PHOTOELECTRIC CONVERSION BY BACTERIORHODOPSIN IN CHARGED SYNTHETIC MEMBRANES

KEHAR SINGH, RAFI KORENSTEIN, HELENE LEBEDEVA, AND S. ROY CAPLAN,
Department of Membrane Research, The Weizmann Institute of Science, Rehovot, Israel

ABSTRACT Photoelectroactivity of oriented purple membrane layers attached to an ion exchange film has been investigated. The action spectrum of the photocurrent followed the absorption spectrum of bacteriorhodopsin. The intactness of structure and function of bacteriorhodopsin was demonstrated by studies of absorption and photocycle kinetics. The direction of the photocurrent suggests that the extracellular surface of purple membrane is more positive. Photocurrents as high as 20 μA cm^{-2} were obtained in some preparations. The dependence of steady-state photocurrents on intensity of illumination and temperature was also studied. The initial rate of build-up of photocurrent depends linearly on the intensity of illumination while the off rate does not exhibit any dependence on the intensity of illumination. With rise in temperature an increase in the steady state photocurrent has been observed. This dependence was found to be linear when increase of the photocurrent due to proton translocation alone was considered.

INTRODUCTION

Purple membrane fragments isolated from *Halobacterium halobium* contain bacteriorhodopsin (1), a protein which acts as a light-driven proton pump as a result of photocycle-associated conformational changes (2–4). The fragments have been incorporated into bilayer lipid membranes (5, 6) stabilized by polymers in some instances (7), or attached to lipid bilayer (8, 9, 10, 11), lipid-impregnated Millipore filters (12, 13, 14, 15) or collodion film (15), with the object of devising electrochemical systems for converting light into electrical energy. Photovoltaic cells, using dried layers of purple membrane oriented by electric field during drying (16), and dried multilayers of air-water interface films of oriented purple membrane and lipids (17, 18), have also been used. A photovoltaic device constructed from bacteriorhodopsin, lipid, and Millipore filter capable of generating electrical potentials high enough to split water into hydrogen and oxygen has recently been reported (19). The photoelectric currents generated by all these membranes are usually very small due to high membrane resistance. To lower the membrane resistance, incorporation of oriented purple membrane fragments in a cation selective hydrogel of acrylic acid and acrylamide was carried out under an electric field (20). Although the extent of orientation achieved was small, high photocurrents could be

---

Dr. Singh is a visiting scientist from the University of Gorakhpur, Gorakhpur (U.P.) India.
Dr. Korenstein is an Incumbent of the Association of Friends of the Weizmann Institute of Science in Israel Career Development Chair.

obtained. Orientation by electric field is an attractive possibility but the electric anisotropy required to produce a reasonably large torque is often too small to produce more than a modest degree of orientation (21). Furthermore, because of the electrolytic nature of the medium containing purple membrane fragments, high electrical fields could not be applied.

Purple membrane fragments spread on a glass slide to form thin layers after drying are efficiently oriented parallel to the supporting surface (22, 23). We have chosen this method to orient the purple membrane on a charged membrane surface to obtain selectivity in electrostatic binding with respect to the two faces of the fragments. The difference in the surface potentials of the faces has already been demonstrated (24) though the nature of the charge of the extracellular purple membrane face could not be adequately determined. The high photocurrents we observed clearly indicate nonrandom orientation of the purple membrane faces. The direction of the photocurrent suggests that the extracellular surface of purple membrane is more positive. The action spectrum of the photocurrent matched the absorption of bacteriorhodopsin and the photocycle was found to remain intact. The dependence of photocurrent on light intensity and temperature has also been studied.

MATERIALS AND METHODS

Preparation of Sulfonated Polysulphone Membrances

Sulfonated polysulphone membranes were prepared using the method described by Broussé et al. (25) with some modifications.

