A NEW APPROACH TO UNDERSTANDING THE INITIAL STEP IN VISUAL TRANSDUCTION

STEVEN J. MILDER AND DAVID S. KLIGER
Division of Natural Sciences, University of California, Santa Cruz, California 95064

ABSTRACT Data from picosecond spectroscopic studies of the formation kinetics of bathorhodopsin upon photolysis of rhodopsin and isorhodopsin was analyzed in terms of the Englman-Jortner theory of radiationless transitions. It was found that low frequency vibrations of the protein and/or chromophore are important in coupling bathorhodopsin to its precursor. The results were consistent with a mechanism for bathorhodopsin formation involving only a simple chromophore isomerization. A similar analysis of the formation kinetics of the K state of bacteriorhodopsin showed that different low frequency vibrations than those calculated for rhodopsin couple it to its precursor. The frequency of these vibrations increases upon deuteration for rhodopsin, while it decreases upon deuteration for bacteriorhodopsin. This points out the importance the specific protein matrix has on the primary photolysis reaction.

The process of visual transduction involves the creation of an activated form of a visual pigment that initiates an enzyme cascade of reactions in the retinal membrane. The activated pigment is in turn formed from a series of reactions initiated by the absorption of light by the pigment. The initial photolysis product is an intermediate whose absorption spectrum is red-shifted relative to the parent pigment and is thus called bathorhodopsin. Because the transformation of rhodopsin to bathorhodopsin triggers the rest of the visual transduction process, much research has been aimed at understanding this reaction. We report here a new approach to analyzing kinetic data on this fundamental photochemical reaction.

Yoshizawa and Kito (1958) first observed the formation of bathorhodopsin by irradiating rhodopsin in a glass at 77°K. A few years later it was observed that at 77°K a photochemical equilibrium occurs between rhodopsin (where the chromophore is a protonated Schiff base of 11-*cis* retinal), bathorhodopsin, and isorhodopsin (where the chromophore has a 9-*cis* conformation), (Yoshizawa and Wald, 1963). This led to the common belief that the photochemical formation of bathorhodopsin involves a *cis* to *trans* isomerization of the chromophore. This simple mechanism went unchallenged until 1972, when the formation kinetics of bathorhodopsin were studied with picosecond spectroscopy at room temperature (Bush et al., 1972). It was found that bathorhodopsin formed within 6 ps of photolysis of rhodopsin. Though theoretical calculations later showed that isomerization of the chromophore might occur in such short times (Honig et al., 1979; Birge and Hubbard, 1980), this result initially raised doubts about whether bathorhodopsin formation involves only a simple chromophore isomerization.

Further picosecond kinetic studies were carried out on the rate of formation of bathorhodopsin at temperatures <40°K. Upon photolysis of rhodopsin, Peters et al. (1977) were able to observe decays that took tens of picoseconds from an unidentified intermediate to bathorhodopsin. Temperature dependence studies of this rate showed that linear Arrhenius behavior was not obtained. Instead, a low temperature limit was observed for the rate of bathorhodopsin formation that was said to be indicative of a tunneling process. These authors also observed non-Arrhenius behavior with a low temperature rate limit for rhodopsin equilibrated with deuterated solvent. Since at all temperatures the deuterium exchanged rhodopsin formed bathorhodopsin at slower rates than in protic solvents, the authors attributed the rate limiting step of the reaction to proton tunneling, or what they called "proton translocation." A number of others have subsequently proposed various mechanisms to explain the rapid formation of bathorhodopsin (Lewis 1978; Honig et al., 1979; Dupuis, et al., 1980).

While the picosecond studies described above suggested that mechanisms other than chromophore isomerization might be responsible for bathorhodopsin formation, other picosecond studies showed that isomerization of the chromophore must still be involved in the rapid formation of bathorhodopsin. In particular, the work of Green et al. (1977) has shown that photolysis of both rhodopsin and isorhodopsin yields bathorhodopsin within a few picoseconds of light absorption. These pigments initially contain chromophores with different *cis* isomers, so bathorhodopsin formation must involve rapid isomerization if the two pigments share the same bathorhodopsin intermediate. Spectra of bathorhodopsins taken on a picosecond time

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scale (Monger et al., 1979) and on a nanosecond time scale (Kliger et al., 1984) indicate that the batho products are indeed similar. In contrast to this, Spalink et al. (1983) have shown that rhodopsin and isorhodopsin appear to have batho products which, at room temperature, have slightly different spectra 85 ps after photolysis. They concluded that formation of batho does not involve only a simple isomerization of the *cis* chromophore to a common distorted all-*trans* chromophore.

