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CIS-TRANS ISOMERIZATION IN THE PHOTOCHEMISTRY OF VISION

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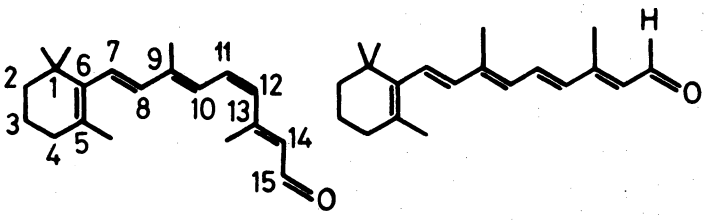
Abstract - Various models proposed for the primary photoevent in vision are critically discussed. It is concluded that the classical picture of a single cis-trans isomerization step is the only one which satisfactorily accounts for all the available experimental data. Experiments are performed showing that this process is temperature independent over a range of 200°C. Photoisomerization yields for the free protonated Schiff base of 11-cis (and all-trans) retinal are measured as a function of the excitation wavelength. In contrast to the efficient and wavelength independent photobleaching of rhodopsin, the yields of the 11-cis→all-trans isomerization of the free chromophore are small, exhibiting a marked dependence on the excitation wavelength. Potential energy curves for both ground and excited states of rhodopsin are derived from the analysis of the accumulated experimental data. In variance with the behavior of model compounds, photoisomerization in the pigment proceeds via the quantitative population of a common, barrierless, thermally relaxed excited state along the 11-12 torsional coordinate separating the 11-cis (rhodopsin) and all-trans (bathorhodopsin) configurations. In the ground state, interactions with the protein destabilize the all-trans isomerization product, leading to storage of a significant fraction of the photon's energy in the primary step.

INTRODUCTION

Visual pigments such as rhodopsin consist of a chromophore, 11-cis retinal, covalently bound to an apo-protein, opsin (1).



The absorption of a photon by the chromophore triggers a sequence of events leading to the release of a transmitter substance (most probably Ca^{++} ions) from the photoreceptor membrane, and thus affects the electrical properties of the cell. (For a review see Ref. 2). Photochemically, light absorption leads to a series of consecutive spectroscopic changes ending in the release of free opsin and all-trans retinal. Most of the available information concerning these reactions is based on continuous or flash photolysis excitation methods, carried out at temperatures which are low enough to inhibit the various thermal decay processes. The photochemical and thermal interconversions between intermediates in the sequence for bovine rhodopsin are summarized in Fig. 1. (For a review see Ref. 3).

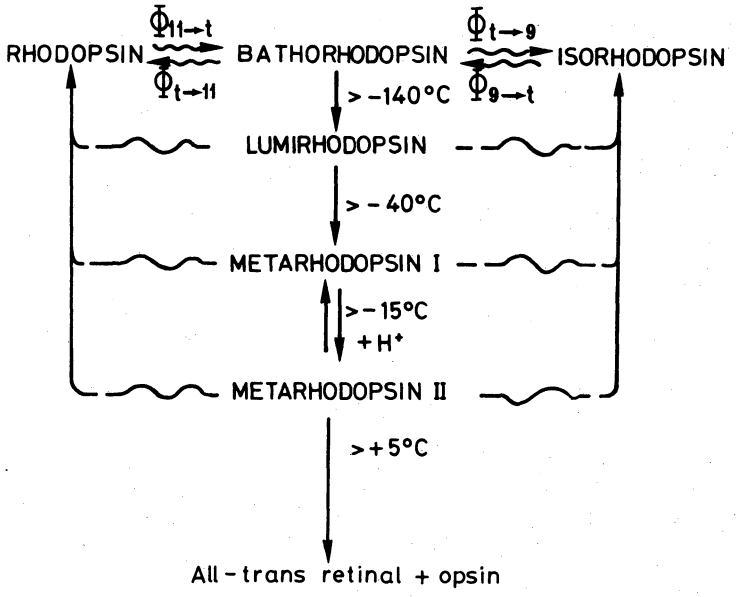


Fig. 1. Light induced (⇝) and thermal (→) interconversion processes between intermediates in the photochemistry of bovine rhodopsin. Temperatures denoted are those below which the various intermediates are stable. (Adapted from Ref. 3).

The exact nature of the chromophore-protein linkage is a crucial determinant of the absorption spectra of these pigments (4). In the last few years, the use of resonance-Raman spectroscopy (5,6) has removed a major source of uncertainty by confirming previous suggestions (7) that the retinylic chromophore of bovine rhodopsin is connected to the protein via a protonated Schiff-base linkage. A second question which attracted considerable attention concerned the variations in the absorption spectra of the pigments (440-580 nm). These were postulated to result from secondary interactions due to charged groups on the protein (8). Recently, it has been shown that such a model, not only quantitatively accounts for the variations, but also explains other spectroscopic details such as the dependence of the bandwidth on the wavelength of maximum absorption (9).

