DISPLACEMENT CURRENT IN BIOLOGICAL MEMBRANES*

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Summary

The functioning of some important membrane bound enzymes of the living systems is connected with charge translocation. A method has been elaborated to measure the displacement current due to the motion of protons on light excitation in the bacteriorhodopsin molecules. From the time distribution and the amplitudes of the current it has been established that the translocation of protons takes five different steps all with different distances inside the proteins.

Introduction

Since the time of Luigi Galvani 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, in the two sides of the membrane. The asymmetric ion distribution—which is measurable—explains the membrane potential easily. The problem now is to understand the underlying molecular mechanism of the pumping process itself. According to our present knowledge the pumps are special proteins embedded in the membranes of the cells or subcellular units and the pumped ions should move inside these units. Moving charges cause a displacement current which may be picked up by electrodes. Registering the time course of displacement current it is possible to learn about the charge translocation.

In this paper a method worked out in our laboratory for the study of a light *driven proton pump* is described and the electric parameters of the pump are discussed.

* Dedicated to Professor Károly Simonyi on the occasion of his Seventieth Birthday

Description of the method

To measure the displacement current oriented systems—in which the protons move in one direction—are needed. Fragments of biological membranes generally are a priori 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 purple membranes of *Halobacterium 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. [2] that the bacteriorhodopsins (*bR*) embedded in purple membranes pump protons during their light-drived photocycle. The protons should therefore move through the molecule, causing a displacement current. In the following we use the expressions moving protons inside *bR* molecules. The discussion, however, is valid for other pumped charges and the movement of charged parts of dipoles of molecules.

A laser flash starts the proton-pumping activity of the *bR* molecules and the protons move in one direction. Figure 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. A time-dependent voltage $V(t)=I(t)\cdot R$ is measured on the resistance R.

Figure 2 shows a set of data [3] showing that the electric signal caused by the moving protons has a complex feature.

Explanation of the electric signal measured in suspension

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. 3a, and the equivalent circuit in Fig. 3b.

The membrane is shunted by R_{pm}, the resistance of the surrounding electrolyte, and is connected to the electrodes by R_{E_1} and R_{E_2}. These resistance 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 $R_{E_1}+R_{E_2}=R_E$.

