PRIMARY PHOTOCHEMISTRY AND PHOTOISOMERIZATION OF RETINAL AT 77°K IN CATTLE AND SQUID RHODOPSINS

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ABSTRACT The relative quantum yields of the photoreactions Rhodopsin ⇌ Bathorhodopsin ⇌ Isorhodopsin over an extended wavelength region have been determined in cattle and squid rhodopsins at 77°K. The quantum yields were found to be wavelength independent and unchanged for samples suspended in D_2O. The rhodopsin-bathorhodopsin forward and backward quantum yields sum to larger than one. These results are consistent with the previous suggestion that the excited singlet potential of rhodopsin has a single minimum along the 11–12 torsional coordinate. The values of the quantum yields are important for evaluating dynamic models of the rhodopsin-bathorhodopsin transition. We conclude that equilibration in the common excited state after excitation of rhodopsin, as previously suggested, does not occur. Models involving molecular excitation trajectories conserving torsional momenta and excited state to ground state surface crossings better fit the data, and a semiquantitative analysis is presented. Probabilities of surface crossings are calculated.

The visual pigment rhodopsin contains the 11-*cis* isomer of retinal as the photochemically active chromophore linked to surrounding apoprotein opsin by a protonated Schiff base. The sole action of light excitation is to form a pigment called bathorhodopsin (also called prelumirhodopsin). While there has been some recent controversy concerning the nature of this primary photochemical event (Rosenfeld et al., 1977; Peters et al., 1977; van der Meer et al., 1976; Lewis, 1978; Warshel, 1978; Favrot et al., 1979; Honig et al., 1979), the evidence strongly favors the original suggestion of Hubbard and Kropf (1958) that the primary step is a *cis-trans* isomerization (see reviews of Honig, 1978; Ottolenghi, 1980). Both at room temperature (Rosenfeld et al., 1977) and at 77°K (Yoshizawa and Wald, 1963), a photoequilibrium can be established between rhodopsin, bathorhodopsin, and isorhodopsin (which contains a 9-*cis* chromophore) according to the scheme:

$$\underset{(11\text{-}cis)}{\text{rhodopsin}} \rightleftharpoons \underset{(\text{all-}trans)}{\text{bathorhodopsin}} \rightleftharpoons \underset{(9\text{-}cis)}{\text{isorhodopsin}}$$

Thus, since bathorhodopsin is a common intermediate between two *cis* isomers and for other considerations (Rosenfeld, et al., 1977), the chromophore of bathorhodopsin is supposed to be a (likely distorted; see Aton et al., 1980) *trans* chromophore.

We study here the quantum yields of these reactions. Previous work on determining quantum yields has been performed by Strackee (1970; 1972) and Hurley et al. (1977; see

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also Rosenfeld et al., 1977). We extend these studies by using a much wider wavelength range, by including squid in addition to bovine visual pigments, and by including deuterated samples. We find that the sum of the forward and backward quantum yields in the rhodopsin-bathorhodopsin photoreactions is somewhat larger than one and are independent of wavelength and deuteration. This is contrary to the result of Rosenfeld et al. (1977) and Hurley et al. (1977), who found the sum equal to one. The discrepancy may be traced to slightly inaccurate photostationary state compositions reported by Oseroff and Callender (1974).

The quantum yields and their wavelength and temperature dependence are very important in understanding the dynamical nature of the primary photochemistry of visual pigments and photochemical isomerization properties of polyene systems generally. It has been previously argued (Rosenfeld et al., 1977; Hurley et al., 1977) that large forward and backward quantum yields which are temperature and wavelength independent strongly suggest a common excited state betwen the 11-*cis* (rhodopsin) and *trans* (bathorhodopsin) configurations characterized by a single minimum. These arguments are unaffected by our results. For the case when the quantum yields sum to one, it was argued (Hurley et al., 1977; see below) that equilibration in this common excited state would occur after excitation from either rhodopsin or bathorhodopsin. As we discuss in more detail below, statements concerning the dynamics of the primary photochemistry are very sensitive to the actual values of the quantum yields. It was this realization, in fact, that prompted the present study. We find that equilibration in the minimum is not complete. Rather, isomerization has occurred in the excited rhodopsin molecule within a few oscillations along the 11–12 double bond torsional coordinate.

