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The primary process of vision and the structure of bathorhodopsin: A mechanism for photoisomerization of polyenes*

(lumirhodopsin/hypsorhodopsin/binding site/concerted twist)

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ABSTRACT **A model for the primary process of vision is proposed, which involves a novel concerted-twist motion. Application of such motions to rhodopsin and bathorhodopsin successfully accounts for the properties of bathorhodopsin and related intermediates, including specific assignment of molecular structures to bathorhodopsin, to lumirhodopsin, and, less specifically, to hypsorhodopsin.**

In this paper we propose a mechanism for *cis-trans* isomerization of polyenes. This mechanism is a special case applicable only to compounds in a constrained medium. Arguments are presented to show that it is the preferred pathway for the primary process of vision.

Background on the primary process of vision

It is generally recognized that bathorhodopsin is the primary photoproduct in the visual process (1–4). The formation and the ensuing dark processes of this unstable intermediate have been extensively studied by low-temperature spectroscopy and fast kinetics. Fig. 1 shows a scheme for the photobleaching process and the characteristics of the intermediates, according to Shichida and Yoshizawa (5).

The existence of an intermediate preceding bathorhodopsin is less definitive. Several reports (6) suggest that the blue-shifted hypsorhodopsin could be either a precursor or a side-product of the photochemical process. Its presence appears to be dependent on the condition of preparation of the rhodopsin sample (6).

Following are a few of the characteristic properties of bathorhodopsin. Most unusual is that the chromophore, while encapsulated by the protein, can be converted to the bathorhodopsin form extremely rapidly (7). Bathorhodopsin in turn is readily converted to the blue-shifted lumirhodopsin (8, 9). The spectroscopic properties of bathorhodopsin are unusual in two ways. Its UV/visible absorption maximum is red-shifted from that of rhodopsin (8, 9) and its Raman spectrum shows bands in the 800–1000 cm^{-1} region that are not observed in derivatives of all-*trans*-retinal (10). The extra bands have been shown to be due to out-of-plane wagging motions of the hydrogens of C-10, -11, and -14 (11). Also noted was the peculiar shift of peaks associated with the out-of-plane wagging motion of the C-12 hydrogen. This was attributed to a specific protein–substrate interaction, a suggestion in agreement with the double point-charge model (12).

Additionally, bathorhodopsin is photochemically convertible to 9-*cis*-rhodopsin and rhodopsin (8, 9). If assuming geometric isomerization to be the primary process of vision (a point to be elaborated later), one is struck by the difference in regioselectivity of photoisomerization of bathorhodopsin (all-*trans* to both 9-*cis* and 11-*cis*) as compared to that of all-*trans*-retinal and its derivatives in nonpolar solvents (predominantly to 13-*cis*) (13, 14).

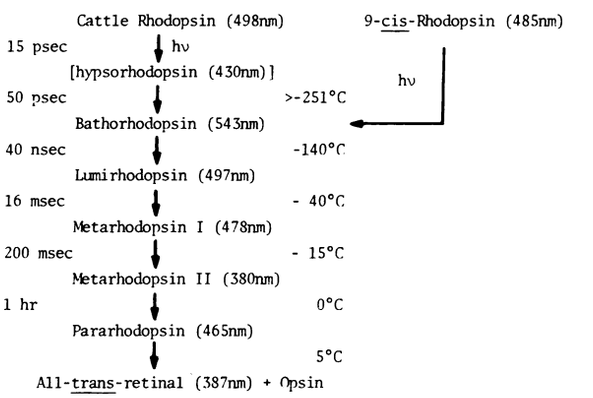

FIG. 1. Photobleaching processes of cattle rhodopsin (5). Transition temperatures between intermediates are listed on the right and decay times of intermediates at room temperature on the left. Absorption maxima are shown in parentheses.

