

Dynamical Theory of Photoisomerization of the Rhodopsin Chromophore: Generation of a Transient Electric Field during Photoisomerization

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Photoisomerization of the rhodopsin chromophore is one of the typical nonadiabatic transitions. We have proposed a new dynamical theory of photoisomerization of the chromophore based on quantum mechanics. We have considered the transient excited state DES (deformed excited state: Ψ_D) of electrons, which has different lengths for stabilization from those in the excited state immediately after the absorption of light, together with the lowest electronic state VGS (virtual ground state: Ψ_V), which has the same bond lengths as Ψ_D . We have also considered the normal vibrational states $\{U_{ij}(\Theta)\}$ associated with these transient electronic states $\{\Psi_i(\Theta)\}$ (Θ represents the angle of *cis*–*trans* isomerization). With these preliminary assumptions, the nonstationary state $\Phi(t)$ of the chromophore has been expressed as an expansion in $\{\Psi_i(\Theta)U_{ij}(\Theta)\}$, and the time-dependent equation for its coefficients $\{b_{ij}(t)\}$ has been derived rigorously by explicitly taking into account the kinetic energy of atomic cores. On these bases, in the present study we calculate a transient electric field caused by the change of the net charges in the chromophore during photoisomerization. To examine the effect of this field on the protein moiety around the chromophore, we calculate by way of example the interaction between formamide and a constant electric field. The results demonstrate that the net charges in formamide are considerably affected by the constant electric field. We then apply this calculation to a bacteriorhodopsin model and also demonstrate the change of the net charges in the amino acids near the chromophore. Finally, we point out that the change of the net charges in the amino acids and the vibrational–vibrational interaction between the chromophore and its surrounding opsin are crucial.

1. Introduction

When light is absorbed by rhodopsin, the rhodopsin chromophore isomerizes from the 11-*cis* to the all-*trans* form during a period of approximately 10^{-12} s.^{1,2} This photoisomerization can be considered to be one of the typical nonadiabatic transitions. According to the Landau–Zener theory,^{3–5} when a nonadiabatic transition takes place in one system, there must be a process of relaxation of energy between the degrees of freedom in that system and the others. Considering the isomerization period, one of the relaxation processes of an excited chromophore is probably the stretching vibration of every bond. That is, the stretching vibrations of bonds promote the nonadiabatic transition from the first excited state to the ground state. Thus, the light-absorbing energy can be partially transferred through interactions between the stretching vibrations of excited chromophore and its surrounding moiety.

On the other hand, it is well-known that the net charge on the atoms of the conjugation system in the chromophore change during photoisomerization. This phenomenon was first reported by Salam and Bruckmann.^{6,7} Subsequently, the polarization of charge in both the first excited state and the ground state were also discussed by Birge *et al.* and Suzuki *et al.*^{8,9} The transient electric field, which is caused by this rapid change in net-charge distribution in the chromophore, gives rise to the change of the electronic state of the amino acid residues near the chromophore and of the vibrational modes in opsin. In short, the change in net-charge distribution during photoisomerization might be one of the critical factors for the relaxation process of the excited chromophore and the conformational change in opsin, together with the above mentioned vibrational energy flow.

To confirm the effect of the transient electric field that the chromophore generates, quantum chemical treatment on the photoisomerization of the rhodopsin chromophore and on the protein opsin must be carried out. Many studies on photoisomerization of the rhodopsin chromophore have been reported, and above all the recent experimental researches give good information on photoisomerization of the chromophore.^{2,10–12} A change in the conformation of opsin during and after isomerization of the chromophore, however, has not been discussed theoretically because the three-dimensional structure of opsin is unknown and there are only a few theoretical studies.^{8,13–21} In the present paper, dynamical treatment of photoisomerization of the rhodopsin chromophore is proposed on the basis of quantum mechanics, and subsequently the time-dependent electronic fields that the chromophore generates during the *cis*–*trans* isomerization are calculated. Their effects on the amino acids around the chromophore are discussed.

First, we have considered the transient excited state DES (deformed excited state: Ψ_D) of electrons, which has different bond lengths for stabilization from those in the excited state immediately after the absorption of light, together with the lowest electronic state VGS (virtual ground state: Ψ_V), which has the same bond lengths as Ψ_D .^{22–25} We have also considered the normal vibrational states $\{U_{ij}(\Theta)\}$ associated with these transient electronic states $\{\Psi_i(\Theta)\}$ (Θ represents the angle of the *cis*–*trans* isomerization).^{26–28} With these preliminary assumptions, the nonstationary state $\Phi(t)$ of the chromophore has been expressed as a linear combination of $\{\Psi_i(\Theta)U_{ij}(\Theta)\}$, and the time-dependent equation for its coefficients $b_{ij}(t)$ has been derived rigorously by explicitly taking into account the kinetic energy of the atomic cores.²⁹

Second, the matrix \mathbf{G} defined by Wilson to determine the normal vibrational states $\{U_{ij}(\Theta)\}$ as functions of Θ have been

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calculated using only the stretching and torsional vibrations, which have the most important role in photoisomerization. Then, noticing that the integrals in the basic equation for $\{b_{rj}(t)\}$ can be stored into two types, A and B, being related to $\{\Psi_r(\Theta)\}$ and $\{U_{rj}(\Theta)\}$ respectively, approximations for calculating the integrals of type A have been proposed. On the other hand, as for the integrals of type B, we have shown that all of the type-B integrals can be exactly reduced to the Franck-Condon factors.³⁰ We have thus been able to calculate the nonstationary state $\Phi(t)$ and expectation value $W(\Theta)$ of energy of the chromophore together with the probability $P_{\text{DES}}(\Theta)$ [$P_{\text{VGS}}(\Theta)$] of finding the chromophore in Ψ_{D} [Ψ_{V}].²⁹

In the present study, our previously obtained results are first presented and then the change of net charges in the chromophore during the *cis-trans* isomerization are calculated. It is thus shown that a transient current is induced in the chromophore by the *cis-trans* isomerization and that the origin of the transient current is attributed not only to a rapid reciprocating motion of the net charges in Ψ_{D} but also to a large polarization current of the net charges in Ψ_{V} . Also, to discuss the effect of the transient current on the protein moiety around the chromophore, the interaction of formamide with a constant external electric field is determined as a preliminary calculation, and it is suggested that the net charge on atom O is especially affected by the electric field. The effect of electric field changes on the protein is calculated by applying it to a more realistic model. The model is a protein bacteriorhodopsin whose structure is known and is similar to rhodopsin.³¹ The result is that the net charges on C, N, and O in amino acids near the bacteriorhodopsin chromophore change together with the change of the net-charge distribution in the chromophore.

