BACTERIORHODOPSIN-LOADED CHARGED SYNTHETIC MEMBRANES

Utilization of light energy to generate electrical current

Michael EISENBACH, Charlotte WEISSMANN, Gerald TANNY* and S. Roy CAPLAN
Department of Membrane Research, The Weizmann Institute of Science, Rehovot, Israel

Received 13 June 1977

1. Introduction

Bacteriorhodopsin from *Halobacterium halobium* is a small protein (mol. wt 25 000 [1]) with unique properties. Upon illumination with light in the visible spectrum it undergoes a series of photocycle-associated conformational changes, which lead to proton pumping from the cytoplasmic to the exterior side of the membrane [2–4]. In addition, the protein is uniquely stable under extreme conditions, such as high temperature [5,6] or extreme pH values [6,7]. These qualities, together with its ease of isolation (in the form of 'purple membrane' fragments [8]) and the low cost of growing these bacteria in large amounts, provided the incentive to attempt its incorporation within a cation selective membrane which would behave as a light energy/electrical current transducer.

Attempts to form synthetic membranes capable of converting light energy to electrical energy have been carried out in several laboratories [9–13]. These attempts utilized modified bilayer lipid membranes, some stabilized by a phospholipid-impregnated Millipore filter [14] or by polystyrene [12]. The resistances of such membranes are in the range 10^8–10^{11} Ω. cm^2. Hence, upon illumination, a potential difference of the order of 100 mV could be measured between solutions on either side of the membranes, but the current generated was only 10^{-12}–10^{-11} A.cm^{-2}. It was the object of the present investigation to attain stable electrical currents several orders of magnitude larger than those observed previously. The approach adopted was to incorporate the bacteriorhodopsin into a cation-exchange hydrogel of acrylamide and acrylic acid, in which the electrical resistance should be very low. However, unlike the situation in lipid bilayer membranes, the gel medium alone does not provide the necessary orientation of purple membrane fragments such that the hydrogen ion pumps are all aligned in the same direction. The solution to this difficulty was to align the bipolar purple membrane fragments in an electrical field during the formation of the gel. To obtain additional ion selectivity, a chlorosulfonated polyethylene film was added in series. On illumination, the composite membrane generated currents (measured under short circuit conditions) of 10^{-6}–10^{-8} A.cm^{-2}. The direction of the proton current through the gel is from the side which was adjacent to the negative electrode towards the side which was adjacent to the positive electrode during the formation period. Thus protons must be released from the more negative sides of the purple membrane fragments.

2. Materials and methods

2.1. *Gel formation*

An aqueous solution of 40% (v/v) acrylamide, 20% acrylic acid (both from BDH Chemical Ltd), 1.8% Bis and 2% Temed (both from Eastman) was freshly prepared at pH 4. The acrylic acid was distilled under vacuum before use, in order to remove the stabilizer.

*Department of Plastics Research, The Weizmann Institute of Science, Rehovot, Israel

Abbreviations: Bis; N, N′- methylenebisacrylamide; MOPS; morpholinopropane sulfonic acid; Temed; N, N, N′, N′-tetramethylethylenediamine

Prior to the polymerization, a concentrated solution of purple membrane (isolated from *H. halobium*, strain M_1, as described by Oesterhelt and Stoeckenius [8]) was added to a final concentration of 13–20 μM. The polymerization was initiated by addition of ammonium persulfate (4% final concentration) (BDH Chemical Ltd.) and a slight heating if required. The polymerization was carried out over both sides of a cotton cloth in a lucite chamber. The cloth was added to increase the mechanical strength of the gel. The chamber, 6.5 mm in width, was constructed of two half-cells each containing a circular platinum electrode and was provided with an electrical field by a Heathkit regulated power supply. The start-up of the electrical field is described in the results. After the initiation of the polymerization the vessel was immersed in an ice-water bath to prevent overheating due to the ongoing reaction.

2.2. *Preparation of the chlorosulfonated polyethylene membrane*

Chlorinated sulfonylchloride polyethylene powder was prepared in the Plastics Department of the Weizmann Institute by reacting SO_2 and Cl_2 under ultraviolet radiation, and was dissolved ($\sim$ 5% w/w) in chlorobenzene with vigorous mixing at 70°C. The solution was poured into a Petri dish and the solvent slowly evaporated in an oven at 70°C, yielding a 45 ± 15 μm thick membrane. To remove the membrane from the dish the sulfonylchloride groups were hydrolyzed at room temperature by immersion in 0.1 M NaOH.

