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DISPLACEMENT CURRENT ON PURPLE MEMBRANE FRAGMENTS ORIENTED IN A SUSPENSION

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The displacement current is measured in a suspension of electric field-oriented purple membranes isolated from *Halobacterium halobium*, the photocycle being driven by a light flash. A simple quantitative theory of the method is presented and used to evaluate the distances the protons move during their way through the bacteriorhodopsin molecules. A lower limit of the velocity of proton movement is also given.

1. Introduction

It is well known that electricity plays an important role in living systems: cells generally sustain a potential difference on their plasma membranes. The membrane potential, its change and its transmission from neuron to neuron are decisive in the nervous system. Studies in the last 20 years have revealed that, in the process of the transduction of the energy of light and food into that of ATP, the membrane potential is an intermediate energy reservoir [1].

The cells and subcellular units (such as mitochondria and chloroplasts) contain special proteins built into their membranes, the function of which is to pump different ions across the membrane to produce an asymmetric ion distribution. Considerable knowledge has accumulated concerning these pumps, their structure and their manner of functioning. The results relate more or less to the equilibrium states, i.e., the initial and final states of the pumps, and not the molecular dynamics involved.

In molecular pumps two different kinds of charge movement may occur: (1) movement of the pumped charge (or charges if more than one charged particle is pumped in one act); (2) movement of charged parts (side chains) or electric dipoles of the pump molecule. While process 1 should naturally appear in every pump process, process 2 may be unobservably small. Clear observation of process 2 in pumps has not yet been reported, but the displacement current observed in the case of Na^+-K^+ channels opened by an electric field [2] is generally attributed to moving charged parts of the channel-forming molecules.

The moving charges or dipoles produce a current through the membranes. For the time behaviour of these currents to be observed, two requirements have to be fulfilled: (i) the pumps or channels should be synchronized by starting them simultaneously in as short a time as possible; (ii) the system must be asymmetric with respect to the current-measuring electrodes. The first requirement has been fulfilled until now by exciting the systems with pulses of electric field, as in the case of neurons, and with short light pulses. The electric field excitation needs electrodes located in or on the surface of the cells. With electrodes (one in the cell, and one outside) the second requirement

is automatically fulfilled, but this is possible only for large cells. We deal in the following with small units not suitable for electrode insertion. In this case three different methods are known for the production of asymmetry:

(a) Light gradient method [3–5]. Closed cells or vesicles containing light-absorbing pigments uniformly distributed on their surface absorb slightly less light at the side more distant from the light source than at the side nearer to the source, because of the absorption in the near side. The pump currents flowing at the two sides are therefore not completely compensated. Electrodes positioned in the direction of the exciting light in the suspension of the vesicles pick up the current.

(b) Membrane-bound systems [6–9]. Closed vesicles and membrane fragments can be adjoined to artificial membranes or layered between water/oil interfacial layers. The electrodes are in the two compartments separated by the membrane of high resistance and capacity. The displacement current caused by the moving charges is coupled capacitatively through the membrane and conductively through the bathing solution to the electrodes. Asymmetry in the case of adhered vesicles is a consequence of the advantageous sensing of charge movement from the adjoining surface or of the oriented attachment in the case of membrane fragments. This method is discussed in detail in ref. 10.

(c) Membrane fragments oriented in suspension [11]. Purple membranes from *Halobacterium halobium* were oriented in suspension by a quasistatic electric field. The oriented system could be used to observe the current due to charge displacement during the photocycle. The displacement current was attributed to protons pumped after laser light pulses [12,13]. This system is very advantageous because the quantity of the working unit is so large that simultaneous electrical and optical measurements can be performed: the events in the proton pumping can be correlated with the photocycle.

The suspension method is probably applicable to membrane fragments of other types. As pointed out [12], even quantitative data on charge displacements can be obtained from such investigations.

It was felt that the novelty of the suspension method and the unusual approach to the explanation of the data (which has only been sketched in our previous work [12]) need further development of the theoretical background.

