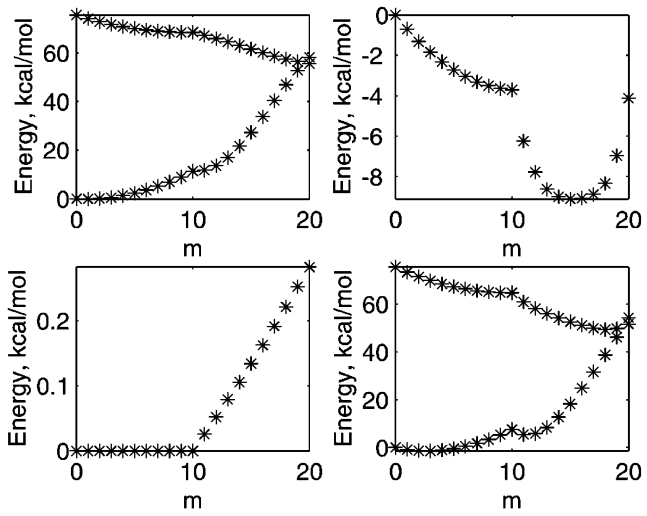

Figure 5. Energy of the reaction path calculated by the ONIOM method including the amino acids Glu113, Ala117, Thr118, Gly121, Glu122, Ser186, Cys187, Ile189, Met207, Phe212, Phe261, Trp265, Tyr268, Ala269, Ala292, and Lys296. SA-CASSCF energy of the chromophore (upper left), van der Waals energy (upper right), energy for twisting γ_1, γ_2, and γ_3 (lower left), and total energy (lower right). *m* represents steps on the reaction path (Supporting Information).

III. Discussion

A. Reaction Path. On the basis of the Hückel method, a dihedral angle close to 90° leads to a decoupling of the two parts of retinal to the left and to the right of the corresponding carbon atoms. Furthermore, the Hückel method yields a crossing between S_0 and S_1 at this angle. We found that the retinal molecule can be essentially maintained in the cavity and meet the conditions of a fast reaction if two other double bonds are twisted apart from C11═C12, but not to perpendicular positions. The major geometrical changes occur in the central part of the molecule when the system moves from the FC point to the AC. C10 moves about 0.7 Å. C20 moves 0.5 Å, and C8, C9, C11, C12, C13, C14, and C19 each move approximately 0.3 Å. The remaining carbon atoms of the polyene chain move less than 0.15 Å. The collective movement of the carbon atoms of the β-ionone ring is about 0.5 Å. Two hydrogen atoms move more than 1 Å, the hydrogen of C10 (1.7 Å) and of C11 (1.4 Å).

The fact that β_2, β_3, and β_4 are twisted in the excited state is consistent with the experiments of Koyama et al.,[40] who showed that the photoproducts of all-trans retinal in different solvents are cis C9═C10, cis C11═C12, and cis C13═C14. This indicates that these bonds are easily twisted in S_1 whereas the C7═C8 and C15═N21 are harder to twist.

The SA-CASSCF PESs of Figures 4 and 5 are characteristic of a fast photoreaction with a downhill reaction path from the FC to the AC region. The simple interpolation method used is most likely the reason behind the small increase in energy (1.5 kcal/mol) at the endpoint of the reaction path.

In Figures 4 and 5, the S_1 energy difference of the chromophore between the FC point and the AC is approximately 17.5 kcal/mol. Because the reaction path is constrained to involve minimal geometrical changes, this energy difference is smaller than obtained in a calculation performed without any constraints where only the C11═C12 bond is twisted, i.e., in a vacuum. Thus, by constraint of the reaction path, potential energy is stored for later use.

A smaller energy difference between the FC point and the AC means, in a classical sense, that the forces acting on the nuclei are smaller, which could slow the reaction. However, since the geometrical changes are minimal along the reaction path, a smaller distance for the nuclei to move compensates the smaller forces acting on the nuclei and the high reaction rate is thereby preserved.

B. Van der Waals Energy. The amino acids closest to the retinal chromophore, i.e., those constituting the immediate steric hindrance against large conformational changes, were included in the calculations. The van der Waals energy profiles in Figures 4 and 5 are very different. What can be said is that the interaction energy of the β-ionone ring and the closest amino acids is relatively constant, meaning that when additional amino acids are included in the calculation (Figure 5) the energy fluctuations have larger amplitudes. It should be noted that the assumption that the amino acids do not move at all during the relaxation in the first excited state is very restrictive. For example, methyl groups on the amino acids can rotate to compensate for an increase in van der Waals energy. With that in mind, we believe that the results of Figures 4 and 5 show a plausible reaction path for the chromophore inside the protein.