30 g of polysulphone resin (Union Carbide Corp., New York) and 204 ml of 1-2 dichloroethane (BDH Chemicals Ltd., Poole, England) were mixed at 50°C. A mixture of dichloroethane and chlorosulfonic acid (170 ml:5 ml) was then added dropwise. The resulting solid was washed with dichloroethane and dissolved in dimethyl-formamide (Fluka A.G., Basel, Switzerland). This solution was slowly added to 10% Na_2HPO_4 (AR) solution. The white precipitate (polymer) obtained was washed repeatedly with distilled water and dried under vacuum for 12 h at 40°C. A 30% solution of the polymer in dimethylformamide was cast on a clean, dry glass plate. The cast solution was dried at 60°C in a ventilated oven for ~30 min. The plate was then immersed in water to remove the film. The film thickness in all preparations was 0.2 mm. The cation transport number, τ_+, for the film estimated from the diffusion potential measured with calomel electrodes in a gradient of $10^{-1}/10^{-2}$ M KCl was found to be 0.98.

Membrane Formation

(The active membrane area in all experiments was 15 cm^2.)

An aqueous supension of purple membrane fragments (isolated from *H. halobium*, strain M_1, as described by Oesterhelt and Stoeckenius [26]) was spread on a polysulphone ion exchange film and allowed to dry under laboratory conditions for a couple of days. The dried purple membrane layer was sandwiched between the supporting film and another similar cation selective polysulphone film by means of acrylic acid and acrylamide gel. A freshly prepared solution of 11.5% (wt/wt) acrylamide, 6% acrylic acid (both from BDH Chemicals Ltd.), 2% Bis acrylamide (Eastman Kodak Co., Rochester, N.Y.) and 2% TEMED (N,N,N',N'-tetra-methylethylenediamine) at pH 4 was polymerised between the two films, each communicating with an aqueous potassium chloride solution. This operation, which was accomplished in the experimental cell, itself permitted equilibration of the gel with the solution during its formation. The danger of mechanical damage to the gel due to changed swelling could thus be obviated. The ion exchange films supporting the gel on either side provided considerable additional mechanical strength and the composite structure could be used for prolonged periods without any difficulty.

The equilibrating solution was renewed before starting the experiment so that its composition remained the same as before equilibration.

Measurement of Short Circuit Current

As described above, the membrane was formed in the experimental cell. This was a double walled lucite cell in which the membrane could be illuminated at right angles to the surface from both sides. The cell could be maintained at the desired temperature by circulating thermostated water. Shortcircuit currents were measured as previously described (20), using a voltage clamp. The experimental cell was equipped with the usual current carrying and potential sensing electrodes. Salt bridges positioned near the membrane faces were connected to saturated calomel electrodes to measure potential difference, while for current measurements salt bridges near the ends of the cells were connected to silver-silver chloride electrodes. Illumination was done with slide projectors each supplying a maximum of 1,750 Wm^{-2} (quartz iodide lamp 24 V, 150 W). For intensity variation, neutral filters were used. To study wavelength dependence Balzers interference broad band filters (50-nm band width) (Balzers Corp., Nashua, N.H.) in combination with neutral density Schott filters were used so as to produce the same photon fluxes at the different wavelengths studied.

For the determination of membrane resistance known potential differences (up to 5 mV) were clamped in either direction and the resulting currents were measured.

Flash photolytic studies were carried out in a conventional flash photometry system. The air-filled flash tubes were operated at 20 KV and gave total energy of ~400 J.

RESULTS AND DISCUSSION

Purple membrane fragments attached to sulfonated polysulphone ion exchange films generated high photocurrents (Fig. 1). All preparations exhibited photoelectroactivity and maintained the same current direction in all cases. The total number of twenty membranes (n = 20) that were studied gave the following results: 2 ± 0.5 $\mu A\ cm^{-2}$ (n = 7), 5 ± 1 $\mu A.cm^{-2}$ (n = 3), 8 ± 0.5 $\mu A.cm^{-2}$ (n = 5), 12 ± 1 $\mu A.cm^{-2}$ (n = 3), and 17 ± 3 $\mu A.cm^{-2}$ (n = 2). The