In this paper we first review the experiments for convenience, and then develop the details of the theoretical background of the method and discuss what type of information can be gained from such investigations.

2. Description of the suspension method

Fragments of biological membranes generally are a prior asymmetric: their internal and external sides are different. The molecules composing the membranes (lipids and proteins) carry charges and one can expect an asymmetric charge distribution at the two sides, i.e., a permanent electric dipole moment perpendicular to the plane of the membrane fragment. The effect of an electric field of sufficient duration on the solution will be to orient the membrane sheets. This is a true orientation: the corresponding sides of all the fragments face in one direction. Cells and closed vesicles are not suitable for true orientation.

The membranes may also be charged, and therefore they would move to one of the electrodes by electrophoresis. To avoid this, the duration of the orientation has to be limited and positive and negative voltages are applied in succession [11].

The purple membranes of *H. halobium* were oriented by a field of approx. 10–20 V/cm to saturation. It is well known from the review of Stoeckenius et al. [14] that the bacteriorhodopsin (bR) embedded in purple membranes pumps protons during their light-drived photocycle. The protons should therefore move through the molecule, causing a displacement current, and they also spend some time in the suspension after release and before uptake. In the following we use the expressions moving protons inside bR molecules, and released protons in the suspension. The discussion, however, is valid for other pumped charges and the movement of charged parts or dipoles of molecules.

The laser flash starts the proton-pumping activity of the bR molecules and the protons move in

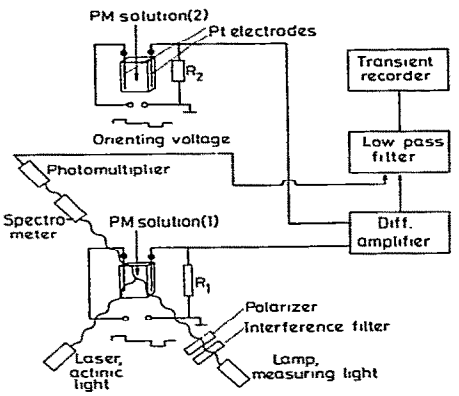

Fig. 1. Scheme of the measuring system. Two identical purple membrane (PM) suspension + R circuits are used to compensate the voltage on R due to the orienting current. The compensation is in the differential amplifier. The polarizer helps to check the orientation of the purple membranes.

one direction (to the negative electrodes). Fig. 1 schematically shows the apparatus. The laser pulse may be timed at any time during the orienting electric field or after it, when a substantial proportion of the orientation is still preserved. In the first case a change in conductivity due to transiently released protons appears, in addition to the current of the moving protons. We omit discussion of the current due to conductivity changes. A time-dependent voltage $V(t) = I(t) \times R$ is measured on the resistance R.

In a suspension, a large number of purple membranes take part in the photocycle; it is therefore easy to measure the absorption kinetics in addition to the registration of $V(t)$. Fig. 2 shows a set of data [12] indicating that the rate constants of the voltage changes coincide with the rate constants of the photocycle determined from absorption kinet-

