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Vibrational Polarization and Opsin Shift of Retinal Schiff Bases: Theoretical Study

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Abstract: The changes in the electronic excitation energy arising from molecular structural displacement induced by external electric field (so-called vibrational polarization) are examined theoretically for the protonated and neutral 11-cis retinal Schiff bases. It is shown that the magnitude of the field-induced structural displacement is significantly large for the protonated species, so that the change in the electronic excitation energy arising from this structural displacement is of the same order of magnitude as that arising from the direct effect of electric field on the electronic wave function. These two effects contribute additively to the field-induced spectral shift. The intensity-carrying mode (ICM) theory is employed to extract a single vibrational mode (called primary infrared ICM) that is most important for the field-induced structural displacement. A simple one-dimensional model is constructed, and the extent to which we can interpret the field-induced spectral shift by such a model is examined. In the case of the neutral species, only a small change in the electronic excitation energy is induced by external electric field, mainly because the vibrational polarizability of this species is small. The meaning of these results in the spectral tuning of visual pigments is discussed.

1. Introduction

Retinal is bound in visual pigments as their chromophore by forming a Schiff base with a lysine residue of the apoprotein. Their functions are initiated by the absorption of light by the chromophore. The conjugated chain is in the 11-cis form, and the Schiff base is protonated in the initial state. Cis−trans isomerizations of the conjugated chain and protonation/deprotonation of the Schiff base occur during the photoreactions.[1−3]

In a previous study, vibrational spectroscopic properties of protonated and neutral (unprotonated) conjugated Schiff bases have been examined theoretically.[4] It has been shown that some of those properties are substantially different between the protonated and neutral species. The most notable difference is that the infrared (IR) intensities of the so-called in-phase C═C stretching mode[5] and the C═N stretching mode of the conjugated chain are significantly enhanced upon protonation.[6−8] It has been suggested[4] that this intensity enhancement is explained by the motion of the positive charge going back and forth from one end of the conjugated chain to the other occurring upon the vibration along the C═C and C═N stretching modes. In other words, the coupling between the electronic and vibrational motions of the conjugated chain is significantly larger in the protonated species than in the neutral species.

In another previous study, vibrational polarization of conjugated π-electron systems has been examined.[9] Vibrational polarization is the contribution of the vibrational degrees of freedom to the electric polarization of a given molecule. Within the harmonic approximation and to the first order in external electric field, the structural displacement[10] along a normal mode Q_p with vibrational angular frequency ω_p is expressed as

$$\delta Q_p = \frac{1}{\omega_p^2}\sum_k \frac{\partial \mu_k}{\partial Q_p} E_k \tag{1}$$

where $\partial\mu_k/\partial Q_p$ is the dipole derivative of the normal mode, and E_k is the external electric field. The dipole moment induced by this structural displacement is given as

$$\delta\mu_i = \sum_p \frac{\partial \mu_i}{\partial Q_p}\delta Q_p \tag{2}$$

Combining eqs 1 and 2, the vibrational polarizability is expressed as[11,12]

$$\alpha_{ik}^{(v)} = \sum_p \frac{1}{\omega_p^2}\frac{\partial \mu_i}{\partial Q_p}\frac{\partial \mu_k}{\partial Q_p} \tag{3}$$

Because the IR intensity of a normal mode is proportional to the square of the magnitude of its dipole derivative, it is expected

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that the molecular structure is likely to be perturbed along strongly IR-active modes by external electric field. The appearance of some strongly IR-active modes as "peculiar" Raman bands in the Raman spectra of streptocyanine dyes observed in solution[13,14] conforms to this expectation.