2.3. *Short-circuit current and resistance measurements*

Short-circuit current was measured by means of a voltage clamp using a transparent, thermostated Ussing-type lucite chamber equipped with salt bridges for the usual current-carrying and potential-sensing electrodes. The potential difference across the membrane was sensed by salt bridges placed close to the membrane surfaces and connected to saturated calomel electrodes. Current was carried by salt bridges placed at the apices of the conical half-cells and connected to silver/silver chloride electrodes. Illumination of the membrane from both sides was accomplished by two slide projectors projecting their light at 45° to the plane of the membrane, each supplying a maximum of 750 W/m^2 (quartz iodide lamp 24 V, 150 W). The maximal light intensity was measured with a YSI Kettering 65 A radiometer, but no convenient technique could be found to measure the light flux actually reaching the membrane in the chamber.

The membrane resistance was determined by clamping potential differences of several mV in either direction across the membrane and measuring the current.

3. Results and discussion

As mentioned in the Introduction, in order to construct a bacteriorhodopsin-containing synthetic membrane with low internal impedance several requirements must be met. To begin with, we selected monomers which could be polymerized to a gel in aqueous media without reducing the 'proton pump' activity of bacteriorhodopsin. The gel had to be transparent to allow light to reach the embedded purple membrane fragments. In addition, the gel was required to possess cation-selective properties so that the current generated by proton pumping would not be diminished by a leak of anions in the same direction. Finally, the method of membrane fabrication had to facilitate the orientation of the purple membrane fragments. All these requirements could be met by preparing a copolymer gel of acrylic acid and acrylamide (1:2, v/v) containing bacteriorhodopsin, and orienting the latter in an electric field during the formation of the gel. The ionic selectively of the system was further increased by attaching a highly-selective, cation exchange membrane, chlorosulfonated polyethylene, to the gel. The cation transport number, τ_+, was calculated from the diffusion potential measured with calomel electrodes in a gradient of $10^{-1}/10^{-2}$ M KCl. For the copolymer gel at pH 7 we found $\tau_+ \simeq$ 0.77, while for the chlorosulfonated polyethylene $\tau_+ \simeq$ 0.93.

To examine the effect of the polymerizing solution on the 'proton pump' activity of bacteriorhodopsin, a combined pH electrode was immersed in a small quantity of the reagents and polymerization was initiated. The resultant gel-coated electrode was illuminated and the light-induced pH changes were followed. No difference was found between the light-induced pH changes observed with the coated

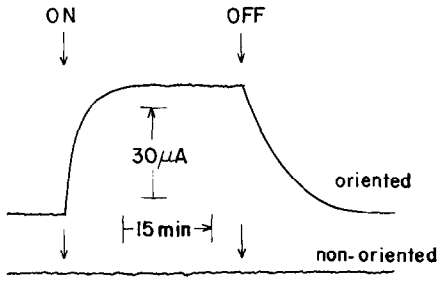

Fig.1. Light-induced short-circuit electrical current in oriented and non-oriented bacteriorhodopsin-containing gels. The preparation of the gel (with 20 μM bacteriorhodopsin), the electrical orientation and the current density measurement with a voltage clamp are described in the text. A chlorosulfonated polyethylene membrane was attached to one side of the gel (in this case the side close to the anode; similar behaviour was obtained with the ion-exchange membrane on the other side). The measurement solution in each half-cell contained 0.1 M KCl and 0.1 M MOPS (pH 7) at 25°C. The surface area of the membrane which was exposed was 7 cm^2.

electrodes dipping in water, and those observed with an uncoated electrode immersed in an aqueous suspension of purple membrane fragments under the same experimental conditions.

