Biophys. Struct. Mechanism 5, 55–73 (1979)

Biophysics of Structure and Mechanism

Molecular Mechanism for the Initial Process of Visual Excitation

III. Theoretical Studies of Optical Spectra and Conformations of Chromophores in Visual Pigments, Their Analogues and Intermediates Based on the Torsion Model*

T. Kakitani and H. Kakitani

Department of Physics, Nagoya University,
Nagoya 464, Japan

Abstract. The torsion model with which we proposed to interpret the specific properties of the photoisomerization reaction of rhodopsin has been developed to apply to isorhodopsin I, isorhodopsin II and some intermediates. Based on this model, optical absorption wavelengths and oscillator strengths, as well as rotational strengths of visual pigments, analogues and intermediates at low temperatures are analyzed by varying twisted conformations of the chromophores. As a result, it was found that most of the optical data could be very well accounted for quantitatively by the torsion model. The twisting characters in the chromophore of rhodopsin are very similar to those of isorhodopsin. The obtained conformations of the chromophores are very similar in rhodopsin and its analogues, and in isorhodopsin and its analogues. Those of the chromophores of bathorhodopsin, lumirhodopsin and metarhodopsin I are similar to one another except that the conjugated chain of metarhodopsin I bends considerably when compared with the other intermediates.

Key words: Optical spectra of visual pigments – Conformations of Chromophores.

Introduction

Visual pigments have the following photochemical properties; one of which is the specific property of optical spectra. When the chromophore, i.e., retinal, is bound to opsin, a large bathochromic shift of optical absorption occurs. The absorption wavelength λ_{max} ranges from 345–575 nm for A_1 pigments, and from 438–620 nm for A_2 pigments (Honig et al., 1976; Hamdorf et al., 1973). Dramatic changes in λ_{max} are also seen in such photoreaction intermediates as bathorhodopsin, lumirhodopsin and metarhodopsin I. Positive circular dichroism (CD) is induced when visual pig-

* A part of this work was performed while one of the authors (T.K.) was a Visiting Investigator of Japan Society for the Promotion of Science at Kyoto University from April, 1977 to March, 1978

0340-1057/79/0005/0055/$ 03.80

ments are formed from retinal and opsin. Furthermore, large negative CD is observed in bathorhodopsin (Yoshizawa and Horiuchi, 1973).

The other photochemical property of visual pigments is the specific photoisomerization reaction. That is, the quantum yield of the photoisomerization of rhodopsin is very large ($\gamma \sim 0.65$, Dartnell, 1968), and it is independent of the excitation wavelength (Dartnall, 1968), and of temperature (Rosenfeld et al., 1977; Hurley et al., 1977). Photoconversion from rhodopsin to bathorhodopsin, in which the chromophore is conceivably isomerized (Rosenfeld et al., 1977; Green et al., 1977), occurs rapidly within 6 ps at room temperature (Busch et al., 1972). The reaction path is very specific; i.e., only all-trans retinal is released from ospin when either rhodopsin or isorhodopsin is photobleached.

There is no doubt that all the above properties are essentially due to specific interactions between the chromophore and opsin. At present, X-ray analysis of the three-dimensional structure of rhodopsin has not been successful. Studies so far have been done under arbitrary assumptions of the role of the protein. With regard to the spectroscopic properties, for example, they include the model of a counter anion which is located near the Schiff-base of retinal (Blatz et al., 1972; Suzuki et al., 1974), the model of two counter anions which are located near the Schiff-base and ionone ring (Honig et al., 1976), the electric field model (Suzuki et al., 1970), the charge transfer model (Akhtar et al., 1968), the microenvironmental polarizability model (Irving et al., 1970), the torsion model (Kakitani et al., 1977), and so on. As for the photoisomerization mechanism, they include the torsion model (Kakitani and Kakitani, 1975), the bicycle-pedal model (Warshel, 1976), barrierless common state model (Rosenfeld et al., 1977), the proton translocation model (Peters et al., 1977), and so on.

At the present stage of our investigation, we cannot determine definitely which model is best. Consequently, it will be necessary to check how much experimental data can be consistently explained by each model. In this paper, we intend to calculate specific spectroscopic data of visual pigments of vertebrates (mostly of cattle) and their intermediates at low temperatures by the torsion model, and to show how closely our theoretical calculations agree with experiments. At the same time we obtained conformations of the chromophores.

Method of Calculation

Torsion Model

The torsion model was originally proposed to interpret the specific properties of the photoisomerization in rhodopsin (Kakitani and Kakitani, 1975). In this model, it was assumed that the binding site of opsin would not have the conformation suitable for the form of 11-cis retinal in solution in such a way as the key and lock fitting, but it would have a cavity with the form suitable for the binding of the chromophore highly twisted around the 11–12 bond. Therefore, when 11-cis retinal is bound to opsin, the 11–12 bond will be twisted to some extent in the ground state by a balance of two forces; one is the force to twist the bond due to a steric hindrance between opsin and the chromophore, and the other the force to keep the bond planar

due to the π-conjugation. On the contrary, in the excited state, the force to twist the bond prevails over the opposing force because of the weaker π-bond, and then the 11–12 bond is twisted by about $\pi/2$. By writing the adiabatic potential as a function of the torsional angle around the 11–12 bond θ_{11-12}, the origins of the large quantum yield and the bathochromic shift of the optical absorption were consistently explained (Kakitani and Kakitani, 1975).

To apply this model to isorhodopsin, the cavity in the binding site of opsin should also fit the conformation with about $\pi/2$ twisting around the 9–10 bond of the chromophore. Then, the 9–10 bond of 9-cis retinal will be twisted considerably by the steric hindrance in forming the pigment. Under these conditions, it will be natural to extend the model so that both of the 9–10 and 11–12 double bonds may be twisted in rhodopsin and isorhodopsin. Another modification is that many of the conjugated single bonds are somewhat twisted, which will be suitable because their twisting force constants are very small.

In order to reproduce the spectroscopic data of visula pigments quantitatively well, we must settle the electronic conditions of the opsin moiety near the chromophore concretely. The retinal forms Schiff-base bonding with the ε-amino group of lysine in opsin (Fager et al., 1972), and the Schiff-base is protonated (Oseroff and Callender, 1974). The counter anion donating a proton to the Schiff-base would be located near the protonated nitrogen. The distance between the nitrogen atom and the counter anion should be less than about 4 Å because the proton donation would not occur beyond this distance. Honig et al. (1976) had estimated the above critical distance as 3.5 Å. Experimentally the pigment formation is found to be rapid (Matsumoto et al., 1978). Then, we presume that the counter anion would be located about 3 Å apart from the protonated nitrogen atom and form an weak hydrogen bond with it. In this condition, the absorption wavelength was estimated to be 440 nm $\sim$ 460 nm by calculations (Suzuki et al., 1974; Honig et al., 1976). This wavelength is similar to the experimental value $\lambda_{max} = 440$ nm for the protonated retinal Schiff-base (PRSB) in ethanol, which is a leveling solvent. The explicit consideration of the counter anion does not mean the alteration in the calculation scheme of our previous studies, because our electronic parameter of the protonated nitrogen was chosen to be the one in ethanol (Kakitani and Kakitani, 1975; Kakitani and Kakitani, 1977b).

In the last, we assume that the shifts of λ_{max}'s in visual pigments from that in PRSB in solution are due to the twistings around the double and single bonds of the chromophore.

Generally speaking, the twisting around the double bond leads to the bathochromic shift and that around the single bond the hypochromic shift. So that, the wavelength regulation of the visual pigments can be achieved by different combinations of the double and single bond twistings. In the present calculation, we assume that the above scheme of the torsion model can be applied systematically to visual pigments, analogues and intermediates.

