A Quantum Chemical Study on the Mechanism of Cis–Trans Isomerization in Retinal-like Protonated Schiff Bases

Guido J. M. Dormans,* Gerrit C. Groenenboom, Wim C. A. van Dorst, and Henk M. Buck


Abstract: The dynamics of the photochemical cis–trans isomerization in retinal-like protonated Schiff bases is studied by means of MNDO/CI calculations. The aim of these calculations is a better understanding of the mechanism which accounts for the highly regioselective and efficient photosomerization of rhodopsin and bacteriorhodopsin in the primary step after light absorption. Calculations on the model compound protonated 1-imino-2,4-pentadiene show that the regioselectivity and efficiency of this reaction can be explained from the intrinsic properties of this molecule. Whereas it is found that the protonated Schiff bases have a lowest \(^1B^+_2\)–like excited state, the second \(^1A^+_2\)–like excited state is particularly photochemically labile. This latter state serves in diminishing (or even removing) the barrier on the potential energy surface of the initially excited state, thus enhancing the rate for the photoisomerization. The transition probability for a radiationless return of the excited molecule to its ground state was evaluated explicitly for the photosomerization around the various double bonds in protonated 1-imino-2,4-pentadiene by means of semiclassical trajectory calculations. The transition probability depends on the energy gap between the ground and excited state and the nonadiabatic coupling between these states for the 90° twisted molecule. The extent of the energy gap is related to the distance from the twisted bond to the nitrogen atom. The role of this electron-deficient nitrogen atom is to stabilize the polarized resonance structure which describes the 90° twisted molecule in the excited state. When this stabilization is too strong, the polarized resonance structure drops below the diradical ground state which results in an increased energy gap and a reduced efficiency of photosomerization. The possibility for a concerted bicycle pedal isomerization around two double bonds is investigated by a calculation of the two-dimensional energy surfaces and nonadiabatic couplings for a combined rotation around these two bonds. A strictly bicycle pedal motion is found to be unfavorable, but a mechanism which involves a complete rotation around one double bond assisted by a partial rotation of the second double bond might provide a route for the photosomerization of the retinylidene chromophore in the confined environment of a protein.

Calculations on a model compound of the protonated Schiff base of retinal show that the extent of the stability of the 90° twisted molecule in the excited state can be directed by locating external point-charges around the molecule. In nature, these point-charges are provided by the protein opsin, and their presence has been used to explain the opsin shift of the various intermediates in the photocycles of rhodopsin and bacteriorhodopsin. Our calculations show that these external point-charges also have an important impact on the energy gap between the ground and excited state and, therefore, on the regioselectivity and efficiency of photosomerization in the retinylidene chromophore. The primary step in the photosomerization in bacteriorhodopsin can be best understood from an external point-charge model with a negative counterion near the protonated nitrogen atom and an ion pair near the cyclohexene ring. A matter of dispute, in particular the configuration around the C\(_14\)-C\(_15\) single bond.\(^{3,6}\) Anyways, the conversion from BR\(_{98}\) to K\(_{610}\) involves the photosomerization of the C\(_17\)-C\(_8\) double bond from cis to cis.\(^{6}\) K\(_{610}\) is stable below -120 °C and its ground-state energy is 16 kcal/mol higher than that of BR\(_{98}\). Some questions that rise are how opsin directs the regioselectivity of the cis–trans isomerization and what conditions are necessary to account for the unusually rapid and efficient reaction. This is especially intriguing when it is borne in mind that the retinylidene chromophore has only a very limited space within the pocket of the surrounding protein. A thorough understanding of these features asks for a detailed knowledge of the intrinsic characteristics of the retinylidene chromophore. Once these are known, it may be deduced how the protein can direct the regioselectivity and increase the efficiency of the photosomerization.

Freedman and Becker\(^{4}\) studied the photosomerization of various isoforms of the n-butylnamine Schiff base of retinal in detail. They

\footnote{(3) Cooper, A. Nature (London) 1979, 282, 531.}

\footnote{(4) There are strong indications that before the ground-state product K\(_{610}\) as an isomer is formed ("I") directly from BR\(_{98}\) within 0.7 ps, which rapidly converts into K\(_{610}\) within a few picoseconds. This latter transformation probably involves conformational changes of the protein. Nuss, N. C.; Zinth, W.; Kaiser, W.; Kölling, E.; Oesterhelt, D. J. Am. Chem. Soc. 1983, 105, 1171.}


found that the quantum yields for the 9-cis, 11-cis, 13-cis, and all-trans isomers are less than 0.01 in hexane. Going from the nonpolar solvent hexane to the polar solvent methanol, the quantum yields for these isomers increase with a maximum of 0.24 for the 11-cis isomer. Whereas the photoproducts of the cis isomers are always the all-trans isomer, irradiation of the all-trans isomer gives a mixture of the cis isomers ($\phi_{\text{cis}} = 0.12$) of which the 11-cis is predominantly formed. Upon protonating the Schiff base, only the 11-cis isomer shows an efficient isomerization to the all-trans isomer ($\phi_{\text{trans}} = 0.24$, independent of solvent polarity). The quantum yield of isomerization of the protonated all-trans isomer was 0.14 in all solvents, being only slightly higher than the quantum yield for the unprotonated species in a polar solvent. Again, the 11-cis isomer was predominantly formed.