Recent models for the primary photochemical process

A series of papers describing different models for the primary process followed reports of many of the observations described above. The hydrogen-transfer model (15, 16) represents a more radical departure from the traditional view (8, 9) of *cis-trans* isomerization. This model has the obvious advantage of requiring only a small reaction volume and explains the red-shifted spectrum of bathorhodopsin and the observed deuterium isotope effect on its rate of formation (16). However, it failed to explain the facile photoequilibration. Substantive argument against the proton-transfer model came from analogue studies. For example, for the phenyl analogue, the absence of reactive allylic hydrogens, whose presence is required for the hydrogen-transfer model, does not seem to affect the overall photobleaching process (17); and analogues without isomerizable 11,12 double bonds fail to undergo photobleaching (18).

Among models that embrace the basic concept of *cis-trans* isomerization, Warshel's bicycle-pedal model (19) is a clever way to permit geometric isomerization within a confined space. However, it fails to account for the photochemical equilibrium. Warshel subsequently disclosed a one-bond *cis* to *trans* isomerization model, supported by results of molecular-dynamics calculations (20). Along with an independent study by Birge and Hubbard (21), the calculations rationalize the facile isomerization process in confined media. The Lewis (22) protein-assisted model included for bathorhodop-

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Abbreviation: CT-*n*, concerted twist at center *n*.

*Photochemistry of Polyenes 22. No. 21 of the series is ref. 14.

sin an energetically untenable structure, the transition state for the bicycle-pedaling process. The model postulated by Honig *et al.* (23) provided an ingenious proton-relocation procedure to account for the red-shift, but lacked specifics on molecular properties of the chromophore. In fact, a weak point of many of the postulated models was the use of vaguely defined terms of protein perturbation of the retinyl chromophore.

In spite of all the recent effort, no definitive structures have been assigned to the bleaching intermediates. Hence, in this regard, little progress has been made beyond the original conclusion by Wald of the formation of the all-*trans* geometry during the bleaching sequence, as indicated by isolation of all-*trans*-retinal (8, 9).

The CT-*n* mechanism for *cis-trans* isomerization

The current model evolved from our recent thoughts on the effect of medium on directions of the photoisomerization of a polyene (24) and was further influenced by the simplicity of the bicycle-pedaling process (19).

It was reported (24) that the distribution of products from photoisomerization of a polyene is determined by regioselective twisting of the double bonds in the planar excited specie. Because of the extremely short lifetime of this specie, usually minor factors, such as relative ease in displacing solvent molecules, become important in determining which double bond is to be twisted. For molecules in a confined medium such as the retinyl chromophore inside the binding site of opsin, the medium effect is expected to be even more dramatic. That the 13-*cis* isomer is not an observed photoproduct from rhodopsin and bathorhodopsin suggests that the binding site is highly restrictive near the Schiff-base portion of the chromophore, possibly due to association of the counter-ion with the protonated nitrogen. The cyclohexenyl ring of the chromophore is, in turn, partially constrained through hydrophobic interaction with the protein (25, 26). That 7-*cis* isomers are not observed in photoreaction of visual pigments could lead to the impression that the binding site is also tightly packed near this end. However, since even in solution the 7-*cis* isomer is either absent or formed in trace amounts (13, 14), the ring terminus need not be as tightly congested as that near the Schiff base. That bathorhodopsin readily gives rhodopsin (11-*cis*) and 9-*cis*-rhodopsin suggests that there is little, if any, protein-induced constraint near the middle portion of the chromophore. Considering that geometric isomerization of any double bond requires twisting of only one end of the double bond, one may further rationalize from the observed photochemistry that the loose pocket of the binding site is specifically localized in the region surrounding C-10 and C-11 of the chromophore.

The above conclusions fit well with the recently postulated tertiary structure of rhodopsin by Hargrave *et al.* (27) and Dratz and Hargrave (28) which followed the disclosure of the primary protein sequence of opsin independently by Ovchinnikov *et al.* (29) and Hargrave *et al.* (27). In this model, the retinyl chromophore is believed to be embedded in seven α-helices of opsin. The only additional stipulation we wish to suggest is that those helices surrounding the middle part of the chromophore (helices 2 and 5 from the NH_2 terminus) (28) must be farther away from the chromophore than those around the 13,14 bond (helices 1 and 6) (28).