Theoretical Method

The electronic spectra of polyenes are very sensitive to the molecular conformation (Suzuki, 1967). So that, it is necessary to use the theory in which the electronic state

and molecular conformation are obtained self-consistently by minimizing the total energy. As such theories, there are the π-electron theory by Warshel and Karplus (1972a, b) based on the Pariser-Parr-Popple's theory (1953) (abbreviated as PPP theory), and the self-consistent HMO theory (Kakitani, 1974b), etc. In this study, we adopt the improved version of the self-consistent HMO theory (Kakitani and Kakitani, 1977b) which is the simplest. This theory was proved to be useful for polyenes with twisted bonds less than 50° (Kakitani and Kakitani, 1977b) and it was also useful for the excited state (Kakitani and Kakitani, 1977a). A simple theory is necessary for our analysis because a lot of conformations of the chromophore must be examined.

The calculated λ_{max}'s of all-trans retinal, RSB and PRSB are 382 nm, 365 nm, and 442 nm, respectively, and these are in good agreement with the experimental values in ethanol 380 nm, 365 nm, and 440 nm, respectively.

The oscillator strength for the electronic transition $i \rightarrow j$ is calculated by the following dipole velocity formalism

$$f_{\nabla}^{i \rightarrow j} = \frac{2\hbar^2}{3\, m(\varepsilon_j - \varepsilon_i)} |\langle j|\nabla|i\rangle|^2 , \tag{1}$$

where ε_i, m, and $\hbar$ are the electronic energy of the i-state, electron mass and $1/2\pi$ of Planck's constant, respectively. The oscillator strength can also be expressed by the dipole length formalism f_r with use of the off-diagonal hypervirial theorem. The calculated values of f_r are systematically $1.8 \sim 2.0$ times larger than those of f_∇ for carotenoids owing to the approximate MO theory (Kakitani and Kakitani, 1978). The experimental value is always between f_r and f_∇. For PRSB, the experimental value is 1.28 times larger than f_∇.

The CD spectra of visual pigments have been calculated either by the coupled oscillator mechanism or by the intrinsic mechanism of the chromophore. According to the former, the experimental CD of rhodopsin could be accounted for by a dipole-dipole interaction if such a residue as tryptophan is located in proper directions at a distance of 4.3 Å for the α-band and 3.5 Å for the β-band from the chromophore (Kropf et al., 1973). According to the latter, the experimental CD's of rhodopsin and bathorhodopsin could be accounted for if the 11—12 and 12—13 bonds are twisted by $30 \sim 40°$ (Kakitani et al., 1977). Now, there is an evidence which implies that the coupled oscillator model will not be plausible. According to the Kuhn's sum rule (1930), the sum of the rotational strengths of all optically active transitions of a molecule is zero. Then, if the positive CD is induced in the visible region by the coupling between the chromophore and the aromatic residues of protein, the CD in the near ultraviolet region should be decreased accompanying with the binding of 11-cis retinal to opsin. Recent experimental data showed that both the far and near ultraviolet CD spectra of the sonicated rod outer segment membranes little changed upon illumination (Rafferty et al., 1977). This fact indicates that the coupled oscillator mechanism is not significant for the induced CD of visual pigments. Thus, we suppose that the intrinsic mechanism will work dominantly in both visual pigments and intermediates. It should be mentioned here about the mechanism producing the intrinsic optical activity. One is that only one enantiomeric form of retinal in solution which has an intrinsic asymmetry can form a visual pigment (Mommaerts, 1969).

The other is that the chromophore is actually twisted in an asymmetric conformation in the binding state. Recent calculations exhibited that the one enantiomeric form of 11-cis PRSB in solution could not explain the experimental-CD's for the α- and β-bands of rhodopsin consistently (Kakitani et al., 1977; Kakitani and Kakitani, unpublished).

The rotational strength in the intrinsic mechanism is expressed as follows:

$$R_{i\to j} = -\frac{\hbar^3 e^2}{2\, m^2 c(\varepsilon_j - \varepsilon_i)} \langle i|\nabla|j\rangle \langle j|r\times\nabla|i\rangle \,, \tag{2}$$

where c and e are the light velocity and the elementary charge, respectively.

Calculation Procedure

In the twisted PRSB's for $\theta < \pi/4$, the second and third allowed transition energies are very close according to the recent calculations (Kakitani et al., 1977). Then, we assume that the β-band in the optical spectra of visual pigments is due to the second and third transitions as follows:

$$\begin{aligned} f_\beta &= f^{n\to n+2} + f^{n-1\to n+1} \,, \\ R_\beta &= R_{n\to n+2} + R_{n-1\to n+1} \,, \\ \lambda_\beta &= (f^{n\to n+2}\lambda_{n\to n+2} + f^{n-1\to n+1}\lambda_{n-1\to n+1})/f_\beta \,, \end{aligned} \tag{3}$$

where $2n$ is the number of π-electrons in the chromophore.

Some basic forms of the chromophores are depicted in Figure 1. For 11-cis pigments, we have chosen 6*s*-cis, 11-cis, 12*s*-trans instead of 6*s*-cis, 11-cis, 12*s*-cis which was observed in crystal (Gilardi et al., 1972). This is because we obtained $f_\alpha < f_\beta$ for the 12*s*-cis form, and $f_\alpha > f_\beta$ for the 12*s*-trans form which is in agreement with the experimental result (Kakitani et al., 1977). Further evidence was presented by the experiment of the binding of 14-methyl retinal (Chan et al., 1974). We have also assumed the trans form of the 9—10 and 11—12 bonds for the photobleaching intermediates, bathorhodopsin, lumirhodopsin and metarhodopsin I, following the result of the critical survey of the photochemical process by Rosenfeld et al. (1977).

As we do have no exact knowledge of the steric interaction between the chromophore and opsin, we treat torsional angles as variable parameters in the analysis. The bond lengths are determined self-consistenly together with the electronic state. All the bond angles in the conjugated chain are taken as 120°. In numerical calculations, we first considered twistings around the four bonds 9—10, 10—11, 11—12, and 12—13. For rhodopsin, its analogues and intermediates, θ_{11-12} was adjusted so that the calculated λ_α might agree with the experimental for each set of the given other three θ's. Then, the optimum conformation was searched in which the calculated λ_β, f_α, f_β, R_α, and R_β were in good agreement with those of the experiment. By such procedures, we selected plausible sets of the four θ's. For isorhodopsin and its analogues, θ_{9-10} was adjusted so that the calculated λ_α might agree with the experi-

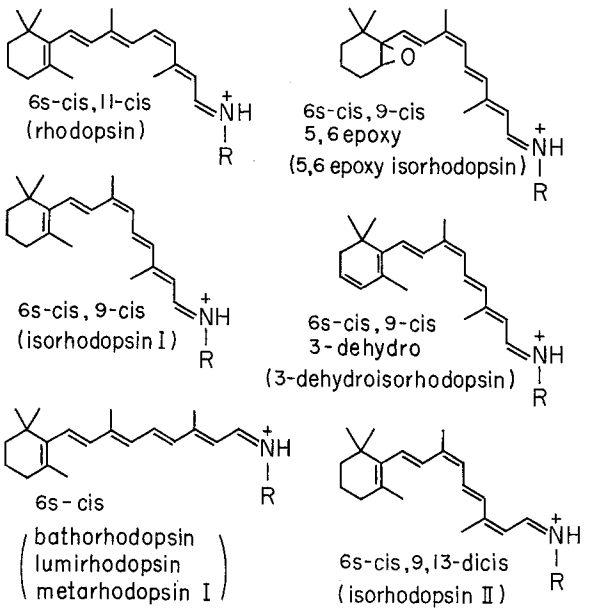

Fig. 1. Basic structures of retinal isomers and these analogues. Pigment in the parentheses are ones which are obtained from the chromophores and opsin

mental data. In the next stage, we also allowed twistings around the 6—7, 8—9, and 14—15 bonds. Varying the seven θ's near the plausible sets of the four θ's considered before, we obtained the most plausible conformation of the chromophore. For isorhodopsin II and intermediates, the twisting around the 13—14 bond was also considered in addition to the above seven θ's.

