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Photocurrents Generated by Bacteriorhodopsin on Planar Bilayer Membranes

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Abstract. When purple-membrane fragments from Halobacterium halobium are added to one aqueous phase of a positively-charged black lipid membrane, the membrane becomes photoelectrically active. Under normal conditions the steady-state photo-current is extremely low, but increases considerably when the lipid bilayer is doped with proton-permeable gramicidin channels or with a lipophilic acid-base system. These findings indicate that the purple-membrane sheets are bound to the surface of the bilayer, forming a sandwich-like structure. The time-behaviour of the photocurrent may be interpreted on the basis of a simple equivalent circuit which contains the conductance and capacitance of the purple membrane in series with the conductance and capacitance of the lipid bilayer. From the dependence of the photocurrent on the polarization of the exciting light the average angle between the transition moment of the retinal chromophore and the plane of the bilayer was calculated to be about 28 degrees. Furthermore, it was shown that chromophore-free apomembrane binds to the lipid bilayer and that its photoelectrical activity can be restored in situ by adding all-trans-retinal to the aqueous phase.

Key words: Photocurrents – Bacteriorhodopsin – Lipid bilayer membranes.

Introduction

Bacteriorhodopsin, a membrane protein from Halobacterium halobium, acts as a light-driven proton pump (Oesterhelt and Stoeckenius, 1973). In the bacterial membrane bacteriorhodopsin forms two-dimensional cristalline patches which may be isolated in form of the so-called purple-membrane fragments. Considerable effort has been spent in the last years to elucidate the structure and the photochemistry of the purple membrane (for recent reviews, see Oesterhelt, 1976; Henderson, 1977). Most of the work so far has been devoted to the analysis of the photochemical reaction cycle of isolated purple membrane fragments in aqueous phase (Oesterhelt and Stoeckenius, 1973; Oesterhelt and Hess, 1973; Lewis et al., 1974; Dencher and Wilms, 1975; Kung et al., 1975; Lozier et al., 1975; Hirsch et al., 1976; Kaufmann

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et al., 1976; Lozier et al., 1976; Sherman et al., 1976; Becher and Ebrey, 1977; Goldschmidt et al., 1977; Marcus and Lewis, 1977; Renthal, 1977; Campion et al., 1977). While these studies have revealed the occurrence of a number of intermediate steps in the overall photocycle, the connection between the single photochemical reaction steps and the proton transfer process is not completely clear. An alternative method to approach this question consists in orienting the purple membrane at an interface and recording the photoelectric response following excitation of the pigment. Various systems and procedures have been used for this purpose such as orientation of purple membrane fragments at the hydrocarbon-water (Hwang et al., 1977) or the teflon-water interface (Trissl and Montal, 1977), binding of lipid vesicles containing purple membrane fragments to lipid-impregnated membrane filters (Blok et al., 1977; Criddle et al., 1977; Blok and Van Dam, 1978; Drachev et al., 1978) or to thick lipid films (Drachev et al., 1974, 1976). A number of studies have also been performed using planar lipid bilayer membranes (Dancsházy and Karvaly, 1976; Herrmann and Rayfield, 1976, 1978; Shieh and Packer, 1976; Packer et al., 1977; Karvaly and Dancsházy, 1977).

In the following we report on experiments in which purple-membrane fragments are combined with planar bilayer membranes using the method of Dancsházy and Karvaly (1976). In this method the interaction of the purple membrane with the lipid bilayer is enhanced by doping the bilayer with a positively-charged detergent (octadecylamine). The results of this study indicate that purple membrane sheets are bound to the surface of the bilayer, forming a sandwich-like structure. A steady-state photocurrent can be observed if the lipid bilayer under the purple membrane is made permeable to protons by the addition of ion channels such as gramicidin A.

Materials and Methods

Purple membrane fragments were isolated according to the method of Oesterhelt and Stoeckenius (1971) from Halobacterium halobium (see also Dencher and Wilms, 1975). The absorbance of the membrane fragments at 280 nm to 568 nm had a ratio of about 1.6 to 1.7. The aqueous suspension of the membrane fragments was stored in the frozen state at −20° C. Before use for the membrane experiments the suspension was sonified for about 3 min at 20° C (Bransonic, Mod. 12). Aliquots of 50 mm^3 of the suspension having an optical density of 0.5 at 570 nm ($l = 1$ cm) were added under stirring to one aqueous compartment of the membrane cell (total volume 10 cm^3). The stirring was continued for about 40 min during the development of photosensitivity. In all experiments bacteriorhodopsin was used in the light-adapted state (Oesterhelt et al., 1973; Sperling et al., 1977) in which the retinal chromophore is present predominantly in the all-trans form.

For the preparation of chromophore-free apo-membrane (Oesterhelt et al., 1974; Becher and Cassim, 1977) a purple-membrane suspension of an optical density of 2 was sonified for 5 min at 20° C (Bransonic, Mod. 12). Thereafter hydroxylamine was added to a final concentration of 0.8 M and the pH was adjusted to 7 by addition of NaOH. The suspension was irradiated at 20° C for about 2 h with light from a 250 W tungsten lamp, filtered through a cut-off filter (Schott OG 530) which removed radiation of wavelengths shorter than 530 nm. The almost colourless suspension was dialysed for about 24 h against distilled water.

Purified gramicidin *A* was a gift of Dr. E. Gross (Bethesda, Maryland, U.S.A.). Carbonylcyanide-p-trifluoromethoxyphenylhydrazone (FCCP) was kindly provided by Dr. Heytler (Dupont de Nemours & Co.). All-trans-retinal was obtained from Fluka (puriss.). All other reagents were analytical grade.