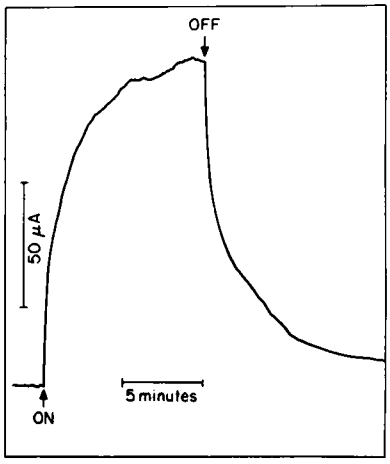

FIGURE 1

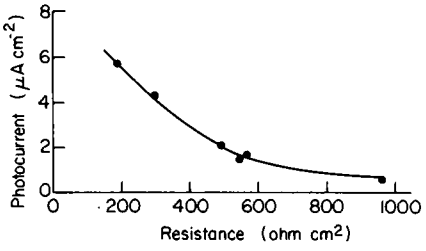

FIGURE 2

FIGURE 1 A typical result showing the build up and decay of photoelectric current. The membrane was equilibrated with 0.1 M KCl and 0.1 M MOPS at pH 7 and illuminated at right angles to its surface from both the sides.

FIGURE 2 Photocurrents obtained from membranes having different resistances. 1 ml of purple membrane suspension (~0.3 mM bacteriorhodopsin) was used during each preparation. Measurements were carried out at 30°C using 0.1 M KCl and 0.1 M MOPS solution.

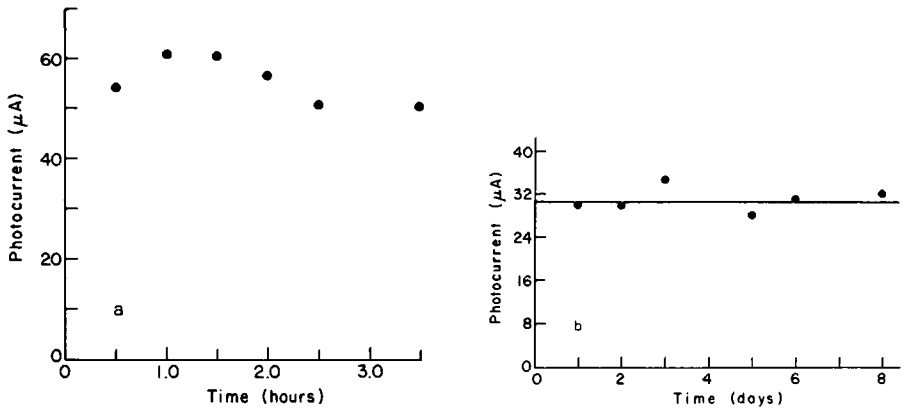

FIGURE 3 Photoresponsiveness of a representative membrane for prolonged periods. Intensity of illumination, 3,750 Wm^{-2}. (*a*) The membrane was subjected to continuous illumination. (*b*) The membrane was illuminated for ~1 h every day to measure photocurrents.

membrane resistances ranged between 10^2 and 10^3 Ω cm^2. The photoelectroactivity seemed to depend on factors such as concentration of purple membrane suspension, the rate of drying, thickness of the purple membrane thin layer, and the resistance of the membrane. That the last factor is important is clearly shown by the data included in Fig. 2 for six membranes prepared under similar conditions. The membranes showed photoresponsiveness for prolonged periods without any appreciable decline in photoelectroactivity (Fig. 3 *a* and *b*).

The action spectrum of the photocurrent followed the absorption spectrum of bacteriorhodopsin (Fig. 4), demonstrating that bacteriorhodopsin is responsible for the proton transport. The intactness of structure and function of bacteriorhodopsin attached to the synthetic membrane matrix was demonstrated by absorption and photocycle kinetic studies. The absorption spectrum of bacteriorhodopsin bound to the polymer matrix matches the one obtained from purple membranes suspended in water. Moreover, it is capable of undergoing a dark light adaptation which corresponds to the 13-*cis* → all-*trans* photoisomerization. The