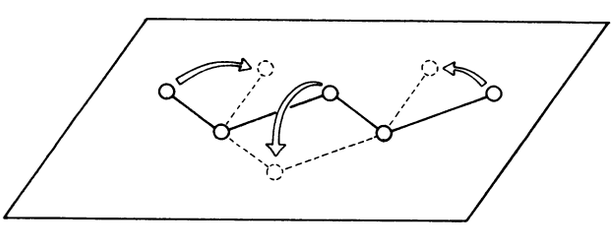
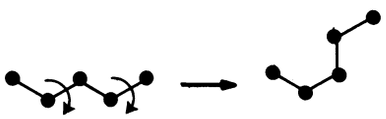


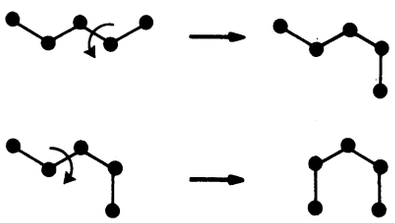
FIG. 2. The W (solid line) to U (dashed line) conversion via the concerted twist process (CT-3).

The simplicity of the bicycle-pedaling process as a mechanism for geometric isomerism of a molecule in a confined space intrigued us. Even though the process does not seem to be operative in the visual process, it is known to take place in ground-state reactions of the annulenes (30) and possibly in the dark adaptation process of bacteriorhodopsin (31, 32). We now wish to propose a new mechanism which bears some resemblance to the bicycle-pedaling process. For a clear distinction among the proposed model, the bicycle-pedaling model, and a related stepwise process, the latter two will be described first.

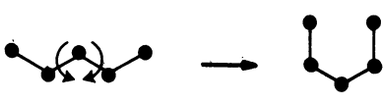
Consider an array of five trigonal-planar (sp^2) atoms arranged in a W-shape. A bicycle-pedaling process leads to a sickle-shaped arrangement of the five atoms.



A stepwise conversion of the W-shape to a U-shape can take place via sequential rotation of the middle bonds to give eventually an *inverted* U.



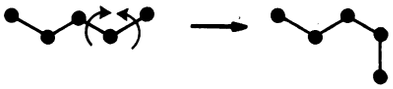
But there exists an alternative pathway for the W to U conversion. A simultaneous twisting motion of the two central bonds in the W-form gives an *upright* U directly (process 1).†



[1]

The salient feature of this concerted twisting process is that only the central atom moves in a sweeping semicircular manner (in and out of the plane of the molecule) while the two terminal atoms translate sideways in the original plane of the molecule (Fig. 2). Therefore, the process retains part of the virtue of the bicycle-pedaling mechanism by requiring a relatively small "reaction volume."

Twisting at different centers leads to different arrangements of atoms. Thus, a similar concerted twist around atom 2 (from right) of the W form gives an inverted sickle (process 2).



[2]

†For clarity, we recommend that the reader build such a five-atom framework. By holding atoms 2 and 4 in two hands one can easily achieve this motion, after a little practice, by applying a gentle continuous twist to the central bonds.

