Quantum Chemistry: Molecular Dynamics Study of the Dark-Adaptation Process in Bacteriorhodopsin

Ilya Logunov and Klaus Schulten*

Contribution from the Beckman Institute and Departments of Chemistry and Physics, University of Illinois at Urbana—Champaign, 405 North Matthews Avenue, Urbana, Illinois 61801

Received September 7, 1995. Revised Manuscript Received July 29, 1996

Abstract: Molecular dynamics simulations and quantum chemistry calculations have been combined to describe the dark adaptation in bacteriorhodopsin (bR). The process involves the reversible thermally activated transformation of retinal from an all-trans to a 13-cis,15-syn configuration. The potential surface governing the thermal isomerization of retinal around two (13→14, 15→N) double bonds has been determined for representative protein configurations taken from molecular dynamics trajectories. CASSCF(8,8)/6-31G level \textit{ab initio} calculations (within Gaussian94) were carried out for this purpose. The charge distributions of all atoms in the protein are represented by partial point charges and explicitly included in the electronic Hamiltonian. Placement of retinal into bR is found to reduce the calculated isomerization barrier. Thermal fluctuations of the protein lead to a further effective reduction of this barrier. The isomerization process is shown to be catalyzed by the protonation of an aspartic acid (Asp85) side group of bacteriorhodopsin.

Introduction

Bacteriorhodopsin (bR) is a protein composed of seven \( \alpha \)-helices which spans the purple membrane of \textit{Halobacterium halobium} and which functions as a light-driven proton pump. It is a member of the retinal protein family, which encompasses proteins with a retinal chromophore bound within the protein interior via a protonated Schiff base linkage to a lysine side chain. Figure 1a shows the chemical structure of retinal and its conventional numbering scheme. The retinal isomer composition in bR is 66% 13-cis and 34% all-trans in the dark-adapted form of the pigment (DA), a ratio which is altered in mutants of bR and in bacterial rhodopsins of other species. The two isomers, denoted bR\(_{548}\) (13-cis) and bR\(_{568}\) (all-trans), absorb at 548 and 568 nm, respectively. Retinal in the bR\(_{568}\) pigment exists in an all-trans, 15-anti configuration and in the bR\(_{548}\) pigment in an 13-cis,15-syn configuration as suggested originally on the basis of semi-empirical quantum chemical calculations and observed by Resonance Raman experiments.

Both the bR\(_{568}\) and the bR\(_{548}\) isomers undergo a characteristic photocycle (see Figure 2) initialized in both cases by a photoisomerization involving rotation around the 13→14 double bond. However, proton pumping is restricted to the photocycle of the bR\(_{548}\) pigment in an 13-cis photoisomerization involving rotation around the 13→14 bond. The photocycle of bR\(_{548}\) does not involve vectorial proton translocation. The photocycle of native bR\(_{548}\) leaks into a mixture of bR\(_{568}\)/bR\(_{548}\) eventually becomes a pure bR\(_{568}\) pigment.

Figure 1. (a) Numbering scheme of the retinal chromophore bound via a protonated Schiff base linkage to a lysine side chain of bacteriorhodopsin; (b) retinal analogue employed in our quantum chemical calculations.

which, accordingly, is called the light-adapted form. The photocycles of bacteriorhodopsin are discussed in detail in recent reviews.

A widely accepted model for the three-dimensional structure of bR has been provided by Henderson and co-workers using electron microscopy at low temperature together with bR’s amino acid sequence. The observations resulted in a structure for the membrane-spanning helical portion of bR at a resolution of 10 Å perpendicular to the membrane. This structure provided an opportunity to explore, by means of molecular dynamics trajectories.

bacteriorhodopsin, processes like photoinduced and thermal isomerization of in situ retinal, as well as proton transfer processes (see Figure 2) require potential surfaces which need to be provided by quantum chemical methods. Transformations important for the function of bacteriorhodopsin, which are beyond the realm of molecular dynamics simulations based on standard force fields, involve the thermally activated isomerization of retinal around one or two of its double bonds. This includes the conversion of 13-cis-retinal to its all-trans isomeric state during the later stages of the bR proton pump cycle and includes also the process of dark-adaptation in bR. An understanding of these processes requires quantum chemical descriptions of the respective ground state potential surfaces of in situ retinal. Due to a lack of an atomic level protein structure, only crude potential energy surfaces were applied in studies of the isomerization of in situ retinal until recently.\(^{25-27}\) The structure of Henderson \textit{et al.}\(^ {14}\) and its refinement by means of molecular dynamics simulations\(^ {17-19,23,28}\) allow one now to explore the electronic properties and chemical transformations of retinal in a model-free environment.\(^ {29}\)

