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FAST ELECTRIC RESPONSE SIGNALS IN THE BACTERIORHODOPSIN PHOTOCYCLE

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The time behavior of flash-induced charge movements during the first steps in the bacteriorhodopsin photocycle was measured on a suspension of purple membranes oriented by an electric field. The experiments were done in the temperature range 80–278 K. During the formation of the intermediate K, two negative (with respect to the direction of the proton pump) components of the response signal are well resolved with time constants $\tau_1 < 3$ μs and $\tau_2 \simeq 150$ μs at 200 K. The distances of the charge displacements responsible for the electric signals are estimated. On the basis of the results the two components are assigned to two steps in the *trans-cis* isomerization of the retinal. A third negative component appears at higher temperatures which is related by time constant measurements to the K → L transition.

Introduction

The retinal of the bacteriorhodopsin molecule from *Halobacterium halobium* undergoes a *trans-cis* isomerization after photon absorption [1,2]. It has been shown that the isomerization occurs before the formation of the L intermediate [3] or even during the bacteriorhodopsin → K transition [4,5]. In discussing models of the bacteriorhodopsin proton pump it is usually assumed that the *trans-cis* isomerization occurs during the bacteriorhodopsin → K transition (in the approx. 10–20 ps time range) [6,7].

The appearance of the fast negative (with respect to the direction of the proton pump) electric signal in response to a flash illumination has already been reported [8–11]. The signal described in Refs. 9–11 was measured as a photocurrent and as a photovoltage in Ref. 8. The rise time of the signal is very short (shorter than 1 μs in all publications) and its decay time depended on the particular measurements but was no longer than 3–5 μs. All the authors assigned the signal as a result of charge displacement in bacteriorhodopsin associated with its photocycle.

In this paper we report a detailed investigation of this negative component. The suspension method outlined in Ref. 10 was used with improved time resolution and to obtain more information data were taken over a wide temperature range (80–278 K). The results show that the negative signal can be decomposed into three well resolved components, two of them corresponding to the bacteriorhodopsin → K transition and the third to the K → L transition.

Results discussed in this paper were previously presented at the Symposium 'Aspekte Molekularer Bioenergetik', 1–2 March, 1982, in Dortmund, dedicated to Professor B. Hess on his 60th birthday.

Materials and Methods

The purple membranes used in the measurements were isolated by the usual procedure from *H. halobium* strain NRL R_1M_1 [12]. The purple membranes were suspended in 40% glycerol-water

mixtures and in water. Measurements of the photocycle with optical monitoring have already been performed on purple membranes in glycerol/water [13,14]. The glycerol was shown to slow down only some steps in the photocycle but not to change it essentially.

The protein concentration was approx. 200 μM. A simple calculation reveals that assuming a diameter of 500 nm of the purple membrane there is $1.6 \cdot 10^{-13}$ cm^3 volume in the suspension for the purple membrane to rotate during orientation while it needs only $6.5 \cdot 10^{-14}$ cm^3. The average distance of the oriented purple membranes as planes in the direction of the orienting field is approx. 700 nm while their thickness is approx. 5 nm.

Samples were light adapted with a focused 200 W Hg arc lamp filtered with water and green glass for several minutes at room temperature.

The apparatus used to make the 278 K measurements in water is the same as reported in Ref. 10 with one important change. The Keithley differential amplifier was replaced with a home-made 5 MHz differential amplifier.

At lower temperatures (80–220 K) a modified system was used to measure the displacement currents. A single cell was placed in an optical cryostat, where the temperature could be controlled down to 80 K. The reference cell was not used when using the cryostat. The sample (in glycerol/water mixture) was placed in a $1 \times 0.5 \times 0.1$ cm cuvette with two Pt electrodes placed 8 mm apart. After light adaptation, the sample was oriented with 7 V (d.c.). With the field still present, the sample was rapidly cooled to 80 K in the dark. The 300–80 K temperature transition took about 2 min. At 80 K the orienting field was removed. To check the orientation one has to measure linear dichroism of the sample. This is practically impossible due to scattering in the cooled suspension. Instead, before cooling, the orientation properties of the suspension were investigated at room temperature. In the glycerol/water mixture orientation (after switching on the orienting field) took about 20 s, and saturation could be achieved with a field of 5–7 V/cm. Due to the solvent no detectable electrophoresis of the negatively charged purple membranes occurred within 2 min. Therefore, during the process of cooling to 80 K the sample was kept in a stable oriented form. Another problem is the stability of the orientation when measurements were done at higher temperatures (approx. 200 K). This was tested indirectly: with repeated flashes we determined that the photoelectric activity, and thus the orientation in the field off case, decreased to half of its original value in about 5–10 min at 275 K. We conclude that at the much lower temperatures where the measurements were performed the orientatoin of the sample was surely constant for the 2–3 min needed for data taking.