In contrast to the considerable progress made in understanding the spectroscopy of the pigments, much less is known about the molecular changes associated with the events of Fig. 1. Even the primary step, originally attributed to a simple cis-trans isomerization (10), has been recently the subject of considerable controversy. In this work the nature of the primary act in vision is critically reconsidered. To this end we review and analyze available experimental data and present new results pertaining to the photochemistry of rhodopsin and to that of a free protonated Schiff base (PRSB) of 11-cis retinal in solution. An analysis is presented showing that a cis→trans isomerization is the only plausible change among those proposed for the primary photoevent in rhodopsin. It is shown that rhodopsin and its primary (trans) photoproduct, bathorhodopsin (or prelumirhodopsin), are interconvertible via a common (barrierless) thermally relaxed excited state. A comparison of the photochemical properties of the pigment and those of model compounds, indicates that the protein plays a major role in directing the excited state processes of the bound chromophore. Finally, we conclude that a considerable fraction of the light energy absorbed, is stored in the primary photoproduct and suggest that the resulting destabilization of the trans ground-state conformation may account for the efficiency of the primary cis→trans event.

METHODS AND RESULTS

Rhodopsin was extracted from bovine rod outer segments with 2% Ammonyx-LO (11) in 67 mM phosphate buffer (pH=6.9). The OD_{280}/OD_{500} ratios in the extracts, indicative of the preparation purity, ranged between 2 and 3. Aqueous-glycerol (1:2) mixtures were used in all experiments. Absorption spectra were measured on Cary 118 and Cary 14 spectrophotometers. A dewar with planar glass windows was used for recording absorption spectra at low temperatures.

Clear glasses at 77°K were obtained by rapid immersion of the sample cell into liquid nitrogen (12). Rhodopsin bleached in the presence of hydroxylamine was used as the baseline at all temperatures. The solvent contraction relative to room temperature was 5% at -78°C and 6.5% at -196°C.

Quantum yields for the bleaching of rhodopsin at -78°C and -196°C, relative to the room temperature value, were determined by measuring the corresponding photosensitivity ratios. The samples were irradiated with a 500 nm line isolated from a tungsten lamp by a 12 nm half-band width interference filter. For low temperature measurements, the sample was cooled, irradiated, and then bleached by warming in the dark to room temperature. Bleaching rates were determined from absorbance changes at 500 nm. At 77°K samples were bleached by less than 2% to prevent substantial light absorption by bathorhodopsin. The sample was uniformly illuminated with constant light intensity. Relative quantum yields were calculated (13), correcting for the temperature dependence of the extinction coefficient integrated over the 12 nm band width of the interference filter. (The wavelength of maximum absorption in the spectrum of rhodopsin in Ammonyx-LO was observed to shift, from 498 nm at room temperature, to 502 nm at -50°C and to 505 nm at -196°C). Absorbance changes due to solvent contraction upon cooling were also taken into account.

The quantum yields obtained for the bleaching of rhodopsin at -78°C and -196°C, relative to the room temperature value are: 0.97±0.06 and 0.93±0.1 respectively. Thus, within the limits of experimental accuracy, there appears to be no temperature effect on the isomerization quantum yield.

The protonated n-butyl-amine Schiff bases of both 11-cis and all-trans retinal were prepared as previously described (14) using freshly prepared solutions for each experiment. The solutions were found to obey Beer's law over the 5×10^{-4}M-5×10^{-7}M concentration range. Continuous irradiation was carried out with emission lines isolated from medium pressure (313, 405, 436, 546, 577 nm) and low pressure (254 nm) Hg arcs, using Corning-glass and solution filters combinations. Uranyl oxalate or ferri oxalate (above 500 nm) actionometers were employed. The initial (i.e. below 15% conversion) cis-trans photoisomerization quantum yields, following the direct irradiation of 11-cis and all-trans PRSB in methanol were determined as previously described (14). For 546 nm excitation, differential irradiations and actionometry (577 nm only vs. both 577 and 546 nm) were carried out. Since the composition of cis isomers formed from the irradiation of the all-trans isomer was not established, the values reported for $\phi_{t\rightarrow cis}$ were calculated assuming that the conversion is exclusively to the 11-cis isomer (14). The values reported in Table 1 were found to be independent of the acid ($HClO_4$) concentration (in the range 10^{-4}-10^{-2}M). The same results were also obtained with trichloroacetic and (saturated) hydrochloric acids. All values were unaffected by dissolved oxygen.

TABLE 1. Quantum yields for the direct-excitation photoisomerization of the 11-cis and all-trans protonated Schiff bases of retinal in acidified methanol.[a]

λ excitation nm	Absorption band	$\Phi_{11\rightarrow t}$	$\Phi_{t\rightarrow cis}$
254	γ	0.25	0.27
313	β	0.15	0.13
405	α	0.005	0.0005
438	α	0.05	0.09
540	α	0.25	---
577	α	0.07	---

[a] See ref. 14 for details of quantum yields determinations. ($\Phi_{11\rightarrow t}$ and $\Phi_{t\rightarrow cis}$ are isomerization quantum yields evaluated, respectively, assuming that the corresponding processes are: 11-cis → all-trans and all-trans → 11-cis).

