PHOTO-INDUCED POTENTIALS ACROSS A POLYMER STABILZIED PLANAR MEMBRANE, IN THE PRESENCE OF BACTERIORHODOPSIN

by

Paul Shieh and Lester Packer

Membrane Bioenergetics Group, Energy and Environment Division, Lawrence Berkeley Laboratory and Department of Physiology-Anatomy, University of California, Berkeley, CA 94720

Received May 27,1976

SUMMARY: Black lipid planar membranes were prepared by incorporating polymers such as polystyrene in a membrane forming solution. The polymer-incorporated planar membranes showed greater stability to applied electric fields and have longer lifetimes. Photopotentials were studied under several conditions; with bacteriorhodopsin in the planar membrane; with bacteriorhodopsin in liposomes; with bacteriorhodopsin fragments in suspension; and with bacteriorhodopsin both in the planar membrane and in liposomes. Skulachev's laboratory has reported that bacteriorhodopsin-liposomes develop potentials across a black lipid planar membrane. In our studies, because the polymer incorporated planar membranes are very stable, it was possible to obtain somewhat larger photopotentials in the presence of bacteriorhodopsin ranging between 30-500 mV. The enhancement of bacteriorhodopsin catalyzed photopotentials, found in the presence of Ca^{++} (or other bivalent cations) and/or applied electric fields, appeared to result from an orientation effect of bacteriorhodopsin at the membrane interface.

INTRODUCTION It is known that a planar phospholipid bilayer containing chlorophyll as a photosensitizer, if separated by two compartments containing solute of differing redox potential, can, upon illumination, cause charge movement and production of a photopotential (1). Although this system could be used in principle, for conversion of light to electrical energy (2), it suffers from instability of the membrane itself and photocatalysts. In the present study a method is described for the stabilization of the lipid membrane using polymers like polystyrene. Also the photopotentials developed across this polymer stabilized lipid membrane tested in the presence of BR* are described. We have chosen BR as the photosensitive pigment, because it is found in an aggregated state in the "purple membrane" areas of Halobacterium halobium (3), where it acts as a light-dependent proton pump (4,5). We have tested BR in several ways; with the pigment in the planar membrane, in liposomes, and as fragments in suspension.

---

* Abbreviation: Bacteriorhodopsin, BR

The results are compared with studies using BR-liposomes and a planar membrane reported from Skulachev's laboratory (6-8).

METHODS

Electrical Measurements. Planar membranes were formed over a 2 mm circular hole in a 10 ml teflon cup which was then placed inside a 20 ml glass cup; membrane potential measurements were made by a pair of calomel electrodes immersed in the aqueous solutions separated by the membrane. The inner compartment was where the measuring electrode was located and the outer compartment was where the grounded electrode was immersed. Both aqueous compartments contained 0.1 M sucrose and 5 mM Tris HCl (pH = 7.3). Illumination was from a projector lamp with a 300 W tungsten filament with an output of 20 mW/cm^2 at the planar membrane surface. Membrane resistance (R_m), membrane thickness (t_m) and the activation energy were calculated after Tien (9).

Test Conditions.

I. Bacteriorhodopsin (BR) incorporated within the planar membrane. The planar membrane forming solution contained: 100 mg polystyrene resin (Dowex-I), 5mg dried purple membrane fragments and 1 ml oxidized cholesterol (0.2 mg/ml) in octane.

II. BR added into inner compartment. The membrane forming solution contained: 100 mg polystyrene/ml of oxidized cholesterol/octane mixture. After a planar membrane was formed, purple membrane fragments containing 0.25 mg BR/ml were added to the inner aqueous compartment.

III. BR incorporated into liposomes. The planar membrane was made as in II. Afterward 0.5 ml liposomes were added to inner aqueous compartment. The stock suspension of liposomes (15 mg egg lecithin/ml) was prepared by sonicating a mixture of egg lecithin and water, then 1 ml purple membrane fragments (5 mg protein/ml H_2O) were mixed with 1 ml stock suspension of liposomes and sonicated for 1 minute.

IV. BR incorporated both in the planar membrane and into liposomes. The planar membrane was made as in I, then 0.5 ml liposomes were added to inner compartment.

RESULTS

Electrical Properties of Polymer Incorporated Planar Membrane.

The electrical properties of planar membranes prepared with various concentrations of polystyrene are summarized in Figure 1. As polystyrene increases in the membrane forming solution there is a fall in capacitance, rise in resistance, and increase in the calculated membrane thickness. Polystyrene also increases the stability of the membrane to breakdown by applied voltage. However, at the higher polystyrene concentrations membrane thickness substantially increases causing the breakdown electric field strength (which is a function of breakdown voltage divided by thickness) to pass through a maximum at about 100 mg/ml. Membranes thinner than 200 A^0 are generally considered as "black" lipid membranes and thus in our other studies membranes were prepared with 100 mg/ml polystyrene in the membrane forming solution; we have found that membranes prepared this way