2. Method

All the calculations of $\{\Psi_r(\Theta)\}$ and $\{U_{rj}(\Theta)\}$ performed by Suzuki *et al.* are based on the model of the rhodopsin chromophore, shown in Figure 1: (a) the negative point charge e is always in the plane of PRSB linkage ret - N₁₆⁺ ops (PRSB representing protonated retinal Schiff-base); (b) its direction from N₁₆⁺ is along the N₁₆⁺ - H bond; (c) the distance of the point charge from N₁₆⁺ is chosen such that the calculated wavelength of the absorption maximum of 11-*cis*-PRSB with e agrees with the observed absorption maximum of 500 nm; (d) both the conjugated chain from C₅ to C₁₁ and that from C₁₂ to N₁₆⁺ are assumed to be coplanar during the *cis-trans* isomerization.

The determination of $\{\Psi_r(\Theta)\}$ by Suzuki *et al.* is based on their own LCAO-ASMO-SCF-CI method for π -electrons, of which the characteristic can be summarized as follows: (1) in each stage of the iteration process for solving the ASMO-SCF equation, each bond length is modified as the function of its bond order; (2) corresponding with this treatment for bond lengths, all the molecular integrals in the ASMO-SCF equation are also modified as the respective functions of electron densities in each stage of the iteration process; (3) a sufficient number of the singly- and doubly-excited configurations are taken into account in the CI calculation.⁹ On the other hand, the normal vibrational states $\{U_{rj}(\Theta)\}$ associated with $\{\Psi_r(\Theta)\}$ have been determined by using three kinds of force constants for pairs of bonded atoms, which were calculated beforehand by means of the same method mentioned above.²⁶⁻²⁸

Let us now denote $\Psi_s(\Theta)$ more precisely by $\Psi_s(\mathbf{r}, \mathbf{R})$, collecting the set of position vectors of electrons or atomic cores conveniently in vector \mathbf{r} or \mathbf{R} . Let us also denote $U_{sk}(\Theta)$ by $U_{sk}(\mathbf{Q}_s)$, transforming the internal displacement coordinate \mathbf{d} of the atomic cores into normal coordinate \mathbf{Q}_s associated with

$\Psi_s(\mathbf{r}, \mathbf{R})$ by the relation $\mathbf{d} = \mathbf{L}_s \mathbf{Q}_s$. The nonstationary state $\Phi(t)$ of the chromophore is then expressed as

$$\Phi(t) = \sum_s \sum_k b_{sk}(t) U_{sk}(\mathbf{Q}_s) \Psi_s(\mathbf{r}, \mathbf{R}) \exp[-(i/\hbar)(E_s^{(0)} + E_{sk})t] \quad (1)$$

denoting the energies of Ψ_s and U_{sk} by $E_s^{(0)}$ and E_{sk} respectively. The equation for the coefficients $\{b_{sk}(t)\}$ is derived to be

$$\begin{aligned} \dot{b}_{rj} = & (i\hbar/2) \sum_s \sum_k \langle \Psi_r | \nabla(\mathbf{d})^T \mathbf{G} \nabla(\mathbf{d}) \Psi_s \rangle \langle U_{rj} | U_{sk} \rangle f_{rsjk} b_{sk} \\ & + i\hbar \sum_s \sum_k \langle \Psi_r | \nabla(\mathbf{d})^T \Psi_s \rangle \mathbf{G} (\mathbf{L}_s^{-1})^T \langle U_{rj} | \nabla(\mathbf{Q}_s) U_{sk} \rangle f_{rsjk} b_{sk} \\ & - \dot{\Theta} \sum_s \sum_k \langle \Psi_r | \partial \Psi_s / \partial \Theta \rangle \langle U_{rj} | U_{sk} \rangle f_{rsjk} b_{sk} \\ & - \dot{\Theta} \sum_k [(\mathbf{L}_r^{-1})^T]_{\Theta} \langle U_{rj} | \nabla(\mathbf{Q}_r) U_{yk} \rangle f_{rrjk} b_{rk}, \end{aligned}$$

$$f_{rsjk} = \exp\{i[(E_r^{(0)} - E_s^{(0)}) + (E_{rj} - E_{sk})]t/\hbar\} \quad (2)$$

Here, the index T denotes the transpose of a vector or matrix; \mathbf{G} is the well-known \mathbf{G} -matrix defined by Wilson,³² Θ is the angle of *cis-trans* isomerization around the C₁₁-C₁₂ bond, $\dot{\Theta}$ is assumed to be constant and is equal to 180°/T, T being the period of the photoisomerization; $[(\mathbf{L}_r^{-1})^T]_{\Theta}$ is a row vector that satisfies the relation $\partial/\partial \Theta = [(\mathbf{L}_r^{-1})^T]_{\Theta} \nabla(\mathbf{Q}_r)$.