THE PRIMARY PHOTOCHEMICAL PROCESS

Proposed models

It is first important to point out that bathorhodopsin, which is the primary photoproduct at 77°K, is also formed at room temperature. Pulsed laser excitation studies have shown that it is generated within 6 psec (15) and decays in about 100 nsec (15-17). Extension of the observation time scale and wavelength range (18) proved that as the sole primary photoproduct of rhodopsin, bathorhodopsin is the precursor of the subsequent thermal intermediates in the room-temperature sequence. It was also shown that during the laser pulse, bathorhodopsin is photochemically converted to either rhodopsin or isorhodopsin (the pigment with a 9-cis chromophore). It therefore appears that the primary photoequilibrium between these three species, originally detected at low temperatures (see Fig. 1), is also applicable under physiological conditions.

The following models have been proposed to account for the molecular changes associated with the formation of bathorhodopsin:
a) The classical picture, involving a cis-trans isomerization about the 11-12 double bond (10). Variations of this model include incomplete cis-trans isomerization (15) and a two bond isomerization, involving the concerted rotation about the 11-12 and 15-16 double bonds (19).
b) A mechanism involving deprotonation of the Schiff base nitrogen (20).
c) Proton transfer from the methyl group at position 5 to a protein heteroatom. This entails shifting of double bonds along the polyene chain with the formation of a "retro" type compound (21).
d) A photoinduced electron transfer from the chromophore to an appropriate acceptor group on the protein.

Evaluation of models

Before discussing the various proposals it is worthwhile to summarize some major observations that must be accounted for by any model for the primary event in vision:
I. The primary process is photoreversible (22).
II. The photoproduct is the same, starting from either rhodopsin or isorhodopsin. Photoreversibility from the bathoproduct leads back to both original pigments (22).
III. The primary batho-photoproduct is red-shifted relative to the parent pigment and is stable at liquid nitrogen temperatures (22).
IV. Resonance Raman studies indicate that the Schiff base linkage in the batho stage is still protonated and that the C=N vibrational frequency is close to that of rhodopsin (5,6).
V. Artificial pigments formed from synthetic retinal derivatives and submitted to photochemical tests have been found to bleach. In all cases tested a batho photo-intermediate has been observed (23).

Mechanism (b) may be unambiguously rejected in light of Resonance Raman experiments showing that in bathorhodopsin the Schiff base nitrogen is still protonated (6). Moreover, deprotonation is expected to cause a substantial blue shift, in variance with the observed red shift associated with the production of bathorhodopsin. Similar arguments also rule out mechanism (c), since the formation of the retro compound would change the C=N bond to a single bond. This should also result in a significant blue shift since the (protonated) nitrogen is no longer conjugated with the chain. The model is furthermore inconsistent with the fact that 5,6 dihydroretinal (24) and 5,6 epoxyretinal (25), in which the ring is not conjugated with the chain, undergo photobleaching as do normal pigments. Finally, the important observation that 5, 9, and 13 desmethylretinals, all bleach via a batho intermediate (23), precludes any mechanism involving proton transfer from methyl groups.

Electron transfer mechanisms such as (d) cannot account for the fact that in bovine rhodopsin the change may be photochemically but not thermally reverted throughout the bleaching sequence. Thermal irreversibility of a light induced electron transfer requires geometrical separation between the donor and the acceptor moieties which would then preclude photoreversibility. (Similar arguments also apply to all proton transfer mechanisms).

It seems to us that the strongest evidence against models which do not involve cis-trans isomerization in the primary step can be found in the original argument of Kropf and Hubbard (10) and Yoshizawa and Wald (22). This is based (see Fig. 1) on the observation that at low temperatures it is possible to establish a photoequilibrium between isorhodopsin (9-cis) and rhodopsin (11-cis) via bathorhodopsin (or lumirhodopsin) as a common intermediate. Excluding a one-photon, two-bond, isomerization in isorhodopsin (leading to an 11-cis conformation in bathorhodopsin), this picture can be rationalized only by assuming isomerization around the corresponding cis double bonds as primary steps in both rhodopsin and isorhodopsin. Although a one-photon, two-bond, isomerization in a visual pigment (9, 13 dicis rhodopsin) has been reported (26), the possibility that bathorhodopsin is still in a cis conformation is extremely implausible. The essentially identical photochemical behavior (reversal to rhodopsin and isorhodopsin) of the bleaching intermediates through metarhodopsin II, which (as it has explicitly been shown in the case of squid rhodopsin (27)) contains an all-trans chromophore, strongly implies that bathorhodopsin is characterized by the same all-trans conformation.

As indicated above it has been suggested that the model of a simple cis-trans isomerization should be modified to include either a partial, or a concerted two-bond isomerization. The former possibility was suggested by Busch et al (15) who observed that the formation of bathorhodopsin occurs within less than 6 picoseconds and argued that complete isomerization is unlikely to take place within this time. However, since frequency factors as high as $\sim 10^{13} sec^{-1}$ have been reported for thermal (ground-state) cis-trans isomerizations (28), this argument will not hold for a barrierless potential energy surface such as that postulated below for the excited state of rhodopsin. In fact, experimental evidence appears to be available, showing that excited-state photoisomerization can compete with vibrational relaxation, and may thus take place within picoseconds. For example, we have previously shown (29) that, following energy transfer into its triplet manifold, 11-cis retinal undergoes isomerization before relaxing to the lowest thermalized triplet state. It appears to us that the major objection to a picosecond isomerization results from viewing the process as a classical rotation involving bulky end groups. This description is probably highly simplified since it assumes that entire end groups follow the rotating bond as a rigid rotor. A more reasonable model would view the process (especially in condensed media) as a set of fast vibrational and translational displacements. In any case, even accepting that isomerization is a relatively slow process, a partial but substantial rotation about the double bond ($\lesssim 90°$), would not modify the time scale required for a complete rotation.