C. Rate of Passing the Avoided Crossing. The low fluorescence quantum yield[41] (on the order of 10^{-5}) and the time constant of 200 fs[7−9] are characteristic for excited-state processes where the system rapidly leaves the Franck−Condon (FC) region following a downhill reaction path. A conical intersection or an AC is reached that offers a decay channel from S_1 to S_0 by internal conversion. In the vicinity of an AC, the energy gap (ΔE) between the PESs is usually small, making the transition between electronic states efficient according to the Landau−Zener approximation[42,43]

$$P = \exp\left(-\frac{\pi^2 \Delta E^2}{h\upsilon \Delta S}\right) \tag{2}$$

where ΔS is the difference in surface slopes in the region of the AC and υ is the nuclear motion velocity in the direction of the reaction coordinate. The energy gap calculated here is about 0.1 eV. Through the use of the calculated ΔE and slopes ΔS and a reasonable value for the speed of the system, the S_1 PES leads to a very small probability for passing the "funnel". However, using eq 2 for actual calculations is almost impossible, particularly since accurate numbers for ΔE cannot be obtained. De Vico et al. calculated a value $\Delta E = 0.025$ eV for a model system of retinal.[21] Using this 4 times smaller value instead of our calculated number ($\Delta E = 0.1$ eV) in the calculation of P (eq 2) leads to a much larger $P = 0.01$. In any case, transition from S_1 to S_0 in every attempt ($P = 1$), as has been suggested, does not seem reasonable. We therefore have to assume that the system spends some time on the S_1 surface before it falls down to S_0.

D. Photorhodopsin and Bathorhodopsin. The most important result of this paper is that it has been established that there is a downhill reaction pathway to the AC region that permits the retinal molecule to remain in the cavity. Earlier ab initio results have been obtained for a reaction path where only the C11═C12 bond is twisted on the way to a typical cis−trans-isomerized geometry. Since a cis−trans-isomerized (in the accepted meaning of this word) molecule cannot fit into the protein cavity after it has started to turn, such a pathway is only relevant in free space, in a solvent, or possibly in a protein if sufficient time is available to allow the protein to respond to the torsion of the retinal molecule. The energy lowering is, of course, greater for the unconstrained pathway than for the constrained one in the protein. Consequently, less stored energy remains in the unconstrained case for the final steps compared to the case in a constrained pathway.

It is not clear from earlier work whether there are two distinct intermediates, photorhodopsin and bathorhodopsin, or only the latter. Photorhodopsin has been assigned as the first product and identified as a 90° twisted excited-state species.[7] In other works, bathorhodopsin, absorbing at 543 nm,[44] has been assigned as the first product and identified as a ground-state species.[8,9] The promptly appearing photoproduct absorbs at a longer wavelength, close to 600 nm.[9] The transient absorption shifts from 600 to approximately 560 nm already during 200 fs but is still shifted to the blue during the next few picoseconds.[9] This indicates the presence of more than one intermediate. Most authors hypothesize that absorption in the 560 to 600 nm region at very short times (35−200 fs) is due to $S_1 \rightarrow S_n$ absorption and that retinal crosses over to the S_0 surface after 200 fs. It is also suggested that later (>200 fs) changes in the spectrum are due to $S_0 \rightarrow S_1$ absorption. We found, however, when the bond lengths were reoptimized in S_0 at AC, that the latter absorption is less than 20 kcal/mol (19.3). This value is likely to be overestimated rather than underestimated in our calculation since excitation energies tend to be overestimated in general using CASSCF. The $S_0 \rightarrow S_1$ excitation energy, after retinal has returned to the ground state, is thus too small to correspond to the 500−600 nm absorption region. It appears more likely that absorption from both photo- and bathorhodopsin is with S_n as the final state of the absorption. Furthermore, our results indicate that retinal spends some time at AC and that absorption at about 200 fs is due to an $S_1 \rightarrow S_n$ transition ($S_0 \rightarrow S_n$ after return to the ground state). In the case of bacteriorhodopsin, it has been proposed on experimental grounds that the absorption at short times is due to $S_1 \rightarrow S_n$ transition at the twisted intermediate at AC (J-intermediate).[45] For rhodopsin, the same mechanism has been suggested on the basis of semiempirical dynamics calculations.[46] The latter authors also reach the conclusion that the excited-state absorption at AC is very similar to the absorption of bathorhodopsin in wavelength and oscillator strength.

With the available results, we cannot decide how much larger than 90° the C11═C12 torsion angle really is in bathorhodopsin compared to the AC structure. In principle, it is possible to use our ground-state structure to begin a molecular dynamics simulation using the ground-state PES.

It is reasonable to propose that the first event (after 35 fs)[9] is due to changed C−C bond lengths in the excited state. This type of relaxation is believed to occur during the first period of the C−C vibrations, and this fits very well with the experimentally measured 35 fs. The torsional angles have not yet had time to respond to the excitation, i.e., the system has not yet left the FC region.

