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A MEASUREMENT OF THE PROTON PUMP CURRENT GENERATED BY BACTERIORHODOPSIN IN BLACK LIPID MEMBRANES

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Summary

The light-induced electrical current generated by black lipid membranes containing bacteriorhodopsin from *Halobacterium halobium* has been measured directly. It is shown that a measurement of membrane potential can also be used to obtain the proton pump current developed during illumination. Evidence is presented that the charge movement across the membrane is associated with the release of protons in the photoreaction cycle of bacteriorhodopsin. The time variation of the pump current when the light is turned on suggests the rapid depopulation of some initially occupied state.

Halobacterium halobium contains in its cell envelope differentiated regions of purple membrane [1,2]. These purple membrane sheets contain a single protein, bacteriorhodopsin, and lipids [1,3,4] and appear to be part of a photophosphorylating system [5]. The photochemical events occurring in the purple membrane sheet have been correlated with the vectorial release and uptake of protons [6]. Further studies have shown a light-dependent translocation of protons in both *H. halobium* cells [7] and in artificial reconstituted systems [8,9]. This proton movement across the membrane can lead to measurable pH gradients and even drive a leucine transport system [10]. Using flash spectrophotometry it has been shown that at least four different states (configurations) of the rhodopsin protein are involved during the uptake and release of protons [11,12].

These previous experiments involving intact *H. halobium* cells and vesicles are limited in that both sides of the purple membrane are not readily accessible for electrical measurements. It has been shown in previous work that a purple membrane sheet can be incorporated into a black lipid membrane of soya bean phospholipid [13]. The light-induced electric potential generated by a black lipid membrane incorporating bacteriorhodopsin is

easily observed. The advantage of this system utilizing a black lipid membrane is that the electrical properties of the light driven pump is easily and directly measurable.

The light-driven proton pumps are really current generators which move charge from one chamber across the black lipid membrane to the other chamber. The rate at which charges are moved determines the pump current i_p. The potential difference V_M between the two chambers under illumination is determined by several parameters including the capacitance of the membrane, the resistance of the membrane and external circuitry. The following paragraphs will describe how the pump current i_p can be measured directly (short-circuit current method) and related to the membrane potential V_M. The time variation of the pump current i_p under illumination can be studied in detail.

Our membranes were formed on a teflon septum with a 1.2 mm hole separating two teflon chambers containing silver-silver chloride electrodes. A glass window is incorporated in one chamber for viewing and illuminating the membrane. The purple membrane samples were provided by J. Lanyi. These samples were centrifuged, the pellet placed in a purified solution of soybean phospholipid in decane (10 mg/ml) and briefly sonicated. The phospholipid was Sigma type II-S, purified according to Szabo et al. [16]. The resulting mixture was used to form a black lipid membrane across the 1.2 mm hole in the septum.

The capacitance C_M and resistance R_M of the bare black lipid membrane and purple membrane-doped black lipid membranes were measured using common techniques. Typical resistances of these membranes were $2 \cdot 10^{10}$ Ω with a capacitance of 1000 pF (no illumination).

The aqueous solutions in the two chambers separated by the artificial membrane contained 100 mM NaCl and 5 mM Tris·HCl (pH 7.0). A 20 W tungsten light (American Optical Illuminator) with glass filters* was used to illuminate the membrane.

A schematic of the circuit used to measure the short circuit current is shown in Fig. 1. Both operational amplifiers were obtained from Analog Devices and have FET inputs. By switching S_1 and S_2 the circuit can be used

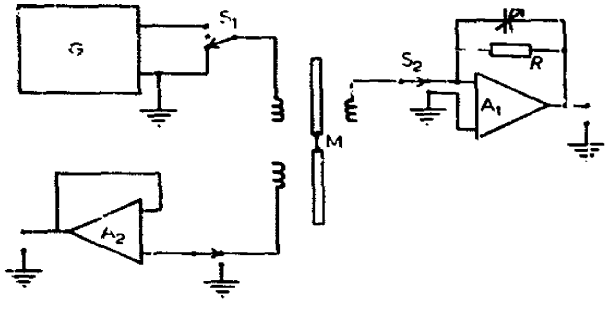

Fig. 1. Circuit used to measure short-circuit current (position of switches S_1 and S_2 as shown) and open-circuit membrane potential under illumination. G is a voltage ramp generator designed after Heubner and Bruner [17]. A_1 and A_2 are electrometer operational amplifiers, Analog Devices models 42L and AD523L, respectively. R is a 10^9 Ω feedback resistor. M represents a membrane.

