## BBA Report

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# The separation of voltage-dependent photoemfs and conductances in Rudin–Mueller membranes containing magnesium porphyrins

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SUMMARY

It is shown that the photoemf in a pigmented membrane is specific to the magnesium-porphyrin conductance channel. A null current method was devised to measure directly the voltage dependence of the photoemf and the magnesium-porphyrin conductance. Their voltage dependence is in agreement with the hypothesis that the magnesium-porphyrin cation is the majority carrier.

Observations of photoresponses from Rudin–Mueller membranes containing chromophores have been reported by several groups of investigators. Tien[1,2] has reported photovoltaic and photoconductive effects of membranes formed from a chloroplast extract. His conclusions have been modified by Hesketh[3]. The magnitude of the responses observed were generally small, mainly because the two aqueous solutions separated by the membrane were symmetrical. Subsequently, Tien[4], and Trissl and Läuger[5] reported larger photoresponses from asymmetrical systems with the pigmented membrane separating solutions of electron donors and acceptors. Similar results were obtained with membranes containing other pigments, *e.g.* β-carotene, retinols and retinals[6,7], and cyanine dyes[8]. Pant and Rosenberg[9] have reported photoelectric effects of a non-pigmented membrane in the presence of light-sensitive inorganic ions. In these experiments, the open-circuit voltage was measured. The photocurrent through the membrane driven by an external high-impedance voltage source was also measured. These methods have two inherent limitations. The "photoemf" so observed is actually the voltage-charging process of the membrane capacitance, driven by a photo-induced emf. The large value of the membrane capacitance distorts the time course of the "photoemf" so observed. The presence of a leakage conductance will also reduce the steady-state voltage. The second limitation arises

from the possibility of a voltage dependence of the photoemf and the membrane conductance. A difference between photocurrents under the same externally applied voltage of opposite polarities may be either due to a voltage-dependent conductance, or due to the presence of a photoemf, or even to the combination of both. Their separation is ambiguous, unless either the conductance or the photoemf is independent of membrane voltage. This fact is difficult to establish *a priori*. To circumvent these limitations, the voltage-clamp method[10] frequently used in electrophysiology is a powerful tool. This method has been used in the study of light-induced changes in the conductivity of Rudin–Mueller membranes in the presence of iodine and iodide ion[11]. Recovery times in the millisecond rage were observed. We have applied this method to the study of Rudin–Mueller membranes containing various pigments and separating aqueous solutions of oxidant and reductant. We have found that these photoresponses are the properties of a large class of compounds, *e.g.* chlorophyll, bacteriochlorophyll and metalloporphyrins. We report here a simple and direct method to measure separately the photoemf and the magnesium-porphyrin conductance of the pigmented membranes, under continuous illumination. Since the detailed effects using pulsed light sources are quite different, they will be reported elsewhere[12]. We also report here a simple experiment establishing the equivalent circuit of the system.

A Rudin–Mueller membrane of 2% egg lecithin and 1% cholesterol in *n*-decane-*n*-amyl ether mixture (10:1, v/v) containing about 10 mM magnesium octaethylporphyrin separated 10 mM solution of potassium ferricyanide and potassium ferrocyanide in 0.1 M NaCl and 1 mM sodium phosphate buffer at pH 7. The continuous light sources were always filtered through 5 cm of 0.1 M $CuSO_4$ solution and suitable yellow Corning filters to exclude infrared and short-wavelength radiation. Electrical signals of the membranes were monitored with a pair of calomel electrodes with saturated KCl bridges immersed in the two solutions. The voltage-clamp circuit consisted of an operational amplifier (Zeltex 133) with a feedback loop of adjustable gain and time constant and a variable voltage source (± 100 mV) in series on the input side. The choice of pigments and redox reagents was suggested by previous work on electron transfer by metalloporphyrin in the ground[13] and excited state[14]. The metalloporphyrins undergo perfectly reversible one-electron oxidations. The cation is stable, and is readily formed from the excited state with a variety of acceptors. Thus we expect the magnesium octaethylporphyrin to be oxidized to the cation at the membrane–ferricyanide interface, and the magnesium octaethylporphyrin cation to be reduced to magnesium octaethylporphyrin at membrane–ferrocyanide interface. In fact, we observed emf and conductance changes both in the dark and during illumination. The necessary control experiments were provided by non-pigmented membranes separating ferricyanide and ferrocyanide. They showed no emf and conductance change in the dark and during continuous illumination. Also, illumination of a pigmented membrane without redox reagents in the aqueous solutions showed only a small photoemf of about 1 mV, which depends on the direction of illumination, similar to that observed by Tien[1,2]. Upon adding redox reagents, much larger responses were observed with a polarity expected from the above redox reactions, namely, the oxidant side becomes

negative with respect to the reductant side.