The opsin shift (the spectral shift of electronic absorption maximum) of visual pigments has been extensively studied for decades. A number of mechanisms have been proposed for the opsin shift, including ring/chain coplanarization,[15,16] interaction between the Schiff base proton and its counterion,[17−19] effects of charged or dipolar amino acid residues near the chromophore,[20−23] and electronic polarization of aromatic residues.[24−26] In recent studies,[27,28] the critical role of the electric field arising from dipolar residues in the spectral tuning of visual pigments has been emphasized. The nature of the protein−chromophore interactions that are important for spectral tuning still remains to be a fundamental issue.[29]

Because some of the normal modes of a protonated conjugated Schiff base have strong IR intensities and the molecular structure is likely to be perturbed along those normal modes by electric field, it is interesting to see how large is the effect of such structural displacement in the spectral tuning of visual pigments. In the present paper, we study this point theoretically by employing ab initio molecular orbital (MO) and density functional (DFT) methods. As a model of the chromophore in visual pigments, we take the Schiff base of 11-cis retinal formed with methylamine as well as its protonated species. We compare the results obtained for the protonated and neutral species to see the difference in the capability of spectral tuning between the two species.

In many situations, introduction of a simple model is helpful to get insight into the main mechanism of the phenomenon in question. In the present case also, it is preferable to introduce such a simple model for clear understanding of the field-induced spectral shift. We have developed the *intensity-carrying mode (ICM)* picture to take a small number of vibrational degrees of freedom important for generation of IR intensities and for molecular structural changes induced by external electric field.[30,31] In the present study, we examine some properties of the one-dimensional model (containing only one vibrational degree of freedom) constructed on the basis of this theory. The extent to which we can interpret the field-induced spectral shift of the protonated retinal Schiff base by using such a simple model is examined.

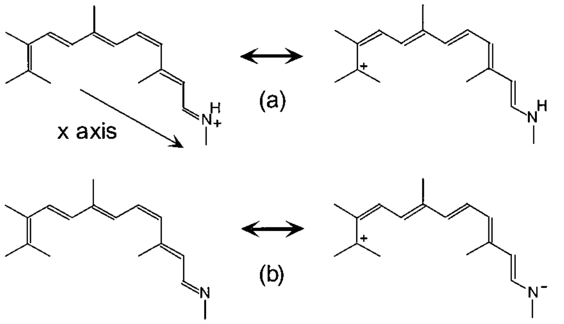

Figure 1. Resonance structures for the conjugated chains of the (a) protonated and (b) neutral 11-cis retinal Schiff bases. An approximate direction of the longest molecular axis (x axis) is also shown.

2. Computational Method

The molecular structure was first optimized for each species in the ground electronic state without applying electric field. The vibrational frequencies and the dipole derivatives of normal modes were calculated for this optimized structure. Those calculations were performed by using Becke's three-parameter hybrid DFT method[32] with the Lee−Yang−Parr correlation functional[33] (B3LYP) and the 6-31G* basis set. (As supplementary calculations, some of the quantities appearing in this paper were also obtained at the Hartree−Fock (HF) level with the 6-31G* basis set.) We can then calculate the structural displacement for a given electric field on the basis of eq 1. Electric field was applied in the direction of the longest molecular axis (hereafter denoted as x axis) shown in Figure 1. Electronic excitation energies were calculated at the configuration interaction singles (CIS) level[34] of ab initio MO method with the 6-31G* basis set in the following three ways: (1) for displaced structures with the corresponding electric field applied (denoted as "F + D"), (2) for displaced structures without applying electric field (denoted as "D only"), and (3) with electric field applied on the structure optimized in the ground electronic state (denoted as "F only"). In the "D only" calculation, the extent of molecular structural displacement is represented by the electric field that induces the displacement, but the electric field is not applied in the actual MO calculation so that the direct effect of electric field on the electronic wave function is removed. In this way, we can examine separately the effect of molecular structural displacement and the direct effect of electric field on the electronic wave function.