Optically black lipid films of an area of about 1.8 mm^2 were formed in the usual way in a thermostated Teflon cell filled with aqueous electrolyte solution (Läuger et al., 1967). The temperature was 25° C throughout. The membrane forming solution contained 1% (w/v) diphytanoyllecithin (L-1,2 -diphytanoyl-3-phosphatidylcholine) and 0.025% octadecylamine (Fluka, puriss.) in n-decane. The lecithin was synthetized by K. Janko (Janko and Benz, 1977); n-decane was from Merck (standard for gas chromatography). After addition of the purple membrane suspension, the black film was usually stable for many hours. Purified gramicidin *A* was added from a methanolic stock solution to the side opposite to the purple membrane compartment. The membrane cell was enclosed in a Faraday shield and was connected to the external measuring circuit via Ag/AgCl electrodes. In order to avoid photoeffects at the Ag/AgCl electrodes, the electrodes were separated from the membrane cell by salt bridges which were filled with the same electrolyte solution (0.5 mM Tris, pH 7.0 and 0.1 M $MgCl_2$ or 0.1 M KCl) which was present in the cell. In some cases photocurrents were measured directly as a voltage drop across the 1 MΩ input of a Tectronix 7633/7A22 storage oscilloscope. For membranes with higher conductance (e.g., in the presence of uncoupler) and for measurements requiring a high time-resolution a Keithley current amplifier (Mod. 427) was used as a preamplifier between membrane cell and oscilloscope. In all cases the internal resistance of the measuring circuit was much smaller than the membrane resistance. Light from a 150 W or 250 W halogen-tungsten lamp (Osram Bellaphot) with heat-protection filter (Balzers, Liechtenstein) was focussed on the membrane. The light beam passed first through the front compartment, whereas the purple membrane suspension was added to the rear compartment. The action spectrum of the photocurrent was determined using a series of narrow-band interference filters (Balzers, B 40, half-width about 10 nm). In the other experiments a broad-band interference filter (Balzers K 4) with peak transmission at 550 nm and a half-width of 50 nm was used. The light intensity was varied with a series of grey filters (Balzers) and was measured with a calibrated Kipp & Zonen thermopile (Mod. CA 1). The maximum light intensity in the plane of the membrane was about 20 mW cm^{-2}. The light was switched on and off with a photographic shutter (rise time about 10 ms).

For the polarization experiments a membrane cell was used in which the membrane plane was inclined at an angle of 45° with respect to the light beam, whereas the front window of the cell was perpendicular to the beam. Polarized light was obtained using a polaroid filter supplied by Spindler & Hoyer (Göttingen).

Results and Discussion

Time Behaviour of Photocurrents

When a purple membrane suspension is added to one aqueous side of the black film, photosensitivity develops in the course of minutes, but continues in increase slowly during several hours. Records of the short-circuit photocurrent obtained about 3 h

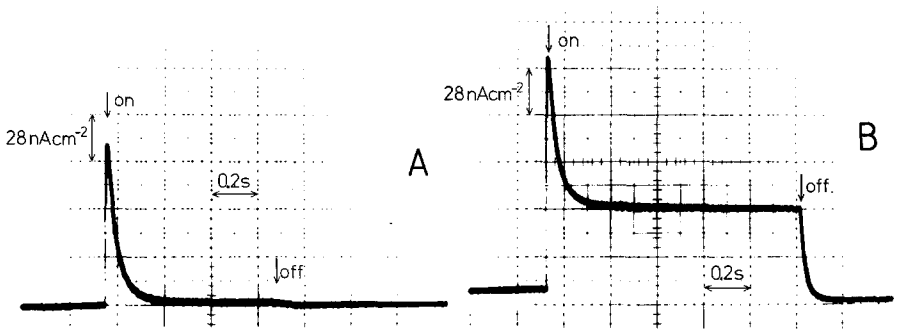

Fig. 1. Short-circuit photocurrent after addition of purple membrane fragments to one aqueous compartment. The aqueous phase contained 0.1 M $MgCl_2$ and 0.5 mM Tris, pH 7.0. The light was filtered through a K4 filter (Balzers, $\lambda_{max} \simeq 550$ nm), yielding a light intensity in the plane of the membrane of about 5.0 mW cm^{-2}. The area of the black film was 1.8 mm^2. **A:** Photocurrent in the absence of gramicidin. The sign of the photocurrent corresponds to a proton transfer toward the bacteriorhodopsin-free side. **B:** After addition of 30 nM gramicidin *A* to the bacteriorhodopsin-free compartment. After switching off the light the current falls to a slightly negative value and approaches zero level (horizontal trace at left) within a few seconds. The record was made about 30 min after the addition of gramicidin; the higher amplitude of the initial current I_0 in record *B* as compared with record *A* is presumably due to the binding of additional purple membrane during this time (a similar increase of I_0 is observed in the absence of gramicidin). The dark conductance of the membrane after addition of gramicidin was about 10 nS cm^{-2}