Our results and analysis are in overall agreement with detailed theoretical calculations of Birge and Hubbard (1980; 1981).

MATERIALS AND METHODS

Preparation of Rhodopsin

Cattle rod outer segments were isolated from retinas (Hormel Co. Austin, Minn.) by shaking in phosphate buffer (0.05 M, pH 7.0). Rod outer segment was purified by the method of Makin et al. (1977). Rhodopsin was purified by a modified method of Ebrey (1971). Rod outer segment was solubilized with 2% Ammonyx LO (Onyx Chem. Co., Jersey City, N.J.) in 0.01 M phosphate buffer (pH 7.0) and applied on hydroxylapatite column equilibrated with 0.75% Ammonyx LO in 0.01 M phosphate buffer. The rhodopsin adsorbed on the column was then washed with 0.01, 0.02, 0.05 M phosphate buffer containing 0.75% Ammonyx LO. Rhodopsin was eluted with 0.1 M phosphate buffer containing 0.75% Ammonyx LO. In the case of D_2O-rhodopsin, the last two steps were done with D_2O containing the same concentrations of detergent and buffer.

Squid (*Todarodes pacificus*) rhodopsin was prepared by the method of Suzuki et al. (1976). Rhodopsin was extracted from microvillar membranes with 2% digitonin and purified with DEAE-cellulose column. The purified rhodopsin was dialyzed against 0.2% digitonin in 0.005 M phosphate buffer (pH 6.8) to reduce the concentration of buffer.

Irradiation of Rhodopsin

Rhodopsin was irradiated at 77°K with the monochromatic light (~50 mW) of argon (Spectra-Physics Inc., Loser Products Div., Mountain View, Calif.) and krypton (Coherent Inc., Palo Alto, Calif.) lasers.

A sample of 0.8 ml vol was uniformly irradiated in a glass Dewar containing liquid nitrogen by rotating sample tube and using a lens system. To produce the photostationary state mixture, cattle rhodopsin was irradiated for 30 min at 530.9, 520.8, 514.5, 488.0, 476.5, and 457.9 nm and squid rhodopsin at 530.9, 520.8, 501.7, 482.5, 476.2, 457.9 nm. We found that >10-min irradiation was sufficient to reach photostationary state under the conditions described above.

Isorhodopsin was made by irradiating rhodopsin for 60 min in liquid nitrogen with the 568.2-nm emission of the krypton laser. We confirmed this sample contained >96% isorhodopsin by a similar analysis to that reported by Yoshizawa and Wald (1964).

The photoconversion from bathorhodopsin to rhodopsin and isorhodopsin was studied as follows. Samples of cattle rhodopsin were irradiated with 457.9-nm light for 30 min in liquid nitrogen to yield mixtures rich in bathorhodopsin. These were divided into two parts, and one part was kept in the dark. Another part was reirradiated with 647.1-nm light for 5 min to convert bathorhodopsin to rhodopsin and isorhodopsin. Squid rhodopsin was irradiated with 457.9-nm light for 1 h then with 647.1-nm light for 20 min with the same procedure as in cattle rhodopsin. The irradiation with 647.1-nm light was carefully done to prevent reverse reactions from rhodopsin and isorhodopsin. Thus the irradiation time using 647.1-nm light was chosen not to reach the photostationary state, i.e., some amount of bathorhodopsin still remained in the mixture. A diffraction grating was used in this irradiation to improve monochromaticity.