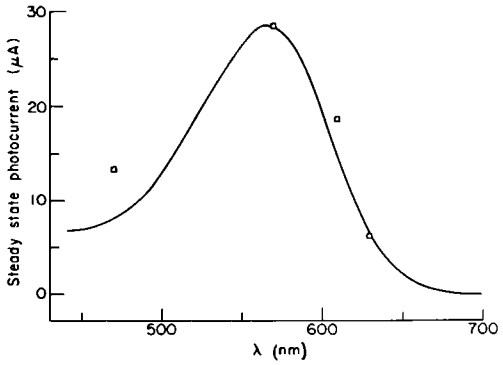

FIGURE 4 Action spectrum of the photocurrent. The intensity of light was regulated by a combination of interference and neutral filters to yield the same photon flux of 180 *n* Einstein. The continuous line is the absorption spectrum of bacteriorhodopsin normalized at 570 nm.

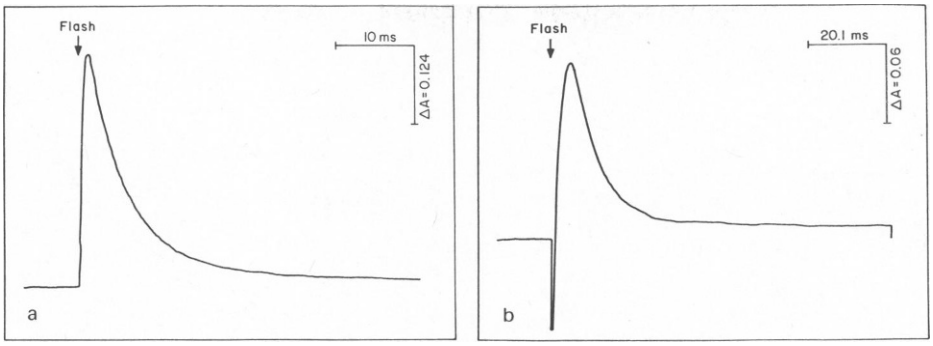

FIGURE 5 The photocycle decay kinetics of the M_{412} and O_{660} phototransients of the purple membrane attached to a synthetic membrane matrix. (*a*) The decay kinetics of M_{412}. (*b*) The decay kinetics of O_{660}.

photocycle kinetics shows the two long-lived intermediates M_{412} and O_{660}. The O_{660} was shown previously (27) to be very sensitive to perturbation, such as temperature, pH, or relative humidity. Its existence in the polymer matrix shows that the microenvironment was not changed much in comparison with aqueous suspensions of purple membrane. The decay kinetics of M_{412} and O_{660} (Fig. 5 *a* and *b*) exhibit similar relaxation times as observed in aqueous suspensions of purple membrane.

The effect of intensity of illumination on photocurrent is shown in Fig. 6. The steady state photocurrent exhibits linear dependence only at low intensities and becomes nonlinear at higher levels of illumination. The initial slope during build-up of the photocurrent increases linearly with light intensity (Fig. 7). It is clear from Fig. 8 that the off rate does not depend on

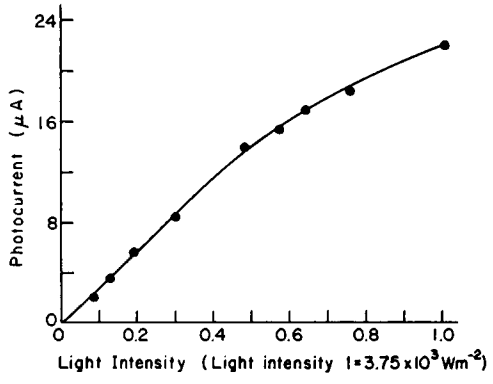

FIGURE 6

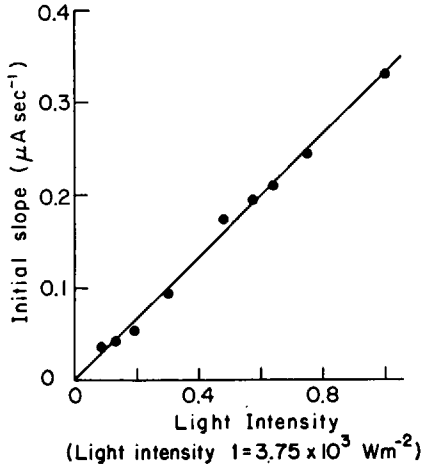

FIGURE 7

FIGURE 6 Dependence of photocurrents on intensity of illumination. The equilibrated membrane was illuminated only from one side with a halogen lamp (24 V, 250 W) having a 3,750-Wm^{-2} maximal intensity of illumination. The intensity was varied using neutral filters.