Freedman and Becker explained these differences from the characteristics of the two lowest excited singlet states of the retinylidene Schiff base. These excited states are related to the two lowest singlet states of the set of trans linear polyenes which have $C_{2v}$ symmetry. One excited state is constructed from the promotion of an electron from the highest occupied $\pi$-MO to the lowest unoccupied $\pi^*$-MO. The symmetry of this singlet ($\pi\pi^*$) excited state is $B_2^+$. The + sign refers to the symmetry of the configurations in the CI expansion of a PPP wave function. This state is described in a CI expansion, this state is described by means of two-photon spectroscopy the location of this $B_2^+$ state became possible, and it has been shown to be the lowest excited singlet state in long-chain linear polyenes. The same level ordering is found for the retinylidene Schiff bases. In valence bond formalism, the wave function of the $B_2^+$ state is dominated by ionic configurations, whereas the $A_2^+$ state is mainly built up from covalent configurations. This indicates that the polarity of the solvent has a different impact on these two states. This prompted Freedman and Becker to explain the observed photochemistry of the isomers of the $\beta$-butylyamine Schiff base of retinal using the quantum chemical formalism, as we have used before for the description of the dynamics of cis-trans isomerization of small polyenes. However, this method can only be applied for one-dimensional isomerizations with a moment of inertia which is independent of the twist angle. In the present case, this restriction does not hold. Especially for the rotation around the central $C_2-C_3$ bond in pentanimine-H$^+$ and most of the motions involving rotations around several bonds simultaneously, the moment of inertia strongly depends on the twist angle. Therefore, the semiclassical trajectory method has been used to study the dynamics of cis-trans isomerization in pentanimine-H$^+$. Similar calculations have been performed by Warshel$^1$ and Birge$^4$ in the study on the photoisomerization of the retinylidene chromophore. However, their calculations were based on a more or less semiemipirical expression for the nonadiabatic coupling. Our calculations make explicit use of calculated nonadiabatic couplings.

In the semiclassical trajectory method, the time-dependent wave function is written as an expansion of the wave functions of the electronic states:

$$\Psi(t = \theta; \mathbf{r}) = \sum_{K} \psi_{K}(\theta) \Phi_{K}(\mathbf{r},\theta) \delta(\mathbf{r}; \theta)$$

In this equation, $\psi_{K}(\theta)$ represents the wave function of state $K$ which depends on the electron coordinates ($\mathbf{r}$) and parametrically on the twist angle $\theta$. $E_K(\theta)$ stands for the potential theory curve of this state. The time-dependent contribution of the electronic state $K$ to the total wave function $\Psi(t = \theta; \mathbf{r})$ equals $\psi_{K}(\theta)$. The contribution of the electronic ground state $\psi_{K}(\theta) = 0$ can be disregarded as the transition probability of the molecule from the excited state to the ground state. The (complex) coefficients $\psi_{K}(\theta)$ are found by substituting eq 1 in the time-dependent Schrödinger equation:

$$ih\frac{d}{d\theta}\Psi(\theta; \mathbf{r}) = H(\theta; \mathbf{r})\Psi(\theta; \mathbf{r})$$

resulting in a set of differential equations:

$$\frac{d}{d\theta}\psi_{K}(\theta) = \frac{1}{i\hbar}(E_K(\theta) - H(\theta; \mathbf{r}))\psi_{K}(\theta)$$

In this equation, $E_K(\theta)$ represents the nonadiabatic coupling function, which can be calculated from the multiconfigurational wave functions of the electronic states (vide infra):

$$E_K(\theta) = \langle \psi(0); \mathbf{r}|\mathbf{r}|\psi(\theta); \mathbf{r} \rangle$$

The nuclear velocity $\mathbf{v}(\theta) = \frac{\partial}{\partial\theta} \psi(\theta)$ was obtained from the classical equations of motion. In these calculations no energy dissipation to the other degrees

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of freedom was assumed. Hence the classical Hamiltonian can be written as:

$$H = \frac{1}{2}J(\theta)^2 + E_k(\theta)$$  \hspace{1cm} (5)$$

The angular dependent moment of inertia $I(\theta)$ needed in eq 5 was evaluated by a calculation of the kinetic energy at a constant $\theta$ of the molecular moving on the potential energy curve of the initially excited state. For all isomerizations, three singlet states were included in the calculations. It was assumed that all trajectories started on the potential energy curve of the first excited state ($\{\alpha_1 D(\theta) = 1\}$), because this state was found to have the largest oscillator strength. In practice, several trajectories were run with various initial conditions corresponding to a different excess kinetic energy of the excited molecule. This approach mimics the situation in which an ensemble of vibrational levels is excited, each with its own excess vibrational energy.

The nonadiabatic coupling (NAC) functions $E_{\text{NAC}}(\theta)$ were obtained from a MNDO14 SCF calculation followed by a configuration interaction with the CIPSI20 algorithm. Starting from a zeroth order wave function including six leading configurations (see Scheme 1) for the three lowest singlet states, the most important configurations were selected from a second-order perturbation after generating all possible configurations single or double excited with respect to the reference space.