*Corning glass filters nos. 4-94 P and 3-70 P.

to measure the open-circuit membrane potential V_M instead of short-circuit current i_p. Outputs were recorded on a strip chart recorder, Heath-Schlumberger EV-205-11, which limited the response time of the system.

No current or membrane potential was observed when a bare black lipid membrane without purple membrane was illuminated. The proton pump current polarity varied between different membranes, as one would expect. The spectral absorption characteristics of purple membrane after mixing with phospholipid-decane solution was measured on a Cary Model 16 spectrophotometer. No change in the absorption spectra was observed when compared with the spectra of an untreated purple membrane sample.

The current i_p flowing through a typical membrane with both sides of the membrane held at zero potential is shown in Fig. 2, curve A. Time zero represents the beginning of illumination. The pH on both sides of the membrane is the same and the salt concentrations on both sides are equal. Therefore, the only current flowing through the membrane is due to charge translocation by bacteriorhodopsin in the membrane under illumination. After a sharp initial rise, the pump current settles down to a steady state value on the order of $2 \cdot 10^{-12}$ A. Some membranes have yielded steady-state currents as large as 10^{-11} A.

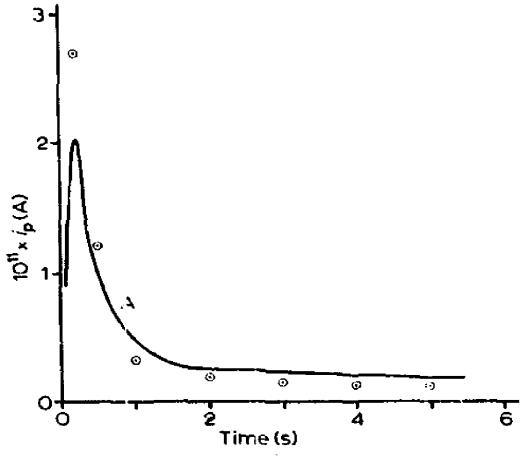

Fig. 2. Proton pump current i_p. Time is that from the start of illumination. Curve A represents i_p measured directly as a short-circuit current, recorded on a chart recorder. The points (⊙) are calculated values of i_p from an open-circuit voltage measurement of the same membrane (Fig. 3, curve A). Constant values for capacitance and resistance, $C_M = 900$ pF and $R_M = 4.8 \cdot 10^{10}\ \Omega$, were used in eqn. 1 of the text. Response time of the strip chart recorder does not allow good resolution of the initial 0.2 s of any curves.

Measurements of the membrane potential V_M developed by the purple membrane under illumination are shown in Fig. 3. Again, illumination begins at time zero. Generally, the shape of the curve for V_M depends on the membrane resistance. High resistance membranes ($R > 10^{10}\ \Omega$) give curves like Fig. 3, curve A. However, lower resistance membranes ($R < 10^9\ \Omega$) give curves like Fig. 3, curve B*. Changing the membrane resistance has little

*The membrane resistance was lowered by the addition of 250 μg valinomycin per ml.

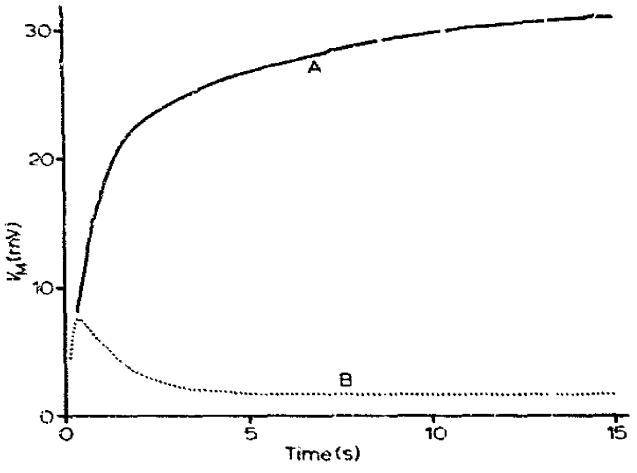

Fig. 3. Open-circuit voltage recordings. Time is from the start of illumination. Curve A (——) is for a membrane of resistance $4.8 \cdot 10^{10}\ \Omega$. Curve B (. . . .) is for a membrane of lower resistance, $9 \cdot 10^{8}\ \Omega$. The pump current for both curves is approximately 10^{-12} A.