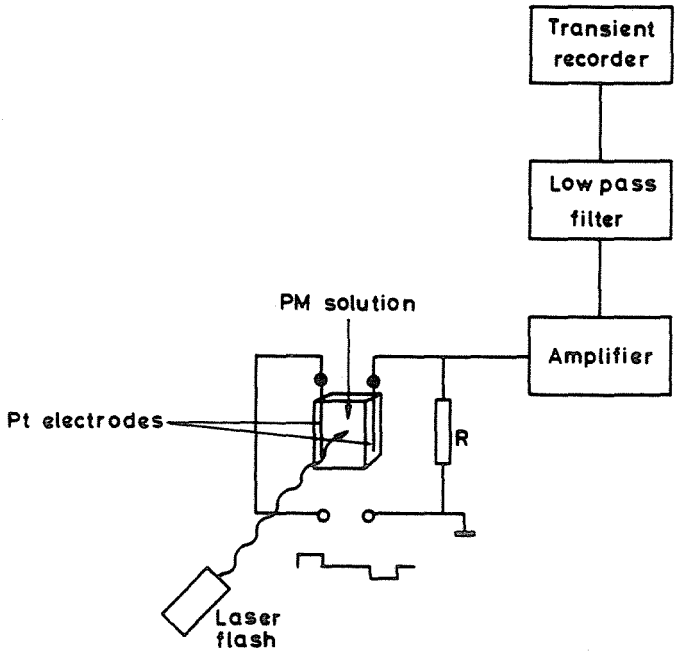

Fig. 1. Scheme of the measuring system

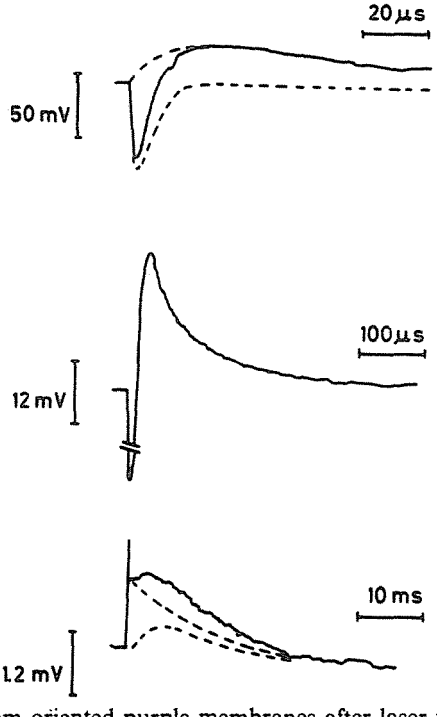

Fig. 2. Electric signals from oriented purple membranes after laser flash excitation. Note the different amplitude and time scales

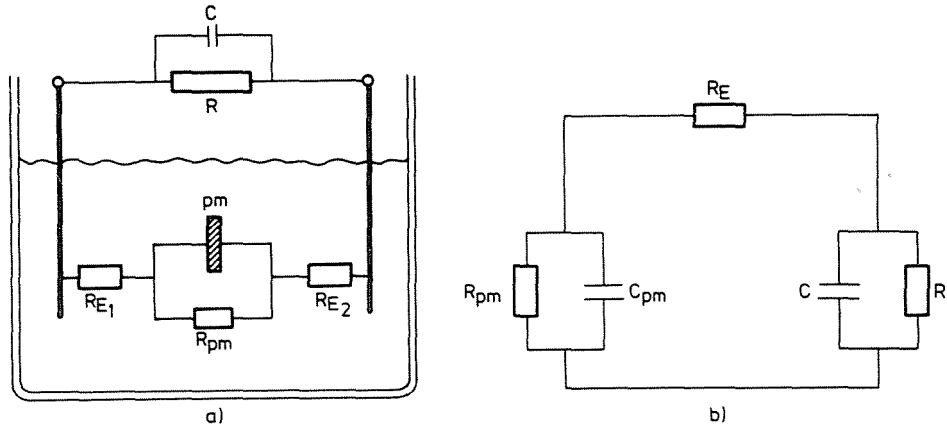

Fig. 3. The actual (a) and the equivalent (b) electric circuit. E_1 and E_2 electrodes, R_{E_1}, R_{E_2} and R_{pm} resistances of the electrolyte, R measuring resistance, C_{pm} capacitance of the purple membrane, C the measuring capacitance

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}.

Let us select a single oriented purple membrane and assume that large planar electrodes are in contact with it (Fig. 4). The medium is considered as a homogeneous isolator. An absorbed photon acts by pushing a proton from point 1 to point 2. According to the Ramo-Shockley theorem of electrody-

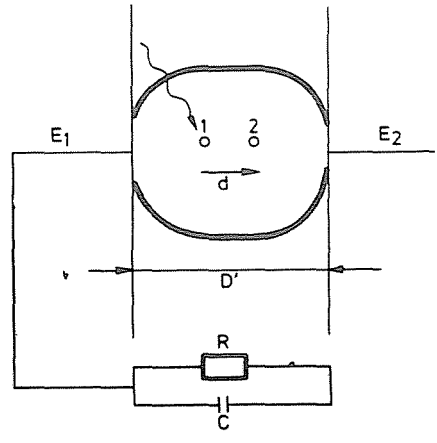

Fig. 4. Assumed elementary act in the measurement of displacement current. Bacteriorhodopsin protein embedded in membrane, charge moves from point 1 to 2; E_1 and E_2 hypothetical electrodes (in reality they represent C_{pm})

namics [4], a current i is induced in the external circuit:

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

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, and ε the dielectric constant of the protein. We assume that $v(t)$ is very large, i.e., the protons jump from point 1 to point 2. Integrating eq. 1 with respect to time:

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

The proton jump induced a charge (Q_{ind}) proportional to the displacement d.

