extract the carotenoid is present in phospholipid micelles. The water-insoluble naphthoquinone is likely to be held in the micelles in relatively close proximity to the carotenoid, a situation not achieved in ethanolic solution. The protection of quinone by transfer of excitation energy to carotenoid will therefore be facilitated.

In studies of respiration, the function attributed to carotenoid is protection of the quinone[10]. Since the extracted carotenoid carries out the same function and has the same absorption maxima as in the membrane-bound state, we believe that the bulk of the pigment is associated with membrane lipid *in vivo*. In viability studies the protective function of carotenoid has been shown to depend not only on chromophore length[21] but also on concentration[22]. Thus the bulk carotenoid seems to be necessary for photoprotection. This does not, however, rule out the possibility of small amounts of carotenoid being protein-bound[23] or of the pigment being associated with lipoprotein, but such states would not be essential for the protective function. We conclude that the lipid-associated carotenoid in *M. luteus* has a role in the photoprotection of naphthoquinone.

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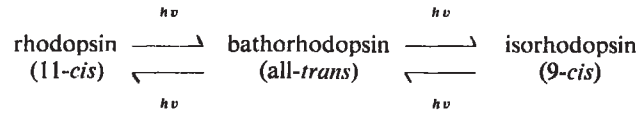
Temperature and wavelength effects on the photochemistry of rhodopsin, isorhodopsin, bacteriorhodopsin and their photoproducts

The primary photochemical event in visual pigments has become a matter of considerable controversy[1–3]. We recently reviewed the various models that have been proposed and argued that the accumulated evidence strongly favours the original suggestion of Kropf and Hubbard that the primary step involves a *cis-trans* isomerisation[4]. Evidence for isomerisation is based on the photoequilibrium that can be established, both at 77 K (ref. 5) and at room temperature[6], between rhodopsin, bathorhodopsin (its primary photoproduct), and isorhodopsin (which contains a 9-*cis* chromophore). The equilibrium can be represented by

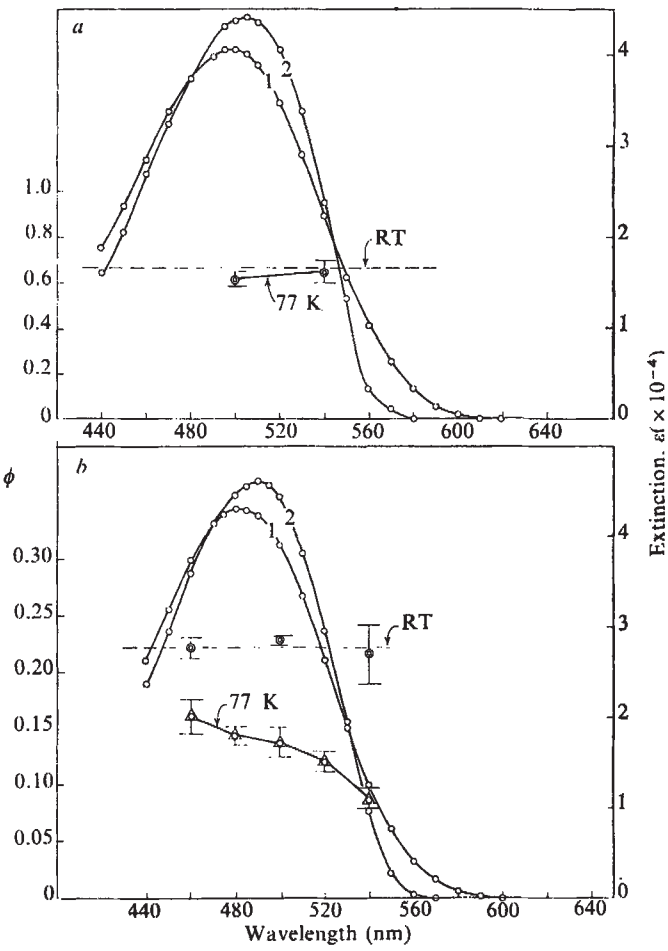


This strongly suggests that the isomeric configuration of the chromophore of the common bathorhodopsin intermediate is *all-trans* retinal, and thus light has isomerised the chromophore from 11-*cis* to all-*trans*.

