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

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**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 photoisomerization 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 $^1B_u^+$-like excited state, the second $^1A_g^-$-like excited state is particularly photochemical 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 photoisomerization. The transition probability for a radiationless return of the excited molecule to its ground state was evaluated explicitly for the photoisomerization 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 diradicalar ground state which results in an increased energy gap and a reduced efficiency of photoisomerization. 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 photoisomerization 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 photoisomerization in the retinylidene chromophore. The primary step in the photoisomerization 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.

## I. Introduction

The first step of the vision process involves the absorption of light by rhodopsin which transduces the light information into a nerve signal.[1] Bacteriorhodopsin acts as a light-driven proton pump in the purple membrane of the halophilic microorganism *Halobacterium halobium*. It converts the light energy into an electrochemical gradient across the cell membrane.[1]

Rhodopsin and bacteriorhodopsin have in common that they are constructed from a covalent linkage between a protonated Schiff base of retinal and the $\epsilon$-amino group of a lysine residue of the apoprotein opsin. In both systems, the primary step after light absorption involves a photochemical cis-trans isomerization of the retinylidene chromophore.[1] This process is known to proceed on a (sub)picosecond time scale with a high quantum yield of cis-trans isomerization (e.g., $\Phi = 0.7$ for rhodopsin[2]).

In rhodopsin, the conformation of the retinylidene chromophore is 11-cis and upon light absorption it is isomerized to the all-trans form (bathorhodopsin). This conversion is characterized by a red shift of the UV absorption maximum from 498 to 548 nm. Bathorhodopsin is stable below −140 °C and its ground state energy is 35 kcal/mol higher than that of rhodopsin[3] so that ca. 60% of the light energy is stored in this way.

Whereas it is well established that the conformation of the light-adapted form of bacteriorhodopsin ($BR_{568}$) is all-trans, the exact conformation of the primary photoproduct[4] $K_{610}$ is still a matter of dispute,[5] in particular the conformation around the $C_{14}$-$C_{15}$ single bond.[5b,c] Anyway, the conversion from $BR_{568}$ to $K_{610}$ involves the photoisomerization of the $C_{13}$-$C_{14}$ double bond from trans to cis.[6] $K_{610}$ is stable below −120 °C and its ground-state energy is 16 kcal/mol higher than that of $Br_{568}$.[7]

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 photoisomerization.

Freedman and Becker[8] studied the photoisomerization of various isomers of the *n*-butylamine Schiff base of retinal in detail. They

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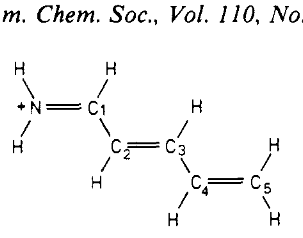
found that the quantum yields for the 9-cis, 11-cis, 13-cis, and all-trans isomers are less than 0.01 in hexane. Going from the nonpolar solvent hexane to the polar solvent methanol, the quantum yields for these isomers increase with a maximum of 0.24 for the 11-cis isomer. Whereas the photoproducts of the cis isomers are always the all-trans isomer, irradiation of the all-trans isomer gives a mixture of the cis isomers ($\phi_{t\rightarrow c} = 0.12$) 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 ($\phi_{c\rightarrow t} = 0.24$, independent of solvent polarity[9]). 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 $C_{2h}$ 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 $^1B_u{}^+$ (the + sign refers to the symmetry of the configurations in the CI expansion of a PPP wave function[10]). The transition to this state is symmetry allowed and is responsible for the strong absorption band in the UV spectra of these polyenes. Additionally, there is an excited state with the same symmetry as the ground state ($^1A_g{}^-$) to which a single photon transition is symmetry forbidden in $C_{2h}$ symmetry so that it is not observed in the UV spectrum. Its presence was first indicated by quantum chemical calculations.[11] In a CI expansion, this state is described by the doubly excited $(\pi$–$\pi^*)^2$ configuration and two singly excited configurations ($\pi_{-1}$–$\pi^*$) and ($\pi$–$\pi^*_{+1}$) in which $\pi_{-1}$ and $\pi^*_{+1}$ refer to the $\pi$-MOs just below the HOMO and just above the LUMO, respectively.

With the advent of two-photon spectroscopy the location of this $^1A_g{}^-$ 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.[12] In valence bond formalism, the wave function of the $^1B_u{}^+$ state is dominated by ionic configurations, whereas the $^1A_g{}^-$ state is mainly built up from covalent configurations.[13] This indicates that the polarity of the solvent and the protonation state of the Schiff base may have a different impact on these two states. This prompted Freedman and Becker[8,9] to explain the observed photochemistry of the isomers of the *n*-butylamine Schiff base of retinal as a function of solvent polarity and protonation state in terms of the mixing of the $^1B_u{}^+$ and $^1A_g{}^-$ states.

By means of INDO–CISD (configuration interaction with single and double excitations) calculations, Birge and Hubbard[14] indicated that particularly the $^1A_g{}^-$ state is photochemically labile for cis–trans isomerization and that its interaction with the $^1B_u{}^+$ state might be responsible for the formation of a barrierless potential energy surface for cis–trans isomerization in this latter state. However, this interaction alone cannot account for the fact that the photoisomerization of the isomers of the (protonated) Schiff bases is confined to one particular double bond. Therefore, we have performed MNDO/CI calculations for the model compound protonated 1-imino-2,4-pentadiene (pentanimine-H$^+$) which has all relevant elements necessary to demonstrate the most important features that control the cis–trans isomerization in protonated Schiff bases.

Potential energy curves of the ground and lower singlet excited states were calculated for the isomerization of the various double bonds in this molecule. The qualitative shape of these curves can be explained from the stability of the resonance structures which



Pentanimine-H$^+$

describe the ground and lowest excited state of the 90° twisted conformations. It is shown that the relative stability of these resonance structures may explain the rate (barrierless potential energy curve) and the quantum yield of isomerization (energy gap and nonadiabatic coupling between the ground and excited state in the 90° twisted region). The nonadiabatic interactions between the various singlet states were calculated explicitly from the multiconfigurational wave functions, which enables the evaluation of the probability for a radiationless transition to the ground state by means of semiclassical trajectory calculations. Finally, some preliminary results are presented which show how the protein can direct the photoisomerization process by supplying external point-charges in the neighborhood of the retinylidene chromophore.

## II. Calculational Method

The dynamics of photoexcited molecules can be studied using a strictly quantum chemical formalism, as we have used before for the description of the dynamics of cis–trans isomerization of small polyenes.[15] 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[16] has been used to study the dynamics of cis–trans isomerization in pentanimine-H$^+$. Similar calculations have been performed by Warshel[17,18] and Birge[14] in the study on the photoisomerization of the retinylidene chromophore. However, their calculations were based on a more or less semiempirical expression for the nonadiabatic coupling. Our calculations make explicit use of calculated nonadiabatic couplings.