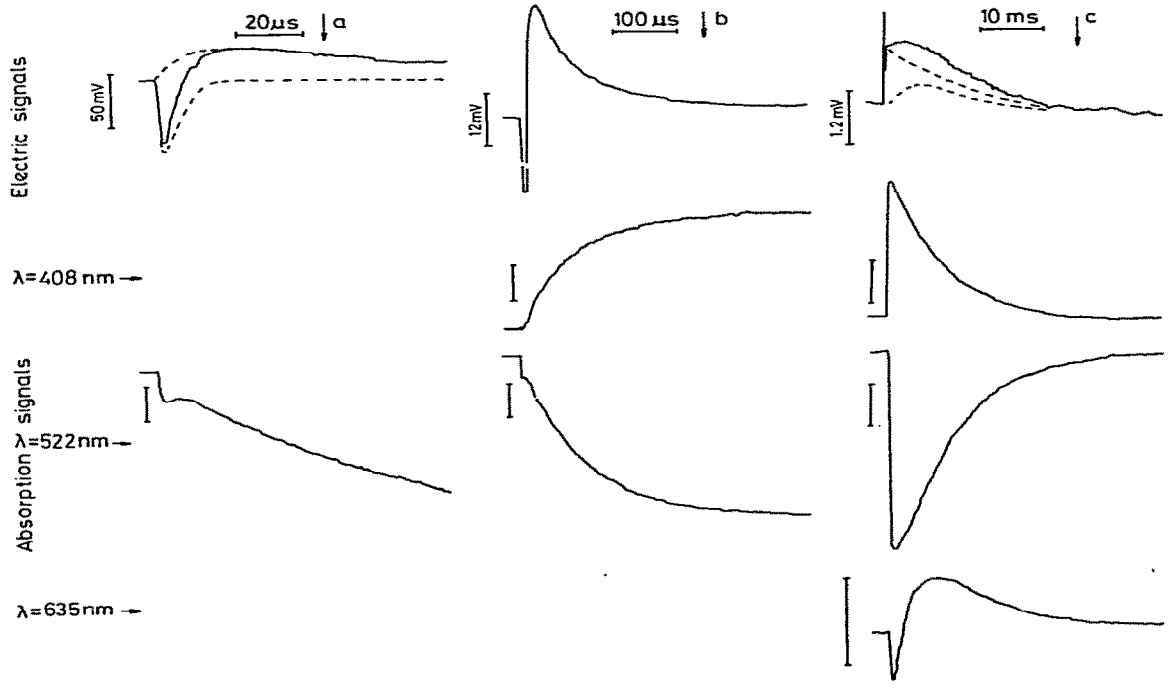

Fig. 2. Comparison of electric and light absorption signals (λ = 408, 522 and 635 nm). (a–c) Signals measured with different time resolutions. The electric signals were measured when the orienting field was switched off, but orientation was still present. Therefore, the signals do not contain the contribution of conductivity change. Dashed lines in a and c denote the decomposition of the electric signals. Purple membranes in H_2O suspension, $A = 1.8$, $T = 20°C$, orienting voltage 8.5 V. The size of bars in absorption signals represents $\Delta I/I = 0.1$; positive signals indicate $\Delta I < 0$.

ics [15]. After more thorough evaluation of the rate constants in cases of purple membranes in H_2O and ^2H_2O solution [12], from the temperature and pH dependences ($4 < pH < 7$) (unpublished data) it was concluded that the rate constants of the four components of the electric signal are the same as those for the transitions of the photocycle. This was the basis for the assignment of the components of the electric signal to the transitions in the bR photocycle.

3. Explanation of the electric signal measured in the suspension

Let us select a single oriented purple membrane and assume that large planar electrodes are in contact with it (fig. 3). The medium is considered as a homogeneous isolator. An absorbed photon acts by pushing a proton from point 1 to point 2. We use the assumption introduced previously that a single proton moves. No real change would be needed if other charge(s) or dipole(s) were to move, because the charge Q can be specified later. According to the Ramo-Shockley theorem of electrodynamics [16], a current i is induced in the external circuit:

$$i(t) = \frac{Qv(t)}{D'} \qquad (1)$$

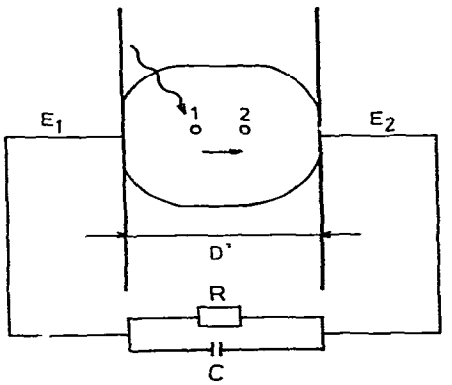

Fig. 3. Assumed elementary act in the measurement of displacement current. bR protein embedded in membrane; charge moves from point 1 to 2; E_1 and E_2 hypothetical electrodes.