It is of considerable interest to obtain a quantitative description of the physical processes involved in the primary photochemical event. Based on an analysis of the temperature and wavelength effects on the photochemistry of rhodopsin and protonated Schiff bases in solution, we proposed a model[4] describing a potential energy curve for the excited state of the visual pigment. The major conclusion of the model is that the protein moiety of the pigment efficiently channels the excitation energy of the chromophore into a single potential minimum along the 11–12 torsional coordinate.

We extend here our original experiments and analysis to the

Fig. 1 *a* Absorption spectra of bovine rhodopsin in 67% glycerol and 2% digitonin at (1) room temperature and (2) 77 K. Corrections were made for scattering by subtracting the spectrum of the same sample bleached in the presence of hydroxylamine. Corrections were also made for a 7.7% solvent contraction at 77 K. The dashed line represents the quantum efficiency for bleaching rhodopsin at room temperature[10,11,12]. The circles represent the average of five measurements of the quantum efficiency at 77 K as described in the text. Standard deviations are given by the error bars. *b*, Isorhodopsin spectra under same conditions as in *a*. Isorhodopsin was prepared by regeneration of opsin with 9-*cis* retinal. Circles represent room temperature quantum efficiencies and triangles represent the quantum efficiencies at 77 K.



artificial pigment, isorhodopsin, and to the purple membrane protein of *Halobacterium halobium*, bacteriorhodopsin. The results for isorhodopsin provide a strong independent confirmation of the original model. Moreover, our analysis of bacteriorhodopsin photochemistry suggests a protein induced *cis-trans* isomerisation for that pigment as well.

Our experimental approach is to test the effects of temperature and excitation wavelength on the absolute yield of forming the bathoproduct from both rhodopsin and isorhodopsin as well as the purple membrane protein. The amount of isorhodopsin and rhodopsin converted to bathorhodopsin by a low dose irradiation at 77 K was determined by warming the sample to room temperature (where the bathorhodopsin decays to all-*trans* retinal and opsin) and measuring the amount of unbleached pigment remaining. The amount of the purple membrane protein converted to its bathoproduct, K, was calculated from the absorption of the mixture formed after a short irradiation using the extinction coefficients of the purple membrane and K (shown in Fig. 2). Quantum yields were calculated according to Dartnall[8,9] using rhodopsin as an actinometer and correcting for changes in extinction coefficient with temperature (Figs 1*a* and 1*b*). Quantum fluxes at longer wavelengths were determined with a rhodopsin calibrated photodiode.

As shown in Figs 1*a* and 2, we found that the quantum yield of the primary photo-event of rhodopsin and the purple membrane protein is, within experimental error, independent of excitation wavelength and temperature over a 220 K range. Moreover, Fig. 2 shows the quantum yield of the photoreversal from the bathoproduct K to the purple membrane pigment is also independent of temperature. On the other hand, the quantum yield for bleaching isorhodopsin (which has no wavelength dependence at room temperature) shows a significant decrease at lower temperatures (Fig. 1*b*). This lower quantum yield at 77 K was also wavelength dependent, the yields being smaller as wavelength increased.

The wavelength and temperature independence of the bleaching or rhodopsin strongly suggests that *cis-trans* isomerisation takes place after complete thermal relaxation and requires no activation energy. This situation may be achieved by efficiently channelling the excited system into a minimum, along a barrierless potential curve connecting the *cis* and *trans* configurations as shown in Fig. 3. This conclusion was also suggested by the observation that the quantum yields for the forward