Calculated Results

Analysis of Optical Spectra

Typical examples of the calculations in determining the conformation of the chromophore are shown in Figures 2 and 3. In these, λ_β varies gently, f_α and f_β vary considerably, and R_α and R_β vary very much with θ_{12-13} or θ_{10-11}. They are not the cases that the variations are too large to determine the plausible value of θ's uniquely, or too small to fit the calculated values to the experimental ones. The plausible value of θ in this graph is depicted by a white arrow. About 10^3 such graphs were drawn for the combinations of the values of θ's which were fixed in each graph. By this method, torsional angles around the double bonds could be uniquely determined within deviation of $\pm$ 5°, and torsional angles around the single bonds within deviation of $\pm$ 10° in most cases. Thus obtained most plausible torsional angles are listed in Table 1. From this table we find that all the torsional angles except for θ_{6-7} are positive for rhodopsin and isorhodopsin. It should be noticed that the 9—10 and 11—12 double bonds are twisted from plane by 20 and 28°, respective-

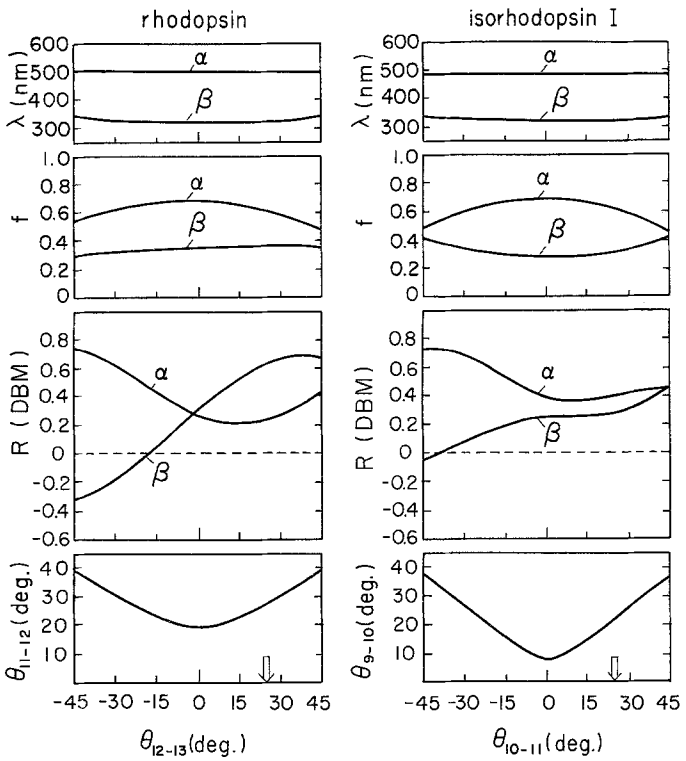

Fig. 2. Graphs of wavelength λ, oscillator strength f, rotational strength R and torsional angle θ_{11-12} (θ_{9-10}) of rhodopsin (isorhodopsin I) as a function of θ_{12-13} (θ_{10-11}). In rhodopsin, θ_{6-7}, θ_{8-9}, θ_{9-10}, θ_{10-11}, and θ_{14-15} are fixed to −40, 15, 20, 10, and 15°, respectively. In isorhodopsin I, θ_{6-7}, θ_{8-9}, θ_{11-12}, θ_{12-13}, and θ_{14-15} are fixed to −30, 10, 25, 20, and 15°, respectively. In the figure, α and β denote the α-band and the β-band, respectively. λ_α of rhodopsin is fitted to 498 nm by adjusting θ_{11-12} and λ_α of isorhodopsin I is fitted to 486 nm by adjusting θ_{9-10} in calculations. The plausible torsional angle is shown by a white arrow

ly in rhodopsin, and by 23 and 25°, respectively in isorhodopsin I. The analogues have considerably similar torsional angles to those of the visual pigments. In bathorhodopsin, all the θ's are negative. Among them, θ_{9-10} and θ_{11-12} are −25 and −38°, respectively. In lumirhodopsin, θ_{9-10}, θ_{10-11}, θ_{12-13}, θ_{13-14}, and θ_{14-15} turn to positive angles. In metarhodopsin, θ_{11-12} turns to positive one.

The calculated values of the absorption wavelengths, oscillator strengths and rotational strengths for the α- and β-bands are shown in Table 2 together with those of the experiment. In λ_α, the agreement between the calculation and the experiment is perfect owing to our calculation procedure. For λ_β, the calculated values are in good agreement with the experimental ones for rhodopsin, isorhodopsin I, isorhodopsin II, and bathorhodopsin, and they are relatively larger than those of the experiments for lumirhodopsin and metarhodopsin I.

In replacing the 9-methyl group with a hydrogen atom, a large spectral shift occurs in the α-band of both rhodopsin and isorhodopsin I (Kropf et al., 1973). In the present calculations, the shift of 37 nm in 9-*dm* rhodopsin was brought about by

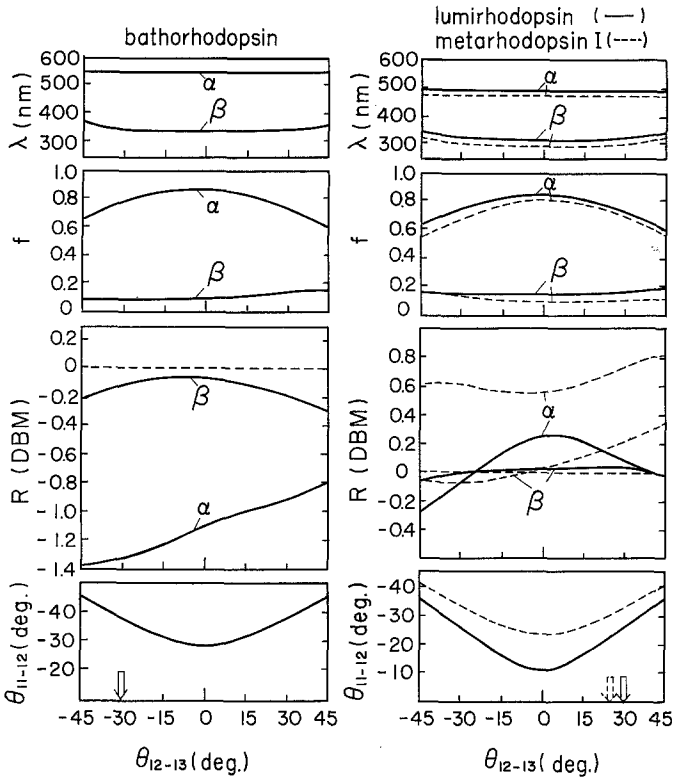

Fig. 3. Graphs of wavelength λ, oscillator strength f, rotational strength R and torsional angle θ_{11-12} of bathorhodopsin, lumirhodopsin and metarhodopsin I as a function of θ_{12-13}. In bathorhodopsin, θ_{6-7}, θ_{8-9}, θ_{9-10}, θ_{10-11}, θ_{13-14}, and θ_{14-15} are fixed to −50, −20, −25, −30, −15, and −15°. In lumirhodopsin, they are fixed to −50, −15, 25, 15, 15, and 15°. In metarhodopsin I, they are fixed to −50, −30, 20, 15, 0, and 15°, respectively. λ_α of bathorhodopsin, lumirhodopsin and metarhodopsin I are fitted to 543 nm, 497 nm, and 478 nm, respectively, by adjusting θ_{11-12} in calculations. The plausible torsional angle is shown by a white arrow

the increases of $|\theta_{10-11}|$ from 10° to 20° and of θ_{12-12} from 25° to 30°. The shift of 28 nm in 9, 13-*dm* isorhodopsin I was brought about mainly by the increase of θ_{10-11} from 25° to 35°. So that, the 9-methyl group is considered to play a role to regulate the torsional angles of some single bonds in the torsion model. In contrast with this, the replacement of the 13 methyl of the chromophore scarcely changes the absorption wavelengths of rhodopsin and isorhodopsin (Kropf et al., 1973). However, in our results of Table 1, small changes in the torsional angles of both the double and single bonds take place accompanying with the replacement of the 13-methyl group.