In the semiclassical trajectory method,[16] the time-dependent wave function is written as an expansion of the wave functions of the electronic states:

$$|\Psi^{el}(r,\theta(t))\rangle = \sum_K a_K(t)|\Psi_K{}^{el}(r,\theta)\rangle e^{-(i/\hbar)\int_0^t E_K(\theta)d\tau} \qquad (1)$$

In this equation, $|\Psi_K{}^{el}(r,\theta)\rangle$ represents the wave function of state K which depends on the electron coordinates ($r$) 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 $|\Psi^{el}(r,\theta(t))\rangle$ equals $|a_K(t)|^2$. The contribution of the electronic ground state $|a_0(t)|^2$ can be interpreted as the transition probability of the molecule from the excited state to the ground state. The (complex) coefficients $a_K(t)$ are found by substituting eq 1 in the time-dependent Schrödinger equation:

$$i\hbar\frac{\partial}{\partial t}|\Psi^{el}(r,\theta(t))\rangle = H^{el}(r,\theta)|\Psi^{el}(r,\theta(t))\rangle \qquad (2)$$

resulting in a set of differential equations:

$$\dot{a}_L(t) = -\dot{\theta}(t)\sum_{K\neq L} a_K(t)g_{KL}(\theta)e^{-(i/\hbar)\int_0^t(E_K(\theta)-E_L(\theta))d\tau} \qquad (3)$$

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

$$g_{KL}(\theta) = \langle\Psi_K{}^{el}(r,\theta)|\partial/\partial\theta|\Psi_L{}^{el}(r,\theta)\rangle, \qquad (4)$$

The nuclear velocity $\dot{\theta}(t)$ was obtained from the classical equations of motion. In these calculations no energy dissipation to the other degrees

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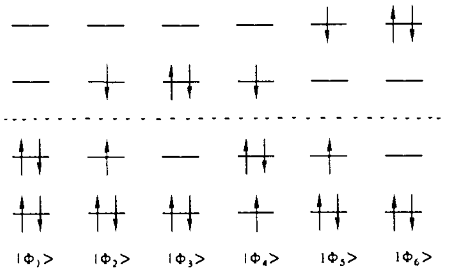
Scheme I




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

$$H = \tfrac{1}{2}I(\theta)\dot{\theta}^2 + E_K(\theta) \quad (5)$$

The angular dependent moment of inertia $I(\theta)$ needed in eq 5 was evaluated by a calculation of the kinetic energy at a constant $\dot{\theta}$ 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 ($|a_1(0)|^2 = 1$), 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 potential energy curves $E_K(\theta)$ were obtained from a MNDO[19] SCF calculation followed by a configuration interaction with the CIPSI[20] algorithm. Starting from a zeroth order wave function including six leading configurations (see Scheme I) 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 conformations. 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 threshold[20] $\eta = 0.0$; 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 ($\mu_{KL}(\theta) = \langle \Psi_K^{el}(r,\theta)|\vec{r}|\Psi_L^{el}(r,\theta)\rangle_r$) and oscillator strengths ($f_{osc} = {}^2/_3\Delta E_{KL}|\mu_{KL}|^2$) from multiconfigurational wave functions.

The nonadiabatic coupling functions $g_{KL}(\theta)$ can be expressed as:[21]

$$g_{KL}(\theta) = g_{KL}^{CI}(\theta) + g_{KL}^{MO}(\theta) \quad (6)$$

In this equation, $g_{KL}^{CI}(\theta)$ involves the differentiation of the CI coefficients and $g_{KL}^{MO}(\theta)$ the differentiation of the molecular orbitals. Both terms were evaluated approximately by a two-point numerical procedure[21] with a stepsize of $\Delta\theta = 0.02°$. The MNDO results were compared[22] with our ab initio calculations[19] 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 ($\pi$–$\pi^*$) excited 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$^+$.

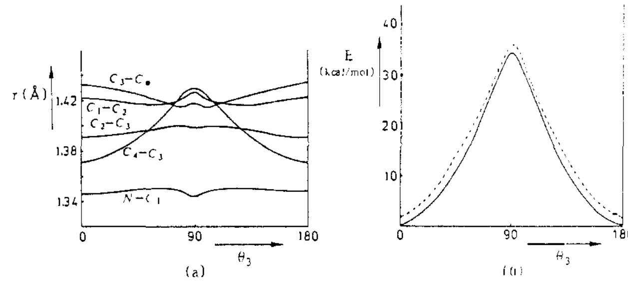



**Figure 1.** Optimal bond lengths (a) for a rotation around the $C_4$–$C_5$ bond in pentanimine-H$^+$ (optimized in $S_0$). Energy curve (b) of the ground state for a rotation around the $C_{4\text{-}C5}$ bond when optimized in $S_0$ (solid line) or $S_1$ (broken line).

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 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 optimal 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 $S_0$ and 34.1 kcal/mol when optimized in $S_1$. 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 ($r_{C_2-C_3} = r_{C_4-C_5} = 1.40$ Å, $r_{C_1-C_2} = r_{C_3-C4} = 1.42$ Å, $r_{N-C1} = 1.35$ Å, $r_{C-H} = 1.10$ Å, $r_{N-H} = 1.00$ 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–$C_1$, $C_2$–$C_3$, and $C_4$–$C_5$ 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 $\theta = 90°$. The rotation around the central $C_2$–$C_3$ bond represents an isomerization from the cis isomer ($\theta_2 = 0°$) to the trans isomer ($\theta_2 = 180°$). 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 maximum[15] ($\theta = 0°$ or $\theta = 180°$). 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 (vide supra), 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 po-

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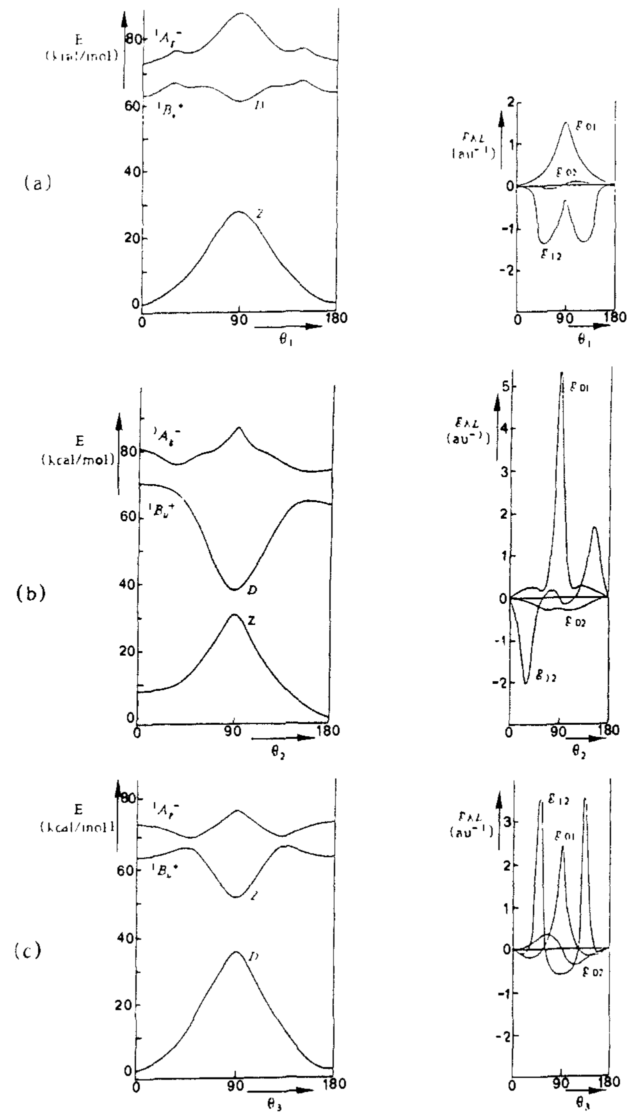