Analysis of Pigment Composition in the Mixture

All samples were warmed to 10°C after irradiation at 77°K. 20 μl of 1 M NH_2OH was added to cattle rhodopsin to convert unstable photoproducts to retinal oxime (final concentration 0.025 M NH_2OH). 20 μl of saturated Na_2CO_3 solution was added to squid rhodopsin (final pH 10.6–10.8) to convert acid metarhodopsin to alkaline metarhodopsin. Absorption spectra were determined at 10°C with Model EU-700-56 spectrophotometer (GCA/McPherson, Boston, Mass.) connected to a PDP 8/e computer (Digital Equipment Corp., Marlboro, Mass.). The wavelength was scanned from 650 to 420 nm in cattle rhodopsin and from 630 to 430 nm in squid rhodopsin. In these regions, the final products (retinal oxime or alkaline metarhodopsin) did not disturb the absorption spectrum of the rhodopsin-isorhodopsin mixture. The data were transferred to a PDP 10 computer for further analysis.

Pigment composition was calculated with the PDP 10 computer using an unirradiated rhodopsin sample to represent 100% rhodopsin base line and a sample formed by irradiation with 568.2-nm light to represent 100% isorhodopsin (when the value of 96% isorhodopsin is used for the analysis, the resultant difference was <1%). A least squares fitting program was used to compare the two controls against the sample and to calculate the amount of rhodopsin and isorhodopsin present. The amount of bathorhodopsin was calculated assuming the percentages of the three components added up to 100.

Calculation of Relative Quantum Efficiencies

When quantum efficiencies are defined:

$$\text{Rhodopsin} \underset{r_2}{\overset{r_1}{\rightleftharpoons}} \text{Bathorhodopsin} \underset{r_4}{\overset{r_3}{\rightleftharpoons}} \text{Isorhodopsin},$$

the following equations are held in photostationary state:

$$[\text{Rh}]\,\epsilon_r r_1 = [\text{Batho}]\,\epsilon_b r_2 \qquad r_1/r_2 = \epsilon_b[\text{Batho}]/\epsilon_r[\text{Rh}] \tag{1}$$

$$[\text{Batho}]\,\epsilon_b r_3 = [\text{Iso}]\,\epsilon_i r_4 \qquad r_3/r_4 = \epsilon_i[\text{Iso}]/\epsilon_b[\text{Batho}], \tag{2}$$

where the ϵ's are the respective absorbances. From the reaction, Rhodopsin + Isorhodopsin + Bathorhodopsin 647.1 nm Rhodopsin + Isorhodopsin, the following equation is obtained:

$$\Delta Rh/\Delta Iso = \epsilon_b r_2 [Batho]/\epsilon_b r_3 [Batho] = r_2/r_3, \quad (3)$$

because only bathorhodopsin absorbs the 647.1-nm light.

TABLE I
PIGMENT COMPOSITION IN PHOTOSTATIONARY STATE MIXTURE OF RHODOPSIN AT 77°K (CATTLE)

Wavelength	Pigment composition			Quantum efficiency $r_1 = 1.0$		
	Rhodopsin	Isorhodopsin	Bathorhodopsin	r_2	r_3	r_4
(nm)	*(%)*	*(%)*	*(%)*			
530.9	35.0	34.2	30.7	0.82	0.08	0.15
520.8	32.9	28.1	40.0	0.77	0.08	0.14
514.5	30.9	23.6	45.4	0.68	0.07	0.15
488.0	29.4	16.4	54.2	0.69	0.07	0.16
476.5	28.6	15.7	55.7	0.76	0.08	0.15
457.9	27.2	14.4	58.7	0.72	0.07	0.14
			Average	0.74	0.08	0.15

In calculation of relative quantum efficiencies the value of $r_2/r_3 = 0.1$ was used (from Table II). Relative absorbancies were referred to Yoshizawa and Wald (1963).