FIGURE 7 Variation of initial slope with intensity of illumination during photocurrent build-up.

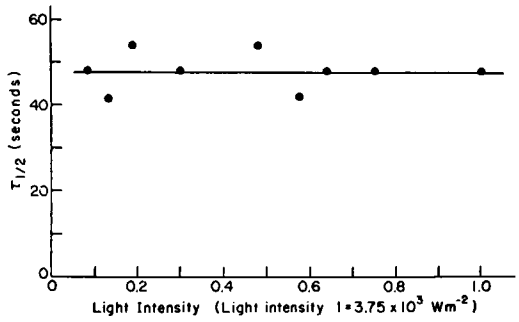

FIGURE 8

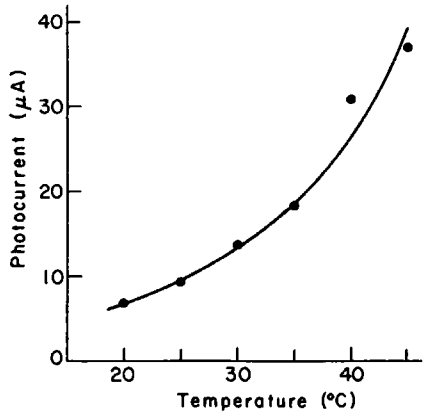

FIGURE 9

FIGURE 8 Dependence of $\tau_{1/2}$ of the off-reaction on the intensity of illumination.
FIGURE 9 Variation of photocurrent with temperature.

the intensity of illumination. Similar conclusions have been derived from photovoltaic studies (17) on oriented purple membrane monolayers.

The dependence of photocurrent on temperature (Fig. 9) shows a nonlinear relationship. This temperature-dependent change in photocurrent can be attributed to two factors: (*a*) change in the resistance of the composite membrane with temperature, and (*b*) the temperature-dependent activity of the proton pump of the bacteriorhodopsin. To distinguish between the two contributions, the dependence of the resistance of the membrane on temperature was studied (Fig. 10). Here, again, a nonlinear dependence was found. To obtain the net contribution of the proton pump of bacteriorhodopsin to the observed photocurrent, the contribution due to the change of resistance with temperature was accounted for at each temperature. The dependence of photocurrent on temperature due to temperature-dependent translocation of protons is shown in Fig. 11. The linear relationship of the photocurrent with temperature yields a positive temperature coefficient of 0.2 μA/deg.

Increase of the photocycle kinetics with temperature has been observed (28). Thus an increase in the turnover of the proton pump is to be expected. Indeed an increase of light-induced pH change with temperature was observed in sub-bacterial particles (29) and in proteoliposomes (30). Moreover, the linear dependence in the temperature region studied by us agrees with a linear region that can be observed in light-induced pH changes in sub-bacterial particles (29).

The present study clearly indicates that during drying the purple membrane fragments are nonrandomly oriented at the ion exchange film. The direction of current in all preparations indicates a light-dependent proton translocation towards the primary supporting film side. Since protons are translocated from the intracellular to the extracellular surface of the purple membrane, it appears that during drying the purple membrane fragments attach with their extracellular side towards the ion exchange film. Since the selective binding is presumably due to electrostatic interactions between the purple membrane face and the negatively-charged support, the extracellular face must bear a more positive surface charge than the intracellular face.