after addition of the bacteriorhodopsin are shown in Figure 1. The first measurement (Fig. 1A) was carried out in the absence of gramicidin *A* or uncoupler. Under these conditions the black film has an extremely low conductance (of the order of 10 nS cm^{-2}) and is virtually impermeable to protons. After switching on the light the current rises within less than 20 ms and thereafter declines with a time constant of the order of 50 ms to a very low level. The sign of the photocurrent was always the same, corresponding to a proton transfer toward the bacteriorhodopsin-free side. In the second part of the experiment (Fig. 1B) the membrane was doped with gramicidin *A* by adding a small amount of the peptide to the bacteriorhodopsin-free compartment. Gramicidin *A* is known to form channels which are permeable to alkali ions and protons and virtually impermeable to anions and divalent cations (Hladky and Haydon, 1972; Bamberg et al., 1977); at the given composition of the aqueous phases (0.1 M MgCl, 0.5 mM Tris) the only permeable ion is the proton. It is seen from Figure 1B that in the presence of gramicidin *A* a large stationary photocurrent is observed after the decline of the early transient. Still higher photocurrents may be obtained by adding more gramicidin to the membrane, although in this case the photocurrent slowly decreases with time. (This slow component presumably results from changes in the H^+ concentration in the unstirred layers adjacent to the membrane). The highest (quasi)-stationary photocurrents at light saturation (see below) were of the order of 100 nA cm^{-2}. Under steady illumination such photocurrents may be observed for at least 20 min. If a continuous monolayer of purple membrane would be present on the planar film, the total charge corresponding to 1 H^+ per bacteriorhodopsin would be about 1.4 μC cm^{-2}. If a current of 100 nA cm^{-2} flows for several minutes, the transferred charge is far in excess of 1.4 μC cm^{-2}. This

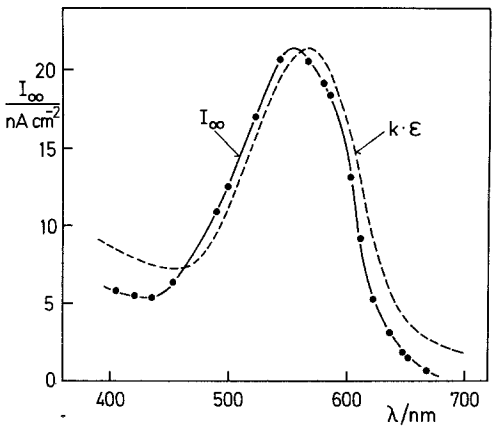

Fig. 2. Action spectrum of the stationary photocurrent I_∞, as measured with a series of narrow-band interference filters. The experimental conditions were similar to those of Figure 1B. Correction factors accounting for the emission spectrum of the lamp and the transmission of the filters were obtained by calibration with a bolometer. The action spectrum was normalized to equal quantum flux density. The dashed line represents the extinction coefficient ε of the purple membrane (Oesterhelt, 1976), normalized to equal peak height by multiplication with a constant factor k

means that a continuous function of the proton pump is observed in these experiments.

Whereas the decay time-constant of the photocurrent in experiments of the type represented in Figure 1A was reproducible, the amplitude I_0 of the current transient varied within a factor of about two from experiment to experiment, when I_0 was measured at a given time after the addition of the purple membrane suspension. This variation of I_0 probably results from differences in the amount of purple membrane adsorbed to the black film.

In Figure 2 the action spectrum of the stationary photocurrent I_∞, as obtained from a measurement with a series of narrow-band interference filters, is compared with the optical absorption spectrum of the purple membrane. The action spectrum is slightly blueshifted (by about 15 nm), but otherwise closely agrees with the absorption spectrum. A simular agreement between the absorption spectrum and the action spectrum of the transient photovoltage has been reported by Dancsházy and Karvaly (1976).

Both the initial current I_0 (which is observed after switching on the light) as well as the stationary current I_∞ saturate with increasing light intensity. This is shown in Figure 3 where the reciprocal values of I_0 and I_∞ are plotted as a function of reciprocal light intensity J. Both I_0 and I_∞ may be represented by the equation

$$I = I^s \frac{J}{J + J_{1/2}} \tag{1}$$

where I^s is the saturation current and $J_{1/2}$ is the half-saturation intensity which is approximately the same for I_0 and I_∞ ($J_{1/2} \simeq 5$ mW cm^{-2}).

The observation that the stationary photocurrent strongly increases upon addition of the channel-forming peptide gramicidin *A* (Fig. 1) argues against the possibility that the purple membrane is incorporated into the black film in such a way that both surfaces come into contact with water. The most likely interpretation of the findings represented in Figure 1 consists in the assumption that purple-membrane sheets are attached to the black lipid film in a preferential orientation (Fig. 4). If the proton permeability of the underlaying black film is low, only a transient capacitive

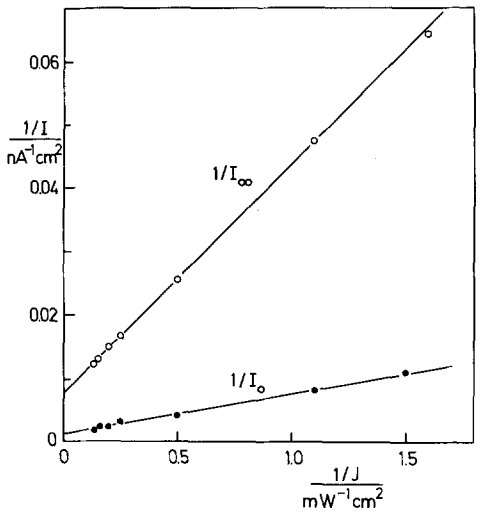

Fig. 3. Reciprocal values of the initial current I_0 (which is measured after switching on the light) and of the stationary current I_∞ as a function of reciprocal light intensity J. White light was filtered with a Balzers K4 filter ($\lambda_{max} \simeq$ 550 nm), the light intensity was varied with a series of calibrated grey filters. All measurements have been performed with the same membrane in the presence of 30 nM gramicidin *A;* the aqueous phases contained 0.1 M $MgCl_2$ and 0.5 mM Tris, pH 7.0