**Figure 2.** Energy curves and nonadiabatic couplings for a rotation around the N–$C_1$ (a), the $C_2$–$C_3$ (b), and the $C_4$–$C_5$ (c) bonds in pentanimine-$H^+$.

tential energy curves decreases. So there are two important features which are necessary to account for a rapid and efficient cis-trans isomerization: a barrierless potential energy curve of the excited state and a minimal energy gap with the ground state for the twisted geometry. These two conditions are best fulfilled for the rotation around the central $C_2$–$C_3$ bond.

Both the shape of the excited-state potential energy curve and the energy gap with the ground state can be explained from the bond densities of the planar geometry and resonance structures of the twisted geometries.

In Figure 3, the charge and bond densities of the carbon skeleton of pentanimine-$H^+$ are shown as calculated from the natural orbitals for each individual electronic state.[25] As indicated in the Introduction, the lowest excited states of (protonated) Schiff bases are related to the low-lying $^1B_u^+$ and $^1A_g^-$ states of trans linear polyenes. For these molecules in $C_{2h}$ symmetry the dipole transition to the former state is strongly allowed, whereas the dipole transition to the latter state is forbidden. Though these symmetry restrictions do not strictly hold for pentanimine-$H^+$, the $^1B_u^+$ state has a large (0.61) and the $^1A_g^-$ state a relatively small (0.11) oscillator strength.

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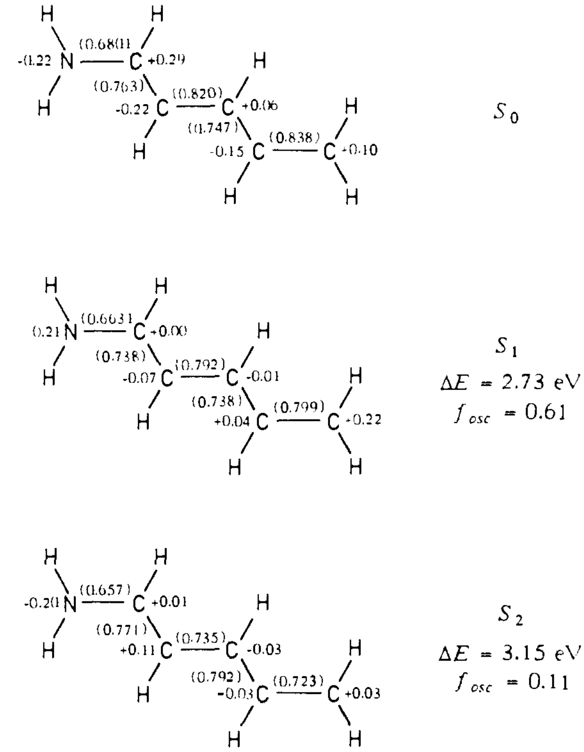
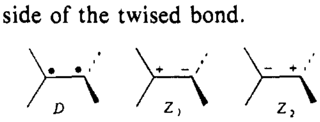



**Figure 3.** Calculated charge and bond densities (in parentheses) of the three lowest singlet states of planar pentanimine-$H^+$.

We found that the $^1B_u^+$ state is the lowest excited state, in agreement with other calculations[14,26] and experimental data[27] 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: $|\Psi_{^1B_u^+}\rangle = 0.89|\Phi_2\rangle + 0.19|\Phi_3\rangle + 0.12|\Phi_4\rangle$; $|\Psi_{^1A_g^-}\rangle = 0.16|\Phi_1\rangle - 0.20|\Phi_2\rangle + 0.55|\Phi_3\rangle + 0.41|\Phi_4\rangle + 0.39|\Phi_5\rangle + 0.18|\Phi_6\rangle$ (the configurations in these CI expansions are those of Scheme I). This mixing explains the relatively large oscillator strength of the $^1A_g^-$ state. The $^1A_g^-$ state has important contributions from the doubly excited configurations $|\Phi_3\rangle$ and $|\Phi_6\rangle$ and, therefore, has a more antibonding character than the $^1B_u^+$ state. This is reflected in the larger decrease in bond densities of the double bonds going from the ground state to the $^1A_g^-$ state compared to the $^1B_u^+$ state (Figure 3). This effect is more accentuated for the $C_2$–$C_3$ and $C_4$–$C_5$ bonds than for the N–$C_1$ bond, which implicates that a rotation around the former two bonds is energetically favored in the $^1A_g^-$ state. As the $^1A_g^-$ state is located above the $^1B_u^+$ 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 $g_{12}$ coupling functions of Figure 2.

The effect of this avoided crossing is that the potential energy curve of the absorbing state ($^1B_u^+$) is bent downwards. *Though the $^1A_g^-$ 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 $^1B_u^+$ 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°. Three singlet configurations can be generated from a distribution of the frontier two $\pi$-electrons over the two centra on each side of the twised bond.