The final CI space was constructed from a collection of the selected configurations at various nuclear configurations. No essential changes (level ordering of states, shapes of the potential energy curves, etc.) were found upon increasing the active MO space from 6 MOs (selection threshold20 $\eta = 0.00$; 170 configurations) to 20 MOs ($\eta = 0.02$; 450 configurations). A program was written to calculate natural orbitals, charge and bond densities, transition dipole moments ($\mu_{\text{CI}}(\theta) = \langle \Psi_{\text{CI}}(\theta)|\hat{p}|\Psi_{\text{CI}}(\theta)\rangle$), and oscillator strengths ($f_{\text{CI}} = \frac{1}{2}\langle \Delta E\Delta E_{\text{CI}}|\mu_{\text{CI}}^2\rangle$) from multiconfigurational wave functions.

The nonadiabatic coupling functions $g_{\theta}(\theta)$ can be expressed as:

$$g_{\theta}(\theta) = g_{\theta}^{(\text{CI})}(\theta) + g_{\theta}^{(\text{MO})}(\theta)$$  \hspace{1cm} (6)$$

In this equation, $g_{\theta}^{(\text{CI})}(\theta)$ involves the differentiation of the CI coefficients and $g_{\theta}^{(\text{MO})}(\theta)$ the differentiation of the molecular orbitals. Both terms were evaluated approximately by a two-point numerical procedure21 with a stepsize of $\Delta \theta = 0.02^\circ$. The MNDO results were compared23 with our ab initio calculations for the cis-trans isomerization of small polyenes. The results of both methods are found to correspond qualitatively well, the main difference being that ab initio calculations tend to overestimate transition energies (especially for the $\sigma$-state) whereas the MNDO calculations underestimate excitation energies.

III. Results

A. Double Bond Rotations in Pentanimine-H$^+$. The aim of this work is to demonstrate some essential characteristics of the cis-trans isomerization in protonated Schiff bases. Special attention has been drawn to the possibility of simultaneous double bond rotations for which we calculated two-dimensional potential energy surfaces of the lowest singlet states and nonadiabatic couplings among them. Considering the large amount of calculations involved, pentanimine-H$^+$ was chosen as the smallest molecule which incorporates all essential elements of a protonated Schiff base.

The relatively small dimensions of this molecule made it possible to optimize all geometrical parameters for each electronic state individually with a reasonably large multiconfigurational wave function. It is known that especially the bond lengths of the carbon-carbon bonds may change drastically upon twisting such bond.23 This is demonstrated in Figure 1 for the twist around the C$_4$-C$_5$ bond of pentanimine-H$^+$.

Because of the electron-withdrawing capacity of the protonated nitrogen atom, already in the planar conformation the picture of alternating single and double bonds has almost vanished. The former double bonds are elongated whereas the single bonds have become shorter. This effect is even more accentuated near the nitrogen atom. When the C$_4$-C$_5$ bond is twisted, that $\pi$-bond is broken and, consequently, the optical bond length increases. The effect of this twist on the optical bond lengths of the other bonds is much smaller.

In the same figure, the effect of a separate geometry optimization in the ground and first excited state on the energy of the ground state is shown. Despite the fact that the geometrical parameters are markedly different in both states, the effect on the energy of the ground state is minimal. The energy barrier for a rotation around the C$_4$-C$_5$ bond is 34.5 kcal/mol when optimized in $S_1$ and 34.1 kcal/mol when optimized in $S_0$. The same effect is found for the excited states and for rotations around other bonds. It is clear that in this case the influence of a separate geometry optimization in the ground and excited states on the general appearance of the potential energy curves is minimal. Therefore, we have used one idealized geometry for all calculations ($\tau_{C1-C2} = 1.40$ Å, $\tau_{C2-C3} = 1.42$ Å, $\tau_{C3-C4} = 1.35$ Å, $\tau_{C4-C5} = 1.10$ Å, $\tau_{C5-C6} = 1.00$ Å, all bond angles 120°). For an extensive study of the dependence of the energy of the ground state and excited state on the carbon-carbon bond lengths we refer to the work of Warshel.24

The potential energy curves and nonadiabatic coupling for the rotations around the N-C$_1$, C$_4$-C$_5$, and C$_5$-C$_6$ bonds are shown in Figure 2. For the N-C$_1$ and C$_4$-C$_5$ bonds, the curves are symmetric (or antisymmetric for certain couplings) with respect to $\theta = 90^\circ$. The rotation around the central C$_4$-C$_5$ bond represents an isomerization from the cis isomer ($\theta = 0^\circ$) to the trans isomer ($\theta = 180^\circ$). The ground state energy of the cis isomer is 7.3 kcal/mol higher than that of the trans isomer. This difference is obviously too high because the steric repulsion is overestimated for the idealized geometry (with all bond angles 120°).

The isomerization rate is determined by the moment of inertia associated with the rotation and, more important, the slope of the potential energy curve of the excited state near the vertical absorption maximum25 ($\lambda = 0^\circ$ or $\theta = 180^\circ$). This slope is markedly different for the three rotations considered, already indicating that there is a large difference to be expected for the dynamics of photochemical cis-trans isomerization around these bonds.

The quantum yield of cis-trans isomerization is related to the nonadiabatic coupling in the region where the potential energy curves of the ground and excited states come close. The extent of the nonadiabatic coupling determines the probability for a radiationless transition (vibrational) and, in its turn, this probability determines the quantum yield.17 Qualitatively, it can be said that the probability increases when the energy gap between two points is small.

