ELECTROGENESIS BY BACTERIORHODOPSIN INCORPORATED IN A PLANAR PHOSPHOLIPID MEMBRANE

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1. Introduction

In 1966, Mitchell [1] put forward the idea of electrogenesis in coupling membrane systems. According to this concept, there are molecular electric generators incorporated in membranes of mitochondria, chloroplasts, photosynthetic and respiring bacteria. These generators were postulated to represent enzyme complexes charging the membranes by electron or proton transport across hydrophobic barriers. According to Mitchell, the transmembrane electric potential and pH differences generated by enzymes of respiratory or photosynthetic redox chains are utilized by a reversible ATPase to form ATP.

Several years ago, Tupper and Tedeschi [2] tried to detect membrane potentials in mitochondria, using microelectrodes, but failed. The size of the mitochondrion is apparently too small to allow an electrode to be introduced into the matrix space without a sharp decrease in the very high electric resistance of the mitochondrial membrane [3]. Some light-dependent electric responses of a complex character were demonstrated by Bulychev et al. [4] with microelectrodes in isolated chloroplast.

The electric phenomena in coupling membranes were also studied by other methods. To measure the membrane potential in mitochondria, Mitchell and Moyle determined K^+ distribution across the membrane of energized mitochondria whose K^+ permeability was increased by valinomycin [5]. Our group, has described the antiport of synthetic penetrating anions and cations across the membranes of energized mitochondria [6], submitochondrial particles [7, 8], subchloroplast particles [9], chromatophores of photosynthetic bacteria [10] and particles of respiring bacteria [9]. The discovery of this phenomenon defined as transmembraneous electrophoresis [11, 12] was considered to support the idea of electric potential generation in coupling membranes.

An independent line of evidence for membrane potential generation in coupling membranes was presented by authors studying electrochromic spectral shifts of some pigments in chloroplasts [13, 14] and bacterial chromatophores [15].

Development of Racker's methods for reconstitution of the oxidative phosphorylation system from enzymic complexes and phospholipids [16–19] permitted a new approach to the study of membrane potential generators to be used. It was shown that reconstituted cytochrome oxidase- and/or ATPase-containing phospholipid vesicles (proteoliposomes) were capable of carrying out transmembrane electrophoresis of synthetic ions [20–22] and of K^+ (+valinomycin) [16, 18, 19]. A similar effect was found in bacteriorhodopsin proteoliposomes, which suggests that they are also capable of forming a membrane potential [23]. If this were so, bacteriorhodopsin would be the simplest biological system for generating electric potentials. Bacteriorhodopsin, a rather small protein (mol. wt. 26 000) containing retinal, was found in *Halobacterium halobium* membranes by Stoeckenius and coworkers [24, 25]. It occupies special areas in these membrane ('purple sheets') in which no other protein can be demonstrated. So, conversion of light energy into a transmembrane electric field, if it occurs, must be carried out by bacteriorhodopsin as the only protein.

The obvious simplicity of the bacteriorhodopsin generator and its ability to be incorporated into recon-

stituted spherical phospholipid membranes provides a unique opportunity to incorporate this protein into planar phospholipid membranes and to measure electrogenesis with ordinary electrometer techniques.

A planar membrane separating two electrolyte solutions of identical composition was formed from a soya bean phospholipid mixture supplemented with the bacteriorhodopsin-containing purple sheets isolated from *H. halobium*. Illumination of the membrane resulted in the appearance of a transmembrane electric potential difference, the maximal values of this difference being about 50 mV. A protonophorous uncoupler sharply reduced this effect.

2. Methods

Purple membrane sheets of *H. halobium* containing bacteriorhodopsin (for procedures, see [23, 24]) were mixed with a decane solution of soya bean phospholipids (azolectin) containing 70 mg of azolectin per ml.

This mixture was applied to an aperture (1 mm diameter) made in the Teflon wall which separated two electrolyte solutions of identical composition. Electric potential difference across the membrane formed were measured by Ag/AgCl electrodes connected to a vibrating capacitor electrometer RFT VA-J-51 and a KSP-4 recorder.

In the dark, there was no electric potential difference between the two aqueous solutions. Illumination was with a 20 W tungsten lamp whose light was focused on the aperture in the Teflon wall. The light intensity was 5×10^{-7} W per mm^2. To measure the action spectrum, interference filters were used.

3. Results and discussion

The results of a typical experiment are shown in fig. 1. It can be seen (fig. 1A) that illumination of the membrane induced the appearance of an electric potential. Repeated illuminations of the same membrane gave rise to stable, reproducible responses. The addition of a small amount of 2,4,6-trichlorocarbonylcyanidephenylhydrazone (CCCP) sharply decreased the electric potential and changed the form of the light- and dark-induced responses (fig. 1B). Similar changes in the form and magnitude of these responses were observed

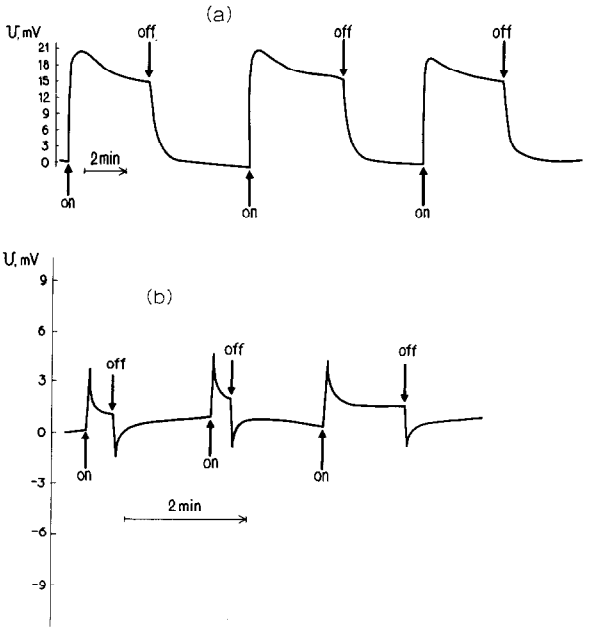

Fig. 1. The light-induced generation of electric potential difference across a planar phospholipid membrane with incorporated bacteriorhodopsin. The membrane separated two solutions of identical composition, namely 0.15 M KCl and 0.01 M Tris–HCl (final pH 6.3). In fig. 1B, the saline solution was supplemented with 2×10^{-7} M CCCP.

when the membrane was shunted with an external resistance.