(26) Schulten, K.; Dinur, U.; Honig, B. *J. Chem. Phys.* **1980**, *73*, 3927. Dinur, U.; Honig, B.; Schulten, K. *Chem. Phys. Lett.* **1980**, *72*, 493. Shoda, T.; Noro, T.; Nomura, T.; Ohno, K. *Int. J. Quantum Chem.* **1986**, *30*, 289.
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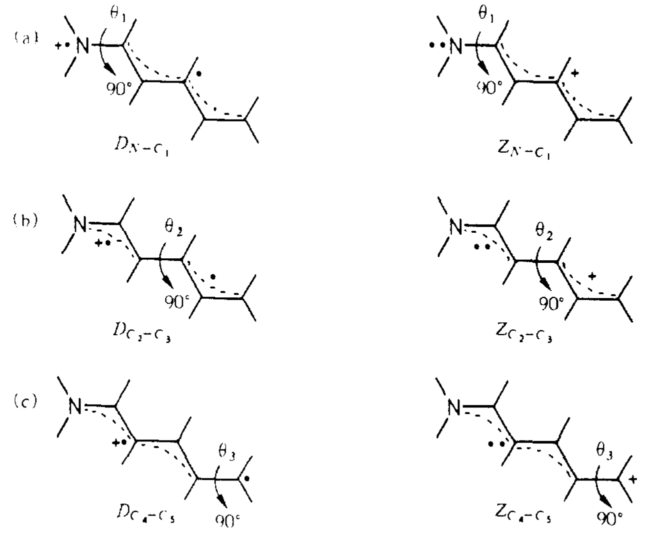

Figure 4. Resonance structures for pentanimine-$H^+$ 90° twisted around the $N-C_1$ (a), the $C_2-C_3$ (b), or the $C_4-C_5$ (c) bond. Because of the positive charge of the molecule, it is difficult to identify a particular resonance structure as a diradical or a zwitterion. For convenience, they are identified as D or Z merely indicating that the two former $\pi$ electrons are located at different sides or at the same side of the twisted bond, respectively. The subscripts indicate the bond which is 90° twisted.

In the first configuration, one electron is located on each side. Therefore, this configuration is a diradical (D) and has no net dipole moment. In the latter two configurations, the two electrons are located on one side of the twisted bond. These configurations are zwitterionic (Z) and have a strong dipole moment. The first time this polarization of charge upon twisting an excited double bond was theoretically described,[28] it was found to occur in a small region around $\theta = 90°$. Therefore, this effect was called "sudden polarization". Many calculations[15,29] at various levels of theory have confirmed this effect, but showed that the polarization already develops in an earlier stage so that the term "sudden" is somewhat confusing.

In the limiting case of a twisted symmetric alkene (like ethylene or *trans*-hexatriene twisted around the central double bond), there is no difference between $Z_1$ and $Z_2$, and the electronic wave functions of $S_1$ and $S_2$ are essentially linear combinations of these two resonance structures. Therefore, the excited states of these symmetric molecules do not have a dipole moment. When this symmetry is broken by introducing a substituent on one side of the twisted bond, the former degeneracy is lifted and one of the resonance structures is energetically stabilized whereas the other is destabilized. As a result, the energy gap between $S_1$ and $S_2$ increases and their wave functions now resemble $Z_1$ and $Z_2$.

Such a situation is fulfilled for (protonated) Schiff bases. The resonance structure with the two electrons at the side of the electron-deficient nitrogen atom (the N end) is strongly stabilized. This stabilization may even become so large that the Z state drops below the D state so that the ground state now has the characteristics of the zwitterionic configuration.[30] For pentanimine-$H^+$ 90° twisted around the $N-C_1$, the $C_2-C_3$, or the $C_4-C_5$ bond, the resonance structures are given in Figure 4. The relative energy

(28) Bonačić-Koutecký, V.; Bruckmann, P.; Hiberty, P.; Koutecký, J.; Leforestier, C.; Salem, L. *Angew. Chem., Int. Ed. Engl.* **1975**, *14*, 575.

(29) See e.g.: Bruckmann, P.; Salem, L. *J. Am. Chem. Soc.* **1976**, *98*, 5037. Baraldi, J.; Bruni, M. C.; Momicchioli, F.; Ponterini, G. *Chem. Phys.* **1980**, *52*, 415. Dormans, G. J. M.; Fransen, H. R.; Buck, H. M. *J. Am. Chem. Soc.* **1984**, *106*, 1213. Pogliani, L.; Niccolai, N.; Rossi, C. *Chem. Phys. Lett.* **1984**, *108*, 597. Kikuchi, O.; Yoshida, H. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 131. Brooks, B. R.; Schaefer, H. F., III *J. Am. Chem. Soc.* **1979**, *101*, 307. Nebot-Gil, I.; Malrieu, J.-P. *Ibid.* **1982**, *104*, 3320. Karafiloglou, P.; Hiberty, P. C. *Chem. Phys. Lett.* **1980**, *70*, 180. Petsalakis, I. D.: Theodorakopoulos, G.; Nicolaides, C. A; Buenker, R. J.; Peyerimhoff, S. D. *J. Chem. Phys.* **1984**, *81*, 3161.

(30) Salem, L.; Leforestier, C.; Segal, G.; Wetmore, R. *J. Am. Chem. Soc.* **1975**, *97*, 479. Bonačić-Koutecký, V.; Köhler, J.; Michl, J. *Chem. Phys. Lett.* **1984**, *104*, 440. Bonačić-Koutecký, V.; Koutecký, J.; Michl, J. *Angew. Chem.* **1987**, *99*, 216.

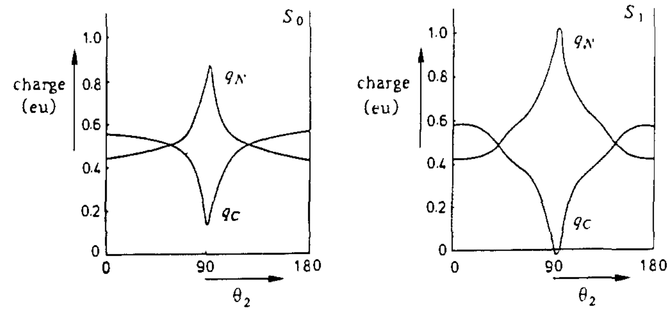


Figure 5. Calculated charge densities of the ground state ($S_0$) and first excited state ($S_1$) for a rotation around the $C_2-C_3$ bond ($\theta_2$) in pentanimine-$H^+$. The charge densities are summed for all atoms at the N end ($q_N$) and the C end ($q_C$) of the molecule.

sequence of the D and Z states can be derived from the stability of the two fragments on each side of the twisted bond:

$$D_{N-C_1} < D_{C_2-C_3} < D_{C_4-C_5}$$

$$Z_{N-C_1} > Z_{C2-C3} > Z_{C_4-C_5}$$

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 ($\theta_2$) 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 ($\theta_2$). It clearly shows the polarization of charge and it follows that at $\theta_2 = 90°$ 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 $|\Phi_3\rangle$ of the planar molecule. Because the ${}^1A_g^-$ state has a large contribution from this configuration, it is obvious that the Z structure at $\theta = 90°$ correlates diabatically with the ${}^1A_g^-$ state at $\theta = 0°$. Therefore, the energy curve of the ${}^1A_g^-$ 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 elecronic state K equals $|a_K(t)|^2$. The contribution of the ground state $|a_0(t)|^2$ 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.[31] We have calculated these contributions $a_K(t)$ for the various isomerizations in pentanimine-$H^+$.