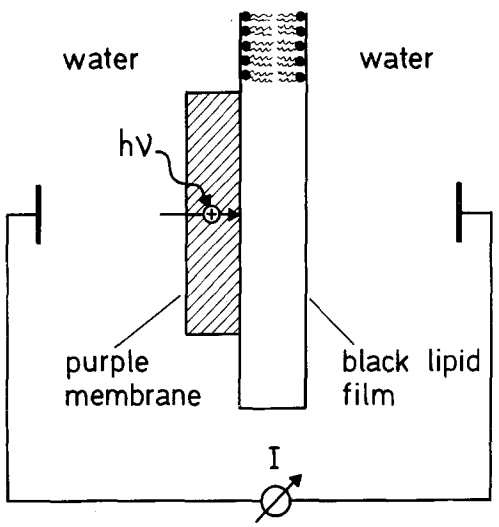

Fig. 4. Proposed structure of the photocurrent generator

photocurrent occurs which creates a voltage drop between the intermediate layer and the aqueous phase. On the other hand, after incorporation of proton-permeable channels into the black film, a permanent photocurrent can flow between the aqueous phases. This model may be analyzed on the basis of an equivalent circuit (Fig. 5) similar to that used by Herrmann and Rayfield (1978) for the interpretation of their experiments with purple membrane vesicles. Light absorbed by bacteriorhodopsin drives a pump current I_p which tends to build up a voltage V_p across the purple membrane. In general, the pump current I_p depends on the voltage V_p; as a first approximation, I_p may be represented as linear function of V_p (Herrmann and Rayfield, 1978):

$$I_p \simeq I_{p0} (1 - V_p/V^*) . \tag{2}$$

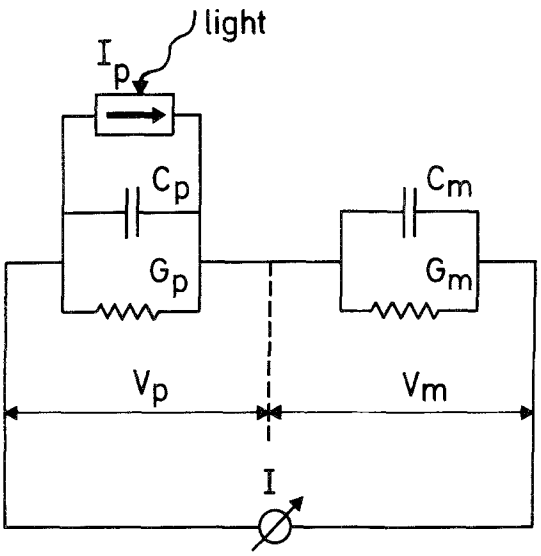

Fig. 5. Equivalent circuit of the model shown in Figure 4. G_p and C_p are the conductance and capacitance of the purple membrane; G_m and C_m are the corresponding values of the underlying part of the black film. Under short-circuit conditions the uncovered areas of the black film can be omitted from the equivalent circuit

I_{p0} is the pump current for $V_p = 0$ and V^* is a constant. (The term V_p/V^* also accounts for gradients of proton activity built-up by the pump which should be roughly proportional to V_p.) Under short-circuit conditions the relation $V_p = V_m$ holds, where V_m is the voltage across that part of the black film which is covered by the purple membrane. G_m and C_m are the conductance and capacitance of the covered part of the black film, and G_p and C_p are the corresponding values of the purple membrane. (Under short-circuit conditions where the total voltage across the membrane vanishes the uncovered parts of the membrane may be omitted in the circuit analysis.) G_p not only contains the conductance across the purple membrane sheet but also accounts for any leakage pathway within the contact layer between black film and purple membrane. For simplicity we disregard any variation of V_p within this layer, considering V_p as a spatial average. With these assumptions, simple circuit analysis may be used to calculate the time course of the externally measured current I and of the voltage V_p after switching on the light at time $t = 0$. The result reads

$$I(t) = I_\infty + (I_0 - I_\infty) \exp(-t/\tau), \tag{3}$$

$$I_0 = I_{p0} \frac{C_m}{C_m + C_p}, \tag{4}$$

$$I_\infty = I_{p0} \frac{G_m}{G_m + G_p + I_{p0}/V^*}, \tag{5}$$

$$\tau = \frac{C_m + C_p}{G_m + G_p + I_{p0}/V^*}, \tag{6}$$

$$V_p = V_p^\infty [1 - \exp(-t/\tau)], \tag{7}$$

$$V_p^\infty = \frac{I_{p0}}{G_m + G_p + I_{p0}/V^*} = \frac{\tau I_0}{C_m}. \tag{8}$$