The longer wavelength of bathorhodopsin was mainly due to a large torsional angle of the 11—12 bond. The experimental values of the wavelengths of 5,6 epoxyrhodopsin and 5,6 epoxyisorhodopsin I seem to be very large in view of the fact that the lengths of their conjugated chains are smaller than that of the natural chromophore by two bonds. In the present calculations, this property was explained by adopting a large value of θ_{11-12} or θ_{9-10}.

Table 1. The most plausible torsional angles θ's for the chromophores of rhodopsin, isorhodopsin, analogues and intermediates. Unit is degree. The numbering of the atom is shown in Figures 4–6. The torsional angles are measured from 6*s*-cis form

	θ_{6-7}	θ_{8-9}	θ_{9-10}	θ_{10-11}	θ_{11-12}	θ_{12-13}	θ_{13-14}	θ_{14-15}	Basic form
Rhodopsin	−40	15	20	10	−152	25	0	15	
9-*dm* rhodopsin	−40	15	20	−20	−152	30	0	15	
13-*dm* rhodopsin	−40	20	15	15	−148	25	0	15	6*s*-cis,
5,6 epoxyrhodopsin		0	20	−20	−147	35	0	15	11-cis
3-dehydrorhodopsin	−40	20	25	−20	−142	40	0	15	
Isorhodopsin I	−30	10	−157	25	25	20	0	15	
13-*dm* isorhodopsin I	−30	10	−153	30	25	20	0	15	6*s*-cis,
9,13-*dm* isorhodopsin I	−30	10	−156	35	25	20	0	15	9-cis
5,6 epoxyisorhodopsin I		10	−144	35	25	−30	0	15	
3-dehydroisorhodopsin I	−30	20	−141	−35	20	30	0	15	
Isorhodopsin II	−30	15	−153	15	15	−25	−170	15	6*s*-cis, 9-cis, 13-cis
Bathorhodopsin	−50	−20	− 25	−30	− 38	−27	− 15	−15	
Lumirhodopsin	−50	−15	25	15	− 26	30	15	15	6*s*-cis
Metarhodopsin I	−50	−30	20	15	29	22	0	15	

For f_α, the calculated values are a little smaller than the experimental ones except for 5,6 epoxy and 3-dehydro anologues, but those multiplied by a factor 1.21 to the calculated values agree with the experimental ones very well. Relatively larger values of f_α in the intermediates are due to the all-trans forms of the chromophores whose conjugated chains spread more than those of 11-cis and 9-cis forms. The f_β's of the intermediates are very small compared with those of rhodopsin and isorho-

Table 2. Calculated and experimental absorption wavelength λ_{max}, oscillator strength f, and rotational strength R of rhodopsin, isorhodopsin, their analogues and intermediates

	λ_{max} (nm)				f				R (DBM)			
	exp[a]		cal		exp[a]		cal[o]		exp[a]		cal	
	α	β	α	β	α	β	α	β	α	β	α	β
Rhodopsin	498[b]	340[b]	498	324	0.75	0.2[n]	0.62 (0.75)	0.37	0.50[p] 0.25[q] 0.33[r]	0.65[p] 0.80[q] 0.63[r]	0.24	0.63
9-*dm* rhodopsin	461[c]	—	461	316	—	—	0.52 (0.63)	0.42	0.44[c]	0.31[c]	0.47	0.39
13-*dm* rhodopsin	495[d]	—	495	321	0.70	—	0.60 (0.73)	0.36	0.22[c]	0.88[c]	0.18	0.80
5,6 epoxyrhodopsin	465[e]	—	465	309	0.54	—	0.56 (0.68)	0.23	0.6[s]	~0[s]	0.85	−0.01
3-dehydrorhodopsin	517[f]	—	517	358	0.49	—	0.52 (0.63)	0.35	1.4[s]	0.3[s]	0.87	−0.11
Isorhodopsin I	486[g]	335[g]	485	326	0.79	0.3[n]	0.64 (0.77)	0.31	0.40[p] 0.30[q] 0.31[r]	0.35[p] 0.45[q] 0.22[r]	0.41	0.29
13-*dm* isorhodopsin I	488[d]	—	488	329	0.74	—	0.61 (0.74)	0.33	0.37[c]	0.25[c]	0.43	0.32
9,13-*dm* isorhodopsin I	458[c]	—	458	320	—	—	0.55 (0.67)	0.37	0.28[c]	0.21[c]	0.33	0.28
5,6 epoxyisorhodopsin I	465[c]	—	465	314	0.54	—	0.56 (0.68)	0.23	0.45[s]	~0[s]	0.49	0.07
3-dehydroisorhodopsin I	500[f]	—	500	360	0.51	—	0.48 (0.58)	0.40	1.2[s]	~0[s]	0.82	0.03
Isorhodropsin II	481[h]	335[h]	481	327	0.79	0.3[n]	0.58 (0.70)	0.20	0.29[r]	0.21[r]	0.43	0.36
Bathorhodopsin	543[i]	340[k]	543	332	0.93	—	0.77 (0.93)	0.06	−1.5[t]	~0[t]	−1.31	−0.03
Lumirhodopsin	497[j]	350[l]	497	326	0.88	—	0.74 (0.90)	0.17	0.1[u]	0.1[u]	0.10	0.04
Metarhodopsin I	478[j]	340[m]	478	313	0.89	—	0.76 (0.92)	0.11	0.7[v]	0.1[v]	0.65	0.15

[a] Experimental values are for cattle pigments; [b] Wald and Brown (1953); [c] Kropf et al. (1973); [d] Nelson et al. (1970); [e] Blatz et al. (1970); [f] Azuma et al. (1973); [g] Hubbard and Wald (1952); [h] Crouch et al. (1975); [i] Yoshizawa and Wald (1963); [j] Hubbard and Kropf (1959); [k] Estimated from the CD spectra obtained by Yoshizawa and Tokunaga (1973); [l] Estimated from the CD spectra obtained by Ebrey and Yoshizawa (1973); [m] Yoshizawa and Horiuchi (1973); [n] Estimated from the ratio of the absorptions between the α- and β-bands in the spectra by Crouch et al. (1975); [o] Values in parenthesis are those multiplied by 1.20 to f_{∇}; [p] Solvent is aqueous buffer. Burke et al. (1973); [q] Solvent is 67% Glycerol. Burke et al. (1973); [r] Solvent is Triton X-100. Ebrey et al. (1975); [s] Obtained from the data by Azuma et al. (1973); [t] Yoshizawa and Tokunaga (1973); [u] Ebrey and Yoshizawa (1973); [v] Yoshizawa and Horiuchi (1973)

dopsin. The reason of this is as follows: The β-band essentially corresponds to the cis-peak in carotenoids, and it is very small in the all-trans form by the symmetrical condition.