As a result of these conflicting studies, there has been much argument in recent years over whether the primary step of vision involves a chromophore isomerization or a proton translocation. To account for all the picosecond results, some investigators have proposed mechanisms for bathorhodopsin formation that include both an isomerization step and a proton translocation (Honig et al., 1979; Birge et al., 1985). In analyzing the low temperature kinetic data for bathorhodopsin, we will show that the observed nonlinear Arrhenius plots, as well as the effect of deuteration, can be understood without the inclusion of a proton translocation.

The curved Arrhenius plots characteristic of batho formation kinetics are similar to those expected for a curve crossing between two harmonic potential surfaces when quantum effects are included in the theoretical description of the kinetics. Englman and Jortner (1970) (EJ) have treated nonradiative decay kinetics from such a quantum theoretical point of view. The EJ theory in the strong coupling limit gives for the nonradiative rate

$$W = \frac{C^2(2\pi)^{1/2}}{\hbar(E_M k_B T^*)^{1/2}} \exp(-E_A/k_B T^*),$$

where E_M is a measure of the energy difference and the relative displacement of the potential surfaces between the two coupled states, C is a measure of the electronic coupling between the two states, and E_a is the energy of the curve crossing point relative to the zero point energy of the reacting state. T^* is an effective temperature given by

$$k_B T^* = \frac{1}{2}\hbar\langle\omega\rangle \coth(\hbar\langle\omega\rangle/2k_B T).$$

The effective temperature is always larger that the actual temperature. This is due to the inclusion of tunneling effects that arise in the quantum treatment used. At low temperatures, the use of an effective temperature will make the rate of crossing between the states faster than classically predicted and will lead to a limiting low temperature rate. Note that the effective temperature is strongly influenced by $\langle\omega\rangle$, the "mean vibrational frequency," and that the larger $\langle\omega\rangle$ is, the larger the effective temperature. The mean vibrational frequency represents an average over the vibrational modes that couple the two states. Thus, $\hbar\langle\omega\rangle$ can be viewed as the "average vibrational energy."

We used two methods to model the low temperature picosecond photolysis data of Peters et al. (1977) for rhodopsin and of Applebury (1984) for isorhodopsin, using the EJ formulation in the strong coupling limit. In the first method, E_a and $\hbar\langle\omega\rangle$ were adjusted to obtain Arrhenius curves that fit the shapes of the published data. Then C' (where $C' = C^2(2\pi)^{1/2}/\hbar(E_M)^{1/2}$) was determined by adjusting the curves to give the best absolute fit to the data. Alternatively, a Simplex algorithm was used to adjust all three parameters (Caceci and Cacheris, 1984). Fig. 1 shows the results of the modeling using the Simplex algorithm. For rhodopsin $\hbar\langle\omega\rangle = 28$ cm^{-1}, with $E_a = 89$ cm^{-1} and $C' = 6.0 \times 10^{13}$ s^{-1}K$^{1/2}$. For isorhodopsin $\hbar\langle\omega\rangle = 38$ cm^{-1}, with $E_a = 36$ cm^{-1} and $C' = 4.8 \times 10^{11}$s^{-1}K$^{1/2}$. For deuterium exchanged rhodopsin $\hbar\langle\omega\rangle = 52$ cm^{-1}, with $E_a = 287$ cm^{-1} and $C' = 1.1 \times 10^{15}$ s^{-1}K$^{1/2}$. Because there are few data points and each point has a large standard deviation, there is some uncertainty in these parameters.

It is clear from Fig. 1 that the EJ theory can adequately account for the curvature in the Arrhenius plots of batho-