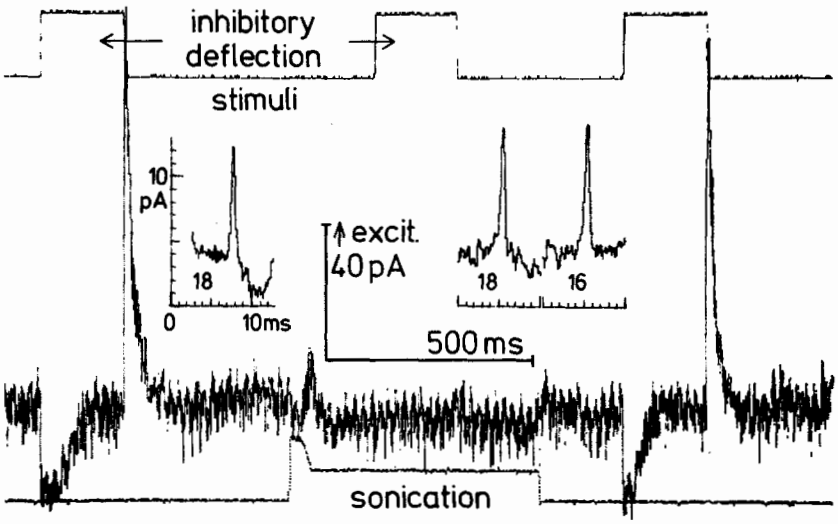

Fig. 6. Suppression of responses (recorded in voltage clamp) to inhibitory deflection stimuli by simultaneous sonication of the cercus; insets: comparison of mean spike amplitudes before, during and after sonication. After the onset of the sonication driving signal the frequency was tuned to the resonance of the transducer. The resulting drop in the envelope signal (due to source output impedance) coincides with an increase in intensity of sonication; "noise" in receptor-current registration mainly due to superimposed spikes. *Upper trace:* deflection stimulus; *medium trace:* receptor current, excitatory upward; *bottom trace:* sonication mark. *insets:* mean shape of all spikes from 200 ms-intervals before, during, and after sonication; numbers of averaged spikes indicated, inverted with respect to current trace

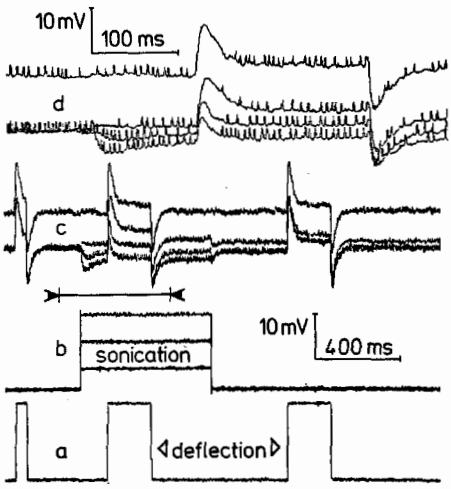

Fig. 7. Partial suppression of responses to inhibitory deflection stimulus by sonication of the hair; **a:** deflection, **b:** sonication, **c:** receptor potentials, *top:* control, **d:** detail above region between arrows in **c:** spike amplitudes in these traces were amplified as indicated in Fig. 4. Note that sonication with increasing intensity first elicits inhibitory responses, but then increasingly excitatory ones, whereas the reduction of the responses to deflection depends monotonously on the sound intensity

However, sonication of the cercus and sonication of the hair (see Methods) diminished the responses to deflection with varying effectiveness and in different ways.

Sonication of the cercus reduced the saturation levels for inhibitory and excitatory stimuli by suppressing the responses to all deflection amplitudes to

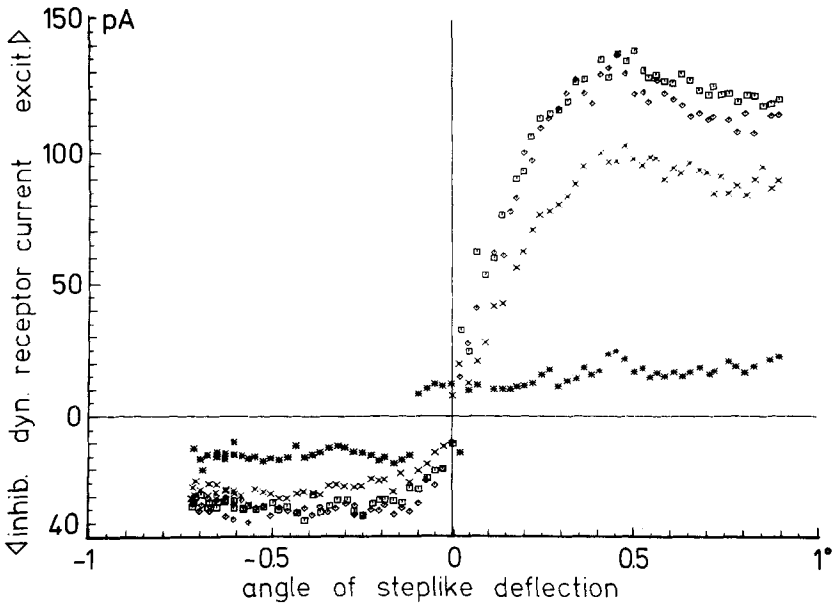

Fig. 8. Dynamic characteristic for two intensities of cercus sonication; stimulus program as in Fig. 6. *Rectangles and rhombi:* control responses without sonication; *crosses:* responses during low-intensity sonication; *asterisks:* responses during high-intensity sonication; remaining deviation from zero (and gap around zero in the other curves) due to noise-peak detection by the evaluating computer program