$$\text{rhodopsin} \xrightarrow{\Phi_{R\to B}} \text{bathorhodopsin}$$

and back

$$\text{bathorhodopsin} \xrightarrow{\Phi_{B\to R}} \text{rhodopsin}$$

reactions nearly sum to unity[4] which implies that most ($\varphi_1 \simeq \varphi_2 \simeq 1$) excited rhodopsin and bathorhodopsin molecules populate a common point on the excited state potential surface. (This can be easily seen since $\Phi_{R\to B} = \varphi_1\gamma_1, \Phi_{B\to R} = \varphi_2\gamma_2$ and $\gamma_1+\gamma_2 = 1$. Thus, $\Phi_{R\to B}+\Phi_{B\to R} = 1$ only when $\varphi_1 \simeq \varphi_2 \simeq 1$).

The general form of the potential curve shown on the left side of Fig. 3 is amenable to more quantitative analysis. Using the absolute and relative quantum yields at 77 K (see Fig. 3) we find $\varphi_1 = 1.0$, $\varphi_2 = 0.9$, $\gamma_1 = 0.67$ and $\gamma_2 = 0.33$. Moreover, the temperature independence of the R→B photoisomerisation shows that these values are accurate at room temperature as well (with the possible exception of φ_2 which cannot be determined at room temperature). Thus, the left side of Fig. 3 provides the first detailed characterisation of the primary photochemistry of rhodopsin.

The fact that $\varphi_2 = 0.9$ (which requires that $\Phi_{R\to B}+\Phi_{B\to R}$ be slightly less than unity) is due to the leakage (φ_3) of bathorhodopsin to the 9–10 torsional coordinate leading to the formation of isorhodopsin. An independent check of the numbers given above requires that an analysis of the B⇌I interconversion yield $\varphi_3 = 1-\varphi_2 \simeq 0.1$. Unfortunately, the temperature dependence of $\Phi_{I\to B}$ and its wavelength dependence at 77 K (due perhaps to a small barrier along its potential surface) preclude an accurate determination of φ_3, φ_4, γ_3 and γ_4. It is possible, however, to use the values of $\varphi_{I\to B} = \varphi_4\gamma_4$ and $\Phi_{B\to I} = \varphi_3\gamma_3$ to estimate φ_3.

Taking an average value of $\Phi_{I\to B} \simeq 0.13$ (Fig. 1*b*) and the ratio $\Phi_{B\to I}/\Phi_{I\to B} = 0.4$ determined previously[4] we find $\varphi_3\gamma_3 = 0.05$. (Thus our model yields $\Phi_{B\to R}/\Phi_{B\to I} = \varphi_2\gamma_2/\varphi_3\gamma_3 \simeq 5$, in complete agreement with the estimate of Yoshizawa and Wald[5].) Simple numerical considerations (see Fig. 3*a*) now lead to the conclusion that φ_3 must be approximately 0.1, thus providing an important consistence check for the analysis of the B⇌R interconversion.

The most striking implication of the above analysis is the complete channelling ($\Phi_1 = 1.0$) into the common minimum from excited rhodopsin molecules (11-*cis* configuration) and almost complete channelling of bathorhodopsin (all-*trans*) ($\Phi_2 = 0.9$) to the 11–12 torsional coordinate accounting for the relationship $\Phi_{R\to B}+\Phi_{B\to R} \simeq 1$. As can be seen from Fig. 2, the same relationship ($\Phi_{PMP\to K}+\Phi_{K\to PMP} = 0.28+0.72 = 1$) also characterises the primary photoevent in light-adapted bacteriorhodopsin, both at 77 K and at room temperature (Fig. 2 and refs 9, 13, 14). Moreover, the photoreversibility of the various intermediates so characteristic of the bleaching sequence of visual pigments[5] is also a feature not only of the K intermediate but also of the 'M' (412 nm) intermediate of the purple membrane protein[10]. These striking photochemical analogies very strongly suggest a *cis-trans* geometry change as the primary photochemical step in bacteriorhodopsin.