Major challenges arise for \textit{ab initio} electronic structure calculations of in situ retinal. A first challenge originates from the strongly correlated nature of the conjugated $\pi$-electron system of retinal. Retinal belongs to the family of polyene dyes, which are well-known for their characteristic structure of alternating single and double $\pi$-electron bonds. Several research groups have studied the electronic structure and potential surfaces of conjugated polyene systems.\(^ {26,30-37}\) A distinct feature of the electronic structure of a conjugated polyene molecule is the existence of near-degenerate excited states and crossings between different potential surfaces for the twisted geometries of a polyene chain.\(^ {38-42}\) The most efficient way of handling near-degenerate states in quantum chemical calculations is through a multiconfigurational (MCSCF) treatment.\(^ {43-46}\) Electronic correlation effects can be taken into account further by performing configuration interaction (CI) calculations.\(^ {47-50}\) However, the use of CI calculations on top of an \textit{ab initio} MCSCF treatment is computationally prohibitive for almost any

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{fig2.png}
\caption{Photocycles of bacteriorhodopsin. Shown are the bR\textsubscript{568} and bR\textsubscript{548} photocycles. Superscripts on intermediates indicate measured absorption maxima of these states. Isomerization configurations of retinal in every state are indicated in brackets. Steps involving photoinduced or thermal isomerization of retinal, as well as proton transfer reactions, are marked accordingly.}
\end{figure}


length of a conjugated polyene. An alternative and very promising approach is the implementation of multireference perturbation methods, such as CASPT2.51,52

Another challenge arises from the need to account for the protein environment when studying the electronic structure of retinal in bR. Protein side groups in the vicinity of the retinal Schiff base strongly affect the bR absorption spectrum as shown through mutation studies.53 These side groups affect also drastically the rates of both thermal54 and photodissociation55,56 in bR. The experiments imply that the protein environment plays a crucial role in determining the physicochemical properties of retinal in situ.

The problem of the proper representation of the environment in electronic structure calculations of a molecule in situ is complex. Initially,7,58 a combination of in vacuo ab initio quantum chemical and classical solution energy calculations were carried out. Later, various techniques were developed which permit the self-consistent ab initio treatment of a molecule embedded in a dielectric continuum.59–61 At present, there exist a number of ab initio as well as semiempirical techniques which try to account for the realistic atomic structure and charge distribution of the environment via explicit incorporation of protein (or solution) point charges in the electronic Hamiltonian.62–69

The present study of the dark-adaptation process in bacteriorhodopsin combines molecular dynamics and ab initio quantum chemistry calculations. Dark-adaptation involves a reversible thermally activated transition between the bRsa and bRss forms which, at room temperature, occurs with a halftime of about 1 h. Dark-adaptation is believed to proceed through the coisomerization around the 13–14 and 15–N retinal double bonds (bicycle-pedal motion). This mechanism is supported by both molecular dynamics studies of the retinal binding site in bR,6 semiempirical calculations of the retinal ground state potential surface in vacuo,7 as well as Resonance Raman measurements.3,4 In the present work we perform in situ ab initio calculations of the retinal potential surface which governs dark-adaptation of bacteriorhodopsin and estimate the overall rate of dark-adaptation. We have also recently completed a combined quantum–MD study of the spectrum of bR.70

Methods

Molecular dynamics (MD) and in situ quantum chemistry calculations were performed independently of each other. All structures were generated by the MD method which utilizes empirical force fields. Z-matrices for the retinal molecule employed in ab initio calculations were constructed on the basis of retinal Cartesian coordinates obtained from snapshots of MD trajectories. Coordinates of all the protein charges (which were explicitly included in the electronic Hamiltonian of mol) were taken from the same snapshots. It should be emphasized that in MD simulations, motions of the retinal molecule itself were governed by the empirical force fields (see below) and not described by a de novo evaluated potential surface.