TABLE II
FORMATION OF RHODOPSIN AND ISORHODOPSIN FROM BATHORHODOPSIN ON IRRADIATION WITH RED LIGHT (CATTLE)

Irradiation		Pigment composition			$\Delta Rh/\Delta$ Iso (r_2/r_3)
		Rhodopsin	Isorhodopsin	Bathorhodopsin	
		(%)	*(%)*	*(%)*	
Exp. 1	476.2	31.5	14.5	54.0	
		31.2	14.9	53.9	
		31.8	14.7	53.9	
	476.2 + 647.1	77.7	20.2	2.1	
		77.4	19.7	2.9	
		75.6	19.7	4.7	0.11
Exp. 2	476.2	30.7	14.7	54.5	
		29.0	15.9	55.1	
	476.2 + 647.1	73.6	19.5	6.9	
		74.5	19.2	6.4	0.09
Exp. 3	476.2	29.2	14.7	56.1	
		29.0	16.3	54.7	
		30.3	14.2	55.5	
	476.2 + 647.1	73.3	19.0	7.7	
		71.6	19.3	9.0	
		70.6	19.3	10.0	0.10
				Average	0.10

Rhodopsin was irradiated with 476.2-nm light for 30 min then with 647.1-nm light for 5 min at 77°K.

TABLE III

PIGMENT COMPOSITION IN PHOTOSTATIONARY STATE MIXTURE AT 77°K (SQUID)

Wavelength	Pigment composition			Quantum efficiency $r_1 = 1.0$		
	Rhodopsin	Isorhodopsin	Bathorhodopsin	r_2	r_3	r_4
(nm)	*(%)*	*(%)*	*(%)*			
530.9	10.7	79.6	9.7	0.41	0.27	0.22
520.8	12.2	76.2	11.6	0.56	0.38	0.18
501.7	16.8	54.4	28.9	0.53	0.35	0.23
482.5	18.2	43.3	38.5	0.58	0.38	0.26
476.2	18.3	40.0	41.7	0.59	0.39	0.26
457.9	17.6	31.0	51.5	0.56	0.38	0.28
			Average	0.54	0.36	0.24

In the calculation of quantum efficiencies the value of $r_2/r_3 = 1.5$ was used (from Table IV). Relative absorbancies were referred to Shichida et al. (1978).

TABLE IV

FORMATION OF RHODOPSIN AND ISORHODOPSIN FROM BATHORHODOPSIN ON IRRADIATION WITH RED LIGHT (SQUID)

Irradiation	Pigment composition			ΔRh/Δ Iso (r_2/r_3)
	Rhodopsin	Isorhodopsin	Bathorhodopsin	
(nm)	*(%)*	*(%)*	*(%)*	
457.9	16.8	29.6	53.7	
	15.6	32.1	52.2	
	16.2	31.3	52.6	
	15.8	31.1	53.2	
	14.8	31.6	53.7	
Average	15.8	31.1		
457.9 + 647.1	37.7	44.6	17.7	
	39.3	45.2	15.6	
	37.1	46.0	16.9	
	37.3	47.8	14.9	
Average	37.8	45.9		1.5

Rhodopsin was irradiated with 457.9-nm light for 1 h then with 647.1-nm light for 20 min at 77°K.

Relative absorbencies of pigments at 77°K were referred to Yoshizawa and Wald (1963) in cattle rhodopsin and to Shichida et al. (1978) in squid rhodopsin.

RESULTS

The results are summarized in Tables I, II, III, and IV. Our methods fix the ratios of the quantum yields. To determine the absolute values, we use 0.67 for the quantum yield of the rhodopsin to bathorhodopsin transformation which has been determined at room temperature (Dartnall, 1972) and at 77°K (Hurley et al., 1977) and has been found to be wavelength

independent. The yields are then:

$$\text{Cattle}\quad \text{Rhodopsin} \underset{0.5}{\overset{0.67}{\rightleftharpoons}} \text{Bathorhodopsin} \underset{0.1}{\overset{0.054}{\rightleftharpoons}} \text{Isorhodopsin}$$

$$\text{Squid}\quad \text{Rhodopsin} \underset{0.36}{\overset{0.67}{\rightleftharpoons}} \text{Bathorhodopsin} \underset{0.16}{\overset{0.24}{\rightleftharpoons}} \text{Isorhodopsin.}$$