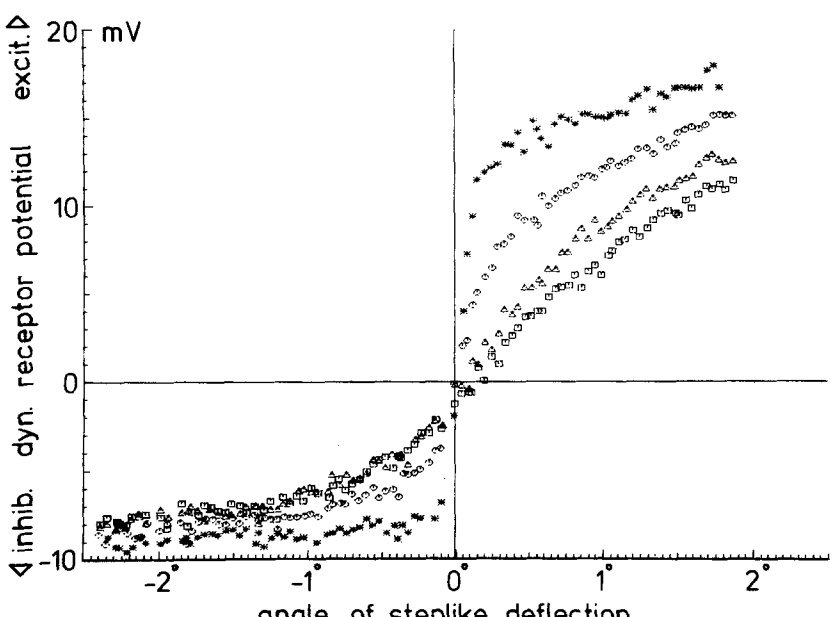

Fig. 9. Dynamic characteristic for three intensities of hair sonication; stimulus program as in Fig. 7. *Asterisks:* control; ellipses, triangles, rectangles: three intensities of sonication during deflection

brane may be estimated to be in the range of 1–5 μF cm^{-2} (as the purple membrane contains only about 25% lipid, the value of $\bar{C}_p$ is probably higher than the specific capacity $\bar{C}$ 1 μF cm^{-2} found for most biological membranes). Using $\bar{C}_p \simeq 2$ μF cm^{-2}, the experimental value of τ_0 ($\tau_0 \simeq 0.2$ s) yields $(\bar{G}_m + \bar{G}_p) \simeq 10$ μS cm^{-2}. $\bar{G}_m$ is much smaller than this value (of the order of 10 nS cm^{-2} for a planar membrane in the absence of gramicidin or FCCP) and may be neglected here so that $\bar{G}_p$ is estimated to be about 10 μS cm^{-2}. As mentioned earlier, G_p contains a contribution from the leakage conductance so that $\bar{G}_p \simeq 10$ μS cm^{-2} should be regarded rather as an upper limit for the specific conductance of the purple membrane.

From the extrapolated value of τ at high light intensity ($\tau \simeq 5$ ms), and using Equation (13), the ratio $\bar{I}^s_{p0}/V^*$ is estimated to be about $5 \cdot 10^{-4}$ A cm^{-2} V^{-1}. $\bar{I}^s_{p0}$ is the short-circuit photocurrent at light saturation, referred to unit area of the purple membrane. From the structure of the purple membrane (Henderson, 1975) a density of bacteriorhodopsin molecules of about $9 \cdot 10^{12}$ cm^{-2} is calculated. If it is assumed that the purple membrane which is attached to the planar bilayer, has a similar turnover rate as in the free state (~ 100 s^{-1}; Lozier et al., 1975), $\bar{I}^s_{p0}$ should be about 100 μA cm^{-2}. This would mean that V^* is of the order of 0.2 V. These values of V^* and $\bar{G}_p$ should be regarded as tentative, however, in view of the approximative nature of the equivalent-circuit description (see below).

From the limiting value of the initial photocurrent at light saturation, I^s_0, the maximum pump current I^s_{p0} of the purple membrane may be calculated according to Equation (4):

$$I^s_{p0} = I^s_0 \left(1 + \frac{C_p}{C_m}\right) = I^s_0 \left(1 + \frac{\bar{C}_p}{\bar{C}_m}\right). \tag{15}$$

With $\bar{C}_m \simeq 0.4$ μF cm^{-2} and an estimated value of $\bar{C}_p \simeq 2$ μF cm^{-2} (see above), the factor $(1 + \bar{C}_p/\bar{C}_m)$ becomes equal to 6. The largest values of I^s_0 which have been observed in these experiments were of the order of 800 nA cm^{-2}. This gives a maximum of $I^s_{p0} \simeq 5$ μA cm^{-2}. This value may be compared with the calculated pump current $\bar{I}^s_{p0}$ of the purple membrane, which is of the order of 100 μA cm^{-2} (see above). Thus, the actual pump current I^s_{p0} is by a factor of about 20 smaller than the pump current $\bar{I}^s_{p0}$ which could be delivered by a black film uniformly covered by a monolayer of purple membrane. There are several possible reasons for this large difference between I^s_{p0} and $\bar{I}^s_{p0}$. a) The rate of the photochemical cycle of the purple membrane bound to the black film may be less than the rate which was observed with purple membrane suspensions (100 s^{-1}) and which was used for the calculation of $\bar{I}^s_{p0}$. b) The fraction γ of film surface covered with purple membrane may be low. An attempt was made to estimate γ by measuring the electrical capacitance of the film before and after the addition of purple membrane. Both capacitances were found to be the same within the experimental error limits (about 3%). This result, however, does not necessarily mean that γ is small, since the purple membrane presumably has a much larger specific conductance and also a larger specific capacitance than the black film (see above). c) It is conceivable that the purple-membrane fragments are bound to the lipid bilayer with either orientation and that there is only a slight preference for one orientation over the other. This would mean that the observed photocurrent is the difference of two almost equal but opposite currents. In

our experiments the direction of the photocurrent corresponds to a preferential binding of the extracellular side of the purple membrane to the bilayer surface. Recently, Fisher et al. (1978) reported that purple-membrane sheets preferentially adsorb with the cytoplasmic side to positively charged, polylysine-treated glass surfaces. On the other hand, they observed that cationic ferritin preferentially binds to the extracellular surface of the purple membrane. These findings suggest that both the cytoplasmic and the extracellular surface of the purple membrane have an affinity for positive charges and that the preference for one or the other orientation in a binding experiment depends on secondary interactions. Furthermore, we cannot exclude that stacks of two or more purple-membrane sheets form on the bilayer surface and that the photoeffects of the single sheets in such a stack partially cancel each other.