Next, confining ourselves to only DES and VGS and expressing them by Ψ_{D} and Ψ_{V} respectively, the basic eq 2 is approximated as follows:²⁹

$$\begin{aligned} \dot{b}_{Dj} = & i[\langle \Psi_{\text{D}} | \partial^2 \Psi_{\text{V}} / \partial \Theta^2 \rangle \sum_k T_{jk}^{(1)} \\ & + \langle \Psi_{\text{D}} | \partial \Psi_{\text{V}} / \partial \Theta \rangle \sum_k T_{jk}^{(2)}] f_{\text{DV}jk} b_{\text{V}k} \\ & - \dot{\Theta} \langle \Psi_{\text{D}} | \partial \Psi_{\text{V}} / \partial \Theta \rangle \sum_k T_{jk}^{(3)} f_{\text{DV}jk} b_{\text{V}k} - \dot{\Theta} \sum_k T_{jk}^{(4)} f_{\text{DD}jk} b_{\text{D}k} \end{aligned} \quad (3)$$

$$T_{jk}^{(1)} = (\hbar/2) G_{18,18} \langle U_{Dj} | U_{\text{V}k} \rangle \quad (4)$$

$$T_{jk}^{(2)} = \hbar \mathbf{G}_{18} (\mathbf{L}_{\text{V}}^{-1})^T \langle U_{Dj} | \nabla(\mathbf{Q}_{\text{V}}) U_{\text{V}k} \rangle \quad (5)$$

$$T_{jk}^{(3)} = \langle U_{Dj} | U_{\text{V}k} \rangle \quad (6)$$

$$T_{jk}^{(4)} = [(\mathbf{L}_{\text{D}}^{-1})^T]_{\Theta} \langle U_{Dj} | \nabla(\mathbf{Q}_{\text{D}}) U_{\text{D}k} \rangle \quad (7)$$

$$f_{\text{DV}jk} = \exp\{i[(E_{\text{D}}^{(0)} - E_{\text{V}}^{(0)}) + (E_{Dj} - E_{\text{V}k})]t/\hbar\} \quad (8)$$

Here $G_{18,18}$ represents the (18,18)-component of matrix \mathbf{G} ; \mathbf{G}_{18} represents the 18th row vector of \mathbf{G} . After the calculations of integrals $\langle \Psi_{\text{D}} | \partial \Psi_{\text{V}} / \partial \Theta \rangle$, $\langle \Psi_{\text{D}} | \partial^2 \Psi_{\text{V}} / \partial \Theta^2 \rangle$, and $\langle U_{Dj} | \partial U_{\text{V}k} / \partial \Theta \rangle$, eq 3 is solved to obtain the probability, $P_{\text{DES}}(\Theta)$ [$P_{\text{VGS}}(\Theta)$], of the chromophore being in DES [VGS]:

$$P_{\text{DES}}(\Theta) = \sum_j |b_{Dj}(\Theta)|^2, \quad P_{\text{VGS}}(\Theta) = 1 - P_{\text{DES}}(\Theta) \quad (9)$$

The net charge $Q_{\mu}(\Theta)$ on the atomic core μ in the chromophore is calculated as a function of Θ in terms of the relation

$$Q_{\mu}(\Theta) = P_{\text{DES}}(\Theta) Q_{\mu, \text{DES}}(\Theta) + P_{\text{VGS}}(\Theta) Q_{\mu, \text{VGS}}(\Theta) \quad (10)$$

where $Q_{\mu, \text{DES}}(\Theta)$ [$Q_{\mu, \text{VGS}}(\Theta)$] is the net charge on the core μ in DES [VGS].

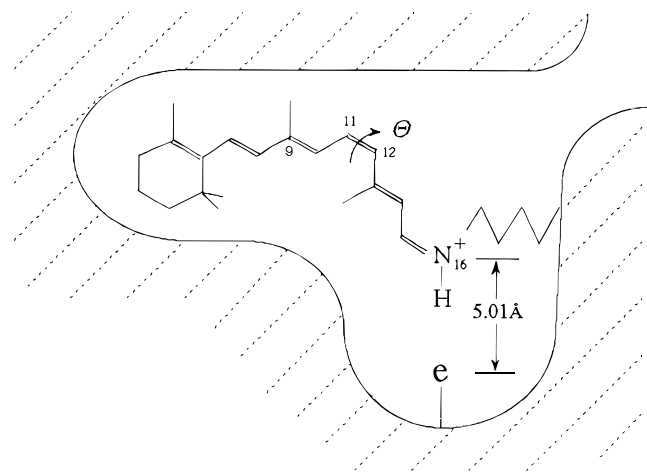


Figure 1. Schematic representation of Suzuki *et al.*'s model for the rhodopsin chromophore. Here, 11-*cis*-PRSB interacts with a negative point charge e (being taken equal to the electronic charge).

In the present paper, to investigate the effect of the change of net-charge distribution in the chromophore on the amino acids around the chromophore, we calculate the electronic state of these amino acids, considering the charges in the chromophore as the perturbation.

First, as a preliminary calculation, the net charge of C, N, or O in formamide is calculated according to the extended INDO-CI method, interacting with the constant external electric field whose values are almost the same as the inside of the protein. Then, the change in net charge of the amino acids is confirmed by applying the same method to a more realistic system, bacteriorhodopsin, whose three-dimensional structure is known and is similar to rhodopsin. Three amino acids THR89, THR90, and PRO91 in helix C and ALA184, TYR185, and PRO186 in helix F, both of which are near the chromophore, are utilized to calculate the change of the electronic structure.

A hydrogen atom is added to the N terminus of THR89 (or ALA184), and a methyl group CH_3 is substituted for a hydroxyl group OH at the C terminus of PRO91 (or PRO186), because the hydroxyl group OH strongly attracts electrons. The three-dimensional structure data (2BRD) from the Protein Data Bank (PDB) is applied to our calculation for these three amino acids. The hydrogen atoms in the amino acids are added and optimized by using the MNDO-PM3 method. Then, maintaining the geometries of the three amino acids with optimized hydrogens, we calculate their electronic states, including the effect of the net-charge distribution in the chromophore, according to the extended INDO-CI method.

