Proc. Natl. Acad. Sci. USA
Vol. 76, No. 6, pp. 2503–2507, June 1979
Chemistry

Photoisomerization, energy storage, and charge separation: A model for light energy transduction in visual pigments and bacteriorhodopsin

(charge separation in proteins/rhodopsin photochemistry/purple membrane/proton pumping)

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Communicated by Cyrus Levinthal, March 22, 1979

ABSTRACT **A simple model for the early events in visual pigments and bacteriorhodopsin is proposed. The model makes use of the likelihood that a negatively charged amino acid forms a salt bridge with the positively charged nitrogen of the retinylic chromophore. The photochemical event is a *cis-trans* isomerization in visual pigments and a *trans-cis* isomerization in bacteriorhodopsin, which in each case cleaves the salt bridge and thus separates charge in the interior of the protein. We propose that this is how the energy of a photon is transduced into chemical free energy of the primary photoproduct. The use of photoisomerization of a flexible chromophore to achieve charge separation provides a general mechanism which may be applicable to other systems. Our model explains many of the fundamental properties of visual pigments and their photoproducts. First, the extraordinarily low rate of thermally populating the ground state of the primary photoproduct, as determined from psychophysical and electrophysiological measurements, is seen as resulting from the large barrier to thermal isomerization about a double bond, perhaps enhanced by electrostatic attraction in the salt bridge. Second, the increase in energy and the spectral red shift that characterize the primary photochemical events are natural consequences of the separation of charge. Proton-dependent processes detected with picosecond techniques are proposed to be ground-state relaxation processes following the primary photochemical event. Finally, the charged groups of the salt bridge, repositioned by photoisomerization, provide a simple mechanism for vectorial proton translocation in bacteriorhodopsin.**

Visual pigments are a class of proteins found in the membranes of photoreceptor cells (for reviews, see refs. 1 and 2). Their chromophoric unit is 11-*cis*-retinal covalently bound in the form of a Schiff base to the ϵ-amino group of a lysine. The absorption of a photon by a visual pigment initiates a sequence of biochemical events that eventually lead to the generation of a neural signal by a photoreceptor cell. The identity of the primary photochemical event has been a subject of considerable interest and controversy. It was originally suggested that the primary event was an isomerization of the chromophore from its 11-*cis* to an all-*trans* conformation (3, 4). The strongest evidence favoring this mechanism was the observation (based on spectral data at low temperature) that an artificial pigment containing a 9-*cis* chromophore had the same photoproduct as rhodopsin itself. It was quite reasonably concluded that the most plausible common photoproduct formed from the two *cis* isomers is a *trans* isomer.

A number of picosecond absorption studies of the primary event have raised widespread doubts as to the validity of the original model. It was found (5) that the primary event is complete in less than a few picoseconds at room temperature, and it was argued that this is too short a time for isomerization to occur [although other picosecond studies have reached the opposite conclusion (6)]. More recent evidence has come from the observation that at low temperature the rate of the process is significantly inhibited by deuterium replacement of the exchangeable protons on the pigment (7). Since only one proton on the chromophore is exchangeable, it is unlikely that this would have a measurable effect on the rate of isomerization. The picosecond measurements have generated numerous models (7–11) whose major feature is a photochemical proton transfer followed by a thermal *cis-trans* isomerization that occurs at a later stage. However, the original evidence upon which the suggestion of a *cis-trans* isomerization was originally based has never been discredited and, thus, it appears necessary to find a molecular model that is consistent with the entire body of available evidence. The purpose of this paper is to present such a model.

There are, in fact, a fairly large number of observations that can be used in the construction and evaluation of alternative models. For example, we have shown, by using simple thermodynamic arguments, that a significant fraction of the photon's energy is "stored" in the primary photoproduct (12). Clearly a mechanism for energy storage must be an important component of any model that is proposed. Another energetic constraint may be derived from psychophysical and electrophysiological measurements of the level of thermal noise in photoreceptor cells. We show below that these observations may be interpreted directly in molecular terms and that they require an unusually high thermal activation energy for the primary event that appears to be inconsistent with many of the models that have been proposed.