In order to analyze the measured photosignals in terms of such electrical parameters as photoemf and photoconductance, it is desirable to first establish the equivalent circuit of the system. Two likely models are depicted in Fig. 1. In Model A, the light-

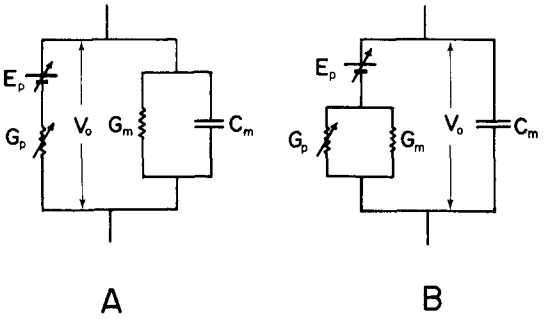


Fig. 1. Two models of equivalent circuit. $E_p$ is the emf and $G_p$ is the magnesium-porphyrin conductance due to the redox reaction of magnesium octaethylporphyrin. $G_m$ is the ionic leakage conductance originally present in a non-pigmented membrane. $C_m$ is the membrane capacitance. Model A predicts that $V_o$, the open-circuit voltage, decreases with increasing $G_m$ according to the relation: $V_o = E_p/(1 + G_m/G_p)$. Model B predicts $V_o$ to be independent of $G_m$. Under the voltage-clamp condition, the change of feedback current, $\Delta i$, when light shines on the membrane, remains constant with increasing $G_m$ in model A. In Model B, it increases with increasing $G_m$, and is proportional to $G_m + G_p$. Arrows indicated that the electrical elements are dependent on light flux.

sensitive emf ($E_p$) is specific to the magnesium-porphyrin conductance in the dark or light ($G_p$) and does not act across the membrane ionic leakage conductance ($G_m$); while the photoemf acts across both conductances in Model B. The voltage-clamp circuit keeps a fixed voltage across the membrane. Thus, on illumination, the change in its feedback current, $\Delta i$, reflects the change of current in the channels where a photoemf and/or a conductance change is present. If $G_m$ increases, we expect an increase of $\Delta i$ in Model B, while $\Delta i$ in Model A should remain constant. Nystatin is known to increase the conductance to anions of Rudin–Mueller membranes[15]. Thus experiments were carried out in which

TABLE I

INDEPENDENCE OF $\Delta i$ ON THE MEMBRANE IONIC LEAKAGE CONDUCTANCE

The light source was an iodine–quartz lamp filtered by 5 cm of 0.1 M $CuSO_4$ solution and a Corning 3-70 filter. Total light flux through the 1-mm$^2$ membrane was 1.2 mW (500–600 nm). Short-wavelength radiation must be excluded to avoid light-induced destruction of nystatin. The data was taken at 25 °C (maintained constant by a water bath and a thermostat) under constant stirring.

| *Nystatin concn (μg/ml)* | $G_m + G_p$ ($n\Omega^{-1}$) | $\Delta i$ (pA) |
|---|---|---|
| 0 | 3.3 | 15 ± 1 |
| 4 | 14 | 18 ± 1 |
| 5 | 190 | 14 ± 2 |

the conductance $G_m$ was varied by means of nystatin, and $\Delta i$ was measured with the membrane clamped at $V = 0$, and the light intensity fixed. The experimental results, given in Table I, show that $\Delta i$ is virtually independent of $G_m$ over two orders of magnitude and thus clearly indicate that Model A is the equivalent circuit consistent with the experimental data, *i.e.* the photoemf is specific to the magnesium-porphyrin channel. Another consequence of this model (A) is that $G_m$ acts as an internal shunt to the photoemf, and the open-circuit voltage should decrease with increasing $G_m$. This has also been observed in the nystatin-treated system, using continuous illumination.

