Biochimica et Biophysica Acta, 591 (1980) 37—52

BBA 47843

PHOTOCURRENT RESPONSE OF BACTERIORHODOPSIN ADSORBED ON BIMOLECULAR LIPID MEMBRANES

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(Received July 9th, 1979)
(Revised manuscript received December 10th, 1979)

Key words: Photocurrent response; Bacteriorhodopsin binding; Lipid bilayer; (Bimolecular lipid membrane)

Summary

The photo response of bacteriorhodopsin adsorbed on a bimolecular lipid membrane has been investigated using short-circuit current measurements. The results revealed a biphasic current vs. time curve for the photocurrent at pH values of approx. 7. This phenomenon could be modified by altering either the value of the external applied electrical field or the proton concentration differences.

The observed effects of the external applied voltage, pH gradient and lipophilic proton carriers enabled us to conclude that the bacteriorhodopsin can be adsorbed in two different states, which give rise to a pumping effect and a flux of protons in opposite directions.

A theoretical analysis of the photocycle in relation to the electrical field which acts on the proton uptake and release is proposed. The main effect of this field is to diminish the pumping rate due to the proton motive force resulting from the creation of space-charge in the vicinity of purple membrane fragments.

Introduction

Purple membrane fragments of *Halobacterium halobium* are formed by a chromoprotein, bacteriorhodopsin (BR), which tends to form a two-dimensional network in the presence of lipids. It was been established unquestionably

Abbreviations: BR, bacteriorhodopsin; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone.

that BR acts as a light-driven proton pump and that, when it is illuminated between 500 and 600 nm, it gives rise to active proton fluxes. At the same time, the photochemical properties of BR have been studied extensively (for a recent review, see Ref. 1). As a result mainly of spectroscopic studies, the existence has been shown of a number of intermediate forms of BR during the photochemical cycle (in the course of which a proton is successively released and taken up). Several attempts have been made to relate these photochemical steps to the mechanism of active vectorial transport of protons across the membrane. A particularly convenient technique for the investigation of this problem is based on the recording of the photocurrent response of oriented BR at the interface between an aqueous solution and a non-aqueous phase.

One expect this type of photochemical measurement to yield two sorts of information: if the non-aqueous phase is permeable to protons, the proton flux can be estimated using photocurrent measurements: here, it is necessary to separate the ion-transfer current from the capacitate current [2]; proton flux is also responsible for the photovoltage recording. When this phase is not permeable to protons as, for instance, with Teflon sheets [3,4], capacitate coupling can give information concerning intramolecular change or dipole displacement during the first few microseconds after illumination. Various systems other than bimolecular lipid membranes have been used, e.g., thick lipid films [5], lipid-impregnated membranes [6,7], polymer membranes [8], planar bimolecular lipid membanes with liposomes [9].

In this study, the photocurrents during and after steady illumination of a planar bimolecular lipid membrane, in which are incorporated purple membrane fragments, are recorded in that wavelength region corresponding to the adsorption of BR. The method of Dancshazy and Karvaly [10] for binding the BR to the planar bilayer was used: the bimolecular lipid membrane was charged positively by the addition of octadecylamine.

Particular attention was paid to correlating the flux of electrical charges with the electrochemical potential differences of the protons, by means of kinetic equations of proton transfer.

Materials and Methods

Bimolecular lipid membranes were prepared and the BR incorporated as indicated, using the same membrane-forming solution as Ref. 10: 2% w/w phosphatidylcholine and 0.025% w/w octadecylamine in *n*-decane. The *n*-decane and the carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), were, respectively, Merck puriss. and Sigma products. Purple membrane fragments were prepared from *Halobacterium halobium* strains (R_1M_1) by the method of Oesterhelt and Stoeckenius [11]. The mother liquor of CCCP was a 10^{-3} M ethanol solution and volumes of this solution were added to the phase without bacteriorhodopsin to make this solution (without BR) to 10 μM CCCP.

All measurements were carried out at 25°C, in an electrically-shielded apparatus. The solutions were not buffered, but the pH of the aqueous solutions was determined using a hanging-drop type of micro-glass electrode. The pH values were adjusted by addition of HCl or NaOH, within a range of pH 4—10. Before use, the suspension of purple membrane fragments (cooled in ice)

was sonified for four periods of 30 s, at 2 min intervals, using an ultrasonic Annemasse apparatus. The solutions were agitated by bubbling nitrogen through.

The cell used was filled with 0.01 M NaCl prepared from three-times distilled water). The electrical apparatus enabled measurement, using a single bimolecular lipid membrane, of both its capacitance, using a coulostatic method [12] to check its bimolecular state, and the photocurrents at a given applied voltage. A Keithley 427 current amplifier was used, connected to a memory 5103 N Tektronic oscilloscope.

An Osram xenon XBO 75 W lamp illuminated the membrane. The light beam was focused on the bilayer by a system of lenses, and a broad band coloured MTO glass filter (DA 557d') was placed in the light path.

The membrane was illuminated only after the voltage had been applied and when the current (resulting from this) had reached a steady value. The light energy was measured with a Centronic FRD1 photodiode connected to an optical glass-fibre inside the cell near the membrane; this filter had a transmission band between 515 and 600 nm.

A high-gain current-to-voltage converter was used to register the current of the photodiode. The mean current was corrected by taking into account the response curve of the diode and the transmission curve of the optical filter. In all the experiments reported here, the membrane was illuminated with nearly constant light power of approx. 60 $\mu W \cdot mm^{-2}$. The effect of modification of illumination will be discussed in a further paper.