The essential feature of the model presented in this paper is that electrostatic interactions between the chromophore and the protein determine many of the important properties of visual pigments and their primary photoproducts. The existence of such interactions is deduced from resonance Raman studies (13–16), which have clearly demonstrated that the Schiff base of the chromophore is protonated. Because buried charges generally appear as members of an ion pair in a salt bridge, this leads to the expectation that a negative counter-ion,[f] presumably the carboxylate group of an aspartic or glutamic acid, will be located at the ionic bond length of about 3 Å from the Schiff base nitrogen (17, 18). The primary photochemical event is

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[f] It is possible, though unlikely, that there is no counter-ion and that the protonated nitrogen is "solvated" by dipolar groups in the opsin which provide a negative environment in the vicinity of the Schiff base. Although we assume in the remainder of this paper that a true counter-ion exists, our discussion would be applicable if the counter-ion would be replaced by formal charges on electrically neutral dipolar groups such as carbonyls.

assumed to be an isomerization that breaks the salt bridge and, as a result, leads in the ground state to the rapid movement of one or more exchangeable protons on the protein. That isomerization can disrupt a salt bridge has been pointed out previously (17–19). The new idea introduced in this work that has not been previously recognized is that the essential goal of photoisomerization is charge separation and that this alone can account for the characteristic properties of the primary event. Indeed, it provides a general mechanism for light energy transduction in other biological systems that make use of a flexible chromophore and, in particular, as discussed below, can readily account for the spectroscopic photochemical and proton pumping properties of the retinal-containing pigment of *Halobacterium halobium*. bacteriorhodopsin (20).

The primary photochemical event

The absorption of a photon drives rhodopsin through a series of spectrally distinct intermediates, leading finally to the dissociation of the all-*trans* isomer of retinal from the apoprotein opsin. The general scheme is given by

$$\text{Rhodopsin} \xrightarrow{h\nu} \text{bathorhodopsin} \xrightarrow{\Delta} \text{all-}trans\text{-retinal} + \text{opsin}.$$

Bathorhodopsin is the first stable species at 77 K and corresponds to the primary photoproduct.

At 77 K a photoequilibrium can be established (4) between rhodopsin, bathorhodopsin, and isorhodopsin (an artificial pigment with 9-*cis*-retinal as its chromophore):

$$\text{Rhodopsin} \overset{h\nu}{\rightleftharpoons} \text{bathorhodopsin} \overset{h\nu}{\rightleftharpoons} \text{isorhodopsin}.$$

That bathorhodopsin is a common intermediate between pigments with different *cis* isomers is the original basis for the suggestion of a *cis-trans* isomerization as the primary photochemical event (3, 4). Several recent studies have provided strong supporting evidence (6, 21, 22).

The major argument that has been put forth against *cis-trans* isomerization is that a molecule the size of retinal cannot isomerize at low temperatures and, in particular, in less than 6 psec. With regard to isomerization times, the equation $\frac{1}{2}I\omega^2 = \frac{1}{2}kT$, in which I is the relevant moment of inertia and ω an angular frequency, shows that molecules the size of retinal, if not subject to a barrier, would have a rotation period at room temperature of this order. Moreover, so long as there is no barrier to the process, isomerization will be temperature independent and can occur in subpicosecond times under the influence of a driving force such as the gradient of a barrierless potential energy surface [like that proposed for the excited state of visual pigments (12, 23); see below]. For example, using Newtonian mechanics and assuming even a shallow potential gradient of 1 kcal/mol-Å (1 cal = 4.184 joules) and a molecular weight of 100 for the fragment that actually moves, we calculate an isomerization time of about 1 psec. It seems worth pointing out in this regard that picosecond studies of other molecules show extremely fast rotation times even for large molecular fragments (24).

It is perhaps more difficult to envision a large conformational change of the chromophore buried inside the protein at low temperature. Nevertheless, photoisomerization at temperatures as low as 4 K from rhodopsin (11-*cis*) to isorhodopsin (9-*cis*) has been clearly demonstrated (22, 25) so that it would appear that the active site has been so designed to facilitate geometric rearrangements that would not normally occur in solution. The actual isomerization need not lead to large displacements of atoms if some combination of torsional motion [perhaps involving the simultaneous rotation about a number of bonds (19) or rotations and translations of the entire chromophore (12)] are involved.