The capacitance of the frozen suspension was about 1 pF, constant within 20% in the temperature range 80–220 K, while the resistance in the same range was always higher than 10^{10} Ω.

The electrodes were shunted with a load resistor (10–100 kΩ) and the induced voltages were amplified with the 5 MHz amplifier. Signals were digitized with an eight-bit, 10 MHz transient recorder (NEO 200-B, KFKI, Hungary) and stored in a multichannel analyzer (ICA 70, KFKI, Hungary). The data were computer fitted.

In the low-temperature measurements, the sample was excited with an Xe flashlamp (rise time of the flash = 1 μs, the flash had a roughly exponential decay with $\tau = 3.6$ μs decay time). To initiate the cycle the flash was filtered with a green-glass filter (530 nm transmission maximum and 70 nm half-width). The back-reactions were induced using a red filter in front of the flashlamp (high-pass filter with 610 nm cutoff). The photon flux in the flashes was approx. $3 \cdot 10^{15}$ photons/cm^2).

At lower temperatures, the photocycle of bacteriorhodopsin is not completed within any reasonable length of time. Below about 170 K the sample could be pumped back quantitatively to the ground state with red light because the photocycle did not yet progress appreciably to the L intermediate within the time to flash it with red light (approx. 2–3 min were needed to read out the data and change the color filters). Above 170 K the K $\rightarrow$ L transition became appreciable within 2–3 min, therefore, the sample was warmed to room temperature to complete the photocycle. Measurements at these temperatures were always performed on samples freshly oriented, quickly cooled down to 80 K and then heated up to the desired temperature.

Results

Fig. 1a shows the signal when the purple membranes are in water at 278 K. The major peak rises within approx. 1 μs, corresponding to the width of the laser pulse and decays with a 0.2–0.3 μs time constant characteristic of the electric circuit and the amplifier. The slower tail which extends beyond the baseline has a lifetime of 7.4 μs. This lifetime corresponds well with the lifetime observed by optical methods for the K → L transition (8.5 μs) (Fig. 1b). The slower part of the electric signal is much better seen in Fig. 1c with time and voltage scales different from those of Fig. 1a. The lifetime of the falling positive signal 650 μs corresponds to the lifetime of L → M transition (625 μs measured optically, but not shown). As explained in Ref. 10 the first positive signal is a mother (K → L) and daughter (L → M) signal which was assigned to charge movement associated with the L → M transition. After decomposition a small negative signal remains with the lifetime of the K → L transition. This is the electric signal associated with the K → L transition. We may conclude from these assignments that the large unresolved negative signal must be associated with the bacteriorhodopsin → K transition. (In Ref. 10 it was not possible to separate the latter two negative signals, and their sum was assigned to only the K → L transition. These results show the decomposition into the signals due to the bacteriorhodopsin → K and K → L transitions.)

To investigate further the bacteriorhodopsin → K transition, the temperature was decreased.

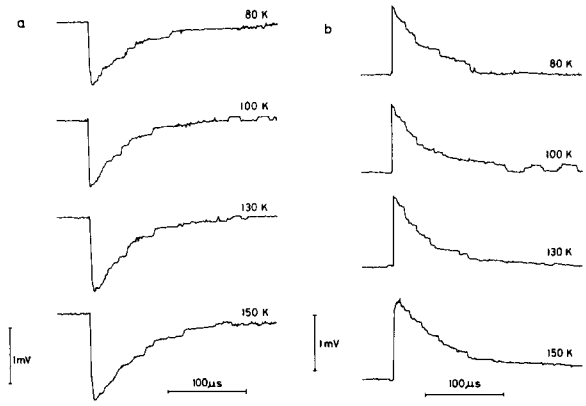

Fig. 2. Protein electric response signal of purple membranes suspended in 40% glycerol/water suspension at 80, 100, 130 and 150 K. (a) The signals initiated with green light from a 3 μs Xe flashlamp. (b) After being pumped with green light, the sample is excited with red light.