For R_α and R_β, the calculations well account for the experimental results. That is, $0 < R_\alpha < R_\beta$ holds for rhodopsin whereas $R_\alpha > R_\beta > 0$ for isorhodopsin I. It should be mentioned here that this property of isorhodopsin I could not be obtained

Table 3. Calculated bond lengths of the chromophores in rhodopsin, isorhodopsin I, isorhodopsin II, and intermediates and of all-trans PRSB. Unit is Å

	R_{5-6} [b]	R_{6-7}	R_{7-8}	R_{8-9}	R_{9-10}	R_{10-11}	R_{11-12}	R_{12-13}	R_{13-14}	R_{14-15}	R_{15-21}
Rhodopsin	1.342	1.489	1.353	1.457	1.373	1.442	1.384	1.453	1.367	1.442	1.313
Isorhodopsin I	1.344	1.477	1.355	1.453	1.374	1.455	1.378	1.451	1.367	1.442	1.313
Isorhodopsin II	1.344	1.477	1.355	1.454	1.380	1.448	1.368	1.458	1.367	1.443	1.312
Bathorhodopsin	1.340	1.498	1.351	1.460	1.377	1.453	1.402	1.445	1.378	1.434	1.319
Lumirhodopsin	1.340	1.498	1.351	1.456	1.378	1.445	1.381	1.458	1.372	1.440	1.314
Metarhodopsin I	1.340	1.498	1.348	1.472	1.369	1.447	1.385	1.449	1.368	1.441	1.314
All-trans PRSB[a]	1.339	1.506	1.349	1.459	1.358	1.451	1.363	1.445	1.369	1.435	1.316

[a] θ_{6-7} is taken as −60°

[b] The numbering of the atom is shown in Figures 4–6

unless we took into account the twisting around the 11–12 bond. In this analysis, it was found that the rotational strengths of isorhodopsin II changed abruptly when the torsional angles were varied a little from those listed in Table 1. So that it seems to be accidental that the CD spectrum of isorhodopsin II is very similar to that of isorhodopsin I. In 9-*dm* rhodopsin, 13-*dm* rhodopsin, 13-*dm* isorhodopsin I, and 9,13-*dm* isorhodopsin I, the calculated values of R_α and R_β are in satisfactorily good agreement with the experimental ones.

In our calculations, R_β's of 5,6 epoxyrhodopsin, 3-dehydrorhodopsin, 5,6 epoxyisorhodopsin I, and 3-dehydroisorhodopsin I are either very small or zero in agreement with the experimental results. The electronic transition corresponding to the β-band is due to the π-electron motion throughout the twisted conjugate chain as well as the α-band. Then, the CD for the β-band cannot be correlated with the specific region of the chromophore such as the ionone ring.

We see that our calculations of R_α and R_β for bathorhodopsin well account for the experimental data. It is important to notice that the large negative CD of the α-band could be reproduced only when most of the torsional angles were chosen to be negative. Even in such a conformation, the calculated R_β is very small. We could also reproduce the small R_α and R_β of lumirhodopsin and the large positive R_α and small R_β of metarhodopsin I.

Conformations of Chromophores

In our calculations, bond lengths of chromophores are obtained simultaneously with the electronic state if the torsional angles are given. These results for rhodopsin, isorhodopsin I, isorhodopsin II, and intermediates are listed in Table 3 together with

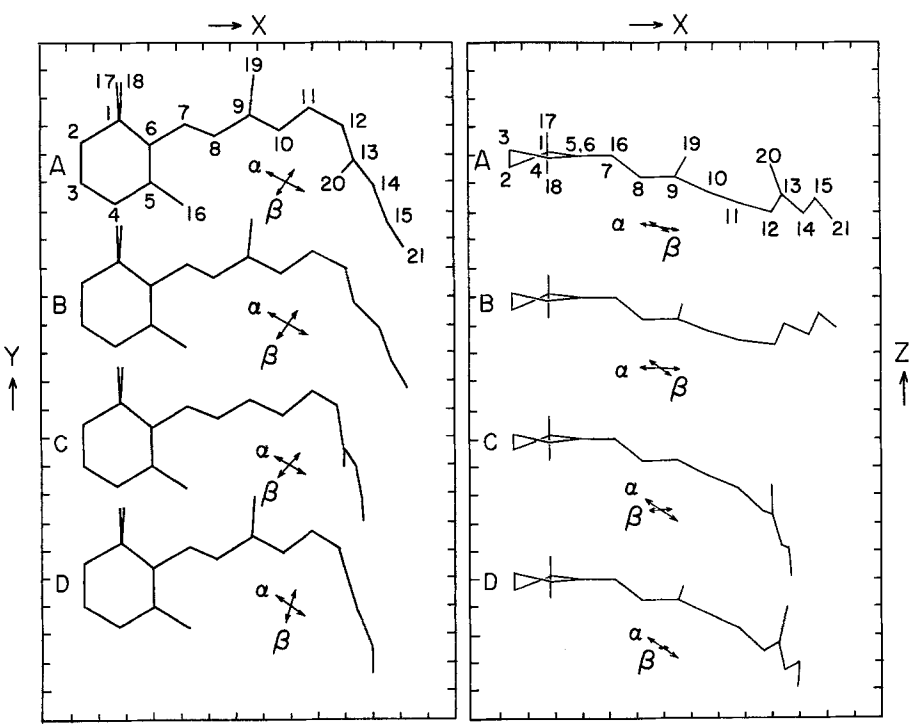

Fig. 4. Conformations of the chromophores of **(A)** rhodopsin, **(B)** 13-*dm* rhodopsin, **(C)** 9-*dm* rhodopsin, and **(D)** 3-dehydrorhodopsin projected to the *X-Y* and *X-Z* planes. One mesh of the coordinate denotes 1 Å. Transition dipole moment vectors of the α- and β-bands are shown by double arrows

the ones for all-trans PRSB. It is seen that large bond alternations are still preserved in all the visual pigments and intermediates. To see in detail, the bond lengths for 9—10 and 11—12 of the visual pigments and the intermediates are fairly larger than those of all-trans PRSB. The bond lengths for 6—7 of rhodopsin, isorhodopsin I, and isorhodopsin II are smaller than those of the others. The $C=N$ bond lengths (R_{15-21}) of all the pigments are quite similar to that of PRSB. These results would be useful in analysing the resonance Raman spectra.

The calculated conformations of the chromophores projected to X-Y and X-Z planes are shown in Figures 4—6. Here, Y-axis is chosen as the direction from C_5 to C_6, X-axis perpendicularly to the Y-axis in the plane C_5-C_6-C_7 in the direction from ionone ring to the Schiff-base, and Z-axis perpendicularly to the X- and Y-axes in the right-handed coordinate system. The conformation of the chromophore of rhodopsin in Figure 4 is considerably different from that of the 12—13 twisted 11-cis retinal. The plane which mostly includes the conjugated chain from C_6 to C_{11} is almost perpendicular to the plane which mostly includes the conjugated chain from C_{12} to N_{21}. The similar features are also seen in the chromophore of isorhodopsin I in Figure 5. The chromophore conformation in 13-*dm* rhodopsin is close to that in rhodopsin, but the conformations in 9-*dm* rhodopsin and 3-dehydrorhodopsin are a little different from that in rhodopsin. The chromophore conformations in 13-*dm* isorhodopsin I and 9,13 *dm* isorhodopsin I are close to that in isorhodopsin I. The chromophore conformation in isorhodopsin II is rather similar to that in isorhodop-