A model describing isomerization processes in visual pigments in terms of concerted rotation about two double bonds ("bicycle-pedal" motion), has been recently proposed (19). In particular, the formation of bathorhodopsin is accounted-for by rotating around both 11-12 and 15-16 (C=N) double bonds. By calculating classical trajectories on excited state potential energy surfaces, important features such as isomerization yields and rates are quantitatively evaluated. At present no experimental methods are available for establishing conformational changes around the C=N double bond. However, in its present form, the model is unacceptable since some of its main predictions contradict important experimental observations:

a) The model predicts an efficient direct photochemical path from isorhodopsin to rhodopsin and allows the reverse process. Neither reaction is known to occur since it is well established that both interconversions occur only via bathorhodopsin as a common photointermediate (22). Moreover, the linearity of the photobleaching curves observed for isorhodopsin at room temperature (8) precludes rhodopsin as an intermediate in the process.

b) The reported trajectories start at a single point on the excited state potential surface (excitation at ~ 56 Kcal mol^{-1}). Although calculations for other excitation energies are not reported, it would appear that both product distribution and quantum yields will be markedly affected by the excitation wavelength, in variance with the wavelength independent quantum yield for the bleaching of rhodopsin (13,30).

c) The predicted energy for bathorhodopsin is ~ 5 Kcal above rhodopsin. This is about 8 Kcal below the lower limit established from available experimental data (see below). Since the calculations are sensitive to details of the potential functions such as gradients and small energy gaps, an uncertainty in energy of this magnitude questions the reliability of the model and its main conclusions.

Summarizing, the picture of a simple cis-trans isomerization seems to account for all presently available experimental observations related to the generation of bathorhodopsin. This does not imply however that the chromophore in bathorhodopsin has the same planar configuration as the free all-trans chromophore. In fact, it is likely that the high free energy associated with the batho photoproduct is at least partially due to significant conformational distortions. Whatever its specific conformation may be, bathorhodopsin has a trans conformation in the sense that it has overcome the barriers to isomerization from either 11-cis or 9-cis isomers.

POTENTIAL ENERGY CURVES IN RHODOPSIN

Excited state

Using available experimental data it is possible to infer that the excited state along the 11-12 torsional coordinate has a single minimum which is reached by exciting either rhodopsin or bathorhodopsin. Most probably, a similar common excited state is also shared between bathorhodopsin and isorhodopsin. This conclusion is based on the wavelength independence of the photochemistry of rhodopsin, on its insensitivity to temperature and on the relative and absolute quantum yields of the rhodopsin $\rightleftarrows$ bathorhodopsin $\rightleftarrows$ isorhodopsin interconversions.

The wavelength independence of both the bleaching of rhodopsin (13,30) and the photosensitivity for seeing (31,32) imply that the quantum yield for forming bathorhodopsin is independent of the vibronic level reached by light absorption. Similar measurements have not been carried out at low temperatures. However, a number of observations show that the main features of the primary act in the photochemistry of rhodopsin are unaltered by cooling to 77°K. First, as reported above, the quantum yield in a 77°K glass is, within the limits of experimental

accuracy, the same as at room temperature. Second, the relative quantum yields for the interconversion of rhodopsin, bathorhodopsin and isorhodopsin at 77°K appear to be wavelength independent. These ratios can be calculated from the composition of their photostationary mixture at 77°K (6), using the low temperature absorption spectra reported by Yoshizawa and Wald (22). Denoting by $\Phi_{11\to t}$, $\Phi_{t\to 11}$, $\Phi_{t\to 9}$ and $\Phi_{9\to t}$, the quantum yields of interconversion between bathorhodopsin (t), rhodopsin (11) and isorhodopsin (9) respectively, we find for both 514.5 and 476.2 nm excitation wavelengths, that $\Phi_{11\to t}/\Phi_{t\to 11} \cong 2.2$ and $\Phi_{9\to t}/\Phi_{t\to 9} \cong 2.5$. It is important to note that these numbers are close to those determined by Hubbard and Kropf (10) for the interconversion between rhodopsin, isorhodopsin and metarhodopsin I. Moreover, Yoshizawa and Wald have noted (22) that the ratio, $\phi_{t\to 11}/\phi_{t\to 9} \cong 5$, is the same for the equilibria involving either metarhodopsin I or bathorhodopsin. Thus, although metarhodopsin I and bathorhodopsin are different trans species, their photochemical behavior appears to be essentially identical.