Lowering the temperature permits us to slow down the cycle and completely resolve the bacteriorhodopsin → K transition from later transitions. The results obtained with glycerol/water suspensions were checked by using water suspensions. The signals from the water samples were smaller, but qualitatively they were the same.

The electric signals induced by green and red flashes from the glycerol/water mixture in the 80–150 K temperature range are shown in Fig. 2a and b. The signals have the same amplitude and time characteristics but are negative for green and positive for red illuminations. Fig. 3a and b contains the voltage forms at temperatures between 80 and 165 K. It is seen that a new component

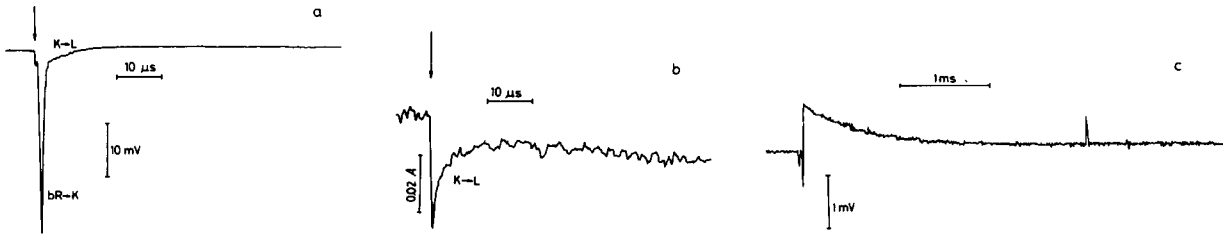

Fig. 1. (a) Protein electric response signal of purple membranes suspended in water at 278 K. The cycle was initiated with a 1 μs flash from a dye laser, λ = 580 nm. The purple membranes were oriented with 13 V, bR, bacteriorhodopsin. (b) Change of absorbance at 525 nm for the same sample. The change corresponds to the K → L transition. (c) The same as a with different time and voltage scales. The arrows indicate the exciting flash.

appears with a different lifetime. It may be restored by red illumination quantitatively at 155 and 165 K but at higher temperatures the quantitative relation is lost because the photocycle progresses further as explained in Materials and Methods. As the temperature rises the signals become faster and have higher amplitudes.

There is a definite difference between the signals appearing at low (signal I) and at higher temperatures (signal II). The dependence of the voltage form on the load resistance R is presented in Fig. 4a and b. In the case of signal I the decay time is roughly proportional to R and the amplitude remains constant. The opposite is true for signal III which is obtained by subtracting signal I from signal II point by point: its amplitude is proportional to R; the decay time, however, does not depend on R.

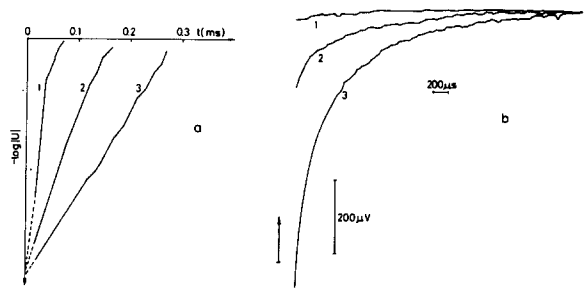

Fig. 4. The dependence of the green flash-induced protein electric response signal on the load resistance. (a) The log of the amplitude of the protein electric response signal at 80 K with (1) $R = 10$ kΩ, (2) $R = 50$ kΩ and (3) $R = 100$ kΩ. (b) The protein electric response signal at 165 K; (1) $R = 10$ kΩ, (2) $R = 50$ kΩ and (3) $R = 100$ kΩ. Only the slow part of signal II is shown. The arrow indicates the time of the exciting flash.

Some of the relevant data from computer evaluation are collected in Table I. The calculated capacitance ($C = \tau_I/R$) is apparently higher at $R = 10$ kΩ. This apparent inconsistency is caused by the fact that the time constant of the measuring circuit and the decay time of the flash are of the same order. At that R value the convolution of $\tau_R = 4.8$ μs calculated with $C = 480$ pF and $\tau_F = 3.6$ μs, the lifetime of the flashlamp results in $\tau_I = 6$ μs.