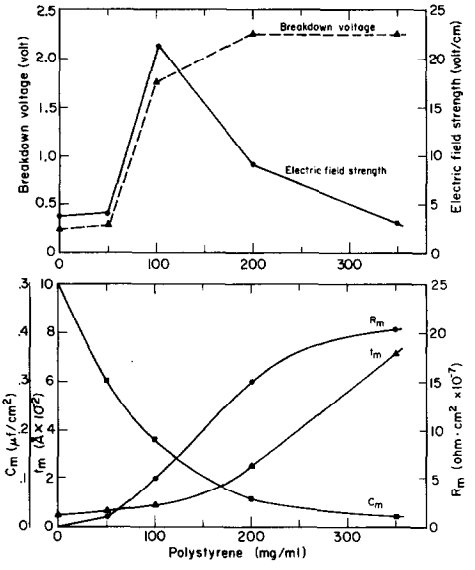

Figure 1. Effect of polystyrene on the electrical properties of planar membranes.

TABLE I

EFFECT OF BACTERIORHODOPSIN AND POLYSTYRENE ON THE STABILITY OF THE BLACK LIPID PLANAR MEMBRANE

Planar Membrane	Membrane Resistance (ohm/cm^2)	Lifetime		Breakdown Voltage (applied volts)
		Dark (hours)	Illuminated (hours)	
Control[a]	$5-8 \times 10^6$	0.50	0.16-0.31	0.20-0.25
BR[b]	$5-6 \times 10^5$	0.16	0.10-0.20	0.15-0.20
Polystyrene[c]	$5-6 \times 10^7$	3-5	0.31-0.80	1.50-2.10
BR + polystyrene	$2-4 \times 10^6$	3-5	0.30-0.80	1.30-1.80

[a] 0.2 mg oxidized cholesterol/ml octane.

[b] 5 mg BR (as purple membrane fragments)/ml added to a.

[c] 100 mg/ml added to a.

have enhanced stability both in terms of lifespan and applied fields (cf. Table I). Similar electrical properties were obtained when 47 mg/ml polyacrylamide was substituted for 100 mg/ml polystyrene, as the polymer in the membrane forming solution.

In a different study King and Steinraut (11) reported that adding glutaraldehyde crosslinked polylysine to a solution bathing a planar lipid membrane increased lifetime at pH 11, but the conductance of such membranes was 70% reduced.

Photopotentials in the Presence of Bacteriorhodopsin (Fig. 2)

I. With BR incorporated in the planar membrane—illumination results in a potential change of about 30 mV which reaches saturation in about 3 seconds; when the light is switched off the potential decays to its dark level in 4 seconds. If 1mM $CaCl_2$ is present (in the inner compartment) the photopotential is about 50 mV.

II. With BR added to inner aqueous compartment—illumination results in a slow rise in potential (with positive polarity in this compartment), followed by a slow decay over one minute. The slow response and small magnitude of the photopotential probably arises from the

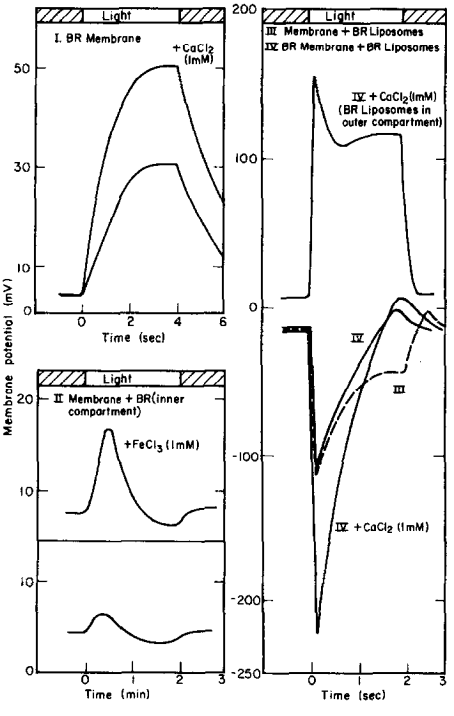

Figure 2. Photoresponses of planar membranes in the presence of BR.

random orientation of purple membrane fragments in suspension. The magnitude of the photopotential is enhanced if the pH is lowered in the outer compartment like when HCl or an aerobic $FeCl_3$ solution is added.

III. With BR incorporated into liposomes--illumination results in a 100 mV transient photopotential. The negative rise in potential in the inner compartment containing BR-liposomes occurs in 150 msec. In this case, decay is incomplete reaching a steady state level above the dark level. However, the signal completely decays when the light is switched off.

IV. With BR both in the planar membrane and liposomes - illumination results in a 100 mV transient photopotential. Like in III, the rise in potential also occurs in 150 msec, but the signal decays to its original dark level even under continuous light.