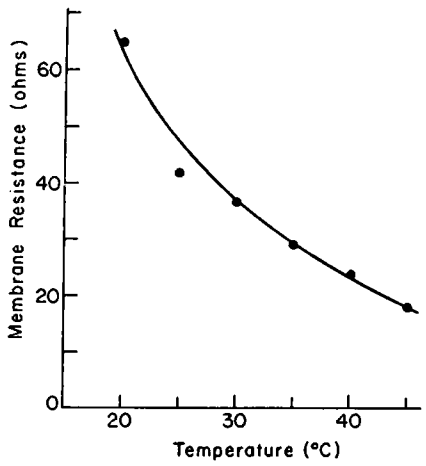

FIGURE 10

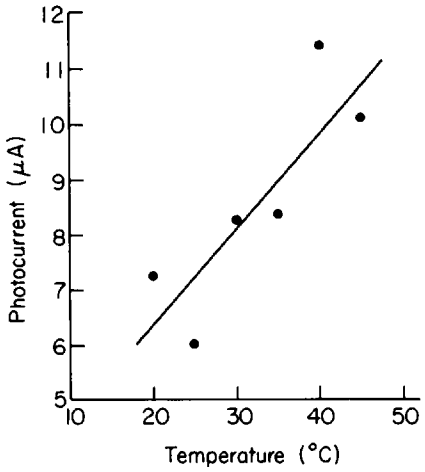

FIGURE 11

FIGURE 10 Variation of the resistance of the equilibrated membrane with temperature.
FIGURE 11 The photocurrent attributed to proton translocation by bacteriorhodopsin plotted as a function of temperature. (Line by least squares.)

The photocurrents obtained in the present investigation are the highest obtained so far (Table I). The values are, however, much lower than a current density of $\sim 10^{-3}$A cm^{-2} theoretically estimated for an idealized case (31) on the basis of the following assumptions: (*a*) The diameter of a purple membrane fragment is 0.5 μm (32) and it contains 10^5 bacteriorhodopsin molecules (33). (*b*) One proton is pumped per photocycle and the turnover of bacteriorhodopsin is 100 s^{-1} (4). (*c*) The purple membrane fragments form a monolayer and are oriented completely nonrandomly. (*d*) No current losses due to ionic migration exist. (*e*) Continuous irradiation by saturating light is used. The big difference between the experimental values and the one calculated for the idealized case arises mainly because of the inapplicability of assumptions *c–e*. In the present study current loss due to coion (Cl^-, OH^-)

TABLE I
PHOTOCURRENTS GENERATED BY BACTERIORHODOPSIN CONTAINING MEMBRANE SYSTEMS

Membrane type	Resistance	Intensity of illumination	Maximal photocurrent	Reference
	$\Omega\ cm^2$	$W\ cm^{-2}$	$A\ cm^{-2}$	
Purple membranes in planar lipid membranes	10^8	2×10^{-2}	10^{-9}	5
	2×10^{10}		10^{-9}	6
Purple membranes attached to bilayer lipid membrane	5×10^7	4×10^{-3}	10^{-10}	8
	10^5	1×10^{-2}	10^{-7}	9
Proteoliposomes attached to lipid membrane	2×10^{10}	2×10^{-2}	10^{-9}	10
	$>10^{10}$	2×10^{-2}	10^{-9}	11
Electrically oriented purple membranes in a hydrogel	2×10^2	15×10^{-2}	7×10^{-6}	20
Purple membranes bound to a cation selective film	10^2	37.5×10^{-2}	20×10^{-6}	Present work

migration in the direction of the light-driven protons has been practically overcome by the use of highly selective cation-exchange films. The leakage due to counter ion (K^+, H^+) migration may, however, still be substantial. For further progress, therefore, a solution to the twin problem of incomplete orientation and current dissipation will have to be found. Enhancement of orientation may be possible if the purple membrane fragments are attached during drying under the simultaneous action of high electric or magnetic fields applied in the appropriate direction. To overcome current losses from counter ion migration, K^+ ion may be replaced by polyvalent counterions that exhibit pronounced reduction in mobility in the ion exchanger (34) by their stronger electrostatic interaction with the charged matrix. We are presently working along these lines.