The normalized amplitude of signal III at different temperatures is shown in Fig. 5. The decay curves have a different character below 190 K (strongly nonexponential) and above 190 K are exponential to a good approximation. In the limited range where the process is exponential and before further transitions distort the signals (190–220 K), the rates can be fitted to an Arrhenius relation to give an activation enthalpy, ΔH, of 35 ± 5 kJ/mol and a preexponential factor of $A = 7 \cdot 10^{12}$ s^{-1} $\pm$ 10%. The same parameters for the K $\rightarrow$ L transition, as reported in Ref. 14, are $\Delta H = 52$ kJ/mol and $A = 6.5 \cdot 10^{14}$ s^{-1} (bacteriorhodopsins in a similar glycerol/water mixture). These very different parameters serve as additional arguments that the observed electric

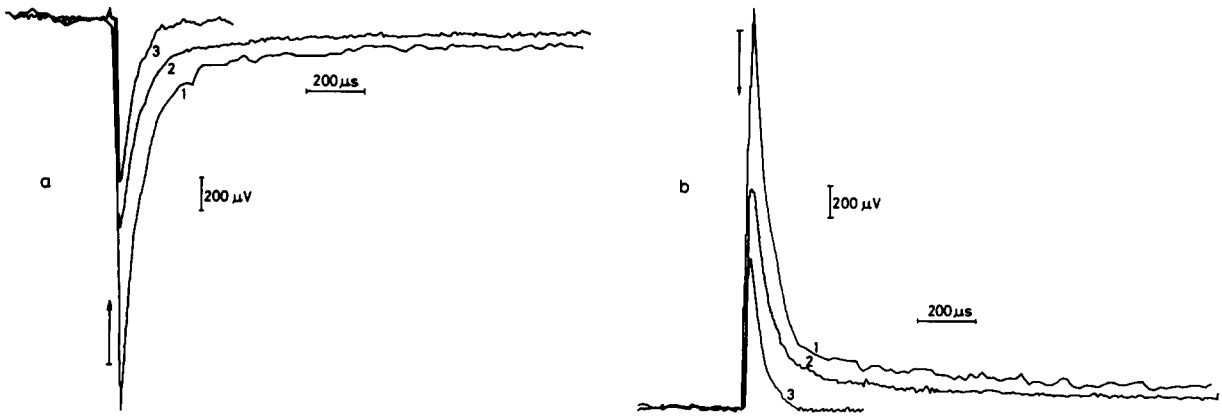

Fig. 3. Protein electric response signal of purple membranes suspended in 40% glycerol/water at 80 (curve 3), 155 (curve 2) and 165 K (curve 1). (a) The sample was in the bacteriorhodopsin state and excited with green light from a 3 μs flashlamp. (b) After being pumped with green light, the sample is excited with red light from a 3 μs flashlamp. $R = 100$ kΩ.

TABLE I

COMPUTER FITS OF THE PROTEIN ELECTRIC RESPONSE SIGNAL FOR THE BACTERIORHODOPSIN → K TRANSITION AND RELATIVE DISTANCES FOR THE CHARGE DISPLACEMENT

Distances were calculated by taking into account that the flashlamp excites the sample to 50% saturation, and in saturation 30% of the bacteriorhodopsin molecules are excited.

Temperature (K) (±3 K)	R (kΩ) (±5%)	C (pF) (±12%)	$Ampl_1$ (mV) (±10%)	$Ampl_2$ (mV) (±10%)	τ_1 (μs) ±10%)	τ_2 (ms) (±10%)	Fd_1 (nm) (±20%)	Fd_2 (nm) (±20%)
Green excitation								
80	10	670	1.3		6.7		0.06	
80	50	480	1.4		24.0		0.04	
80	100	430	1.5		43.0		0.04	
100	100	490	1.4		49.0		0.04	
130	100	470	1.5		47.0		0.04	
150	10	670	2.0		6.7		0.08	
150	50	490	1.4		28.0		0.05	
150	100	480	1.7		48.0		0.05	
190	100	500	1.5	0.91	50.0	0.40	0.05	0.22
200	10	670	1.6	0.27	6.7	0.15	0.04	0.25
220	10	670	1.6	2.4	6.7	0.018	0.04	0.27
Red excitation								
80	10	710	1.3		7.1		0.06	
80	100	500	1.4		50.0		0.04	
100	100	470	1.4		47.0		0.04	
130	100	490	1.4		49.0		0.04	
150	100	490	1.5		49.0		0.05	