The wavelength and temperature independence of the photochemistry of visual pigments implies that cis-trans isomerization takes place after complete thermal relaxation and requires no activation energy. This situation may be achieved by populating one or more minima in the excited state potential energy surface from which radiationless transitions to cis and trans ground-state conformers take place. Using the room-temperature value (33) of 0.67 for the temperature-independent quantum yield for the photobleaching of rhodopsin, we have $\Phi_{11\to t} = 0.67$. With $\Phi_{11\to t}/\Phi_{t\to 11} \cong 2$ this yields $\Phi_{11\to t} + \Phi_{t\to 11} \cong 1$. Neglecting in a first approximation the small yield of converting bathorhodopsin to isorhodopsin, this strongly suggests that a single potential minimum is populated (both from excited rhodopsin and bathorhodopsin) from which the ground-state 11-cis and all-trans configurations are formed with a 1:2 ratio (curve I in Fig. 2).

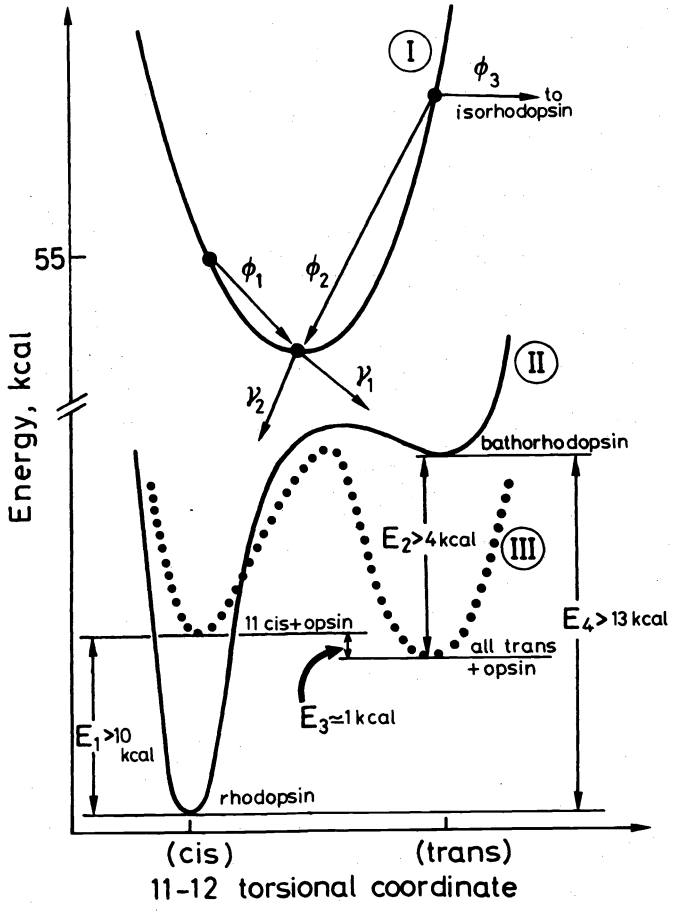


Fig. 2. Schematic potential energy curves and energy relationships in rhodopsin. I. Excited state of rhodopsin and bathorhodopsin. II. Ground state of rhodopsin and bathorhodopsin. III. Ground state of isolated

chromophore. Various symbols discussed in text. E_2 is estimated from the equilibrium constant K_2 = [bathorhodopsin]/[all-trans retinal][opsin] < 10^{-2}. E_1 is estimated from the equilibrium constant K_1 = [rhodopsin]/[11-cis retinal][opsin] > 10^7. (See text and Ref. 25).

A quantitative analysis of this model should also consider the interconversion with isorhodopsin. In Fig. 2 ϕ_1 and ϕ_2 denote the yields of reaching the single potential minimum along the 11-12 coordinate, while γ_1 and γ_2 are the yields of crossing from this minimum to the all-trans and 11-cis, ground state, conformations. Thus, $\Phi_{11\to t} = \phi_1\gamma_1$, $\Phi_{t\to 11} = \phi_2\gamma_2$ and $\gamma_1 + \gamma_2 = 1$. The value $\phi_1 = 1.0$ given in Fig. 2 is based on the assumption that upon excitation of rhodopsin, all excess vibrational energy relaxes in the 11-12 torsional coordinate. Since $\Phi_{11\to t} = 0.67$ this implies that also $\gamma_1 = 0.67$ and thus $\gamma_2 = 0.33$. Taking the value $\Phi_{11\to t}/\Phi_{t\to 11} = 2.2$ we find $\Phi_{t\to 11} = 0.30$ and thus $\phi_2 = 0.9$. This value leaves a low probability channel ($\phi_3 \cong 0.1$) for torsional motion around the 9-10 double bond, accounting for the direct formation of isorhodopsin from bathorhodopsin as observed experimentally.
It is of interest to apply the same model to the interconversion between bathorhodopsin and isorhodopsin. With ϕ_3, ϕ_4, γ_3 and γ_4 replacing respectively ϕ_2, ϕ_1, γ_2 and γ_1, we now have: $\Phi_{t\to 9} = \phi_3\cdot\gamma_3$ and $\Phi_{9\to t} = \phi_4\cdot\gamma_4$. Assuming (as for ϕ_1) that $\phi_4 = 1.0$, and using the observed (10) room temperature value $\Phi_{9\to t} = 0.2$, we obtain $\gamma_4 = 0.2$ and $\gamma_3 = 0.8$. Thus, with $\phi_3 = 0.1$, it is predicted that $\Phi_{t\to 9} = 0.08$. The value of the ratio $\Phi_{9\to t}/\Phi_{t\to 9} = 2.5$ obtained from this analysis is identical to that evaluated above from experimental data at 77°K. Moreover, we also obtain $\Phi_{t\to 11}/\Phi_{t\to 9} \cong 4$ which is in good agreement with the value of 5 approximated by Yoshizawa and Wald (22). It should be pointed out that, since $\phi_3 << \phi_2$, the analysis in the case of the isorhodopsin-bathorhodopsin interconversion is very sensitive to the absolute magnitude of ϕ_3 which only slightly affects the picture along the 11-12 coordinate. Thus, the above model, especially in relation to the 9-10 coordinate, awaits final confirmation by a detailed and accurate determination of the various quantum yields and their wavelength dependence at 77°K. However, the data presently available provide strong support for the scheme of Fig. 2. Of particular interest is the fact that in rhodopsin the excitation energy is quantitatively channeled to a single minimum in the 11-12 coordinate. As will be shown below this behavior is not shared by any retinal derivative when free in solution. It thus appears that the protein environment in visual pigments plays a major role in directing the photochemistry of the chromophore.