Results and Discussion

Short-circuit current during and after illumination

The short-circuit currents appear several minutes after the addition of purple membrane fragments to one of the aqueous solutions. These currents increase slowly with time, owing to the binding rate of the purple membrane fragments to the bimolecular lipid membrane, but may easily differ in a ratio of as much as 1—3 between experiments. Owing to certain uncontrolled factors, it is difficult to obtain very good reproducibility, and the evolution with time of this short-circuit photocurrent depends greatly on the pH of the aqueous solution (Fig. 1). The initial current corresponded to the passage of positive charges from the side containing the BR to the other and it decreased exponentially with time. When the pH difference across the membrane is such that the pH is 4.1 on the BR side and 6.25 on the other side, a small steady-state current was recorded (Fig. 1c). With pH 4.8 or 6.5 on each side of the membrane, the current, respectively, decreased to zero (Fig. 1a) or become at first negative and then returned to zero (Fig. 1b). This biphasic variation in the latter case was accentuated when the pH was increased. All these responses to the light illumination were obtained with the same light intensity (60 $\mu W \cdot mm^{-2}$).

When the light was extinguished in the first two cases with no difference in pH, the current became negative, returning exponentially to zero with a time constant of approx. 0.5 s. This type of behaviour of the photocurrent has been observed by Hermann and Rayfield [2] with liposomes containing BR bound

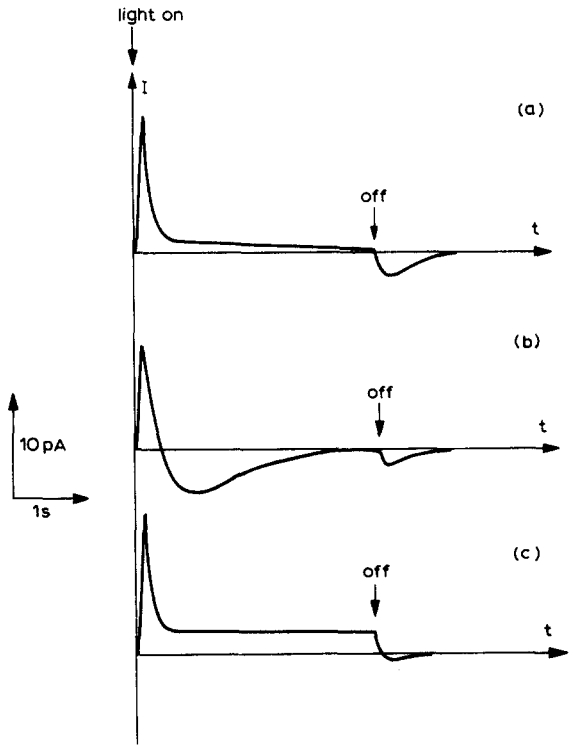

Fig. 1. Variation of the photocurrents with time at different pH in the two aqueous solutions under steady illuminations (60 $\mu W \cdot mm^{-2}$): (a) pH 4.8—4.8; (b) pH 6.5—6.5; (c) pH 4.1 on the side with bacteriorhodopsin and 6.25 on the other side.

to planar bimolecular lipid membrane. When there is a pH difference, after switching off the light source, the current seems to remain positive decreasing from the steady-state value to zero, with a very slight, negative 'off-response' current.

The shape of the short-circuit currents was also modified by the addition of a lipophilic proton carrier: Before the addition of CCCP at pH < 5.5, the type of response obtained was generally monophasic (full line in Fig. 2, left side); at pH > 6, it appeared to be biphasic (full line Fig. 2, right side). In each of these cases, if one adds to the solution on the aqueous side (which does not contain purple membrane fragments) CCCP (to 10 μM) in an alcoholic solution, the response to the illumination is transformed (dotted line in Fig. 2, left side and right side).

It will be noted that there is a slight change in the value of the initial current. Bamberg et al. [13], using diphytanoyl phosphatidylcholine as a bilayer-forming lipid, obtained a photocurrent at pH 7 which decreased exponentially without becoming negative, whereas, using gramicidin, they observed a steady, positive current. They interpreted these results as being due to the binding process of the purple membrane fragments on the surface of the lipid bilayer. This representation of the incorporation of BR in bimolecular lipid membranes seems to be the only possible explanation of the appearance of a steady-state current on the addition either of channel-forming substances, like gramicidin,

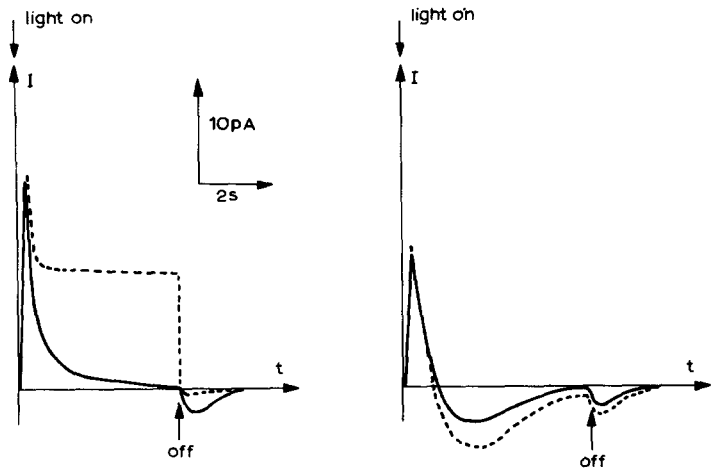

Fig. 2. Variation of the photocurrent with time before and 5 min after the addition of CCCP on the side without bacteriorhodopsin under steady-state illumination (60 $\mu W \cdot mm^{-2}$); pH 4.8—4.8 on the left side; pH 6.5—6.5 on the right side. ———, without CCCP; - - - - - -, after addition of CCCP.

or lipophilic proton carriers, such as CCCP or FCCP. Moreover, this adsorption model is in agreement with the lowering of the photovoltage observed when a proton carrier is added [9]. It also explains the shape of the photovoltage variations when a resistance is connected across the two electrodes [9].