Enhancement of Photopotentials

Since polymer incorporated planar membranes are able to withstand applied fields without breakage, experiments were performed to determine how this would effect photopotentials. A linear relationship between the photopotential and the applied field was found when purple membrane fragments were located in one aqueous compartment and absent in the planar membrane. Figure 3 shows that the magnitude of photopotential increased with the applied field. A maximum photoresponse of

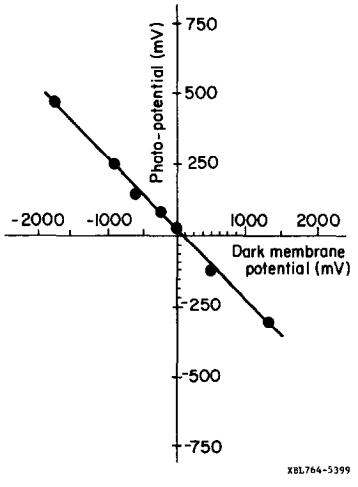

Figure 3. Relation between the photoresponse of polymer incorporated planar lipid membrane and applied electrical field in the presence of purple membrane fragments in suspension.

500 mV was obtained when the applied field was up to 1.5-2 volt. At zero applied field a photopotential of 25 mV was observed.

Ca^{++} ions are known to reduce the electrostatic repulsion of charged phospholipid head groups and to promote vesicle fusion (10). Skulachev's laboratory (10) showed that Ca^{++} permitted BR containing liposomes to interact with a black lipid planar membrane and photopotential development. We find that a two-fold increase in the magnitude of photoresponse to 200 mV occurs if 1 mM $CaCl_2$ is added to the compartment having BR liposomes (test condition III). In this case, the photoresponse was dependent upon the time of dark incubation, with the maximum photoresponse occurring after 60 minutes of dark pre-incubation with Ca^{2+}. The transient photopotential in III disappeared and became a saturated photopotential (IV, Fig. 2) when BR liposomes and 1 mM $CaCl_2$ were in the outer compartment. The saturated photopotential observed when BR-liposomes and $CaCl_2$ were added to the outer compartment may be explained by a similar direction of H^+ pump activity across the planar membrane by BR (in the planar membrane and in liposomes when they are located in the outer compartment).

No photopotentials were found in the absence of purple membrane fragments and the photovoltaic action spectrum of polymer incorporated membrane closely coincided with the absorption spectrum of the purple membrane fragments. This implies that BR is the pigment that is catalyzing charge transfer across the planar membrane.

DISCUSSION

Using crosslinked polymers like polystyrene in the membrane forming solution, black lipid planar membranes can be prepared with electrical properties similar to control black lipid and natural membranes, but manifest greater stability in terms of duration and of ability to withstand applied voltage of $\geq$ 1 volt without breakage. Thus our findings will enhance the usefulness of the planar membranes, particularly for reconstitution studies because the incorporation of large protein complexes, confer even greater instability to planar membranes. Using the polymer stabilized membrane we have shown that large photopotentials can be developed by BR (added in the form of "purple membrane fragments") when it is incorporated into the planar membrane.

The presence of BR in egg lecithin liposomes also allows large photopotentials to be developed across the planar membrane, but only if Ca^{++} is present to cause interaction of liposomes with the planar membrane. Since the photoresponse was enhanced by pre-incubation

of the liposomes with the planar membrane in the dark in the presence of Ca^{++}, this suggests that time was required for interaction of the two components. However, Ca^{++} may also effect the orientation of purple membrane fragments at the membrane interface (Fig. 2,I). Applied electric fields also resulted in an increased photoresponse (with a polarity opposite to the applied field) up to 500 mV. Further studies in the mechanisms of photopotential generation, by both Ca^{++} and electric fields, including the role of orientation effects are in progress.

ACKNOWLEDGEMENTS: The authors would like to thank Dr. Janos Lanyi for providing purple membrane preparations and Dr. Tetsuya Konishi for providing the bacteriorhodopsin containing vesicles; also, for their helpful discussion of this work. This research was supported by the U. S. Energy Research and Development Administration.

REFERENCES

1. Tien, H.T. (1968) J. Phys. Chem., 72, 4512.
2. Shieh, P., and Tien, H.T. (1974) J. Bioenergetics, 6, 45-55.
3. Blaurock, A.E., and Stoeckenius, W. (1971) Nature New Biology 233, 152.
4. Lozier, R., Bogomolni, R., and Stoeckenius, W. (1975) Biophysical Journal 15, 955.
5. MacDonald, R.E., and Lanyi, J.K. (1975) Biochemistry, 14, 2882.
6. Kayushin, L.P., and Skulachev, V.P. (1974) FEBS Letters, 39, 39.
7. Drachev, L.A., Kaulen, A.D., Ostroumov, S.A., and Skulachev, V.P. (1974) FEBS Letters, 39, 43.
8. Skulachev, V.P., et al. (1974) Doklady Akademi Nauk, SSSR 218, No. 2, 481.
9. Tien, H.T. (1974) Bilayer Lipid Membrane, Chapter 11, p. 496, Marcel Dekker, Inc., New York.
10. Drachev, L. A., Jasaitis, A. A., Kaulen, A. D., Kondrashin, A. A., Liberman, E. A., Nemecek, L. B., Ostroumov, S. A., Semenov, A., Yu, A., and Skulachev, V. A. (1974) Nature, 249, 321.
11. King. T. E., and Steinrauf, L. K. (1972) Biochem. Biophys. Res. Comm. 49:1433.