Ground state: Excitation energy storage

It is of major interest to consider at this point the free energy changes associated with the primary step in vision. These are summarized in Fig. 2 for both ground and excited states. Neglecting entropy contributions, which are likely to be small in a cis-trans chromophore rearrangement at 77°K, the diagrams may be taken to reflect the corresponding enthalpy changes. In contrast to the 11-cis and all-trans isomers of a free protonated Schiff base which should be relatively close in free energy (E_3 in Fig. 2 is about the same as in retinals, for which a value of 1Kcal/Mol. has been measured (34)), Yoshizawa and Wald (22) and Rodieck (35) have noted that bathorhodopsin is considerably higher in free energy than rhodopsin. It is possible to establish a lower limit for this energy difference, showing that the primary photoproduct in bovine rhodopsin lies at least 13 Kcal/mole above the parent pigment (E_4 in Fig. 2). We arrive at this number by taking the lower limit of 10^7 estimated by Kropf et al. (25) for the equilibrium constant of the reaction:

11-cis retinal + opsin $\rightleftarrows$ rhodopsin

which corresponds to a free energy difference (E_1) of at least 10 Kcal/Mol. The equilibrium constant of the reaction:

all-trans retinal + opsin $\rightleftarrows$ bathorhodopsin

is not known. However, conservatively assuming that less than 1% of bathorhodopsin remains at the end of its bleaching process at room temperature (usually assumed to go to completion in a 10^{-5}M solution), a value of at least 4 Kcal/mole is obtained for the energy difference (E_2) between bathorhodopsin and all-trans retinal + opsin (see Fig. 2). Thus, the sum $E_4 = E_2 + E_1 - E_3$ is at least 13 Kcal, with an upper limit set by the energy of the absorbed photon, ca. 50 Kcal/Mol.

This result provides an explanation for the interesting observation that the (trans) "batho" form of the chicken pigment iodopsin, rapidly reverts in the dark at -140°C to its (cis) parent pigment (36). Since the barrier for cis-trans isomerization in retinals is of the order of 20 Kcal/mole (34), the large lower limit established for E_4 explains the rapidity of this process in terms of the significant reduction of the activation energy for the t → 11 thermal reaction (see Fig. 2).

In variance with the 11-cis → all-trans photochemical transformation characteristic of rhodopsin, there are related systems such as the squid pigment retinochrome (37) and bacteriorhodopsin (see below) which base their primary photochemistry on an all-trans retinal chromophore. In the case of retinochrome it is the 11-cis isomer (which does not complex with the protein) which is the unstable photochemical product storing (part) of the excitation energy (37).

It is possible that in rhodopsin destabilization of the trans ground-state configuration may affect the relative rates of relaxation (γ_1 and γ_2 in Fig. 2) to the corresponding (trans and cis) minima after internal conversion to the (vibrationally excited) ground state. With other factors (e.g. shapes of potential energy curves etc.) being equal, thermalization to the higher energy potential minimum will be favoured, thus accounting for the relatively high value of γ_2. It should be pointed out that of the energy (E_4) stored in bathorhodopsin, E_2 is the part which becomes available for driving the bleaching sequence and may thus be utilized for producing the photoreceptor response. According to this picture, a significant fraction of the photon's energy is stored in bathorhodopsin, so that light not only triggers the visual cycle but is actually its main driving force.

It is of interest to consider the mechanism by which energy might be stored in the primary photoproduct. Whatever model is assumed, it must also account for the observation that all "batho" pigments appear spectrally red-shifted with respect to the parent pigment. It is important to note, however, that energetically the lower limit for the destabilization of the ground state (13 Kcal/Mol.) is considerably larger than the bathochromic shift ($\sim$ 5 Kcal/Mol.). Thus, upon light absorption, bathorhodopsin must reach a higher energy (Franck-Condon) excited state, on an absolute scale, than rhodopsin. This may be achieved by some asymmetry in the excited state potential surface as shown in Fig. 2. One possible model for energy storage in the batho product involves steric constraints, forcing the all-trans chromophore into a strained geometry (see also ref. 22). Since the barrier to isomerization about double bonds is of the order of 25 Kcal/mole, a twisted conformation about one or more of these bonds might account for the considerable destabilization in bathorhodopsin. Such twisting would be expected to cause a red shift. An alternative possibility may involve isomerization affecting the electrostatic interactions between the chromophore and the protein, for example, by moving the protonated Schiff base away from its counter ion. Thus, unfavorable electrostatic interactions between the chromophore and protein in bathorhodopsin might be the major source of its instability.