Both the sign and the magnitude of the photoinduced electric potential varied from membrane to membrane. Maximal values of the light-dependent electric potential were of about 50 mV at a current of about 5×10^{-13} A. Green light was found to be most effective in generating the electric potential. The effect disappeared when the spectral maximum of the illumination was shifted from 570 nm to shorter or longer wave-lengths, indicating that the action spectrum of the photoeffect observed was similar to that of bacteriorhodopsin [23].

Thus, the above experiments have demonstrated that planar bacteriorhodopsin–phospholipid membranes are capable of converting light energy into electric energy. The photoelectromotive force produced by this generator is apparently higher than the maximal values of 50 mV measured. It is highly probable that electric potentials measured were, in fact, the result of the simultaneous actions of many bacteriorho-

dopsin sheets incorporated into the phospholipid membrane. Random orientation of the sheets in the membrane would result in some generating an electric field in one direction and others in the opposite direction. So, the values of the measured membrane potential represents the difference in voltage of the sets of opposed batteries.

Direct measurements of the electric parameters of planar bacteriorhodopsin–phospholipid membranes have confirmed the conclusion reached from studies of the PCB^- responses of bacteriorhodopsin proteoliposomes [23]. There is an obvious similarity in the data obtained by these two methods. In particular, the CCCP-induced modifications of the form of the light responses of proteoliposome and planar membranes were essentially the same (cf. fig. 1B of this paper and fig. 2B of ref. [23]).

These results demonstrate the adequacy of the PCB^- probe for detecting transmembrane electric potentials and create a precedent for the direct measuring of the electric potential formation by a coupling membrane protein.

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References

[1] Mitchell, P. (1966) Chemiosmotic Coupling in Oxidative and Photosynthetic Phosphorylation (Glynn Research, Bodmin).
[2] Tupper, J.T. and Tedeschi, H. (1971) Proc. Natl. Acad. Sci. U.S. 63, 370, 713.
[3] Liberman, E.A. and Skulachev, V.P. (1970) Biochim. Biophys. Acta 216, 30.
[4] Bulychev, A.A., Andrianov, W.K., Kurella, G.A. and Litvin, F.F. (1971) Fisiol. Rast. 18, 248.
[5] Mitchell, P. and Moyle, J. (1969) Eur. J. Biochem. 7, 471.
[6] Bakeeva, L.E., Grinius, L.L., Jasaitis, A.A., Kuliene, V.V., Levitsky, D.O., Liberman, E.A., Severina, I.I. and Skulachev, V.P. (1970) Biochim. Biophys. Acta 216, 13.
[7] Liberman, E.A., Topaly, V.P., Tsofina, L.M., Jasaitis, A.A. and Skulachev, V.P. (1969) Nature 222, 1076.
[8] Grinius, L.L., Jasaitis, A.A., Kadziauskas, J.P., Liberman, E.A., Skulachev, V.P., Topali, V.P., Tsofina, L.M. and Vladimirova, M.A. (1970) Biochim. Biophys. Acta 216, 1.
[9] Grinius, L.L., Il'ina, M.D., Mileykovskaya, E.I., Skulachev, V.P. and Tikhonova, G.V. (1972) Biochim. Biophys. Acta 283, 442.
[10] Isaev, P.I., Liberman, E.A., Samuilov, V.D., Skulachev, V.P. and Tsofina, L.M. (1970) Biochim. Biophys. Acta 216, 22.
[11] Skulachev, V.P. (1970) FEBS Letters 11, 301.
[12] Skulachev, V.P., Energy Accumulation in the Cell (Nauka Press, Moscow, 1969).
[13] Junge, W. and Witt, H.T. (1968) Z. Naturforsch. 23b, 244.
[14] Witt, H.T. (1972) J. Bioenerg. 3, 47.
[15] Jackson, J.B. and Crofts, A.R. (1969) FEBS Letters 4, 185.
[16] Kagawa, Y. and Racker, E. (1971) J. Biol. Chem. 246, 5477.
[17] Racker, E. and Kandrash, A. (1971) J. Biol. Chem. 246, 7069.
[18] Hinkle, P.C., Kim, J.J. and Racker, E. (1972) J. Biol. Chem. 247, 1338.
[19] Racker, E. (1972) J. Membrane Biol. 10, 221.
[20] Jasaitis, A.A., Nenečék, I.B., Severian, I.I., Skulachev, V.P. and Smirnova, S.M. (1972) Biochim. Biophys. Acta 275, 485.
[21] Skulachev. V.P. (1972) FEBS Symp. 28, 371.
[22] Hinkle, P.C. (1973) in: Abstracts of Conf. on Mechanism of Energy Transduction in Biol. Systems, Abstr. No. 7, New York Acad. Sci., New York.
[23] Kayushin, L.P. and Skulachev, V.P., FEBS Letters 39–42.
[24] Oesterhelt, D. and Stoeckenius, W. (1971) Nature New Biol. 233, 149.
[25] Blaurock, A.E. and Stoeckenius, W. (1971) Nature New Biol. 233, 152.