In fact, it seems unlikely that bathorhodopsin is a pure all-*trans* chromophore but rather, as has been suggested, it exists in a distorted all-*trans* conformation (4, 12). A conformation that is twisted about single bonds appears to us to be the easiest way of alleviating any strain that might be present. In the absence of data that allow a more accurate description, it seems most reasonable to use the term "transoid" to characterize the conformation of the chromophore of bathorhodopsin.

One other important property of bathorhodopsin should be emphasized. As implied by its name, bathorhodopsin is red shifted relative to rhodopsin, and this behavior has been noted in every visual pigment that has been examined. Moreover, bacteriorhodopsin exists in two isomeric forms and each has a red-shifted primary photoproduct stable at 77 K (26, 27). Thus, a spectral red shift seems to be a universal characteristic of the primary photoproduct of retinylic chromophores, and this should be incorporated in any model of the primary event.

Thermal stability: Evidence from psychophysics and electrophysiology

The role of visual pigments as light transducer molecules depends on their thermal stability as well as on their photolability. The photobleaching of a single rhodopsin molecule can excite a rod. Thus, it is necessary that the probability of thermally populating the bathorhodopsin ground state be extremely low; otherwise thermal noise would obscure the signal initiated by the photon. From psychophysical measurements, Barlow (28) estimated the level of thermal noise in rods; assuming all the noise is due to thermal excitation of the pigment, he obtained an upper limit for the rate constant of thermal bleaching of rhodopsin of less than 10^{-10} sec^{-1}. Assuming a preexponential factor in the range of 10^9–10^{13} (29), we estimate the activation energy in going from rhodopsin to bathorhodopsin to be at least 30 ± 3 kcal/mol. Recently, Ashmore and Falk (30) identified a component of noise in dogfish bipolar cells which they show arises from thermal isomerization of the pigment. By studying its temperature dependence, they determine an activation energy of 36 kcal/mol. Using this value and an activation energy for the back reaction of 7 kcal/mol [an estimate for the chicken cone pigment iodopsin (31)], we obtain a rough upper limit for the enthalpy difference between rhodopsin and bathorhodopsin of 29 kcal/mol, consistent with our previous estimate of greater than 13 kcal/mol (12) (see Fig. 1).

The barriers to thermal isomerization of free retinals are known from Hubbard's work (29), but similar numbers are not available for the protonated Schiff bases. These are expected to be somewhat smaller since the increased pi electron delocalization in these systems somewhat weakens their double bonds (18). It is interesting that the rather large activation energies measured for the thermal isomerization of retinal (29) approach the values determined for the pigments. It would seem that a photoisomerization about a double bond was chosen by evolution not only because it can occur so readily in the excited state, but because it occurs so rarely in the ground state, thus assuring a low level of thermal noise. However, the rate of thermal isomerization in retinals is still about 2 orders of magnitude higher than the upper limit for the rate of thermal isomerization of rod pigments. Thus, the interaction with the opsin must enhance the inherent stability of the ground state conformation of the retinal chromophore.

Interaction of Schiff base with counter ion accounts for thermal stability, energy storage, and spectral red shift

In this section, we show that an isomerization event provides a simple and direct explanation of many important properties of rhodopsin and bathorhodopsin if the counter ion is explicitly taken into account.