For the rotation around the $C_4-C_5$ bond (Figure 2c), there is a strong nonadiabatic interaction between the two excited states at $\theta_3 = 50°$ and $\theta_3 = 130°$, as can be seen from the peaks in the nonadiabatic coupling function $g_{12}(\theta_3)$ at these angles. As a result, we found that the contribution of the second excited state to the total wave function $|a_2(t)|^2$ increased to a value of 0.60. On the other hand, the contribution of the electronic ground state $|a_0(t)|^2$ 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

(31) The quantum yield of isomerization can be calculated from the expression[17] $\Phi = (1 - f)/(2 - |a_0(t)|^2)$, where $f$ 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 $C_4$–$C_5$ bond, a calculation of the contributions of the electronic states for a rotation around the central $C_2$–$C_3$ bond showed that in this case the transition probability to the ground state is much larger ($|a_0(t)|^2 = 0.30$). This is a direct result of the small energy gap and correspondingly large nonadiabatic coupling at $\theta_2 = 90°$ (see Figure 2b). From the value of 0.30, a quantum yield of isomerization of 0.59 is calculated[31] (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 $S_0$ and $S_1$, and the slope of the diabatic energy curve of the ${}^1A_g{}^-$ state. In its turn, the shape of this curve determines the shape of the energy curve of the absorbing ${}^1B_u{}^+$ 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 allylimine,[32] and it gives a possible explanation for the photochemical behavior of the protonated Schiff bases of retinal.[8,9]

**B. Multiple Double Bond Rotations in Pentanimine-H⁺.** It has been argued by several research groups[18,33] 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" model[18]) or the simultaneous isomerization of a double bond and an adjacent single bond (the "hula twist" model[33]). From an inspection of the bond densities of the excited states of pentanimine-$H^+$ (Figure 3), it follows that particularly a simultaneous rotation around the two carbon–carbon double bonds should be energetically favorable.[34] Therefore, we performed MNDO/CI calculations for the two-dimensional reaction surfaces for the twist around these bonds ($\theta_2$ and $\theta_3$). The results are shown in Figures 6 and 7. The energy curves for the twist around the $C_2$–$C_3$ and $C_4$–$C_5$ bonds as shown in Figure 2 are essentially the sections of the two-dimensional surfaces which correspond to $\theta_3 = 180°$ and $\theta_2 = 180°$, respectively.

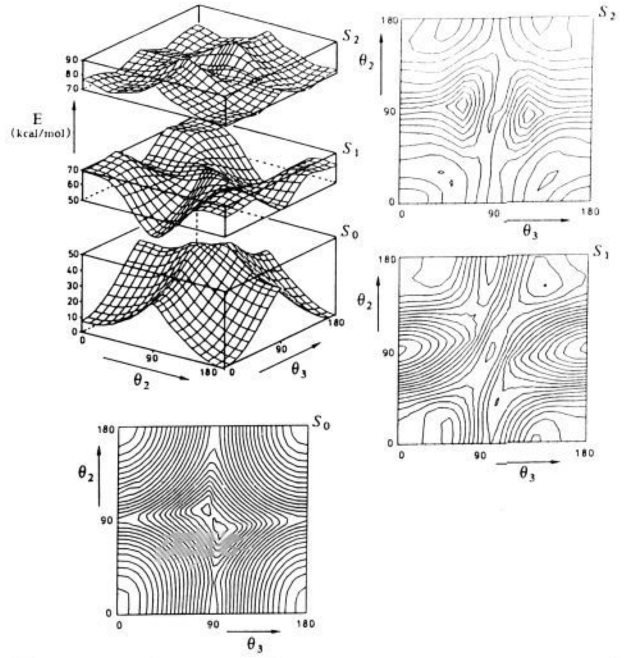


**Figure 6.** Potential energy surfaces and corresponding contour maps of $S_0$, $S_1$, and $S_2$ for a simultaneous rotation around the $C_2$–$C_3$ ($\theta_2$) and $C_4$–$C_5$ ($\theta_3$) bonds. The energy difference between two contour lines in the contour maps is 2 kcal/mol.

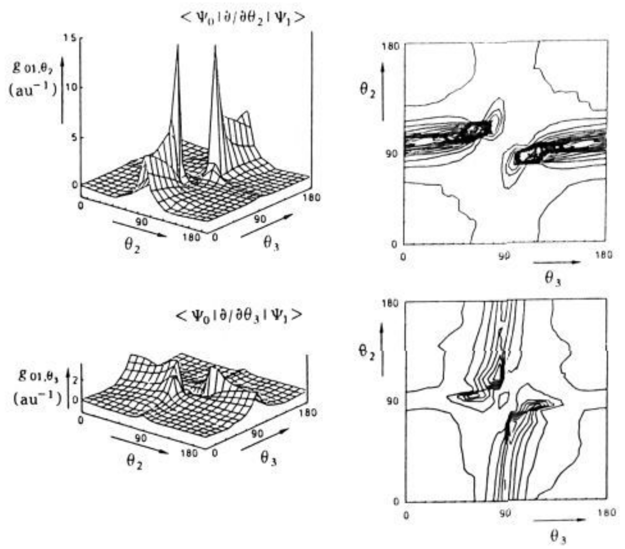


**Figure 7.** Two-dimensional plot of the nonadiabatic couplings $g_{01,\theta_2}$ and $g_{01,\theta_3}$ and corresponding contour maps for a simultaneous rotation around the $C_2$–$C_3$ ($\theta_2$) and $C_4$–$C_5$ ($\theta_3$) bonds. The difference between two contour lines in the contour maps is 0.5 au$^{-1}$.

From an inspection of Figure 6 it is seen that there is a region near $\theta_2 = \theta_3 = 90°$ where the energy of $S_1$ 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 $S_0$ and $S_1$ going from $\theta_2 = \theta_3 = 0°$ (180°) to $\theta_2 = \theta_3 = 90°$. To demonstrate this, the potential energy curves representing a simultaneous isomerization for the two bonds out of phase ($\theta_2 = -\theta_3$) are given in Figure 8.

This latter isomerization is especially interesting to describe the cis–trans isomerization of the retinylidene chromophore, where

(32) Childs, R. F.; Dickie, B. D. *J. Chem. Soc., Chem. Commun.* **1980**, 1268.

(33) Liu, R. S. H.; Asato, A. E. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 259. Liu, R. S. H.; Mead, D.; Asato, A. E. *J. Am. Chem. Soc.* **1985**, *107*, 6609. Liu, R. S. H.; Matsumoto, H.; Asato, A. E.; Mead, D. *Ibid.* **1986**, *108*, 3796. Liu, R. S. H.; Browne, D. *Acc. Chem. Res.* **1986**, *19*, 42.

(34) A concerted rotation around a double bond and an adjacent single bond seems to be unlikely in the excited state, but it might be feasible for the ground-state processes which follow the primary photoisomerization of the retinylidene chromophore.