Actually, when the chromophore isomerizes and its geometry changes, the structure during the isomerization is still not known. We can discuss the approximate effect of the charge distribution in the chromophore, however, by using the electric field. Therefore, the interaction of the three above-mentioned amino acids with the external electric field produced by the chromophore is studied. The positive direction of the electric field is from C_{12} in the chromophore to C_α in THR90 (703CA) in the case of THR89-THR90-PRO91, and from C_8 to C_α in TYR185 (1433CA) in the case of ALA184-TYR185-PRO186. Namely, the net-charge distributions of the chromophore during isomerization are described as the changes of the electric fields and are discussed below.

3. Results

The Θ -dependence of $\langle \Psi_D | \Psi_V / \partial \Theta \rangle$, together with the adiabatic potentials of Ψ_D and Ψ_V , calculated by Suzuki *et al.* for

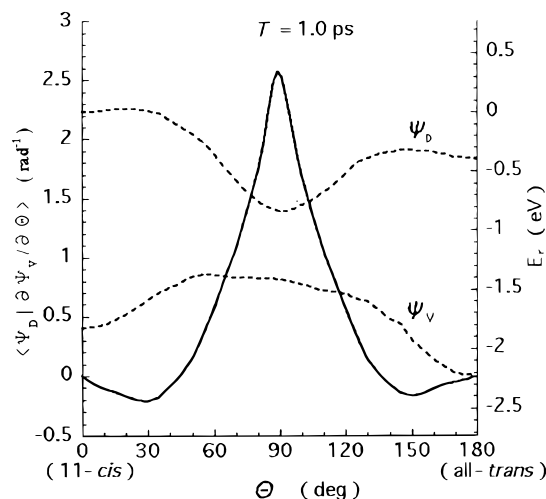


Figure 2. (Solid curve) value of $\langle \Psi_D | \partial \Psi_V / \partial \Theta \rangle$ calculated as a function of Θ . (Broken curves) adiabatic potentials of Ψ_D and Ψ_V . The vertical axis on the right-hand side is used for the broken curves, choosing the total energy of DES of the 11-*cis* form to be zero.

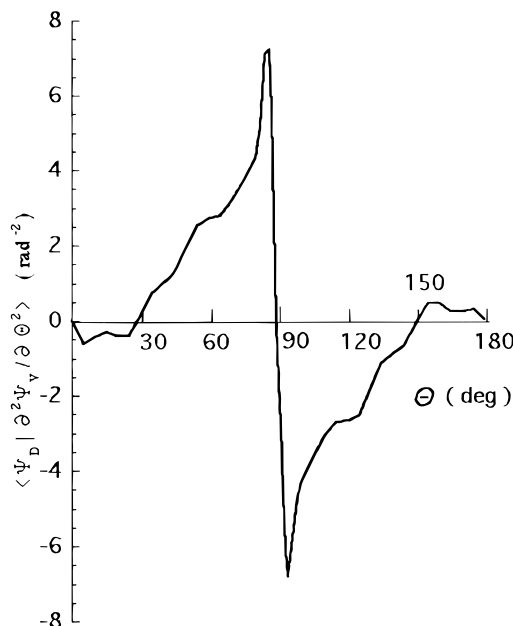


Figure 3. Value of the integral $\langle \Psi_D | \partial^2 \Psi_V / \partial \Theta^2 \rangle$ calculated as a function of Θ .

the *cis*–*trans* isomerization of 11-*cis*-PRSB with e (see Figure 1) are shown in Figure 2. The integral $\langle \Psi_D | \partial \Psi_V / \partial \Theta \rangle$ has high values in the vicinity of $\Theta = 90^\circ$ and hence has a very important role in the nonadiabatic transition from Ψ_D to Ψ_V . The calculations for the adiabatic potentials around the other bonds (not C_{11} – C_{12}) were also performed, but in these cases, it was found that the nonadiabatic transitions from Ψ_D to Ψ_V cannot occur.⁹ The dependence of $\langle \Psi_D | \partial^2 \Psi_V / \partial \Theta^2 \rangle$ on Θ is also shown in Figure 3, indicating that this integral is as important as $\langle \Psi_D | \partial \Psi_V / \partial \Theta \rangle$ (note that the discard of $\langle \Psi_D | \partial^2 \Psi_V / \partial \Theta^2 \rangle$ is equivalent to the neglect of the significant vibronic interaction $\langle \Psi_r | \nabla(\mathbf{d})^T \mathbf{G} \nabla(\mathbf{d}) \Psi_s \rangle$ in the equation (2)).³⁰

Table 1 shows some of the values of $T_{jk}^{(1)}$ (in s^{-1} units), $T_{jk}^{(2)}$ (in s^{-1} units), $T_{jk}^{(3)}$, or $T_{jk}^{(4)}$ (see eq 4–7) which we calculated at $\Theta = 0^\circ$ or 70° , taking into account the lowest 50 vibrational states of both Ψ_D and Ψ_V (each vibrational state is constructed from 22 modes of the stretching-torsional vibrations). The values of $T_{jk}^{(3)}$ just represent those of the Franck–Condon factors; the respective terms including $T_{jk}^{(1)}$, $T_{jk}^{(2)}$, and

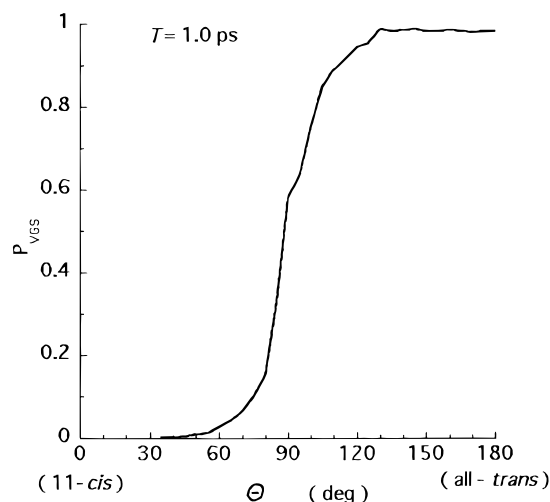


Figure 4. Probability of the nonadiabatic transition from DES to VGS calculated as a function of Θ for the case of $T = 1.0$ ps in eq 2.