Figure 1. Optimal bond lengths (a) for a rotation around the C$_4$-C$_5$ bond in pentanimine-H$^+$ (optimized in $S_0$). Energy curve (b) of the ground state for a rotation around the C$_4$-C$_5$ bond when optimized in $S_0$ (solid line) or $S_1$ (broken line).

(22) van Dorst, W. C. A.; Dormans, G. J. M., unpublished results.
Cis-Trans Isomerization in Schiff Bases


We found that the $1B^+$ state is the lowest excited state, in agreement with other calculations and experimental data for protonated Schiff bases. The energy splitting between the two states is calculated to be 0.42 eV (9.7 kcal/mol) and their wave functions are slightly mixed: $\langle \Psi_{1A^+} \rangle = 0.16|\psi_1\rangle + 0.20|\psi_2\rangle + 0.55|\psi_3\rangle + 0.41|\psi_4\rangle + 0.39|\psi_5\rangle + 0.18|\psi_6\rangle$ (the configurations in these CI expansions are those of Scheme I). This mixing explains the relatively large oscillator strength of the $1A^+_i$ state. The $1A^+_i$ state has important contributions from the doubly excited configurations $\langle \Psi_{0} \rangle$ and, therefore, has a more antibonding character than the $1B^+$ state. This is reflected in the larger decrease in bond densities of the double bonds going from the ground state to the $1A^+_i$ state compared to the $1B^+$ state (Figure 3). This effect is more accentuated for the C2-C3 and C4-C5 bonds than for the N-C1 bond, which implicates that a rotation around the former two bonds is energetically favored in the $1A^+_i$ state. As the $1A^+_i$ state is located above the $1B^+$ state for the planar geometry, the energy difference between the two states will decrease upon twisting. Because there is no symmetry in the molecule, the potential energy curves will exhibit an avoided crossing. This is indeed the case as can be seen from the peaks in the $g_{ij}$ coupling functions of Figure 2.

The effect of this avoided crossing is that the potential energy curve of the absorbing state ($1B^+$) is bent downwards. Though the $1A^+_i$ state is not directly involved in the absorption of protonated Schiff bases, it plays an important role in lowering (or even removing) the energy barrier in the $1B^+$ state.

The description of the electronic structures of the 90° twisted molecule is intimately related to the description of twisted alkenes. For symmetric alkenes, the HOMO and LUMO become degenerate for $\theta = 90^\circ$. Three singlet configurations can be generated from a distribution of the frontier two $\pi$-electrons over the two centra on each side of the twisted bond.

\[ \text{Roos, S. In Computational Techniques in Quantum Chemistry and Molecular Physics} \]
\[ \text{(Procedures of the NATO Advanced Study Institute); Diercks, G. H. F.; Sutcliffe, B. T.; Veillard, A., Eds.,} \]
\[ \text{1974, Vol. 15, p 251.} \]

\[ \text{(26) Schulten, K.; Dinur, U.; Honig, B. J. Chem. Phys. 1980, 73, 3927.} \]

Figure 2. Energy curves and nonadiabatic couplings for a rotation around the N-C1 (a), the C2-C3 (b), and the C4-C5 (c) bonds in pentanimine-H⁺.

Figure 3. Calculated charge and bond densities (in parentheses) of the three lowest singlet states of planar pentanimine-H⁺.
The fact that the direction of these energy sequences is opposite for the D and Z states indicates that there might be a bond for which the D and Z states come close in energy. For this bond, the energy gap between \( S_0 \) and \( S_1 \) is minimal. Twisting a bond which is closer to the N end of the molecule increases this energy gap because Z is too strongly stabilized. Twisting a bond which is closer to the C end of the molecule increases the energy gap because Z is not enough stabilized.

Our calculations show that the optimal condition for stabilization and destabilization occurs for the twist \( \theta \) around the central C\(_2\)-C\(_3\) bond. Figure 5 gives the charge densities on each side of this twisted bond for \( S_0 \) and \( S_1 \) as a function of the twist angle \( \theta \). It clearly shows the polarization of charge and it follows that at \( \theta = 90^\circ \) the ground state ("Z") bears the positive charge at the C end of the molecule. From the situation for twisting in ethylene, it is known that Z correlates with the doubly excited configuration \( |S_1\rangle \) of the planar molecule. Because the \( 1^3\Sigma^- \) state has a large contribution from this configuration, it is obvious that the Z structure at \( \theta = 90^\circ \) correlates diabatically with the \( 1^3\Sigma^- \) state at \( \theta = 0^\circ \). Therefore, the energy curve of the \( 1^3\Sigma^- \) state shows a negative slope for a twist around a double bond for which the 90° twisted structure is strongly stabilized in the excited state. The size of the slope depends on the extent of this stabilization. As indicated before, the time-dependent probability of finding the molecule in a certain electronic state K equals \( |\psi_K(t)|^2 \). The contribution of the ground state \( |\psi_0(t)|^2 \) can be interpreted as the probability for a radiationless transition. This value can then be used to evaluate the quantum yield of cis-trans isomerization.\(^{11}\) We have calculated these contributions \( |\psi_K(t)|^2 \) for the various isomerizations in 

\[ \text{for the rotation around the } C_2-C_3 \text{ bond (Figure 2c), there is a strong nonadiabatic interaction between the two excited states at } \theta_1 = 50^\circ \text{ and } \theta_2 = 130^\circ, \text{ as can be seen from the peaks in the nonadiabatic coupling function } g_{12}(\theta), \text{ at these angles. As a result, we found that the contribution of the second excited state to the total wave function } |\psi_2(t)|^2 \text{ increased to a value of } 0.60. \]