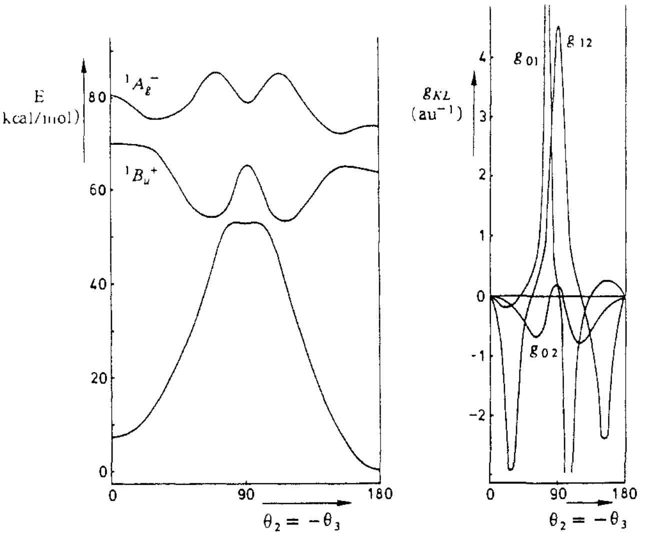


Figure 8. Potential energy curves and nonadiabatic couplings for a simultaneous out-of-phase rotation ($\theta_2 = -\theta_3$) around the $C_2$–$C_3$ and $C_4$–$C_5$ bonds in pentanimine-$H^+$.

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 this motion is almost identical with that for the rotation around the $C_2$–$C_3$ bond alone. Evidently, the orientation of the terminal $CH_2$ fragment does not influence this moment of inertia significantly. However, the effect of a combined rotation will be much larger in the situation where the molecule is linked to a heavy fragment or a protein.

The relevant resonance structures for the doubly twisted structure can be deduced from the occupation of the MOs for this geometry (Figure 9). For $S_0$ and $S_1$ the bond between $C_2$–$C_3$ and $C_4$–$C_5$ has become a real double bond. The resonance structures for these states differ mainly by the location of the positive charge in the molecule. This already indicates that these states will be differently influenced by a charged environment.

The potential energy curve for the out-of-phase rotation of the two double bonds (Figure 8) shows that this motion is unfavorable in both the ground and the excited state. The potential energy barrier in the excited state is 11.2 kcal/mol. Besides, there is a very strong nonadiabatic coupling between the two states for a twist angle less than 90°. The dynamic calculations for this combined rotation showed that the contribution of the ground state ($|a_0(t)|^2$) reaches a value of 0.60 for this twist angle. When the molecule crosses over to the ground state at this angle, the potential energy curve of this state will direct it back to the initial conformation. The number of trajectories which will cross the energy barrier on the potential energy curve of $S_1$ and enter the second region of strong coupling is smaller. A crossing to the ground state in this second region will lead to the isomerized product. Therefore, the quantum yield of cis–trans isomerization via this coupled motion will be relatively small.

In Figure 7 the nonadiabatic couplings $g_{01,\theta_2}(\theta_2,\theta_3) = \langle\Psi_0|\partial/\partial\theta_2|\Psi_1\rangle$ and $g_{01,\theta_3}(\theta_2,\theta_3) = \langle\Psi_0|\partial/\partial\theta_3|\Psi_1\rangle$ between the ground and first singlet excited state are shown for the two-dimensional reaction surface. It can be seen that $g_{01,\theta_2}$ depends more strongly on $\theta_2$ than on $\theta_3$. The opposite holds for $g_{01,\theta_3}$. In regions where the nonadiabatic coupling is large, the probability for a radiationless transition is relatively large (provided that the nuclear velocity is large as well; vide supra). Therefore, any combined rotation bringing the molecule in such a region may represent a trajectory which is favorable for a radiationless transition. A trajectory is imaginable in which the $C_2$–$C_3$ bond isomerizes from $\theta_2 = 0°$ to $\theta_2 = 180°$ while in the meantime the $C_4$–$C_5$ bond rotates partly to a twist angle of approximately 60° and then rotates back.[35] Such a mechanism would generate a route with a large transition probability. It has the advantage that the overall reaction only involves the isomerization of one double bond, as is observed for rhodopsin. The other 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 more 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 $BR_{568}$ to dark-adapted $BR_{548}$.[36] Resonance Raman spectra[5a] 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–$C_1$ and $C_2$–$C_3$ 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.[5b]

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 (the two former $\pi$ electrons at 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 $S_0$ and $S_1$ 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 $S_1$ and $S_0$, 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. Freedman and Becker[8,9] 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 $^1B_u^+$ state into the $^1A_g^-$ 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. For the protonated *n*-butylamine Schiff base of retinal this seems to be the $C_{11}$–$C_{12}$ double bond[8] ($\Phi_{c\to t} = 0.24$, $\Phi_{t\to c} = 0.14$). When the chromophore is embedded in opsin, the quantum yield of isomerization in rhodopsin is strongly increased[37] ($\Phi_{c\to t} = 0.67$, $\Phi_{t\to c} = 0.50$), whereas for bacteriorhodopsin it is found that,

(35) Warshel, A.; Barboy, N. *J. Am. Chem. Soc.* **1982**, *104*, 1469.

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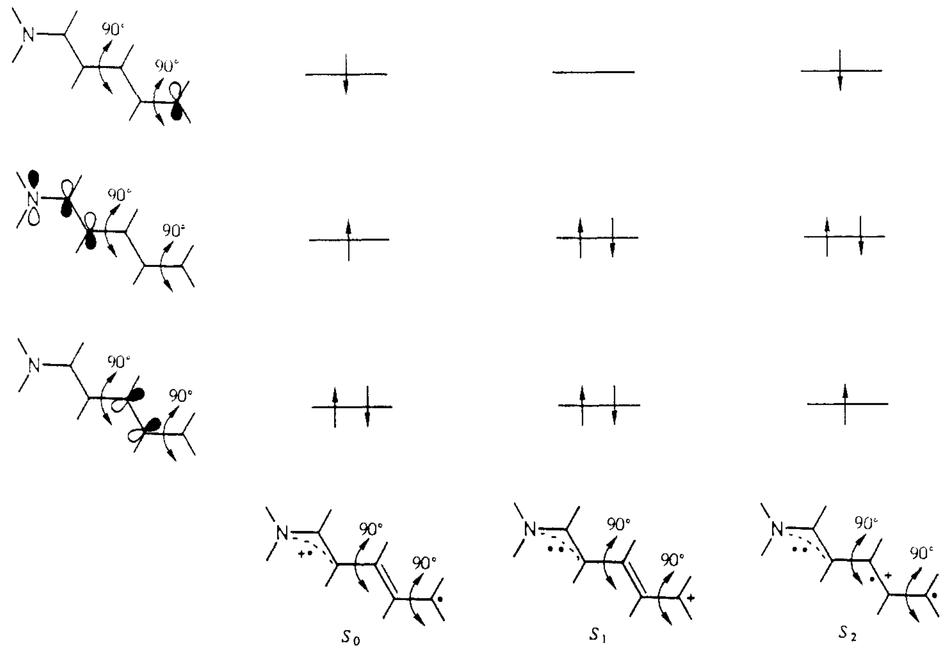
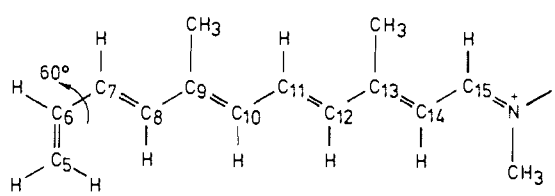