Molecular Dynamics Simulations. Molecular dynamics simulations reported here are based on the refined and equilibrated structure of bR reported in Humphrey et al.17 Following Humphrey et al.,17 the present description involves explicit hydrogens, includes sixteen water molecules placed and equilibrated within the protein interior and assigns standard protonation states to all side groups, except to Asp-96 and Asp-115, which are assumed to be protonated.19,71 The water molecules are modeled using TIP3P parameters.72 The program X-PLOR73 with the CHARMM force field74 was used for all simulations. bR was modeled in vacuum at a temperature of 300 K. A cut-off distance of 12 Å and a dielectric constant of ε = 30 were used for the evaluation of Coulomb forces. All simulations used the standard X-PLOR protein topology file topallh22x.pro and parameter file parallh22x.pro.

Partial charges of retinal atoms, employed in our MD simulations, were determined with Gaussian9275 using a Mulliken population analysis at the MP2/6-31G level. (We favored Mulliken charges to the alternative ESP-based charges,76–79 since the distribution of retinal charges was not used only in MD simulations, but also as a tool to study effects of fluctuating protein environment on the retinal electronic structure.) The retinal topology and parameters for the equilibrium (bRsa) configuration are the same as those in Humphrey et al.,17 with the exception of the dihedral angle rotations for the “single” and “double” bonds, which were modified to enhance the planarity of retinal and, thus, assure better convergence of the employed CASSCF quantum chemical calculations. The increased torsional rigidity of retinal for its single bonds is justified on account of the fact that double bond rotations, i.e., around the 13–14 and 15–N bond, increase the double bond character of the single bond, rendering torsional barriers higher. This effect was originally demonstrated by semiempirical MINDO3/MNDO calculations3 and by our ab initio calculations at the CASSCF-(8,8) level. Non-additivity of the retinal torsional potentials makes a parametrization of the retinal ground state potential surface difficult and cannot be captured well by empirical force fields as employed in MD simulations.

Quantum Chemical Calculations. The quantum chemistry packages Gaussian92/9475 were employed to study the electronic structure and potential surface of in situ retinal. Since a treatment of the complete retinal is computationally prohibitive, calculations have been performed on a retinal analog (Figure 1b) with the β-ionone ring and the C-9

methyl groups removed. CASSCF(8,8)/6-31G level ab initio calculations have been carried out. Molecular orbitals obtained from the unrestricted Hartree–Fock SCF calculations have been used as an initial guess in the CASSCF calculations. The four HOMO’s and four LUMO’s of retinal displaying a strong \( \pi \)-character have been included in a complete active space (CAS) description. The convergence criterium of \( 10^{-4} \) hartree was used in all the CASSCF calculations.

The protein environment was represented through explicit point charges in the electronic Hamiltonian using the CHARGE statement within Gaussian.

Computation of the Isomerization Potential Surface. In situ isomerization of retinal has been modeled by following a predefined reaction coordinate. Retinal was gradually rotated around the 13–14 and 15–N bonds by altering the respective dihedral angles simultaneously, i.e., bicycle peddle style, in steps of 5° and constraining these values, permitting the motions of all remaining retinal degrees of freedom to minimize the employed empirical potentials. All protein degrees of freedom were minimized according to the empirical CHARMM force field. In calculating the \( \text{in vacuo} \) potential surface of retinal, the same sequence of retinal conformations as in the \text{in situ} case had been chosen except that charges of the protein environment were not taken into account. Multiple isomerization barriers were calculated by determining the energy difference between the conformations in which retinal is co-rotated around the 13–14 and 15–N bonds by 90° and 0°, respectively. All calculations of the \text{in situ} potential surfaces and isomerization barriers were computed for two different protonation states of Asp-85, since it has been suggested that the protonation state of this residue controls the rate of dark-adaptation.54

Results and Discussion

The refined structure of bacteriorhodopsin17 was equilibrated during 100 ps using the CHARMM22 force field and new retinal parameters depicted in Methods. Another 10 ps of molecular dynamics simulations were performed to generate protein structures utilized in the calculations of potential surfaces and the rate of dark-adaptation. We first describe briefly structural and dynamical properties of the bR binding site and then consider energetics and kinetics of the dark-adaptation process.