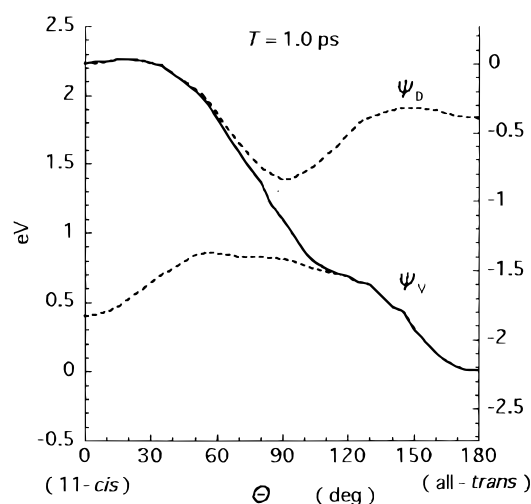


Figure 5. Expectation value $W(\Theta)$ of energy calculated as a function of Θ . The broken curves represent the same adiabatic potentials as shown in Figure 2. The vertical axis on the right (left)-hand side is used for the solid curve (broken curves), choosing the total energy of DES of the 11-*cis* form (VGS of all-*trans* form) to be zero.

$T_{jk}^{(3)}$ are concerned with the transition probability from Ψ_D to Ψ_V ; the term containing $T_{jk}^{(4)}$ has to do with the transition between the vibrational states associated with Ψ_D and Ψ_V . It is found from Table 1 that the values of $T_{jk}^{(1)}$ and $T_{jk}^{(2)}$ are on the order of 10^{11} s^{-1} and that $T_{jk}^{(3)}$ and $T_{jk}^{(4)}$, which are coupled with $\dot{\Theta} = 180^\circ/T$ (note that $1 \text{ ps} \leq T < 10 \text{ ps}$ in our calculation), have values on the order of 10^0 . Therefore, when we calculate the probability of the nonadiabatic transition from Ψ_D to Ψ_V , we have to take into account the respective terms including $\langle \Psi_D | \partial \Psi_V / \partial \Theta \rangle$ and $\langle \Psi_D | \partial^2 \Psi_V / \partial \Theta^2 \rangle$.

Figure 4 shows the transition probability from Ψ_D to Ψ_V (i.e., the probability $P_{VGS}(\Theta)$ defined by eq 9) calculated for the case of $T = 1 \text{ ps}$. On the basis of this result, the expectation value $W(\Theta)$ of energy for the nonstationary state $\Phi(t)$ is calculated (the result is shown in Figure 5).²⁹ Thus, we find that the radiant energy absorbed by the chromophore is transferred to opsin during and after photoisomerization to induce its conformational change.

Figure 6 shows the change in net charges $\{Q_\mu(\Theta)\}$ of eq 10 during the photoisomerization, denoted by Q_{5-11} [Q_{12-16}], the fractional sum of net charges on C₅ to C₁₁ [C₁₂ to N₁₆]. This result indicates that a transient current is induced in the chromophore by the *cis*–*trans* isomerization and that the origin

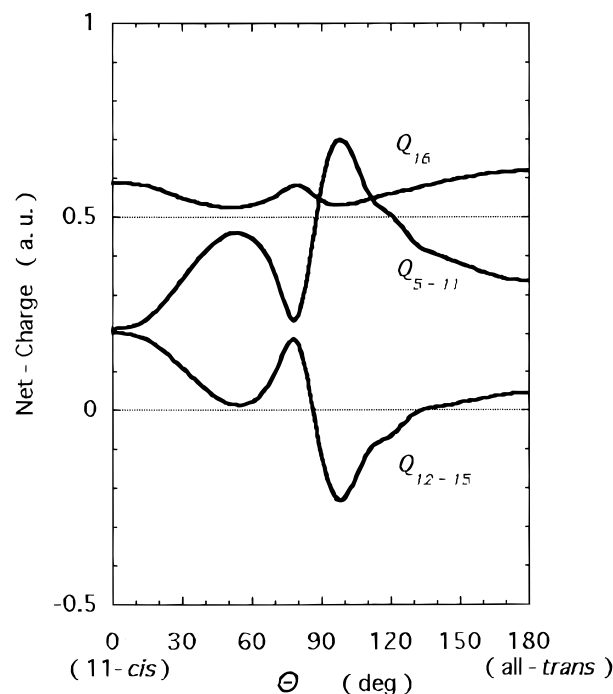


Figure 6. Change in net-charge (in atomic units) distribution during the photoisomerization of the chromophore. See the text for the definitions of Q_{5-11} and Q_{12-16} .

of this transient current is attributed not only to a rapid reciprocating motion of the net charges in Ψ_D but also to a large polarization current of the net charges in Ψ_V .⁹

Then, Θ -dependence of the transient electric field $\mathbf{E}(\mathbf{r}, \Theta)$ at point \mathbf{r} which is produced by the net charges $\{Q_\mu(\Theta)\}$ is calculated according to the relation

$$\mathbf{E}(\mathbf{r}, \Theta) = \sum_{\mu=1}^{16} Q_\mu(\Theta) [\mathbf{r} - \mathbf{R}_\mu(\Theta)] / |\mathbf{r} - \mathbf{R}_\mu(\Theta)|^3 \quad (11)$$

where $\mathbf{R}_\mu(\Theta)$ is the position vector of the atomic core μ . In this calculation the geometric configuration of the chromophore model is assumed as shown in Figure 7, where the β -ionone ring side is rotated around the C₁₁–C₁₂ bond and the point at which the X-component E_x of \mathbf{E} has a large value is denoted by A, B, or C. The respective Θ -dependencies of E_x are thus calculated at the three points. Figure 8 shows the difference between the maximum and minimum values in each of the Θ -dependencies is approximately $2 \times 10^7 \text{ V/cm}$. Thus, the nearer the observation point of E_x is to the chromophore, the greater the magnitude of E_x .