The ab initio MO and DFT calculations were performed by using the Gaussian 98 program.[35] The calculations of displaced structures

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Table 1. Field-Induced Displacement and the Displacement along the Primary IR Intensity-Carrying Mode in the Conjugated π-Electron System of the Protonated and Neutral 11-cis Retinal Schiff Bases[a]

	protonated			neutral
coordinate	field-induced displacement/au	displacement along primary IR ICM/10^{-3} au	ratio/10^{-3}	field-induced displacement/au
$r(N{=}C_{15})$	−7.96	−4.03	0.507	−1.87
$r(C_{15}{-}C_{14})$	7.85	4.43	0.565	2.46
$r(C_{14}{=}C_{13})$	−7.75	−3.50	0.451	−1.48
$r(C_{13}{-}C_{12})$	8.93	3.85	0.432	1.02
$r(C_{12}{=}C_{11})$	−7.47	−3.75	0.502	−0.95
$r(C_{11}{-}C_{10})$	8.18	3.57	0.436	1.25
$r(C_{10}{=}C_{9})$	−7.50	−3.50	0.467	−1.19
$r(C_{9}{-}C_{8})$	8.00	2.86	0.357	0.66
$r(C_{8}{=}C_{7})$	−5.69	−2.85	0.502	−0.40
$r(C_{7}{-}C_{6})$	7.65	2.28	0.297	1.48
$r(C_{6}{=}C_{5})$	−4.68	−2.22	0.474	−0.53

[a] Calculated at the B3LYP/6-31G* level.

and vibrational polarizabilities based on eqs 1−3 as well as the calculations based on the ICM theory described in section 3B were carried out with our original programs on an XP1000 workstation in our laboratory.

3. Results

A. Field-Induced Displacement of Molecular Structure and Changes in the Electronic Excitation Energy. The field-induced structural displacement in the conjugated chain calculated for the protonated and neutral species, expressed as $\delta R_q/E_x$ with

$$\delta R_q = \sum_p \frac{\partial R_q}{\partial Q_p}\delta Q_p = \sum_p \frac{1}{\omega_p^2}\frac{\partial R_q}{\partial Q_p}\frac{\partial \mu_x}{\partial Q_p}E_x \tag{4}$$

where R_q is the qth internal coordinate, is shown in atomic units on the second and fifth columns in Table 1. It is clearly seen that the extent of bond alternation is enhanced (in the sense that the C=C and C=N bonds shrink and the C−C bonds stretch) by the electric field in the $+x$ direction. This result is most easily understood by inspecting the resonance structures of the conjugated chain, which are shown in Figure 1.[36] The system is stabilized under the electric field in the $+x$ direction when it is polarized in the same direction. As shown in Figure 1, such a polarization is induced when the resonance structure on the left-hand side has a larger weight than the other. Because this resonance structure has a "normal" bond-alternation pattern in the conjugated chain, the electric field in the $+x$ direction gives rise to enhancement of the extent of bond alternation. On the contrary, the extent of bond alternation is reduced by electric field in the $-x$ direction, because the weight of the resonance structure on the right-hand side (with the reversed bond-alternation pattern in the conjugated chain) becomes larger.

Table 2. Total IR Intensities (in km mol^{-1}) in Specified Frequency Regions Calculated for the Protonated and Neutral 11-cis Retinal Schiff Bases[a]

frequency region	protonated	neutral
>2000 cm^{-1}	872	1094
2000−500 cm^{-1}	12581	1059
<500 cm^{-1}	97	54

[a] Calculated at the B3LYP/6-31G* level.

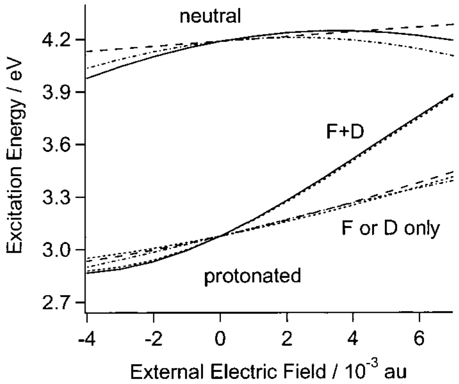

Figure 2. Calculated changes in the electronic excitation energies of the protonated and neutral 11-cis retinal Schiff bases induced by external electric field. Long dashed lines, only structural displacement is taken into account ("D only"); dot−dashed lines, only the direct effect of electric field on the electronic wave function is taken into account ("F only"); solid lines, both of these effects are fully taken into account ("F + D"). The dotted lines are the results obtained by using the ICM picture described in section 3B.