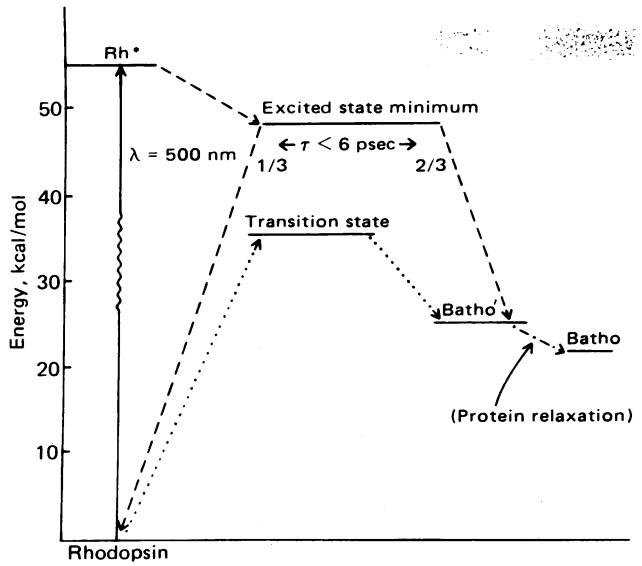

FIG. 1. Energy level diagram for primary processes in vision. Batho' is the transient observed by Peters *et al.* (7); bathorhodopsin is the longer lived species. The chromophore of rhodopsin has an 11-*cis* conformation. Batho' and Batho have "transoid" chromophores; they differ by a small conformational change in the protein, involving the movement of a proton(s). The excited state minimum and the transition state are configurations twisted by approximately 90° about the 11=12 double bond: (---) pathway of radiationless de-excitation involving torsional motion about the 11=12 double bond; (·····) pathway of thermal population of bathorhodopsin through the transition state. The activation energy is determined from the thermal noise in rods (see text): (-·-) relaxation of the protein (proton transfer) after *cis-trans* isomerization.

A fundamental difference between the counter ion in the protein and one in solution is that the latter is mobile while the former is not. In the opsin matrix, the counter ion would not be expected to follow the Schiff base nitrogen during isomerization. Thus, an ionic bond has to be broken before isomerization can occur, and this is energetically unfavorable in a low dielectric medium. This, then, provides the first important property of our model—a straightforward explanation of the increased resistance of visual pigments to thermal isomerization relative to protonated Schiff bases in solution.

After photoisomerization has occurred, the chromophore finds itself in a new conformation in which the nitrogen has been removed from the vicinity of the counter ion. A rigid body rotation of 11-*cis*-retinal with the counter ion kept fixed would lead to a charge separation of about 7 Å, compared to the 3-Å ionic bond length before isomerization.[g] However, as discussed above, isomerization can occur without large displacements of atoms. Assuming only a 5 Å charge separation and a distance-dependent dielectric constant (32) for the protein interior of $\epsilon = 1.0$ at $r = 3$ Å and $\epsilon = 2.5$ for $r = 5$ Å and a charge of 0.5 on the nitrogen (18), the increase in internal energy of the protein upon isomerization could be as large as 40 kcal/mol. Although this rough estimate is clearly too large, it does demonstrate that significant energy storage can be obtained by charge separation alone in a low dielectric medium.

Finally, we note that separating a protonated Schiff base from its counter ion should always lead to a red shift (18, 33) and provides an immediate explanation of the spectral red shift seen in the formation of all primary photoproducts.

These consequences of an isomerization event are not limited to a particular starting *cis* or *trans* isomer. *Any isomerization can induce a charge separation of the protonated Schiff base of the chromophore from its counter ion,* so that the mechanism will work equally well for the 11-*cis* to all-*trans* isomerization in visual pigments, *trans* to *cis* isomerization in light-adapted bacteriorhodopsin, and *cis* to *trans* isomerization in dark-adapted bacteriorhodopsin (34). The photochemical event depicted in Fig. 2 refers to rhodopsin, but the general mechanism is applicable to any pigment that has a flexible chromophore even though the specific bond that rotates and the direction of isomerization may vary.

[g] We assume here that the bulky ring end of the chromophore undergoes only small displacements during the isomerization.

Evidence that proton transfer is a ground-state process

After excitation of either bovine rhodopsin at very low temperatures or bacteriorhodopsin at room temperature, Rentzepis and coworkers (7, 35) have detected a transient absorption that appears in less than the 6-psec resolution time of their apparatus at all temperatures. The transient state decays to the bathorhodopsin ground state via a temperature-dependent process that is resolvable below 20 K and is slowed down by deuterium exchange. It was suggested that the transient corresponds to the first singlet excited state, S_1, of the parent pigment so that the measured absorption would have to arise from a transition to a higher singlet, ($S_1 \rightarrow S_n$).