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

$$V_1(t)=\frac{Qd}{\varepsilon CD'}\frac{R_{pm}}{R_E}\left(e^{-t/R'C}-e^{-t/R_{pm}C_{pm}}\right),$$
$$R'=R\frac{R_E}{R+R_E}. \tag{3}$$

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

In the real case N_0 protons move. Charge is induced in the external circuit only when a transition from state 1 to 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:

$$\rho(t)=kN_0e^{-kt}, \tag{4}$$

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:

$$V_{N_0}(t)=\frac{N_0Qd}{\varepsilon CD'}\frac{R_{pm}}{R_E}k\int_0^t e^{-kt'}\cdot e^{-(t-t')/R'C}\,\mathrm{d}t'=$$
$$=\frac{N_0Qd}{\varepsilon CD'}\frac{R_{pm}}{R_E}\frac{kR'}{1-kR'C}\left(e^{-kt}-e^{-t/R'C}\right). \tag{5}$$

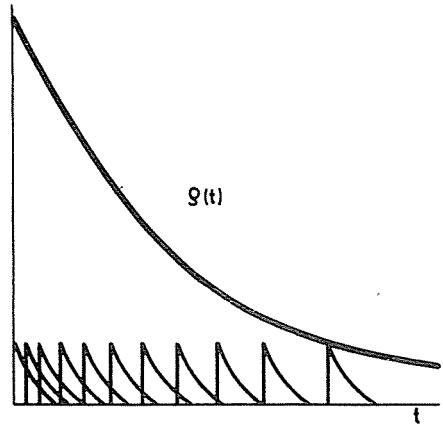

Fig. 5. Every induced charge from a decaying state produces a voltage $V_1(t)$ (eq. 3). The time density of incidence is given by $\rho(t)$ (eq. 4). $V_{N_0}(t)$ at a given t value is obtained by summing all the $V_1(t)$ values from decays at times $t' < t$

In this equation $V_{N_0}(t)$ is measured in the experiment, N_0 the number of excited photocycles may be determined by optical methods [3], Q is the elementary charge, C, R', k can be measured, $R_{pm}/R_E \approx D'/D$, $\varepsilon \simeq 2$ for proteins. This means that one can determine the distance d the protons make. In practice, however, eq. 5 only gives the right order of magnitude for d because R_{pm}/R_E is surely greater than D'/D, the value of ε is uncertain too, and the orientation may not be complete. Therefore it is important to combine the deviation of R_{pm}/R_E from D'/D, a factor A expressing the degree of orientation, and ε in one factor F, which can be determined by normalizing the sum of the separately determined d values Σd_i to the thickness of the membrane D which is known to be 5 nm from X-ray diffraction measurements. This way eq. 5 is rewritten for the i-th component of the charge translocation:

$$V^i_{N_0}(t) = \frac{N_0 Q F}{CD} d_i k_i \frac{R'}{1 - k_i R' C} (e^{-k_i t} - e^{-t/R'C}) . \tag{6}$$

Application

Equation 6 has been applied to calculate the distance the protons move during their path through the *bR* molecules after light excitation (3). 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 assuming $F = 1$. In Table 1 the different components of the electric signal

Table 1

Proton displacements (in nm) during the photocycle of bacteriorhodopsin

Transition	Distances	Calculated via eq. 6.	Normalized
bR→K	d_1	−0.26	−0.13
K→L	d_2	−0.04	−0.02
L→M	d_3	+1.0	+0.5
M→0	d_4	+6.2	+3.1
0→bR	d_5	+3.0	+1.5

are assigned to the known transitions of the photocycle (2) on the correspondence of the lifetimes. The meaning of the distances has been discussed in ref. [5].

Appendix

We write the differential equation of the equivalent circuit (Fig. 3b). 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

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

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

$$I_{pm}R_{pm}=IR+I_ER_E\,.$$

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

$$\dot{Q}=-Q\left(\frac{1}{RC}+\frac{1}{R_EC}\right)+Q_{pm}\frac{1}{R_EC_{pm}} \tag{A1}$$

$$\dot{Q}_{pm}=-Q\frac{1}{R_EC}-Q_{pm}\frac{1}{R_{pm}C_{pm}}\,. \tag{A2}$$

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

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

from which $V_1(t)=Q/C$ as given in eq. 3.

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