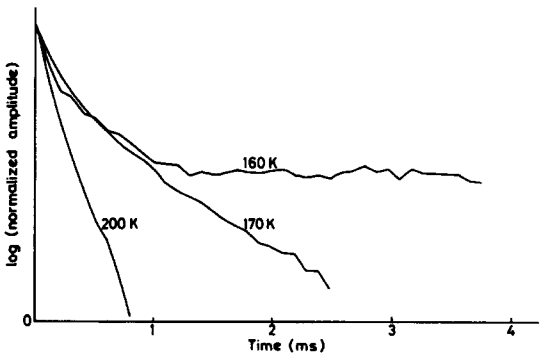

Fig. 5. The log of the amplitude of the protein electric response signal for the second process observed in the bacteriorhodopsin → K transition at 160, 170 and 200 K. The amplitudes were normalized for easier comparison of the rates. At 160 and 170 K the decays are nonexponential. At 200 K the decay has relaxed to a single exponential.

signals belong to processes before the decay of the K intermediate.

The slower process represented by signal III is not exponential in time below 190 K (Fig. 5). It also cannot be fitted with two exponentials. The nonexponential behavior can be explained by the frozen protein structure. Protein molecules have been shown to exist in nonidentical configurations [15], and the different configurations are believed to freeze in at low temperature. Therefore, even a single process will take place in a nonexponential way due to differences in the frozen conformational substates [16]. Instead of a single activation enthalpy, a distribution of barriers determines the kinetics. At higher temperatures, where the protein is free to fluctuate, an 'averaged barrier' or equivalently, a single exponential is seen. This change of behavior happens in our case around 190 K. Using optical absorption spectroscopy, we have also found that the K → L transition is nonexponential in the same temperature range, only the rates are much slower (unpublished observations).

Discussion

Before discussing the results, we review and extend the interpretation of a protein electric response signal [10]. Let us select a single oriented bacteriorhodopsin molecule and assume that electrodes are in contact with the protein which is considered as a homogeneous isolator. An absorbed photon acts by displacing a charge Q from point 1 to point 2 inside the protein. According to the Ramo-Shockley theorem of electrodynamics [17], a current i is induced in the external circuit switched to the electrodes:

$$i = \frac{Qv}{\varepsilon D'} \tag{1}$$

where v is the velocity of the charge movement, D' the distance of the electrodes, in this case, the thickness of the purple membrane, and ε the dielectric constant. The individual charge translocations are very fast, the jumping time being of the order of 1 ps [18]. Integrating Eqn. 1 with respect to time:

$$Q_{\text{ind}} = \int_0^\infty i\,\mathrm{d}t = \frac{Q}{\varepsilon D'}\int_0^\infty v\,\mathrm{d}t = \frac{Qd}{\varepsilon D'} \tag{2}$$

The jumping charge induces a charge Q_{ind} proportional to the distance d between points 1 and 2.

In the real sample the bacteriorhodopsin molecule in the oriented purple membrane is in the frozen medium of high resistance. We consider it a homogeneous dielectric with electrodes at a distance D. The induced charge now appears on these electrodes which are loaded by a capacitance C (stray capacitances of wires and input capacity of amplifier) and the resistance R. This way D' in Eqn. 2 may be replaced by D and the voltage form induced by a single charge jump is:

$$V_1(t) = \frac{Qd}{\varepsilon DC} e^{-t/RC} \tag{3}$$

where ε is the dielectric constant of the protein.

The photons in a real experiment produce N excited states at $t = 0$. In a simple exponential decay the number of states decaying in unit time is:

$$\rho(t) = \frac{1}{\tau} N e^{-t/\tau} \tag{4}$$

where τ is the lifetime. Every induced-charge displacement produces a voltage as given in Eqn. 3. To obtain $V_N(t)$ we have to sum the $V_1(t)$ functions for all times $t' < t$. In calculation this means the folding of Eqns. 3 and 4:

$$V_N(t) = \frac{NQd}{\varepsilon CD}\frac{1}{\tau}\int_0^t e^{-t'/\tau} e^{-(t-t')/RC}\,\mathrm{d}t'$$

$$= \frac{NQd}{\varepsilon D}\frac{R}{\tau - RC}\left(e^{-t/\tau} - e^{-t/RC}\right) \tag{5}$$