To investigate the role of the transient electric field E_x (see Figure 8), we first consider the interaction of formamide with the external electric field and then calculate the change of net charge Q_C , Q_N , or Q_O on atom C, N, or O in formamide as a function of the magnitude of the constant electric field E_x applied along the X-axis according to the extended INDO-CI method, assuming the geometric configuration as shown in Figure 9. The results obtained, shown in Figure 10 indicate that the net charge Q_O on atom O is especially affected by the constant electric field E_x in which the magnitude is approximately 10^8 V/cm .

The same calculations are then applied to a more realistic model, bacteriorhodopsin. The two groups of three amino acids, which are near the chromophore in bacteriorhodopsin, THR89-THR90-PRO91 in the C helix and ALA184-TYR185-PRO186 in the F helix, are now discussed (see Figure 11). Focusing on

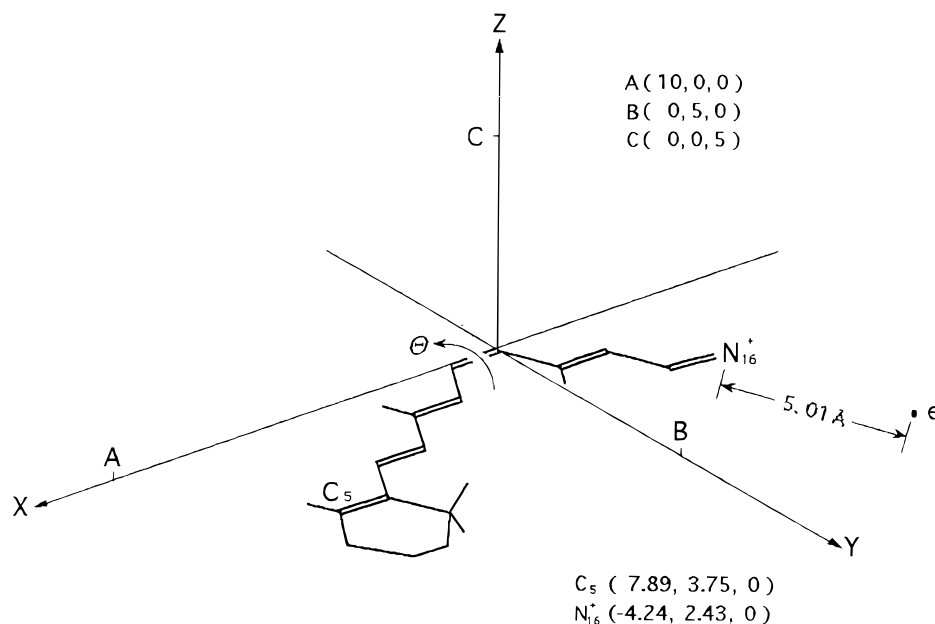


Figure 7. Rectangular coordinates (in Å units) of point A, B, or C, at which the X-component E_x has high values.

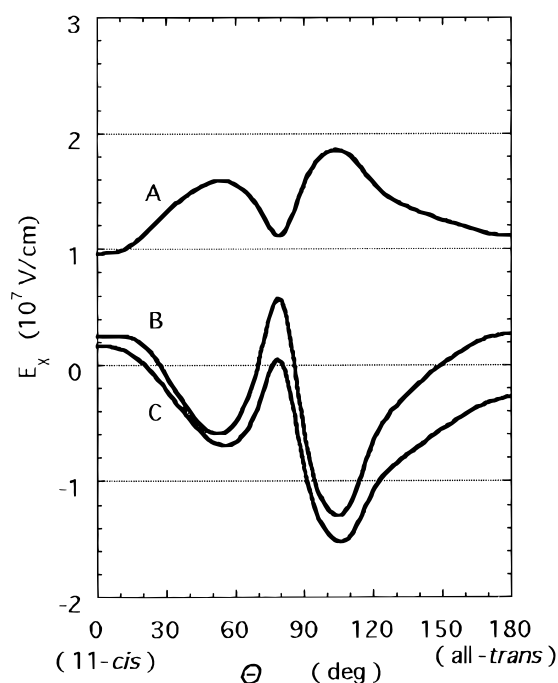


Figure 8. Dependence of E_x on Θ at the respective points A, B, and C.

the middle amino acid of the three, the changes of net charges in THR90 are shown in Figure 12, and those of TYR185 in Figure 13. It is found that the net charge on atom O is especially affected. The net charges on O of the main chains, except THR90 and TYR185, are also shown. The distance between THR90 and the chromophore is nearly equal to that between TYR185 and the chromophore. As for the changes of net charges without O, however, it is evident that TYR185 is influenced by the electric field more strongly than THR90. The changes of net charges at the aromatic ring in TYR185 are of particular interest as they may be concerned with energy relaxation of the excited chromophore. In addition, this change and the change of net charge on O in ALA184 are also expected to give rise to a change of vibrational modes in this part of the protein. These changes might be associated with the mechanism of the proton pump at the next stage.