where Q and v are the charge and velocity of protons, respectively, and D' the distance between the electrodes, in this case the membrane thickness. We assume that $v(t)$ is very large, i.e., the protons jump from point 1 to point 2. (We shall return to this later.) Integrating eq. 1 with respect to time:

$$Q_{ind} = \int_0^\infty i(t)dt = \frac{Q}{D'}\int_0^\infty v(t)dt = \frac{Qd}{D'}. \qquad (2)$$

The proton jump induced a charge (Q_{ind}) proportional to the displacement d. The same expression is derived without using the Ramo-Shockley theorem [10]. Q_{ind} charges the capacitance C of the electrodes and of the external circuit. The capacitance discharges through a resistance R. The voltage for one charge displacement is

$$V_1(t) = \frac{Q_{ind}}{C} e^{-t/RC}, \qquad (3)$$

if the inductance of the circuit is negligible.

In the real case the electrodes are immersed in the suspension in a distance D and N_0 charges are moving with a certain time distribution. The calculation involves two processes: (i) to determine $V_1(t)$ for electrodes in a distance D and (ii) to sum up of the $V_1(t)$ functions for N_0 particles. In the following two treatments will be elaborated for process i.

(a) We assume that the conductivity of the suspension is very small. This approximation means that the hypothetical electrodes in fig. 3 are

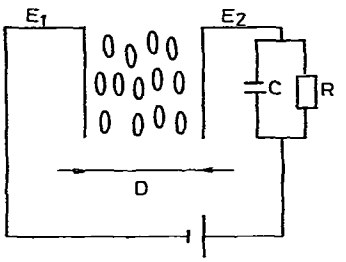

Fig. 4. The displacement currents caused by the elementary acts in the bR proteins in purple membranes (small ellipses) are summed by electrodes E_1 and E_2 at a distance D on resistance R. C is the capacitance of the measuring system (i.e., capacitances of electrodes, wirings, input capacitance of the amplifier, etc.). Typical value of $C = 100$ pF.

now removed from the surface of the purple membrane to a distance D. (We consider – as an approximation – the macroscopic electrodes infinitely large compared to purple membranes (fig. 4). The error involved in this approximation will be discussed later.) In this case D' can simply be replaced by the distance of the immersed electrodes D in eq. 2. Because the thickness of a purple membrane $D' \simeq 5$ nm and $D \simeq 1$ cm this means an approx. 10^6-fold decrease in Q_{ind}.

Charge is induced in the external circuit only when a transition from state 1 to state 2 occurs. We assume a simple exponential decay of the N_0 states excited at $t = 0$ then the number of states decaying in unit time is:

$$\rho(t) = kN_0 e^{-kt}. \tag{4}$$

where k is the rate constant. Every induced charge displacement produces a voltage as given in eq. 3. To obtain $V_{N_0}(t)$ we have to sum the N_0 uncorrelated $V_1(t)$ functions for all times $t' < t$ (fig. 5). In calculation this means the folding of eqs. 3 and 4:

$$V_{N_0}(t) = \frac{N_0 Q dk}{DC} \int_0^t e^{-kt'} \cdot e^{-(t-t')/RC} dt'$$

$$= \frac{N_0 Q dk}{D} \cdot \frac{R}{1 - kRC} (e^{-kt} - e^{-t/RC}). \tag{5}$$

If $k \ll 1/RC$, then:

$$V_{N_0}(t) = \frac{N_0 Q d}{D} \cdot Rke^{-kt}. \tag{6}$$

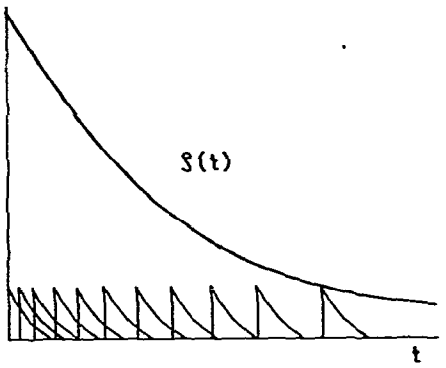

Fig. 5. Every induced charge from a decaying state produces a voltage $V_1(t)$ (eq. 3). The time density of incidence is given by $\rho(t)$ (eq. 4). $V_{N_0}(t)$ at a given t value is obtained by summing all the $V_1(t)$ values from decays at times $t' < t$.