**Figure 9.** Frontier molecular orbitals, occupation of these molecular orbitals and derived resonance structures for the $S_0$, $S_1$, and $S_2$ states of pentanimine-$H^+$ 90° rotated around the $C_2$–$C_3$ and the $C_4$–$C_5$ bond.

instead of the $C_{11}$–$C_{12}$ double bond, the $C_{13}$–$C_{14}$ 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[1,38-40] have been put forward by several research groups to explain the shift of the UV absorption maximum (opsin shift[38]) for the various intermediates in the photocycles of rhodopsin and bacteriorhodopsin, the vibrational spectra of these intermediates,[5b] and the isomerization barriers in the ground state.[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-$H^+$) representing




RET-$H^+$

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.[42] This restricted molecule is found to be a suitable model for the study of the properties of the protonated Schiff base of retinal.[23]

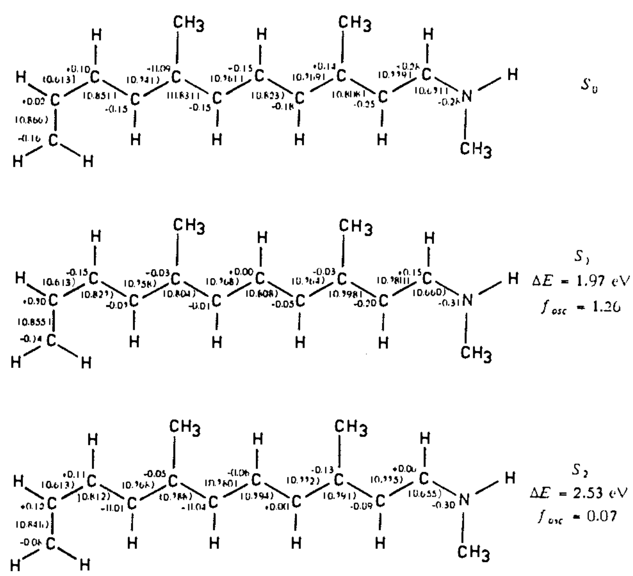



**Figure 10.** Calculated charge and bond densities (in parentheses) for the three lowest singlet states of RET-$H^+$.

Figure 10 shows the charge and bond densities of the lowest singlet states of RET-$H^+$. We found that the $^1B_u^+$ 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.[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 $^1B_u^+$ and the $^1A_g^-$ state is 0.56 eV which is considerably smaller than the energy gap calculated by Birge et al.[44] (1.54 eV) but agrees much better with the observed value of ca. 0.25 eV for 11-cis-rhodopsin.[44] The calculated oscillator strengths show that the one-photon absorption will take place almost entirely to the $^1B_u^+$ state.

(38) Nakanishi, K.; Balogh-Nair, V.; Arnaboldi, M.; Tsujimoto, K.; Honig, B. *J. Am. Chem. Soc.* **1980**, *102*, 7945. Motto, M. G.; Sheves, M.; Tsujimoto, K.; Balogh-Nair, V.; Nakanishi, K. *Ibid.* **1980**, *102*, 7947.

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(41) Seltzer, S. *J. Am. Chem. Soc.* **1987**, *109*, 1627.

(42) Most crystal structures of retinal analogues reveal an angle of 40–60° between the polyene chain and the cyclohexene ring: Simmons, C. J.; Asato, A. E.; Liu, R. S. H. *Acta Crystallogr., Sect. C* **1986**, *342*, 711. For bacteriorhodopsin, NMR data of ref 43 argue for a 6-s-trans chromophore. One idealized geometry was used for all calculations: $r_{C=C} = 1.40$ Å; $r_{C-C} = 1.42$ Å; $r_{H_3C-C} = 1.50$ Å; $r_{H-C} = 1.10$ Å; $r_{N-C} = 1.35$ Å; $r_{N-H} = 1.00$ Å; $r_{H_3C-N} = 1.46$ Å; $\theta_{H_3C-C-C} = 122°$; $\theta_{H-C-C} = 116°$; $\theta_{H-N-C} = 117°$; $\theta_{H_3C-N-C} = 126°$.

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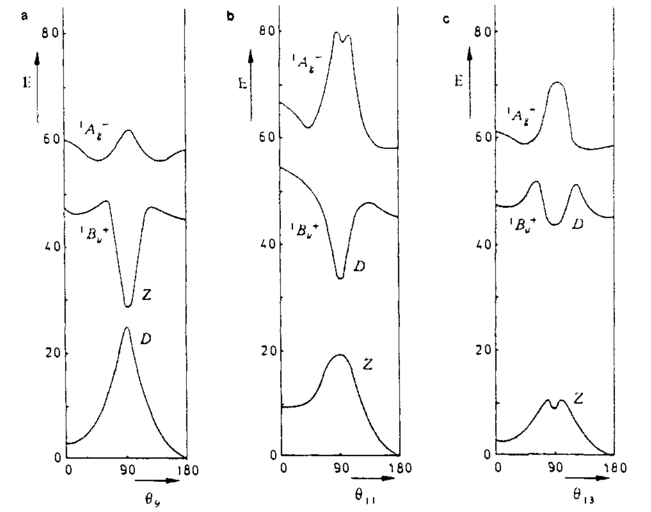


Figure 11. Potential energy curves for the rotation around the $C_9$–$C_{10}$ (a), the $C_{11}$–$C_{12}$ (b), and the $C_{13}$–$C_{14}$ (c) bonds in RET-H$^+$. Energies are in kcal/mol.

An inspection of the changes in the bond densities going from the ground state to the $^1B_u^+$ ($S_1$) and $^1A_g^-$ ($S_2$) states shows that it is the latter state which has the most antibonding character. Therefore, the $^1A_g^-$ 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 $C_{14}$–$C_{15}$ single bond for which the bond density slightly decreases ($S_0 \rightarrow S_2$). 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[45] for the primary photoreaction in bacteriorhodopsin. On the other hand, a simultaneous twist around two double bonds[18] seems plausible from these calculations.

The calculated potential energy curves for a rotation around the $C_9$–$C_{10}$, the $C_{11}$–$C_{12}$, and the $C_{13}$–$C_{14}$ 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$^+$. Both the energy barrier on the potential energy curve of the first excited state and the energy gap between $S_0$ and $S_1$ for the 90° twisted molecule strongly depend on the distance from the twisted bond to the nitrogen atom. The energy barriers for the trans ($\theta$ = 180°) to cis ($\theta$ = 0°) 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° 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° twisted around the $C_9$–$C_{10}$ 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° twisted around the $C_{11}$–$C_{12}$ and $C_{13}$–$C_{14}$ bonds, the Z structure is so strongly stabilized that it has become the ground state.