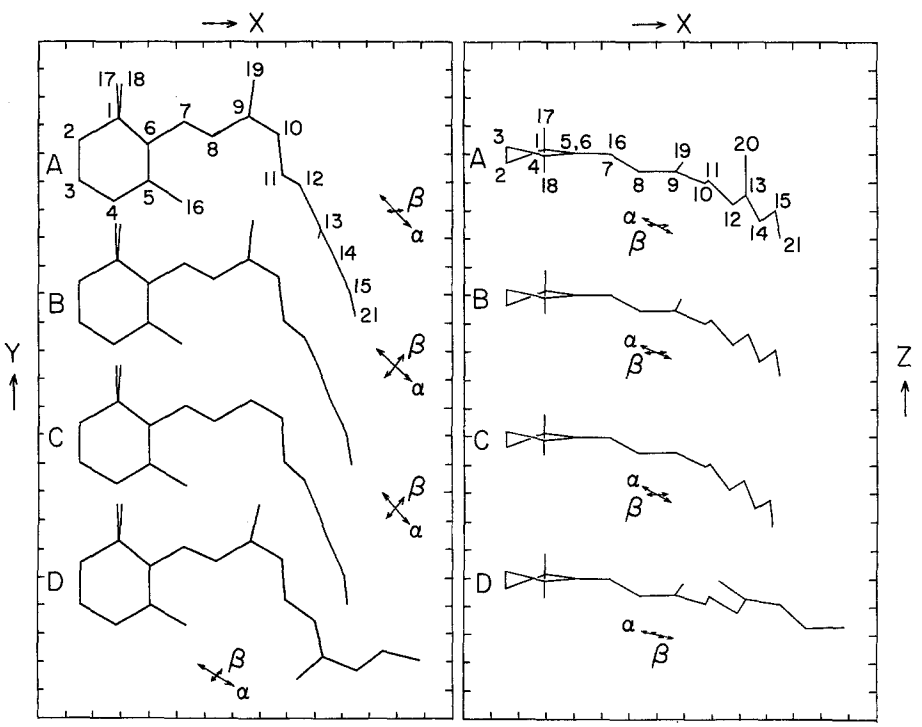

Fig. 5. Conformations of the chromophores of (**A**) isorhodopsin I, (**B**) 13-*dm* isorhodopsin I, (**C**) 9,13-*dm* isorhodopsin I, and (**D**) isorhodopsin II projected to the X-Y and X-Z planes

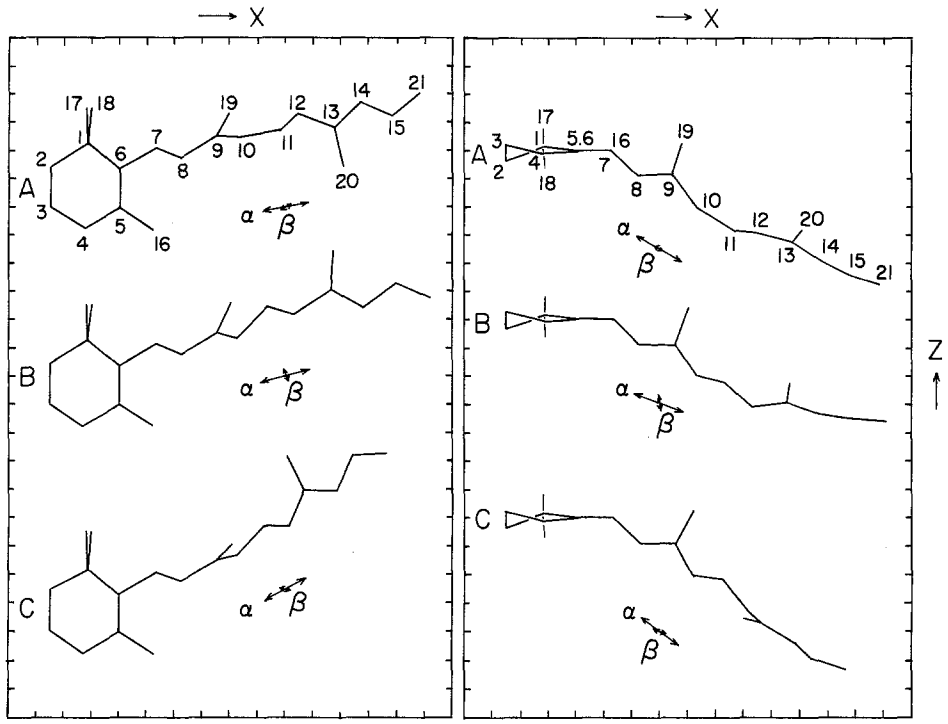

Fig. 6. Conformations of the chromophores of (**A**) bathorhodopsin, (**B**) lumirhodopsin, and (**C**) metarhodopsin I projected to the X-Y and X-Z planes

sin I in the X-Z plane but differs considerably in the X-Y plane. The length of the chromophore in isorhodopsin I in the X direction is a little smaller than that in rhodopsin. The chromophore conformation in bathorhodopsin in Figure 6 is rather similar to that of lunirhodopsin, but a little different from that in metarhodopsin I owing to the fact that the conjugated chain of metarhodopsin I bends back.

Discussions

Since the optical spectral parameters such as the rotational strength are sensitive to the electronic wavefunctions used in the calculation, it will be valuable to check whether the present results are significantly altered when calculations are made by using the other electronic theory. For this object, we recalculated λ, f, and R by the PPP theory, using the chromophore conformations determined in the present study. The effective nuclear charge on the protonated nitrogen was taken to be 1.65. The Coulomb repulsion integral was calculated with use of the Mataga-Nishimoto formula (1957). An empirical relationship between the bond order and bond length was satisfied. The configuration interaction was considered for the lower nine singly excited states. These calculated results are listed in Table 4. Most of the calculated values by this theory are fairly similar to those in Table 2. So that, at least qualitative

Table 4. Calculated values of λ, f, and R by the PPP-CI method

	λ (nm)		f		R (DBM)	
	α	β	α	β	α	β
Rhodopsin	501	306	0.55	0.13	0.22	0.09
Isorhodopsin I	507	311	0.58	0.15	0.24	0.31
Bathorhodopsin	516	316	0.75	0.03	−1.25	0.03
Lumirhodopsin	491	301	0.73	0.02	0.19	−0.13
Metarhodopsin I	480	307	0.75	0.02	0.57	0.08

results of the present study are expected to be unchanged if the calculation is made by the usual PPP theory.

Recently Birge et al. (1975) demonstrated that the $^1A_g^-$ like level lies close to the ^1B_u like level in the excited state of 11-cis retinal from the PPP calculations with all the single and double excitations. The $^1A_g^-$ state appears at the lower energy level only when double excitations are included in the CI calculation. At present, experimentally it is not known whether the $^1A_g^-$ level is near the ^1B_u level or not in the visual pigment. Furthermore, there is no guarantee at present that the rotational strength can be rightly calculated using the wavefunction with such a large number of CI's. Under these situations, as the first approximation, we neglected the effect of the $^1A_g^-$ state in the present analysis.

It is general that the mixing of σ- and π-orbitals occurs when a conjugated bond is twisted. In order to check the validity of the π-electron approximation in our torsional problem, we examined a degree of the mixing at $\theta \sim 30°$ by the CNDO/s method. As a result it was found that the mixing is small when σ-MO levels are apart more than 1 eV from the π-MO level. In PRSB, σ-orbital levels located just below the second highest occupied π-MO level and above the fourth lowest unoccupied π-MO level according to the CNDO/s calculation. Then, it is expected that the α-band will be little affected by the σ-π mixing but the β-band will be considerably affected. Now, it is generally known that the ordering of the energy levels of π- and σ-orbitals in the CNDO scheme is not necessarily good. In our case, it seems that the highest occupied σ-MO level is too high compared with the higher occupied π-MO levels. So that, the above examination should not be so conclusive.