The magnitude of the field-induced structural displacement of the protonated species is significantly larger than that of the neutral species because the vibrational polarizability is larger for the former. The xx component of the vibrational polarizability ($\alpha_{xx}^{(v)}$) of the protonated species is calculated to be 213 au,[37] which is nearly 1 order of magnitude larger than that of the neutral species (35 au). This enhanced vibrational polarizability of the protonated species arises from the significantly large dipole derivatives of the normal modes in the 2000−500 cm^{-1} region, especially the so-called C=C stretching modes in the 1600−1500 cm^{-1} region and the C−C stretching modes in the 1300−1200 cm^{-1} region. As shown in Table 2, the total IR intensity, which is proportional to the sum of the squares of the dipole derivatives, of the protonated species is 1 order of magnitude larger than that of the neutral species in this frequency region. The difference between the two species is not so large in the other frequency regions.

The changes in the electronic excitation energies (of the lowest strongly allowed transition) induced by external electric field are shown in Figure 2. Because the CIS method (including only singly excited configurations) is used for the excited electronic state, the electronic excitation energies (calculated as the CIS−HF energy differences) are calculated to be a little too large. However, it is expected that the *changes* in the electronic excitation energies are obtained in reasonable accuracy, considering that the lowest strongly allowed excited states of conjugated π-electron systems are dominated in general by the contribution of the $\pi\pi^*$ singly excited wave function.

In the case of the protonated species, the electronic excitation energy increases noticeably by the electric field in the $+x$ direction. From the results of the "D only" and "F only" calculations (shown in a long dashed line and a dot−dashed

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Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B. G.; Chen, W.; Wong, M. W.; Andres, J. L.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98*; Gaussian, Inc.: Pittsburgh, PA, 1998.

(36) A similar figure is shown in ref 4 for a shorter chain.

(37) For a shorter protonated conjugated Schiff base [1-(*N*-methylamino)-2,4-hexadienylium cation, $CH_3{-}CH{=}CH{-}CH{=}CH{-}CH{=}NH^+CH_3$], the value of $\alpha_{xx}^{(v)}$ calculated at the B3LYP/6-31G(2d,p) level is 34 au, which is almost the same as the value calculated at the MP2/6-31G(2d,p) level (33 au). It is therefore expected that calculations at the B3LYP level are sufficient for calculating vibrational polarizabilities of this type of compound.

line, respectively), it is recognized that the increase in the electronic excitation energy originates both from the molecular structural displacement induced by electric field and from the direct effect of electric field on the electronic wave function, and *these two effects are of the same order of magnitude*. In other words, both of these effects are considered to be important for the field-induced changes in the electronic excitation energy. As seen in the result of the "F + D" calculation (shown in a solid line), a larger change is obtained when both effects are taken into account.

The increase in the electronic excitation energy arising from the molecular structural displacement is rationalized by considering that the extent of bond alternation of the conjugated chain is reduced at the optimized structure in the excited electronic state,[21] especially in the central part of the chain. As described above, the structural displacement induced by the electric field in the $+x$ direction enhances the extent of bond alternation. Therefore, the excited electronic state is destabilized by such displacement, and hence the electronic excitation energy increases. By contrast, the increase in the excitation energy due to the direct effect of electric field is rationalized by considering that the molecular dipole moment, which is almost parallel to the x axis and oriented in the $+x$ direction, is larger in the ground electronic state than in the excited electronic state.[38,39] This is because the wave function in the excited electronic state has a larger weight of the resonance structure on the right-hand side in Figure 1 than that in the ground electronic state.[21] As a result, the ground electronic state is more stabilized by the electric field in the $+x$ direction than the excited electronic state, and hence the electronic excitation energy increases.