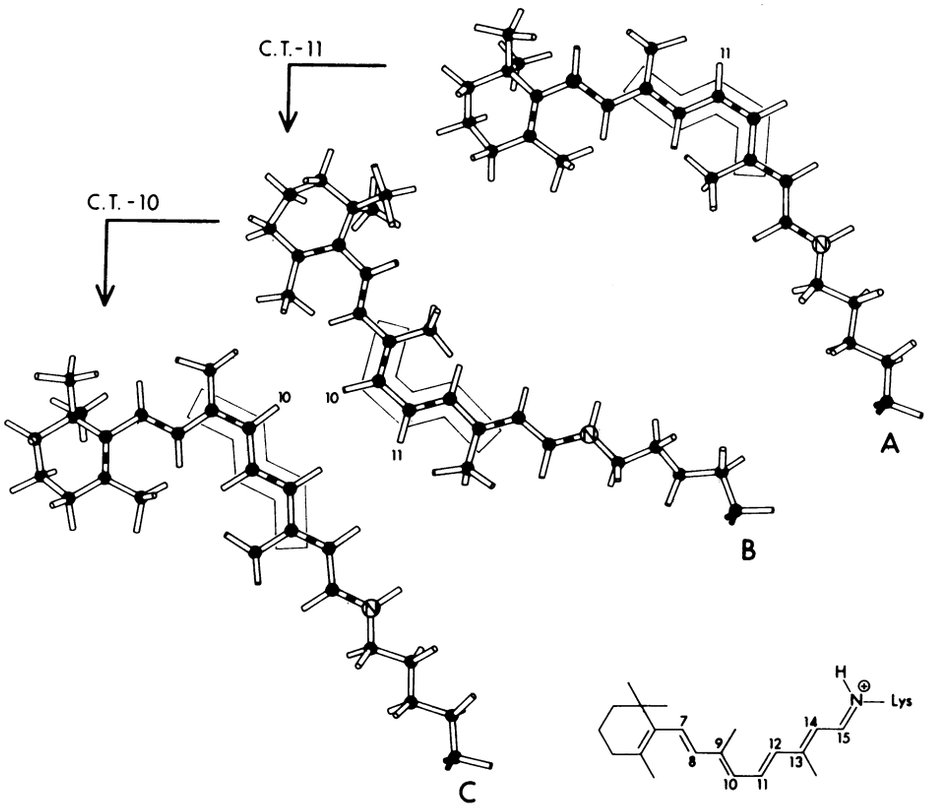


FIG. 3. Molecular models showing consequences of CT-*n* transformations of the chromophore of rhodopsin. Structure A: Molecular model of the chromophore bonded to the butylamino group of Lys-296. To approximate the longitudinal restrictions of the binding site, the α-carbon of Lys-296 was affixed to a table top and the motion of the cyclohexenyl ring was arbitrarily restricted by a metal loop attached to the C-2—C-3 bond. With the loop lying next to C-2, the side arm of the loop was affixed onto the table top. The box marks the inverted sickle formed by C-9 to C-13. Structure B: A new structure (that for bathorhodopsin) was obtained after application of the CT-11 motion to structure A. The C-9 to C-13 atoms now define a different sickle. Only the hydrogen on C-11 has translocated to the opposite side of the polyene chain. A bond-line structure of bathorhodopsin is shown in the lower right corner. Structure C: The structure for 9-*cis*-rhodopsin was obtained after applying the CT-10 motion to structure B. The C-9 to C-13 portion now appears in the shape of a W. The C-10 hydrogen is the only atom translocated.

A successive concerted twist at atom 3 now gives an upright sickle (process **3**), whereas a concerted twist at atom 4 leads to an inverted U (process **4**).

[3]

[4]

For want of an appropriate existing name for such a concerted twisting process, we propose the term CT-*n* (concerted twist at center *n*).‡ The motions described in processes **1**–**4** are therefore designated as CT-3, CT-2, CT-3, and CT-4 respectively (numbering from right to left).

Application of CT-*n* to the chemistry of visual pigments

The CT-*n* motion involves simultaneous twisting of two adjacent bonds. When applied to a conjugated polyene, it means geometric isomerization accompanied by conformational (180°) change of the adjacent single bond. On the other hand, the bicycle-pedaling process leads to geometric isomerization of two double bonds (not observed in experiments) and stepwise rotation involves nonconcerted geometrical or conformational changes.