observable effect on the short-circuit current measurements. In Fig. 3, curve A, the high membrane resistance R_M means the steady-state value of V_M is large since $V_M = i_p R_M$. At steady state, the leakage current of sodium and chloride ions is equal to the pump current i_p. Fig. 3, curve B reflects the fact that the initial value of the pump current can be large and yield a high transient V_M. The steady state value of V_M is small since the pump current is compensated by a relatively large leakage current for small V_M.

The proton pump current can be derived from the shape of the V_M versus time curve. The net rate at which charge is transferred across the membrane is

$$i = \frac{dQ}{dt} = C_M \frac{dV_M}{dt}$$

The net current i is due to two competing current terms: the pump current i_p and the leakage current $i_L = V_M/R$. Thus we may write

$$i_p = C_M \frac{dV_M}{dt} + \frac{V_M}{R} \tag{1}$$

and i_p can be determined from the shape of V_M versus time after the start of illumination. A result of this analysis yield the points shown in Fig. 2, which are consistent with the short-circuit measurement of i_p (Fig. 2, curve A) of the same membrane. When the light is turned off, i_p goes to zero and V_M decays with a time constant $R_M C_M$. During steady state illumination $dV_M/dt = 0$ and $i_p = V_M/R \approx 10^{-12}$ A. Generally, C_M depends somewhat on the membrane voltage V_M, as does R_M. Thus, the use of curves like those in Fig. 3 may not prove as useful as the more direct short-circuit current measurements.

Using the steady-state value of i_p, one can estimate the average number

of pumps involved if one takes the average cycling time of a pump to be about 10^{-2} s [11]. This yields approximately $5 \cdot 10^4$ pumps assuming a pump current of 10^{-12} A. This is consistent with the number of pumps expected in one purple membrane sheet*.

The very rapid rise of $i_p(t)$ to a value much in excess of i_p (steady state) when the light is turned on suggests the rapid depopulation of some initially occupied state. For example, one might speculate that in the dark, the four states of the protein observed in flash spectroscopy [11] are unoccupied except for the ground state (bR_{570}). A short time after the initiation of illumination, the intermediate states bR_{610}, bR_{550} and bR_{415} begin to fill up, even though the branching ratio of ($bR_{610} \rightarrow bR_{550}$) relative to ($bR_{610} \rightarrow bR_{570}$) is small †. The initial burst of current finally decays away as bR_{570} begins to depopulate, finally approaching a steady state in which the occupation of the various states is constant in time.

Another observation can be made based on the initial rapid rise of the pump current i_p after illumination. This feature is directly associated with the movement of charge across the membrane. The initial step in the photoreaction cycle ($bR_{610} \rightarrow bR_{550}$) discussed in the previous paragraph is coupled with the release of protons [11]. Therefore, we propose that the actual movement of charge across the purple membrane is associated with the release of protons in the photoreaction cycle of bacteriorhodopsin.

Preliminary experimental results have indicated that the purple membrane pump depends to some degree on the voltage bias across the membrane. Furthermore, the light intensity has an effect on the shape of the pump current versus time curve, as one might expect. A careful investigation of these curves may provide useful information on the rate constants in the photoreaction cycle. Better resolution of the leading edge can be achieved by faster recording methods and signal averaging techniques.

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*The area of a typical purple membrane sheet is about 10^8 Å^2 [14,15]. The area occupied by a single pump is about 1000 Å^2. Therefore, the number of pumps in a single sheet is of order 10^5.

†It should be noted that the $bR_{570} \rightarrow bR_{610}$ reaction is reversible, while the $bR_{610} \rightarrow bR_{550}$ reaction is not [11].

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