In the case of the neutral species, external electric field induces only a small change in the electronic excitation energy. One reason for this is that the vibrational polarizability of this species is smaller than that of the protonated species, and hence a smaller structural displacement is induced by electric field, as shown in Table 1. Another reason is the nonlinear behavior of the electronic excitation energy due to the direct effect of electric field on the electronic wave function, as shown in the result of the "F only" calculation (drawn in a dot−dashed line) in Figure 2. This nonlinear behavior originates from the larger electronic polarizability in the excited electronic state than in the ground electronic state. Because of this electronic polarizability, the excited electronic state is stabilized when sufficiently large electric field is applied, whether the electric field is in the $+x$ direction or in the $-x$ direction.

B. Intensity-Carrying Mode Picture. To extract a small number of vibrational degrees of freedom important for molecular structural changes induced by external electric field, we use the ICM theory developed in our recent studies.[30,31] This procedure is useful to construct a one-dimensional model (containing only one vibrational degree of freedom) described below in section 4B.

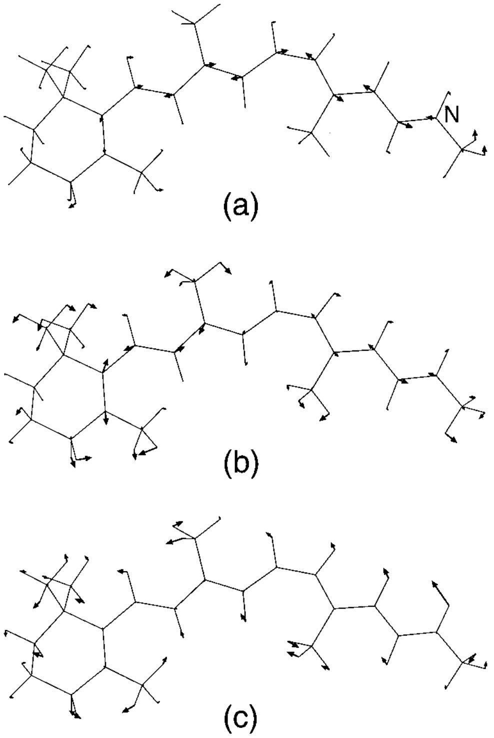

Figure 3. Vibrational patterns of the IR ICMs calculated for the protonated 11-cis retinal Schiff base. (a) The primary IR ICM that generates the largest dipole derivative (16.8 D Å^{-1} amu$^{-1/2}$), (b) the IR ICM generating the second largest dipole derivative (3.5 D Å^{-1} amu$^{-1/2}$), and (c) the IR ICM generating the smallest dipole derivative (2.6 D Å^{-1} amu$^{-1/2}$).

The explicit forms of the IR ICMs are obtained by diagonalization of an $n \times n$ matrix $\boldsymbol{M}$ defined as[30]

$$M_{pq} = \sum_{k=1}^{3} \frac{\partial \mu_k}{\partial Q_p} \frac{\partial \mu_k}{\partial Q_q} \tag{5}$$

where n is the number of normal modes. On the basis of an algebraic property of the IR intensity formula,[40] it is proved that there are at most three nonzero eigenvalues for this matrix.[30] The eigenvectors for the nonzero eigenvalues represent the IR ICMs and are responsible for generating the whole dipole derivatives of the system. In general, each of these IR ICMs is not equal to any single normal mode, but is represented by a linear combination of normal modes. The mode represented by the eigenvector of the largest eigenvalue is called *primary IR ICM*, and the square root of this eigenvalue is equal to the maximum magnitude of dipole derivative that can be generated by one vibrational degree of freedom.

In some cases, it is convenient to work in a smaller space of vibrational motions to calculate the IR ICMs. In the present study, we have eliminated the C−H and N−H stretching motions before calculating the IR ICMs, because the skeletal vibrational motions of the conjugated chain are mostly responsible for the vibrational polarization.