Simultaneous twisting of a formal double bond and a formal single bond is an energetically prohibitive process for a ground-state molecule. However, this process is not as difficult in the excited state because of reduction of the π-bond order of formal double bonds. This has been demonstrated elegantly with self-consistent-field molecular orbital (SCF MO) calculations by Simmons (33) and recently was substantiated by Birge *et al.* (34) in INDO (intermediate neglect of differential overlap) calculations of excited retinal. In the latter case, the π-bond orders for the 9,10; 10,11; and 11,12 bonds (those of interest here) were shown to be nearly identical for either the $^1A_g^-$ state or the close-lying $^1B_u^+$ state for all-*trans*-retinal (34). Therefore, in the excited state, the CT-*n* process should be no more difficult than that of bicycle-pedaling, which has been calculated to proceed exothermically (19). In fact, we suspect that the transition state of CT-*n* could be stabilized by homo-allylic resonance interaction.

Based on the above consideration of the shape of the binding site, it should be clear that only the CT-10 and CT-11 processes are likely to take place. But, before one can apply such transformations to the visual pigments, an appropriate model first must be constructed. Fig. 3 is a reproduction of a molecular model constructed on the basis of a longitudinal restriction of the binding site of opsin (35, 36). The length of the butyl side-chain of Lys-296 as well as the length of the chromophore have now been taken into consideration.

Structure A in Fig. 3 shows the retinyl chromophore with one end anchored via a protonated imine linkage to the ε-amino group of Lys-296 (27, 29) and the other end in the hydrophobic pocket around the ring. The 12,13 bond assumes the *s-trans* conformation (36, 37). The enclosed five-atom region (C-9 to C-13) takes the shape of a sickle. Of the two possible CT-10 and CT-11 processes, only the latter causes *cis* to *trans* isomerization. It corresponds to twisting of the middle carbon of the five-carbon fragment shown above (process **3**) to give an inverted sickle. The product from such a process is structure B in Fig. 3, which we believe is the structure in bathorhodopsin. Not surprisingly, the structure contains the all-*trans* configuration, but additionally it reveals the presence of the 10-*s-cis* conformation.

With this new structural feature in mind, we can now account for the "unusual" spectral properties of bathorho-

‡The research group at Hawaii prefers to use the term HT-*n*: hula twist at center *n*.

dopsin. In addition to any possible protein-induced redshift, we now have to contend with the intrinsic property of the 10-*s-cis* conformation. Bathochromic shifts associated with such a linkage in a π-chromophore are well known. In fact, it is an integral part of the Woodward rule for UV-absorption properties of unsaturated systems: an extra 39 nm is added to the base values of homoannular dienes (38). Similar redshifts have recently been reported for *s-cis*-1,3-butadiene and 2-*s-cis*-1,3,5,7-octatetraene (39, 40) from the corresponding *s-trans* conformers. Also affecting the red-shift could be changes in the extent of the protein–substrate interaction that contributed to the red-shifted property of rhodopsin.

The difference in the Raman spectra of bathorhodopsin and model all-*trans* analogues can now be explained on the basis of the involvement of different conformers (10-*s-cis* and 10-*s-trans*). Furthermore, we suggest that the shifted band of the wagging mode of the C-12 hydrogen is not due to any specific interaction with the protein but due to perturbation of this motion by steric interaction with the 9-methyl hydrogens.

A CT-10 of rhodopsin is precluded because of the formation of the extremely crowded 9-*cis*,10-*s-cis*,11-*cis* isomer. [It differs from the pigment derived from 9-*cis*,11-*cis*-retinal (41) by its 10-*s-cis* conformation.] It is not surprising that the process is competitively not as favored as CT-11.

The secondary photochemical reactions of bathorhodopsin can also be readily explained by the CT-10 and CT-11 processes. In this case, both twisting processes are observed. The CT-11 process is clearly the reverse of the primary process, hence rhodopsin is regenerated. However, when CT-10 is applied to the C-9–C-13 sickle of bathorhodopsin, a W-shaped fragment is generated (reverse of process 2) corresponding to 9-*cis*,10-*s-trans*,11-*trans*, i.e., the stable conformer of 9-*cis*-rhodopsin (Fig. 3, structure C). Therefore, it should be no surprise that these species are photochemically interconvertible and that their formations are in the order observed in experiments.