It may be stressed that the method of membrane formation we have used in the present study has two distinct advantages over the method reported earlier (22): (*a*) the structure possesses acceptable mechanical stability and manipulability, and (*b*) the dried purple membrane fragments are bound between cation selective films. This facilitates the migration of protons translocated by light, which reveals high photocurrents.

We are grateful to Dr. G. Tanny for providing facilities for the preparation of sulfonated polysulphone films.

This study was supported by grants from the National Council for Research and Development, Israel, and the KFA Jülich, W. Germany, and from the United States-Israel Binational Science Foundation (BSF), Jerusalem, Israel.

Received for publication 1 April 1980.

REFERENCES

1. OESTERHELT, D., and W. STOECKENIUS. 1971. Rhodopsin-like protein from the purple membrane of *Halobacterium halobium*. *Nat. New Biol.* **233**:149–152.
2. OESTERHELT, D., and W. STOECKENIUS. 1973. Functions of a new photoreceptor membrane. *Proc. Natl. Acad. Sci. U.S.A.* **70**:2853–2857.
3. STOECKENIUS, W., and R. H. LOZIER. 1974. Light energy conversion in *Halobacterium halobium*. *J. Supramol. Struct.* **2**:769–774.
4. LOZIER, R. H., R. A. BOGOMOLNI, and W. STOECKENIUS. 1975. Bacteriorhodopsin: a light-driven proton pump in *Halobacterium halobium*. *Biophys. J.* **15**:955–962.
5. DRACHEV, L. A., A. D. KAULEN, S. A. OSTROUMOV, and V. P. SKULACHEV. 1974. Electrogenesis by a bacteriorhodopsin incorporated in a planar phospholipid membrane. *FEBS Lett.* **39**:43–45.
6. HERRMANN, T. R., and G. W. RAYFIELD. 1976. A measurement of the proton pump currents generated by bacteriorhodopsin in black lipid membranes. *Biochim. Biophys. Acta.* **443**:623–628.
7. SHIEH, P., and L. PACKER. 1976. Photo-induced potentials across a polymer stabalized planar membrane, in the presence of bacteriorhodopsin. *Biochem. Biophys. Res. Commun.* **72**:603–609.
8. DANCSHAZY, Z., and B. KARVALY. 1976. Incorporation of bacteriorhodopsin into bilayer lipid membrane: a photoelectric spectroscopic study. *FEBS Lett.* **72**:136–138.
9. BAMBERG, E., H. J. APELL, N. A. DENCHER, W. SPERLING, H. STIEVE, and P. LAÜGER. 1979. Photocurrents generated by bacteriorhodopsin on planar bilayer membranes. *Biophys. Struct. Mech.* **5**:277–292.
10. DRACHEV, L. A., V. N. FROLOV, A. D. KAULEN, E. A. LIBERMAN, S. A. OSTROUMOV, V. G. PLAKUMOVA, A. YU. SEMENOV, and V. P. SKULACHEV. 1976. Reconstitution of biological molecular generators of electric current. *J. Biol. Chem.* **251**:7059–7065.
11. HERMANN, T. R., and G. W. RAYFIELD. 1978. The electrical response to light of bacteriorhodopsin in planar membranes. *Biophys. J.* **21**:111–125.
12. SKULACHEV, V.P. 1976. Conversion of light energy into electric energy by bacteriorhodopsin. *FEBS Lett.* **64**:23–25.
13. BLOCK, M. C., K. J. HELLINGWERF, and K. VAN DAM. 1977. Reconstitution of bacteriorhodopsin in a millipore filter system. *FEBS Lett.* **76**:45–50.