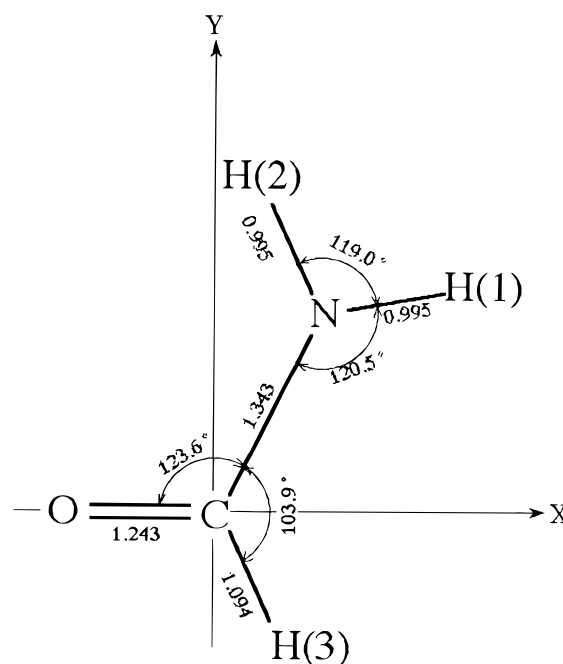


Figure 9. Bond lengths (in Å units) and bond angles of formamide taken from the microwave analysis.³³

4. Discussion

To understand how the protein rhodopsin performs its role as a receptor, its three-dimensional structure might be determined at the atomic level. Upon absorbing light, the apoprotein opsin undergoes a change in conformation that allows it to bind and activate the G-protein, transducin. The first step of this change in conformation is photoisomerization of the rhodopsin chromophore. Accordingly, it is likely that the electronic structure and the vibrational modes at the amino acid residues in opsin change during and after the isomerization of the chromophore.

In the present paper, we confine ourselves to an examination of the change in π -electronic net charge on the conjugated system of the chromophore and calculate the transient electric field that the chromophore generates during and after its isomerization. The results indicate that the change of magni-

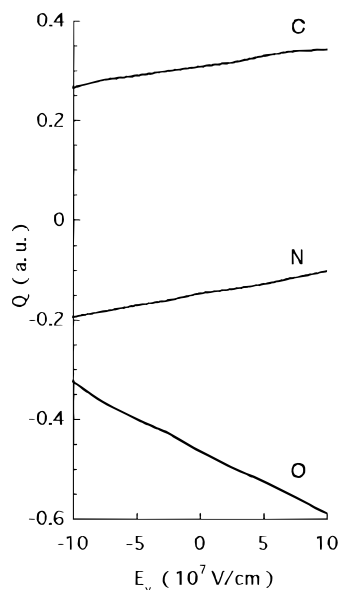


Figure 10. Change of net charge Q_C , Q_N , or Q_O (in atomic units) on atom C, N, or O in formamide of Figure 9 calculated as a function of the magnitude of the constant electric field E_x applied along the X -axis.

tude, which is approximately 2×10^7 V/cm, occurs at the points that are 5 Å from the conjugated systems. As for the chromophore model shown in Figure 7, that the β -ionone ring side is rotated around the C_{11} – C_{12} bond is not significant from the viewpoint of theoretical simulation. The point charge e in Figure 7 is not likely to be a specified amino acid. The structure of the chromophore during photoisomerization is still unknown. All we know is that we can explain adiabatic potentials which lead to the nonadiabatic transition from DES to VGS, using the point-charge model which Suzuki *et al.* have proposed. Therefore, how the magnitude of the change in the electric field affects the amino acid residues near the chromophore is significant, and a virtual electric field, estimated from Figure

8, is used to evaluate the change of the electronic state of the amino acids in opsin. Furthermore, the concrete structural change in the chromophore during isomerization is another issue to consider.

Thus, to investigate the change in the electronic structure of an amino acid due to an external electric field, the change of net charges on atoms in formamide due to the interaction with an electric field is examined according to the extended INDO-CI method³⁴ for valence-shell electrons and the perturbation theory. The bond lengths and bond angles of formamide are shown in Figure 9, and the external electric field is directed along the x -axis. As shown in Figure 10, the net charge Q_O or Q_N on atom O or N is quite remarkable. This figure also indicates that in the case of rhodopsin, the interaction between opsin and the transient current in the chromophore exerts a considerable influence not only on the electronic structure of opsin but also on the internal electric field acting on the chromophore, that is, the reaction field.

In the case of bacteriorhodopsin, TYR185 is influenced. It is found from the results of this calculation that the amino acid residue TYR185 has different vibrational modes following isomerization of the chromophore. From a theoretical approach, the proton pump mechanism cannot be explained. The mechanism can be attributed to the conformational change in bacteriorhodopsin, however, and the change in the conformation of rhodopsin will also be similar.

The mechanism of the conformational change might be as follows: (i) the period of photoisomerization of the chromophore is, at most, 1 ps; as such, the stretching vibrations in the chromophore will interact with the same vibrational modes in opsin and a part of the light-absorbing energy will be transferred. (ii) The chromophore acts on its surrounding moiety, opsin, in the process of the change of net-charge distribution, and has lower electronic energy, changing its structure from 11-*cis* to all-*trans*. On the other hand, opsin absorbs the energy in processes i and ii. The energy in i confers some specific