Atomic Structure of bR and Its Dynamical Properties. A stereo- view of the retinal Schiff base region is shown in Figure 3a. The figure shows the relative position of active groups in the binding site, as well as the positions and orientations of water molecules in the region. The counterion complex of the positively charged protonated retinal Schiff base is composed of two negatively charged aspartic acids, Asp-85 and Asp-212, a positively charged Arg-82, and a few neutral water molecules. One of the water molecules (\( w_1 \)) plays the role of the primary Schiff base contact, forming a hydrogen bond with the Schiff base NH group.

Isomerization barriers of retinal are influenced by the arrangement of the surrounding side groups. All such groups, as well as retinal itself, are in thermal motion, which should play a significant role for the properties of \text{in situ} retinal. Shown in Figures 4a,b are time-dependent conformational properties. Figure 4a presents the motion of the 13–14 and 15–N retinal double bonds. The dihedral angles of these bonds fluctuate within a range of 180° in a strongly coherent fashion. The strong coherence of the torsions around the 13–14 and 15–N bonds shows that the protein can accommodate co-rotation around the 13–14 and 15–N retinal bonds and supports the notion that dark-adaptation occurs via a “bicycle peddle” type motion. Figure 4b shows the time dependence of the distance between the Schiff base nitrogen and the C\(_\gamma\) of Asp-85 and Asp-212. Both distances fluctuate within approximately 1.5 Å, which implies that Coulomb interactions between retinal and the rest of the protein are properly accounted for only after exhaustive sampling.

Time Dependence of the Isomerization Barriers. The dark-adaptation isomerization barriers have been determined for 25 snapshot structures taken every 2 ps along a 50 ps molecular dynamics trajectory. An isomerization barrier for each structure has been computed as described in the Methods section. The observed time dependence of the isomerization barriers in case of deprotonated and protonated Asp-85 is shown in Figures 5a and 5b, respectively. It is seen that the
barrier height values fluctuate significantly, ranging from 20 to 70 kcal/mol. The majority of points lie between 30 and 60 kcal/mol. For the deprotonated state of Asp-85, the average isomerization barrier is 51 kcal/mol, while for the protonated state the average barrier height is 43 kcal/mol. It can also be noticed that the difference between \textit{in situ} and \textit{in vacuo} barriers is not very significant, varying in sign and magnitude, and rarely exceeding 10 kcal/mol. Thus, one should conclude that the barrier height is controlled mainly by the internal geometry of retinal.

The internal degrees of freedom of retinal, which undergo large changes due to the thermal fluctuations and could affect considerably isomerization barriers, are the torsions around retinal’s single and double bonds. The time dependence of retinal’s dihedral energy is presented in Figure 6a for both protonation states of Asp-85. It is seen that dihedral energy fluctuations constitute about 6 kcal/mol which is approximately 30\% of the average value of retinal’s dihedral energy. It should be noticed that the average values of retinal’s dihedral energy, in case of deprotonated and protonated states of Asp-85, are different, measuring respectively 19 and 16 kcal/mol. The lower average value of the dihedral energy for deprotonated Asp-85 correlates well with the lower average value of the dark-adaptation isomerization barrier. Thus, one can conclude that a higher degree of retinal twist inhibits, on average, rotation around the 13–14 and 15–N double bonds.

\textbf{Potential Surface for Dark-Adaptation}. We will first consider the character of the potential surface governing dark-adaptation, and later discuss how fluctuations of the isomerization barrier control the rate of the dark-adaptation process. Given in Figure 7 are two sample surfaces, one for protonated and one for deprotonated Asp-85. Respective structures of the BR binding site are shown in Figures 3a,b. These two particular structures are chosen to demonstrate possible effects of retinal torsions and of the protein environment on the nature of the dark-adaptation potential surface.

The \textit{in vacuo} potential curve shown in Figure 7b is much flatter than the one shown in Figure 7a and displays the isomerization barrier which is 18 kcal/mol lower. This difference is due to the different conformations assumed by retinal in the binding sites containing protonated and deprotonated Asp-85. The effect can be understood in terms of the strongly correlated nature of the π-electron system of retinal: rotations around particular bonds are strongly coupled to rotations around the other bonds and, thus, to the overall conformation of retinal. Distributions of dihedral angles along retinal’s backbone for the structure presented in Figure 3 are shown in Figure 6b. No specific quantitative relationships between the distribution of dihedral angles along retinal and the values of isomerization barriers can be drawn. However, the general tendency for the more twisted retinal to have larger isomerization barrier is confirmed.