14. BLOCK, M. C., and K. VAN DAM. 1978. Association of bacteriorhodopsin-containing phospholipid viscles with phospholipid-impregnated millipore filters. *Biochim. Biophy. Acta.* **507**:48–61.
15. ABDULAEV, N. G., M. YU-FEIGINA, A. V. KISELEV, YU. A. OVCHINNIKOV, L. A. DRACHEV, A. D. KAULEN, L. V. KHITRINA, and V. P. SKULACHEV. 1978. Products of limited proteolysis of bacteriorhodopsin generate a membrane potential. *FEBS Lett.* **90/2**:190–194.
16. NAGY, K. 1978. Photoelectric activity of dried, oriented layers of purple membrane from *Halobacterium halobium. Biochem. Biophys. Res. Commun.* **85**:383–390.
17. HWANG, S.-B., J. T. KORENBROT, and W. STOECKENIUS. 1977. Proton transport by bacteriorhodopsin through an interface film. *J. Memb. Biol.* **36**:137–158.
18. HWANG, S.-B., J. T. KORENBROT, and W. STOECKENIUS. 1978. Transient photovoltages in purple membrane multilayers: charge displacement in bacteriorhodopsin and its photointermediates. *Biochim. Biophys. Acta.* **509**:300–317.
19. SHIEH, P. K. 1978. Photovoltaic cell constructed from bacteriorhodopsin: a direct solar energy conversion into electricity. *Neng Yuan Kan* 8(2), 7–16.
20. EISENBACH, M., C. WEISSMANN, G. TANNY, and S. R. CAPLAN. 1977. Bacteriorhodopsin-loaded charged synthetic membranes: Utilization of light energy to generate electrical current. *FEBS Lett.* **81**:77–80.
21. SHINAR, R., S. DRUCKMANN, M. OTTOLENGHI, and R. KORENSTEIN. 1977. Electric field effects in bacteriorhodopsin. *Biophys. J.* **19**:1–5.
22. KORENSTEIN, R., and B. HESS. 1978. Immobilization of bacteriorhodopsin and orientation of transition moment of purple membrane. *FEBS Lett.* **89**:15–20.
23. HEYN, M. P., R. J. CHERRY, and V. MULLER, 1977. Transient and linear dichroism studies on bacteriorhodopsin: determination of the orientation of the 568 all-trans retinal chromophore. *J. Mol. Biol.* **117**:607–620.
24. FISHER, K. A., K. YANAGIMOTO, and W. STOECKENIUS. 1978. Oriented adsorption of purple membrane to cationic surfaces. *J. Cell. Biol.* **77**:611–621.
25. BROUSSE, C. L., R. CHAPURLAT, and J. P. QUENTIN. 1976. New membrane for reverse osmosis. I. Characteristics of the base polymer: sulphonated polysulphones. *Desalination.* **18**:137–153.
26. OESTERHELT, D., and W. STOECKENIUS. 1974. Isolation of the cell membrane of *Halobacterium halobium* and its fractionation into red and purple membrane. *Methods Enzymol.* **31**:667–678.
27. KORENSTEIN, R., and B. HESS. 1977. Hydration effects on the photocycle of bacteriorhodopsin in thin layers of purple membrane. *Nature (Lond.)* **270**:184–186.
28. KORENSTEIN, R., W. V. SHERMAN, and S. R. CAPLAN. 1976. Kinetic isotope effects in the photochemical cycle of bacteriorhodopsin. *Biophys. Struct. Mech.* **2**:267–276.
29. EISENBACH, M., H. GARTY, E. P. BAKKER, G. KLEMPERER, H. ROTTENBERG, and S. R. CAPLAN. 1978. Kinetic analysis of light-induced pH changes in bacteriorhodopsin-containing particles from *Halobacterium halobium. Biochemistry.* **17**:4691–4698.
30. RACKER, E., and P. C. HINKLE. 1974. Effect of temperature on the function of a proton pump. *J. Membr. Biol.* **17**:181–188.
31. SINGH, K., and S. R. CAPLAN. 1980. Purple membrane and solar energy conversion. *Trends Biochem. Sci.* **5**:62–64.
32. BLAUROCK, A. E., and W. STOECKENIUS. 1971. Structure of the purple membrane. *Nat. New Biol.* **233**:152–155.
33. LANYI, J. K. 1978. Light energy conversion in *Halobacterium halobium. Microbiol. Rev.* **42**:682–706.
34. HELFFERICH, F. 1962. Ion Exchange. McGraw-Hill, New York. 304.