Effects of an applied external voltage

When an external voltage is first applied across the bimolecular lipidic membrane in the presence of purple membrane fragments, one observes a change in the short-circuit photocurrent values. However, on subsequent applications of this voltage, the reproducibility of these photocurrents is greatly improved. In all likelihood, this is due to the effect of the electrical field on the adsorption of the purple membrane fragments: the application of an external voltage perhaps stabilizes the binding forces between this substance and the surface of the bilayer.

In view of this in the measurements undertaken here, the bimolecular lipid membranes were submitted to a prepolarization cycle (from zero to +100 mV then to —100 mV passing through zero, and finally back to zero). A better reproducibility was thus obtained for the same membrane, whatever the applied voltage and on condition that the interval between two subsequent illuminations was greater than 15 s.

The applied voltage gave rise to a variation in the dark current and, in all cases, the light was switched on after the dark current had reached a steady state. This constant (non-zero) value represents the base line of the photocurent. It varied linearly with the applied potential in the —100 to +100 mV range. It can be considered as measure of an activated transport, limited by diffusion or interfacial processes.

Fig. 3 shows the effect of positive- and negative-applied voltages on the photocurrent in the case of a pH gradient (pH 4.1—6.25) as in Fig. 1. Under

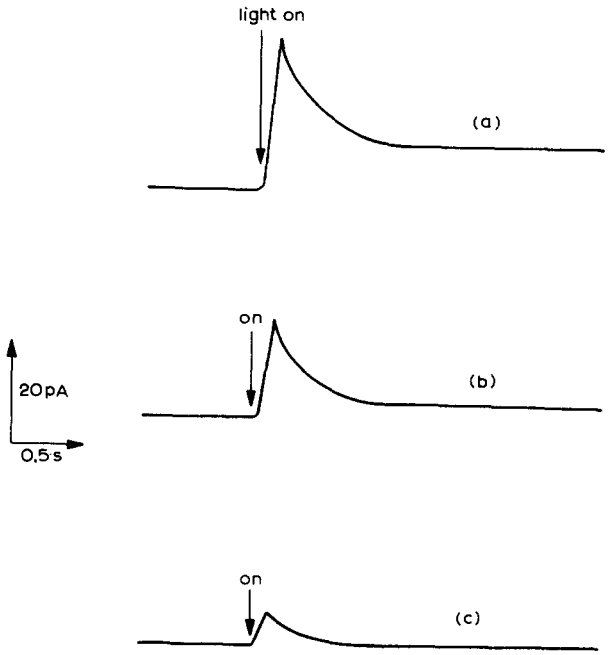

Fig. 3. Variation of the photocurrent with time when the pH of the two solutions are 4.1 on the bacteriorhodopsin side and 6.25 on the other, under steady-state illumination (60 $\mu W \cdot mm^{-2}$) but different external applied voltages (the polarity of the applied voltage is given with respect to the side without bacteriorhodopsin; (a) +60 mV; (b) 0 mV; (c) —60 mV.

these conditions, the photocurrents were always positive, but the initial value, respectively, increased and decreased when the applied potential was positive or negative.

When the pH was the same on both sides of the membrane (pH 6.5), a biphasic response was obtained and the applied voltage, polarity of which is referred to the side without bacteriorhodopsin, modifies the shape (Fig. 4). At +100 mV, the photocurrent remained positive and had a shape corresponding to a monophasic time response but, nevertheless, a transient negative current was recorded when the light was switched off. At the negative extreme (—100 mV), the negative part of the biphasic variation with the time was more pronounced. On switching off the light source, a transient negative response was observed the maximum value of which was greater than that of the current at the end of the illumination.

The maximum negative value of the photocurrent, I_m, increased continuously as the applied voltage becomes more negative (Fig. 5), at pH 6, whereas the initial current, I_0, maintained a steady value. On the other hand, for positive applied voltages, I_0 increased linearly with the voltage. The fact that the initial current did not disappear indicates that the electrical field cannot reverse completely the flux of the BR pump in this case.

Influence of the duration of the time interval between two illuminations

In all measurements, the system was submitted to two successive illuminations of identical light intensity, but separated by a variable interval