If there are many components of charge movement in series, then we have to taken into account that the expression for the number of decaying states (eq. 4) has several exponential components with different preexponential factors. These functions were first calculated by Bateman for the radioactive decay series [17] and were recently given, specialized for the case of the bR photocycle [9,10]. Denoting this function by $f(k_1, \ldots, k_i, t)$, we obtain a simple expression for the i-th transition:

$$V_{N_0}^{(i)}(t) = \frac{N_0 QR}{D} d_i k_i \; f(k_1, \ldots, k_i, t). \tag{7}$$

This is the equation used for data evaluation [12].

The equation needs two modifications. We have to take into account that the charges in the proteins move in a dielectric medium of permittivity ϵ_i which may depend on the position inside the protein. Further, factors A and B are introduced, expressing the degree of orientation and the error involved in the approximation that the electrodes are infinitely large, respectively. The modified equation is:

$$V_{N_0}^{(i)}(t) = \frac{N_0 QRAB}{D} \cdot \frac{d_i k_i}{\epsilon_i} \; f(k_1, k_2, \ldots, k_i, t) \tag{8}$$

The model correctly expresses the time behaviour of the voltage form, and gives the right order of magnitude for the sum of the successive displacements for an assumed proton movement in the bR molecule. (The introduced factors A and B are expected to be near unity (see p. 403 and 404), and the order of magnitude estimation has been obtained with values $A = 1$ and $B = 1$). Nevertheless, because of the crudeness of the basic assumption (the negligible conductivity of the suspension), another model has been elaborated which takes into account the conductivity of the solution and pays more attention to the electronic circuitry.

(b) The purple membranes are suspended in a conductive electrolyte medium. To understand the behaviour of the system we shall consider a single purple membrane under this condition. The actual electric circuit is shown in fig. 6a, and the equivalent circuit in fig. 6b.

The membrane is shunted by R_{pm}, the resistance of the surrounding electrolyte, and is con-

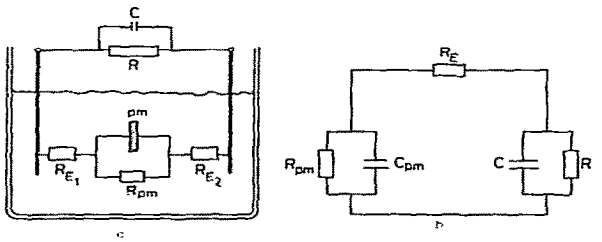

Fig. 6. The actual (a) and equivalent (b) electric circuit. E_1 and E_2, electrodes: R_{E_1}, R_{E_2} and R_{pm}, resistances of the electrolyte: R, measuring resistance: C_{pm}, capacitance of purple membrane; C, the measuring capacitance.

nected to the electrodes by R_{E_1} and R_{E_2}. These resistances are proportional to the distances in question. As the thickness D' of the purple membrane is much smaller than the distance of the electrodes, D, the resistance of the suspension is $R_{E_1} + R_{E_2} = R_E$. The measured value of $R_E \approx 2 \times 10^5\ \Omega$ in case of distilled water suspension falls to $R_E = 1\text{–}2 \times 10^4\ \Omega$ if the pH is set by buffers. The order of magnitude of the ratios of $R_{pm}/R_E = D'/D = 10^{-6}$, therefore $R_{pm} = 10^{-1}\text{–}10^{-2}\ \Omega$.