The other hand, the contribution of the electronic ground state \( |\psi_0(t)|^2 \) did not exceed a value of 0.06. When it is assumed that molecules cross over to the ground state with conservation of the direction of rotation, it follows that only a small fraction of the molecules will reach the isomerized structure in the ground state after the first passage of the 90° region. The rest of the molecules will start to oscillate back and forth in the energy minimum with an equal probability to cross over to the ground state in either direction of the isomerization (provided the energy of the system does not decrease via intramolecular energy dissipation or collisions with the environment). The result is an almost 1:1 distribution of the

\[ \Phi = (1 - f)/(2 - |\psi_0(t)|^2), \text{ where } f \text{ is the fraction of the molecules which do not reach the crossing region.} \]
cis and trans isomers in the ground state.

Another important factor controlling the dynamics is the energy barrier on the potential energy curve of the first excited state. Because of this barrier, a number of trajectories corresponding to vibrational levels with low energies will not reach the crossing region. Therefore, molecules with such low vibrational energies will not isomerize but exhibit other processes (such as fluorescence and other photochemical processes). This will reduce the quantum yield of cis-trans isomerization.

In contrast to the isomerization around the C2=C3 bond, a calculation of the contributions of the electronic states for a rotation around the central C2=C3 bond showed that in this case the transition probability to the ground state is much larger (θg=θt=0.30). This is a direct result of the small energy gap and correspondingly large nonadiabatic coupling at θg=θt=90° (see Figure 2b). From the value of 0.30, a quantum yield of isomerization of 0.59 is calculated30 (assuming that no other processes occur). This value may be compared with the value of 0.7 observed for rhodopsin and indicates that this latter system must also have a barrierless potential energy surface in the excited manifold and a similar small energy gap and correspondingly large nonadiabatic coupling with the ground state for the twisted structure.

The transition probability is found to be almost identical for the cis → trans isomerization and the trans → cis isomerization. However, for this latter isomerization the energy barrier on the potential energy curve of the first excited state will decrease the number of trajectories which reach the crossing region. Therefore, the quantum yield for an isomerization of the sterically hindered cis isomer will be larger than for the isomerization of the trans isomer. Such a difference in quantum yield between these two directions of isomerization is frequently found and must thus be contributed to the absence of a barrier on the potential energy surface in the excited state as a result of steric hindrance for one of the two isomers.

In summary, it is found that there is a relation between the distance from the twisted bond to the N end of the protonated Schiff base, the energy gap between S0 and S1, and the slope of the diabatic energy curve of the 1A2 state. In its turn, the shape of this curve determines the shape of the energy curve of the absorbing B1 state. For pentanimine-H+ the optimal conditions occur for the isomerization around the central double bond for which the stabilization of the 90° twisted structure in the excited state leads to both a barrierless potential energy curve and a minimal energy gap and correspondingly large transition probability to the ground state. Evidently, protonated Schiff bases have an intrinsic control of the regioselectivity and efficiency of cis-trans isomerization, which depend on the distance between the twisted bond and the nitrogen atom. Such a regioselectivity is found indeed for the photoisomerization of protonated allylamine32 and it gives a possible explanation for the photochemical behavior of the protonated Schiff bases of retinal.9,33

B. Multiple Double Bond Rotations in Pentanimine-H+. It has been argued by several research groups32,33 that the isomerization around one double bond cannot occur for the retinylidene chromophore in the confined pocket of the surrounding protein. Therefore, mechanisms have been proposed which involve the simultaneous isomerization of two double bonds (the "bicycle pedal" model33) or the simultaneous isomerization of a double bond and an adjacent single bond (the "hula twist" model32). From an inspection of the bond densities of the excited states of pentanimine-H+ (Figure 3), it follows that a simultaneous rotation around the two carbon-carbon double bonds should be energetically favorable.34 Therefore, we performed MNDO/Cl calculations for the two-dimensional reaction surfaces for the twist around these bonds (θ2 and θ1). The results are shown in Figures 6 and 7. The energy curves for the twist around the C2=C3 and C2=C3 bonds as shown in Figure 2 are essentially the sections of the two-dimensional surfaces which correspond to θ1 = 180° and θ2 = 180°, respectively.

From an inspection of Figure 6 it is seen that there is a region near θ1 = θ2 = 90° where the energy of S1 increases, whereas the top of the energy surface of the ground state levels off. This is a result of a crossing of the wave functions describing S1 and S2 going from θ1 = θ2 = 0° (180°) to θ1 = θ2 = 90°. To demonstrate this, the potential energy curves representing a simultaneous isomerization for the two bonds out of phase (θ1 = -θ2) are given in Figure 8.

This latter isomerization is especially interesting to describe the cis-trans isomerization of the retinylidene chromophore, where
the N end is anchored to the protein via a covalent linkage, because the motion of the N end in this concerted isomerization is minimal.