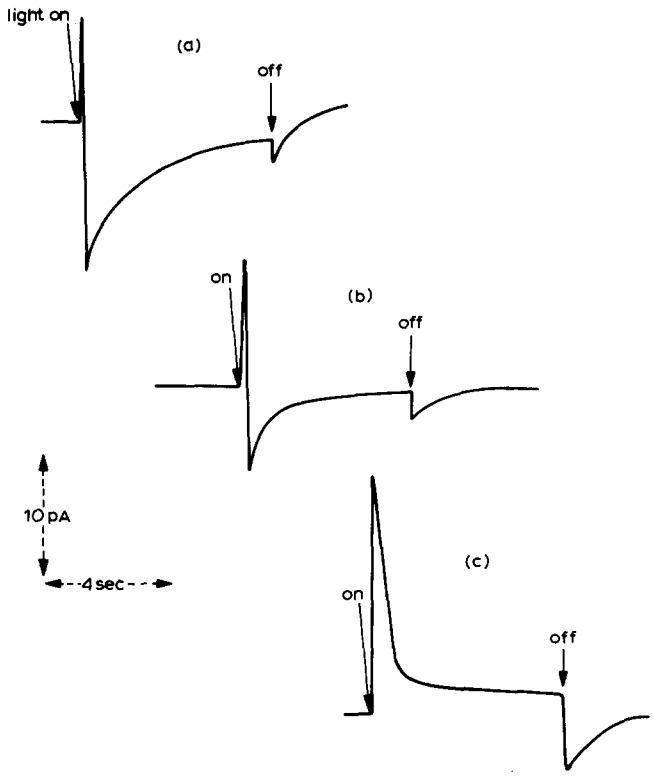

Fig. 4. Variation of the photocurrent with time at pH 6.5 on both sides of the membrane, under steady-state illumination (60 $\mu W \cdot mm^{-2}$) but different external applied voltages (the polarity of the applied voltage is given with respect to the side without bacteriorhodopsin). (a) —100 mV; (b) 0 mV; (c) +100 mV.

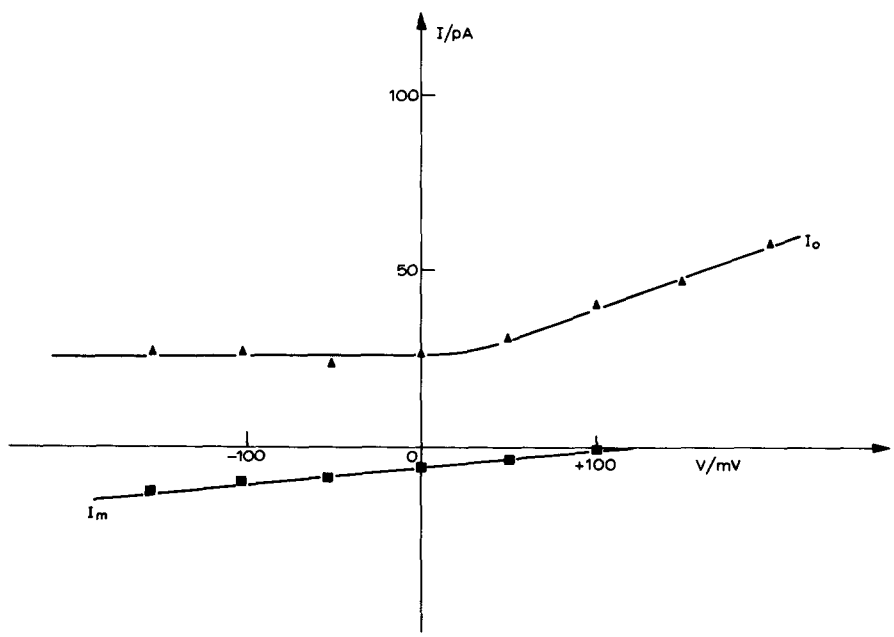

Fig. 5. Variation at pH 6 with the applied voltage whose polarity is given with respect to the side without bacteriorhodopsin, of the photocurrent peak values I_0 and the minima I_m for biphasic photo-response under steady-state illumination (60 $\mu W \cdot mm^{-2}$).

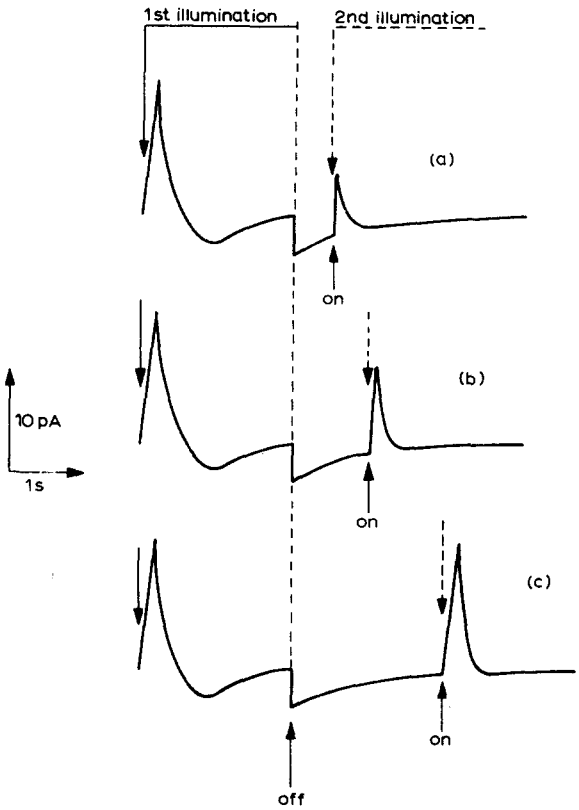

Fig. 6. Variation of the photocurrent, pH 6.5 on both sides, with time for different interval of time between periods of illumination of identical light intensity (60 $\mu W \cdot mm^{-2}$).

of time. The initial current after the second illumination was always positive but depended strongly on the duration of the time interval between the two illuminations. The curves shown in Fig. 6 were obtained with the same membrane for which it was established that the response at the end of a series of illuminations was identical to that at the beginning. It can be seen that the initial current, after the second illumination, increases with the intercal of time between subsequent illuminations.