The vibrational patterns of the three IR ICMs calculated for the protonated species are shown in Figure 3. These modes generate dipole derivatives of the magnitude 16.8, 3.5, and 2.6 D Å^{-1} amu$^{-1/2}$, respectively. The sum of the squares of these

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(38) The molecular dipole moment is calculated to be 14.9 D (at the B3LYP/6-31G* level) or 19.8 D (at the HF/6-31G* level) in the ground electronic state and 11.0 D in the excited electronic state (at the CIS/6-31G* level).

(39) Although the dipole moments observed in solution in ref 20 contain the contribution of the counterion, the *difference* in the dipole moments between the ground and excited electronic states may be compared between experiment and calculation. The observed value of $\Delta\mu$ (12.0 D) is in reasonable agreement with the value of $\Delta\mu$ calculated as a CIS−HF difference (8.8 D).

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dipole derivatives is equal to the total IR intensity in the 2000−0 cm^{-1} region shown in Table 2 after appropriate conversion of units. These dipole derivatives are oriented in mutually orthogonal directions.[30] The primary IR ICM shown in Figure 3a is mostly responsible for the vibrational polarization along the x axis. In the normal mode picture, this ICM may be expanded mainly by strongly IR-active normal modes in the 2000−500 cm^{-1} region.

In Figure 3, the vibration along the bond-alternation mode is seen only in the primary IR ICM. The displacement in the conjugated chain along this primary IR ICM is shown in the third column in Table 1 in atomic units. It is clearly seen that this displacement is roughly proportional to the field-induced displacement, which is shown in the second column. The ratios of the values in the second and third columns are shown in the fourth column. From this result, it may be reasonable to consider that the unit displacement along the primary IR ICM is equivalent to $\sim 4.5 \times 10^{-4}$ of the field-induced displacement.

Assuming this conversion ratio, we calculated electronic excitation energies for structures displaced along the primary IR ICM, with the corresponding electric field applied ("F + D") and without applying electric field ("D only"). The results are shown in dotted lines in Figure 2. It is seen that they follow rather faithfully the electronic excitation energies of field-induced displaced structures calculated in the way described in section 3A. This result indicates that the primary IR ICM provides a good approximation to the field-induced structural displacement.[41]

One merit of employing the ICM picture is that the ICMs may be treated, in contrast to the field-induced structural displacement itself, as genuine vibrational modes, because they are related to normal modes by an orthogonal transformation and have the same dimension $[(\text{length}) \times (\text{mass})^{1/2}]$.[31b] Therefore, when we construct a few-dimensional model (e.g., a one-dimensional model described below) on the basis of the ICM picture, the physical meaning of the vibrational coordinate(s) employed in the model will be clear.

4. Discussion

A. Protonated State of Retinal Schiff Base in Relation to the Spectral Tuning of Visual Pigments. The above results clearly show that the electronic absorption maximum of the protonated retinal Schiff base is more sensitive to the electric field from the surrounding residues than the neutral one. This is because the two factors, that is, the effect of molecular structural displacement and the direct effect of electric field on the electronic wave function, are working additively in the protonated state. It may be said, therefore, that the protonated state of the retinal Schiff base in visual pigments is suitable for spectral tuning. However, this result is not inconsistent with the suggestion made recently[29,42] that the chromophores in some UV-sensitive visual pigments may be deprotonated.

In relation to this point, it should be noted that, when the protonated retinal Schiff base is dissolved in polar solution, a certain magnitude of electric field is expected to operate on the solute as the reaction field from the solvent. For example, if we assume a spherical cavity of radius $a \approx 10$ au (5 Å) with the dipole moment of $\mu \approx 6$ au (15 D, in the $+x$ direction) at the center and the surrounding continuum of dielectric constant $\epsilon \approx 10$, we get the reaction field of $|\boldsymbol{E}| \approx 5 \times 10^{-3}$ au in the $+x$ direction. Although the protonated retinal Schiff base is not at all spherical (the reaction field is expected to decrease for an ellipsoidal cavity[43,44]) and the surrounding medium is not dielectric continuum but has some molecular character, it is reasonable to expect that the reaction field of this order of magnitude is operating on the protonated retinal Schiff base in polar solutions. As a result, the absorption spectrum is blue-shifted (on the order of a few tenths of eV as expected from Figure 2) in polar solutions as compared to that of an isolated molecule.[45] A similar situation is expected also in visual pigments, with the amino acid residues playing the role of solvent molecules. However, the electric field on the protonated retinal Schiff base will vary depending on the locations and the orientations of the charged or polar residues around it.[46] Accordingly, the electronic absorption maximum will shift to the red or blue side as compared to that in polar solutions.