No effect due to deuterating the sample was found. The relationships were found to be independent of wavelength except that, of course, the value of r_2/r_3 was determined only at 647.1 nm. We assume that r_2/r_3 is also wavelength independent because r_1, r_2, and r_3/r_4 are observed to be wavelength independent, and it would be highly accidental to have the wavelength dependence of r_4 match that of r_3. Nevertheless, it should be pointed out that Hurley et al. (1977) found r_4 (bovine) to be wavelength dependent with values ranging from 0.09 to 0.16. We assume below that the yields are constant. This has little effect on our analysis; the important observation is that r_3 and r_4 are small. The values for the quantum yields are averages of many separate runs at each wavelength. The standard deviation for r_2, r_3, r_4, (r_1 fixed at 0.67) was 4% for the bovine samples and 6% for the squid samples.

Our cattle results are close to those inferred by Strackee (1970, 1972) who used rhodopsin extracts and isolated retinas. Our Ammonyx LO extracts purified on hydroxylapatite column are almost completely delipidated. It thus appears that the quantum yields are independent of lipid binding and detergent.

DISCUSSION

The values of the quantum yields have important consequences concerning the mechanism of photoisomerization in the primary process of visual pigments and for polyene chemistry generally. It has been strongly argued that the excited state potential curve of rhodopsin was characterized by a single common minimum and, further, that excitation energy is channeled to this minimum regardless of whether or not the retinal chromophore was rhodopsin (11-*cis*) or bathorhodopsin (*trans*) (Rosenfeld et al., 1977; Hurley et al., 1977). It was further argued that the molecule equilibrates in the common minimum after excitation. The key evidence for this latter concept was that the quantum yields for the rhodopsin to bathorhodopsin (r_1) and bathorhodopsin to rhodopsin (r_2) transformations summed to one. The measurements by which the quantum yields were calculated are primarily from photostationary state results of bovine samples at liquid nitrogen temperatures reported by Oseroff and Callender (1974). Our more extensive photostationary measurements here over an expanded wavelength region are in slight disagreement with these results. While this disagreement has little effect on the study and purposes of Oseroff and Callender (1974), it can have a major impact on models for the photochemistry of visual pigments. We find that the forward and backward reaction quantum yields ($r_1 + r_2$) sum to more than one, and this raises serious difficulties with the concept that the excitation of rhodopsin or bathorhodopsin produces an equilibrated common excited state complex that subsequently decays to form ground state products.

We analyze your results in terms of the following model. The excited singlet state of the retinal chromophore is viewed as having a single common minimum (Hurley et al., 1977; see Fig. 1) along the 11–12 and 9–10 torsional coordinates. Isomerization of rhodopsin to