The photocurrent appears to be related to the polarization, as in the case of electrochemical systems. Polarization is usually induced by the application of an overvoltage, which, in this case, is opposed to the photoactivated proton transfer. To determine whether the electrochemical polarization is due to a change in the proton concentration within the BR-bimolecular lipid membrane system or to a transmembrane electrical field induced by a modification of the capacitance, the influence on the photocurrent of the addition of a proton carrier and of the applied field was the considered.

When CCCP was added to a system giving rise to a monophasic response in the absence of this carrier there were two main modifications in the type of curve (see Fig. 7, as compared with Fig. 1c): firstly, the steady-state current was higher than in absence of CCCP; secondly, the initial currents recorded

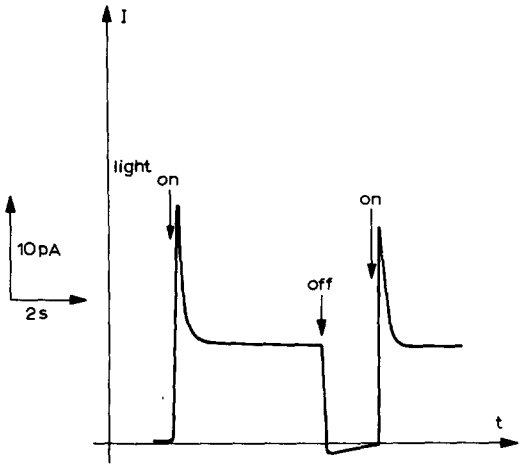

Fig. 7. Variation of the photocurrent with time after the addition of CCCP such that the aqueous solution without bacteriorhodopsin becomes 10 μM in CCCP, during and after illumination and during a further period of illumination: pH 4.1 on the side with bacteriorhodopsin, pH 6.2 on the other side.

during the first and the second illumination were greater than in the absence of CCCP, and this for an identical time interval of illumination and the same membrane. Nevertheless, the current peak for the second illumination remains lower than the first one (Fig. 7). This result indicates that the polarization depends on the concentration of protons and that it can be compared to the concentration polarization in a classical (metal-solution) electrochemical system, or at the membranesolution interface.

Electrochemical kinetics of proton fluxes

To interpret these results, we refer to a simple model for a proton-pumping process associated with the BR photochemical cycle. This model takes into account the two possible orientations of the BR adsorbed on the surface of the bimolecular lipid membranes, as well as the two directions of flux of the protons during the uptake and release steps. For a given orientation, this model is represented schematically in Fig. 8.

The fundamental light-adapted BR is designated by BRH^+. It corresponds to BR_{570}, in which the Schiff's base is protonated and all double bonds in the

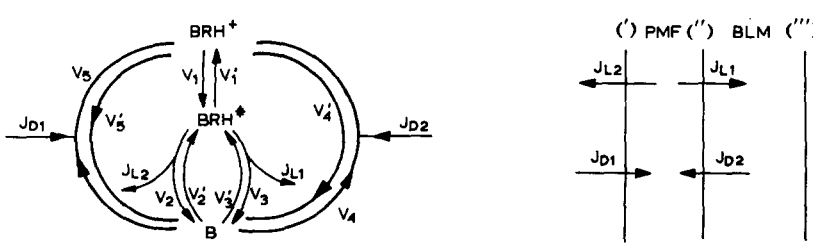

Fig. 8. Photochemical cycle of bacteriorhodopsin under illumination and schematic representation of the release and uptake of protons. BRH^+, ground state of bacteriorhodopsin; BRH^{+*}, excited state of bacteriorhodopsin; B, deprotonated state of bacteriorhodopsin.

retinal chain are in the trans configuration. The excited form can return either in the dark or when illuminated to the initial state with the release and uptake of a proton, passing through a sequence of intermediate states. Several of these intermediates have been identified (L_{550}, M_{412}, N_{520}, O_{640}) but there is some lack of agreement as to the actual steps which are which of these reactions are light-dependent or not. As for the deprotonated form of BR, it is given as B.

It is generally considered that proton movement through the purple membrane fragments takes place through channels [14,15], Schiff's base acting as a binding site with a dissociation constant depending on the form in which the BR exists, the conformational change to which it is submitted, as well as to changes in the profile of the energy barrier. The electrical field is one of the parameters which can affect the shape and height of the energy governing proton release and giving rise, therefore, to vectorial proton movements.

The proton release consequent on the light deactivation gives rise to a proton flux, J_A, which can be defined in terms of four fluxes. In the kinetic expression for proton-transfer processes through the BR (Fig. 8), one sees that, in the most general case, it is necessary to take into consideration four fluxes, as the uptake and release of protons can, a priori, take place in either of two directions

BR → solution
or
BR → bimolecular lipid membrane

These four fluxes are the following:
J_{L1} proton release, purple membrane fragments → bimolecular lipid membrane
J_{L2} proton release, purple membrane fragments → solution
J_{D1} proton uptake, solution → purple membrane fragments
J_{D2} proton uptake, bimolecular lipid membrane → purple membrane fragments

For a time unit corresponding to a whole number of photochemical cycles, one can define for each pump element, i, a net flux, J_A:

$$J_A = J_{L1} + J_{D2} = J_{L2} + J_{D1}$$

with the added condition that

$$|J_{L1}| + |J_{L2}| = |J_{D1}| + |J_{D2}|$$

These fluxes are such that J_{L1} and J_{D1} are positive and the others, with the same assumptions mode earlier concerning the sign, negative. The same applies for the purple membrane fragments.