Implications of the CT-*n* model

The assigned structure of bathorhodopsin further implies certain structural properties of the subsequent intermediate (lumirhodopsin) and possibly its precursor (hypsorhodopsin). The 10-*s-cis* conformation in bathorhodopsin is clearly highly unstable. It should undergo ready conversion to the more stable 10-*s-trans* form, hence the blue-shifted lumirhodopsin is formed.

After examination of molecular models, one might further speculate on the structure of hypsorhodopsin. The CT-*n* motion, while not demanding a large volume, does involve substantial translational motion of the polyene chain in the plane of the chromophore (Fig. 4). This motion should be permissible within the space defined by the vertically arranged α-helices of opsin (27), especially if the plane of the chromophore parallels the axes of the helices. It is further facilitated by the tether-like function of the butyl group of Lys-296. Examination of models shows that, if not anchored, there is an increase in the overall length from the ring to the α-carbon of Lys-296. For the chromophore and the lysine tether to be accommodated in the binding site, the conformation of either the chromophore or the butyl group must change (Fig. 4). Since the butyl group is in the tightly packed protein pocket whereas a portion of the chromophore is not, one can envision the formation of an initial photoproduct with a conformationally distorted 10-*s-cis*,all-*trans*-retinyl chromophore and a conformationally unchanged butyl group. This is hypsorhodopsin. Upon a slight relaxation of the protein near the butyl group, bathorhodopsin is formed, with a relatively planar chromophore and a conformationally readjusted butyl group (Fig. 4). The rate of this protein-relaxation process could be sensitive to hydrogen isotopes as observed in experiments (16). Furthermore, since the existence of hypsorhodopsin depends on the rigidity of the binding site, its detection becomes dependent on the method of sample preparation (6).

Furthermore, the formation of photo-metarhodopsin-II-465, thought to contain the 13-*cis* geometry (42), can be readily rationalized on the basis of the current model. With the chromophore not fully enclosed by the protein at the meta-II stage (42), the photochemistry of the retinyl chromophore no longer proceeds by way of the CT-*n* motion but rather adopts the single-twist motion expected for unconstrained polyene systems. Hence, the 13-*cis* isomer is the major photoproduct (13, 14).

The proposed mechanism of isomerization and the structure of bathorhodopsin suggest several interesting experiments. Lumi- and meta-I-rhodopsins should not give anomalous Raman bands. Conformationally rigid 10-*s-cis* retinal analogues and related model compounds could be used to shed light on the Raman data for and photochemical properties of bathorhodopsin. Some results with model compounds support the CT-*n* model. For example, the "anomalous" Raman data obtained with the batho intermediate of 5-demethylrhodopsin but not with that of 9-demethylrhodopsin (43) must be due to the difference in steric crowding in the respective 10-*s-cis* intermediates. The photoproduct from 7-*cis*-rhodopsin obtained at 79 K (44) either is not the primary photoproduct or has spectral properties deceptively similar to those of bathorhodopsin. In the same vein, the identity of the primary photoproduct from 9-*cis*,11-*cis*-rhodopsin (41) is unknown.

In this paper, we have emphasized discussions on the bovine visual pigment. The close similarity of other visual systems (5, 9) and bacteriorhodopsin suggests that CT-*n* motions might also be operative in these systems. It was brought to our attention during preparation of this paper that a process similar to CT-14 has been suggested for the primary process of bacteriorhodopsin and has been shown by