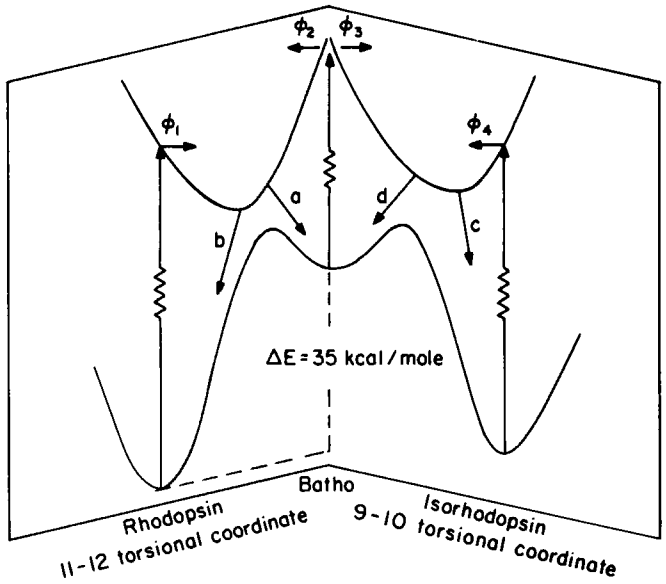

FIGURE 1 Potential energy diagrams for rhodopsin, bathorhodopsin, and isorhodopsin. The quantum yield for rhodopsin isomerization, r_1, involves the value of the branching probability ϕ_1 to oscillate along the 11–12 torsional coordinate and the value of surface crossing probability a and similarly for the other quantum yields (see text). We assume $\phi_1 = \phi_4 = 1$ and $\phi_2 + \phi_3 = 1$. The energy curves are more or less to scale using the results of Honig et al. (1979) and Cooper (1979). Wavy lines indicate photoreactions.

bathorhodopsin occurs first by excitation of rhodopsin to the 11-*cis* side of the excited state common 11-*cis* to *trans* potential well. The molecule then oscillates between the 11-*cis* and *trans* coordinates. With each pass we define a probability of crossing from the excited state surface to the ground state surface. The probabilities are a and b, respectively, for crossing from excited state 11-*cis* to ground state *trans* and excited *trans* to ground 11-*cis*. To conserve torsional motion in what could be called a trajectory of the excitation of the molecule, no reversal of motion is allowed. Thus our excited rhodopsin molecule populates the ground state of bathorhodopsin with probability a on the first pass of the common minimum and repopulates its own ground state with probability b on the second pass and so on until all the excitation energy is used up. Excitation of bathorhodopsin yields rhodopsin in the same manner; and similar surface crossing probabilities, c and d, are defined for the 9-*cis* to *trans* torsional coordinate. The numbers ϕ_1, etc., are branching numbers that define the probability of the system oscillating along the defined coordinates after excitation.

This approach to photoisomerization of retinal systems has been previously developed theoretically (Warshel and Karplus, 1975; Birge and Hubbard, 1980). However, the analysis here has been simplified since our static measurements do not yield sufficient information to fully examine the theory. In general the values of a, b, c, and d depend upon the energy of the oscillating coordinate and so depend on the particular pass and whether one enters the excited state from rhodopsin (or isorhodopsin) or bathorhodopsin (Birge and Hubbard, 1980). Furthermore, it is likely that bathorhodopsin production from rhodopsin involves not only isomerization but also proton movements (Peters et al., 1977; Honig et al., 1979). Thus the

excited state of the system may be slightly different for rhodopsin and bathorhodopsin. The values of a, b, c, and d derived below should be thus viewed as "average" values to provide a semiquantitative framework describing the dynamics.

In terms of the parameters of Fig. 1, we find that

$$r_1 = \frac{a\phi_1}{1 - (1 - a)(1 - b)}$$

$$r_2 = \frac{b\phi_2}{1 - (1 - a)(1 - b)}$$

$$r_3 = \frac{c\phi_3}{1 - (1 - c)(1 - d)}$$

$$r_4 = \frac{d\phi_4}{1 - (1 - c)(1 - d)}$$

so that

$$a = 1 + \frac{(r_1 - \phi_1)\phi_2}{\phi_1 r_2}$$

$$b = 1 + \frac{(r_2 - \phi_2)\phi_1}{\phi_2 r_1}$$

$$c = 1 + \frac{(r_3 - \phi_3)\phi_4}{\phi_3 r_4}$$

$$d = 1 + \frac{(r_4 - \phi_4)\phi_3}{\phi_4 r_3}$$

We assume that $\phi_1 = \phi_4 = 1.0$ and that $\phi_2 + \phi_3 = 1$ (Hurley et al., 1978). This amounts to assuming that there exists no significant fluorescence or nonradiative processes channeling the system in directions other than that depicted by Fig. 1. There is substantial evidence to support this (see, for example, Honig, 1978). Since ϕ_2 and ϕ_3 are not separately known, there is some ambiguity in the results. However, because the yields for the 9-*cis* coordinate are small for both cattle and squid pigments, the possible range in the values for a, b, and d is quite well determined and is obtained using the constraint that $0 < c < 1$ which fixes the range of values of ϕ_3 (and hence ϕ_2). We find for cattle: $a = 0.38$, $b = 0.30$, $0 < d < 0.1$, and $0.940 < \phi_2 < 0.946$. Similarly, for squid, we find: $0.30 < a < 0.35$, $0.21 < b < 0.26$, $0.1 < d < 0.16$, and $0.71 < \phi_2 < 0.76$.