The placement of retinal inside the protein in one case (Figure 7a) increases and in the other case (Figure 7b) reduces the isomerization barrier governing the thermal isomerization of retinal around two (13–14 and 15–N) double bonds, by about 10 kcal/mol with respect to the \textit{in vacuo} calculations. The question arises whether this reduction can be explained on the basis of first-order perturbation energy invoking chromophore–protein electrostatic interactions. Figures 7c,d show the effect of Coulomb interactions at the retinal binding site on the isomerization potential surface computed exactly and in the first-order perturbation theory approximation.

We denote by \(\Delta E_{q}\) the sum of electrostatic interactions between retinal and the apoprotein evaluated in first-order perturbation theory using the conventional MD point charge model. We denote by \(\Delta E_{q}\) the difference between \textit{in situ} and \textit{in vacuo} potential surfaces evaluated quantum chemically, e.g., as shown in Figures 7c,d; \(\Delta E_{q}\) includes the polarizing effects of the protein environment. Unlike \(\Delta E_{q}\), the contribution \(\Delta E_{q}\) appears to have a negligibly small effect on the isomerization process. It can be concluded that the standard MD force field, which neglects changes of retinal’s electronic structure induced by the protein environment, fails to represent the potential surface of \textit{in situ} retinal.

The effect described by \(\Delta E_{q}\) can be rationalized as being due to the polarization of retinal’s electronic system by the counterion complex of the BR binding site. A factor which affects significantly retinal’s electronic structure is hydrogen bonding to the Schiff base NH group.

\textbf{Figure 4.} (a) Time dependence of the dihedral angles of the 13–14 and 15–N bonds. (b) Time dependence of the distance between the Schiff base nitrogen and the \(C_{\alpha}\) carbons of Asp-85 and Asp-212. (c) Autocorrelation function of the dihedral angles of the 13–14 and 15–N bonds. (d) Autocorrelation function of the distance between the Schiff base nitrogen and the \(C_{\alpha}\) carbons of Asp-85 and Asp-212.
that a water molecule is engaged in such bonding (see text).

In our bR structure, a water molecule is engaged in such bonding (see Figure 3). A stronger hydrogen bonding to the retinal Schiff base increases electronic density in the C=N bond and should increase the isomerization barrier. This is confirmed by the two cases shown in Figures 3 and 7. In the case of deprotonated Asp-85, the strong hydrogen bonding to the Schiff base (the distance between the Schiff base hydrogen and oxygen of water w1 measures 1.8 Å) increases the isomerization barrier by 9 kcal/mol. In the case of protonated Asp-85, the hydrogen bonding to the Schiff base is weak (the distance between the Schiff base hydrogen and oxygen of water w2 measures 2.2 Å), and the isomerization barrier in situ is 10 kcal/mol lower than that in vacuo.

Rate of the Dark-Adaptation Process. We have demonstrated that the potential barrier for dark-adaptation depends on the momentary structural rearrangements within the bR binding site. To test this hypothesis that the dark-adaptation process in bR is catalyzed by protonation of the Asp-85 side group (see Figure 8), we evaluated isomerization rates for both unprotonated and protonated states of Asp-85. Isomerization barriers were computed for 25 different structures for the two protonation states of Asp-85 (see Figure 5). We determined $E_{\text{iso}} = 29$ kcal/mol for unprotonated Asp-85 and $E_{\text{eff}} = 22$ kcal/mol for protonated Asp-85, i.e., protonation of Asp-85 lowers the effective barrier of dark-adaptation by 7 kcal/mol. This effect of Asp-85 protonation implies an increase of the reaction rate by four orders of magnitude. Our calculations are in a qualitative agreement with the experimental observation that transition from high pH (Asp-85 unprotonated) to low pH (Asp-85 protonated) is associated with a 1000-fold increase in the rate of dark-adaptation.