For pentanimine-H⁺, the angle-dependent moment of inertia for the bond between C2-C3 and C4-C5 has become a real double bond. The resonance structures for this state will be differently influenced by a charged environment. Freedman and Becker⁵ argued that the variety in quantum yields of isomerized products upon irradiation of the n-butylamine Schiff base of retinal as a function of protonation state and solvent polarity could be explained from the mixing of the \( B_{2u} \) state into the \( A_{1u} \) state. The charge stabilization in the excited state of the twisted molecule now introduces an additional property which will strongly influence the shape of the potential energy surface and, therefore, the dynamics and quantum yields of the various photoisomerizations. Moreover, it offers an explanation for the regioselectivity of isomerization in the irradiated reactant. For the free (protonated) Schiff base, the optimal condition will be fulfilled for the isomerization around one particular double bond. The other double bond only assists the reaction in that it brings the molecule in a region of large coupling, whereas the volume needed for the isomerization is smaller. A complete treatment of this concerted mechanism asks for the solution of trajectory equations in two dimensions. Present work is aimed at performing such calculations.

A similar concerted bicycle pedal motion has been proposed for the thermal isomerization of light-adapted BR\(_{148}\) to dark-adapted BR\(_{148}\).⁵⁶ Resonance Raman spectra show that this transformation involves the isomerization from all-trans-retinal to 13,15-dicis-retinal. From the bond densities given in Figure 3, it follows that a simultaneous rotation around the N-C₁ and C₂-C₃ bonds is unfavorable for pentanimine-H⁺ in the excited state. Because of the complementarity of the thermal and photochemical processes, this mechanism may provide a route for the thermal process.⁵⁶

In conclusion, the cis-trans isomerization in pentanimine-H⁺ is found to be most effective via an isomerization of the central double bond. However, a partial rotation of the adjacent carbon–carbon double bond may assist the reaction in bringing the molecule into a region of strong nonadiabatic coupling while reducing the volume needed for rotation.

IV. Influence of External Point Charges

From the calculations for pentanimine-H⁺ it follows that the regioselectivity and efficiency of photoisomerization in protonated Schiff bases is directly related to the bond densities of the reactant in the excited state and the relative stability of the resonance structures describing the ground and excited state of the 90° twisted molecule. This stability was shown to depend on the distance from the nitrogen atom to the twisted bond. For a bond which is too close to the nitrogen atom the ground state, which is described by a Z structure (through the N end of the molecule, the C fragment positively charged), is too strongly stabilized. The excited state, now described by a D structure (one of the former \( \pi \) electrons at each side of the twisted bond, the N fragment positively charged) is so strongly destabilized that the energy minimum on the excited-state potential energy surface becomes less deep. In this situation, the energy gap between \( S₂ \) and \( S₃ \) is relatively large. On the other hand, twisting a bond which is too far from the nitrogen atom results in an opposite stabilization of the Z and D structures (now describing \( S₂ \) and \( S₃ \) respectively). However, the effect is the same because the minimum on the excited-state potential energy surface is less deep and the energy gap with the ground state relatively large.

The determining factor in this description is the stabilization of the positive charge in either of the two fragments on each side of the twisted bond. This feature indicates that the various twisted structures will be differently influenced by a polarizable (solvent) or charged (surrounding protein) environment.
instead of the C\textsubstitue{11}–C\textsubstitue{12} double bond, the C\textsubstitue{13}–C\textsubstitue{14} double bond isomerizes. These differences can now be understood by assuming that the surrounding protein provides external charges at different locations relative to the chromophore. Such external point-charge models\textsuperscript{1,18–40} have been put forward by several research groups to explain the shift of the UV absorption maximum (opsin shift\textsuperscript{39}) for the various intermediates in the photocycles of rhodopsin and bacteriorhodopsin, the vibrational spectra of these intermediates,\textsuperscript{40} and the isomerization barriers in the ground state.\textsuperscript{23,24,41}

To demonstrate the influence of a charged environment on the relative stabilities of the D and Z resonance structures, we performed calculations for a model compound (RET-H\textsuperscript{+}) representing the protonated Schiff base of retinal. In this model compound, the cyclohexene ring of retinal is replaced by a vinyl group which is arbitrary twisted by 60° out of the plane of the polyene chain.\textsuperscript{42} This restricted molecule is found to be a suitable model for the study of the properties of the protonated Schiff base of retinal.\textsuperscript{23}