It has been discussed[29] that the short-wavelength visual pigments pose a particular challenge in the understanding of retinal−protein interactions, because many of the absorption spectra are blue-shifted as compared to the protonated Schiff base in solution. As shown above, if there is sufficiently strong electric field in the $+x$ direction, the absorption spectrum will be blue-shifted because of the effect of molecular structural displacement and the direct effect of electric field on the electronic wave function. This result will help understand the retinal−protein interactions that are responsible for the spectral tuning in visual pigments.

B. One-Dimensional Model. For a clear understanding of the field-induced spectral shift, we examine some properties of the one-dimensional model constructed on the basis of the ICM picture. The primary IR ICM is taken as the vibrational coordinate in the model.

In this model, the structural displacement induced by electric field along the x axis is given as

$$\delta S = \frac{1}{k_S} \frac{\partial \mu_x}{\partial S} E_x \tag{6}$$

where S is the vibrational coordinate of the primary IR ICM, and k_S is the force constant for this coordinate. As noted above, we have $\partial\mu_x/\partial S \cong 8.15 \times 10^{-2}$ au (16.7 D Å^{-1} $\text{amu}^{-1/2}$) as a property of this ICM. The value of k_S is calculated to be 4.20×10^{-5} au (1.19 mdyn Å^{-1} amu^{-1}) by using the vibrational pattern of this ICM and the force constant matrix obtained from

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(41) Because of the one-dimensional nature of the picture based on the primary IR ICM, the effects of mechanical and electrical anharmonicities may be easily examined. When we expand the potential energy as $V = V_0 + ({}^1/{}_2)k_S S^2 + ({}^1/{}_6)f_S S^3 + ... - (\partial\mu_x/\partial S)SE_x - ({}^1/{}_2)(\partial^2\mu_x/\partial S^2)S^2E_x - ...$, where S stands for the primary IR ICM, the structural displacement induced by electric field E_x (along the x axis) is expressed as $\delta S = \delta S_\text{h} + \delta S_{\text{anh}-\text{m}} + \delta S_{\text{anh}-\text{e}}$, where $\delta S_\text{h} = (1/k_S)(\partial\mu_x/\partial S)E_x$ is the displacement obtained within the harmonic approximation, and the second and the third terms are the mechanical and electrical anharmonic corrections expressed as $\delta S_{\text{anh}-\text{m}} = -({}^1/{}_2)(f_S/k_S)(\delta S_\text{h})^2$ and $\delta S_{\text{anh}-\text{e}} = (\partial^2\mu_x/\partial S^2)\delta S_\text{h} E_x$. In the case of the protonated 11-cis retinal Schiff base treated in the present study, $k_S = 4.20 \times 10^{-5}$ au, $f_S = 9.9 \times 10^{-8}$ au, $\partial\mu_x/\partial S = 8.15 \times 10^{-2}$ au, and $\partial^2\mu_x/\partial S^2 = -2.0 \times 10^{-4}$ au. Therefore, the values of δS_h, $\delta S_{\text{anh}-\text{m}}$, and $\delta S_{\text{anh}-\text{e}}$ for $E_x = 5.0 \times 10^{-3}$ au (a typical value in the present study) are estimated as $\delta S_\text{h} = 9.7$ au, $\delta S_{\text{anh}-\text{m}} = -0.11$ au, and $\delta S_{\text{anh}-\text{e}} \approx -10^{-5}$ au, indicating that the harmonic approximation is reasonable.