Catalysis via Protonation of Asp-85. To test the hypothesis that the dark-adaptation process in bR is catalyzed by protonation of the Asp-85 side group (see Figure 8), we evaluated isomerization rates for both unprotonated and protonated states of Asp-85. Isomerization barriers were computed for 25 different structures for the two protonation states of Asp-85 (see Figure 5). We determined $E_{\text{iso}} = 29$ kcal/mol for unprotonated Asp-85 and $E_{\text{eff}} = 22$ kcal/mol for protonated Asp-85, i.e., protonation of Asp-85 lowers the effective barrier of dark-adaptation by 7 kcal/mol. This effect of Asp-85 protonation implies an increase of the reaction rate by four orders of magnitude. Our calculations are in a qualitative agreement with the experimental observation that transition from high pH (Asp-85 unprotonated) to low pH (Asp-85 protonated) is associated with a 1000-fold increase in the rate of dark-adaptation.

Matching this expression to the conventional Arrhenius expression

$$k = k_0 \exp(-E_{\text{eff}}/kT)$$

yields

$$E_{\text{eff}} = -kT \ln\left(\frac{1}{\sum_{i=1}^{N} \exp(-E_i/kT)}\right)$$

Absolute Rate of Dark-Adaptation. In order to determine the absolute rate of dark-adaptation, one must obtain an estimate for the pre-exponential factor $k_0$. We define the characteristic frequency $k_0$ as the inverse of the decay time $t_0$ of the autocorrelation function for the torsional motions around the 13–14 and 15–N double bonds of retinal. The autocorrelation function is depicted in Figure 4c. From Figure 4c follows $t_0 \approx 2 \times 10^{-14}$ s. Thus, we obtain $k_0 \approx 5 \times 10^{13}$ s$^{-1}$. According to eq 3 and using $E_{\text{eff}} \approx 22$ kcal/mol (see above), the rate of dark-adaptation, in the case of protonated Asp-85, is then $k_{\text{prot}} = 6 \times 10^{-3}$ s$^{-1} \sim 10^{-2}$ s$^{-1}$. Comparison with the observed maximum rate $k_{\text{max}} = 4 \times 10^{-2}$ s$^{-1}$ of bR dark-adaptation at low pH values, i.e., for protonated Asp-85, shows that our calculations give the right order of magnitude for the absolute rate.

In evaluating the rate constant for the dark-adaptation process, a static distribution of the activation barriers was assumed. This assumption is equivalent to the postulate that the elementary act of retinal isomerization occurs on a time scale which is fast compared to the structural rearrangements within the bR binding site. To test this assumption, the characteristic time scale for the torsional motions of retinal around retinal’s 13–14 and 15–N double bonds and compared them with the autocorrelation functions for the distances between the Schiff base nitrogen and C$_x$ carbons of Asp-85 and Asp-212, two key amino acids in the retinal binding pocket. Figure 4c,d shows that the autocorrelation function for the retinal dihedral torsions decays five times faster than the autocorrelation function of the distance between the Schiff base and aspartic acids. The observation, that fluctuations along the reaction coordinate (rotation around retinal’s 13–14 and 15–N bonds) occur on a shorter time scale compared to the fluctuations

of the side group positions within the bR binding site, lends support to the procedure employed for evaluating $E_{\text{eff}}$. However, a rigorous prove of eq 4 requires analysis of the autocorrelation function for the isomerization barrier $E(t)$ itself. Such a procedure is currently computationally prohibitive.

**Elementary Act of Dark-Adaptation.** The characteristic time of the elementary microscopic act of dark-adaptation can also be monitored. Activation of the torsional degrees of freedom from their equilibrium values to the twisted conformations in a transition state (see Figure 8) is a microscopic time reversal of the relaxation from the transition state to the equilibrium. Thus, simulation of such a relaxation process provides information about the elementary isomerization act.81,82

Figure 9 portrays the behavior of the 13–14 and 15–N dihedral angles in the course of their relaxation from the transition state to equilibrium. It is seen that complete energy dissipation from the 13–14 and 15–N torsional degrees of freedom to other protein degrees of freedom occurs within a picosecond. However, the major change of the dihedral angle values occurs within 50 fs. This implies that, although energy accumulation in the reaction coordinate takes about 1 ps, the sharp jump along the reaction coordinate happens within 50 fs. This time is to be compared with the characteristic time of 200 fs for the noticeable rearrangement of protein side groups within the retinal binding site (Figure 4d). One can conclude that the assumption concerning time scale separation between the elementary isomerization act and environmental fluctuations within the retinal binding site is justified.