The equivalent circuit of the purple membrane fragments-bimolecular lipid membrane system is represented in Fig. 9. Plane (′) corresponds to the proton reaction plane in the aqueous solution while the plane (″) is that of contact between purple membrane fragments and bimolecular lipid membrane. The purple membrane fragments are equivalent to a capacitor, C_F, in parallel with a generator associated with the flux, J_A. The equivalent circuit of an individual pump is thus the sum of all the elementary circuits of the two adsorbed BR forms. In the dark, the equivalent circuit for the purple membrane fragments is always constituted by an impedance, Z_P, in parallel with a capacitance, C_F. For the bimolecular lipid membrane, the equivalent circuit can be considered

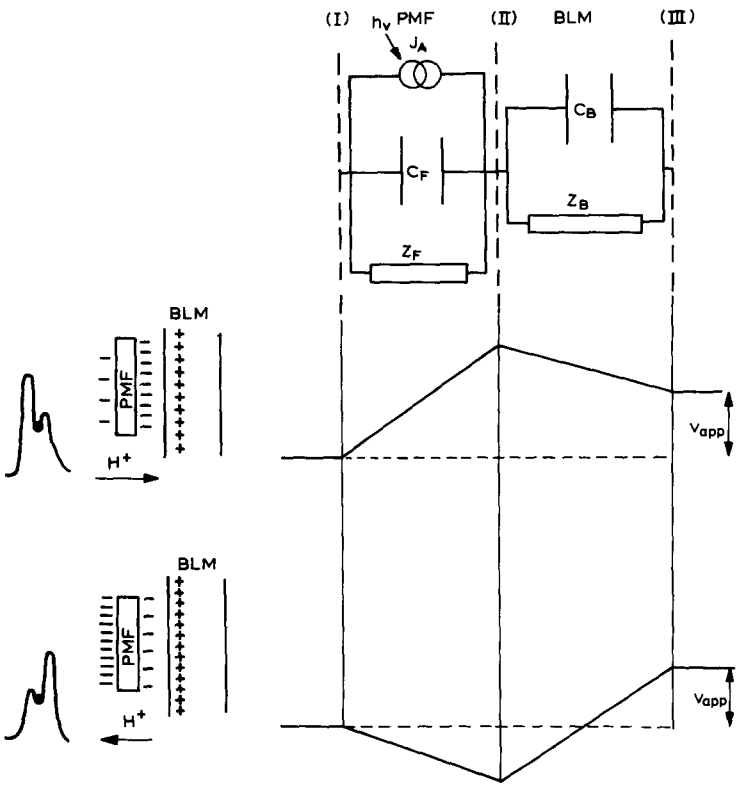

Fig. 9. Schematic representation of the equivalent circuits of purple membrane fragments adsorbed on BLM.

as a capacitor in parallel with an impedance, corresponding to the passive flux of protons, J_P, though the bimolecular lipidic membrane. The plane (''') is that of closest approach of protons in the second aqueous solution. The electrical profile is represented in Fig. 9.

As in all cases only transient currents due to the photoactivity of the system are taken into consideration, it is not necessary to take into account the uncovered part of the bimolecular lipid membrane surface. The measured current, I, in both parts of the system is the algebraic sum of the proton transfer current and a capacitative current associated with the variation of the charge of C_F and C_B due to the accumulation, or depletion, of protons on the plane ('). If V', V'', V''' are the mean values of the electrical potentials on the corresponding planes:

$$I = F \sum_{i=1}^{n} J_A + C_F \frac{d(V'' - V')}{dt} = FJ_P + C_B \frac{d(V''' - V'')}{dt} \quad (1)$$

The law of conservation of matter on the plane (") gives, if no lateral diffusion takes place,:

$$\frac{dn''_{H^+}}{dt} = \sum_{i=1}^{n} J_A + J_P \quad (2)$$

n being the number of elementary pumps of BR. One notes that whatever the sign of the applied voltage, in the case of a biphasic response the initial photocurrent is positive. This reveals the vectorial character of proton release, the number of BR releasing protons in the direction purple membrane fragments → bilayer tending to predominate. However, in some cases, and mainly at higher pH, the current can be negative.

The passage of protons gives rise to an accumulation or a depletion of protons on the plane ('') and results in the charge of the capacitances, C_F and C_B. Thus, the potential at this plane will become either greater or smaller than V' or V''', depending on the form of the BR adsorbed on the bimolecular lipid membrane. The passive flux of protons across the bilayer is given by the classical electrochemical equation of ion transfer across an unmodified bimolecular lipid membrane

$$J_P = n''_{H^+} k_f \exp[f\alpha_P(V'' - V''')_L - n'''_{H^+} k_b \exp[-f(1-\alpha_P)(V'' - V''')_L] \quad (3)$$

with $f = F/RT$.

This takes into account the change of potential with light.

From this equation, it can be seen that if the number of protons on plane ('') either increases or decreases there results a charge or discharge of the capacitance C_B. This leads to a change in the absolute value of $(V'' - V''')$ and, hence, in the number of protons because of

$$\sum_{i=1}^{n} J_A$$

which gives rise to a passive flux, J_P.

The kinetic equations for the photochemical cycle can be written thus:

$$BRH^+ \underset{k'_1}{\overset{k_1}{\rightleftharpoons}} BRH^{+*} \quad (4)$$

$$BRH^{+*} \underset{k'_2}{\overset{k_2}{\rightleftharpoons}} B + H^{+\prime} \quad (5)$$

$$BRH^{+*} \underset{k'_3}{\overset{k_3}{\rightleftharpoons}} B + H^{+\prime\prime} \quad (6)$$

$$B + H^{+\prime\prime} \underset{k'_4}{\overset{k_4}{\rightleftharpoons}} BRH^+ \quad (7)$$

$$B + H^{+\prime} \underset{k'_5}{\overset{k_5}{\rightleftharpoons}} BRH^+ \quad (8)$$

Reactions 6 and 8 correspond to positive fluxes, while Reactions 5 and 7 correspond to negative fluxes.