It follows from the energy curves that both the size of the energy barrier in $S_1$ and the energy gap with $S_0$ favor an isomerization around the $C_9$–$C_{10}$ double bond for RET-H$^+$. However, experiments[8] for this compound show a preferred isomerization of the $C_{11}$–$C_{12}$ double bond. This discrepancy probably raises from the fact that the influence of the solvent is not included in this calculation (vide supra). A solvent will increase the transition energy to the $^1B_u^+$ state and, consequently, increase the energy gap for RET-H$^+$ 90° twisted around the $C_9$–$C_{10}$ double bond and decrease this value for RET-H$^+$ 90° twisted around the $C_{11}$–$C_{12}$ and $C_{13}$–$C_{14}$ double bonds.

A comparison of the energy curves for the cis isomers ($\theta$ = 0°) shows an additional element which directs the photoisomerization. Because of the steric hindrance between the methyl group at $C_{13}$ and hydrogen atom at $C_{10}$, the energy curve for the isomerization from 11-cis ($\theta_{11}$ = 0°) to all-trans ($\theta_{11}$ = 180°) is barrierless in the excited state. The calculated effect is overestimated (as is obvious from the energy difference of 9.3 kcal/mol between the 11-cis and the all-trans isomers[47]) because the molecule was not optimized for the 11-cis isomer. No such steric hindrance is present for the 9-cis and 13-cis isomers so that this feature offers an additional reason for the efficient cis—trans isomerization in the 11-cis isomer of the protonated Schiff base of retinal.[8]

It is generally accepted that the proton at the Schiff base of the retinylidene chromophore interacts with a negatively charged counterion.[1] The presence of this negative charge is of great importance for the stability of the resonance structures of the twisted molecule. To demonstrate this, we calculated[48] the energy curves for a rotation around the $C_9$–$C_{10}$, the $C_{11}$–$C_{12}$ and the $C_{13}$–$C_{14}$ double bonds of RET-H$^+$ with a negative charge at a distance of 3 Å from the proton at the nitrogen atom in the all-trans conformation (see Scheme II). These calculations mimic the situation for bacteriorhodopsin. During the rotation of the double bonds, the negative point-charge was fixed at its initial position. The calculated energy gaps between $S_0$ and $S_1$ in the various orthogonal conformations are given in Table I.

The effect of the negative point-charge is to stabilize the D structures (with the N end positively charged) and to destabilize the Z structures (with the C end positively charged). As a matter of course, this stabilization is more effectuated for small distances between the twisted molecule and the negative charge. This distance decreases going from the molecule 90° twisted around the $C_9$–$C_{10}$ double bond to the molecule 90° twisted around the $C_{13}$–$C_{14}$ double bond. Therefore, the effect of the negative charge is largest for this latter isomerization. The energy gap between $S_0$ and $S_1$ for RET-H$^+$ 90° twisted around the $C_{13}$–$C_{14}$ double

Scheme II

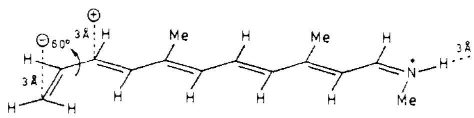


Table I. Influence of External Point-Charges on the Energy Gap between $S_0$ and $S_1$ for RET-H$^+$ (1) 90° Twisted around the $C_9$–$C_{10}$, $C_{11}$–$C_{12}$, and $C_{13}$–$C_{14}$ Bonds, RET-H$^+$ with a Negative Charge Fixed at 3 Å from the Proton at the Nitrogen Atom in the All-Trans Isomer (2), an Additional Negative Charge at 3 Å above $C_5$ (3), and an Additional Positive Charge 3 Å above $C_7$ (4) (see Scheme II)[a]

| | $C_9$–$C_{10}$ | $C_{11}$–$C_{12}$ | $C_{13}$–$C_{14}$ |
|---|---|---|---|
| **1** | 3.1 (Z) | 13.5 (D) | 34.8 (D) |
| **2** | 6.1 (Z) | 8.9 (D) | 14.9 (D) |
| **3** | 16.8 (D) | 22.8 (D) | 37.3 (D) |
| **4** | 16.9 (Z) | 4.1 (Z) | 12.3 (D) |

[a] The energies are in kcal/mol. D and Z refer to the character of the 90° twisted molecule in the excited state (see text).

(45) Schulten, K.; Tavan, P. *Nature (London)* **1978**, *212*, 85.
(46) Huppert, D.; Rentzepis, P. M. *J. Phys. Chem.* **1986**, *90*, 2813.

(47) A separate geometry optimization of both isomers yields an energy difference between the all-trans and 11-cis isomers of ca. 4 kcal/mol. The energy difference with the 9-cis and 13-cis isomers is ca. 1 kcal/mol.[20]
(48) The effect of external point-charge was introduced by adding so-called sparkles to the molecular frame. The sparkles represent pure unpolarizable ionic elements with an integer nuclear charge of +1 or −1. The calculations with sparkles were performed with the AMPAC program package, using the MNDO parametrization.[15] AMPAC (QCPE 506): Dewar, M. J. S.; Stewart, J. J. P. *QCPE Bull.* **1986**, *6*, 24.

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 $C_{13}$–$C_{14}$ double bond (as is the case for the photoisomerization of $BR_{568}$ to $K_{610}$). A much smaller effect is observed for the isomerizations around the $C_9$–$C_{10}$ and the $C_{11}$–$C_{12}$ 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.,[38] 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 $S_0$ and $S_1$ 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 $C_5$ (**3**) shows that this is indeed the case (Table I). The energy gap for the $C_{13}$–$C_{14}$ 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.[40] have argued that the negative charge near the $C_5$ position in the cyclohexene ring is accompanied by an additional positive charge near $C_7$. Their conclusions were based on the opsin shift of some dihydro derivatives of retinal in bacterioopsin and are supported by NMR experiments of the same groups.[43] A calculation of the energy gap between $S_0$ and $S_1$ for RET-$H^+$ with a negative charge near the protonated nitrogen atom, a negative charge 3 Å above $C_5$, and a positive charge 3 Å above $C_7$ (**4**) shows that the isomerization around the $C_{13}$–$C_{14}$ double bond now becomes favorable again (see Table I). A similar effect is found for the $C_{11}$–$C_{12}$ 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 steric restrictions specific for protein substrate interaction. Some information regarding Schiff base–protein interaction can be substracted from the NMR data of Harbison et al.[49] 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[40,43] 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 relative 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 ($^1B_u^+$) excited state, the second ($^1A_g^-$) 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 diradicalar 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 → trans and trans → 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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