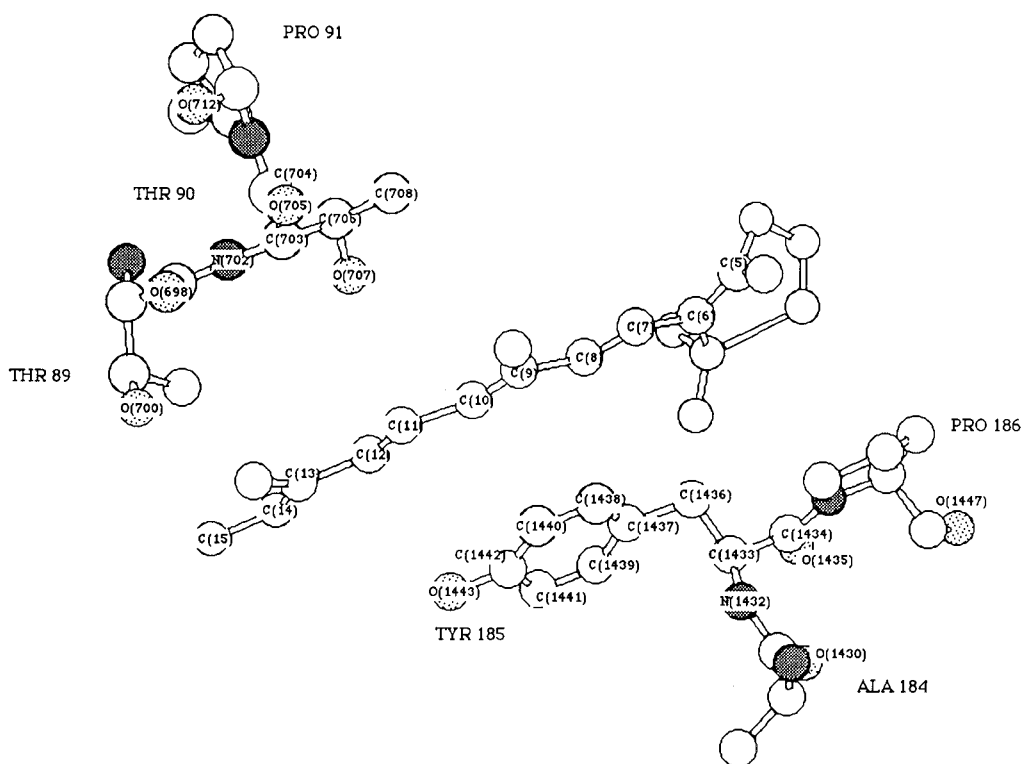


Figure 11. Relation between the positions of the chromophore, THR89-THR90-PRO91, and ALA84-TYR185-PRO186 in bacteriorhodopsin on the basis of the Protein Data Bank.

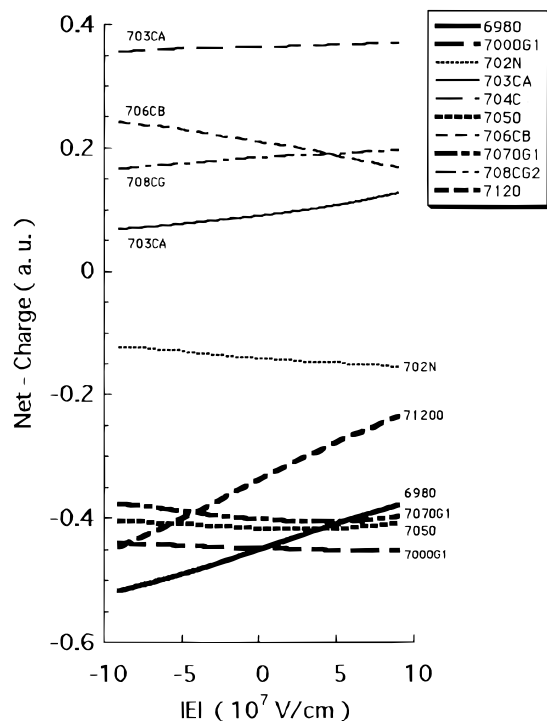


Figure 12. Change of net charge (in atomic units) on atom C, N, or O in THR90 and on atom O in THR89 or in PRO91 in Figure 11 calculated as a function of the magnitude of the constant electric field. The positive direction of the external electric field is from C(12) in the chromophore to C(703) in THR90 in Figure 11.

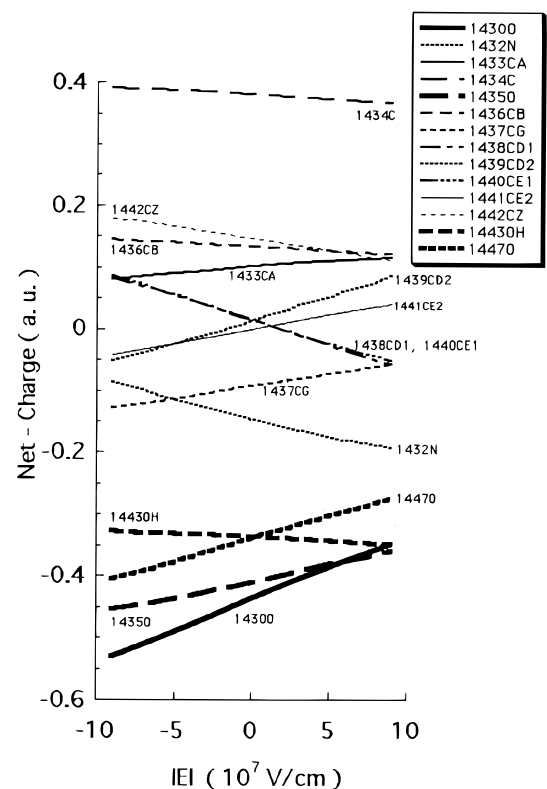


Figure 13. Change of net charge (in atomic units) on atom C, N, or O in TYR185 and on atom O in ALA184 or in PRO186 in Figure 11 calculated as a function of the magnitude of the constant electric field. The positive direction of the external electric field is from C(8) in the chromophore to C(1433) in TYR185 in Figure 11.

vibrational modes in opsin due to a nonlinear interaction, and the conformational change results. At the time, the change of the electronic state of the amino acids in the process it will

also affect the nonlinear interaction. The study of process it must include the chromophore and its surrounding opsin (Suzuki *et al.*, in preparation).

When light is absorbed by rhodopsin, the rhodopsin chromophore isomerizes from the 11-*cis* to the all-*trans* form. The changes in the electronic structure of some specific amino acids and the change in the vibrational modes in opsin probably lead to a conformational change, and the relations between them must be a well-devised mechanism. Thus, it remains the challenge of quantum chemistry to disclose the mechanisms of the conformational change of opsin.

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