Very little detail is known at the present time regarding the structure of bathorhodopsin. It is reasonable to believe that it still fits into the protein cavity. Probably, it can be said at the same time to have a highly twisted all-trans geometry,[47−49] although the cavity seems well-shaped to fit a cis form of a linear molecule. Through calorimetry, it was found that bathorhodopsin stores 32 kcal/mol of strain energy,[18,19] which is in agreement with a twisted chromophore in the ground state. To calculate this number, we optimized bond lengths and bond angles in the ground state and gained as much as 13.7 kcal/mol. In summary, our calculations lead to a S_1 state energy 75.6 kcal/mol above the ground state at FC. Relaxation on the S_1 PES to the AC region is calculated to 17.5 kcal/mol. The energy gap between S_0 and S_1 is found to be 2.6 kcal/mol. Since the relaxation in the ground state at AC is 13.7 kcal/mol, there remains $75.6 - (17.5 + 2.6 + 13.7) = 41.8$ kcal/mol. Since this value is about 10 kcal/mol larger than 32 kcal/mol, one would surmise that further relaxation of the dihedral angles within the cavity would lead to the final energy lowering of 10 kcal/mol. However, this would be unreasonable. The greatest error in ab initio calculations is normally in excitation energies. Better estimates can be obtained using the experimental value for the $S_0 \rightarrow S_1$ energy (56.1 kcal/mol). This leads to a final stored strain energy of only 22.4 kcal/mol. To obtain 32 kcal/mol, we obviously need to scale down the calculated relaxation energies as well. The reason for this is likely too large of a calculated difference between C−C bond lengths in the ground and excited states. This is consistent with experience from retinal and polyenes when different methods are compared.[50,51]

If only calculated numbers are used, then we calculate that 55.4% of the energy of the absorbed photon is stored. If an experimental value is used for the excitation energy (56.1 kcal/mol), then we calculate that 40% is stored. In these numbers, further relaxation of the dihedral angles to some strained "trans form" of retinal is not included. The experimental value is $32/56.1 = 57\%$. Clearly, our calculated relaxations due to bond lengths and bond angles are overestimated, possibly also the energy decrease of S_1 from FC to AC.

At the calculated AC structure, we also calculated a preliminary value of the $S_0 \rightarrow S_1$ excitation energy (19.3 kcal/mol = 6750 cm^{-1}). This is too small to justify the $S_0 \rightarrow S_1$ assignment of the absorption at approximately 560 nm as has been done in a number of papers. If full relaxation is permitted to the trans form, regardless of protein strain, then we obtain an excitation energy close to the original from the cis form. In that case, the stored energy would be absent.

Our hypothesis regarding photorhodopsin is consistent with the original interpretation by Callender et al.[7] that the prompt absorption is due to excited-state absorption. It also agrees reasonably well with the model by Mathies et al.[8,9] in which the photoproduct is on the ground-state PES. However, the picture given by Mathies et al. is that the photoproduct absorption is from the ground-state PES to the first excited-state PES.

Mathies et al. claim that the molecule after it has reached the AC region immediately continues to the ground-state PES. There appears to be some difficulties with this model, however. The most important one is that, in any case, the energy loss on the S_1 surface cannot be much less than that calculated in our minimal motion model, i.e., 17.5 kcal/mol. At the AC, the excited-state energy is down to $56.1 - 17.5 = 38.6$ kcal/mol. Only approximately 7 kcal/mol remains for further relaxation to be consistent with the 32 kcal/mol stored energy. This means that the S_1 PES has to rise very steeply to obtain an excitation energy at 52.7 kcal/mol, corresponding to 543 nm. From published pictures, this steep increase of S_1 to the right of AC has been "anticipated", but unfortunately, there is no experimental evidence to support such a sharp increase. We are therefore forced to conclude that the electronic transition causing the 543 nm excitation wavelength of bathorhodopsin is not due to the $S_0 \rightarrow S_1$.

IV. Conclusions

It has been assumed and accepted for a long time that the retinal molecule performs a cis−trans isomerization as a primary reaction after excitation by light and thereby initiates a series of events that produce the proper signal to the visual nerve. About 15 years ago, it was found that the primary process of vision required only approximately 200 fs to be completed.[7−9] We have shown that a downhill reaction path exists that can explain the photophysical data while still observing the steric

constraints of the protein cavity. A cis−trans isomerization around one single double bond leads to large displacement of the nuclei, which is inconsistent with the short time measured for the primary process. It cannot be excluded that torsion around the C11−C12 bond in its batho form is closer to 180° than to 0°, but by and large the batho intermediate has the same shape as in the ground state, which perfectly fits the protein cavity.

Due to the constrained motion in the protein, potential energy is stored in the chromophore for later use in the isomerization process. The short reaction path leads to a fast primary reaction. In solution, however, the minimum free-energy path is very likely along the C11−C12 torsion angle. Most atoms of the retinal molecule then have to move a considerable distance, and this leads to a slower primary process.

What is the nature of the bathorhodopsin intermediate? In agreement with most other researchers in the field, we believe that this intermediate is on the ground-state PES, but the existing experimental evidence on the structure seems to converge to a very constrained form that is still located in the protein pocket. This is consistent with our results. The problem to decide whether the C11−C12 torsion angle is still 90° or closer to 180° might be solved in the future using computational methods or low-temperature crystallographic methods. Some mechanism must be found that leads to the trans form in 67% of the cases.[52] It is also desirable to determine exactly the character of the excited states involved in the photochemical measurements.

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Supporting Information Available: Details of the internal coordinates interpolation method, AMBER force field, and ONIOM calculations. This material is available free of charge via the Internet at http://pubs.acs.org.

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