The photoemf and the photoconductance are defined as the values of the emf and the conductance, respectively, in the light minus that in the dark. In fact, the photoconductance of the magnesium-porphyrin channel is very small. This is most clearly seen upon illuminating a symmetrical membrane, *i.e.* one having equal concentrations of oxidant and reductant on *both* sides. Since no net photoemf can be generated under this condition, any change of feedback current upon illumination at a non-zero clamping voltage will reflect the change of conductance. The results show that the magnesium-porphyrin conductance ($G_p$) increases by less than 10% upon illumination at clamping voltages of ± 100 mV. With this fact established, we can separate the photoemf ($E_p$) and the magnesium-porphyrin conductance ($G_p$) as functions of clamping voltage by means of a null current method. When the membrane voltage is clamped at a fixed value, continuous illumination causes a change in the feedback current, $\Delta i$. This current is brought back to the original level in the dark state by changing the clamping voltage. If the photoemf and the magnesium-porphyrin conductance are not voltage dependent, the change in clamping voltage would give the true photoemf. However, if the photoemf and the magnesium-porphyrin conductance are voltage dependent, analysis show that the difference in clamping voltage is a close approximation to the true photoemf at the original clamping voltage; the error is related to the dependence of the conductance and the emf on the membrane voltage, and is less than 10% in these experiments. Division of $\Delta i$ by the photoemf ($E_p$) gives the conductance ($G_p$) of the magnesium-porphyrin channel. The results (Fig. 2) show that both the photoemf and the magnesium-porphyrin conductance are voltage dependent. These results are similar to one reported by Hesketh[3]. Voltage dependence of the dark emf (approx. 30 mV) is more difficult to measure accurately, but a similar non-linearity is observed. It should be pointed out that both the emf and the magnesium-porphyrin conductance increase with an electric field in the direction that enhances the migration of the majority carrier, magnesium octaethylporphyrin cation, in the forward direction. The effect is not large, most likely because of a concentration-gradient build-up, *i.e.* polarization, at the interfaces. In fact, the rate of stirring of the aqueous solution influences some of these measurements. The data of Fig. 2 were obtained from the same membrane with constant stirring.

The steady state photoemf under continuous illumination depends on a complicated equilibrium involving the concentration gradient of redox reagents at the interfaces, the migration of magnesium porphyrin and its cation inside the membrane and their coupling with redox reagents at the interfaces. The stable steady state magnesium-porphyrin

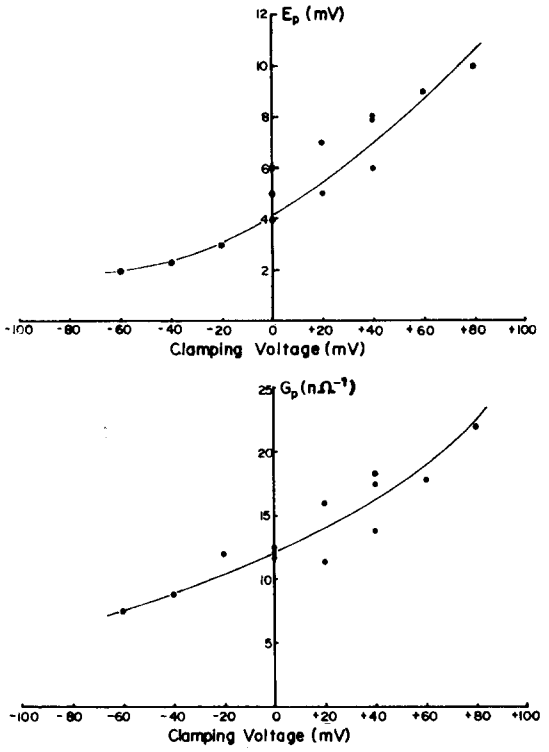


Fig. 2. Voltage dependence of the photoemf ($E_p$) and the magnesium-porphyrin conductance ($G_p$). The clamping voltage is defined as the potential of oxidant side minus that of reductant side. Illumination was from a 100-W tungsten lamp, filtered by 5 cm of 0.1 M $CuSO_4$ solution and a Corning 3-72 filter. Total light flux through the 2-mm$^2$ membrane was 1.6 mW (450–600 nm). The data were taken from the same membrane at 25.8 °C under constant stirring. The spread of the points represents repeated measurements at a given clamping voltage.

conductance depends on the capability of the magnesium porphyrin to complete a cycle of reaction at both interfaces. In the intensity range 5 to 50 mW of the continuous light used (530–600 nm), the photoemf is found to vary linearly with light intensity; while the magnesium-porphyrin conductance remains nearly constant.

Our interpretation of the photoreactions in pigmented membranes which we have just discussed is analogous to the scheme proposed by Grünhagen and Witt[16] to explain observations on photo-induced ion movement across photosynthetic membranes. Our voltage-clamp experiments on model membranes allow us to dissect these photoreactions into their specific components, and our experiments with pulsed light[12] show that the rise time of the photoemf is indeed extremely fast.

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