As Reactions 5, 6, 7 and 8 are heterogeneous and concern an electrical charge, the kinetics must be influenced by the transmembrane electrical field. The energy barrier contains an electrical term which is a fraction of the applied voltage, so that the kinetic laws have the same form as all electrode reactions which are potential dependent.

The fluxes due to the light and to the electrochemical gradient can be written as:

$$J_{L1} = v'_3 - v_3 \tag{9}$$

$$J_{L2} = v'_2 - v_2 \tag{10}$$

$$J_{D1} = v'_5 - v_5 \tag{11}$$

$$J_{D2} = v'_4 - v_4 \tag{12}$$

where v corresponds to the rate of reaction and will be defined below.

It is also necessary to take into consideration all the intermediates of the photochemical cycle, i.e.

$$\frac{dn_{BRH^+}}{dt} = -v_1 + v'_1 + v_5 - v'_5 + v_4 - v'_4 \tag{13}$$

$$\frac{dn_{BRH^{+*}}}{dt} = v_1 - v'_1 + v'_3 - v_3 + v'_2 - v_2 \tag{14}$$

$$\frac{dn_B}{dt} = v_3 - v'_3 + v_2 - v'_2 - v_5 + v'_5 - v_4 + v'_4 \tag{15}$$

The reaction rates are given by the classical equations:

$$v_1 = I_L \sigma_{BRH^+} n_{BRH^+} \tag{16}$$

$$v'_1 = I_L \sigma_{BRH^{+*}} n_{BRH^{+*}} \tag{17}$$

where I_L corresponds to the light intensity [16], and σ the adsorption coefficient corrected for quantum efficiency.

$$v'_2 = k'_2 n_B n'_{H^+} \exp[f\alpha_2(V'' - V')] \tag{18}$$

$$v_2 = k_2 n_{BRH^{+*}} \exp[-f(1-\alpha_2)(V'' - V')] \tag{19}$$

$$v'_3 = k'_3 n_B n''_{H^+} \exp[-f(1-\alpha_3)(V'' - V')] \tag{20}$$

$$v_3 = k_3 n_{BRH^{+*}} \exp[f\alpha_3(V'' - V')] \tag{21}$$

$$v_4 = k_4 n_B n''_{H^+} \exp[-f(1-\alpha_4)(V'' - V')] \tag{22}$$

$$v'_4 = k'_4 n_{BRH^+} \exp[f\alpha_4(V'' - V')] \tag{23}$$

$$v_5 = k_5 n_B n'_{H^+} \exp[f\alpha_5(V'' - V')] \tag{24}$$

$$v'_5 = k'_5 n_{BRH^+} \exp[-f(1-\alpha_5)(V'' - V')] \tag{25}$$

The parameters, α_2, α_3, α_4, α_5, are not transfer coefficients as in the case of the kinetics at a metalsolution interface, but they indicate that the preceeding reactions depend on only a part of the electrical potential difference across the purple membrane fragments. The law of conservation of matter in the photochemical cycle is given as:

$$n_{BRH^{+*}} + n_{BRH^+} + n_B = \overline{n_{BRH^+}} \tag{26}$$

The bar over the last term serves to indicate the number of molecules in that form, before the switching on of the light. Neither the value of the electrical

potential nor that of the proton concentration near the purple membrane fragments is known. Nevertheless, in the ordinary case where the normal active proton flux is in the positive direction, the rate of Reaction 6 must be much greater than that of Reaction 5. This leads to a value for k_3 which must also be much greater than k_2 even if the potential V'' becomes greater than V', and this is the case if protons are released on plane ($''$), then the proton gradient and the electrical field must lead to a decrease in the difference between the fluxes ($J_{L1} - J_{L2}$). Similarly, under normal conditions, $J_{D1} >> J_{D2}$ and, therefore, $k_5 >> k_4$. The effect of the change in proton concentration and of the potential difference is, therefore, as described above.

It is also necessary to complete the above set of relations by the laws of conservation of matter on planes ($'$) and ($'''$)

$$\frac{dn'_{H^+}}{dt} = \sum_{i=1}^{n} J_A \pm D_{H^+}\left(\frac{\partial C}{\partial x}\right)_{x'} \tag{27}$$

$$\frac{dn'''_{H^+}}{dt} = J_P \pm D_{H^+}\left(\frac{\partial C}{\partial x}\right)_{x'''} \tag{28}$$

where C corresponds to the proton concentration in the aqueous solutions. In these equations, neither the lateral diffusion nor the effect of the double layer at the planes ($'$) and ($'''$) are taken into consideration. D_{H^+} is the diffusion coefficient of protons in the aqueous solution; it is assumed to be independent of the concentration of protons in these two phases.

This mathematical approach is not dependent on the orientation of the BR of the purple membrane fragments, but the same kinetic relations must also be written for the other form of adsorbed purple membrane fragments. These sets of equations must be accompanied by the expressions for $(V'' - V')$ and $(V'' - V''')$, which are functions of the number of protons n''_{H^+} on plane ($''$). It is difficult to solve mathematically this set of equations, but they enable us to come to certain conclusions.