Figure 10 shows the charge and bond densities of the lowest singlet states of RET-H\textsuperscript{+}. We found that the 1\textsubscript{B\textsuperscript{2}}\textsuperscript{+} state is the lowest excited state. The calculated vertical excitation energy to this state is 1.97 eV (630 nm) which is too low compared with the experimental value of about 500 nm, but in agreement with the INDO-CISD calculations of Birge et al.\textsuperscript{44} for a similar compound. The difference between the theoretical (gas phase) and experimental (solvent) values can be explained from the interactions of the molecule with the solvent which will (partly) shield the positive charge of the protonated nitrogen atom thereby inducing a blue shift of the UV absorption maximum. The calculated energy gap between the 1\textsubscript{B\textsuperscript{2}}\textsuperscript{+} and the 1\textsubscript{A\textsuperscript{2}}\textsuperscript{+} state is 0.56 eV which is considerably smaller than the energy gap calculated by Birge et al.\textsuperscript{44} (1.54 eV) but agrees much better with the observed value of ca. 0.25 eV for 11-cis-rhodopsin.\textsuperscript{44} The calculated oscillator strengths show that the one-photon absorption will take place almost entirely to the 1\textsubscript{B\textsuperscript{2}}\textsuperscript{+} state.
An inspection of the changes in the bond densities going from the ground state to the $B^+$ (S) and $A^-$ (S) states shows that it is the latter state which has the most antibonding character. Therefore, the $A^-$ state is expected to be especially photochemical labile. The antibonding character of the double bonds in this state is found to increase going from the N end of the molecule to the vinyl group. This implies that a photoisomerization is expected for those double bonds close to the vinyl group (vide supra). The bond densities of the single bonds are found to increase upon excitation. From this observation it is suspected that rotations around these single bonds do not contribute to a decrease in energy of the excited states in the protonated Schiff bases of retinal. Therefore, it seems that the hula-twist model of Liu et al.\(^{(33)}\) does not apply for photochemical isomerizations in these molecules.\(^{(34)}\)

In this calculation an exception seems to be the C$_1$-C$_11$ single bond for which the bond density slightly decreases (S$_0$  $\rightarrow$ S$_1$). This suggests that a rotation around this particular single bond might be energetically favorable in the excited state. Such a rotation has been proposed by Schulten and Tavan\(^{(2)}\) for the primary photoreaction in bacteriorhodopsin. On the other hand, a simultaneous twist around two double bonds\(^{(34)}\) seems plausible from these calculations.

The calculated potential energy curves for a rotation around the C$_9$-C$_{10}$, C$_{11}$-C$_{12}$, and C$_{13}$-C$_{14}$ double bonds in RET$^-$H$^+$ are shown in Figure 11. They clearly show the characteristics of a protonated Schiff base as demonstrated for pentanimine-H$^+$.\(^{(46)}\) Both the energy barrier on the potential energy curve of the first excited state and the energy gap between the $B^+$ and $A^-$ states (S$_0$  $\rightarrow$ S$_1$) are in kcal/mol.

The calculated energy gaps at $\theta = 90^\circ$ isomers of ca. 4 kcal/mol. The energy barriers for the trans ($\theta = 180^\circ$) to cis ($\theta = 0^\circ$) isomerizations are 3.2 (C$_9$-C$_{10}$), 2.2 (C$_{11}$-C$_{12}$), and 5.9 (C$_{13}$-C$_{14}$) kcal/mol, respectively. These values lie in the order of the value of 2 kcal/mol obtained by Huppert and Rentzepis\(^{(46)}\) from luminescence measurements for the isomerization of the protonated Schiff base of retinal.

The calculated energy gaps at $\theta = 90^\circ$ are 3.1 (C$_9$-C$_{10}$), 13.5 (C$_{11}$-C$_{12}$), and 34.8 (C$_{13}$-C$_{14}$) kcal/mol, respectively. For RET$^-$H$^+$ 90$^\circ$ twisted around the C$_{10}$-C$_{11}$ bond, the ground state is found to be a D structure (the N end positively charged) and the first excited state a Z structure (the C end positively charged). For RET$^-$H$^+$ 90$^\circ$ twisted around the Z ground state is so strongly stabilized that it has become the excited state.

It follows from the energy curves that both the size of the energy barrier in S$_0$ and the energy gap with S$_1$ favor an isomerization around the C$_9$-C$_{10}$ double bond for RET$^-$H$^+$. However, the calculated energy gaps at $\theta = 90^\circ$ twisted around the C$_9$-C$_{10}$, C$_{11}$-C$_{12}$, and C$_{13}$-C$_{14}$ bonds, RET$^-$H$^+$ with a Negative Charge Fixed at 3 Å from the Proton at the Nitrogen Atom in the All-Trans Isomer (1), an Additional Negative Charge at 3 Å above C$_1$ (2), and an Additional Positive Charge 3 Å above C$_7$ (4) (see Scheme II).\(^{\text{a}}\)

<table>
<thead>
<tr>
<th>Isomer</th>
<th>C$<em>9$-C$</em>{10}$</th>
<th>C$<em>{11}$-C$</em>{12}$</th>
<th>C$<em>{13}$-C$</em>{14}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>RET$^-$H$^+$</td>
<td>3.1 (Z)</td>
<td>13.5 (D)</td>
<td>34.8 (D)</td>
</tr>
<tr>
<td>1 (Z)</td>
<td>6.1 (Z)</td>
<td>8.9 (D)</td>
<td>14.9 (D)</td>
</tr>
<tr>
<td>2 (D)</td>
<td>16.8 (D)</td>
<td>22.8 (D)</td>
<td>37.3 (D)</td>
</tr>
<tr>
<td>4 (Z)</td>
<td>16.9 (Z)</td>
<td>4.1 (Z)</td>
<td>12.3 (D)</td>
</tr>
</tbody>
</table>

\(^{\text{a}}\) The energies are in kcal/mol. D and Z refer to the character of the 90$^\circ$ twisted molecule in the excited state (see text).
bond decreases from 34.8 kcal/mol in the absence of a negative charge to 14.9 kcal/mol (see Table I). Consequently, the counterion near the protonated Schiff base will increase the quantum yield for an isomerization around the C11-C12 double bond as is the case for the photoisomerization of BR580 to K580. A much smaller effect is observed for the isomerizations around the C6-C7 and the C11-C12 double bonds. Note that the effect of the counterion on the energy gap is probably overestimated because these are gas-phase calculations. A polarizable environment will diminish the effect of the counterion.