The extremely simple photochemical behaviour of both rhodopsin and purple membrane protein should be contrasted with the complex patterns that characterise the photoisomerisation of retinal analogues[15] and other well studied systems such

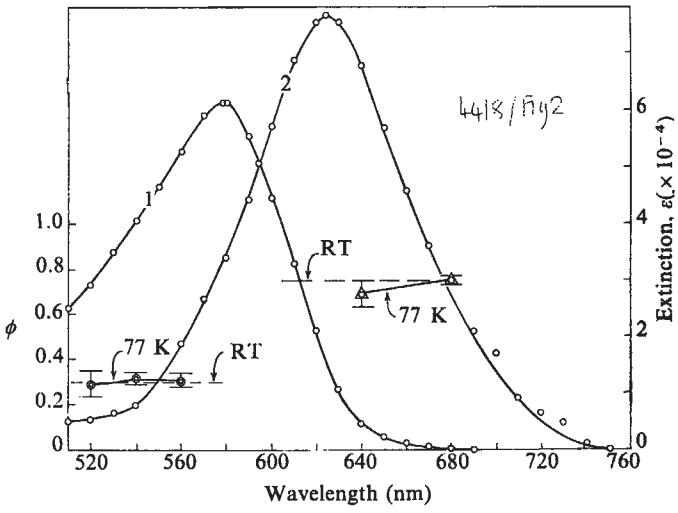


Fig. 2 Absorption spectra at 77 K in 67% glycerol of (1) the purple membrane protein and (2) its primary photoproduct, the K intermediate. The K spectrum was determined from the spectrum of the 500 nm photosteady-state mixture, assuming it to be 28%K. This was determined by warming the mixture under conditions[9] which allow the complete conversion of K to M(412), revealing the percentage of pigment unconverted in the photosteady-state mixture. The circles represent the purple membrane protein quantum efficiency and the triangles represent the K quantum efficiency both at 77 K. The dashed lines represent the corresponding quantum efficiencies for the pigment (at −40 °C (ref. 9); a similar value is seen at room temperature)[14] and for the bathoproduct (at room temperature RT).