ε has in general a temperature and position dependence inside the protein. The temperature dependence is not problematic if we relate the data at a given temperature. The position dependence is not known – the best we can do is to assume that ε is constant inside the protein. We must bear in mind, however, that the final data carry the problems involved in this assumption. Eqn. 5 is valid if all the bacteriorhodopsins are oriented completely. The degree of orientation, the dielectric constant and the deviations from ideal homogeneous dielectric properties of the suspension may be condensed in a factor F. Then:

$$V_N(t) = \frac{NQFd}{D}\frac{R}{\tau - RC}\left(e^{-t/\tau} - e^{-t/RC}\right) \tag{6}$$

A general description of the ideas involved in the above derivation – not restricted to the suspension – may be found in Ref. 18.

The two limiting cases of Eqn. 7 are:

$$V_N(t) = \frac{NQFd}{D}\frac{1}{C}e^{-t/RC}, \quad \text{if } \tau \ll RC \tag{7}$$

$$V_N(t) = \frac{NQFdR}{\tau D}e^{-t/\tau}, \quad \text{if } \tau \gg RC \tag{8}$$

A charge-displacement process with a much shorter lifetime than the circuit lifetime, RC, should induce an electric signal with a fast rise and an exponential decay having a lifetime RC. The amplitude is independent of R. Processes with much longer lifetimes than the circuit lifetime should induce a signal decaying with the lifetime of the process τ and an amplitude proportional to R and $1/\tau$. One can state that in the case of Eqn. 7 the photovoltage and in that of Eqn. 8 the photocurrent are measured.

Signals I and III satisfy the criteria of Eqns. 7

and 8, respectively.

The distances of the charge displacements (multiplied by the factor F and with the essential assumption that one positive elementary charge is moving) were calculated. These results are also listed in Table I.

The distances given in Table I are in a ratio of $d_1/d_2 \simeq 0.2$ (assuming F is constant). The absolute value of the displacement can be estimated from measurements where all the distances representing charge movement appear and a normalization to the membrane thickness is possible. This has been done in Ref. 10. The basic assumption is that a single proton moves in every step of the photocycle. From the data reported in this paper the distance involved in the L → M transition is $d_4 =$ 0.5 nm. The negative signals are caused by a charge displacement of -0.15 nm. According to the present data the large unresolved negative component in Fig. 1a corresponds to $d_1 + d_2 = -0.13$ nm. (The lifetime of the second process – calculated from the Arrhenius parameter – is approx. 0.7 μs at 278 K in the glycerol/water mixture. It is generally smaller in water and cannot be resolved with our measuring system.) From the small negative component related to the K → L transition, $d_3 = -0.02$ nm is calculated.

From the simple geometry of the *trans-cis* isomerization of the retinal in purple membranes a displacement of the protonated Schiff base of 0.16 nm is calculated in the direction of the membrane normal (the direction of charge movement measured with the protein electric response signal). The good agreement of the measured and the calculated distances of charge movement during the bacteriorhodopsin → K transition corraborates the model outlined in Refs. 6 and 7 which assume that the photon energy is transformed into electrostatic energy by displacing the protonated Schiff base from a negative charge being near to the Schiff base. The displacement and its stabilization are the result of the photoinduced *trans-cis* isomerization of the retinal.

Our data indicate two steps in the charge movement connected with the isomerization: The first step is small, $d_1 \simeq 0.02$ nm, and fast ($\tau_1 < 3$ μs in these experiments and $\tau_1 < 1$ ns from our separate unpublished observation using picosecond laser excitation), the second step is large, $d_2 \simeq 0.11$ nm, and its lifetime is thermally controlled.

Two independent optical investigations indicate similar events. The flash photolysis experiments of Kuschmitz and Hess [5] reveal the fast ($\tau < 200$ ns) appearance of negative absorption peaks at 280, 400 and 430 nm and positive absorption changes from 290 to 375 nm which deviate from the difference spectrum between 13-*cis*- and all-*trans*-retinal of bacteriorhodopsin. They consider the results as indicative of the appearance of a twisted 13-*cis* isomer. The absorption peak at 296 nm has a component with a half-time of 0.5–0.9 μs, faster than the K → L transition. They claim that this is caused by a proton displacement from the neighborhood of a tryptophan residue. The lifetime of this step agrees well with the lifetime of the second step in the charge movement estimated from the Arrhenius parameters $\tau_2 \simeq 0.5$ μs (water solution and 274 K).