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the DFT calculation. From these values, we obtain $\delta S/E_x \cong 1.94 \times 10^3$ au, which is in good agreement with the inverse of the "conversion factor" mentioned above in section 3B (4.5×10^{-4} au). Therefore, it may be said that the field-induced structural displacement is reasonably estimated from the dipole derivative and the force constant of the primary IR ICM.

To obtain an estimate of the changes in the electronic excitation energy induced by this structural displacement, we need $\partial V_{eg}/\partial S$, where V_{eg} is the (vertical) electronic excitation energy. We should therefore include the excited electronic state into the model in an appropriate way. In the two-state model,[9] where only two electronic states are taken into account, we have $\partial V_{eg}/\partial S = 2k_S S_{min}$, where S_{min} is the value of S at the potential energy minimum on the ground electronic state. (In this case, the origin of S is taken at the midpoint between the potential energy minima of the two diabatic electronic states included in the model, which give rise to the ground and excited adiabatic electronic states after electronic coupling.) Although it is hard to estimate the value of S_{min} straightforwardly, it is true that $2S_{min}$ is on the same order of magnitude as the structural difference between the ground and excited (adiabatic) electronic states.

To have the value of $(\partial V_{eg}/\partial S) \times (\partial S/\partial E_x) = 1.56$ au as obtained in the calculation described in section 3A, S_{min} should be 8.4 au [taking into account that $(\partial S/\partial E_x)^{-1} \approx 4.5 \times 10^{-4}$ au as obtained in section 3B and $k_S = 4.20 \times 10^{-5}$ au as obtained above]. For this value of S_{min}, the displacements along internal coordinates corresponding to $2S_{min}$ are ~0.03 Å, considering the values of $\partial R_q/\partial S$ shown in the third column in Table 1. This value is in the middle of the CIS−B3LYP and CIS−HF structural differences obtained from the optimized structural parameters at each theoretical level. Therefore, it may be said that a structural difference of this magnitude is most likely between the ground and excited electronic states. Further theoretical studies are needed on this point.

Experimentally, the dimensionless displacement parameters have been estimated for bacteriorhodopsin, which contains protonated all-trans retinal Schiff base, from its resonance Raman excitation profile.[47] For a normal mode located at 1527 cm^{-1}, the dimensionless displacement parameter has been estimated to be as much as 0.700, which is equivalent to 8.4 au and explains about one-half of the structural difference described in the previous paragraph. Because a few other normal modes also contribute to the primary IR ICM, the theoretical discussion described above seems to be consistent with this experimental result.

5. Summary

In the present study, the changes in the electronic excitation energy arising from molecular structural displacement induced by external electric field (vibrational polarization) have been examined for the protonated and neutral 11-cis retinal Schiff bases by using ab initio MO and DFT methods. The conclusions may be summarized as follows: (1) The magnitude of the field-induced structural displacement of the protonated species is significantly larger than that of the neutral species. The bond-alternation pattern (including the sign) of this structural displacement is explained by the resonance structures of the conjugated chain shown in Figure 1. (2) The change in the electronic excitation energy arising from the field-induced structural displacement is of the same order of magnitude as that arising from the direct effect of electric field on the electronic wave function. These two effects contribute additively to the field-induced spectral shift. (3) The vibrational mode that is most important for the vibrational polarization along the x axis is obtained as the primary IR ICM. (4) The vibrational pattern of this ICM and the structural difference between the ground and excited electronic states are related to the sign of the field-induced spectral shift arising from vibrational polarization.

The results obtained in the present study clearly show that the protonated state of the retinal Schiff base in visual pigments is suitable for spectral tuning. In visual pigments, the electric field arising from each charged or polar amino acid residue depends on its location and orientation. In this sense, detailed information on the protein structure is required to elucidate the protein−chromophore interactions that are important for spectral tuning.

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