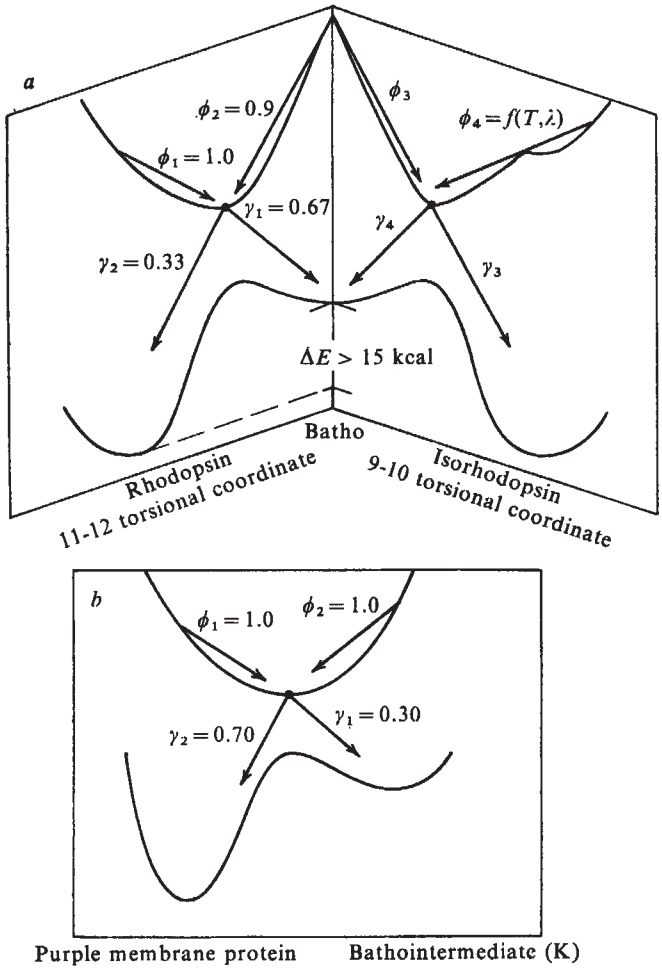


Fig. 3*a* Potential energy diagrams for the rhodopsin, bathorhodopsin, isorhodopsin interconversions. The quantum efficiency for rhodopsin isomerisation is $\varphi_{R\to B} = \varphi_1\gamma_1$, where φ_1 is the probability of reaching the bottom of the potential well of the excited state and γ_1 is the probability of going to bathorhodopsin from the bottom of the well. Similar relations hold for $\varphi_{B\to R}$, $\varphi_{B\to I}$, and $\varphi_{I\to B}$. Quantum yields for the 11–12 coordinate are calculated from the 77 K data presented here and using $\varphi_{R\to B}/\varphi_{B\to R} = 2.2$ (ref. 4). For the 9–10 coordinate, our room and low temperature data set limits for φ_4 between 0.22 and 1.0 leading to $0.22 < \gamma_4 < 0.59$, $0.41 < \gamma_3 < 0.78$ and then using $\varphi_3\gamma_3 = 0.06$ we find $0.07 < \varphi_3 < 0.14$. Thus, all values of φ_3 are consistent with $\varphi_2 \simeq 0.9$. *b*, Hypothetical energy level diagram for the purple membrane protein and its bathoproduct K. This diagram ignores the observed fluorescence which is thought to come from another excited state (ref. 19).

as stilbenes[16]. In contrast to the pigments, the quantum yields of the model compounds are strongly wavelength-dependent and can be 1–2 orders of magnitude less than those of the pigments. Thus, it seems that the protein facilities isomerisation in the chromophore by altering the excited state potential energy surfaces and/or by modifying rates of radiationless deactivation. It is interesting in this context that the artificial pigment isorhodopsin seems to undergo a less efficient and more complex photochemistry than does rhodopsin.

The protein can also influence the ground state energy surfaces. Although free 11-*cis* and all-*trans* retinal have very similar free energies[17], the opsin modifies the equilibrium conformation of the chromophore so that rhodopsin is at least 10 kcal mol^{-1} lower in free energy than the final products, opsin and free all-*trans* retinal. Moreover, bathorhodopsin decays spontaneously to opsin + all-*trans* retinal and, thus, must be still higher in free energy than these products; this free energy must be obtained from the photon absorbed by rhodopsin. A hypothetical ground state energy diagram is also included in Fig. 3.

The bathoproduct of the purple membrane protein must also contain a significant fraction of the absorbed photon's energy since the spontaneous decay of this photoproduct back to the original pigment is used to power a proton pump across the cell membrane[18]. In view of the basic similarity of this pigment and rhodopsin with respect to the chromophore structure and binding and their primary photochemistry, it is reasonable that similar energy storage mechanisms are operative in both pigments.

Recently, Peters *et al.*[20] have observed a deuterium-dependent decay of a transient species following picosecond stimulation of rhodopsin; they have proposed that light does not isomerise the chromophore, but rather initiates a proton transfer to it (see also ref. 3). We do not, however, believe that their data in any way rules out *cis-trans* isomerisation; indeed many interpretations of the data, other than the one they give, are possible. For example, the transient species may be due to a time-dependent shift in the absorption spectrum of bathorhodopsin, due to a relaxation of the protein/chromophore following isomerisation; that is, it could arise if the proton of the protonated Schiff base changes its orientation with respect to its counter ion or even switches its counter ion as a result of *cis-trans* isomerisation. This could account for the temperature and deuterium effects of the rate of formation of bathorhodopsin. In any case, the models Peters *et al.* propose for the primary event are incompatable with the known properties of bathorhodopsin discussed above and in ref. 4. Moreover, it is difficult to see how proton tunnelling as the only primary event would result in a stable species (at 77 K) which would not revert back to rhodopsin. Finally, Green *et al.*[21] have recently provided evidence that isomerisation in rhodopsin and isorhodopsin can occur in picoseconds at room temperature.

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