**Accuracy of Performed Calculations.** The accuracy of the performed calculations is determined by the level of ab initio calculations employed, by the way in which the protein environment is accounted for, by the procedure chosen to calculate the isomerization barriers of dark-adaptation, as well as by the limited statistics employed for evaluation of the overall rate of dark-adaptation. The CASSCF(8,8)/6-31G level of ab initio calculations has been employed in the present work. Our sample calculations with a double-polarized basis set (6-31G**) performed on a smaller retinal analog, have demonstrated that rotation barriers around the double bonds of protonated Schiff bases essentially do not change when one uses basis sets larger than 6-31G at the CASSCF(8,8) level. Improvements in the basis set combined

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with improvements in the \textit{ab initio} level (via increase of the complete active space and implementation of configuration interaction or perturbation theory on top of CASSCF) could further improve the barriers for dark-adaptation, but such calculations are computationally prohibitive at present.

The role of the environment was taken into account by explicitly including all protein partial charges in the Hamiltonian. Improvements of the representation of the environment should account for the protein polarizability, as well as for protein atoms in direct van der Waals contact with retinal.

The co-rotation around retinal’s 13–14 and 15–N double bonds has been chosen as the isomerization reaction pathway. This choice of the reaction coordinate is well justified. On the one hand, this “bicycle peddle” motion is favored by the protein environment, since it invokes the smallest possible perturbation of the surrounding groups. The \textit{in vacuo} \textit{ab initio} calculations also favor co-rotation over the sequential isomerization, e.g. all-trans $\rightarrow$ 13-cis $\rightarrow$ 13-cis,15-syn. Calculations performed for the planar optimized retinal geometry yield isomerization barriers of 34 kcal/mol for the rotation around the 13–14 bond, 47 kcal/mol for rotation around the 15–N bond, and 47 kcal/mol for the co-rotation around the 13–14 and 15–N bonds.

The main shortcoming of the described calculations is that retinal geometries are computed by empirical potentials, while energies are determined by \textit{ab initio} calculations. While complete \textit{ab initio} minimization of \textit{in situ} retinal would be more preferable, it is
computationally prohibitive. The in situ ab initio calculations performed in the study are already computationally very expensive. The evaluation of an isomerization barrier height for a given structure requires approximately 1 day on an HP-735/125. Thus, we had to restrict ourselves to a very limited number of data points.

Conclusions

Experimental determination and theoretical refinement of the bacteriorhodopsin structure have led to a point where detailed simulations of elementary processes occurring in the retinal binding pocket have become feasible. In the present work we have examined the dark-adaptation process in bacteriorhodopsin which involves the thermally activated isomerization around two (13–14 and 15–N) retinal double bonds. Ab initio in situ quantum chemical calculations were performed on an ensemble of protein structures. The calculations provide important insight into the regulation of in situ transformations of retinal by the protein environment. The employed level of ab initio calculations (CASSCF(8,8)) proved to be adequate for describing the ground state potential surface of in situ retinal. The calculated absolute rates of the dark-adaptation process are in accord with observations.

Our study supports the notion that steric and Coulomb interactions are the two major factors that govern chemical transformations in a protein active site. However, our simulations also demonstrate that the effect of steric interactions is not restricted to a mere “key-lock” relationship and that Coulomb interactions cannot be properly described within first-order perturbation theory accounting for the retinal—protein electrostatic interactions. We observe that steric interactions between retinal and the protein cause conformational changes of the chromophore resulting in a noticeable change of its electronic structure and isomerization potential surface. Explicit incorporation of protein charges in the electronic Hamiltonian of retinal is of crucial importance, since the protein charge environment polarizes the chromophore and alters substantially its electronic structure.

Acknowledgment. The authors express their gratitude to Chuck Martin for helpful discussions and to William Humphrey for assistance in the preparation of the manuscript. The research was carried out at the Resource for Concurrent Biological Computing at the University of Illinois, funded by the National Institutes of Health (grant P41RR05969). The research has been supported also by the Roy J. Carver Charitable Trust. The majority of simulations were carried out on Hewlett-Packard and Silicon Graphics workstations operated by the Resource. I. Logunov received a graduate fellowship from the University of Illinois.

JA953091M