An alternative hypothesis is that the transient is not an excited state species, but rather corresponds to a ground-state precursor of bathorhodopsin (22, 23). About 33% of the excited rhodopsin molecules and 70% of the excited bacteriorhodopsins fail to form the batho photoproduct and simply repopulate their own ground state (12, 23). The picosecond data (7, 35) suggest that this process is complete in less than 6 psec at all temperatures because no transient absorption changes corresponding to ground-state repopulation have been detected at longer times. Thus, the observed transient (which, for example, decays at 10 K with a half life of 29 psec) is unlikely to be the first singlet excited state of rhodopsin because the apparent decay kinetics of the first singlet (repopulation of the rhodopsin ground state in less than 6 psec at all temperatures) do not match those of the transient.

Further evidence is based on the finding (12, 23) that there is only a single minimum along the excited state coordinate connecting rhodopsin and bathorhodopsin (Fig. 1). Repopulation of both ground states from this common excited state requires that they appear in identical times, corresponding to the lifetime of the excited state. Thus, if the rhodopsin ground state is repopulated in less than 6 psec, as suggested above, it follows that the transient is also a ground-state species which we call Batho'. Batho' is formed in less than 6 psec at all temperatures and decays to bathorhodopsin in a temperature-dependent and deuterium-dependent process (7). The sequence of events we suggest then (Fig. 1) is rhodopsin* → Batho' → Batho, in which the first step, which is complete in less than 6 psec at all temperatures, is a photoisomerization of chromophore with the concomitant charge separation. The second, deuterium-dependent process, involves a ground-state relaxation of the protein induced by the isomerization and may take from less than 6 psec to 36 psec, depending on temperature. This relaxation, we propose, gives rise to the transient.

Isomerization and proton transfer

The data of Rentzepis and coworkers (7, 35) suggest that proton translocation is a rate-limiting step in the formation of bathorhodopsin.[h] We view this as part of the relaxation process that might involve the formation or disruption of hydrogen bonds that accompany protein conformational changes or the motion of a single proton close to the cleaved salt bridge. Because Raman studies (13–16) show that the Schiff-base proton does

[h] See next page for footnote.

