PHOTOCURRENTS OF DARK-ADAPTED BACTERIORHODOPSIN ON BLACK LIPID MEMBRANES

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1. Introduction

The main constituent of purple membranes from *Halobacterium halobium* is bacteriorhodopsin, the light-driven proton pump [1]. The addition of purple membranes (i.e., bacteriorhodopsin) to one side of a lipid bilayer induces on illumination photocurrents [2–6]. The purple membranes are not integrated in the lipid bilayer, but they form, with the supporting black lipid membrane, a sandwich-like structure. This membrane system has been used for measurements under stationary light conditions [2–5]. Photocurrent kinetics induced by a laser flash have been studied: Single steps of the photocurrent could be assigned to the photocycle of bacteriorhodopsin [6]. Bacteriorhodopsin exists in a dark-adapted form, the chromophore, retinal, is in ~1:1 equilibrium between the 13-*cis* and the all-*trans* configuration [7–9], whereas in the light-adapted form almost all of the chromophore exists in the all-*trans* form [10,11]. Here, the change of the photocurrent amplitude during the dark-adaptation process of purple membranes attached to a black lipid membrane is reported.

2. Materials and methods

Purple membranes were a gift of N. A. Dencher (Basel). Lipid bilayer membranes were formed in the usual way in thermostatted teflon cell filled with aqueous electrolyte solution [13] at 30°C. The membrane-forming solution contained 1.2% (w/v) diphytanoyllecithin and 0.025% octadecylamine (Riedel-d'Haen reinst) in *n*-decane. The lecithin was synthezised by K. Janko. After addition of the purple membrane suspension to the aqueous phase the final absorbance was 0.04. The membrane was excited by laser pulses of ~10 ns duration, 575 mm (Dye-laser, Molectron DL 10/UV 12). The energy flash was monitored via a semi-transparent mirror by which half of the light intensity was deflected to a Joulemeter J 3 (Molectron). Therefore, photocurrent and laser flash could be measured simultaneously. This was necessary because the light intensity of the laser flash was fluctuating within a factor of 2. The low light intensity was adjusted to ~60 $\mu J/cm^2$ which corresponds to 1.7×10^{14} quanta/cm^2. Since the absorption cross-section of bacteriorhodopsin is $\sim\sigma = 2.1 \times 10^{-16}$ corresponding to an extinction coefficient $\epsilon = 54\,000$ $M^{-1} . cm^{-1}$ [12] means that $\sigma . n = 3.6 \times 10^{-2}$, so that ~3% of the present bacteriorhodopsin molecules can be excited by a single flash.

Platinized electrodes were used throughout and protected from light. Photocurrent signals were measured by a Burr Brown 3554 amplifier which was directly mounted above the membrane cell inside the Faraday cage. The time resolution of the registration system was set to 100 kHz to reduce the noise level. This low noise level was necessary because a single flash with low light intensity was required for experiments on dark-adapted purple membranes, so the expected photocurrents are small (details in [5]).

3. Results

After addition of purple membrane suspension (50 mm^3) to one side of a preformed bilayer the photocurrent was maximal in ~30 min. After establishment of a maximal photocurrent the membrane was left for 4 h in the dark. After 4 h in the dark, a laser pulse with a light intensity of 10 $\mu J/cm^2$ was applied

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to the membrane system and the resulting photocurrent transient was stored on an oscilloscope. For these experiments the light intensities of the laserflash was chosen such, that only 3% of the bacteriorhodopsin molecules were hit by a photon (see also section 2). The low intensity test pulse was necessary in order to prevent conversion of the dark-adapted form into the light-adapted form. That means <3% of the present bacteriorhodopsin molecules can be converted by the laser test pulse to the light-adapted form.

Fig.1 shows an experiment where photocurrent and light intensity were measured simultaneously. The upper trace represents the photocurrent (A) and the lower trace shows the time course of the light response of the calibrated Joulemeter (B). The peak value of the photocurrent was taken for evaluation. After the measurement of the photocurrent response of dark-adapted bacteriorhodopsin, the membrane system was illuminated with stationary saturating white light (light intensity 10 mW/cm^2). A few seconds after turning off the stationary white light, the membrane was flashed again with the calibrated laser pulse. The value of the peak current was increased by factor of 2 compared to the photoresponse of the dark-adapted form. Fig.2 shows the photoresponse of purple membranes during the dark-adaptation process. After light adaptation, the membrane was put in the dark and in the minute time range intervals the membrane was flashed with the test laser pulses (60 μJ/cm^2).