Although the equivalent circuit of Figure 5 approximately describes the current transient after switching on the light, it does not account for the observed off-response. For undoped membranes, i.e., in the absence of gramicidin or FCCP, $G_m/(G_m + G_p)$ is of the order of 10^{-3} and $C_m/(C_m + C_p)$ of the order of 10^{-1}; Equation (9), together with Equation (8) and (14), would then predict that after switching off the light a negative current-transient should be observed with an amplitude of $-I_0\tau/\tau_0$ and a decay time-constant of $\tau_0 = \tau_{off}$. In the experiment represented in Figure 1A, no such off-response is observed, however. In other experiments with undoped membranes, positive or negative current transients of small amplitude occured after switching off the light, but in a irreproducible manner. The reason of this erratic behaviour of the off-response is not known at present.

Polarization Effects

When the exciting light is linearly polarized, the rate of light absorption is proportional to the average value of $\cos^2 \psi$ where ψ is the angle between the electrical vector of the radiation and the transition moment of the chromophore. Therefore, at small light intensities (outside the saturation region) the photocurrent should be proportional to $\cos^2 \psi$. Assuming that the purple-membrane sheets have no preferential orientation within the plane of the bilayer, the calculation of $\cos^2 \psi$ leads to the following expression for the photocurrent I (Steinemann et al., 1972):

$$I(\theta) = I^*(A + B \cos^2\theta) , \tag{16}$$

$$A \equiv \frac{1}{2} \sin^2\alpha \sin^2\beta + \frac{1}{4} \cos^2\beta (1 + \cos^2\alpha) , \tag{17}$$

$$B \equiv \frac{1}{4} \sin^2\alpha (3 \sin^2\beta - 1) . \tag{18}$$

θ is the angle between the electrical vector of the light and the plane of incidence (Figure 8), α the angle between the normal n to the membrane and the light beam, β the angle between the transition moment and the plane of the membrane, and I^* is a constant. In the derivation of Equations (16)–(18) it has been assumed that the angle β is identical for all chromophores, i.e., the possibility that the directions of the transition dipoles are distributed over a certain range (Frehland, 1976) is excluded.

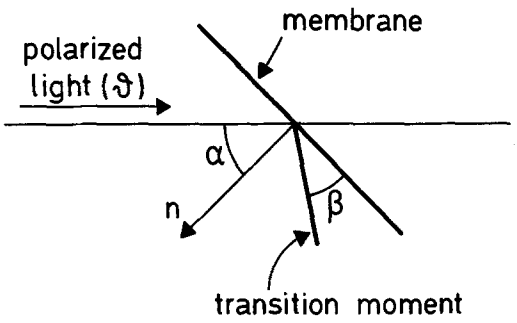

Fig. 8. Excitation with polarized light. n is the normal to the membrane, θ is the angle between the electrical vector of the light and the plane of incidence (the plane formed by n and the light beam)

Furthermore the effects of exciton coupling are neglected (Heyn et al., 1977). From Equations (16)–(18) the socalled dichroic ratio D may be calculated:

$$D \equiv \frac{I(0)}{I(90°)} = 1 + \sin^2\alpha\,(2\tan^2\beta - 1)\,. \tag{19}$$

In all experiments the tilt angle of the membrane with respect to the light beam was $\alpha = 45°$. For the representation of the results it is convenient to plot the ratio $I(\theta)/I(45°)$ which is obtained for $\alpha = 45°$ from Equations (16)–(18) as

$$\frac{I(\theta)}{I(45°)} = 1 + \frac{3\sin^2\beta - 1}{3 - \sin^2\beta}\cos^2\theta. \tag{20}$$

In Figure 9 experimental values of $I(\theta)/I(45°)$ have been plotted as a function of $\cos^2\theta$. In these experiments the bilayer membrane was doped with gramicidin A. The steady-state photocurrent I_∞ was measured at low light intensity J where I_∞ was proportional to J. From the slope of the straight line in Figure 9 the tilt angle between the transition moment and the plane of the bilayer membrane was calculated to be $\beta = 27.5°$ using Equation (20). This value may be compared with previous dichroism studies of oriented samples of purple membrane. Bogomolni et al. (1977) determined a value of $\beta = 23.5° \pm 2°$ from experiments with molecular films of purple membrane at the air-water interface, whereas linear-dichroism studies with oriented multilayers on a glass or quartz substrate gave $\beta = 19° \pm 4°$ (Heyn et al., 1977) and $\beta \leqslant 27°$ (Korenstein and Hess, 1978). Thus, the value of β determined from the bilayer experiments is somewhat larger than β values obtained from purple-membrane layers on water or on a solid substrate. A possible explanation may be that the orientation of the chromophore is changed by the interaction of the purple membrane with the lipid film. A more trivial explanation should also be discussed, however. Although the experiments have been carried out in such a way as to minimize straight light in the membrane cell, we cannot completely exclude the possibility that the exciting light was depolarized to a small degree. According to Equation (20) this would mean that the true value of β would be somewhat lower than 27.5°.