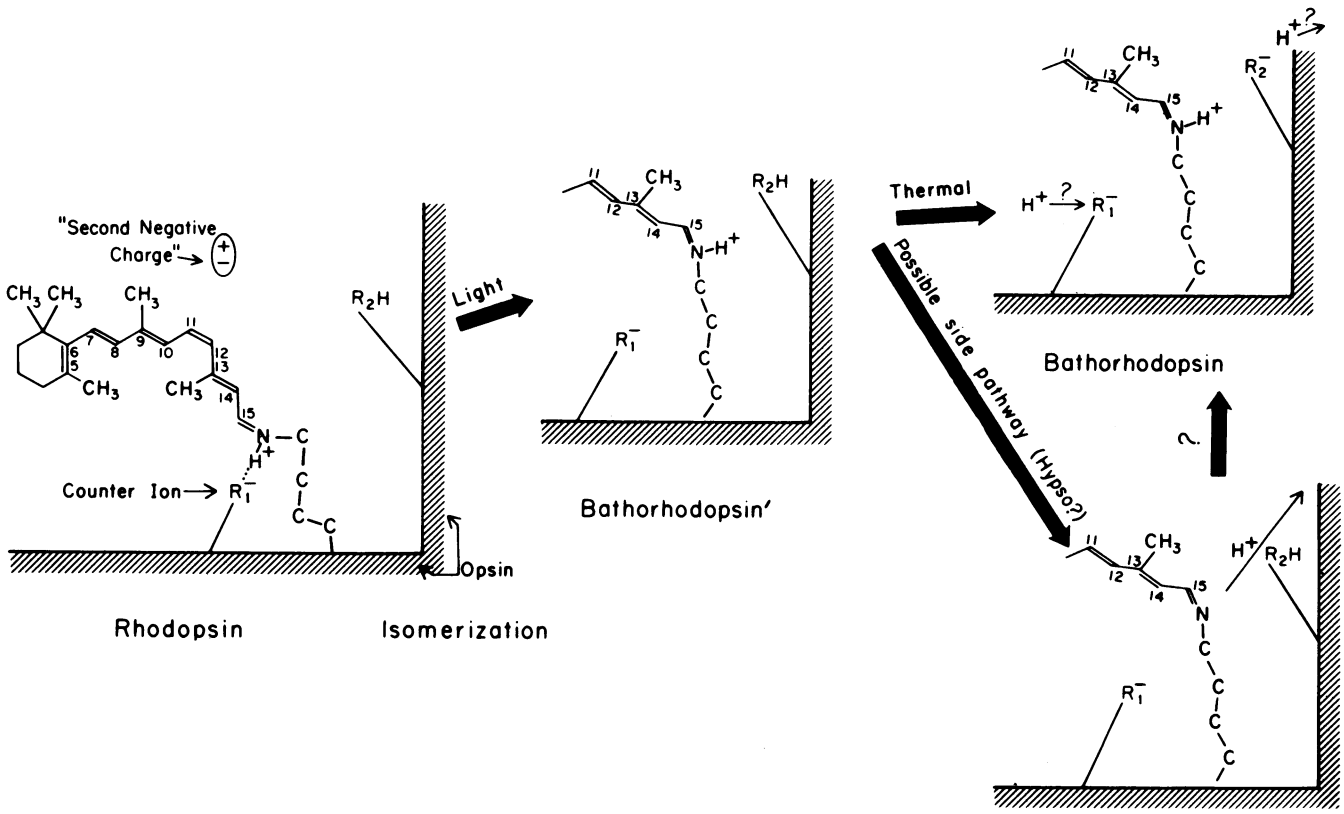

FIG. 2. Model for the early events in visual excitation. The 11-*cis* chromophore of rhodopsin is depicted with its Schiff base forming a salt bridge with a negative counter ion. The additional charge pair near the 11=12 double bond represents the group (or groups) that regulate the absorption maxima of different pigments (18). The photochemical event is an isomerization about the 11=12 double bond in rhodopsin (probably about the 13=14 bond in bacteriorhodopsin), but any isomerization in any direction will produce charge separation as shown in the first step in the figure. The pK values of the Schiff base and those of other groups on the protein, such as R_1 and R_2, are strongly affected by photoisomerization because a salt bridge is broken, a positive charge has moved near R_2, and R_1 is now a bare negative charge. Possible proton transfer steps resulting from charge separation are depicted. We speculate that in some pigments, hypsorhodopsin can be formed if a transfer of a proton from the Schiff base is possible. For bacteriorhodopsin, the isomerization is *trans-cis* rather than *cis-trans*, but all other events are assumed to be equivalent. The proton transferred in the Batho'-Batho transition could be pumped in bacteriorhodopsin, as might the Schiff base proton if it were released at a later stage. The number of protons pumped in bacteriorhodopsin has not been firmly established.

not change its state of protonation as a result of the primary event, this cannot be the proton involved.

The model for the isomerization event in Fig. 2 shows the reason that another proton near the chromophoric site might be transferred in the ground state. The charge separation that occurs as a consequence of the primary photochemistry must significantly alter the pK values in the vicinity of the Schiff base because the counter ion has remained in the form of a buried negative charge, which will become more basic, while the protonated nitrogen will become more acidic. We suggest that the separation of these groups induces a ground-state proton transfer which corresponds to the step Batho' → Batho mentioned above. There are a number of ways that a proton transfer could occur as a result of a photoisomerization; two possibilities are shown in Fig. 2.

Fig. 2 also provides a plausible explanation of the blue-shifted species, hypsorhodopsin (25). Because the pK of the Schiff base will be significantly reduced by isomerization, the Schiff base might itself transfer a proton and become deprotonated (Fig. 2), thus accounting for the large spectral blue shift that is observed. Although the model given in Fig. 2 is not unique, it serves to emphasize the important point that a variety of proton translocations are possible as a consequence of the isomerization event and the accompanying charge separation at the chromophore site. Further conformation changes would be expected to be generated by the unstable product of the photoisomerization. For example, lumirhodopsin or metarhodopsin I or both might result if a new salt bridge is formed by the protonated nitrogen (say with R_2^-).