The charges on the purple membrane discharge through resistances R_{pm} and $R_E + R$, connected in parallel. The working unit is therefore an RC circuit consisting of the purple membrane with a capacitance C_{pm} and the shunt resistance R_{pm}. C_{pm} for one purple membrane with an average diameter of 0.5 nm is approx. 2×10^{-15} F (assuming a 1 μF capacitance for 1 cm^2 usual for biological membranes [18]). The purple membranes can be considered as uncorrelated units because the volume for one purple membrane even in the most dense suspension we used ($\approx 280\ \mu$M concentration of bR [12]) is around twice the volume needed for a purple membrane of 0.5 nm diameter to rotate freely. Expressing it in another way, the average distance between ordered discs in their plane is approx. 140 nm.

It is easy to determine the function $V_1(t)$ for the equivalent circuit when a charge Q_{ind} (eq. 2) appears on C_{pm} at $t = 0$. The result of a simple calculation (see the appendix) is

$$V_1(t) = \frac{Qd}{CD'} \frac{R_{pm}}{R_E} (e^{-t/R'C} - e^{-t/R_{pm}C_{pm}}), \tag{9}$$

$$R' = R \frac{R_E}{R + R_E}.$$

It may be seen that $V_1(t) = 0$ at $t = 0$ and has a maximum at $T \approx 2R_{pm}C_{pm}$. $R_{pm}C_{pm}$ is much smaller than $R'C$ and therefore the second term is negligible and $T = 0$. Folding eqs. 4 and 9 yields

$$V_{N_0}(t) = \frac{N_0 QD}{D'} \cdot \frac{R_{pm}}{R_E} \frac{kR'}{1 - kR'} (e^{-kt} - e^{-t/R'C}) \tag{10}$$

Eq. 10 is essentially the same as eq. 5 because in first approximation $R_{pm}/R_E = D'/D$ and $R' \approx R$ if $R_E \gg R$, which means that the realistic treatment leads to the conclusion that the simple assumption is a good model of the system. (The usual value of R is approx. 10–50 kΩ.)

It is difficult to calculate R_{pm}/R_E exactly; it is surely greater than D'/D. Therefore, only an order of magnitude value of Σd_i is expected from eqs. 5 and 8. This is what has been found by us earlier [12]. The method of obtaining d_i values seems to be to combine the dielectric constant ϵ_i, the factor A expressing the degree of orientation and the deviation of R_{pm}/R_E from D'/D in one factor, F_i which can be determined by normalizing Σd_i to D'. With the approximation $k_i \ll 1/R'C$ the final equation is

$$V_{N_0}^{(i)}(t) = \frac{N_0 Q d_i R'}{D} F_i k_i f(k_1, k_2, \ldots, k_i, t). \tag{11}$$

4. The velocity of the moving charge

In deriving eq. 2 it was assumed that the velocity $v(t)$ of the moving charge is very large. In this section we investigate the necessary modifications of eq. 11 for the final value of $v(t)$.

Let us write the velocity of the moving charge as

$$v(t) = d\lambda e^{-\lambda t} \tag{12}$$

where $1/\lambda$ is the mean time for the charge to move a distance d. The function $V_1(t)$ can now be

calculated by integration:

$$V_1(t) = \frac{Q}{C}\frac{d}{D} F\lambda \int_0^t e^{-\lambda t'} e^{-(t-t')/R'C} dt$$

$$= \frac{Qd}{D} F\lambda \frac{R'}{1-\lambda R'C}(e^{-\lambda t} - e^{t/R'C}). \quad (13)$$

Folding eq. 13 with eq. 4, we obtain

$$V_{N_0}(t) = \frac{N_0 Qd}{D} F\lambda \frac{R'k}{1-\lambda R'C}\left[\frac{1}{\lambda - k}(e^{-kt} - e^{-\lambda t}) - \frac{R'C}{1-kR'C}(e^{-kt} - e^{-t/R'C})\right]. \quad (14)$$