SOME COMMENTS ON BACTERIORHODOPSIN

The rhodopsin-like purple membrane protein of the bacterium H. halobium (bacteriorhodopsin) (38), which has been recently the subject of considerable interest, is similar to rhodopsin in its photochemical behavior. When the light-adapted (568 nm) form of this pigment is illuminated, a cycle is initiated in which (in contrast to vertebrate visual pigments) the pigment returns within a few msec to its original state, without the (all-trans) chromophore being detached from the protein (38). In the cycle, the pigment goes through a set of spectral intermediates similar to those observed in the bleaching sequence of rhodopsin. Since the ("batho") photoproduct of the bacterial pigment shares many properties with bathorhodopsin [red shifted relative to the parent pigment (λ_{max} = 630 nm), stable at liquid nitrogen temperatures (38), formed within less than 10 picoseconds (39)], it is of interest to consider the extent to which the primary events in the two pigments are related. Points I, III and IV above, which have been used to exclude mechanisms which do not assume significant geometry changes in the rhodopsin chromophore, are also applicable in the case bacteriorhodopsin. Although this might suggest that a trans → cis isomerization should also occur as the primary photoevent in the bacterial pigment, no evidence for such a process has yet been obtained.

However, the ground state potential energy surface derived for rhodopsin (see Fig. 2) allows a fast thermal reversal of cis → trans isomerizations (e.g., see the above discussion for iodopsin). Thus, it is not unlikely that in bacteriorhodopsin a trans → cis isomerization around a single or even a double bond (including the C=N bond) occurs photochemically, followed by a rapid reversal during the cycle to the original all-trans conformation. If this is actually the case, light energy [subsequently used for driving its proton pump (38)] will be stored in the batho product of bacteriorhodopsin via mechanisms similar to those postulated for rhodopsin.

STUDIES OF MODEL COMPOUNDS

Comparison of the photochemical properties of free and opsin-bound protonated Schiff bases

The photochemical properties of molecules related to the visual chromophore (e.g. retinol, retinal, the n-butylamine Schiff base, the oxime, etc.) have been studied in detail under the assumption that they might simulate the behavior of the pigments (40). These properties turned out to be complex, depending on solvent, isomer and end group (41). In contrast to the behavior of the pigments, the photoisomerization quantum yields are relatively small (< 0.15), exhibiting a marked wavelength and temperature dependence. Another property which is characteristic of visual pigments and not of the model molecules, is the high specificity for the isomer composition of the photoproducts. For example, it appears that in pigments one-photon, two-bond, photoisomerization can efficiently take place. Thus, it has been shown that for bovine opsin pigments (using Triton X-100 as a detergent) the all-trans isomer is the sole photoproduct, not only of the 11-cis and 9-cis but also of the 9,13 dicis pigment (26). In contrast, the photolysis of 9,13-dicis retinal leads to little or no all-trans photoproduct (42). Multiple-bond, single photon, isomerizations have also been observed for the squid pigment retinochrome, where the 11-cis isomer appears to be the prominent photoproduct when starting not only with all-trans but also with 9-cis or 13-cis isomers (37).

Since it is now clear that the chromophore in rhodopsin is a protonated Schiff base, the free form of this molecule (PRSB) is likely to be the most appropriate model system for visual pigments. In a recent study we reported quantum yields for the triplet-sensitized photoisomerization of various PRSB isomers (14). We now present direct-excitation quantum yields for 11-cis and all-trans PRSB in methanol solutions.

As shown in Table 1, the isomerization yields of the protonated Schiff bases of 11-cis and all-trans retinal are small and markedly dependent on the excitation wavelength. The variations in quantum yields are observed not only among the three main absorption bands (α, 445 nm; β, 320 nm; γ, 280 nm), but also within the intense α band itself. In contrast to the high ($\sim$ 0.67 (33)) and wavelength-independent (13,30) value of the photobleaching of rhodopsin [note that also the photosensitivity for seeing is wavelength independent (31,32)], the isomerization yields of protonated Schiff bases of retinal in solution are small and markedly dependent on the excitation wavelength. It is therefore evident that the presence of the opsin moiety dramatically affects the photochemical properties of the 11-cis chromophore.

Is a triplet-state populated in the photochemistry of rhodopsin?