The shape of the biphasic photocurrent corresponds to the sum of two exponential curves with different time constants, with initial currents of opposite sign. The time constant of the negative part of the signal is always greater than that of the positive current. A possible explanation of this result may be proposed by taking into consideration, as described above, two possible adsorbed states for the purple membrane fragments in order to account for a net negative flux with a different time constant, as found experimentally. This difference in the time constants can be accounted for by taking into consideration the profile of the energy barrier which must depend on the local electrical field. The shape of this barrier is different for the release or the uptake of protons in the two adsorption states. A difference in the values of the coefficient, α, may lead to a variation of that of the active field and, hence, of v_2 and v'_2. Now J_{L2}, which depends on α_2, can have a time constant greater than J_{L1}, if $\alpha_3 > \alpha_2$. The resulting flux is then the sum of the active fluxes due to the two adsorption states, as is shown by Eqn. 2.

As a result of the above calculation developed for one adsorption state, one can envisage that, depending on the number of protons n''_{H^+} or n'_{H^+} for this other state (which can reach high values), a very negative applied voltage can

actually reverse the direction of pumping, as is tacitly implied in the relation given by Hermann and Rayfield [2] for the variation of the current with the voltage.

Biphasic photocurrents are generally obtained for pH values near neutral. Their existence can be explained on the basis of two orientation states for the purple membrane fragments adsorbed on the bimolecular lipidic membranes. However, the fact that the initial currents are always positive must, under this hypothesis, mean that the number of molecules which are of the orientation which leads to $J_A > 0$, is greater than those which give rise to a flux in the opposite direction. It may also mean that, even in the absence of an applied electrical field, the rate constant of Reaction 5 is greater than that of Reaction 6.

The existence of two possible orientation states of the adsorbed purple membrane fragments can be explained thus: firstly, the bimolecular lipidic membrane is positively charged, while the polypeptide chain of the protein in the BR has a certain number of negative charges, which, however, are not uniformly distributed. (This means that one external side of the α-helix can be regarded as more negatively charged than the other.) Secondly, the local pH on the two sides of the purple membrane fragments can also modify the dissociation constants of the amino acid groups, which may even lead in certain cases to a change in the distribution of the negative charges on the protein, as well as the bimolecular lipidic membranes.

In view of these two arguments, it can be assumed that the probability of the purple membrane fragments being adsorbed on the side of the bimolecular lipidic membrane with the apparent smaller negative charge is not zero. The energy barrier profile of this adsorption state is different from that of the other adsorbed state, so that the release and uptake of protons do not have the same kinetics. The possibility of two orientations can also serve to explain the stabilization of the photocurrent after the polarization of the membrane. The effect of the applied voltage is to contribute to the fixation of the purple membrane fragments on the bimolecular lipidic membrane and the purple membrane fragments are orientated in such a manner that its adsorption either on the one side or on the other is facilitated.

The entirely negative photocurrents sometimes registered at basic pH values can also be interpreted by a preferential orientation of the BR in a state which corresponds to an easier neutralization of the electric charges of the purple membrane fragments and of the lipids of the bimolecular lipidic membrane (which contains octadecylamine at these pH values).

This theoretical approach tends to emphasise the importance of the electrical proton gradient on the working of the proton pump, the main effect of this gradient being to decrease the net flux of the released protons. The set of equations given serves to show that an active positive flux cannot remain constant because of the increase and decrease of the number of protons, respectively, on the ($''$) and ($'$) planes decreasing J_{L1} and increasing J_{D2}. This results in a charge change of C_F and an increase in the electrical potential difference $(V'' - V')$, which in turn leads to the rates of the Reactions 6 and 7 becoming, respectively, smaller and greater. If the photocurrent on illumination remains zero for a long period of time, this may be due to the fact that the fluxes, J_{L1}

and J_{D2}, are equal and compensate one another. Although the pump functions continuously, the net proton flux is zero. The electrical field, on the other hand, affects the fluxes, J_{D1} and J_{L2}, and when the latter increases, the former decreases.

The positive stationary current increase with the applied voltage up to 100 mV can also be attributed to an increase in the fluxes J_{D2} or J_{D1} because of the increase in the absolute potential difference $(V'' - V')$ and the corresponding decrease in the rates of Reactions 5 and 6. The off-photoresponse can be regarded as a decrease in the electrical potential $(V'' - V')$, which gives rise to a decrease of the proton number n''_{H^+} on the ('') plane, increasing the flux J_{D2}. Moreover, the transport of matter by diffusion will increase the number of protons on the (') plane. The effect of the time between the switching off and on of the light on the height of the peak of the photocurrent of the on response can also be attributed to differences in $(J_{L1} - J_{D2})$ or $(J_{L2} - J_{D1})$, which increase with time. J_{L1} is a maximum when J_{D2} has returned to a value very close to zero, which leads to the largest value for the active flux.

In conclusion, a two-state adsorption for the purple membrane fragments can account for the shape of the photocurrent in the case of a biphasic response, but the dependence of the electric field on the flux of the protons does not allow one to eliminate the contribution of the reverse flux J_{L2} during the illumination. Furhtermore, it is necessary to assume that the probability of existence of the purple membrane fragments state leading to a negative flux of released protons must be less than that of proton release from the other state. This is a necessary condition for obtaining first a positive, and then a negative, photocurrent. Nevertheless, at pH values in the basic region, some photocurrents which remain negative within the whole range-time have been observed. Here, the time constant is greater than that of the positive current. This can be explained on the basis of the adsorption of the purple membrane fragments on the bimolecular lipid membranes such that there is a predominant coverage by the active form leading to an active negative current.

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