In the section of *theoretical method*, we assumed that the chromophore of bathorhodopsin would be the 6*s*-cis form. This assumption is found to be authentic from our theoretical analysis. That is, calculating the rotational strengths for the chromophore in the 6*s*-cis, 11-cis or 6*s*-cis, 9-cis form, we could find no conformation whose R_α was negatively very large and R_β was very small. In addition to this, the large value of f_α could be scarcely realized in the 6*s*-cis, 11-cis or 6*s*-cis, 9-cis form of the chromophore although it is rather easily obtained in the 6*s*-cis form. The latter fact also held true in lumirhodopsin and metarhodopsin I.

The wavelength of the CD maximum generally differs from that of the absorption maximum. In most visual pigments and analogues, the difference amounts to about 10 nm for the α-band (Kropf et al., 1973). Phenomenological investigation of

this problem was made by Kropf et al. (1973) using the coupled oscillator model. In the intrinsic model, this problem is solved as follows. By taking account of the electron-vibration interaction, the electronic wavefunction of the chromophore can be expressed by the Herzberg-Teller expansion. Those terms which contain nuclear displacements make different contribution to the electric transition dipole moment from that to the magnetic transition dipole moment. This results in different spectral shapes between the absorption and CD. Then, the maximum wavelengths differ from each other. Quantitative calculations are now in progress.

Recently Mathies et al. (1977) obtained the experimental data that the resonance Raman spectrum of rhodopsin is very similar to that of 11-cis PRSB in solution, and the resonance Raman spectrum of isorhodopsin I to that of 9-cis PRSB in solution. From this, they presumed that the conformation of the chromophore in rhodopsin and isorhodopsin I would be very similar to those of 11-cis PRSB and 9-cis PRSB in solution, respectively. Therefore, the twisted conformations of the chromophores in visual pigments were thought to be unsuitable (Callender and Honig, 1977). In relation to this, we recently calculated vibrational frequencies of the twisted chromophores using the formalism of the molecular force constant developed by the self-consistent HMO theory (Kakitani, 1974a). As a result, it was found that many of the important vibrational frequencies of the chromophores in isorhodopsin I were rather similar to those in 9-cis PRSB. The vibrational frequency of the ethylenic stretching mode in isorhodopsin I was considerably smaller than that in 9-cis PRSB. The vibrational frequency of the $C = N^{+}H$ stretching mode in isorhodopsin I was similar to that in 9-cis PRSB. Also for rhodopsin and 11-cis PRSB, the similar results were obtained. All these facts are consistent with the experimental results. So that, the Mathies et al.'s data of the resonance Raman spectra do not necessarily preclude the torsion model. The detailed analysis will be published elsewhere (Kakitani and Kakitani, 1979).

The torsional angles obtained in this study may be overestimated. This is because we took into account only the steric hindrance effect between the chromophore and opsin in analyzing the optical data, even though there is a possibility that some electronic interactions between the protein moiety and the chromophore might simultaneously affect the optical spectra considerably. If the electronic interaction works in cooperation with the steric interaction for the absorption wavelength, our calculated angles around the double bonds would be reduced. However, even if so, our opinion is that the main factor which most affects the optical spectra will be the steric effect in visual pigments.

In succeeding papers, locations of the chromophores of visual pigments and intermediates in opsin will be investigated using the present results of the chromophore conformations. Furthermore, the adiabatic potential surfaces of the chromophore will be drawn in the θ_{9-10} to θ_{11-12} plane. Using these results, the specific photoisomerization properties of visual pigments could be accounted for. It will also be demonstrated how the highly twisted conformations of the chromophores in visual pigments are realized and how the large strain energies are supplied. These will be useful for the torsion model to become more realistic.

Acknowledgements. The authors are much indebted to Prof. S. Yomosa for helpful discussions. Thanks are due to Prof. T. Yoshizawa and the colleagues of his laboratory for their kind hospitality and valuable discussions during the stay of one of the authors (T.K.) at Kyoto University. They also thank

to Dr. F. Tokunaga and Mr. Y. Shichida for their valuable discussions on the CD spectra. This work was partly supported by a grant from the Japanese Ministry of Education to T.K. The numerical calculations were carried out by FACOM 230-75 at the Computation Center of Nagoya University.

References

Akhtar, M., Bolsse, P. T., Dewhurst, P. B.: Studies on vision: the nature of the retinal-opsin linkage. Biochem. J. **110,** 693–702 (1968)

Azuma, M., Azuma, K., Kito, Y.: Circular dichroism of visual pigment analogues containing 3-dehydroretinal and 5,6-epoxy-3-dehydroretinal as the chromophore. Biochim. Biophys. Acta **295,** 520–527 (1973)

Birge, R. R., Schulten, K., Karplus, M.: Possible influence of low lying covalent excited state on the absorption spectrum and photoisomerization of 11-cis retinal. Chem. Phys. Lett. **31,** 451–454 (1975)

Blatz, P., Dewhurst, P. B., Balasubramaniyan, B., Balasubramaniyan, P., Lin, M.: Preparation and properties of new visual pigment analogues from 5,6-dihydroretinal and cattle opsin. Photochem. Photobiol. **11,** 1–15 (1970)

Blatz, P., Mohler, J., Navangul, H.: Anion-induced wavelength regulation of absorption maxima of Schiff bases of retinal. Biochemistry **11,** 848–855 (1972)

Burke, M. J., Pratt, D. C., Faulkner, T. R., Moscowitz, A.: An analysis of the absorption and circular dichroism of some visual pigments. Exp. Eye Res. **17,** 557–572 (1973)

Busch, G. E., Applebury, M. L., Lamola, A. A., Rentzepis, P.: Formation and decay of prelumirhodopsin at room temperatures. Proc. Natl. Acad. Sci. USA **69,** 2802–2806 (1972)

Callender, R., Honig, B.: Resonance Raman studies of visual pigments. Annu. Rev. Biophys. Bioeng. **6,** 33–55 (1977)

Chan, W., Nakanishi, K., Ebrey, T., Honig, B.: Properties of 14-methylretinal, 13-desmethyl-14-methylretinal, and visual pigment formed therefrom. J. Am. Chem. Soc. **96,** 3642–3644 (1974)

Crouch, R., Purvin, V., Nakanishi, K., Ebrey, T.: Isorhodopsin II: artificial photosensitive pigment formed from 9,13-dicis relinal. Proc. Natl. Acad. Sci. USA **72,** 1538–1542 (1975)

Dartnall, H. J. A.: The photosensitivities of visual pigments in the presence of hydroxylamine. Vision Res. **8,** 339–358 (1968)

Ebrey, T. G., Yoshizawa, T.: The circular dichroism of rhodopsin and lumirhodopsin. Exp. Eye Res. **17,** 545–556 (1973)

Ebrey, T. E., Govindjee, R., Honig, B., Pollock, E., Chan, W., Crouch, R., Yudd, A., Nakanishi, K.: Properties of several sterically modified retinal analogs and their photosensitive pigments. Biochemistry **14,** 3933–3941 (1975)

Fager, R. S., Senjnowsky, P., Abrahamson, E. W.: Aqueous cyanohydridoborate reduction of the rhodopsin chromophore. Biochem. Biophys. Res. Commun. **47,** 1244–1247 (1972)

Gilardi, R., Karle, I. L., Karle, J.: The crystal and molecular structure of 11-cis retinal. Acta Crystallogr. **B28,** 2605–2612 (1972)

Green, B. H., Monger, T. G., Alfano, R. R., Aton, B., Callender, R. H.: Cis-trans isomerization in rhodopsin occurs in picoseconds. Nature **269,** 179–180 (1977)