A final feature of our model is that it provides a simple mechanism for the proton pump of bacteriorhodopsin. The essential mechanistic requirement for a proton pump is a light-driven conformational change that increases the net flux of protons in a given direction (36). This could most easily be accomplished by a *trans-cis* isomerization that we have proposed, based on photochemical analogies to visual pigments, to be the primary event in bacteriorhodopsin (12, 23, 36). Recently, additional evidence has been obtained which tends to support this conclusion (34, 37). As discussed above (see also ref. 36 and Fig. 2), cleavage of the salt bridge should lead to the ejection of a proton (or protons) from a group near the chromophoric site.

Proton transfer models

In this section we briefly consider a number of other recent models for the primary event (7–11). All of these models have in common the suggestion that light initiates an excited state proton transfer and that *cis-trans* isomerization is a later ground-state process that somehow results from the change in the protein induced by proton translocation. [In two of the

[h] With regards to the back reaction, the existence of a common minimum implies that the location of the proton has little or no effect on the shape of the excited-state surface, at least at the crossover point to the ground state. In fact, it is possible that in the bathorhodopsin → rhodopsin back reaction, proton transfer is an excited-state process.

models (9, 10), proton transfer also occurs in a vibrationally excited region of the ground-state surface.] Thus, each of these models implies that proton transfer precedes repopulation of the rhodopsin ground state and, as such, they are in conflict with the evidence discussed above which suggests that ground-state repopulation is the faster process.

The large thermal barrier for the primary event (approximately 30 kcal/mol) provides an additional constraint on possible models that has not been previously recognized. Thus, for example, the thermal barrier for the small displacement of a proton along a hydrogen bond between the Schiff base and an imidazole group is unlikely to be more than a few kcal/mol. This would appear to preclude models (8, 11) in which the rhodopsin–bathorhodopsin transition is activated by such a displacement. Another class of models involves light-induced proton transfer from one group on the protein to another (9, 10). Here again it is difficult to see how an extremely large activation energy could be obtained, assuming the normal range of pK values for amino acid side chains. Indeed, one model (10) specifically incorporates an activation energy of only 6 kcal/mol, a value that would lead to the rapid thermal bleaching of rhodopsin.

Models (7, 8, 11) that involve a change in the state of protonation of the Schiff-base proton appear to be inconsistent with the Raman evidence (13–16) which shows no change in the vibrational frequency of the C=N stretching mode in going from rhodopsin to bathorhodopsin. Moreover, these models assume that in rhodopsin the Schiff-base proton is only hydrogen bonded to the nitrogen, yet the large (about 25 cm^{-1}) frequency shifts observed in deuterated samples suggest that it is covalently bonded (16). Additional arguments against specific proton transfer models have been previously discussed (12).

Concluding remarks

We have presented a model for the primary event in visual pigments and bacteriorhodopsin that involves a photochemically induced charge separation caused by a geometric change in the chromophore. Our model provides a general mechanism for the conversion of light into chemical energy in retinal-based pigments (and, indeed, in any pigment in which a chromophore can undergo photoisomerization) and thus removes a difficulty that has always been inherent in the model of a simple *cis-trans* isomerization. The chromophore is viewed here as a means of achieving charge separation photochemically while ensuring that both the forward and reverse thermal reactions are suitably slow. Delbrück (38) has recently pointed out that chlorophyll makes use of its rigidity to achieve rapid transfer of an electron and to preclude back reaction while retinal and other flexible chromophores such as phytochrome function by imposing a conformational change on the protein. We believe our model now extends these concepts by emphasizing the common goal of both types of systems, which is to generate the separation of charge.

This work was supported by grants from the National Science Foundation (PCM 77-06728 at City College; PCM 76-82764 at University of Illinois), the National Institutes of Health (EYO 1323 and EYO 0025 at University of Illinois), the United States-Israel Binational Science Foundation, and the City University PSC-BHE Faculty Award Program.

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