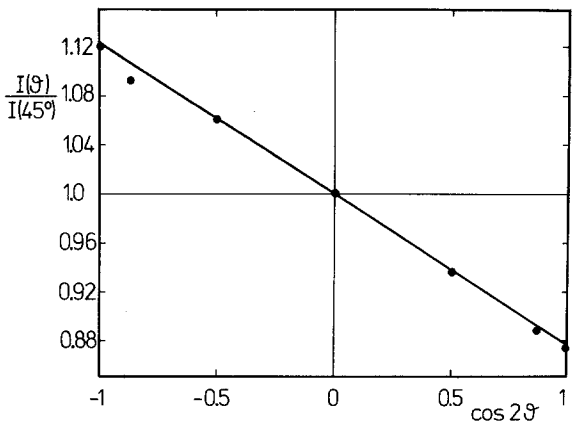

Fig. 9. Photoelectric effect with linearly polarized light. $I(\theta)$ is the steady-state photocurrent (I_∞) at low light intensity (0.05 mW cm^{-2}) and θ is the angle between the electrical vector of the light and the plane of incidence (Fig. 8). The light was filtered through a Balzers K4 filter ($\lambda_{max} \simeq 550$ nm). To avoid light scattering in the cuvette, the light beam was focussed and centered on the membrane plane. The diameter of the light beam was about 0.8 mm, whereas the membrane diameter was 1.8 mm. θ was varied by rotating the polaroid filter which was interposed between light source and membrane. The aqueous solutions contained 0.1 M $MgCl_2$ and 0.5 mM Tris, pH 7.0. 30 nM gramicidin *A* was added to the bacteriorhodopsin-free compartment. The level of the aqeous phases on both sides of the membrane was carefully controlled in order to keep the membrane planar. The experimental data were corrected for the residual polarization of the light source and the optical system in the following way. The membrane was replaced by a bolometer and the bolometer signal $S(\theta)$ was measured as a function of the rotation angle θ of the polaroid filter. The ratio $I(\theta)/I(45°)$ was corrected by multiplying the measured value of $I(\theta)/I(45°)$ by the factor $S(45°)/S(\theta)$. The correction was opposite to the polarization effect on the photocurrent and the deviation of $S(45°)/S(\theta)$ from unity was less than 0.06. $I(\theta)$ was symmetrical with respect to $\theta = 0$ within the limits of experimental error; the data points represent the average of $I(+\theta)$ and $I(-\theta)$

Apo-Membrane Experiments

When an apo-membrane suspension was added to the membrane cell under the same conditions as in the purple-membrane experiments, only a small photosensitivity developed within 3–4 h. This residual photoeffect probably resulted from small amounts of unbleached bacteriorhodopsin still present in the preparation. When all-trans-retinal was added to the aqueous compartment where the apo-membrane had been introduced before, a pronounced photosensitivity developed within about 10 min (Fig. 10). Addition of all-trans-retinal without previous addition of apo-membrane had no effect. In agreement with previous findings with membrane suspensions (Oesterhelt et al., 1974; Becher and Cassim, 1977) this experiment demonstrates that the photosensitivity of the bleached membrane is restored by addition of retinal. The reactivation takes place in a much shorter time than the development of the photosensitivity after addition of purple-membrane suspension to the cell. This strongly suggests that apo-membrane bound to the planar bilayer is reactivated in situ.

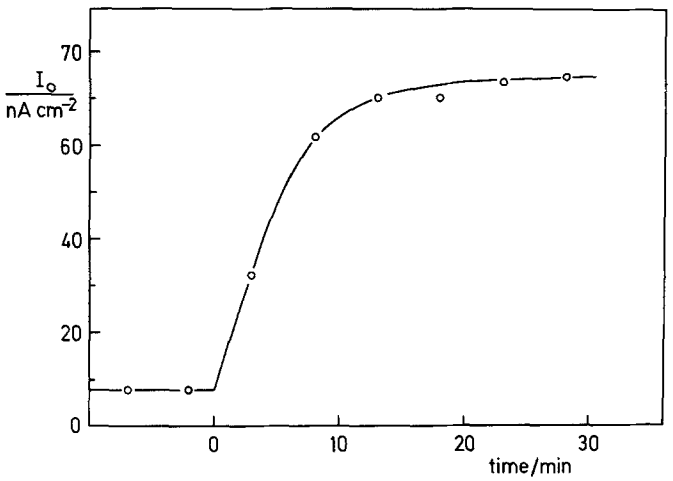

Fig. 10. Reactivation of photoactivity of the bleached membrane. At time $t = -3.5$ h a suspension of apo-membrane fragments was added to one aqueous phase to a final concentration of about 5 μg/cm^3. The aqueous solutions contained 0.1 M $MgCl_2$ and 0.5 mM Tris, pH 7. During the period of 3.5 h only a small photosensitivity developed. I_0 is the amplitude of the photocurrent transient measured under short-circuit conditions after switching on white light of a intensity of about 3.0 mW cm^{-2}. At time $t =$ 0, 10 mm^3 of a 2 mM solution of all-trans-retinal in ethanol were added under stirring to the same compartment (volume about 10 cm^3) where the apo-membrane suspension has been introduced before

Conclusion

The experiments described above indicate that the interaction of purple-membrane fragments with positively-charged planar bilayers leads to the formation of a sandwich-like structure in which the purple membrane is bound to the surface of the bilayer. The main result leading to this conclusion is the finding that the steady-state photocurrent is extremely low under normal conditions, but increases considerably when the lipid bilayer is rendered proton permeable by addition of a proton transport system, such as gramicidin *A* or a weak lipophilic acid. These observations would be difficult to explain assuming that the purple membrane is incorporated in the lipid bilayer in such a way that both surfaces are in contact with water. From the dimensions of the gramicidin *A* channel which has a length of about 2.5–3.0 nm and a diameter of about 1.5–2.0 nm and from the tight lattice structure of the purple membrane it seems rather unlikely that gramicidin channels may form within the purple membrane. The main effect of gramicidin presumably consists in providing a pathway for protons through the lipid bilayer supporting the purple membrane. In this way continuous proton pumping under steady illumination becomes possible.

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