Figure 1 compares the electrical response (measured under short-circuit conditions as described under Materials and methods) of electrically-oriented and non-oriented bacteriorhodopsin-containing gels. It is seen that the gel containing oriented bacteriorhodopsin produced a current of 40 μA (equivalent to 5.7 $\mu A.cm^{-2}$) within 5–10 min, while no current could be measured with the gel containing non-oriented bacteriorhodopsin. The current of the 'oriented gel' was stable for 1.5 h or more , and decreased to zero upon turning off the light. These on/off cycles were repeated several times without any observable decrease in the electrical activity. It should be mentioned that the current shown in fig.1 is one of the largest measured, and a considerable variability in gel performance between different preparations existed, with currents ranging from 0.05–7 $\mu A.cm^{-2}$, and membrane resistances between 2×10^2–3×10^4 $\Omega.cm^2$. Nevertheless, these measured currents were orders of magnitude higher than the values reported in the literature (cf. Introduction) and the internal impedance was correspondingly lower.

Upon illumination the direction of the current generated was always the same: the solution on that side of the gel connected to the positive electrode during the electrical orientation step became positive upon illumination. The direction of the current was independent of the direction of the illumination and was not dependent on the side of the gel to which the chlorosulfonated polyethylene had been attached. As the more negatively-charged side is the side which extruded the protons from the membrane, and since the protons are known to be extruded from the external side of the bacteria [15], we conclude that the external side of the bacterial membrane is the more negative side.

The large variability in the current densities measured with different gel preparations indicates the difficulty involved in their preparation, probably because not all the parameters which influence the current are known and under control. One of these parameters is probably the optimal viscosity for electrical orientation. The electrical field (100 $V.cm^{-1}$ for 1–2 min) was applied after the initiation of the polymerization, when the suspension appeared viscous enough to prevent the migration of the acrylic acid molecules and purple membrane fragments in the field, but fluid enough to permit rotation of the fragments. Since no objective criteria were applied, our choice of conditions was necessarily haphazard; fortunately, our results indicate that the hypothesis behind the experiment was correct. The mechanical stability of the gel is also unsatisfactory, as its shape becomes distorted with time, probably because of inhomogeneous swelling or shrinking. Our present research, therefore, is proceeding in two main directions:
(1) To define the optimal conditions and parameters.
(2) To improve the mechanical strength and selectivity of the host synthetic membrane gel.

Acknowledgements

We would like to thank Mr Benjamin Bikson for the generous supply of sulfonylchloride polyethylene powder. This study was supported by a grant from the National Council for Research and Development, Israel and the KFA Jülich, GSF München, GFK Karlsruhe, GKSS Geesthacht-Tesperhude, Germany. One of us (M.E.) is indebted to 'The Bat-Sheva de Rothchild Foundation for the Advancement of Science in Israel' for a research grant.

References

[1] Bridgen, J. and Walker, I. D. (1976) Biochemistry 15, 792–798.
[2] Stoeckenius, W. and Lozier, R. H. (1974) J. Supramol. Struct. 2, 769–774.
[3] Lozier, R. H., Bogomolni, R. A. and Stoeckenius, W. (1975) Biophys. J. 15, 955–962.
[4] Dencher, N. and Wilms, M. (1975) Biophys. Struct. Mech. 1, 259–271.
[5] Mendelsohn, R. (1976) Biochim. Biophys. Acta 427, 295–301.
[6] Eisenbach, M., Garty, H., Rottenberg, H. and Caplan, S. R. (1977), submitted.
[7] Kanner, B. I. and Racker, E. (1975) Biochem. Biophys. Res. Commun. 64, 1054–1061.
[8] Oesterhelt, D. and Stoeckenius, W. (1974) Meth. Enzymol. 31, 667–678.
[9] Drachev, L. A., Kaulen, A. D., Ostroumov, S. A. and Skulachev, V. P. (1974) FEBS Lett. 39, 43–45.
[10] Drachev, L. A., Frolov, V. N., Kaulen, A. D., Liberman, E. A., Ostroumov, S. A., Plakunova, V. G., Semenov, A. Yu and Skulachev, V. P. (1976) J. Biol. Chem. 251, 7059–7065.
[11] Herrmann, T. R. and Rayfield, G. W. (1976) Biochim. Biophys. Acta 443, 623–628.
[12] Shieh, P. and Packer, L. (1976) Biochem. Biophys. Res. Commun. 71, 603–609.
[13] Dancshazy, Z. and Karvaly, B. (1976) FEBS Lett. 72, 136–138.
[14] Skulachev, V. P. (1976) FEBS Lett. 64, 23–25.
[15] Lozier, R. H., Niederberger, W., Bogomolni, R. A., Hwang, S. B. and Stoeckenius, W. (1976) Biochim. Biophys. Acta 440, 545–556.