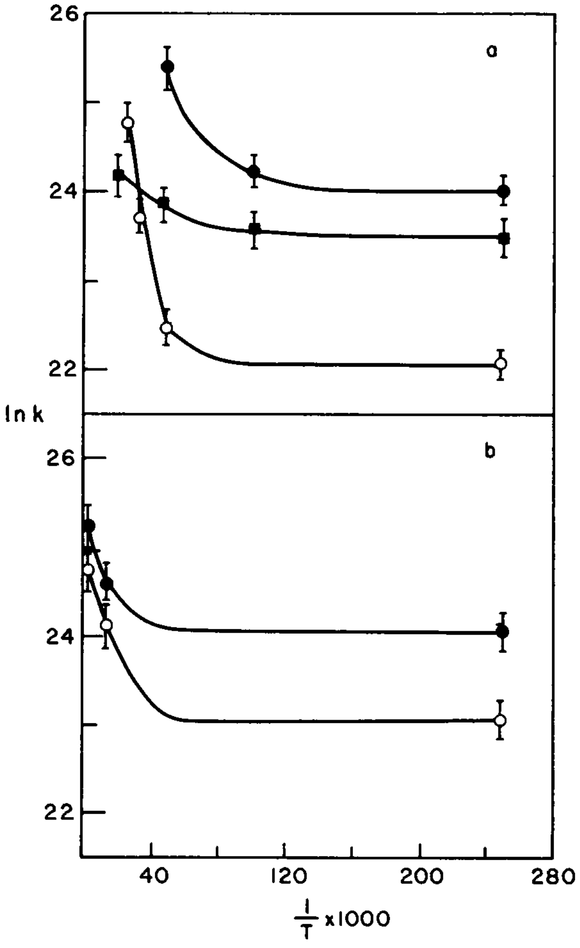

FIGURE 1 Arrhenius plots for the decay of the precursor state to the batho state. Curves through the data points represent the calculated fits using the Englman-Jortner equations. (*a*) Data correspond to rhodopsin (●), deuterium-exchanged rhodopsin (○), and isorhodopsin (■). (*b*) Data correspond to bacteriorhodopsin (●) and deuterium-exchanged bacteriorhodopsin (○).

rhodopsin formation kinetics. We note that the EJ theory is approximate and includes a number of adjustable parameters. Thus, the meaning of the parameters in terms of the physical phenomena being studied should be taken with some care. With this in mind, it is interesting to look at the parameters in light of what one might expect from proton translocation processes. The mean vibrational energy for the formation of bathorhodopsin from its precursor state is 28 cm^{-1}. This is significantly less than the energy of an N-H stretch or an O-H stretch, the energy one might expect the mean vibrational energy to be if proton translocation was the process that leads the precursor state to bathorhodopsin. Furthermore, for deuterium-exchanged rhodopsin $\hbar\langle\omega\rangle$ is 52 cm^{-1}, a value larger than for rhodopsin. If movement of a particular proton was directly involved in connecting the precursor state to bathorhodopsin, one would expect the mean vibrational energy to decrease after rhodopsin undergoes deuterium exchange. For isorhodopsin $\hbar\langle\omega\rangle = 38\ cm^{-1}$, a value somewhat higher than for rhodopsin. No data is available for the calculation of the mean vibrational energy for the decay of the precursor to bathorhodopsin for deuterium-exchanged isorhodopsin. It would be interesting to see if deuterium exchange in isorhodopsin led to an increase in $\hbar\langle\omega\rangle$ as it does in rhodopsin.

These results point to the possible importance of low frequency motions of the protein in and around the chromophore pocket rather than individual proton movements in facilitating bathorhodopsin formation. Deuteration of ionizable protons may have a significant effect on the rigidity of the chromophore/protein interaction via hydrogen bonding and other forces. It is possible that a proton (deuteron) within the chromophore pocket is coupled to the vibration(s) that connect the precursor state and bathorhodopsin. Also, long range effects of the deuterated solvent environment may significantly affect rhodopsin and thus the rigidity of the protein near the chromophore pocket. Recent resonance Raman results for myoglobin, for example, have shown significant shifts in heme vibrational stretches when the solvent environment around the protein is changed from H_2O to D_2O (Argade et al., 1984).

In addition to the modeling of rhodopsin and isorhodopsin kinetics described above, we also modeled the temperature dependence of the rate of reaction of the precursor J state to the K (batho) state of bacteriorhodopsin given by Applebury et al. (1978). The fits to this data are also shown in Fig. 1. In this case there are only three points in the Arrhenius plots for bacteriorhodopsin in protic or deuterated solvent, so there is quite a bit of uncertainty in the actual parameters. Nevertheless, the best fits for bacteriorhodopsin in protic solvent and in deuterated solvent are $E_a = 114\ cm^{-1}$ and 104 cm^{-1}, $\hbar\langle\omega\rangle = 92\ cm^{-1}$ and 70 cm^{-1}, and $C' = 2.3 \times 10^{12} s^{-1} K^{1/2}$ and $1.3 \times 10^{12} s^{-1} K^{1/2}$, respectively. These values differ significantly from the values obtained for rhodopsin or isorhodopsin. This might be expected, since the low energy vibration(s) of the protein/chromophore that couple the precursor and batho states are dependent on the protein's specific structure. It is interesting that for bacteriorhodopsin the value of $\hbar\langle\omega\rangle$ decreases by about the square root of two upon deuterium exchange. This ratio is what one might expect if a direct proton translocation process is involved in the formation of K. Though the values of $\hbar\langle\omega\rangle$ are much lower than the energy of an O-H or N-H stretch, this effect of deuteration is interesting in light of the known role of bacteriorhodopsin as a proton pump.

In conclusion, we emphasize that the published kinetic data of the temperature dependence of the rate of formation of the batho state from its precursor state can be modeled with the EJ theory of radiationless decay. Within this model, the calculated mean vibrational energies fit with a mechanism in which the precursor state couples to the batho state via low energy vibrations of the protein and/or chromophore. Isomerization of the chromophore during the formation of the batho state is consistent with this. No proton translocation step is needed to explain the available kinetic data.

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