In the original point-charge model of Nakanishi et al., it was assumed that in the case of bacteriorhodopsin there is a second negative charge near the cyclohexene ring. Such a negative charge will stabilize the Z structures and destabilize the D structures. Therefore, the energy gap between S0 and S1 will increase by this additional negative charge for all twisted conformations where the ground state has Z character. A calculation for RET-H+ with an additional negative charge at 3 Å above C5 (3) shows that this is indeed the case (Table I). The energy gap for the C13-C14 double bond increases from 14.9 kcal/mol to 37.3 kcal/mol, which disagrees with the observed photoisomerization of this double bond in bacteriorhodopsin.

However, Lugtenburg et al. have argued that the negative charge near the C5 position in the cyclohexene ring is accompanied by an additional positive charge near C7. Their conclusions were based on the opsin shift of some dihydro derivatives of retinal in bacteriorhodopsin and are supported by NMR experiments of the same groups. A calculation of the energy gap between S0 and S1 for RET-H+ with a negative charge near the protonated nitrogen atom, a negative charge 3 Å above C5, and a positive charge 3 Å above C7 (4) shows that the isomerization around the C13-C14 double bond now becomes favorable again (see Table I). A similar effect is found for the C11-C12 isomerization. The proposed field from the ion pair may, in fact, also be created by protein dipoles.

Of course, for a more precise evaluation of the effect of external point-charges, more information is needed about the nature of the counterions and the structure of the protein with respect to local ionic restrictions specific for protein substrate interaction. Some information regarding Schiff base–protein interaction can be obtained from the NMR data of Harbison et al. However, the present results clearly show how the electronic interactions which induce the opsin shift of the retinylidene chromophore also have an important effect on the energy gap between the ground and excited state and the related dynamics of the photoisomerization. The external point-charge model with an ion pair near the cyclohexene ring is found to be the best model to explain the observed behavior of bacteriorhodopsin.

V. Summary

The aim of this work was to demonstrate which intrinsic properties of protonated Schiff bases and which external feature account for the highly regioselective and efficient cis-trans isomerization observed for the retinylidene chromophore in rhodopsin and bacteriorhodopsin.

MNDO/CI calculations for pentanimine-H+ revealed that the intrinsic properties can be understood from the bond densities of the reactant in the excited states and the related stability of the resonance structures describing the 90° twisted molecule in the ground and excited state. Whereas the light absorption takes place to the first (B1) excited state, the second (A2) excited state is found to be particularly photochemically labile. It serves in diminishing (or even removing) the energy barrier on the potential energy curve of the first excited state via an avoided crossing.

The electron-deficient nitrogen atom causes a strong stabilization of the polarized structure of the 90° twisted molecule in the excited state, resulting in a decrease of the energy gap between the ground and excited state. When this stabilization is too strong, the polarized Z structure drops below the diradical D structure, thereby increasing the energy gap again. This energy gap is found to depend on the distance from the twisted bond to the nitrogen atom, which explains the intrinsic regioselectivity for cis-trans isomerization in free protonated Schiff bases.

Dynamical calculations on the isomerization of the double bonds in pentanimine-H+ by means of semiclassical trajectory calculations made it possible to evaluate explicitly the transition probability of the excited molecule to the ground state. This transition probability depends on the energy gap and the extent of the nonadiabatic coupling between the ground and excited state. The largest transition probability (0.30) was found for the central double bond in pentanimine-H+. The difference between the cis to trans and trans to cis isomerizations arises from the presence of an energy barrier on the potential energy curve for this latter reaction. This causes a number of molecules with low vibrational energies in the excited state not to reach the region of strong coupling. As a result, the quantum yield of this isomerization is smaller than for the reverse reaction.

From an inspection of the bond densities of pentanimine-H+ it follows that a simultaneous (bicycle pedal) rotation of the two double bonds should be energetically feasible. A calculation of the transition probability for this concerted motion shows that a radiationless transition is most likely to occur for a twist angle less than 90° so that the molecule will return to the initial conformation. However, a calculation of the potential energies and nonadiabatic couplings for the two-dimensional reaction surface of the two double bonds shows that a rotation around the central double bond can be assisted by a partial rotation of the other double bond. This mechanism may be important for the isomerization of the retinylidene chromophore in the confined environment of the protein.

Finally, we described the influence of external point-charges on the mechanism of cis-trans isomerization. Such external point-charge models have been proposed in literature to account for the opsin shift of various intermediates in the photocycles of rhodopsin and bacteriorhodopsin. Our calculations on a model for the retinylidene chromophore show that these point-charges also have a very important impact on the stability of the resonance structures of the twisted molecule and, therefore, on the efficiency of photoisomerization. From these calculations it follows that the primary step in the photoisomerization of bacteriorhodopsin can best be understood from an external point-charge model with a negative charge near the protonated nitrogen atom and an ion pair near the cyclohexene ring. This type of calculations can be very helpful in elucidating the mechanism of the strikingly efficient cis-trans isomerization in the retinylidene chromophore and in developing model systems for an experimental verification of this mechanism.

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