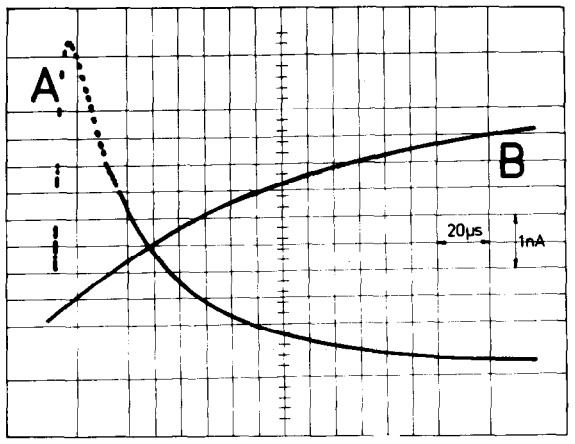

Fig.1.(A) Photocurrent of dark-adapted purple membranes after a laser-flash (60 μJ/cm^2): 0.1 M NaCl; 5 mM Tris (pH 7.0); T = 30°C; illuminated membrane area 5 × 10^{-4} cm^2. (B) Simultaneous determination of the laser-flash energy (see section 2).

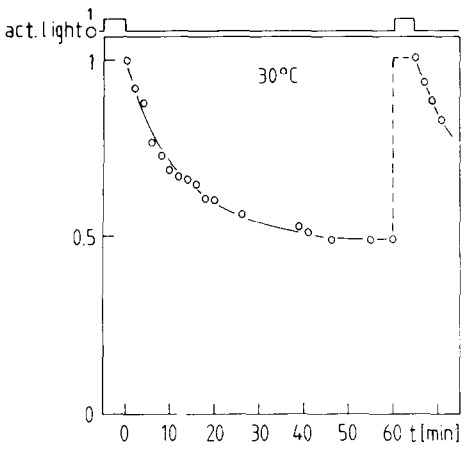

Fig.2. Time course of the change of photocurrent amplitudes during the dark-adaptation process of purple membranes. The amplitude of the photocurrents is normalized to 1. The membrane was first illuminated for 5 min with saturating white light (10 mW/cm^2). After reaching a new stationary photocurrent amplitude the membrane was again flashed with saturating white light. Other experimental conditions as in fig.1.

The photocurrent decays to the half in the absence of light. Fig.3 shows a semilogarithmic plot of the time course of the decay of the photocurrents. A characteristic decay time constant of 14 min 30°C was determined.

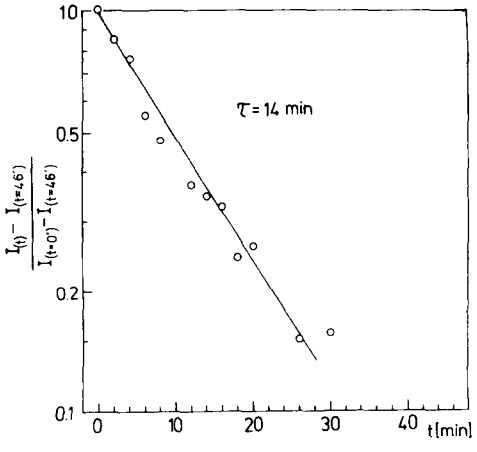

Fig.3. Semilogarithmic plot of the time course of the current amplitude change during the dark-adaptation process. Data taken from fig.2.

4. Conclusions

Obviously, the measurement of proton pump activity of bacteriorhodopsin on black lipid membranes during the dark-adaptation process was possible. Photocurrents reflect directly the pumping mechanism. These photocurrent measurements can be made with high precision [3–5] compared to ΔpH measurements in suspension of purple membranes or purple membranes containing vesicles [10,11]. The reduction of the photocurrents by a factor of 2 during dark-adaptation suggests strongly that bacteriorhodopsin with its retinal in the *cis* configuration does not contribute to the proton movement in the membrane. Furthermore this conclusion is supported by the time course for the reduction of the photocurrent, which is very close to the kinetics of the spectroscopically determined dark-adaptation process.

From these data, it can be derived that a response to a low intensity laser-flash dark-adapted bacteriorhodopsin moves less charges than light-adapted bacteriorhodopsin. During the dark-adaptation process the all-*trans* configuration of retinal is shifted to a new equilibrium between the all-*trans* and the 13-*cis* configuration with a ratio of 1:1. The characteristic time of this process was determined within ~15 min [7], which is in close agreement with the time course of the reaction of the photocurrent described here.

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