Before attempting to rationalize the differences between the photochemistry of visual pigments and those of model compounds, it is important to discuss the nature of the (common) excited state from which photoisomerization takes place in the pigments. One possibility which has repeatedly been proposed involves a triplet state intermediate. This would be consistent with the observations that for all retinal derivatives the triplet sensitized isomerization yields are substantially higher than those associated with direct excitation in the singlet manifold (41). The effect is particularly pronounced in the case of the 11-cis PRSB where the maximum yield of unity is obtained by triplet sensitization (14), in contrast to the relatively small numbers reported in Table I. Moreover when rhodopsin is treated with the unique triplet sensitizer trimethyl-1,2-dioxetane, the protein is denatured and the chromophore isomerized (42). Since the intersystem crossing yield in PRSB has been shown (14,43) to be beyond the experimental detection limit (< 0.001), a triplet-state model would require that in the pigment the rate of triplet formation increases by at least three orders of magnitude. An additional difficulty arises from the fact that radiationless transitions from the lowest triplet (T_1) to the ground state singlet (S_o) are relatively slow processes. In the particular case of systems where triplet mechanisms have been invoked to account for thermal ground-state cis-trans isomerization, frequency factors for crossing from S_o to T_1 ($k_{T_1 \rightarrow S_o}$) of the order of 10^6-10^8 sec^{-1} were postulated. Thus, it is highly unlikely that in rhodopsin $k_{T_1 \rightarrow S_o}$ is of the order of 10^{12} sec^{-1}, as implied by the picosecond generation time of bathorhodopsin (15).

In fact, the application of either optical (15-18) or magnetic (44) detection methods in the photolysis of rhodopsin produced no evidence for the formation of a triplet state. Recently however, transient e.s.r. signals following the low temperature illumination of rhodopsin, attributed to the triplet state of tryptophane, have been reported (45). A serious difficulty with this study is that the energy of the tryptophane triplet state [$\sim$ 24,000 cm^{-1} (46)] is higher than that of the light quanta used for exciting rhodopsin in its α absorption band. In any case, a relatively long-lived triplet state could play no role as a precursor in the ultrafast generation of prelumirhodopsin.

The role of the protein in the photoisomerization of the visual pigment chromophore

The outstanding features of the photochemistry of rhodopsin are its wavelength and temperature independence and the fact that the isomerization yields in both directions almost sum to unity. The unusually simple picture led us to the conclusion that the excitation energy is quantitatively channeled into the 11-12 torsional coordinate, leading to the population of a common potential energy minimum from which isomerization takes place. It is interesting that a common excited state has been postulated for stilbenes, considered as classical model systems for the study of cis-trans isomerization (47). However, in the case of stilbenes the population of the common state is a complicated process, associated with thermally activated intersystem crossing and with competing internal conversions to the ground state. The simple behavior in the excited states of visual pigments is also in contrast to the complicated photoisomerization patterns in PRBS, as suggested by the small and wavelength dependent yields of Table I. It is thus of interest to consider the ways in which the protein may induce this transformation.

Excluding dramatic effects on intersystem crossing rates, the data presented in Table I can be interpreted in terms of vibronic effects on singlet excited-state processes such as internal conversion, isomerization, and vibrational relaxation. Alternatively, it is in principle possible that the observed wavelength effects in PRSB are due to an absorption band composed of overlapping transitions of several ground state conformers, each with a wavelength independent isomerization yield. In order to explain on this basis the low quantum yields at both ends of the α band, it would be necessary to assume that different conformers have different absorption bandwidths and very different photochemical activity with respect to isomerization about the 11-12 double bond. Since the bandwidth is determined primarily by stretching vibrations (9) which are not coupled to torsional motion, we believe that different ground state conformations cannot be responsible for the observed wavelength effects.

Thus, the data of Table I must be rationalized in terms of excited state processes. The fact that the sum $\Phi_{11\to t} + \Phi_{t\to cis}$ for the free protonated Schiff base is smaller than unity and depends on the excitation wavelength, precludes a mechanism in which the only excited state process is the population of a common (barrierless) state as postulated for the opsin bound chromophore. The wavelength dependence in the free chromophore and its removal in the pigment can arise in two ways:

a) The lowest excited state of the isolated chromophore has a significant barrier between the 11-cis and the all-trans configurations. The complicated wavelength effects on the quantum yields may then arise from a complex competition scheme between isomerization and vibrational or electronic relaxation. According to this interpretation, the chromophore-protein interactions eliminate this intrinsic barrier or reduce it to a size allowing thermal equilibration between cis and trans conformations during the excited state lifetime. A plausible mechanism responsible for such effects may involve electrostatic interactions between charged groups on the protein and a highly polar excited state of the chromophore (48,49). Alternatively, it is possible that the protein, without necessarily affecting barrier heights, causes an inversion of two energy levels so that a relatively high-energy barrierless state in the free chromophore becomes the lowest one in the pigment. Such levels may be the ^1B_u state, responsible for the main absorption band, and the closely spaced $^1A_g^-$ state which has been detected in polyenes (50), and whose relative position appears to be sensitive to molecular conformation (51).

b) A barrierless curve along the 11-12 coordinate is also appropriate to the isolated protonated Schiff base. However, in the free chromophore, deactivation processes to the ground state (either from vibrationally excited levels of the 11-12 torsional mode or via paths involving other coordinates) may efficiently compete with relaxation to this minimum.

At present it is impossible to discriminate between these and other alternatives which might account for the complicated patterns of the data of Table I. Additional work will be required to reach a quantitative understanding of the mechanism by which the protein enhances and simplifies the photoisomerization of the visual chromophore.

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