Hamdorf, K., Paulsen, R., Schwemer, J.: Photoregeneration and sensitivity control of photoreceptors of invertebrates. In: Biochemistry and physiology of visual pigments. H. Langer (ed.), pp. 155–166. Berlin, Heidelberg, New York: Springer 1973

Honig, B., Greenberg, A. D., Dinur, U., Ebrey, T. G.: Visual-pigment spectra; implication of the protonation of the retinal Schiff base. Biochemistry **15,** 4593–4599 (1976)

Hubbard, R., Wald, G.: Cis-trans isomers of vitamin A and retinene in the rhodopsin system. J. Gen. Physiol. **36,** 269–315 (1952)

Hubbard, R., Kropf, A.: Molecular aspects of visual excitation. Ann. N.Y. Acad. Sci. **81,** 388–398 (1959)

Hurley, J. B., Ebrey, T. G., Honig, B., Ottolenghi, M.: Temperature and wavelength effects on the photochemistry of rhodopsin, isorhodopsin, bacteriorhodopsin and their photoproducts. Nature **270,** 540–542 (1977)

Irving, C. S., Byers, G. W., Leermakers, P. A.: Spectroscopic model for the visual pigments. Influence of microenvironmental polarizability. Biochemistry **9,** 858–864 (1970)

Kakitani, T.: Theoretical study of optical absorption curves of molecules. III. – Force constant and bond-bond interaction in conjugated molecules –. Prog. Theor. Phys. **51,** 656–673 (1974a)

Kakitani, T.: Theoretical study of optical absorption curves of molecules. IV. – Improvement of self-consistent HMO method using a new resonance integral and investigation of a relation between bond alternation and wavelength of optical absorption –. Prog. Theor. Phys. **52,** 1721–1736 (1974b)

Kakitani, T., Kakitani, H.: Molecular mechanism for the initial process of visual excitation. I. Model of photoisomerization in rhodopsin and its theoretical basis by a quantum mechanical calculation of adiabatic potential. J. Phys. Soc. Jpn. **38,** 1455–1463 (1975)

Kakitani, H., Kakitani, T., Yomosa, S.: Molecular mechanism for the initial process of visual excitation. II. Theoretical analysis of optical activity in rhodopsin and bathorhodopsin. J. Phys. Soc. Jpn. **42,** 996–1004 (1977)

Kakitani, T., Kakitani, H.: Self-consistent HMO theory for the excited state of conjugated molecules. – Molecular geometry, molecular vibration, and optical spectra –. J. Phys. Soc. Jpn. **42,** 1287–1295 (1977a)

Kakitani, T., Kakitani, H.: Application of self-consistent HMO theory to heteroconjugated molecules. Theor. Chim. Acta **46,** 259–275 (1977b)

Kakitani, T., Kakitani, H.: An empirical relation between oscillator strengths calculated from the dipole length and dipole velocity formalisms in the optical absorption of conjugated molecules. Theor. Chim. Acta **48,** 155–163 (1978)

Kakitani, H., Kakitani, T.: Theoretical analysis of resonance Raman spectra of rhodopsin and isorhodopsin. Submitted to J. Phys. Soc. Jpn. (1979)

Kropf, A., Whittenberger, B. P., Goff, S. P., Waggoner, A. S.: The spectral properties of some visual pigment analogs. Exp. Eye Res. **17,** 591–606 (1973)

Kropf, A., Whittenberger, B. P., Goff, S. P., Waggoner, A. S.: The spectral properties of some visual pigment analogs. Exp. Eye Res. **17,** 591–606 (1973)

Kuhn, W.: The physical significance of optical rotatory power. Trans. Faraday Soc. **26,** 293–308 (1930)

Mataga, N., Nishimoto, K.: Electronic structure and spectra of nitrogen heterocycles. Z. Phys. Chem. **13,** 140–157 (1957)

Mathies, R., Freedman, T. B., Stryer, L.: Resonance Raman studies of the conformation of retinal in rhodopsin and isorhodopsin. J. Mol. Biol. **109,** 367–372 (1977)

Matsumoto, H., Horiuchi, K., Yoshizawa, T.: Effect of digitonin concentration on regeneration of cattle rhodopsin. Biochim. Biophys. Acta **501,** 257–268 (1978)

Mommaerts, W. F. H. M.: Circular dichroism and the conformational properties of visual pigments. In: The retina. B. R. Straatsma et al. (eds.), pp. 225–234. Los Angeles: University of California Press 1969

Nelson, R., deRiel, J. K., Kropf, A.: 13-desmethyl rhodopsin and 13-desmethyl isorhodopsin: Visual pigments analogues. Proc. Natl. Acad. Sci. USA **66,** 531–538 (1970)

Oseroff, A. R., Callender, R. H.: Resonance Raman spectroscopy of rhodopsin in retinal disk membranes. Biochemistry **13,** 4243–4248 (1974)

Pariser, R., Parr, R. G.: A semi-empirical theory of the electronic spectra and electronic structure of complex unsaturated molecules: I. J. Chem. Phys. **21,** 466–471 (1953)

Pople, J. A.: Electron interaction in unsaturated hydrocarbons. Trans. Faraday Soc. **49,** 1375–1385 (1953)

Peters, K., Applebury, M. L., Rentzepis, P. M.: Primary photochemical event in vision; proton translocation. Proc. Natl. Acad. Sci. USA **74,** 3119–3123 (1977)

Rafferty, C. N., Cassim, J. Y., McConnell, D. G.: Circular dichroism, optical rotatory dispersion and absorption studies on the conformation of bovine rhodopsin in situ and solubilized with detergent. Biophys. Struct. Mech. **2,** 277–320 (1977)

Rosenfeld, T., Honig, B., Ottolenghi, M., Hurley, J., Ebrey, T. G.: Cis-trans isomerization in the photochemistry of vision. Pure Appl. Chem. **49,** 341–351 (1977)

Suzuki, H.: Electronic absorption spectra and geometry of organic molecules, Chapt. 12. New York, London: Academic Press 1967

Suzuki, H., Takizawa, N., Komatsu, T.: Optical properties of carotenoids in photoreceptor systems. Prog. Theor. Phys. [Suppl.] **46,** 16–39 (1970)

Suzuki, H., Komatsu, T., Kitajima, H.: Theory of the optical property of visual pigment. J. Phys. Soc. Jpn. **37,** 177–185 (1974)

Yoshizawa, T., Wald, G.: Pre-lumirhodopsin and the bleaching of visual pigments. Nature **197,** 1279–1286 (1963)

Yoshizawa, T., Horiuchi, S.: Studies on intermediates of visual pigments by absorption spectra at liquid helium temperature and circular dichroism at low temperature. In: Biochemistry and physiology of visual pigments. H. Langer (ed.), pp. 69–81. Berlin, Heidelberg, New York: Springer 1973

Yoshizawa, T., Tokunaga, F.: Molecular basis of photosensation. Kagaku **43,** 610–620 (1973) [in Japanese]

Wald, G., Brown, P. K.: The molar extinction of rhodopsin. J. Gen. Physiol. **37,** 189–200 (1953)

Warshel, A., Karplus, M.: Calculation of ground and excited state potential surfaces of conjugated molecules. I. Formulation and parametrization. J. Am. Chem. Soc. **94,** 5612–5625 (1972a)

Warshel, A., Karplus, M.: Calculation of $\pi\pi^*$ excited state conformations and vibronic structure of retinal and related molecules. J. Am. Chem. Soc. **96,** 5677–5689 (1972b)

Warshel, A.: Bicycle-pedal model for the first step in the vision process. Nature **260,** 679–683 (1976)

Received June 6, 1978/Accepted November 7, 1978