From laser Raman data, Braiman and Mathies [19] concluded that the primary photochemical event in the proton pumping cycle of bacteriorhodopsin is a photoisomerization about the $C_{13} = C_{14}$ bond, which produces a chromophore with a twisted, nonplanar conformation. This state, which is the only one existing at 80 K, relaxes showing different lines at $t = 50$ ns after excitation at room temperature (Braiman and Mathies, personal communication). Similar changes were reported in the fingerprint region on a time scale of approx. 100 ns at room temperature [4].

It seems safe to accept the existence of an intermediate K′ on the basis of the ultraviolet, Raman and our photoelectric observations before the K intermediate. The sequence of the intermediates before L could be the following – using the results of ultrafast photolysis experiment of Applebury et al. [20] and the notation in Ref. 21:

$$\text{bacteriorhodopsin} \xrightarrow{h\nu} \text{I} \xrightarrow{1\text{ ps}} \text{J} \xrightarrow{11\text{ ps}} \text{K}' \xrightarrow{200\text{ ns}} \text{K} \xrightarrow{3\,\mu\text{s}} \text{L}$$

The lifetime values are approximate for room temperature.

We summarize the data and interpretations related to formation of K′ and K intermediates from Raman spectroscopy (Refs. 4 and 24; and Braiman and Mathies, personal communication), ultra-

violet spectroscopy [5] and the present study (protein electric response signal).

K′: (Raman) twisted 13-*cis* isomer, the only appearing form at 80 K after excitation; (ultraviolet) neither all-*trans* nor 13-*cis*, is faster than 200 ns at 274 K; (protein electric response signal) a small charge displacement involved in its formation of $d_1 \simeq 0.02$ nm against the proton pump direction, faster than 1 ns at room temperature.

K: (Raman) relaxed form of K′ within approx. 100 ns at room temperature; (ultraviolet) during its formation the retinal bends into the 13-*cis* isomer, a large charge shift occurs in the neighborhood of a tryptophan residue, and the lifetime of the transition is approx. 0.5–0.9 μs at 274 K; (protein electric response signal) a large charge displacement $d_2 \simeq 0.11$ nm occurs, and the lifetime is estimated to be 0.5 μs at 274 K from Arrhenius parameters.

The theoretical model of Schulten [22] has relevance to these results. He postulates two steps in the *trans-cis* isomerization: in the fast part a proton moves to the $C_{13} = C_{14}$ bond and the large movement involved in isomerization occurs more slowly due to steric hindrances. The proton near to the $C_{13} = C_{14}$ bond lowers the barrier, i.e., the steric hindrance to isomerization. The quantum chemical calculation [23] yields an activation enthalpy of 60 kJ/mol for the K′ → K transition, i.e., for isomerization. Our experimental value is 35 ± 5 kJ/mol, determined for bacteriorhodopsins in a glycerol/water mixture. Considering the problems involved in calculations of numerical values and that the Arrhenius parameters may depend on viscosity [14], a deviation can be understood.

This model surely requires that the K′ intermediate is not yet the *cis* isomer, not even its twisted form. There are, however, important consequences of both models which are probably measurable. In Schulten's model the Schiff base is unprotonated in K′ and (probably) becomes again protonated in the K intermediate. The data obtained until now (Ref. 24; and Braiman and Mathies, personal communication) are in favor of the protonated Schiff base in K′.

The second process (K′ → K) is a thermally agitated transition according to all experiments. Therefore, the photon energy should have already been transduced into another form of energy in the formation of K′. If the energy is immediately transduced into electrostatic energy, then a (probably) proton should be displaced by 0.2–0.3 nm [6]. Its component in the transmembrane direction is, however, only 0.02 nm. The energy should be transformed into some other form. In the Schulten model the energy is confined to the lateral charge displacement, while in the twisted 13-*cis* model for K′ a substantial part of the photon energy should be first transduced into mechanical energy which 'relaxes' during the K′ → K transition into electrostatic energy. We consider the question of energy transduction and storage to be very important, therefore, we plan to check the lateral charge displacement.

It is important to consider the back-reaction from K. If the back-reaction is initiated with red light the sequence is the same as that of the forward reaction: a fast process followed by a slow process. The slow process has the same rate in both directions. Therefore, the slower reaction is initiated by the fast reaction, both being completely reversible. Any model must take this into consideration.

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