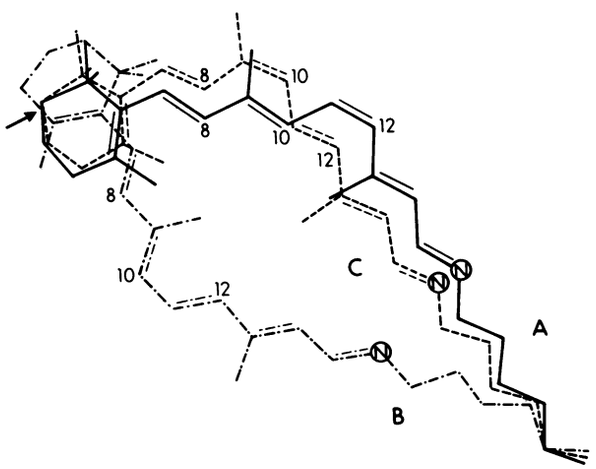


FIG. 4. An abbreviated sketch showing only the carbon-carbon bonds of superimposed structure A–C (see Fig. 3). ——, Rhodopsin (A); ·····, bathorhodopsin (B); ------, 9-*cis*-rhodopsin (C). When freed at both ends, the model showed that the overall lengths of rhodopsin and 9-*cis*-rhodopsin were approximately the same, whereas that of bathorhodopsin was longer by about 5%. Therefore, when the model was affixed as described in the legend to Fig. 3, the conversion of rhodopsin to bathorhodopsin led to a spontaneous readjustment of the conformation of the model by rotating the cyclohexenyl ring and twisting the butyl group (see text for additional discussion). Arrow indicates position of the metal ring described in the legend to Fig. 3.

MINDO/3 (MINDO, modified intermediate neglect of differential overlap) calculations to involve a low-energy pathway (32, 45).

CT-*n* as a general mechanism for geometric isomerization

It is interesting to speculate whether the CT-*n* motion can be a general process for *cis-trans* isomerization. The implications of geometric isomerization accompanied by a conformational change of the adjacent bond cannot readily be tested under ordinary conditions. For conformationally homogeneous polyenes, the resultant photoproducts necessarily are conformationally unstable; hence, their detection is extremely difficult. Entropically, the double-twist motion is not likely to be competitive with the single-twist motion normally described for geometric isomerization. Only when the more space-demanding single-twist motion is blocked might one anticipate possible intervention of the CT-*n* process. The recent report of detection of the 2-*s*-*cis* conformer upon irradiation of 1,3,5,7-octatetraene at <10 K (40) could be a case of CT-2. The CT-2 process predicts that the conformational change will be accompanied by degenerate geometric isomerization at the 1,2-bond.

Conclusion

Application of the proposed two-bond concerted-twist mechanism for photoisomerization of confined polyenes to rhodopsin and bathorhodopsin led to successful explanation of the following observations: (*i*) The rapid rate of the primary photochemical process. (*ii*) The facile photoequilibration of bathorhodopsin, rhodopsin, and 9-*cis*-rhodopsin. (*iii*) The difference in UV/visible absorption maxima of rhodopsin, bathorhodopsin, lumirhodopsin, and possibly hypsorhodopsin. (*iv*) The unusual Raman spectra of bathorhodopsin, batho-5-demethylrhodopsin but not batho-9-demethylrhodopsin. (*v*) The difficulty in detecting hypsorhodopsin in a consistent manner. (*vi*) The photochemical behavior of metarhodopsin-II. The model led to the following structures of bathorhodopsin and lumirhodopsin: the 10-*s*-*cis* and 10-*s*-*trans* conformers of the all-*trans*-retinyl chromophore, respectively. Furthermore, hypsorhodopsin with a highly distorted 10-*s*-*cis*,all-*trans*-retinyl chromophore has been inferred to be a precursor of bathorhodopsin.

The thoughts elaborated in this paper are rooted in the early graduate study of R.S.H.L. He recalls with fond memory the inspirational direction of G. S. Hammond. These thoughts are also an outgrowth of subsequent work done at the University of Hawaii. R.S.H.L. acknowledges the effort of all researchers who participated in the polyene program there, in particular, H. Matsumoto, M. Denny, V. Ramamurthy, A. Kini, V. J. Rao, and D. Mead. Both authors thank Prof. P. Vollhardt for pointing out the analogy between annulene isomerization and the bicycle-pedaling mechanism. They thank Prof. R. Birge for communicating to them additional bond-order data. Support of the polyene program at the University of Hawaii has been provided by grants from the U.S. Public Health Service (AM17806) and the National Science Foundation (CHE12686 and CHE16500).

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