We thus find that both systems are quite similar in their characteristics except that the branching of the squid pigment towards the 9-*cis* coordinate upon bathorhodopsin excitation is substantially larger than in the cattle pigment.

It is instructive to use the previous estimate of the quantum yields for the bovine used in the analysis (Rosenfeld et al., 1977; Hurley et al., 1977). Here we have $r_1 = 0.67$, $r_2 = 0.30$, and $\phi_2 = 0.9$. Using these values we find $a = 0.01$ and $b = 0.005$, very different from the present findings. A very large number of oscillations would have occurred before significant isomerization. This represents the case of equilibration of torsional motion in the excited

electronic potential surface as reported by Rosenfeld et al. (1977) and Hurley et al. (1977). It is evident that models for the dynamical behavior of the isomerization process are quite sensitive to the values of the quantum yields.

It should be noted that the conclusion that the dynamics do not involve equilibration of torsional motion in the excited state is not affected by experimental error. We will obtain small values of a and b when r_1 and r_2 sum to one. In the present case, this occurs for the smallest possible values of r_1 and r_2 within experimental error. Taking the smallest value of $r_1 = 0.63$ as measured by Dartnall (1972) and decreasing the resulting r_2 by 1 SD (4%) in the bovine case, we find $a = 0.22$ and $b = 0.17$, about half the values found for the measured values of r_1 and r_2.

In this regard, the quantum yields of bacteriorhodopsin should be contrasted to those of rhodopsin. Bacteriorhodopsin is similar to rhodopsin in many of its properties. The chromophore of the bacteriorhodopsin protein is retinal attached to the apoprotein by a protonated Schiff base, and a red-shifted species, called K, is produced from the parent pigment, bR568, upon absorption of a photon. However, the quantum yield of this process is smaller, 0.30, than that of rhodopsin and the backward reaction larger, 0.70 (Hurley et al., 1977). There is also no analogous iso-pigment formed. The fact that the quantum yields add to one and following the arguments above indicate equilibration of torsional motion in the excited electronic potential surface before ground state product is formed (Hurley et al., 1977). These rather significant differences of photochemical behavior between visual pigments and bacteriorhodopsin are interesting and need further study.

Birge and Hubbard (1980) have calculated the internal conversion probability (a) using INDO-CISD molecular orbital theory and semiempirical molecular dynamics procedures. The a parameter was calculated to be dependent on the trajectory pass and ranged from 0.19 to 0.62. An average value of 0.39 was obtained which compares well with our value of 0.38 (assumed constant for all passes). More recently, Birge and Hubbard (1981) have calculated values of the forward and backward quantum yields of 0.62 and 0.48, respectively, for the rhodopsin-bathorhodopsin transformation compared to 0.67 previously determined (Dartnall, 1972; Hurley et al., 1977) and 0.5 found here. Our overall conclusions and theirs are in substantial agreement although the exact details of the process likely need further work.

The fact that the quantum yields are independent of sample deuterations is consistent with our proposal that the primary event in visual pigments is *cis-trans* isomerization and that the proton movements observed in picosecond kinetic experiments (Peters et al., 1977) are a subsequent event caused by the isomerization. In this case the determining factor in the quantum efficency is the mechanism of isomerization. The only chromophore exchangeable proton is the proton associated with the chromophore-protein protonated Schiff-base linkage. Isotope substitution of this proton would have little influence on retinal's electronic structure (and thus the forces driving the isomerization) and the moment of inertia about the retinal 11–12 or 9–10 double bonds.

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