Eq. 14 reproduces eq. 10 if $\lambda \gg k$ and $1/R'C$. We are more interested in the changes in $V_{N_0}(t)$ if $\lambda = k$. It is easy to investigate $V_{N_0}(t)$ when λ, $k \ll 1/R'C$:

$$V_{N_0}(t) = \frac{N_0 Qd}{D} FR' \frac{\lambda k}{\lambda - k}(e^{-kt} - e^{-\lambda t}). \quad (15)$$

The form of eq. 15 when $\lambda = k$ is

$$V_{N_0}(t) = \frac{N_0 Qd}{D} FR'k^2 te^{-kt} \quad (16)$$

This has a maximum at $T = 1/k$. The rate constant k of a transition is known from light absorption measurements. If the measured function $V_{N_0}(t)$ is of the form given in eq. 15 or 16 then λ can be determined. From the analysis of our measurements [12], $\lambda > 10k$ was obtained for the transition $M \rightarrow 0$. It is estimated that by careful measurement and computer evaluation a limit of $\lambda > 100k$ can be obtained.

5. Applications

Eq. 7 has been applied to calculate the distance the protons move during their path through the bR molecules after light excitation [12]. The basic assumption is that the protein electric response signal (PERS) results from proton movement. As can be seen from table 1, the value $\Sigma d_i = 10$ nm has been found. The crude assumptions – i.e., the medium is a homogeneous isolator, the electrodes considered to be infinitely large – worked surprisingly well because Σd_i was only twice as large as the accepted membrane thickness (5 nm [14]). In our earlier paper [12], we stated it to be very probable that the electric signals correspond to displacement currents evoked by the movement of two protons during the photocycle. The more realistic eqs. 8 and 11, however, show that because of uncertainties we have to be satisfied that the calculations reproduce the right order of magnitude of Σd_i. The uncertainties are the following: (i) In eq. 8 a nearly complete saturation of orientation means $A \approx 1$, but the values of the dielectric constant of the protein segments, ϵ_i, and the factor B arising from the approximation that the medium is a homogeneous isolator and the electrodes are infinitely large compared with the purple membranes cannot be determined. We may assume that the protein is a homogeneous dielectric with $\epsilon = 2$, and $B = 1$. Then $\Sigma d_i = 20$ nm. In the case when only one proton moves $B = 4$.

(ii) In the more realistic eq. 11 the problem of dielectric constant remains the same; we may again assume that $A \approx 1$, then Σd_i depends on R_{pm}/R_E. It is very hazardous to determine R_{pm} by calculations in such a complex system as the purple membrane suspension. (Even if we assume ideal circumstances, the value of R_{pm} must be calculated by a definite integral with a limit close to the divergence, which causes the results to be quite

Table 1

Proton displacements (in nm) during the photocycle of bacteriorhodopsin

Transition	Distances	Ref. 12		New assignment (normalized)
		Calculated via eq. 7	Normalized	
bR → K	$d_1' + d_2''$	–	–	−(0.02+0.11)
K → L	d_2	−0.30	−0.15	−0.02
L → M	d_3	+1.0	+0.5	+0.5
M → O	d_4	+6.2	+3.1	+3.1
D → bR	d_5	+3.0	+1.5	+1.5

unreliable.) If we accept that one proton is displaced during the photocycle then $R_{pm}/R_E = 4 \times D'/D$ ($\epsilon = 2$).

We have to conclude that because of the above uncertainties the value of Σd_i is reproduced by the equations only in order of magnitude. Therefore, the PERS measurement does not seem to be suitable for decisions about stoichiometry [19].

The dielectric constant may be different in different sections of the protein (from transition to transition when the protons move). Since the values of ϵ_i cannot be determined without knowing the tertiary structure of the protein, the best that we can do is to assume their constancy.

