INCORPORATION OF BACTERIORHODOPSIN INTO A BILAYER LIPID MEMBRANE; A PHOTOELECTRIC-SPECTROSCOPIC STUDY

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1. Introduction

Much research has been done in the past five years on purple membrane fragments (PM) containing a rhodopsin-like protein–pigment complex bacteriorhodopsin (BR) from *Halobacterium halobium*. In particular biochemical properties of PM have been intensively examined [1–3]. It now seems evident that BR functions as a high-efficiency photoelectric energy transducer, converting light energy to electric and chemical energies; it works as a light-driven proton pump and directs a new type of photophosphorylation [4–7]. However, such basic problems as the role of the organized arrangement of BR molecules in membrane-electric phenomena, the origin of the protons and the mechanism of proton translocation under illumination have persistently eluded all attempts at elucidation.

Attempts to make model photoreceptor membranes are known in the literature [8–11]. The disadvantages of these reconstituted membranes were their extremely short lifetime, or they were stable only under non-physiological conditions when the bilayer structure might have been drastically modified. As first Skulachev's group was able to incorporate BR into thick (not bimolecular) lipid membranes using proteoliposomes [12,13]. Recently Shieh and Packer have reported similar experiments on lipid membranes stabilized by polystyrene [14].

We describe here a new method for the reconstruction of cell membrane structure and function in bimolecular lipid membranes (BLM) with functionally active PM. These BLMs with PM were very stable and exhibited both large-amplitude photovoltaic and photoconductive effects. The method developed for the incorporation of BR into BLMs certainly offers new possibilities for putting proteins and protein–pigment complexes into bilayer membranes. Photoelectric-spectroscopic studies of such reconstituted membranes might open new vistas in modeling photosynthetic and visual membrane processes as well.

2. Materials and methods

The PM used in these investigations were extracted from cell culture of a *H. halobium* R_1 strain grown in standard medium, as described [1]. Since BLMs from lecithin, oxidized cholesterol and azolectin did not bind any detectable amount of the protein–pigment complex a slightly modified membrane-forming solution (1% w/w lecithin in decane, with 0.025% w/w octadecyl-amine) was used. The membrane forming solution was left to age for several days at 0°C. The positively charged BLMs from this solution were made over a 2 mm diameter hole in a Teflon wall separating 0.1 M NaCl solutions. These octadecylamine-lecithin BLMs thinned down within a few minutes when the bathing solutions were vigorously stirred, and were stable for hours. The electric break-down potential was dependent upon the salt concentration of the bathing solution, usually ranging between 100 and 180 mV. After the black membrane had been formed, a small quantity (10–20 μl) of concentrated suspension of PM was introduced into either aqueous phase and both compartments were continuously well stirred. During the incorporation the BLM resistance changed by about one order of magnitude

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(R_m = 5 X 10^7 ohm·cm^2). A few minutes after addition of PM to the bathing solution a small photoelectric response could be detected upon white-light illumination. The amplitude of the photovoltaic signal gradually developed in time and became time-independent in one to two hours. For the electric measurements a Keithley Type 604 differential electrometer and a Keithley Type 610 CR electrometer were employed. All the measurements were carried out at room temperature.

3. Results and discussion

BLM containing BR (BR–BLM) exhibited both a large-amplitude fast photovoltaic effect and photoconduction (figs.1 and 2). The polarity of the compartment with the BR was negative upon illumination, indicating that, probably protons crossed the membrane, as suggested by several authors [4,5,11]. The amplitude of the photovoltaic response was of the order of 20–60 mV, depending upon the pigment concentration and the intensity of light. The photoresponses of BR–BLMs exhibited a sigmoid-like dependence upon the light intensity in a semilogarithmic plot, for both white-light and monochromatic excitation.