It is well known from absorption measurements in the ultraviolet that amino acid side chains take part in the bR photocycle (ref. 20 and references cited therein). Therefore, the motions of charged side chains can in principle contribute to the measured displacement current. The arguments that this can produce only small perturbations are the following:

(a) The side chains – if they move – return to their initial positions by the end of the photocycle, because the original bR is re-established.

Therefore, the sum of their displacement current is zero. The non-zero integral of the measured displacement current can originate only from net unidirectional charge motion, i.e., charge translocation during the photocycle. The 'charged side chain current' may only influence the individual distances.

(b) A time-dependent photoselection study of the ultraviolet absorption signals of the bR demonstrated that – at least – the tryptophan and tyrosine side chains are severely restricted in their photo-induced motions [20].

It turned out when the PERS was measured at low temperatures that the first negative signal (fig. 2) was erroneously assigned to the K → L transition. It really corresponds to the bR → K and K → L transitions [21,22]. The K → L transition has a very small negative signal; the distance associated with it is $d_2 = -0.02$ nm. The bR → K transition has two components d_1' and d_1''.

The normalized distances (table 1) confirm our basic assumption that the PERS is evoked by proton movements:

(a) It is very probable that the bR → K transition involves the 13-*cis* isomerization of the all-*trans*-retinal [23]. From the simple geometry of *trans-cis* isomerization a displacement of 0.16 nm of the Schiff base is expected in the direction of the membrane normal, compared with the displacement of 0.13 nm calculated from the data.

(b) The displacements are a good means of locating the origin of protons, i.e., to determine the site of protonated Schiff base across the membrane. Its distance from the internal surface is $d_5 = 1.5$ nm, and from the external surface $-(d + d_2) + d_3 + d_4 = 3.4$ nm, in agreement with structural determinations [14].

(c) Study of the back-photoreaction from the M state [13] has shown that the protons move forward to the M state after a green flash and are taken back to the original position by illuminating the molecules in the M state with a blue flash. The algebraic sum of the displacements is zero.

It is expected that the suspension method which works well in the case of purple membranes in suspension will find many other applications. The measurement of displacement current and the theoretical evaluations outlined in this paper – though they show the timing of charge motions – do not contain information about the nature of the moving charge. It may be positive or negative, bound to the protein or freely jumping, or it may be a rotating dipole. The equations contain the product dQn, where n is the number of simultaneously displaced charges; their individual values cannot be determined from this evaluation. Independent studies are needed to clear up these very important points in every case of application. The case of bR offers the plausible assumption of proton movement because of its proton-pumping function.

Appendix

A1. Calculation of eq. 9

We write the differential equation of the equivalent circuit (fig. 6b). Let us denote the charge on capacitances C and C_{pm} by Q and Q_{pm}, and the current on R, R_E and R_{pm} by I, I_E and I_{pm},

respectively. Then

$$I + \dot{Q} = I_E$$

$$I + \dot{Q} = I_{pm} + \dot{Q}_{pm}$$

$$I_{pm} R_{pm} = IR + I_E R_E.$$

From these equations with $I = Q/RC$ and $I_{pm} = Q_{pm}/R_{pm}C_{pm}$ we obtain the coupled differential equations for Q and $\dot{Q}_{pm}$:

$$\dot{Q} = -Q\left(\frac{1}{RC} + \frac{1}{R_E C}\right) + Q_{pm}\frac{1}{R_E C_{pm}} \quad \text{(A1)}$$

$$\dot{Q}_{pm} = -Q\frac{1}{R_E C} - Q_{pm}\frac{1}{R_{pm}C_{pm}} \quad \text{(A2)}$$

The solution of this equations for Q with initial conditions of $Q_{pm} = Q_{ind}$ and $Q = 0$ at $t = 0$ and neglecting some extremely small terms is:

$$Q = Q_{ind}\frac{R_{pm}}{R_E}\left(e^{-t/R'C} - e^{-t/R_{pm}C_{pm}}\right), \quad \text{(A3)}$$

from which $V_1(t) = Q/C$ as given in eq. 9.

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