The photovoltaic action spectra of the BR–BLMs revealed some deviations from the absorption spectrum of BR in aqueous solution (fig.3). Interestingly, these deviations coincide with the absorption bands of intrastructural carotenoids [15] indicating that some

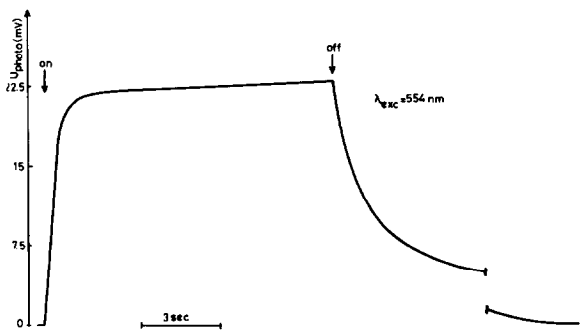

Fig.1. The time-course of the open-circuit photovoltaic response of a bimolecular lipid membrane containing bacteriorhodopsin in the presence of 0.1 M NaCl bathing solution. White-light illumination of 4 X 10^{-3} W. cm^{-2} was used.

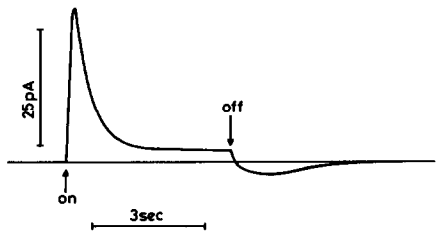

Fig.2. The time-course of the short-circuit photo-response of a bimolecular lipid membrane containing bacteriorhodopsin in the presence of 0.1 M NaCl bathing solution. White-light illumination of 4 X 10^{-3} W. cm^{-2} was used.

carotenoids may in part be involved in a fairly effective shielding or quenching of the photovoltaic process (see also ref. [16]). The mechanism of the action of minute amounts of carotenoids is not clear at all. It is, however, worth mentioning that similar spectral anomalies can be found in the sensory function of BR too [17].

The present preliminary results clearly demonstrate that the purple protein–pigment complex from *H. halobium* (and probably other charged proteins) can be incorporated into oppositely charged lipid bilayer membranes in an oriented fashion and in a functionally active state. The BR–BLMs exhibit large-amplitude photovoltaic response and they supply also steady-state short-circuit photocurrent, i.e., they

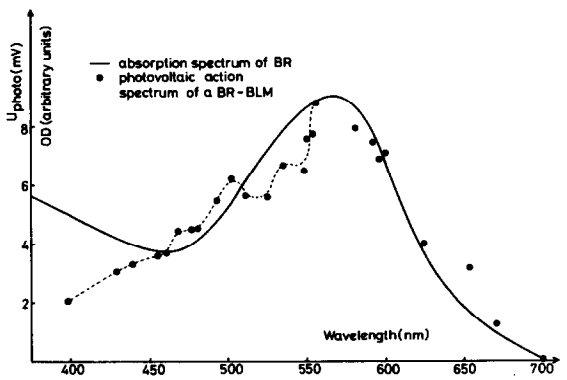

Fig.3. Open-circuit photovoltaic action spectrum of a bimolecular lipid membrane containing bacteriorhodopsin. The action spectrum has been corrected to correspond to equal quantum numbers of incident monochromatic light. Excitation intensity was 10^{13} quanta cm^{-2} s^{-1} at any wavelength. The absorption spectrum of the purple membrane in aqueous dispersion is indicated by the full line.

are functioning as light-energy converters, even in the absence of any further additional substances as electron donors and acceptors and senzitizers. Obviously, the photoelectric processes described above cannot be, for the time being, directly connected to the photochemical cycle of BR; correlating the light-induced membrane-electric phenomena and photochemistry of BR can be expected from flash experiments which are now in progress. This reconstituted membrane-system provides a simple and powerful model for studying the initial photoelectric events in BR-driven photosynthesis and the visual process, and it offers a promising approach for bioanalogous solar cell performance as well.

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